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ABSTRACTS

Summaries of invited lectures, oral and poster presentations given at the Eleventh Hellenic Phytopathological Congress Preveza, Greece, October 1–4, 2002

The 11th National Phytopathological Congress, organised every two years by the Hellenic Phytopathological Society (HPS), was held in Preveza, Greece on October 1–4, 2002, celebrating the 25th anniversary of the HPS. This meeting was attended by more than 400 participants, and 60 oral presentations, 51 posters and 5 invited speeches were presented dealing with plant diseases caused by fungi, bacteria, viruses, and non-parasitic disorders and with disease control. In addition, one round-table discussion was held on "Applied plant pathology: long-lasting problems and possible solutions". Abstracts of the oral presentations, the invited papers and the posters of the congress are presented in this issue.

Biological and integrated control

Invited lectures

The methyl bromide crisis: lessons to be learnt and potential solutions. J. Katan. *Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel.*

Soil pests such as bacteria, fungi, nematodes, parasitic plants, arthropods and other organisms frequently cause heavy losses to major crops, affecting both yield and quality. In severe cases, they may totally destroy the crop, forcing the farmer either to abandon the land or to shift to less susceptible, but often less profitable crops. In intensive agriculture, especially, protected crops are frequently planted and replanted in the same land, sometimes for years in succession, thus leading to a rapid buildup of pest populations in the soil, especially those causing root diseases. There is, therefore, a need to develop effective soil-pest control methods to ensure crop productivity and yield stability. In addition to being effective, these methods have to be economically, environmentally and technologically sound.

Soil disinfestation is one way to control soilborne pathogens causing root and other diseases, and is especially common with high-value crops. It is a sophisticated and expensive, but effective method, which has great advantages, but also some limitations. The basic principle is to eradicate a wide spectrum of harmful agents in the soil before planting, usually by drastic chemical or physical means, while minimizing the damage to beneficial microorganisms.

Methyl bromide (MB) is the major soil fumigant in use worldwide and is highly effective in controlling a variety of soilborne pests, especially in intensively grown crops. However, because of its role in the depletion of the ozone layer, international agreement has been

reached to reduce MB consumption and to phase it out completely by the year 2005 in developed countries. Hence there is a need to develop means to reduce its use now, and to find alternatives to it. MB has several advantages: it has a broad spectrum of control against a wide variety of pests, it can be applied under a range of conditions and has behind it several decades of experience during which methods of its application were developed to a number of intensively grown crops. Because of its high effectiveness, MB was widely adopted and the search for alternatives was often, though fortunately not always, neglected. Many intensively grown crops thus became wholly dependent on MS. If by 2005 a suitable alternative to MB is not found, a severe loss by soilborne pests can be expected. Strawberry grown in California and other places is such an example.

One way of reducing MB dosages without loss of effectiveness is by using plastic sheets that are less impermeable to MB, thus delaying its escape into the atmosphere. Lower dosages can also be achieved by improving the method of application and by combining MB with other methods of control.

Many potential and existing alternatives to MB, both non-chemical and chemical, are available, but no single alternative can totally replace MB in all circumstances. Since MB controls a wide range of pests, an integrated pest management (IPM) approach is called for. IPM aims at integrating all the available, effective and environmentally acceptable methods of management, using them only when necessary with due regard to the environmental, social, economic and legal requirements of each. A combination of methods, which is the heart of IMP, can give a wide spectrum of effective control. A suitable combination can become an alternative to MB. However, it will be necessary to have different combinations for different situations, and that is less convenient than having only one method. Potential methods to be used in such combinations are fumigants at low dosages combined with solarization or cultural or biological control methods, soil solarization alone, biological control, the use of resistant plant varieties, grafting and particular cultural practices. For the future, a dependence on any single method of control should be avoided, in favour of alternating and/or combined approaches, and non-chemical methods of control should also be included. The alternatives that will be developed have to be made known and available, and education and extension tools also need to be further developed, to ascertain that they are appropriately introduced for the farmers to use.

The task of developing effective, economically feasible and safe alternatives to MB for the various crops is difficult and complex, but seems realisable with correct planning and the necessary investment in research, development and manpower. It is not known, however, whether MB alternatives for all major crops will be ready by 2005. Success in this task will serve as a model for solving similar problems with other pesticides in the future, while failure will result in severe economic losses. *We should make every effort to avoid replacing MB with chemicals or other alternatives that may have negative or unknown consequences for human health or the environment.* It is essential to avoid replacement of MB with methods that may be more damaging than MB itself.

Chemical and non-chemical alternatives to Methyl Bromide in Italy. G. Cartia. *Dipartimento di Agrochimica e Agrobiologia, Università degli Studi Mediterranea di Reggio Calabria, Piazza S. Francesco 2, 89061 Gallina (Reggio Calabria), Italy.*

In intensive agriculture, from the point of view of crop protection, vegetables are continuously planted and replanted in the same field, leading to a rapid buildup of soilborne pests (SPs) attacking the plant roots. Because of our climatic conditions, many pathogens induce diseases in more than one major crops, causing damage and economic losses. Heavy damage is also caused by nematodes in the genera *Meloidogyne*, *Dytilenchus* and *Pratylenchus*, and by weeds.

To ensure stable crop production and yield it is necessary to treat soil with steam or fumigants so as to reduce disease damage to below tolerance limits. The high cost of soil disinfestation by steam restricts its use to high-value crops, therefore soil fumigation has become the most common approach for SP control. Chemicals allowed for soil-disinfestation prior to planting are very few (methyl bromide, metham sodium, 1,3 dichloropropene and dazomet) and of these methyl bromide (MB) was the most widely used, due to its relatively short treatment periods and high effectiveness against pests. Unfortunately MB has been found to contribute to stratospheric ozone depletion, so it will gradually be phased out (except for critical use) from now until 2005. Researchers have to play an important role in ensuring the continued safe use of MB at progressively lower dosages until its complete ban. It has been found that in soil naturally infested with pathogens and nematodes, MB is still active at dosages of 40 and 20 g m^{-2}, when the soil is covered with films virtually impermeable (VIFs) to gas (Cartia and Minuto, 1998).

In Italy, studies on controlling diseases of tomatoes and vegetables with methods alternative to MB started in 1980, with soil solarization (SS) (Garibaldi and Cartia, 1991). Studies carried out in our climatic conditions over a twenty-year period, showed that this method was effective in reducing the population density of many pathogens and nematodes.

To optimize alternatives to MB and improve knowledge and techniques of soil solarization, a project (POM, A12) was carried out. The project was implemented under the supervision of the Department of Agrochemistry and

Agrobiology and financed by the European Community, and was designed to be carried out in various regions, and to involve five research institutes and researchers from many different fields (pathologists, nemathologists, physicists, engineers) as well as the extension services from Apulia, Calabria and Sicily (Di Primo *et al.,* 1999). Nurserymen, fumigation and pest control companies, mulching film manufacturers, producers of machinery applying films to the field, film recycling industries and farmers were also involved. Central to the project was SS applied alone or as part of an integrated system (IPM) including: low dosages of chemicals; manure (biofumigation); biological antagonists; resistant/tolerant varieties and rootstocks. The project aims to reduce the cost and increase the quality and environmental safety of agricultural products.

During the three-year period 1998–2001 studies were carried out on:

- thermal regimes in soils subject to solarization (Gutkowski and Terranova, 1991);
- the effectiveness of innovative mulching films intended to enhance SS effects (Di Primo and Cartia, 1998);
- the effectiveness of biological agents against root rot (Cartia and Causarano, 1996);
- the efficacy of SS and IPM in controlling soilborne pathogens and nematodes (Cartia *et al.,* 1997).

Biofumigation (BF), combining SS with organic amendments, offers additional options for SP management in its chemical, physical and biological aspects. Additives such as chicken manure releasing volatile ammonia and increasing soil temperature, controlled overwintering forms of SPs more effectively, than soil solarization alone (Di Primo and Cartia, 1998).

Mulching film is an important factor in creating favourable conditions for SS and in reducing soil reinfestation. Recent studies in this area indicate the important role of coextruded plastic film with different photometric properties and color, in comparison with traditional PE mulching film. Green coextruded film (GCF), which also inhibits weeds, can be left in the field after solarization to reduce reinfestation by pathogens in the solarized soil (Cascone *et al.,* 2000).

Ethylene tetrafluoroethylene (ETFE) is a greenhouse cover film that improves soil thermal regimes and SS effectiveness (Cascone *et al.,* 2001).

Space structural solarization (SSS) is a promising disease management technique that is a complementary soil disinfestation to control inoculum surviving on greenhouse structures. Control was achieved in closed tunnel or greenhouses, during July– August, when air temperatures reached as high as 60–65°C.

To verify the effectiveness of alternative methods to MB, various experimental trials to control SPs of tomato, melon, onion, and carrot were carried out. The main results are given below.

Tomato

Fresh greenhouse tomatoes are a major vegetable crop in Italy. Among SPs of tomato, the most harmful are, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), *Pyrenochaeta lycopersici* and nematodes (*Meloidogyne* spp.), causing Fusarium crown and root rot (FCRR), corky root, and root-knot respectively.

Preliminary studies in the open field showed that 12 days of SS significantly reduced the survival of FORL propagules. Control effectiveness was improved by combining SS with manure, or by extending SS treatment to 27 days. In closed greenhouses SS and BF with bovine manure effectively reduced the viability of FORL chlamydospores, lowered disease incidence and increased commercial yield. Susceptible tomato plants grafted on FCRR-tolerant hybrid rootstock (Heman), even when cropped in a severely FORL-infested soil, remained healthy during the growing season and gave a profitable yield (Di Primo and Cartia, 2001a).

Trials to test the effectiveness of SS as compared with MB to control *P. lycopersici* and nematodes, were performed in greenhouses where the soil was covered with different films. Mulching soil with EVA, GCF, and black coextruded films (BCF) for 373, 265 and 68 hours respectively resulted in soil temperatures over 42.5°C. Corky root and root-knot symptoms appeared with very low frequency in MB and in the solarized plot mulched with EVA and GCF, and differred statistically from BCF and from the untreated untreated control (Cascone *et al.,* 2000).

Five biological agents were evaluated for control of FCRR on 1700 seedlings grown in nine greenhouses. Because of the high incidence of FCRR, only Fus Più controlled this disease, and no yield increase was recorded (Polizzi and Catara, 2001).

Melon

Fusarium oxysporum f. sp. *melonis* (FOM), the causal agent of muskmelon wilt, causes serious damage to crops grown in unheated tunnels. The effectiveness of SS and BF in the control of FOM propagules placed in the soil at depths of 15 and 30 cm was tested in field and in tunnel trials. In the field short solarization (12 days), caused a mortality of 52 and 65% to propagules placed at 30 and 15 cm depth respectively; a combination of SS with chicken manure (1 kg m^{-2}), or extending SS treatment to 27 days, improved pathogen control. In the tunnel trials the chlamydospores were set at the same depths and the soil was treated with SS, BF and MB. SS for 35 days killed all propagules at 15-cm depth and induced 83% mortality at 30-cm depth. BF and MB caused 100% mortality at both soil depths. All treatments reduced disease incidence and increased yield in melon plants (Di Primo and Cartia, 2001b).

Onion

White rot caused by the fungus *Sclerotium cepivorum* is most common on onion crops; the sclerotia remain viable for many years and become a negative factor in infested soils. In preliminary field trials carried out in Calabria, SS significantly reduced white rot by 89%, and increased marketable yield by 64% (Polizzi *et al.,* 1995). Soil solarization for 27 days completely destroyed sclerotia buried at depths of 15 and 30 cm. BF adding chicken manure to the soil significantly enhanced pathogen imncidence as early as 12 days after treatment. Sclerotium mortality at the two tested depths reached 100%. SS of a field amended with organic substances gave better and faster control of sclerotia of *S. cepivorum*, than either treatment alone (Di Primo and Cartia, 1998).
Good results were also obtained by mulching soil with F-clean and GCF. Viability of sclerotia, placed in the soil at 20 cm depth, decreased by 26 % with F-clean, and by 21% with GCF, compared with 10 days for SS alone, and by 100 % after 20 days (Agosteo and Cartia, 2001).

Carrot

The effectiveness of two fenamiphos formulations in combination with SS (August 13–September 25) to control *Ditylenchus dipsaci* of carrots was studied in trials carried out in Sicily. When carrots were sown in October, all control plots and only one solarized plot showed many dead or stunted and yellowing plants in early March. Because of the several generations produced by the nematode, no significant differences were observed in the numbers of nematodes in the soil and in the carrot tap roots at the end of March. However, yield parameters showed highly significant differences. At the end of March the average carrot tap root weight was 58.5 g in plots that were only solarized, 62.5–77.2 g in plots solarized and treated with fenamiphos and only 30.7 g in the control plots. At harvest (17 April) the carrot tap root yield/0.8 m^2 was 3.6 kg in the controls and as much as 8–9.7 kg in the treated plots, while the average tap root weight was 38.7 g and 92–97g, respectively (Greco and Cartia, 2000).
Trials carried out in Apulia, Calabria and Sicily, during a three-year period showed that in specific crops and situations, various chemical and non-chemical methods are able to replace MB to control SP activity. In our climatic conditions, SS treatment is effective in summer, particularly against pathogens sensitive to heat, and SS in combination with other control methods (IPM) is more effective, and can also be extended to regions located in northern Italy. Researches are now evaluating problems related to MB use, and at the same time assessing if chemical alternatives to MB will increase together with environmental problems.
The project "Toxicological impact of agricultural and energetic activities" is carried out in Sicily to determine which chemicals alternative to MB are being used in the Ragusa area to control SPs. Preliminary investigations found that 1,3 dichloropropene is extensively employed to control soilborne nematodes. Its use appears promising; so far no chemical residues have been found in treated soil or in the cropped vegetables.

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Oral and poster presentations

Integrated disease management in greenhouse-grown vegetables in Cyprus. N. Ioannou, M. Ioannou and P. Polykarpou. *Agricultural Research Institute, 1516 Nicosia, Cyprus.*

This work is part of a broader interdisciplinary project that was initiated in 1998–99 at the Zygi Experimental Station with the primary objective to develop IPM/ICM programs for tomato, cucumber and pepper grown in greenhouses. The study is carried out in two identical greenhouses with the same cropping and disease history. One greenhouse receives conventional plant-protection treatments, including: soil fumigation with methyl bromide, preventive fungicide treatments applied on schedule, and manual regulation of the greenhouse environment. The other greenhouse receives IPM practices, including soil solarization, fungicide treatments based on disease monitoring and on prescribed economic or action thresholds, automatic mechanical regulation of the greenhouse environment, removal of infected plants or plant parts, and the use of insect-proof netting and yellow sticky traps to control virus vectors. Both greenhouses are equipped with a heating system maintaining a min. temperature of 14ºC, and both use healthy planting material and standard phytosanitary measures. Results so far show that diseases were effectively controlled under both regimes and that fruit yields were about equal in both greenhouses, for all three crops. However, IPM enabled a reduction of fungicide applications by about 50% in tomato and cucumber, and by about 30% in pepper. The only major problem in the IPM greenhouse was powdery mildew, which appeared to be favoured by the lower relative humidity. This problem may be resolved by the installation of electric sulfur applicators in the IPM greenhouse.

Integrated management of soil-borne diseases in greenhouse-grown cherry tomato. G. Neophytou[1,2], N. Ioannou[1] and D.J. Wright[2]. *[1]Agricultural Research Institute, 1516 Nicosia, Cyprus. [2]Imperial College, Silwood Park, Ascot, Berks, SL5 7PY, UK.*

This study was undertaken in 2000–01 in order to investigate the effectiveness of solarization in combination with various soil amendments to control root-knot nematodes (RKN) and other soil-borne pathogens in greenhouse-grown cherry tomato (cv. Bar 138-8). At the end of the crop season, root galling caused by RKN was reduced by approximately 47% and 66% with the 4 and 8-wk solarization treatments respectively (applied in a hermetically-sealed greenhouse). In contrast, no significant differences were observed between soil amendments and the control treatment. Both solarization treatments significantly reduced *Fusarium oxysporum* populations and eliminated most soil-borne fungal diseases, including corky root rot, Fusarium root and crown rot, and Fusarium and Verticillium wilts. The total fruit yields increased by ~ 25%, compared to that obtained from uncovered soil. In a preliminary study at the same greenhouse site, the effectiveness of the organic fertilizer Agrobiosol (based on dry mycelium of *Penicillium chrysogenum*) alone or in combination with 4-wk solarization was investigated. No significant differences in the control of RKN were observed between *P. chrysogenum* and the control treatment. According to the study, solarization is an important plant protection tool for the control of RKN and other soil-borne diseases of greenhouse grown tomato, under the climatic conditions of Cyprus.

Alternatives for the control of *Fusarium oxysporum* f. sp. *niveum* in watermelon crops in Cyprus. C. Poullis[1], N. Ioannou[2] and J. Heale[3]. *[1]Department of Agriculture, Lefkosia, Cyprus. [2]Agricultural Research Institute, Lefkosia, Cyprus. [3]King´s College, London University, London, UK.*

Fusarium wilt of watermelon, caused by *Fusarium oxysporum* f. sp. *niveum,* FON), is a limiting factor for watermelon production in Cyprus. Trials carried out in recent years with 45 reportedly resistant or tolerant cultivars showed that all were susceptible to local isolates of FON. In order to identify the prevailing races in Cyprus, laboratory pathogenicity tests were carried out with 20 local isolates of unknown race designation, comparing them with isolates from USA and Israel known to belong to races 0, 1 and 2. Three watermelon cultivars, Sugar baby (susceptible), Crimson sweet (partially resistant), and P.I. 296341–FR (resistant) were

used as differentials. The majority of local isolates belonged to race 2, a highly aggressive race that attacks all commercial resistant cultivars produced so far. In view of these results, efforts to control the disease were directed towards the use of resistant rootstocks on which the susceptible watermelon cultivars would be grafted. A number of native cucurbits and several imported rootstocks were evaluated in the laboratory for resistance to FON and for compatibility with the main watermelon cultivars. These tests resulted in the preselection of 10 promising rootstocks which were then evaluated in five field trials, with the following results: (a) All rootstocks resistant to FON provided 100% protection from the disease. Some rootstocks, however, notably *Cucubita maxima* and *C. ficifolia* were susceptible to other soil-borne pathogens, such as *Rhizoctonia solani* and *Pythium* spp. (b) Growth and yield of grafted plants were double that of the non-grafted controls, even in soils not infested with FON. The quality of watermelons was not affected in any way by grafting. (c) The most promising rootstocks were RS 841 and Early M among the imported ones and *Lagenaria siceraria* "clavata" and *Cucurbita pepo* "melopepo" among the native ones.

Isolation of vine epiphytic yeasts and evaluation of their antagonistic activity against fungi of the *Aspergillus niger* group, causing the sour rot of grapes. M.G. Demakopoulou, E.C. Tjamos, S.E. Tjamos, P.P. Antoniou and S.C. Chatzinicolaou. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.*

Sour rot of grapes caused by fungi of the *Aspergillus niger* group is a serious problem in Greece and worldwide both in wine production and raisin grape varieties. Besides crop losses due to sour rot several species of the *A. niger* group produce ochratoxin A, a well known nefrotoxic and carcinogenic substance with additive action in ppb. Research on methods to control the pathogen is therefore needed. Some fungicides may be effective in reducing disease severity but an interesting alternative strategy is the collection of vine epiphytic yeasts and using them as antagonists against fungi of the *A. niger* group to reduce or eliminate the pathogen and the infection it causes. Yeasts were collected from the phyllosphere of various grape cultivars from several parts of Greece, grown under certain plant protection systems. Leaf and cane sampling was conducted during spring 2002 from the vine varieties Fraoula (at Attica Rodhes), Athiri (Santorini), Asyrtiko (Limnos), Limnio (Naoussa), Muscat of Alexandria Debina (Chalkidiki), and Cabernet Sauvignon (Epirus). The sampling method consisted in washing the leaves and canes and transferring the washings to yeast malt agar (YMA). We report on preliminary experiments to develop methods of evaluating the antagonistic activity of yeasts against *A. carbonarius*. Berries of the varieties Fraoula and Sultanina were wounded by making a hole in them 2 mm in diameter and 3 mm in depth. The berries were embedded in yeast suspension of 5×10^6–10^7 cfu ml^{-1}, and after 24 hrs were inoculated with a 10^4 cfu ml^{-1} conidial suspension of *A. carbonarius*. The rate of the growth of the pathogen, the density of sporulation and the dispersal of rot were determined daily for 4–5 days. Preliminary data indicated that epiphytic yeasts restricted or delayed rot development. More research is needed for a more detailed evaluation and taxonomic identification of the antagonistic yeasts.

Control of *Clavibacter michiganensis* subsp. *michiganensis* with the antagonistic rhizospheric bacteria *Bacillus subtilis* 5-127, *Paenibacillus alvei* K-165 and fluorescent *Pseudomonas* sp. PF-17. P.P. Antoniou, S.M. Christoglou, and E.C. Tjamos. *Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.*

Bacterial canker is one of the most serious diseases of tomato. An attempt was made to control the soil phase of this disease with biological agents. Experiments were conducted both in a glasshouse and in the field. During 1999–2001 glasshouse experiments were carried out to induce systemic resistance in tomatoes to *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) with rhizospheric bacteria belonging to *Pseudomonas* sp. fluorescent isolates Pf-17 and Pf-34, and the K-165 strain of *Paenibacillus alvei*. The tomato hybrid NOA F1 was drenched at the stage of four fully expanded leaves. The *Pseudomonas* isolates Pf-17 and Pf-34, and *P. alvei* K-165 were applied as a soil drench (30 ml 10^7–10^9 cfu ml^{-1}). Seven to ten days after application the plants were inoculated with *Cmm*. Inoculum at a concentration of 10^8 cfu ml^{-1} was introduced by injection into the stem and the wound was protected with vaseline. The experiment was repeated three times and 20 plants per treatment were used. K-165 significantly reduced disease symptoms (down to 52.89% compared with 72.78% for the untreated control), while Pf-17 (63.02%) and Pf-34 (65%) were rather ineffective. Several antagonistic bacteria were also evaluated in the field against *Cmm*. The rhizosphere *Bacillus subtilis* strain 5-127, the *P. alvei* strain K-165 and the fluorescent *Pseudomonas* sp. strain Pf-17 were tested against strain 4007 of *Cmm*. Tomato stems 2–3 cm long infiltrated with bacterial inoculum were buried at sites to which the Garnet F1 tomato hybrid was transplanted. Plants were drenched with 30 ml 10^8 cfu ml^{-1} *Cmm* per plant. Two weeks later the plants were drenched for a second time and then again after further ten and twenty days. The percentage of the dead plants was 5% in the untreated control and 0% with the treatments. Further

symptom assessment of the percentage of diseased leaves revealed that K-165 significantly reduced disease symptoms, down to 18.8% compared with 31.5% in the untreated control, while Pf-17 (24.65%) and 5-127 (29.8%) were less effective. However, when fruit setting and the mean number of marketable size tomato fruits were determined, it was found showed that control plants carried only 3.55 fruits per plant, while K-165-treated plants carried 10.17; 5-127-treated plants 11; and Pf-17-treated plants 9.88. In general strain K-165 was the most promising biocontrol agent against bacterial canker of tomato.

Control of bacterial canker of tomato by BABA or the antagonistic bacterium *Paenibacillus alvei* K-165. C. Arampatzis[1], P.P. Antoniou[1], E.C. Tjamos[1] and P. Katinakis[2]. [1]*Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.* [2]*Agricultural University of Athens, Laboratory of Biochemistry, 75 Iera Odos str., 11855 Athens, Greece.*

Systemic resistance in tomato plants to *Clavibacter michiganensis* subsp. *michiganensis*, induced with the rhizospheric *Paenibacillus alvei* isolate K-165 and the chemical factor BABA. The tomato hybrid NOA was drenched at the stage of two fully expanded leaves. *P. alvei* K-165 was applied as soil drench (100 ml 10^8 cfu ml^{-1}). After seven days the plants were inoculated with *C. michiganensis* subsp. *michiganensis*. The inoculum was injected into the stem at a concentration of 10^6 cfu ml^{-1} and the wound was protected with vaseline. The experiment was repeated three times and 15 plants per treatment were used. K-165 reduced disease by 30–40%, compared to the untreated control. Statistical analysis revealed significant differences between the biocontrol agent and the untreated control. These data were consistent with previous results showing that K-165 induces systemic resistance against other vascular pathogens. In the second approach, systemic resistance was induced using the chemical β-aminobutyric acid (BABA) against *C. michiganensis* subsp. *michiganensis*. The tomato hybrid NOA was drenched at the stage of two fully expanded leaves with 60 ml water solution of BABA at a concentration of 2 mg ml^{-1}. Seven days after the application of BABA the plants were inoculated with *C. michiganensis* subsp. *michiganensis* at a concentration of 10^6 cfu ml^{-1}. The inoculum was injected into the stem and the wound was also protected with vaseline. The experiment was repeated three times and 15 plants per treatment were used. BABA reduced disease by 50–60% compared to the untreated control. Statistical analysis showed significant differences between the chemical agent and the untreated control. Although after root drenching with BABA plants developed stress symptoms such as chlorosis, necrotic spots and wilting, they recovered later.

Effect of the biocontrol agents *Pseudomonas fluorescens* WCS365, *P. chlororaphis* PCL1391 and *Fusarium oxysporum* Fo47 on the colonization of tomato roots by the fungus *F. oxysporum* f. sp. *radicis-lycopersici*. A.L. Lagopodi[1], G.V. Bloemberg[2], A.F.J. Ram[2], A.H.M. Wijfjes[2], G.E.M. Lamers[2], C.A.M.J.J. Van den Hondel[2] and B.J.J. Lugtenberg[2]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 54006 Thessaloniki, Greece.* [2]*Institute of Molecular Plant Sciences, Leiden University, Wassenaarseweg 64, 2333 AL, Leiden, The Netherlands.*

Crown and root rot of tomato caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici* can be reduced by *Pseudomonas fluorescens* WCS365, *P. chlororaphis* PCL1391 and the non-pathogenic *Fusarium oxysporum* strain Fo47 in the rhizosphere. Using different auto-fluorescent proteins to mark these micro-organisms allowed simultaneous study of the pathogen and each biocontrol agent on the tomato roots, by confocal laser scanning microscopy. The gfp-labelled pathogenic *Fusarium* were easily distinguished from the morphologically identical, non-pathogenic strain of *Fusarium* marked with cfp. Microscopic examination revealed that competition for colonization sites on the roots and niche exclusion is a feature of the biocontrol affect in this biocontrol pair. Microscopic analysis of the interactions between the pathogenic *Fusarium* marked with gfp and the Pseudomonas biocontrol strains marked with cfp revealed that the fungus and the bacteria competed also for specific colonization sites on the root. In addition, the biocontrol bacteria form microcolonies in the space between the root tissue and the hyphae preventing the pathogenic fungus from penetrating and colonizing the hyphae directly. The interaction between the pathogenic fungus and the biocontrol agents revealed important aspects of the biocontrol mechanism and can help in the development of more effective biocontrol systems.

Three weeks of soil solarization with impermeable plastics singly or together with soil fumigants or nematocides is effective against soilborne pathogens. E.C. Tjamos[1], P.P. Antoniou[1], S.E. Tjamos[1], N.P. Fatouros[2], J. Giannakou[3], A. Paraskeuopoulos[4] and K. Papachristos[2]. [1]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.* [2]*Plant Protection Service of Preveza county, Greece.* [3]*Aristotelian University of Salonica, Salonika, Greece.* [4]*Plant Protection Service of Tryfilia region, Greece.*

The effect that three weeks of soil solarization with impermeable plastics, singly or in combination with methyl bromide 25 g m^{-2}, condor or rugby, had on soil-borne pathogens of tomato in plastic houses at Preveza and on soil-borne pathogens of cucumber in plastic

houses at Kyparissia was studied in 2000–2001. Preveza: All combinations controlled the major tomato pathogens in a winter plantation of the tomato variety Jumbo: *Clavibacter michiganensis* subsp. *michiganensis, Pyrenochaeta lycopersici, Phytophthora* sp. and *Meloidogyne* sp. No nematode infection was noticed, possibly due to the low temperatures. During the same period other tomato plantations in Preveza county suffered from early or late *P. lycopersici* infections in plastic houses where only chemical soil disinfection was applied. In these plantations crop production was also only to 2–2.5 kg per plant compared with 4 kg per plant in the experimental plastic houses studied here. In August 2001 a summer plantation of the *Meloidogyne* sp.-resistant tomato hybrid 622 established in the same plastic houses without any further soil disinfestation had a root knot infection of only 2.5–5%, regardless of the application followed during summer 2000. The lower rate of infection should be attributed not only to the use of the resistant cultivar but also to a long-term effect of soil solarization. Indeed, summer plastic house plantations with the same tomato hybrid established in soil chemically disinfected two years before developed very strong root-knot symptoms on 80% of all plants. Kyparissia: The effectiveness of three weeks of soil solarization with impermeable plastics singly or in combination with methyl bromide 25 g m^{-2}, or condor against soilborne pathogens of cucumber (*Fusarium oxysporum* f. sp. *radicis-cucumerinum* and *Meloidogyne* sp.) was evaluated in plastic houses at Kyparissia in 2000. The susceptible cucumber cultivar Z 14 were planted or grafted on Power, a Fusarium-resistant rootstock. Plants grafted on the resistant rootstock showed no Fusarium symptoms while in the non-grafted plants disease incidence ranged from 2 to 22%. No rootknot symptoms were observed in any of the experimental plots. It seems that soil solarization can be a valuable alternative to methyl bromide fumigation in Greece and elsewhere if impermeable plastics are used singly or in combination with nematicides.

The fumigant Condor (1,3-dichloropropene) as an alternative to methyl bromide. C. Mavrotas[1], C. Fotiadis[2] and N. Tsiboukis[2]. *[1]Dow AgroSciences Export S.A.S., Athens, Greece. [2]K+N Efthimiadis SAS, Thessaloniki, Greece.*

The gradual phasing out of methyl bromide has created a gap in the protection of crops, especially greenhouse crops. The main problems with greenhouse soil in Greece are (in order of importance): nematodes, soil fungi, weeds and soil insects. Condor is a broad-spectrum soil fumigant that can be applied as a water dilution in a drip irrigation system. It acts on contact, controlling all nematode species and soil insects, and it indirectly controls soil fungi, bacteria, viruses and weeds as well. K+N Efthymiadis S.A. in cooperation with Dow AgroSciences has for the past three years developed a program to control these pest problems. The program is based mainly on the nematicide Condor (1,3 dichloropropene), which controls nematodes completely and on Condor in combination with other pesticides, in which nematodes, fungi, and weeds were controlled completely. By means of Condor in Greece it was possible: (a) to cultivate again the areas that had been heavily infested with nematodes (Chrissoupoli-sugarbeets, Ierapetra-greenhouses); (b) to reduce both the population of the easy weeds and the need for soil fungicides. (c) to contol nematodes, fungi and weeds when this gumigant was combined with solarization. Finally, Condor in a protective program was an alternative to methyl bromide.

Control of tomato plant pathogens with antagonist fungi and a commercial biological product. F.T. Gravanis[1], N. Pagidas[1], S. Xifilidou[1] and I.K. Vagelas[2]. *[1]Technological Education Institution of Larissa, 41110 Larissa, Greece. [2]The University of Reading, Earley Gate, P.O. Box 236, Reading, RG6 6AT, Berkshire, UK.*

Two fungal isolates acting as biological agents, *Gliocladium virens* (*Gv*) and *Trichoderma viride* (*Tv*) and a commercial biological product (BCC), derived from plant substances and recommended as a plant defense promoter against plant pathogens and averse abiotic factors, were tested singly as was a combination of *Gv*+BCC, to determine their effectiveness against tomato soil-borne diseases. Two fungal isolates, *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) and *Verticillium dahliae* (*Vd*) and two bacterial strains, *Pseudomonas syringae* pv. *tomato* (*Pst*) and *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) were employed in this study. Twenty-day-old tomato seedlings were transplanted to 9-cm-diameter pots filled with a mixture of equal volumes of the commercial substrate Potgrond P and peat. Seedlings were inoculated with the pathogens at transplanting and the biological agents were applied afterwards with the irrigation water. The fungi were applied once only, as spore suspensions (10^6 spores ml^{-1}, 20 ml $plant^{-1}$). BCC was applied in two doses, each of 40 mg $plant^{-1}$, diluted in water; the first dose upon transplanting and the second a week later. Treatments were given in all possible combinations with pathogens, biological agents and controls, with 10 replicates per treatment. The plants were kept in a greenhouse and the disease assessment was made 21 days after transplanting. The height, net weight, dry weight, stem diameter and overall appearance of plants were assessed. All biological agents and their combinations controlled all pathogens as far as the plant dry weight was concerned, with no significant differences between treatments. The combination *Gv+BCC,* however, gave the best results in terms of the overall appearance of plant stem diameter and plant net weight.

Control of *Leveillula taurica* (Lev.) Arnaud in pepper by soil incorporation of sulphur combined with *Thiobacillus* spp. V.A. Bourbos[1], E.A. Barbopoulou[1] and K. Venetis[2]. [1]*NAGREF, Institute of Olive Tree and Subtropical Plants of Chania, Laboratory of Plant Pathology, Agrokipio, 73100, Chania, Greece.* [2]*Intrachem Hellas LTD, 31 Kiffisias str., 11523 Athens, Greece.*

The work examined whether powdery mildew (*Leveillula taurica*) in a greenhouse pepper crop (cv. Drago) could be controlled with the soil-improving product bearing the commercial name Acidam AVC 50 at a dose of 100 g m^{-2}. Acidam contains 50% sulphur and microorganisms of the genus *Thiobacillus*. The product was incorporated into the soil two weeks before the plantlets were transplanted to their final position. The fungicide triadimenol was used as a reference product, at a dose of 175 ml hl^{-1} of the commercial product Bayfidan 050 EW. Acidam effectiveness was evaluated by counting the lesions per leaf. In experimental plots where Acidam was applied, the pathogen was controlled with 93.98–96.42% effectiveness, which was significantly different from that of the reference product (99.22–99.71%).

Control of soil-borne plant pathogens with the nematode symbiont entomopathogenic bacterium *Pseudomonas oryzihabitans*. I.K. Vagelas[1], F.T. Gravanis[2] and S.R. Gowen[1]. [1]*The University of Reading, Earley Gate, P.O.Box 236, Reading, RG6 6AT, Berkshire, UK.* [2]*Technological Education Institution of Larissa, 41110 Larissa, Greece.*

Pseudomonas (*Flavimonas*) *oryzihabitans,* a symbiont bacterium of the entomopathogenic nematode *Steinernema abbasi,* significantly inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici (Fol), F. oxysporum* f. sp. *radicis-lycopersici (Forl), Rhizoctonia solani (Rs)* and *Pythium ultimum (Pu)*, *in vitro*. Studies using bacterial filtrates confirmed the fungistatic effect of the cell-free solutions on mycelia of *Fol, Forl,* and *Rs*, and on spores of *Fol* and *Forl*. In a glasshouse experiment *P. oryzihabitans* suppressed *Fol* and *Rs* in the soil. In the tomato rhizosphere, *P. oryzihabitans* colonized mycelia of *Fol*. The population of *P. oryzihabitans* increased from Log_{10} 3.52±0.124 (in the absence of *Fol*) to Log_{10} 4.82±0.011 (in the presence of *Fol*). *P. oryzihabitans* also controlled Fusarium wilt of tomato, enhanced plant growth and increased stem height and fresh and dry weight, acting as a plant growth promoting rhizobacterium.

Effect of compost and compost extracts on damping-off caused by *Rhizoctonia solani* on tomato. G.S. Karaoglanidis[1], K. Klonari[1], N. Barbayiannis[2] and G. Diamantidis[3]. [1]*Plant Pathology Laboratory,* [2]*Soil Laboratory,* [3]*Laboratory of Agricultural Chemistry, Aristotelian University of Thessaloniki, Faculty of Agriculture, P.O. Box 269, 54006, Thessaloniki, Greece.*

Damping-off caused by *Rhizoctonia solani* is a major seedling disease in nurseries, greenhouses and outdoor crops. Although damping-off and the yield losses it causes are controlled by various fungicides, these are not always economically or environmentally sustainable. One alternative to fungicides used for disease management may be the amendment of soils with composts. The objective of this study was to evaluate how well damping-off was suppressed by a compost prepared from grape marcs, cotton residues and spent mushroom substrates, and by extracts of this compost also containing humic and fulvic acid. Compost and compost extracts were tested for their control of tomato damping-off in growth-chamber experiments, while compost extracts were also tested *in vitro* for their inhibition of the mycelial growth of *R. solani*. Compost extracts containing humic acid significantly inhibited ($P<0.05$) mycelial growth of the fungus, but extracts containing fulvic acid at any of the concentrations tested did not show any inhibitory effect ($P>0.05$) on mycelial growth of *R. solani*. Amendment of the container media with solid compost significantly lowered Rhizoctonia damping-off severity on tomato plants. Compost extracts containing humic acid also suppressed the disease to some degree, but no suppression was observed with treatments containing fulvic acid. A combination of compost amendment and cultural or even chemical control measures may provide approaches to integrated disease management of damping-off.

Natural volatile substances of grapes of the Isabella variety for the control of *Botrytis cinerea*. E.K. Kulakiotu[1], C.C. Thanasoulopoulos[1] and E.M. Sfakiotakis[2]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Plant Pathology, P.O. Box 269, 54006 Thessaloniki, Greece.* [2]*Aristotle University of Thessaloniki, School of Agriculture, Laboratory of Pomology, 54006 Thessaloniki, Greece.*

Volatile substances produced by grapes of the Isabella variety (sp. *Vitis labrusca*) were studied as providing a possible alternative method for the post-harvest control of the fungus *Botrytis cinerea*. A bioassay method, that of the closed Mariotte system, was used to quantify the biological action of these volatile substances on the growth of *B. cinerea*. The following treatments were tested *in vitro*: (i) volatile substances excreted by grapes of the resistant variety Isabella, (ii) volatile substances excreted by grapes of the susceptible variety Roditis, and (iii) no volatile substances at all. Volatile substances of the resistant variety of grapes had a fungistatic effect on the sporulation of the fungus and on the creation of sclerotia, and volatile substances from the susceptible variety stimulted sporulation of the fungus. In the *in vivo* experiments, an interactive model was used

consisting of volatile substances from grapes of the Isabella variety and *B. cinerea*-clusters of the Roditis variety. The results confirmed the antibiotic action of the volatile substances from the grapes of the Isabella variety as they limited the incidence of infection, by considerably reducing both the amount of inoculum and the activity of the pathogen.

Control of powdery mildew of tomatoes (*Leveillula taurica*) with a formulation of a plant extract from *Reynoutria sachalinensis*. N.E. Malathrakis[1], E. Markellou[2], E. Siranidou[4], M.N. Fanouraki[1], A-M. Kasselaki[1], A. Schmitt[3], N. Petsikos-Panayotarou[2] and S. Konstantinidou-Doltsinis[4]. *[1]School of Agricultural Technology, Technological Education Institute of Crete, 71500 Heraklion, Greece. [2]Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece. [3]BBA Institut für biologischen Pflanzenschutz Heinrich-str. 243 D-64287 Darmstadt Germany. [4]National Agricultural Research Foundation, Institute of Plant Protection, Amerikis and National Road, 26004 Patras, Greece.*

In the framework of an EU-funded project, a study was carried out to determine the effectiveness of Milsana® (liquid formulations of *Reynoutria sachalinensis* extract) applied as a stand-alone treatment or in combination with other biocontrol agents, against tomato powdery mildew (*Leveillula taurica*), in four large-scale greenhouse trials conducted in Peloponessse and Crete (1999–2001). In two of the trials, the effectiveness of Milsana® was studied in relation to its rate of application, whilst in the others, Milsana® was studied in combination with the fungus *Pseudozyma flocculosa* (former *Sporothrix floculossa*) and the bacterium *Brevibacillus brevis*. The above-mentioned biocontrol agents were applied alone or in double combinations and were compared with wettable sulfur or other fungicides as reference treatments. Milsana® significantly reduced the percentage of infected leaf area in comparison to the controls. Its efficacy was similar to that of sulphur and the fungicide penconazole, but lower than that of the fungicide treatments (applied on an as needed basis). A combination of Milsana® with the microbial antagonists did not significantly improve the effectiveness of Milsana®. Despite the level of disease reduction on the leaves in all treatments, yield (number and weight of harvested fruits) was not affected. This was attributed to the ability of tomatoes to produce abundant new phylloplane, which enables them to tolerate infections by *L. taurica* without significant impact on yield. Comparative analysis of the results indicated that the efficacy of Milsana® against powdery mildew was influenced by the time of onset of the epidemic in relation to the host growth stage and the infection rate. These results along with other results from other trials conducted in the framework of the above-mentioned EU Project, showed that Milsana® can be a useful tool to control *L. taurica* in organic agriculture and/or integrated crop systems of tomatoes.

Effect of the growth medium on the *in vitro* sensitivity of *Botrytis cinerea* strains to pyrimethanil. Lowered sensitivity of the pathogen to pyrimethanil after repeated applications to tomatoes grown under cover. E. Markellou[1,3], D. Kyriakopoulou[1], V. Mavroidis[1], A.E. Kalamarakis[1,2], N.E. Malathrakis[3] and N. Petsikos-Panayotarou[1,2]. *[1]Benaki Phytopathological Institute, Department of Pesticides Control and Phytopharmacy, 8 St. Delta str., 14561 Kifissia, Athens, Greece. [2]National Agricultural Research Foundation (N.AG.RE.F.), Plant Protection Institute, Athens, Greece. [3]School of Agricultural Technology, Technological Education Institute of Crete, 71500 Heraklion, Greece.*

The effect of the anilinopyrimidine fungicide pyrimethanil on one wild-type and four strains of *Botrytis cinerea* resistant to the benzimidazoles and/or the dicarboximides and with wild-type insensitivity to diethofencarb was studied *in vitro* and *in vivo*. *In vitro*, when pyrimethanil was incorporated into media containing asparagine and inorganic salts, or glucose and agar, it was very effective against the mycelial growth of the *B. cinerea* strains (EC_{50} from 0.005 to 0.04, and from 0.02 to 0.19 μg ml^{-1} respectively). In more complex media (such as MEA) *B. cinerea* activity was reduced. *In vivo,* preventive applications of pyrimethanil completely protected young cucumber plants and fruits that were inoculated with a conidial suspension (1×10^6 spores ml^{-1}) or mycelial plugs (17 h old) of the *B. cinerea* strains. The effectiveness of pyrimethanil against grey mould was also tested in greenhouse tomatoes in relation to a) the type of infection on different plant parts and b) the response of the naturally occurring *B. cinerea* population to the selection pressure caused by eight successive applications of this fungicide. Before any treatment, the *B. cinerea* population was found to be resistant to the benzimidazoles and the dicarboximides, and the wild-type was insensitive to diethofencarb, and sensitive to dichlofluanid and anilinopyrimidines (phenotype $Ben^{HR}Dic^{MR}$-$Pcm^{R}Dichl^{S}Ani^{S}$). Pyrimethanil effectively controlled grey mould on the leaves, fruits and stems but it did not significantly reduce the number of dead plants or fruits with ghost-spot symptoms. The effectiveness of pyrimethanil on the leaves declined progressively during the spraying period and was inferior to that of the reference mixture after the 6th application. This was attributed to the development of a low level of resistance in the *B. cinerea* population (Rf=7.7) which was detected *in vitro* after the 8th application. With the use of linear models (logistic) pyrimethanil delayed the onset of the mould but it did not reduce the rate of infection. The risk of *Botrytis* developing resistance to the anilinopyrimidines in practice is discussed.

Integrated control of cucumber damping-off with bacterial peat inocula and reduced rates of fungicides. D.G. Georgakopoulos[1] and N.E. Malathrakis[2]. [1]*Agricultural University of Athens, Dept. of Agricultural Biotechnology, Laboratory of General and Agricultural Microbiology, 75 Iera Odos str., 11855 Athens, Greece.* [2]*Technological Educational Institute of Crete, School of Agricultural Technology, P.O.Box 140, 71500 Heraklion, Greece.*

The bacteria *Pseudomonas fluorescens* B5 and X and *P. corrugata* R117 satisfactorily protect cucumber seed and seedlings against damping-off caused by the pathogenic fungus *Pythium ultimum*. These bacteria were formulated into peat inoculum for seed treatment with good results. Peat inoculum was combined with seed treatment with fungicides (thiram, propamocarb, mancozeb, fosetyl-Al, captan and metiram) at lower dosages to achieve better protection against damping-off. The fungicides tested were not toxic to the bacteria *in vitro* in experiments with plates and broth, nor after seed coating, except for captan. The initial rate of application was 70 mg g^{-1} seed, equal to the commercial rate. In the integrated treatments, fungicide levels were reduced from 1/4 to 1/256 of the commercial rate, which drastically reduced the effectiveness of most of them. Integrated seed treatment with thiram at 1/16 the commercial rate and mancozeb at 1/64 the commercial rate were comparable in effectiveness to commercial fungicide treatments. However, no reproducible results were obtained with metiram. When Propamocarb was reduced even to as little as 1/256, it remained almost as effective as at the commercial rate, showing that the effectiveness of this integrated treatment was due to the fungicide. Fosetyl-Al was not effective, and captan was not used because of its toxicity.
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Biological and integrated control of sugar beet damping-off in the field. D.G. Georgakopoulos[1], R. Tilcher[2] , U. Fischer[2], F. Ioannidis[3], K. Doulias[3] and N.E. Malathrakis[4]. [1]*Agricultural University of Athens, Dept. of Agricultural Biotechnology, Laboratory of General and Agricultural Microbiology, 75 Iera Odos str., 11855 Athens, Greece.* [2]*KWS Saat AG, Grimsehlstrasse 31, D-37555, Germany.* [3]*Hellenic Sugar Industry S. A., Factory of Platy, 590 32 Platy, Greece. Hellenic Sugar Industry S. A., Factory of N. Orestiada, 682 00 N. Orestiada, Greece.* [4]*Technological Educational Institute of Crete, School of Agricultural Technology, P.O.Box 140, 71500 Heraklion, Greece.*

The bacteria *Bacillus subtilis* B6 and *Pseudomonas fluorescens* B5 and X were selected on the basis of small-scale experiments as biological antagonists of the pathogenic fungus *Pythium ultimum*, causing damping-off of sugar beet seed and seedlings. Bacterial inoculum was incorporated into sugar beet seed pellets in accordance with the commercial method of production. Pellets either contained bacteria alone (biological treatment) or the bacteria combined with the fungicides thiram and hymexazol reduced to 50% of the commercial dose, and with the insecticide imidacloprid at the full dose (integrated treatment). Initial populations per pellet were 1×10^5 cfu $seed^{-1}$ for *B. subtilis* and 2.9×10^4 cfu $seed^{-1}$ for *P. fluorescens* B5 and X, which were reduced to 33% (*B. subtilis*) and 10% (*P. fluorescens*) because of pellet drying during production. The biological and integrated treatments along with the appropriate controls (commercial pellets, pellets with reduced fungicide, pellets with no protectants at all) were evaluated in field trials (randomized complete block design with 4–6 blocks) in Mavrodendri (Kozani) and N. Orestiada (Evros). Due to high temperatures, infection occurred only in the N. Orestiada trial, where the biological treatments were not effective, although *P. fluorescens* X showed a positive trend. The integrated treatments were not different from the commercial treatment, since the lower fungicide dose was as effective as the commercial dose. *P. fluorescens* X (alone and combined at the lowered dose) was also evaluated in trials at Platy (Imathia). Lack of infection did not allow proper evaluation. It seems probable that pellets were ineffective because of the low bacterial populations in the pellets, since 10^7 bacteria per seed are reported to be necessary for infection to occur, and this population size is difficult to achieve with the current method of pellet production.
The work was supported by the grant FAIR-CT96-1373 from the European Union to the T.E.I. of Crete and other partners.

Study on the effect of combinations of biocontrol agents with a plant extract against powdery mildew of cucumber (*Sphaerotheca fuliginea*) in Greece and The Netherlands. S. Konstantinidou-Doltsinis[1], E. Markellou[2], A. Kalamarakis[2], E. Siranidou[1], A. Schmitt[3], A.J. Dik[4] and N. Petsikos-Panayotarou[2]. [1]*National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, Amerikis and National Road, 26004 Patra, Greece.* [2]*Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece.* [3]*BBA Institut für biologischen Pflanzenschutz, Darmstadt, Germany.* [4]*Applied Plant Research (PPO) Section Horticulture, Naaldwijk, The Netherlands.*

In the framework of an EU-funded project, the effectiveness of combinations of Milsana® (a plant extract from *Reynoutria sachalinensis,* which enhances plant resistance) and the biological control agents *Brevibacillus brevis* and *Pseudozyma flocculosa* (ex *Sporothrix flocculosa*) (that have a direct effect on the pathogen) was studied *in planta*. Five greenhouse trials were conduct-

ed in Greece and the Netherlands during 2000 and 2001, along with preliminary *in vitro* trials in Greece. In the greenhouse trials all treatments with Milsana® (whether alone or combined) reduced disease severity on the leaves as compared with the water controls. In Greece, the effectiveness of Milsana® was not enhanced when the biocontrol agents were combined with it or used instead of it. In the Netherlands, all agents significantly controlled powdery mildew and in one trial the combination of *B. brevis* with Milsana® was significantly better than either agent acting alone. Applications of Milsana® alone or of *P. flocculosa* alone (only in 1 trial, in The Netherlands) significantly increased yield (number and weight of harvested fruits) as compared with the control. The combination of Milsana® and *S. flocculosa* (Greece) or *B. brevis* (The Netherlands) also led to increased yield. In conclusion, Milsana® is a useful tool for the control of cucumber powdery mildew, but the effectiveness of the antagonists *B. brevis* and *P. flocculosa* was not consistent, and their combination with Milsana® mostly did not improve the efficacy of Milsana® alone.

Effectiveness of plant extracts against powdery mildew of cucumber (*Sphaerotheca fuliginea*) and cereals (*Erysiphe graminis*). S. Konstantinidou-Doltsinis, E. Roehner[2], E. Siranidou[1], E. Markellou[3] and H. Buchenauer[2]. [1]*National Agricultural Research Foundation, Plant Protection Institute, 26004 Patras, Greece.* [2]*Institut für Phytomedizin, University of Hohenheim, Stuttgart, Germany.* [3]*Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kiffisia, Athens, Greece.*

In the framework of a Greek-German collaboration programme, plant extracts from a) *Reynoutria sachalinensis* leaves, b) *Cassia septentrionalis* pods and c) *Calendula officinalis* flowers were studied for their effectiveness against powdery mildew of cucumber and cereals (wheat and/or barley), in small-scale experiments in Greece and Germany. Aqueous extracts of *R. sachalinensis* (0.5% w:v), *C. septentrionalis* (1%) and *C. officinalis* (3%), on cucumber plants with only one true leaf reduced powdery mildew severity by >70% when applied two days before inoculation. Mixtures of these extracts or the addition of saponin did not further increase their effectiveness. Extracts from *R. sachalinensis* and *C. septentrionalis* were highly effective when applied prophylactically (-2 to 0 day before inoculation). On the contrary, *C. officinalis* was most effective (ca. 60%) when applied up to 2 days after inoculation. In greenhouse trials (with potted cucumber plants), extract effectiveness ranged from 34–65% when they were applied at 7-day intervals. At longer intervals extracts were less effective. In preliminary trials, extracts from *C. septentrionalis* and *R. sachalinensis* had no systemic activity. Indications of translaminar movement of these extracts were initially obtained. In wheat and barley, preventive application of *C. septentrionalis* extract (2.5% w:v) reduced the leaf area infected with *E. graminis*. The severity of powdery mildew was inversely correlated with the length of time between treatment and inoculation (-4 to -1 d.). A high effectiveness of 97% and 80% against powdery mildew was obtained only with the *Cassia* extract applied 1 and 2 days before inoculation respectively, while the effectiveness of this extract decreased rapidly with increasing pre-infectional lengths of time. No curative effect against powdery mildew in either was obtained when *Cassia* extract was applied 1–4 days after inoculation. Both *C. septentrionalis* and *R. sachalinensis* extracts (2.5%) completely controlled *E. graminis* (effectiveness >95%) on barley when applied 1 day before inoculation. *C. officinalis* (3%) did not reduce disease severity. None of the extracts tested on either host produced systemic activity. Effective concentrations that would reduce powder mildew by 50% (EC_{50}) were estimated for the two effective extracts. The potential of these extracts under commercial conditions should be investigated further in large-scale trials.

Biological activity of *Gliocladium virens* and *Trichoderma* spp. against soil-borne plant pathogens. I.K. Vagelas[1], F.T. Gravanis[2] and S.R. Gowen[1]. [1]*The University of Reading, Earley Gate, P.O. Box 236, Reading, RG6 6AT, Berkshire, UK.* [2]*Technological Education Institution of Larissa, 41110 Larissa, Greece.*

An isolate of *Gliocladium virens* taken from disease-affected soil in a commercial tomato greenhouse in the Larissa region (Greece) was highly antagonistic *in vitro* to the soil-borne fungi *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) and *Rhizoctonia solani* in dual-cultures. A significant improvement in tomato yield was obtained when *G. virens* and other fungal isolates active as biological agents were added to the soil of potted tomatoes artificially infected with *Fol*., *G. virens, Trichoderma harzianum* and *T. viride* significantly reduced tomato wilt caused by *Fol* and significantly increased stem fresh weight and yield as compared with infected and untreated control. *G. virens* and *T. harzianum* were most effective against Fusarium wilt of tomato. *T. viride* was somewhat less effective.

Potential use of ionized water in the spraying solution to control *Spilocaea oleagina* (Cast.) Hugh. E.A. Barbopoulou and V.A. Bourbos. *National Agricultural Research Foundation (N.AG.RE.F), Institute of Olive Tree and Subtropical Plants of Chania, Laboratory of Plant Pathology, Agrokipio, 73100, Chania, Greece.*

Peacock spot of olive tree, attributed to the fungus *Spilocaea oleagina* (Cast.) Hugh., causes leaf-drop, and when severe, may lead to complete fruitlessness. This work examines whether the pathogen can be controlled using

water ionized with Cu ions in the spraying solution and a reduced dose of fungicide. The trial was conducted on 23-year-old olive trees of the Kalamon variety. Bouillie Bordelaise 20 WP, a copper sulphate-based fungicide was used as the reference product and applied at the recommended dose of 500 g hl^{-1}. The same fungicide was also used with ionized water in the "Superior Aqua" system, at fungicide doses of 250 and 125 g hl^{-1}. To determine the effectiveness, the number of spots per leaf in a sample of 100 leaves was counted, and the percentage of leaf-drop in 8 shoots per tree was calculated. Ionized water alone controlled the pathogen with an effectiveness of 28.7 to 34.8%. The fungicide at the recommended dose with natural water had an effectiveness from 89.6 to 99.5%. Ionized water with the fungicide at half and at 1/4 of the recommended dose had an effectiveness from 82.9 to 98.5% and from 80.0 to 96.1% respectively.

Biological control of *Penicillium* spp. by the use of the biocontrol yeast *Pichia anomala*: ecophysiological studies and their impact on inoculum quality and shelf-life. S. Mokiou and N. Magan. *Applied Mycology Group, Biotechnology Centre, Cranfield University, Silsoe, Bedford MK45 4DT, UK.*

For BCAs to be successfully used, several aspects of their development, including the economic mass production of ecologically effective inoculum and elucidation of their mode of action need to be understood. *Pichia anomala* is being assessed as a commercial agent to control *Penicillium roqueforti* and the mycotoxigenic *P. verrucosum* in moist cereals. Manipulating yeast physiology by changing the water stress [water activity (a_w), 0.98/0.96] of cane molasses and rich defined (NYDB) media using different compatible solutes/sugars and NaCl markedly affected yield and quality of cells. The endogenous water potential of cells (Ψ_c), and the sugar/sugar alcohol contents underwent significant changes. The accumulation/synthesis of trehalose or sugar alcohols was affected by the type of medium and the solutes used. Interestingly, there was an intracellular accumulation of the desiccation protectant trehalose when proline, glucose and sorbitol were added to media for the first time. When compatible solutes/sugars and NaCl were added to the media, there was an accumulation/synthesis of glycerol and varying amounts of arabitol. The ecological competence of the yeast treatments was examined by plating on non-stressed (0.995 a_w) and water-stressed media (0.96 a_w). Adding proline or NaCl to molasses media improved viability. Subsequently, the best cell treatments were suspended in isotonic PEG 200 (0.98/0.96 a_w) solutions and then stored as wet pastes at 22ºC and 4ºC for up to six months. Cells with high trehalose levels had better viability; wet pastes stored at 4ºC gave the best results. Studies on the mode of action of the biocontrol yeast *P. anomala* are in progress.

Biological control of root knot nematodes (*Meloidogyne* spp.). G. Neophytou[1,2], N. Ioannou[1], D. Kleanthous[1] and D.J. Wright[2]. *[1]Agricultural Research Institute, 1516 Nicosia, 22016 Cyprus. [2]Imperial College, Silwood Park, Ascot, Berks, SL5 7PY, UK.*

In a pot trial, selected bio-nematicides were evaluated for control of root knot nematodes (RKN) on the susceptible tomato cultivar Graziella. All bio-nematicides, especially bionem (*Bacillus firmus*) and DiTera (*Myrothecium verrucaria*), alone or in combination, reduced the galling index compared with untreated plants. Though significant control of RKN was achieved, plants treated with bionem also showed a significant reduction in growth, suggesting that bionem had some phytotoxic properties. A second study was a small-scale greenhouse trial carried out in naturally RKN-infested soil, to evaluate selected bionematicides for control of RKN disease on the susceptible cherry tomato 'Bar 138-8'. At the end of the crop season, root galling caused by RKN was reduced by ~21% and ~9% with bionem and DiTera respectively, while Intercept (*Burkholderia cepacia*) applied alone had no effect on the galling index. In contrast, bionem+Intercept at lower doses reduced the galling index by 26%, suggesting a synergistic effect between these two bio-nematicides. No phytotoxic symptoms were observed. Plant yield increased with all bio-nematicide treatments, compared to the control. The increase in yield may result from the reduction in RKN infestation, as well as from changes in the nutrient status of the soil, and the stimulation of beneficial microorganisms. The results of this study showed a partial reduction in RKN galling severity and an increase in plant yield, indicating that bio-nematicides have an important role in integrated crop management.

Biological control of the weed *Convolvulus arvensis* (L) with the fungus *Erysiphe convolvuli* de Candolle ex St. Amans *in planta*. V. Saltzis[1], A. Tzavella-Klonari[2] and P. Lolas[3]. *[1]Technological Education Institute of Epirus, Faculty of Agricultural Technology, Department of Plant Production, P.O.Box 110, 47100 Arta, Greece. [2]Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Plant Pathology, P. O. Box 269, 54006 Thessaloniki, Greece. [3]University of Thessaly, Department of Agriculture Plant Production and Agricultural Environment, Laboratory of Weed Science, Fytoko, 38446 Volos, Greece.*

In the spring and summer of 1999 and 2001, *Convolvulus arvensis* plants were inoculated with the fungus *Erysiphe convolvuli,* in order to determine its efficacy as a biological control agent of the weed. The inoculum was obtained from field-grown *C. arvensis* naturally infected with the fungus. Seedlings of *C. arvensis* were grown outdoors in pots. The first inoculation was car-

ried out when the *C. arvensis* seedlings were at the 2–6 true-leaf stage. The above-ground parts of the plants were sprayed with a conidial suspension of *E. convolvuli* at a concentration of 10^6 spores ml^{-1} and the inoculated plants were placed in a growth chamber with a temperature of 19–20°C, RH 91–92% and a 15-hour day for 25 days. After 5–6 days the white mildew appeared. A second inoculation was performed 7–8 days after the first, using conidial suspensions at the same concentration and covering the newly expanded foliage while the plants were kept in the same condition. The inoculated plants were totally covered with mildew 10–11 days after the first inoculation and more than 75% of the leaves were dead after 18–20 days.

Production of the entomopathogenic biocontrol agent *Metarhizium anisopliae* in liquid culture: the effect of environmental parameters on spore production and quality. I. Ypsilos and N. Magan. *Applied Mycology Group, Biotechnology Centre, Cranfield University, Silsoe, Bedford, MK45 4DT, UK.*

One of the major bottlenecks in the development of biocontrol agents is the sensitivity of these agents to changes in relative humidity and temperature, which limit their control potential. For optimal ecological fitness these limitations need to be addressed. In this study production of *Metarhizium anisopliae* in liquid culture was examined in a range of environmental regimes and nutrient statuses. After the optimal physicochemical conditions for blastospore production had been determined different water stress conditions were imposed using ionic solutes (NaCl, KCl). With intermediate water stress (0.98 a_w=-3.0 MPa water potential) the production of blastospores significantly increased. Analysis of the endogenous reserves in blastospores showed that there were also greater amounts of glucose and erythritol. Greater water stress (0.96 a_w=-5.6 MPa) further increased in endogenous glucose and erythritol levels, but blastospore production decreased. Erythritol can act as a compatible solute reducing the intracellular water potential and enabling faster germination at a wider range of environmental relative humidity. Subsequent viability assays simulating different water stresses showed that germination of such characterized blastospore treatments was faster and germ tube extension was improved. Washing the blastospores in water caused a significant loss of endogenous sugars and sugar alcohols with a concomitant loss in germinability. However, if isotonic solutions were used for washing, the blastospores retained their endogenous reserves (sugars and sugar alcohols) and this may enable better germination and establishment. These studies could have significant implications for developing formulations with longer viability and shelf life.

New technologies in plant protection

Invited lecture

Systemic acquired resistance: frontline news and prospects for applications. J-P. Métraux. *Département de Biologie, Université de Fribourg, 1700 Fribourg, Switzerland.*

Plants protect themselves against pathogens by constitutive barriers and by a number of inducible defense mechanisms deployed after contact with a pathogen. A first infection with a fungal, bacterial or viral pathogen often induces resistance towards subsequent infections. To a certain extent, plant immunization of this sort is analogous to immunization in animals and humans. Induced resistance may be expressed locally at the site of infection, or systemically, in uninfected parts of the plant. This latter phenomenon is termed systemic acquired resistance (SAR) to denote the capacity of the plants to acquire resistance after an initial infection even in plant parts remote from the first infection. Many plants express this type of resistance against a wide variety of pathogens, including pathogens unrelated to the initial causing agent. SAR is explained as being caused by the production of a signal released from an infected leaf and translocated to other parts of the plant where it induces a defense reaction. Some non-pathogenic root-colonizing bacteria also induce a SAR in the leaves. When the biochemical nature of the changes induced in infected plants was closely examined, it led to the discovery of a number of proteins termed pathogenesis-related proteins (PRs). It was also observed that a simple phenolic compound, salicylic acid (SA), induced PRs in tobacco and protected the plant against TMV. Plants accumulate SA locally, at the site of infection, but also in the phloem sap and in uninfected systemic leaves. It was suggested that SA was an endogenous signal for SAR. This suggestion opened the way for molecular investigations into induced resistance. A further advance was marked when SAR was found to operate in the genetically tractable system *Arabidopsis thaliana*.

Physiological changes associated with SAR

Generally, the success of an induced defense mechanism depends on the outcome of a race between the invading pathogen and the reaction of the plant. In compatible interactions, the virulent pathogen is often recognized too late and the plant is infected. In the case of incompatible interactions, plants rapidly recognize the avirulent pathogen and resistance mechanisms efficiently block the invader. A first infection leads to many changes, some of which eventually lead to the formation of barriers that block the invading pathogen. These barriers include modifications of the

cell wall such as by the deposition of lignin or papillae at sites of attempted penetration. In addition, antimicrobial metabolites or phytoalexins are produced. Phytoalexins have a broad unspecific activity and form a toxic barrier to invaders. The activation of genetically determined programmed cell death, similar to apoptosis in animals, also prevents the spread of invaders. The synthesis of novel proteins after pathogen attack is perhaps the most fully documented reaction. These host-encoded, pathogenesis-related proteins are induced locally and systemically after pathogen infection. They have been found in most plants where they have been looked for and have various biochemical activities. Some PRs were enzymes such as β-1, 3-glucanases, chitinases or proteinases capable of hydrolyzing the cell wall of invading fungal pathogens, while the function of others, for example PR-1, is still unclear. Combinations of PRs (for example glucanase and chitinase) tend to be more efficient and different types of PRs target different pathogens. With the advent of genome-wide analysis of gene expression using gene chips in the model plant *Arabidopsis thaliana*, it has become apparent that the expression of many genes is modified by pathogen infection. Thus the family of PRs is likely to be larger than hitherto thought. The question now is to understand how these genes function and are regulated.

The sequence of reactions taking place in a leaf attacked for the first time by a pathogen has been extensively studied using various mutants of Arabidopsis. After initial recognition of the pathogen by the plant, a cascade of early events is induced that include ion fluxes, phosphorylation events, and the generation of nitric oxide and active oxygen species. SA acts as a secondary signal molecule and is required for increased expression of resistance and various defense-related proteins such as the PRs. Depending on the inducing microorganism, the signal transduction pathway takes a different course depending on the nature of the initial interaction (virulent versus avirulent pathogen, rhizobacteria). Resistance to a given pathogen may be activated via different signal transduction pathways. For example, the infection with leaf pathogens that induce resistance to *P. syringae* depends on a pathway involving SA, while rhizobacteria-induced SAR act via the plant hormone ethylene and jasmonic acid. The complexity of these signaling pathways is further illustrated by the occurrence of crosstalk or interferences between pathways. For instance, both the induction of PR-1 and the resistance to *P. syringae* are strongly dependent on the light signal transduction pathway.

Given the central role of SA in pathogen-induced signaling for induced resistance, studies have been focused on the regulation of its production and on its molecular mode of action. In tobacco, cucumber and potato, evidence indicates that SA is produced from phenylalanine via coumaric and benzoic acid. In Arabidopsis a different pathway operates and SA is made from chorismic acid via an isochorismate synthase. More work is needed to understand the regulation of SA biosynthesis and its localization after pathogen attack, both locally and systemically.

The mode of action of SA was investigated by searching for SA-binding proteins. SA was found to bind to H_2O_2-scavenging enzymes such as catalase and ascorbate peroxidase, and this inactivation it was suggested could lead to the formation of a radical involved in lipid peroxidation. Lipid peroxidation products can activate defense gene expression providing a link between SA and defense. It remains to be shown that such phenolic radicals form sufficient lipid peroxides in the right time frame for the defense response to take place. More work is needed to complete our understanding of how SA acts at the molecular level.

The responses induced by SA include the transcriptional activation of genes. This is controlled by protein phosphorylation events that eventually lead to the regulation of proteins involved in the control of gene expression.

The importance of SA for SAR was shown by various correlative studies, but most compellingly by transgenic plants overexpressing a bacterial salicylate hydroxylase gene (the *NahG* gene). When it is expressed in the plant, this enzyme degrades SA. Transgenic plants carrying the *NahG* gene do not display a SAR. Similarly, mutants of Arabidopsis with an impaired capacity to synthesise SA are also less able to cause a SAR. A variety of experiments suggest that SA has a role as a systemic signal but do they not exclude the possibility that another putative systemic signal besides SA might be involved in systemic signaling during a SAR.

The systemic responses could be clearly separated from the reactions taking place in the infected parts of the plant, and it was clear that the systemic signal triggered defense-related reactions before contact with the challenging pathogen. In contrast, other reactions such as changes in cell wall lignification were only detected after challenge infection of the upper leaf but with faster induction kinetics: the systemic signal conditioned the tissue to respond faster. Future experiments should investigate more closely how the systemic signal conditions the induced leaves.

Prospects

Substantial progress has been achieved in the study of SAR in the last few years. An increasing number of new components in the signal transduction pathway for induced resistance responses has been discovered and their number will undoubtedly increase still further with the advent of large-scale investigations of gene expres-

sion. Fascinating questions about SAR include those concerning its systemic signal, its regulation and its mode of action. SA has been implicated in this process initially, and its role as a key signal in pathogen-induced SAR is well documented. Its function as a translocated systemic signal remains a matter of debate. In the near future, more will be learned about the regulation and localisation of SA synthesis and its mode of action. The signal transduction involved in the regulation of the SAR response turns out to be far from a linear chain of events and can more correctly be described as a network. It appears that several pathways interact in part, leading to sets of responses targeted to specific pathogens.
Compounds such as SA are interesting model structures that can be used to develop non-antibiotic crop protectants, which trigger the natural potential for resistance in various plants. Such compounds can be compared to immunostimulants by analogy to certain drugs used in humans. A good example is BION®, which has recently been released on the market. Further study is required on the effect of various environmental parameters on the plant response to pathogens. An example is the role played by light in SA-dependent defense responses. This type of knowledge will be of great importance in improving the transfer of SAR to field conditions.

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Oral and poster presentations

Molecular investigation of the defense pathway of a rhizobacterium *Paenibacillus* sp. antagonistic to a virulent fungus and a bacterium. A.S. Venieraki[1,2], E.C. Tjamos[1] and P. Katinakis[2]. [1]*Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.* [2]*Agricultural University of Athens, Laboratory of Molecular Biology, 75 Iera Odos str., 11855 Athens, Greece.*

The capacity of the antagonistic rhizobacterium *Paenibacillus* sp. (strain K-165) to induce systemic resistance against the wilt pathogen *Fusarium oxysporum* f. sp. *raphani* (*For)* and the foliar pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) was investigated. The model plants tested were the *Arabidopsis* ecotype Col-0 (wild-type), the mutants *etr-1* (ethylene response mutant), *npr-1* (non-expressed pathogenesis related proteins, PRs), *jar-1* (jasmonate response mutant), and the transgenic NahG (that cannot accumulate salicylic acid). All these plants were individually sown according to a system ensuring spatial separation of the inducing agent and the challenging root pathogen. Symptom development in the K-165 treated plants was reduced by up to 50% compared with the control (untreated Col-0 plants), 30 days after inoculation. Similar reductions were observed with the *etr-1* and the *jar1* mutants and the NahG transgenic plants. With the *npr1 ArabidoPs* mutant on the other hand there were no significant differences between plants treated with K-165 and untreated plants. The absence of resistance in the *npr1* mutants showed that the resistance caused by K-165 depends on NPR1 regulator protein. The second part of the experiment investigated the capacity of K-165 to induce resistance to *Pst* by studying the gene expression of the ISR-involved genes *PR-1, PR-2, PR-5, Hel, Atvsp, Pdf1.2* and *Tub,* which were amplified with PCR, subcloned and sequenced. Leaf tissues were harvested on different days after inoculation with *Pst*, for RNA analysis. Gene expression was analyzed by RT-PCR. The RT-PCR results showed that these genes were potentiated, leading to enhanced expression after challenge inoculation with *Pst,* and that PR genes had a vital role in the development of induced resistance. It is therefore concluded that pathogen-induced systemic resistance is associated with a potentiation of PR genes. The results with both host-pathogen systems studied showed that the pathogenesis-related proteins caused the induced resistance of *Paenibacillus* sp. strain K-165 to *Fusarium oxysporum* f. sp. *raphani* and *Pseudomonas syringae* pv. *tomato*.

Detection of *β-avr* genes in *Pseudomonas savastanoi* pv. *phaseolicola*. D.S. Tsaltas, G. Tsiamis and J.W. Mansfield. *Molecular Plant Pathology Laboratory, Imperial College at Wye, University of London, Ashford, Kent, TN25 5AH, UK.*

A 154-kb plasmid was cured from race 7 strain 1449B of the phytopathogen *Pseudomonas savastanoi* pv. *phaseolicola* (*Pph*). The cured strain, named RW60, lost virulence towards bean, causing a hypersensitive reaction (HR) in the previously susceptible cultivars Canadian Wonder and Tendergreen. Removal of the 154-kb plasmid revealed the presence of a second category of *avr* genes. Jackson *et.al*. (1999, PNAS 96, 10875–10880) suggested that the function of virulence factors is to suppress the HR induced by this new category of *avr* genes, which they called *β-avr* genes. Our aim was to identify such *β-avr* genes. We made use of transposon mutagenesis with a mini-Tn*5* construct. Mutants were infiltrated into leaves of the cv. Tendergreen (10^8 cfu ml^{-1}) and any changes from the RW60 HR phenotype were recorded. Of a total of 987 putative mutants, 15 non-auxotrophic strains generated altered phenotypes; nine of these caused a weaker HR and six a null response. The six null mutants also caused null reactions on the leaves of the cv. Canadian Wonder and Red Mexican, but they caused a variable HR on cv. A43. The genes disrupted by the Tn*5* insertions were characterized. Sequencing revealed that one mutation was in *hrpZ* while the other disrupted genes were similar to *fabB* (β-ketoacyl-ACP synthase), to the GTP-binding protein in thiophene and furan oxidation (x2), to *trpB* (tryptophane synthase), and to phosphoserine phosphatase. None of these genes showed any characteristics of known *avr* genes and they most likely contributed to basic physiological mechanisms. Recent experimental data on the role of the *β-avr* genes will be discussed.

Quantification of *Verticillium dahliae* microsclerotia in the soil using competitive PCR. E.J. Paplomatas, G.D. Dimou and A. Tzima. *Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.*

Knowing the number of *Verticillium dahliae* microsclerotia in the soil is very important to be able to predict the amount of disease and to decide on control measures. The most popular methods that have been devised for this purpose are wet sieving and dry plating of the soil on semi-selective culture media. Comparative studies have concluded in favor of either one or other of these methods, indicating methodological flaws such as the failure to distinguish between colonies of *V. dahliae* and of the saprophytic or non-pathogenic *V. tricorpus*, the failure of all microsclerotia to germinate equally on a medium, and the bias of individual researchers in the application of the method. As a result, the estimates of *V. dahliae* density in the soil published so far are unsatisfactory. The aim of the present study was to develop a molecular method of *V. dahliae* quantification in the soil that would overcome the weaknesses of the classical methods. The method chosen was competitive PCR with the ribosomal RNA gene of *V. dahliae* as a target. An advantage of this method was that the rDNA gene is present in a high copy number in the cell, something that would increase the sensitivity of the method. After a search in an electronic database, primers amplifying a 347 bp fragment of *V. dahliae* rDNA were designed. Since competitive PCR requires the simultaneous amplification of an internal standard (a competitor fragment) with the target DNA, a "linker primer" within the target sequences was also designed. In this way, an internal standard fragment of 257 bp (90 bp smaller than the target DNA) could be co-amplified. The method developed was evaluated on ten genomic DNA samples from an increasing number of *V.* dahliae microsclerotia in the soil (from 10 to 100). It was found that competitive PCR showed quantitative differences among the samples that were not detectable with simple PCR. Application of this method to *V. dahliae* DNA isolated from the soil will be discussed.

Molecular study of the resistance of *Botrytis cinerea* to new botrycides. A. Tzima[1], E.J. Paplomatas[1] and V.N. Ziogas[2]. *Agricultural University of Athens, Laboratories of [1]Plant Pathology and [2]Pesticide Science, 75 Iera Odos str., 11855 Athens, Greece.*

The RAPD technique was employed to study the genetic diversity and relatedness among nine isolates of *Botrytis cinerea* that differed in their sensitivity to the fungicides cyprodinil, fludioxonil and fenhexamid. The dendrogram that resulted after phylogenetic analysis of the data showed that resistance to these fungicides was associated with genetic changes that could be detected by this technique, and that specifically enabled the mutated resistant strains to be distinguished from the parent wild-strain. In order to develop molecular markers that differentiated *B. cinerea* strains resistant to the fungicides tested, four RAPD-PCR DNA fragments produced from strains representing each of the fungicide groups were cloned. The clones obtained will be evaluated either as DNA probes or to design specific DNA primers. Finally, the cystathionine-β-lyase gene from both *B. cinerea* strains that were sensitive to cypronidil and strains that were resistant to it was cloned. This enzyme is considered a putative target site of anilinopyrminidines. Comparison of gene sequences of strains sensitive and resistant to cyprodinil could give further insight into the mode of action of anilinopyrimidines and the mechanism of resistance of *B. cinerea* to this group of fungicides.

***Agrobacterium tumefaciens*–mediated transformation of *Verticillium dahliae* with the enhanced green fluorescent protein (EGFP) gene.** E.J. Paplomatas[1], E.C. Tjamos[1], D.F. Antonopoulos[1], P. Antoniou[1] and S. Kang[2]. [1]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos str., 11855, Athens, Greece.* [2]*The Pennsylvania State University, Department of Plant Pathology, University Park, PA 16802, USA.*

Agrobacterium tumefaciens–mediated transformation has long been used to transfer genes to plants and has recently also served to transform fungi. As regards fungi, *A. tumefaciens* can transform protoplasts, spores, hyphae and blocks of mushroom mycelial tissue. The purpose of this study was to transform the soilborne plant pathogenic fungus *Verticillium dahliae* with the enhanced green fluorescent protein (EGFP) gene. EGFP will be used as a cytological marker in future studies of *V. dahliae* pathogenesis and the penetration and ramification of the fungus in the plant tissue. The EGFP-labeled pathogen will also be used to study the interaction between the fungus and several potential biocontrol agents. For this purpose, binary vectors for fungal transformation have been constructed. The vectors harbor the bacterial hygromycin B phosphotransferase gene (*hph*) under the control of the *Aspergillus nidulans trpC* promoter as a selectable marker and the EGFP or EYFP (enhanced yellow fluorescent protein) gene under the control of the *Cohliobulus heterostrophus* GAPD promoter. Vectors were inserted into strain AGL–1 of *A. tumefaciens*. Fungal transformation was carried out by cocultivating *A. tumefaciens* strain AGL-1 carrying an appropriate vector with *V. dahliae* conidia at a concentration of 10^6 ml^{-1}. An attempt was made to transform isolates of *V. dahliae* from various hosts, including tomato (race 1 and 2), cucumber, cotton and eggplant. Transformed fungal cells expressing the green or yellow fluorescent protein were visualized under a microscope with UV light at 450–490 nm. Fluorescence was observed in the conidia, mycelia and microsclerotia of *V. dahliae*. When a GFP-transformed *V. dahliae* isolate was used to inoculate eggplants, it was found to be equally pathogenic as the wild-type strain. Moreover, under fluorescence microscopy the vascular bundles of small eggplant roots were observed to emit green fluorescent light.

Expert systems in plant protection. B.N. Ziogas[1] and A.E. Kalamaraki[2]. [1]*Laboratory of Pesticide Science, Agricultural University of Athens, 75 Iera Odos str., 18855 Athens, Greece.* [2]*Benaki Phytopathological Institute, Department of Pesticides Control and Phytopharmacy, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

Expert systems are artificial intelligence applications that emulate the logic and problem-solving proficiency of a human expert. They are most successful in addressing problems in which the knowledge and the experience of a human expert is required. The prediction, diagnosis, control and classification of plant phytoparasites are suitable areas for developing an expert system. The basic components of an expert system are: the user interface, the knowledge base, the database and the inference mechanism. The development of an expert system proceeds through several stages, including problem selection, knowledge acquisition, knowledge representation, programming, testing, evaluation and release into agricultural production. The power of an expert system depends on the knowledge of the experts. The knowledge that the expert uses to solve a problem is written in dependency networks, which are coded into the computer. Expert systems can offer solutions with differing certainty values even when an incomplete set of data is given. The conclusion process, which is used by the system to resolve a problem, is recorded and provides the user with an explanation for how the conclusion was reached.

An empirical model for prognosis of loss assessments in eggplant crops (*Solanum melongena* L.). C.C. Thanassoulopoulos[1], F.A. Bletsos[2] and A.M. Moustafa[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Lab., 54006 Thessaloniki, Greece.* [2]*National Agricultural Research Foundation (N.AG.RE.F), Agricultural Research Center of Macedonia and Thrace, 57006 Thermi, Thessaloniki, Greece.*

A model of Verticillium wilt prognosis in the early stages of eggplant crops has been made based on the results of a three-year experiment. Eggplants were planted in soil infested with *Verticillium* inoculum, and in soil fumigated with methyl bromide used as control, for three consecutive years. Disease incidence was determined at the end of the first month after transplanting and the total yield at the end of the cultivating period was correlated. The model resulted from the integration of the areas line below the regression curve and the ordinates axis on axis X from X_0 to X_5 which are the limits of % yield losses. According to these computations the final proposed model is:

$$\int_0^{100} f(y)\,dy = A = (0.5/n\,(0\times n_0 + 2\times n_1 + 4\times n_2 + 6\times n_3 + 8\times n_4 + 5\times n_5)$$

where n is the total number of counted plants, $n = n_0 + n_1 + n_2 + n_3 + n_4 + n_5$, and the area A has limits 0 to 5. The algorithm which is the results of the model is interpolated in the data of yield losses of each rank of the symptom index found in the experimental work, and the resulting number is the final prognosis for future losses. The model was verified with several results from other experiments, and was highly credible.

The sequence of phases during the feeding cycle of the plant-parasitic nematode *Paratrichodorus anemones*, and its significance for successful tobravirus transmission. E. Karanastasi. *Department of Entomology and Plant Zoology, Laboratory of Nematology, Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

The plant-parasitic, soil-inhabiting nematode *Paratrichodorus anemones* is one of the most efficient vectors of *Tobacco rattle virus* (TRV). Nevertheless, its efficiency as a TRV vector has always been perplexing, since it has been shown that the majority of root cells become irreversibly disorganised when attacked by these nematodes, and since all viruses require living tissues to establish an infection. Cells attacked by *P. anemones* should therefore be no longer suitable for tobravirus establishment. To investigate this contradiction, living *P. anemones* specimens feeding on tobacco seedlings were examined in real-time using video-enhanced interference light microscopy. It was concluded that the feeding cycle of this nematode may be divided in four distinct phases: (i) root exploration, (ii) cell exploration, (iii) cell sampling, and (iv) cell feeding, followed by a period of quiescence. Before commencement of phase (iv), an average of four cells were perforated, but then immediately rejected, so that these cells remained fully functional. Additionally, during phase (iv) 5% of the attacked cells remained alive, providing conditions that are suitable for virus infection. In the study, it was also observed that each feed on a single cell is divided into four phases in a way similar to what has been reported for *Trichodorus similis*, i.e. (i) cell wall perforation, (ii) salivation, (iii) ingestion and (iv) withdrawal.

Mapping the biogeographical distribution and development of electronic databases for the macrofungi of Greece. G. Zervakis[1], D. Dimou[2] and E. Polemis[1,2]. *[1]National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute of Kalamata, 85 Lakonikis str., 24100 Kalamata, Greece. [2]Agricultural University of Athens, Laboratory of General and Agricultural Microbiology, 75 Iera Odos str., 11855 Athens, Greece.*

In recent years the detailed study of the diversity of macrofungi in Greece has increased the number of recorded species to ca. 1300; even so, however, this seems to be less than 25% of the existing number of species. Data on the geographical distribution of almost all mushroom groups are still limited, and there is practically no information to permit a chronological assessment of their abundance in specific regions. The mapping of the fungal diversity is performed by dividing the map of Greece into square grids each with an area of 100 square kilometers; these grids are based on a scale modification of the Chorological Grid Reference System (CGRS); initially only a limited number of selected species are mapped in them. The establishment of an organized monitoring network of macromycetes together and the evaluation of the data collected enables their frequency of occurrence to be estimated, risk assessments to be made, red-data lists to be compiled and measures for the protection of biodiversity to be taken. In parallel, all the information collected is inserted in an electronic database (Claris, FilemakerPro software) which includes fields on the taxonomic arrangement of the fungus, the location of its occurrence, the nature of the host and substrate, photographs, encoding for the exsiccata kept in herbaria, ecomorphological data, etc. Of great importance is the ex-situ conservation of fungal genetic resources and their evaluation for purposes of environmental biotechnology and organic farming programs.

Control of sporulation and germinability of *Botrytis cinerea* Pers. in tomato greenhouses covered with the plastic film TUV 3935. V.A. Bourbos[1], E.A. Barbopoulou[1] and M.V. Kykrilis[2]. *[1]National Agricultural Research Foundation (N.AG.RE.F), Institute of Olive Tree and Subtropical Plants of Chania, Laboratory of Plant Pathology, Agrokipio, 73100 Chania, Greece. [2]Plastika Kritis S.A., P.O. Box 1093, 71110 Heraklion, Greece.*

Botrytis cinerea may cause serious losses of tomato grown in unheated greenhouses. The aim of this work was to study the effect of TUV 3935, a new plastic film for roof covering with a special UV permeability, on the sporulation and viability of *Botrytis cinerea* in comparison with common PE plastic film. Trials were carried out in 0.2-hectare greenhouses in Crete, during two consecutive cultivation periods. *Botrytis* spores in the greenhouse were collected using Petri dishes containing maltose nutrient medium. After incubation for 16 hours at 23°C, the total number of spores, and spores having a germination tube, were counted. There were 10 spore collections at 15 days intervals. The number of infected fruits per plant were also counted before each spore collection. The number of *Botrytis* spores was lower in greenhouses covered with TUV 3935 plastic film (10.3–14.4 per Petri dish, vs. 20.1-26.2 in greenhouses covered with PE plastic film) and the germination of spores was also markedly reduced (12.8–14.7%, vs. 89.9–97.8%). The percentage of infected fruit per plant under TUV 3935 ranged from 13.4 to 16.5% and was statistically lower than that under PE film, which was from 23.8 to 27.0%.

Phytoalexin accumulation during hypersensitive reaction in *Phaseolus vulgaris* challenged by *Pseudomonas savastanoi* pv. *phaseolicola*. D.S. Tsaltas, M. Bennet and J.W. Mansfield. *University of London, Imperial College, Department of Agricultural Sciences, Wye, Ashford, Kent UK.*

The resistant reaction of French bean to strains of *Pseudomonas savastanoi* pv. *phaseolicola* (*Psph*) is visibly

different in terms of the timing of the plant cell collapse, and the browning of the tissue during the hypersensitive reaction (HR). We developed HPLC-based methods to quantify the isoflavonoid phytoalexins (PAs) phaseollin, phaseollinisoflavan and phaseollidin in pod tissue. Analysis of PA accumulation provided a biochemical phenotype of the reactions. Changes occurring in PAs were used to characterise reactions to race 7 of *Psph* and the plasmid-cured variant RW60. In the cultivar Red Mexican the slow development of the HR in response to RW60 was associated with very high levels of each of the isoflavonoids. The PAs were only detected in cells undergoing the HR, not in the surrounding green tissue. The HR elicited by RW60 differed from that activated during gene-for-gene interactions, indicating that it may not be induced by proteins commonly recognised as the product of *avr* genes.

Diseases of fruit trees and grapevine

Invited lecture

The future of plant quarantine in Europe. I.M Smith. *European and Mediterranean Plant Protection Organization, 1 Rue Le Nôtre, 75016 Paris, France.*

The European plant quarantine system, which is enshrined in the relevant EU Directives and in the current regulations of the EU Member States arose as a synthesis of the phytosanitary regulations of individual European countries. Its main purpose remains to exclude from the whole EU region a long list of what EPPO calls 'A1 quarantine pests', and to ensure that the European countries take a common approach in both their regulatory systems and their operational work. These measures taken in relation to so-called third countries have had considerable success. However, the EU system also has to operate in its own single market, so that member states can no longer protect themselves in the same way as before from quarantine pests already present in the EU. In particular, it is no longer possible to take control measures at the borders between member states. Instead, the emphasis now is on producing healthy plant material, certified by 'plant passports', in all parts of the EU. Material moving into certain areas, known as 'protected zones', may be made subject to more stringent requirements before a plant passport can be issued. This internal EU system has the advantage of improving the phytosanitary quality of planting material within the EU, whether traded within or between the member states, and ensuring that nurseries, which have to be duly registered, work to high standards. Nevertheless, it also increases the volume of material moving between member states and thereby increases the possibility for certain quarantine pests to spread that were contained by the old system. However, the problems which have arisen since the new system was introduced have mainly concerned 'EU' pests that are new to certain parts of Europe (*Liriomyza huidobrensis*, *Bemisia tabaci*, tomato yellow leaf curl, *Ralstonia solanacearum*, pepino mosaic), and that therefore require new internal measures and difficult negotiations between the member states. These 'domestic' disputes tend to obscure other important aspects of plant quarantine.

The future of the European system will depend on the success with which such internal measures are handled in future. The problems that arise are illustrated by *Erwinia amylovora* (fireblight) a very dangerous pest that is subject to the protected zone system, and by various citrus pests entering Europe from outside, or moving within Europe. The EU system will also in future have to justify itself in relation to international agreements under the World Trade Organization and the International Plant Protection Convention. In general, the European countries can claim that their phytosanitary measures are technically justified and non-discriminatory to other countries. However, in a world of increasingly frequent trade disputes, countries have to be ready to justify and defend themselves, and this includes performing Pest Risk Analyses according to international standards. National Organizations are accordingly faced with an increasing demand for information on pests, on the losses caused by them, and on the effectiveness of control measures. The fact that at least 10 other European countries are likely to become members of the EU in the next few years also means that they have to accept the EU approach (which thus becomes virtually the standard for Europe, whether wider organizations like the EPPO like it or not), and that the EU system has to consider the concerns of these new members. At present the main concern on the part of the EU is that the new member states should be able to take key phytosanitary measures so as to control high-profile, recently arrived pests. However, the success of the system will also be judged by the degree to which it ensures the future phytosanitary security of the new members.

Finally, it should be pointed out that plant quarantine services in Europe are increasingly concerned with new biological problems that are not a traditional plant quarantine but which now have a high political profile: these problems concern genetically modified organisms, invasive species, and organisms that are harmful to biodiversity. What used to be simply the exclusion of plant pests at the border thus becomes a more general policy to regulate the global movement of organisms, in which plant quarantine services are involved because they have the structure in place to certify and inspect plant material moving in trade, which is one of the principal pathways by which micro-organisms are spread. The purposes of plant quarantine used to be to protect agriculture, but now it also has to protect the environment, and the human consumer.

Oral and poster presentations

Trifloxystrobin ®– the new dimension in plant disease control. A. WITZENBERGER. *BCS – PM – Fungicides*.

Trifloxystrobin is a second-generation strobilurin fungicide of the chemical class of oximino-acetates and offers a very broad and balanced spectrum of plant disease control. It is very effective against ascomycetes, basidiomycetes, deuteromycetes and oomycetes in a large number of temperate, tropical and subtropical crops. Trifloxystrobin inhibits mitochondrial respiration. In general it has a strong fungicidal effect on spore germination and other early stages of fungal development; only in a few specific cases (e.g. *Venturia* spp.) does it inhibit later stages of the pathogen. Consequently trifloxystrobin can be viewed as a foliar fungicide for preventive use. Trifloxystrobin exhibits a unique pattern of distribution and re-distribution on and in plants for which the term 'mesostemic' has been proposed. In short that means that: it has a high affinity to plant surfaces giving it excellent persistence and rainfastness; small amounts of it penetrate the plant tissues and display translaminar and curative activity against specific pathogens; its re-distribution by superficial vapour activity and by surface water ensures significant protection of untreated tissue; and its lack of transport in the vascular system of the plant prevents the dilution of its activity. Trifloxystrobin has a very favourable toxicological and ecotoxicological profile and is safe to operators, consumers and the environment. Consequently it has been given reduced-risk status in the USA, allowing fast-track registration and label extensions. Tailor-made formulations have been prepared to meet specific requirements. These include various solo-products and co-formulations with active ingredients from other chemical classes. Global registration of trifloxystrobin is expected for 2003.

Tristeza in Greece: the progress of eradication. P.E. KYRIAKOPOULOU. *Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece*.

With the further expansion of the EU in 1994, and the consequent abolition of phytosanitary regulations between EU countries, the danger that the destructive *Citrus tristeza virus* (CTV) would be introduced into Greece appeared immediate. As a result, in 1995 the Ministry of Agriculture, in collaboration with the citrus-growing districts and prefectures, started indexing citrus for a possible occurrence of the virus in Greece. As part of this effort, it was discovered in 2000 that in 1994 Lane Late orange seddlings had been illegally introduced from Spain into the Argolis and Chanea prefectures, three of these seedlings were CTV-infected, while their scions had been used for topworking some orchard trees to provide some variety. Since then, an anti-tristeza campain was started in Greece, to eliminate the virus from the country. In Argolis, the battle is systematic and intensive and seems to be successful. Extensive surveys for new cases are being pursued aggressively, and any trees (there have been very few so far) found to be infected are immediately destroyed. Unfortunately, however, some CTV-infected citrus propagation material from Spain and Italy have managed to evade the controls and entered the country. One case, in which such material was introduced illegally through the port of Patras is especially alarming. Attention should therefore be given by the port customs authorities to find and destroy any such attempted introductions. Nevertheless, in new citrus orchards recently established in Argolis with introduced 'certified' citrus seedlings from abroad, some trees were found to be CTV-infected. Unfortunately 'Certified' in this context does not mean 'absolutely virus-free', but only: 'with very limited infection'. However, this grade of less-than-absolute certification is not satisfactory for Greece, which is at the moment basically CTV-free. There is therefore a strong need for EU legislation to be changed so as to prohibit the introduction of any citrus propagation material from other EU countries into Greece. Citrus growers should scrupulously avoid buying any citrus propagation material imported from outside Greece, whether legally or illegally. Fortunately, the rest of the Peloponnese, apart from Argolis, has not shown any cases of CTV. In Crete, however, the situation is very different. A significant number of trees were topworked with infected scions from the two original Lane Late trees imported into the Chanea prefecture in 1994, and some of the growers are objecting to their infected orchards being destroyed. In Crete, besides the Chanea prefecture, the virus has been detected in isolated cases in the Rethymnon prefecture and in one orchard of the Heraclion prefecture. Special phytosanitary measures for Crete to counter the threat should be taken immediately. The Ministry of Agriculture should also proceed with the certification of citrus propagation material.

Tristeza in Argolis and problems arising from the introduction of citrus propagation material. D. DIMOU[1], I. DROSSOPOULOU[2], E. MOSCHOS[2], K. SPANOU[1] and P. DERMATAS[1]. *[1]Argolis Direction of Agricultural Development, Department of Plant Protection, 21100 Nauplion, Greece. [2]Aspropyrgos Control Station of Vegetative Propagation Material, 19300 Aspropyrgos, Greece*.

Tristeza, the most destructive disease of citrus, was detected for the first time in Greece (Argolis, June 2000), in young orange trees, variety Lane Late, of Standard quality (C.A.C.), illegally imported from Spain in 1994. Of those trees, (originally 50 in number), 18 survived, 7 of which were infected with *Citrus tristeza virus* (CTV). Those trees served as inoculum sources, both as mother

trees for obtaining budsticks, and through aphid vectors, for orchards of the Argolic plain (Argos, Dalamanara, Argoliko, Aghia Triada, Aghios Adrianos, Iria, Kallithea Drepanou). All trees of the initial introduction, as well as trees that subsequently became infected through aphids in the field (18) or through grafting, were uprooted when they were identified (74 in all). The surveys were carried out during the spring and autumn and the specimens collected were tested with DAS-ELISA and immunoprinting. During the last surveys (May–June 2002), eight additional trees were found to be CTV-positive in an orchard in Kallithea Drepanou, as well as seven more in a neighbouring orchard; these are additional data providing evidence of aphid transmission. Unfortunately, problems caused by the introduction of citrus propagation trees from other EU countries continue. In the spring of 2001, 1126 duly certified trees of a new clementine clone (Clemenpons) were introduced from Spain and established, but despite certification seven of these trees turned out to be CTV-infected. In April of 2002, there was an attempt to introduce some trees illegally from Italy (Sicily). These trees were of C.A.C. quality, but their accompanying certification label was blue instead of yellow, which was misleading, since blue labels are for 'certified' stock material. These data make clear that CTV-testing of all citrus propagation material introduced from EU countries is imperative. Citrus certification within Greece is also of course vital.

Evaluating the resistance of young olive cultivars and rootstock suckers cv. Amfissis and Lianolia Kerkyras to *Verticillium dahliae*. P.P. Antoniou[1], S.E. Tjamos, E. Markakis, E.J. Paplomatas and E.C. Tjamos. *Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.*

While investigating rapid methods of evaluating the resistance of olive varieties to Verticillium wilt, differences were found between two-year-old trees of the most susceptible cv. Amfissis and the tolerant cv. Kalamon, after the branches were inoculated with a *V. dahliae* conidial suspension (100 μl of 10^8 spores ml^{-1}). Application of this method in suckers of established orchards of the cv. Amfissis in Fthiotis county demonstrated the occurrence of Verticillium-resistant olive rootstocks (4 trees out of 65 inoculated). Since several trees of the corresponding rootstock suckers were infected with the pathogen while the rootstocks remained healthy, a detailed study was conducted by injecting a conidial suspension of the pathogen into two-year-old trees of the Verticillium-susceptible cv. Amfissis and the tolerant cv. Kalamon to elucidate the observed phenomenon. Cultivars were inspected at 10–90 day intervals. The distribution of the pathogen several cm above and below the site of infection was determined. The pathogen was isolated more often and farther from the injection site in the susceptible than in the tolerant cultivar. Differences in symptom development between the cultivars may be due not so much to the difficulty in conidial movement in the vessels of Kalamon variety as to variations in the containment of the pathogen in that variety. A downward movement could be due to the negative pressure observed in the suckers and this would explain why the pathogen ascends further up in those cv. Amfissis trees that developed Verticillium wilt. Similar resistance evaluation experiments were also carried out on olive rootstock suckers on established olive groves of the Verticillium-tolerant variety Lianolia Kerkyras in Preveza county. Of 15 suckers that were injected twice over a period of two years, one remained symptomless and the pathogen was not isolated, but from all the other 14 the pathogen was isolated. Rooted cuttings from suckers of the resistant rootstock will soon be inoculated with *V. dahliae* microsclerotia to evaluate the resistance through soil infection.

Comparative study of isolates of the genus *Camarosporium* from pistachio and olive using RAPDs. E.J. Paplomatas[1], I. Pantelides[1], A. Tzima[1] and A. Chitzanidis[2]. *[1]Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855 Athens. [2]14 Kanari str., 10674 Athens, Greece.*

The fungus *Camarosporium pistaciae* attacks pistachio panicles, shoots and leaves. The anamorph stage *Camarosporium* is rare: usually, the pycnidiospores are of the *Macrophoma* or *Fusicoccum* type. Olive fruits are attacked by the fungus *Macrophoma dalmatica*, which under certain conditions also produces spores of the *Camarosporium* type. This polymorphism, which is usual in *Coelomycetes*, causes a confusion in taxonomy and nomenclature of these two fungi. Their perfect stage is not known, but it is believed that it belongs to the genus *Botryosphaeria*. The aim of the present study was to compare 10 isolates of the fungus from pistachio and 7 from olive using molecular markers. RAPDs markers were selected based on random decamer primers in PCR reactions. After preliminary screening, five primers that generated highly polymorphic bands of the RAPD-PCR products were selected for testing. The electrophoretic profiles of the RAPD bands were scored on a binary basis, where the presence of a band was recorded as '1', and its absence as '0'. These data were used as input in a software package to infer phylogenetic relations. Isolates were divided into two groups that differed by 20%. Each group mostly contained isolates of one host. One group included 7 of the 10 pistachio isolates. The second group was split into two sub-groups, a main subgroup that contained 6 of the olive isolates, and a smaller one that hosted the seventh olive isolate plus the 3 remaining pistachio isolates. It is concluded from these findings that fungal isolates exhibit genetic variation within and between the two plant hosts.

Cypress canker and its control in Greece. P. Tsopelas and S. Xenopoulos. *National Agricultural Research Foundation (N.AG.RE.F), Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 11528 Athens, Greece.*

Cypress canker, caused by the fungus *Seiridium cardinale* (Wag.) Sutton and Gibson, is a very important disease in many Mediterranean countries. In Greece, the disease is widespread. It is most severe in areas with a humid climate, but its incidence is very low in dry climates. A high incidence of cypress canker has been recorded on the west coast, and in the Ionian islands and many areas of the Peloponnese, Sterea Hellas and the island of Euboia. The incidence of cypress canker is also very high in many parts of northern Greece. The main host of the disease in Greece is *Cupressus sempervirens* L., which has been devastated by the pathogen in certain areas. The disease also infects other species of *Cupressus* as well as species of *Thuja* and *Cupressocyparis*, which are used as ornamentals, and lately it has also been found to infect species of *Juniperus* in natural forests of Greece. The most susceptible host is *Cupressus macrocarpa* Hartweg; the variety Goldcrest of this species has recently been widely planted in Greece, contributing to the spread of the disease. Sanitation measures are beneficial in areas with a very low incidence of the disease. Nurseries for the production of Cupressaceae should not be established in areas with high levels of cypress canker. Chemical control with benzimidazolic fungicides (benomyl, carbendazim), can be applied only in nurseries, in spring and in autumn. In areas with a high incidence of the canker, planting resistant trees is the only method of control. In Greece there are more than 100 resistant clones of *C. sempervirens*, of both the *pyramidalis* and the *horizontalis* varieties, which are mainly reproduced by grafting. The species *Cupressus glabra* Sud. and *C. arizonica* Gr. can also be used, since they too have shown resistance to the disease.

Vegetative compatibility groups of *Cryphonectria parasitica* in Greece. C. Perlerou and S. Diamandis. *National Agricultural Research Foundation (N.AG.RE.F), Forest Research Institute, 57006 Vassilika Thessaloniki, Greece.*

Cryphonectria parasitica, the causal agent of chestnut blight, has caused considerable damage in orchards and chestnut forests in several European countries over the last five decades. In Greece the disease was first detected in 1963, on Mount Pelion. A recent survey has revealed that the blight has now spread to almost the whole country. An investigation was carried out into the vegetative compatibility (vc) groups of *Cryphonectria parasitica* in Greece. Twelve widely separated subpopulations distributed all over the country were sampled and a total of 611 isolates were tested. Each isolate was assigned to a single vc group. Four vc groups were identified. Pairing with European vc group testers revealed that the most common vc group in Greece is EU-12, which occurred in all the subpopulations and comprised 88.4% of all isolates. EU-2 was detected in 4 subpopulations and the other two groups, EU-1 and EU-10, were found only in one subpopulation each. Vc group EU-12 has already been reported as the dominant vc group in southern Italy and eastern Europe, while in central Europe the dominant groups are EU-1 and EU-2. In view of the quite small number of vc groups in Greece, we believe that biological control of chestnut blight is possible. The state however should take all possible precautions to pevent the introduction of new vc groups.

Occurrence, distribution and ochratoxigenic capacity of fungi of the *Aspergillus niger* group causing the sour rot of grapes of wine-producing cultivars in Greece. E.C. Tjamos, S.E. Tjamos, P.P. Antoniou, D.F. Antonopoulos and E.J. Paplomatas. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.*

Sour rot of grapes, caused mainly by *Aspergillus niger*, *A. carbonarius* and *A. ochraceum* is a serious disease both in Greece and worldwide in both wine-producing and raisin grape varieties. Besides crop loss due to sour rot, several species of the *A. niger* group also produce ochratoxin A, which negatively affects the quality of fresh grapes, wine and raisins. Within the framework of an EU research project to examine sour rot in general, we decided to investigate the occurrence, distribution and ochratoxin-producing capacity of some fungi of the *A. niger* group collected from wine-producing grape varieties all over Greece. As a side project the sour-rot situation with Corinth raisins was also studied. During 2001 we sampled the wine-producing varieties Xinomauro of Naousa roditis, Cabernet Sauvignon and Sauvignon Blanc from Chalkidiki, Athiri and Cabernet Sauvignon from Mountain Atho, Limnio and Muscat of Alexandria from Limnos and Agiorgitiko and Corinth raisins from Nemea. In 2002 the varieties Mandilaria, Athiri, Cabernet Sauvignon and Grenache were also sampled from Rhodes and the varieties Aidani, Athiri, and Asyrtiko from Santorini. Grapes sampled during fruit setting, veraison and harvesting in selected vineyards of the above-mentioned regions showed that the prevailing *Aspergillus* spp. isolated in the selective media (DRBC and CZ) belonged to the *A. niger* group, and that *A. niger* and *A. carbonarius* were the dominant species. The frequency of isolation of individual *Aspergillus* spp. and the composition of the population depended on the stage of sampling, the variety, the applied control measures and to a lesser extent to the latitude of the vineyard region. Data obtained from ochratoxin A quantification using ELISA and HPLC showed that *A. carbonarius* isolates were very strong

ochratoxin A producers, whereas *A. niger* isolates produced little ochratoxin A.

The role of fungal pathogens associated with substandard grapevine propagation material of cultivars and rootstocks in Greece. I.C. Rumbos, I. Adamopoulos, A. Tourtouri and A. Chatzaki. *National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute of Volos, 38001 Volos, Greece*.

Decline symptoms associated with pathogenic fungi are often observed in young grapevines during the first few years after planting. It was hypothesized that decline may be caused by contaminated or sub-standard grape propagation material produced in Greece or imported from abroad. In the period January–May 2002, 15,000 samples from Greek and foreign nurseries were sent to our laboratory for phytopathogenic fungal identification. Four types of vine material were examined: (a) non-rooted cuttings of rootstocks from both Greek and foreign mother plantations, (b) rooted rootstock cuttings from Greece and abroad, (c) canes of various cultivars used for grafting, and (d) rooted grafted plants ready for planting out. In total, 20,000 petri dishes were used and 80,000 isolations were carried out. Isolations yielded a low incidence (up to 3%) of the fungi *Botryosphaeria dothidea, Cylindrocarpon destructans, Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. In the rootstock mother blocks a basidiomycete sp. was isolated with a high incidence (7–30%); its role is under study. The most prominent result of this study was the low incidence of the pathogenic fungi isolated, although brown wood discoloration was frequent (2–100%). It was assumed that abiotic causes, such as cuts made in the nursery during preparation processes as well as improper storage and transportation of the propagated material, may have contributed to worsening the decline.

Production of standard grapevine propagation material from local cultivars in Cephalonia. I.C. Rumbos, A. Chatzaki, S. Milla, G. Dracopoulos and A.I. Rumbou. *National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute of Volos, 38001 Volos, Greece*.

The lack of certified grapevine propagation material of Greek cultivars combined with the need for healthy propagation material to establish new plantations (EU directive 1493/99) makes it interesting to develop quick procedures for the production of such material. In some grapevine-growing areas of Greece (Cephalonia, Rodos, Paros, Santorini, Samos) attempts have been made to carry out the clonal and sanitary selection of genotypes. In Cephalonia the selection of genotypes from the most important cultivars (Robola, Mavrodafni, Muscat of Cephalonia, Vostilidi) was carried out in the summer of 2000. Sanitary control was directed against viruses, fungi and bacteria that could be disseminated by the propagation material. Virus detection was done by ELISA and the following six viruses were found: *Grapevine fanleaf virus* (GFV), *Grapevine leafroll associated virus* (GLRaV) 1 and 3, *Grapevine virus* A and B, and *Grapevine fleck virus* (GFkV). In total, 855 vines from 81 vineyards and 22 local cultivars were tested. One hundred and ten vines (13%) were virus infected. The most widespread virus was GLRaV-1 (92 vines; 11%); seven vines were infected with GVA, five with GLRaV-3, four with GFV and four with GFkV. Canes from vines that were free of the above six viruses were used to establish mother plants in the local nursery.

A new virus of the genus *Closterovirus* in grapevine. C.I. Dovas and N.I. Katis. *Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 54124 Thessaloniki, Greece*.

Grapevine leafroll disease is widespread all over the world and causes serious crop losses. The production of virus-free propagative material is the only way to combat this disease. However, the fact that at least eight viruses are implicated in its etiology does not allow a reliable and low-cost detection method to be applied. For this reason, we developed a nested-spot polymerase chain reaction (PCR), using degenerate primers, for the simultaneous detection of grapevine closteroviruses. This method was successfully applied in 80 grapevine samples resulting from clonal selection for the detection of viruses associated with grapevine leafroll disease. The same samples were also tested serologically by ELISA using polyclonal antibodies prepared against *Grapevine leafroll-associated virus -1,-2,-3,-5,-6,-7* (GLRaV -1,-2,-3,-5,-6,-7). In grapevine plants cv. Debina from the area of Zitsa (Epirous), a new closterovirus was detected by PCR only. The amplicon of 482 bp was cloned and sequenced, showing a sequence similarity of 80% with GLRaV-5, and of 74% with GLRaV-4. Two new primers were designed and specifically detected the new virus by PCR, in grapevine samples of the cultivars Debina, Repsodebina and Kontokladi from the area of Zitsa.

The new virus disease 'grapevine angular mosaic' is caused by the homonymous new ilarvirus. S.M. Girgis[1] and P.E. Kyriakopoulou[2]. *[1]National Agricultural Research Foundation (N.AG.RE.F), Athens Grapevine Institute, 1 S. Venizelou str., 14123 Lycovryssi, Attica, Greece. [2]Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece*.

A new virus-like symptomatology of grapevine was described in 1994 on the grapevine hybrid Baresana×Baresana in the ampelographical collection of the Grapevine Institute of Athens, in Lykovryssi of

Attica. This symptomatology consisted of a striking angular mosaic on the leaves, connected with the veins and the vein angles, leaf malformation, small leaves and reduction of vine size, flower drop, small berries, small wrinkled and non-germinating seeds, and fruitlessness. Since then, symptoms have gradually become more severe, and two of the original seven symptomatic vines have now died, whereas the remaining five are gradually declining and have become completely fruitless. From all these vines a virus was isolated by mechanical inoculation, originally on *Gomphrena globosa*, and from there to other indicators. On the basis of its seed and pollen transmissibility, its parasphaerical particles and their diameter (29 nm), and the cloning and sequencing of the part of its genome that was characteristic for ilarviruses, the virus was identified as a species of the genus *Ilarvirus* (data presented at the 10th Hellenic Phytopathological Congress). Using pollen of *Chenopodium quinoa* infected with pure culture of the virus as inoculum, the virus was transmitted mechanically to healthy grapevine seedlings from tissue culture, which reacted showing the original field-symptoms on the leaves. In these leaves the virus was detected by DAS-ELISA, using homologous antiserum. With these experiments, the full cycle of Koch's postulates was fulfilled for this virus, so that we can state that it is the cause of the disease originally described. Based on these data, we called this virus *Grapevine angular mosaic virus* (GAMV), after the disease. In preliminary graft-transmission experiments of the virus on grapevine (*Vitis*) indicators we obtained a positive reaction on *V. rupestris* du Lot St. George, with leaf symptomatology similar to the original field symptomatology.

Susceptibility to iron deficiency of some peach rootstocks grown in nutrient solutions. A. Assimakopoulou[1] and C.D. Holevas[2]. *[1]Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece. [2]4 Kanari str., 15121 Athens, Greece.*

The susceptibility of eleven peach rootstocks to iron deficiency was studied in synthetic nutrient solutions (hydroponics). Young rootstocks were grown with iron deprivation (no iron added to the nutrient solution), as well as with reduced iron availability (nutrient solution contained: 3 mM FeEDDHA, 10 mM $NaHCO_3$ and 0.5 g $CaCO_3$ l^{-1}). On the basis of the intensity of leaf chlorosis, three experimental peach-almond hybrids, PR 204/84, Stylianidis K and K.I.D 2, from the Pomology Institute of Naoussa (Greece), showed the same or even greater tolerance to iron deficiency than the French peach-almond hybrid GF 677. The Greek peach-almond hybrid Retsou×Nemaguard, the plum-almond hybrid Myrandier 617 and the peach seedlings GF 305, ID S 37 and Wild Greek Seedling, showed the greatest susceptibility, whereas the plums St Julien GF655/2 and M29C ranked intermediate. Chemical analysis of the leaves showed that iron deprivation decreased concentrations of N and Fe, and increased concentrations of P, K, Mg, Mn, Zn, Cu and B, whereas the addition of bicarbonates decreased not only concentrations of N and Fe but also those of P, Mn and Zn, and increased concentrations of K, Mg, Cu and B. The synergistic and antagonistic relationships observed between leaf nutrient elements both under real iron deprivation, and under low levels of iron caused by a relatively high concentration of bicarbonates - as is usual in calcareous soils - can be a means to detect the early stages of iron deficiency.

Control of Mg deficiency in cherries by using the clones 'F.12/1' (*Prunus avium L.*) and 'Colt' (*P. avium*×*P. pseudocerasus*). Y. Troyanos[1] and N.A. Hipps[2]. *[1]Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece. [2]HRI, East – West Malling, ME19 6BJ, Kent, UK.*

Cherries are susceptible to Mg deficiency which causes defoliation and may reduce plant growth. Differences in susceptibility to Mg deficiency between different cherry varieties have been found in field experiments where *Prunus avium* seedlings showed Mg deficiency symptoms, whereas hardwood cuttings of 'Colt' did not. In order to investigate whether these differences are due to genetic differences, 'F.12.1' and 'Colt' cherry rootstocks from tissue culture were grown in a flowing solution culture system. The results of the experiments showed that 'F.12/1' required a three times greater minimum inflow rate of Mg than did 'Colt'. The minimum inflow rate of Mg in 'F.12/1' was supported by a smaller root system (weight and length) and a greater concentration (500 μM) of Mg in the nutrient solution than with 'Colt' (50 μM). The inhibitory effect of an increased concentration of K in the nutrient solution was also investigated. 'F. 12/1' had a higher concentration of K in the leaves and a greater inflow rate of K. The absorption of Mg in both species was affected similarly by the increasing concentration of K in the nutrient solution. From the results of these experiments it seems that the greater susceptibility of 'F.12/1' to Mg deficiency is due to genetic differences in the size of the root system; 'Colt' consequently had a larger root system than 'F.12/1'. These differences could be used as a guide for the genetic improvement of 'F.12/1' towards larger root system in order to increase its resistance to Mg deficiency.

A serious *Pseudomonas syringae* complex outbreak in apricot trees cv. Early Tirynth and Bebecou in the Argolis region of Greece. P.P. Antoniou, S.M. Christoglou, E.C. Tjamos and A.C. Gregoriou. *Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855, Athens, Greece.*

An unusual bacterial outbreak on apricot trees was ob-

served in the Argolis region of Greece during the spring of 2002. The outbreak was fairly extensive along a stream running through apricot orchards of the villages Sterna and Lyrkia. The damage was serious in the whole area of orchards regardless of the cultivar (Early Tirynth or Bebecou) but it was more severe along or near the stream banks. Heavy rainfall in March, 2002, created very humid conditions. Major symptom development resembled a brown rot epidemic: however, closer field surveys and laboratory examinations showed that *Monilia laxa* was not involved, and in any case fungicide sprays (but no copper compounds) had been extensively used against brown rot. The most striking symptom was necrosis of the leaves and branches as a result of short or elongated cankers and dead areas that formed on the twigs, the bark of the large branches and the trunks. Gummosis was evident in most of the cankers, although in some instances little or no gum was present and the bark became brown, moist and sour smelling. Shriveled flowers and unopened buds were also observed, as were brown necrotic flowers, as a result of which fruit setting was drastically affected. Although the apricot tree was highly susceptible to this disease, records of bacterial infection on apricot are rare in Greece. As regards the identification of the causal agent, laboratory tests (cell morphology, gram stain, oxidase reaction, catalase reaction, acid from glucose, hypersensitivity reaction) as well as fatty acid analysis and Biolog (GN2) tests carried out by BCCM/LMG, showed that most of the isolated bacteria were members of the *Pseudomonas syringae* complex. Two pathovars of *Pseudomonas syringae* (pv. *syringae* and pv. *morsprunorum*) are thought to cause bacterial canker on prunus trees. To check the pathogenicity of the main bacterial isolates obtained, Koch postulates will be applied in the spring of 2003. Previous reports of this disease attributed it to *Pseudomonas* sp., so this is the first report of the epidemic appearance of the *Pseudomonas syringae* complex in Greece.

Molecular detection and characterization of *Citrus tristeza virus* in Greece. C. Varveri. *Benaki Phytopathological Institute, Laboratory of Virology, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

In Greece, *Citrus tristeza virus* (CTV, family Closteroviridae, genus *Closterovirus*) was first identified in Argolis, in 2000, on young Lane Late navel orange trees of Spanish origin. The virus was graft-transmitted to the indicators, sweet orange cv. Madame Vinous and Mexican lime, where CTV was detected by immunoprinting and by IC-RT-PCR, amplifying a 520 bp fragment of the virus 3' end genome between the p20 and p23 genes. RFLP analysis of the PCR product gave the same profile as that produced by the known T385 isolate which belongs to the virus group III strains. The same results were also obtained in the other cases where the virus was isolated: a 20-year-old navel orange tree in Argolis, some Lane Late navel trees in Chania, Crete, and some certified Clemenpons mandarin trees, also of Spanish origin. The phylogenetic nucleotide sequence analysis of the PCR product of the first isolate confirmed its close similarity to the Spanish strains, which is consistent with the historical background of the disease.

Grapevine chlorotic vein banding and grapevine yellow mottle, two new virus diseases. S.M. Girgis[1] and P.E. Kyriakopoulou[2]. *[1]National Agricultural Research Foundation (N.AG.RE.F), Athens Grapevine Institute, 1 S. Venizelou str., 14123 Lycovryssi, Attica, Greece. [2]Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.*

In 1996, in the Amaroussion annex (Sygrou farm) of the grapevine variety collection of the Athens Grapevine Institute, the following two new diseases were observed and were named as follows: i) Grapevine chlorotic vein banding. All ten vines of the white wine grapevine variety Petsariko (origin Kozani, Greece) showed uniform chlorotic bands on both sides of the leaf veins. Using tissue from these vines as a scion, a series of grapevine indicators were grafted (R110, 41B, 5BB, and SO4) in March 2000 and maintained in the greenhouse. In August 2001, the grafted indicator 5BB showed an extended bright-yellow discoloration, in the form of sectors with the veins as axes. To our knowledge this reaction is new to grapevine virology. It should be mentioned that 5BB (*Vitis berlandieri*×*V. riparia*) is a known indicator of grapevine Kober stem grooving disorder, but that disease has a completely different symptomatology. We therefore consider this case as new and worth further study, since 5BB may be a new indicator of a known grapevine virus, or of a new virus of grapevine. In 2002, a virus was mechanically transmitted from the symptomatic vines of the vineyard to the indicator *Chenopodium quinoa*, which showed intense local and systemic mottle, top leaf necrosis and top necrosis. This was therefore a well reacting herbacous host that will help in the study of the virus. ii) Grapevine yellow mottle. In May–June, all ten vines of the coloured wine grapevine variety Lehonitis (origin Magnesia prefecture, Greece) showed a clear yellow mottle in the form of an oak-leaf pattern, which became diffuse later in the summer. In March 2000, using tissue from these vines, the grapevine indicators R110, 41B, 5BB and SO_4 were grafted, of which SO_4 showed wrinkling and chlorosis of the leaves after one year, in May 2001. One year later, in May 2002, the symptomatology of this indicator was more intense. To our knowledge, this indicator reaction is here reported in grapevine virology for the first time. The infection is under further study.

Influence of grape volatiles cv. Isabella in formation of sclerotia by *Botrytis cinerea*. E.K. Kulakiotu[1], C.C. Thanasoulopoulos[1] and E.M. Sfakiotakis[2]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Plant Pathology, P.O. Box 269, 54006 Thessaloniki, Greece.* [2]*Aristotle University of Thessaloniki, School of Agriculture, Laboratory of Pomology, 54006 Thessaloniki, Greece.*

The influence of volatile substances produced by grapes (*Vitis labrusca*) cv. Isabella, on the formation of sclerotia by *Botrytis cinerea* was studied *in vitro*. Since this was a bioassay method, use was made of the closed Mariotte system. To determine the anti-fungal action of the volatile substances the following tests were carried out: (i) using volatile substances excreted by grapes of the resistant cv. Isabella, (ii) using volatile substances excreted by grapes of the susceptible variety Roditis (*V. vinifera*), and (iii) using no volatile substances at all. The action was studied at various temperatures (21, 10 and 0ºC). Volatile substances from cv. Isabella inhibited sclerotium formation. This indicates that the resistance of cv. Isabella to *B. cinerea* is related to the volatile substances secreted by the grape berries, although other factors may also be involved. It also indicates that volatile substances can have an important role as inhibitors or stimulators of an organism.

First report of *Alternaria* spp. as a foliar pathogen of the strawberry tree (*Arbutus unedo*) in Europe. I.A. Laidou and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 26, 54006 Thessaloniki, Greece.*

Leaves of strawberry trees were found to be heavily spotted in the spring of 1999, 2000 and 2001 in the area of Thessaloniki, northern Greece. Small, brown necrotic spots were observed on the leaves, and, where spotting was intense, extensive defoliation. The same symptoms were also noted on the leaves of plants in florists' shops throughout the year. Isolations from the leaves on PDA consistently yielded three isolates that belonged to the genus *Alternaria*. Pathogenicity tests were carried out on healthy plants, which were inoculated with a sterile water suspension containing 6.4×10^3 conidia ml^{-1} plus 0.05% Tween 20. Control plants received only sterile distilled water and 0.05% Tween 20. After incubation in plastic bags for 24 h, the plants were returned to the greenhouse. Symptoms identical to those originally observed appeared ten days after inoculation, on all plants except the control plants. The frequency of reisolation of those fungi from leaf spots was more than 65%. From the three isolates, one belonged to group 4, one to group 6 (according to Simmons' taxonomy), and one did not sporulate. This is the first report of *Alternaria* spp. causing leaf spotting of strawberry tree in Europe.

Infection of olive trees with the fungus *Fomitiporia punctata* (*Phellinus punctatus*). E.J. Paplomatas[1], K. Elena[2], P. Tsopelas[3], A. Tzima[1], A. Paraskevopoulos[4] and A. Papanikolaou[5]. [1]*Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.* [2]*Benaki Phytopathological Institute, Department of Plant Pathology, Laboratory of Mycology, 8 St. Delta str., 14561 Kifissia, Athens, Greece.* [3]*National Agricultural Research Foundation (N.AG.RE.F), Institute of Mediterranean Forest Ecosystems, Alkmanos str., 11528 Athens.* [4]*Prefecture of Messinia, Directorate of Agriculture and Husbandry of Trifyllia, Department of Plant Protection, 24500 Kyparissia.* [5]*Prefecture of Messinia, Directorate of Agriculture and Husbandry of Messinia, 24100 Kalamata, Greece.*

In the last few years, a serious disease of olive trees has been observed mainly in the areas of Messiniaki Mani and Kyparissia. The disease seems to be spread by chainsaws used in olive pruning. The infection is mostly located at the trunk wood and in the main twigs. The most common symptoms of the attack are a brown discoloration leading to wood rot and necrosis of the bark, usually on one side of the trunk, and resulting in cankers. Wood eventually becomes soft and shows symptoms similar to those caused by esca on grapevine. In many cases, fruiting bodies of *Fomitiporia punctata* Murrill [*Phellinus punctatus* (P. Karst.) Pilat] appear on the surface of the trunk and the main twigs. Fruiting bodies are perennial, woody, with a porous, brown, smooth (velvet) surface that expands superficially (resupinate). The fungus has been isolated from the infected wood of several samples as well as from the fruiting bodies, and formed orange brown colonies on PDA. Identification of the pathogen was confirmed by Dr. M. Fischer, Weinbauinstitut, Freiburg, Germany, (personal communication). The pathogenicity of the isolates was tested with artificial inoculations on three-year old olive trees cv. Koroneiki, Amphisis and Kalamon. About three months after inoculation, the infection progress was checked in some of the inoculated trees by removing the bark, inspecting for wood discoloration and re-isolating the fungus. On a trunk cross section, browning of the wood was observed extending 4–5 cm from the inoculation site and *F. punctata* was isolated from wood at 1 and 2.5 cm from the infection site. Because of the slow growth of this fungus, disease development in the rest of the inoculated trees is still continuing. Although this fungus had been recorded on olive trees in Greece before (Plank 1980, Ann. Inst. Phytop. Benaki; F. Kotlaba and J. Klan 1994, Czeck Mycol.), this is the first report of *F. punctata* on olive trees causing economic damage.

Grapevine downy mildew epidemics in the Ionian islands: what has population genetics taught us? A.I. Rumbou[1,2], C. Gessler[2], I.C. Rumbos[1] and I. Adamopoulos[1]. *[1]National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute of Volos, 38001 Volos, Greece. [2]ETH-Zentrum, Institute of Plant Sciences, Universitätstr. 2, 8092, Zürich, Switzerland.*

The role of sexual and asexual reproduction of the oomycete *Plasmopara viticola* in causing the spread of grapevine downy mildew, as well as the distance and means of sporangia dispersal were studied with the help of population genetics. During the period of May to November 2001, leaf lesion samples were collected in non-treated plots in Cephalonia, Lefkada and the Zakynthos islands. Initially, all the primary lesions in each plot were collected first. Every time the environmental conditions favored a new spread of the disease, further sampling was done. In total, three samplings were carried out in Cephalonia (147 lesions), four in Lefkada (640 lesions) and two in Zakynthos (210 lesions). Each leaf lesion was deemed to have derived from a single zoospore and to constitute a single 'isolate' of the population. Microsatellite markers were used to characterize each isolate genetically. Isolates presenting the same allele pattern were taken to be clones while isolates with different allele patterns were assumed to derive from different oospores. In all three epidemic cases, there was a gradual decrease of the population genotypic diversity (Gst); this was because of the clonal reproduction of some isolates. At the same time the populations were enriched with new genotypes, due to sporangia migration from nearby vineyards or late oospore germination (until the middle of July). The three island populations did not share any genotype, meaning there was no sporangia exchange among them. On the other hand, the populations shared the majority of their alleles.

Identification, detection and transmission of a virus associated with the Strawberry Pallidosis disease. I.E. Tzanetakis[1], W.M. Wintermantel[2] and R.R. Martin[1,3]. *[1]Molecular and Cellular Biology Program, Department of Botany and Plant Pathology and Center for Gene Research and Biotechnology, Oregon State University, Corvallis, OR 97331. [2]USDA-ARS, Salinas, CA 93905, USA. [3]USDA-ARS, Corvallis, OR 97330, USA.*

Pallidosis is a disease of strawberry first identified in 1957. Graft transmissibility and the existence of dsRNA in infected plants indicate the viral nature of the disease. DsRNA was extracted from 22 infected plants and cloned. After sequencing about 100 clones we identified eight genes of a virus with homology to *Lettuce infectious yellows virus*, the type member of the *Crinivirus* genus. The major coat protein is most closely related to *Cucurbit yellows stunt disorder virus*, another member of the group. Using the sequence information we developed a sensitive RT-PCR test that detected 37 of the 38 isolates of the virus available to us. We cloned and expressed the major coat protein of the virus and obtained antibodies to the virus for developing a reliable immunological test. We have also started transmission studies with the whitefly species known to transmit criniviruses and we have identified one of them as being a vector, while we are continuing to study the remaining species.

Diseases of vegetable, industrial and ornamental crops

Oral and poster presentations

***Phytophthora* sp. causing stem rot of lettuce. A new disease in Greece.** A. Grigoriou[1] and K. Elena[2]. *[1]Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece. [2]Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

A severe disease of lettuce (*Lactuca sativa* L.) was observed for the first time in Greece, on February and March 2000 in a one-acre field at Marathona, Attika county. The percentage of infected plants was 60%. This disease was observed in the same and in neighboring fields in January, February and March of 2001 and 2002 with similar percentages of diseased plants. Wilt initially affected the lower leaves, then progressed along the upper leaves as well. The plants became chlorotic, declined and died. A brown-black rot appeared along the infected stems starting from the root area and progressing upwards in the stem. A fungus of the genus *Phytophthora* was isolated from all infected plants. On solid and liquid culture media the fungal strains formed semipapillate sporangia, hyphal swellings and coilings of mycelium. Aplerotic oospores formed abundantly with amphigynous and paragynous antheridia. The maximum growth temperature was less than 25°C. Classification of the fungus and pathogenicity tests are in progress. The morphological and physiological characteristics resembled those of *Phytophthora porri* Foister. To our knowledge, this is the first report of the genus *Phytophthora* infecting lettuce in Greece.

Classification of fungi of the genus *Alternaria*. I.A. Laidou and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 26, 54006 Thessaloniki, Greece.*

Different nomenclatures of species of the genus *Alternaria* and different systems of taxonomy have created problems in the taxonomy of this genus. The taxonomi-

cal systems of Saccardo, Elliott, Neergaard, Joly, Ellis, and Simmons have been consulted. The system of Simmons is the best as it distinguishes between species of the genus *Alternaria,* which it divides into six groups based on morphological characters and it defines the similarities and differences between taxa. Isolations of fungi of the genus *Alternaria* in Greece from field-infected cotton, beetroots, parsley and celery plants were classified according to Simmons and assigned to different groups. In this way, of the 31 isolations from cotton, 32.22% belonged to group 1, 9.67% to group 2, 19.35% to group 4, 3.22% to group 5, and 29% to group 6, while 6.45% did not produce any spores. Of the 23 isolations from celery, 26% belonged to group 1, 30.43% to group 2, 4.35% to group 4, 34.78% to group 6, and 4.34% did not produce any spores. Of the 5 isolations from beetroots, one belonged to group 1, one to group 4 and three to group 6. Lastly, of the 4 isolations from parsley, one belonged to group 1 and one to group 5, while two of them did not sporulate.

Characterization of iprodione-resistant isolates of *Alternaria solani*. I. Vloutoglou, I. Aspromougos and T. Katsamakis. *Benaki Phytopathological Institute, Plant Pathology Department, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

Four *Alternaria solani* isolates from naturally infected tomato, and seven from naturally infected potato, all growing in southern Greece (Peloponnese) were examined for sensitivity to iprodione. All isolates were iprodione-sensitive (IS) with EC_{50} values ranging from 0.40 to 2.40 μg ml^{-1}. None of the isolates grew on V-8 agar amended with 10 or 100 μg ml^{-1} of iprodione. However, when mycelial plugs of these isolates were incubated for 3 to 10 days beyond the standard 4 days of the sensitivity test, some of them developed resistant sectors. Isolates randomly selected from these sectors and designated as *in vitro*-derived iprodione-resistant isolates (IR) grew well on V-8 agar amended with 100 μg ml^{-1} of iprodione. The EC_{50} values for these IR isolates ranged from 182 to 614 μg ml^{-1}, whereas the EC_{50} values for their original wild-type IS counterparts were less than 1 μg ml^{-1}. The linear growth as well as the colony morphology of the IR isolates differed from those of their corresponding wild-types, when grown on unamended V-8 agar. The *in vitro* sporulation and pathogenicity of the IR isolates on tomato plants were compared with those of the IS isolates. Studies on a possible genetic differentiation between IR and IS isolates are in progress.

Bacterial bract spot of artichoke caused by *Xanthomonas cynarae*. D.E. Goumas[1], A. Tsagkarakou[1] and S. Splini[2]. *[1]National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, P.O. Box 2228, 71003 Heraklion, Crete, Greece. [2]Technological Education Institute, P.O. Box 1939, 71003 Heraklion, Crete, Greece.*

A bacterial disease of the bract leaves has recently been observed on artichoke in Crete. Symptoms of the disease are restricted to the bracts of the capitulum. Small water-soaked spots appear mainly on the external bracts which progressively coalesce to form larger lesions that are dark-green to brown. The symptoms occur during warm and humid periods and mainly in crops irrigated with an overhead system. The disease reduces the quality of the market crop. Circular, yellow and glistening bacterial colonies are consistently isolated on NAG medium. Morphological, physiological and biochemical tests, coupled with a specific PCR test, identified the eight tested isolates of the bacterium from Crete as members of the species *Xanthomonas cynarae*. Koch postulates have been fulfilled on a bract of a detached capitulum of the plant.

Transmission of *Tomato spotted wilt virus* (TSWV) in relation to *Thrips tabaci* Lindeman host specificity. E.K. Chatzivassiliou[1], P.C. Brunner[2], D. Peters[3], J.E. Frey[2] and N.I. Katis[1]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 54124 Thessaloniki, Greece. [2]Swiss Federal Research Station, P.O. Box 185, CH-8820 Waedenswil, Switzerland. [3]Wageningen Agricultural University, Department of Virology, Binnenhaven 11, 6709 PD Wageningen, The Netherlands.*

Thrips tabaci Lindeman is the most erratic vector of *Tomato spotted wilt virus* (TSWV). Although it is a polyphagous species, its scientific and trivial names (tobacco and onion thrips, respectively) imply that within its wide host range, tobacco and onion/leek are probably its preferred host plants. *T. tabaci* samples were collected from tobacco and leek in different countries, in order to study the vectoring ability and host specialization of each. Thrips populations, from tobacco and leek, showed significant differences in the transmission of TSWV: leek populations did not transmit or transmitted inefficiently, while tobacco populations were highly efficient vectors. In choice tests, the thrips population preferred its natural host, while in infection tests leek populations failed to infect tobacco plants. In preliminary tests, a tobacco and a leek population did not interbreed. Additionally, phylogenetic analysis of mitochondrial cytochrome oxidase I (COI; ~450bp) sequences separated *T. tabaci* populations by their host plant preferences. When other thrips species from previous studies were added, both types of *T. tabaci* formed a monophyletic group. COI sequence variation within different types was low. In contrast, genetic variation between the two host types was high, being comparable to distances among species and representing convincing evidence for genetic struc-

turing within *T. tabaci*, according to host-plant type. So far, results from the biological experiments and genetic analysis suggest a strong polymorphism among *T. tabaci* populations. The question whether *T. tabaci* variants is a cryptic group of sibling species (i.e. whether *T. tabaci* is a species complex) is discussed.

A nested multiplex polymerase chain reaction method for the detection of *Tomato infectious chlorosis virus* and *Tomato chlorosis virus* and the implication of these viruses in epidemics of a yellowing disease of tomato in Greece. C.I. Dovas[1], V. Malioga[1], A.D. Avgelis[2], P.E. Kyriakopoulou[3] and N.I. Katis[1]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. 269, 54124 Thessaloniki, Greece.* [2]*National Agricultural Research Foundation (N.AG.RE.F), Crop Protection Institute, Plant Virology Laboratory, 71110 Heraklion, Crete, Greece.* [3]*Agricultural University of Athens, Faculty of Crop Sciences, Department of Plant Pathology, 75 Iera odos str., 11855 Athens, Greece.*

Since 1997, a yellowing disease has been observed in greenhouse tomato crops in Greece. By 2001, this disease was widespread throughout the country and also affected tomato field crops; in most cases disease incidence was 80–90%. Epidemics were mainly associated with high populations of the whitefly species *Trialeyrodes vaporariorum* and *Bemisia tabaci* (Homoptera: Aleyrodidae). The main symptoms were severe yellowing, downward leaf rolling and brittle leaves. Samples from symptomatic plants were analysed by RT-PCR and were found to be infected with *Tomato infectious chlorosis virus* (TICV) and *Tomato chlorosis virus* (ToCV) (family Closteroviridae, genus *Crinivirus*). TICV was found in 164 of 183 symptomatic samples, while ToCV was less common (25/183). Sequence comparisons of the amplified 229 bp and 466 bp products revealed 99 and 100% identity with the reported sequences of TICV and ToCV respectively. Multiplex RT-PCR using a simple sample-preparation procedure was developed to allow rapid, specific, and simultaneous detection of both ToCV and TICV sequences in two steps. The method involves a one-tube RT-PCR step, in which a pair of degenerate primers amplifies part of the heat shock protein (HSP) region from both ToCV and TICV, and is followed by multiplex nested PCR amplification. This is the first report of TICV and ToCV in Greece, and of TICV in Europe.

Study of a severe NTN isolate of *Potato virus Y*. C. Varveri and N. Vassilakos. *Benaki Phytopathological Institute, Laboratory of Virology, 8 St. Delta str., 14561 Kifissia, Greece.*

Biological, serological and molecular characterization of an NTN isolate (PVY-24) of *Potato virus Y* (PVY, family Potyviridae, genus *Potyvirus*), originating from potato of the Psahna area, Evia, was undertaken. PVY-24 induced severe vein necrosis in *Nicotiana tabacum* cv. Samsun, indicative of PVY^N strains. It also induced severe symptoms of the potato tuber necrotic ringspot disease (PTNRD) on tubers of *Solanum tuberosum* cv. Hermes, indicative of PVY^{NTN} strains, which are considered to form a subgroup inside the PVY^N strain group. Serologically, however, PVY-24 reacted positively with monoclonal antibodies specific to the PVY^O group; this being the first time such a reaction is reported for a PVY^{NTN} isolate. At the molecular level, in RT-PCR PVY-24 reacted positively with specific primers from the 5' region of PVY^{NTN} strains. However, nucleotide sequencing of a fragment of the same region and comparison with those of other isolates led to its clustering with PVY^O strains. By contrast, when nucleotide sequences of a coat protein fragment at the 3' virus genomic region were compared, they were similar to those of the PVY^N strains. PVY-24 is therefore a variant isolate that probably emerged after recombination between different strains. Cloning of its genome for sequence determination is under way.

The effect of infection by a third virus to the specific resistance exhibited by transgenic plants and that induced through cross protection. N. Vassilakos, C. Varveri and F. Bem. *Benaki Phytopathological Institute, Department of Phytopathology, Laboratory of Virology, 8 St. Delta str., 14561 Kifissia, Greece.*

Gene silencing is a general regulation mechanism associated with the inherent ability of plants to control virus infections and transposon DNA elements. In particular, the branch that involves the sequence-specific RNA degradation mechanism (post-transcriptional gene silencing-PTGS or RNA silencing) has been used to produce transgenic plants resistant to viruses. Plant viruses for their part counter by encoding proteins that are suppressors of gene silencing. The present work investigated the effect that infection with two viruses has on resistance to *Tobacco rattle virus* (TRV) exhibited by *Nicotiana tabacum* transgenic plants carrying the 59K region of the replicase gene of TRV. The two viruses used were *Potato virus Y* (PVY) and *Cucumber mosaic virus* (CMV) both of which encode well-characterized gene-silencing suppressor proteins. In these experiments the resistance of the transgenic plants was overcome, since they were infected with TRV at least on the inoculated leaves. There is also evidence that gene silencing is involved in the resistance that is induced through cross protection. In correspondence to the transgenic plants, PVY infection was examined in a cross-protection scheme, in which the mild CMV isolate S (CMV-S) conferred resistance on the virulent CMV-4. *N. tabacum*

plants were inoculated with CMV-S, and 15 days later inoculated with PVY, and then with CMV-4, and a small number of these plants became infected with CMV-4. The significance of these observations for the effectiveness of the above strategies, which are basic for the control of virus diseases, as well as a possible explanation, are discussed.

Incidence of insect-borne viruses of cucurbits in Cyprus. L.C. Papayiannis[1], I.N. Boubourakas[1], C.I. Dovas[1], N. Ioannou[2] and N.I. Katis[1]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 54124 Thessaloniki, Greece. [2]Agriculture Research Institute, 1516 Nicosia, 22016 Cyprus.*

A survey was carried out in order to determine the incidence of cucurbit viruses in Cyprus. About 3000 samples were collected, tested with ELISA, and samples with yellowing symptoms also with RT-PCR. In watermelon crops, the main viruses detected were *Zucchini yellow mosaic virus* (ZYMV) (90%) and *Papaya ringspot virus* (PRSV) (19%). ZYMV was likewise the most prevalent in melon (77%), followed by *Cucurbit aphid-borne yellows virus* (CABYV) (20%) and PRSV (7%). In squash, the predominant viruses identified were ZYMV (59%), PRSV (40%), CABYV (38%) and *Watermelon mosaic virus 2* (WMV-2) (19%). In cucumber crops, *Cucurbit yellow stunting disorder virus* (CYSDV) and *Beet pseudo-yellows virus* (BPYV) were associated with yellowing symptoms with CYSDV being the most widespread, whereas ZYMV, PRSV and *Cucumber yellow vein virus* (CVYV) were detected in only a limited number of samples. *Cucumber mosaic virus* (CMV) and *Squash mosaic virus* (SqMV) were not detected in this survey. Nucleotide sequence analysis of a 791-bp capsid protein fragment from a Cypriot ZYMV zucchini isolate revealed 99% sequence similarity with the Austrian isolate Berlin-1. Finally, sequence analysis of heat shock protein-70 from a cucumber isolate of CYSDV revealed a high similarity with isolates from Spain and Israel.

Criniviruses associated with a yellowing disease of cucurbits in Greece and development of a multiplex polymerase chain reaction for their detection. I.N. Boubourakas[1], A.D. Avgelis[2], P.E. Kyriakopoulou[3] and N.I. Katis[1]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture Plant Pathology Lab. 54124 Thessaloniki, Greece. [2]National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, Plant Virology Laboratory, 71003 Heraklion, Crete, Greece. [3]Agricultural University of Athens, Faculty of Crop Sciences, Department of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.*

Since 1992, a novel disease has been observed in glasshouse cucumber crops. The disease consists of chlorotic angular spots on the leaves that evolve to interveinal yellowing. Large whitefly populations of *Trialeyrodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) were observed in affected plants, suggesting the possible involvement of whitefly-transmitted criniviruses. Moreover, similar symptoms are also caused by *Cucurbit aphid-borne virus* (CABYV). In 2000 and 2001 the disease was widespread with an incidence of 50–80%, and sometimes as much as 100%. Identification of *Beet pseudo-yellows virus* (BPYV) and *Cucurbit yellow stunting disorder virus* (CYSDV) was carried out with the reverse transcription-polymerase chain reaction (RT-PCR) and the sequencing part of the HSP70 gene analogue. A simplified method of multiplex RT-PCR was also developed, using as template crude plant extract, for the rapid, sensitive and simultaneous detection of BPYV, CYSDV and CABYV. The results showed that BPYV was the predominant virus in cucumber and melon crops, whereas CYSDV was isolated only in Rhodes and Leonidio. This is the first report of CYSDV in Greece. CABYV was detected only in four cucumber crops. BPYV also infected arable weeds such as *Amaranthus retroflexus*, *Selosia cristata* and *Sonchus oleraceus* surrounding cucurbit crops.

Relationships between Aphis spiraecola Patch and Cucumber mosaic virus (CMV). K.E. Kapetanopoulou and P.E. Kyriakopoulou. *Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.*

In 1998, in an extended epidemiological investigation on *Cucumber mosaic virus* (CMV) in the tomato-processing area of Eleia prefecture, a high correlation was found between twice-weekly catches of the aphid *Aphis spiraecola* in Moericke traps and the frequency of CMV infection. The correlation prompted us to study this aphid species as a vector of CMV, using analytical transmission experiments in the laboratory. In a previous study of this aphid species as a vector of CMV in Israel, field catches of the aphid were used as experimental vectors. Three clones of the aphid, two from Attica (from sweet orange and *Spiraea media*) and one from Corinthia (from sweet orange), raised in the laboratory on *Spiraea media*, were used. Three isolates of CMV, one from Gastouni, Eleia and two from Corinthia were used in the transmission experiments, and the *Nicotiniana tabacum* cv. Xanthi and Samsung were the virus-sources and indicator plants. The classical aphid virus vector *Myzus persicae* Sulzer (raised on pepper) was used for comparison. The experiment was carried out in an air-conditioned greenhouse. DAS-ELISA was used for virus detection in the experimental plants. The results showed that *A. spiraecola* was a vector of CMV. Transmissibility ranged from 2.8 to 28% in the various treatments. The highest values were

obtained with acquisition feeding periods of 1–6 minutes as compared with the longer periods of 60, 120 and 240 minutes. This was expected, considering the non-persistent transmission of CMV (optimum, a few minutes). No differences in transmission efficiency were observed between aphid clones and virus isolates, but efficiency differed depending on the number of aphid individuals per indicator plant: transmission never occurred with one aphid/plant, but increased with increasing number of aphids/plant. Five to seven aphids/plant gave an easily measurable transmission rate (12.5–28% of experimental plants infected). The transmission efficiency of *A. spiraecola* was slightly lower than that of *M. persicae*. Transmission of the virus by *A. spiraecola* occurred as an erratic phenomenon with low reproducibility, this was also the case with *M. persicae*. In a comparative experiment on tobacco, with mixed infections of CMV and *Potato virus Y* (PVY) as the virus source, *A. spiraecola* (5–7 individuals/test plant) was a much more efficient vector of PVY (92%) than of CMV (14%). The present work showed that *A. spiraecola* participated in the natural transmission of CMV, as well as in epidemics of this virus, depending on its populations and their movements. The epidemiological significance of *A. spiraecola* in the particular experimental field of 1998 in Savalia of Eleia was obvious, since the twice-weekly aphid captures carried out at the height of tomato plants (Moericke traps) yielded individuals of this species almost exclusively throughout the season and in very high numbers.

Resistance of rice to *Fusarium oxysporum* f. sp. *oryzae*. M. Papadopoulou. *Technological Education Institute of Kalamata, Faculty of Agricultural Technology, Antikalamos 24100 Kalamata, Greece.*

Root rot is a very serious and widespread disease of rice. A study of rice plantations in Kazakhstan showed that parasites belonging to the genus *Fusarium* (especially *F. oxysporum* Schlecht f. sp. *oryzae* Bilai) cause root rot of rice. Among the measures to protect the plants we singled out the use of varieties resistant to the disease. To determine the resistance of rice to root rot in the laboratory, five-day-old seedlings were tested. Inoculation was performed by dipping the plant root system into a 10^6 ml^{-1} spore suspension of the fungus or by placing rice seeds with the fungal culture on Petri dishes containing an agar medium. Field inoculation was performed by incorporating into the soil 30–50 g per 100 m^2 of fungal culture grown on oat seeds. A total of 1500 samples of rice were checked, 850 of which came from the collection of the National Bank of Genetic Material of the N. Vavilov Institute of Plant Growth (Petersburg, Russia). None of the rice varieties proved immune to the fungus, only 19 varieties proved highly resistant, 10.6% of varieties showed low sensitivity to root rot, 33.5% medium sensitivity, and 30.4% high sensitivity. The resistant varieties were tested in various kinds of crossings. Those varieties tolerant to cold were also resistant to the fungus. It was also found that disease resistance was cytoplasmically inherited.

The fungus *Dematophora necatrix* on cotton. K. Elena. *Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

In August 1994, in the Fthiotis area, cotton plants were found with roots severely damaged by *Dematophora necatrix* Hartig (perfect state *Rosellinia necatrix* Prill.). In August 2001 the disease appeared again on cotton plants var. Vulcano in Evia Island. Plants in the field had yellow leaves and progressively wilted and died. The disease caused decline, sudden wilting or apoplexy of the plants. The central root was severely attacked, rotted, covered with cottony white mycelium, and infected tissues turned brown. The symptoms appeared in the middle of July. The disease was severe in only one field, in which cotton had been cultivated successively in previous years. The aim of this study was to isolate and identify the causal agent of the disease in Evia Island and to investigate the pathogenicity of isolates of *D. necatrix* to cotton plants of different varieties and ages. Isolates from diseased roots did not form the conidial or the teleomorph stage. The mycelium was white and had pear-shaped swellings adjacent to the septa. In two weeks, the cultures became dark-brown as the fungus formed dark-brown sclerotia-like globose structures. The fungus was identified as *D. necatrix* by its morphological characteristics. Three representative *D. necatrix* isolates were used to inoculate seeds or seedlings of the cotton varieties Acala SJ2, Christina and Myrto using two inoculation methods. All three strains proved to be highly pathogenic to 13 and 30-day-old plants of all varieties. It is clear that this disease of cotton caused severe damage in the field where it occurred. The fungus isolates, as was demonstrated in the greenhouse pathogenicity experiments, attack and destroy cotton seeds and seedlings, causing the same symptoms. In the summer, when the disease appears in the field, it can cause severe losses.

Host plants of *Ralstonia solanacearum* in the Kalavryta area. A.S. Alivizatos[1], P.E. Glynos[1], C. Karafla[1], C. Ziaziari[2] and F. Stathopoulos[2]. *[1]Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece. [2]Regional Plant Protection and Quality Control Centre of Patras, 23 Zaimi str., 26221 Patras, Greece.*

The quarantine bacterium *Ralstonia solanacearum* (Rs) was isolated from 3 out of 6 samples of tomato plants that had symptoms of bacterial wilt collected from the

area of Petsaki Village, Kalavryta. Subsequently the occurrence of Rs in the Kalavryta area was studied by laboratory-testing 39 samples of ware potato tubers, collected from the areas of 11 villages, two samples of egg-plants, one sample of pepper plant, 14 samples of weeds from the banks of the river Selinous and 13 water samples from this river. Rs was detected in 19 potato tuber samples from 7 areas and in one eggplant sample, but not in any samples of pepper, weeds or water. Isolates of Rs from all positive potato, tomato and eggplant samples were identified on the basis of morphological, physiological, biochemical, immunological (IF test) and pathogenicity tests according to EC Directive 98/57/EC. This is the second report of Rs on ware potato, and comes 40 years after the first. Instructions on measures to be taken for the eradication of Rs from potato, tomato and eggplant fields, and for avoiding the undesirable consequences to the area and the country as a whole, were given to the growers (Directive 98/57/EC, PD 255/2000).

Watermelon fruit spot caused by *Pseudomonas syringae* pv. *syringae*. P.E. Glynos[1], A. Paraskevopoulos[2], A.S. Alivizatos[1] and C. Karafla[1]. [1]*Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece.* [2]*Messinia Prefecture, Trifilia Direction of Agriculture and Animal Husbandry, Department of Plant Protection, 24500 Kiparissia, Greece.*

Small necrotic spots surrounded by a halo, and small oily spots were observed on watermelon fruits (*Citrullus vulgaris* Schrad.), late in May 2001 in the area of Agrili, Filiatra, and Trifilia. When the epidermal tissues were cut, the oily spots were found to have progressed and spread deeper into the rind. Transversally cut fruits showed a spread of the infection to the flesh as far as the center of the fruit. Infected tissues had an oily appearance and brown discoloration at the edge. These symptoms appeared in a 2-ha field located in a larger 120-ha watermelon-growing area, following favorable conditions created by a hailstorm and high relative humidity. Affected tissues were not watery or rotted. Bacteria of the genus *Pseudomonas* were constantly isolated in pure culture from these oily spots. Morphological, physiological, biochemical characters and pathogenicity tests (on tobacco leaves and watermelon fruits) identified four of the isolates as *Pseudomonas syringae* pv. *syringae* (Pss) (members of the Ia group of the LOPAT tests). To our knowledge this is the first report of Pss on watermelon in Greece and worldwide.

Physiological stress on clover due to tropospheric ozone toxicity. D. Velissariou[1], A. Assimakopoulou[2] and A. Kolokoutsas[1]. [1]*Technological Education Institute (TEI) of Kalamata, School of Technology-Agronomy, 24100 Antikalamos, Kalamata, Greece.* [2]*Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

The effect of tropospheric ozone on the growth and physiology of clover plants was studied at an experimental site at Benaki Phytopathological Institute, Kifissia, Athens, in the summer of 2001. Two special biotypes of clover (*Trifolium repens*) were used. These two biotypes are broadly used as ozone bioindicators, one being sensitive and the other resistant to ozone damage. Twenty plants of each biotype were grown in pots and harvested every 28 days, to measure the above-ground dry weight of both biotypes. The visible symptoms of ozone toxicity were scored on each biotype before each harvest. In addition, stomatal conductance and net assimilation rate were measured between harvests, using an Lci 2003 (ADC) Infra Red Gas Analyzer. The results showed persistent statistically significant loss of dry biomass and higher ozone toxicity scores for the sensitive biotype than for the resistant one. As well, stomatal conductance of the sensitive biotype was in general lower than that of the resistant one. The net assimilation rate showed no differences between the two biotypes. Clover plants, as bioindicators, recorded highly phytotoxic levels of ozone in the area, and this was confirmed by ozone levels officially monitored by a State air-pollution monitoring station at a close distance to the experimental site. The effects on growth and the differences in physiological responses between the two biotypes indicate that the vegetation growing in a photochemically polluted climate, is subject to long-term, stresses that are often without visible symptoms but can have severe effects on their performance.

Identification of *Phytophthora palmivora* isolates from Dieffenbachia and evaluation of their sensitivity to metalaxyl. K. Elena[1], G. Matthaiadou[2] and A.C. Pappas[2]. [1]*Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece.* [2]*University of Thessaly, School of Agriculture, Plant Production and Agricultural Environment, 38446 N. Ionia, Volos, Greece.*

From *Dieffenbachia maculata* plants showing soft foot rot, in the spring of 2001, a fungus of the genus *Phytophthora* was constantly isolated on PDA medium. A fungus with similar morphological and physiological characteristics had also been isolated from diseased Dieffenbachias originaing from the same glasshouse in 1994. On the basis of morphological and physiological characteristics such as chlamydospores, antheridia, oospores, sporangia, maximum temperature limit for mycelial growth, and the rate of colony development, the 2001 isolates were identified as *P. palmivora* Butler. This species has been a severe pathogen on Dieffenbachia in various states of the USA, since it was first reported in 1947. This is its first report of this fungus on Dieffenbachia plants outside the USA. Twelve ran-

domly selected isolates were tested for their sensitivity to phenylamides. These isolates were obtained from a Dieffenbachia crop where the mixture metalaxyl 7.5% + mancozeb 56% was applied as a soil drench (1 g l^{-1}). Applications were made at 3-month intervals, following each transplanting, during the period 1989–2001. Evaluations of the mycelial growth and sporangia production on PDA media amended with various concentrations of metalaxyl revealed that all isolates were sensitive to phenylamides. Concentrations of 0.1 and 0.5 mg l^{-1} of metalaxyl completely inhibited sporangia production and mycelial growth, respectively. Although the mixture of metalaxyl plus mancozeb has been continuously applied for 12 years, it still offers satisfactory control of Phytophthora soft foot rot of Dieffenbachia.

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *basilici*, a new and serious disease of basil (*Ocimum basilicum*) in Greece. D. Biris[1], D.J. Vakalounakis[2] and E. Klironomou[2]. [1]*National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, 38001 Volos, Greece.* [2]*National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, 71003 Heraklion, Crete, Greece.*

Fusarium wilt is a disease of basil (*Ocimum basilicum*) new to Greece, recognized for the first time in 1999 in Volos. In the immediately following years it was found in all greenhouse and field crops of the small-leaved cultivars of basil around Volos, becoming epidemic, as well as in Agrinion and Heraklion, Crete. The causal agent, *Fusarium oxysporum* f. sp. *basilici*, was identified at the species level on the basis of morphological characteristics, and at the *forma specialis* level using pathogenicity tests and by vegetative compatibility grouping (VCG). All the Greek isolates of the pathogen examined were found to belong to VCG 040, which is the only VCG of the pathogen found so far worldwide. This suggests that the pathogen was probably introduced to Greece through imported organic substrates or seeds from some other country, in which it had already been established. In pure cultures on PDA, the fungus grows in a wide range of temperatures, with an optimum close to 27°C. The fungus is a soil-borne parasite, but it can also infect the above-ground parts of the host, acting as a foliage parasite and then can move to the crown and the root of the plant. The disease can appear at all the growth stages of basil. In the seed bed and the young seedlings the disease is manifested as damping-off. In older plants, the first symptoms are those of drought, i.e. a dull light-green coloring of the foliage, withering, shriveling and partial leaf fall. Wilt can be restricted to only some branches of the plant (hemiplegia), but may gradually extend to the whole plant, which finally dries up. The symptoms often appear suddenly throughout the whole plant which withers and dies very soon (apoplexy). The interior of the infected tissues turns dark in color, while their surface under high-humidity conditions is covered by a cream-colored mycelium and conidia of the fungus. This, and the possibility of infection of the plants through the foliage facilitate the spread of the disease. The fact that the fungus can also act as a foliage pathogen, which is unusual for a soil-borne pathogen, requires a new strategy for disease control.

Effect of nitrogen concentration on the levels of micronutrients in spinach. A. Assimakopoulou, G. Troyanos and A. Vitoratos. *Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

The effect that seven concentrations of nitrogen, 1, 3, 6, 10, 14, 16 and 22 mM N, in the nutrient solution had on the concentrations of Fe, Mn, Zn and Cu in the leaves and roots of the curly leaved spinach cv. Viroflay, was studied in a hydroponic system. Nitrogen was added as nitrate (NO_3-N), except at the concentration of 14 mM N, where it was added as ammonium at a ratio 3:1 (NO_3-N:NH_4-N). At the 14 mM N concentration the growth of leaves was significantly higher than with all other N concentrations. Increasing the N concentration past 14 mM did not affect the level of Fe, but decreased that of Mn (r=-0.66), Zn (r=-0.48) and Cu (r=-0.68). At the 14 mM N concentration, root concentrations of Fe and Cu increased, whereas that of Mn decreased; no significant differences were found among Zn concentrations at any N concentrations. The total plant dry weight was significantly correlated with their content in Fe (r=0.91), Mn (r=0.82), Zn (r=0.76) and Cu (r=0.85). At the 14 mM N concentration, the uptake of Fe, Mn, Zn and Cu, expressed as mg of element g^{-1} d wt of root, was found higher than that with other N concentrations.

Occurrence of *Tomato yellow leaf curl virus* on tomato crops in Greece. A.D. Avgelis[1], N. Roditakis[1], C.I. Dovas[2], N.I. Katis[2], C. Varveri[3], N. Vassilakos[3] and F. Bem[3]. [1]*National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, 71003 Heraklion, Crete, Greece.* [2]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 54124 Thessaloniki, Greece.* [3]*Benaki Phytopathological Institute, Laboratory of Virology, 14561 Kifissia, Athens, Greece.*

In the late summer of 2000, tomato (*Lycopersicon lycopersicum* Mill.) crops grown under greenhouses in Ierapetra, Tympaki and Chania (Crete) showed leaf curling, reduced leaf size, yellowing, short internodes and bushy appearance. More than 30 ha of tomato greenhouses were affected and the disease incidence ranged from 15 to 60%. Similar symptoms were observed in tomato samples from Marathon (Attiki) and the south-

ern Peloponnese, while in 2001 an outbreak was observed in Rhodes island. All greenhouses with infected plants were infested by dense populations of *Bemisia tabaci* (Gennadius), which were also observed outside the greenhouses on several weeds. Tomato symptoms were similar to those caused by *Tomato yellow leaf curl virus* (TYLCV). The assumed virus could not be transmitted mechanically but successful transmission was obtained by grafting on healthy tomato plants. Over 100 samples of symptomatic tomato plants collected from Crete and the southern Peloponnese gave a positive reaction when tested by ELISA using monoclonal antibodies (Adgen Ltd, TYLCV-European). The serological results were confirmed by PCR using two pairs of primers, amplifying different parts of the virus genome. The RFLP analysis (*Alu*I, *Hae*III and *Taq*I) of the 541 bp amplicon obtained with the first primer pair, showed patterns similar to the Israeli species of TYLCV (TYLCV-Is). The second pair of primers gave the expected 348 bp product which was sequenced. Sequence comparisons revealed 99% identity with TYLCV-Is (X15656, X76319). Identity with other isolates of the Israeli cluster (Dominician Republic-AF024715, Cuba-AJ223505, Portugal-AF105975, Iran-AJ13271, Spain-AF071228) was at least 97.7%. This is the first time that TYLCV-Is caused damage in tomato cultivation in Greece.

Production of transgenic plants carrying part of the RNA polymerase gene of *Cucumber green mottle mosaic virus* (CGMMV) and their resistance to the virus. N. Vassilakos, A.S. Rota and F. Bem. *Benaki Phytopathological Institute, Department of Phytopathology, Laboratory of Virology, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

Plant transformation using parts of viral RNA polymerase genes has been used for the production of plants resistant to the relative viruses. This approach was followed in the present study for the production of transgenic plants resistant to *Cucumber green mottle mosaic virus* (CGMMV, genus *Tobamovirus*). First, primers were designed according to the published sequence of CGMMV isolate SH, for the amplification of the 57K part of the RNA polymerase gene of the Greek CGMMV isolate GR-7. Amplification was carried out on total RNA isolated from infected cucumber plant. Primers incorporated *Xba*I and *BamH*I restriction sites for the insertion of the PCR product in pKSII and subsequently *Escherichia coli* DH5a cells were transformed. Recombinant pKSII/57K plasmid was subcloned into the plant transformation vector pWRok2 and mobilized into *Agrobacterium tumefaciens* strain LBA4404 by triparental mating using the helper plasmid pRK2013. *Nicotiana benthamiana* plants were then transformed and their resistance to CGMMV is under study.

Sudden decline of *Washingctonia filifera* caused by *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi*. D.E. Goumas[1] and S. Magoufi[2]. *[1]National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, P.O. Box 2228, 71003 Heraklion, Crete, Greece. [2]Technological Education Institute, P.O. Box 1939, 71004 Heraklion, Crete, Greece.*

A sudden decline of some Washingctonia trees (*Washingctonia filifera)* has been observed over the last few years in the city of Heraklion, Crete. Declined trees are usually less than ten years old. The inner new leaves initially show a pale green discoloration; later all leaves gradually become yellowish. Yellowing starts from the crown periphery and moves downward. A brown discoloration and rot of the inner tissues of leaf stalk, stem and roots also occurs with the whole plant usually declining and dying within two months. Bacteria have been consistently isolated from diseased tissues. So far, six isolates from declining trees have been identified by their morphological, physiological and biochemical profile (tests API 20E and 50CHE): they are members of the species *Erwinia carotovora* subsp. *carotovora* and *E. chrysanthemi*. The symptoms of the disease have been reproduced on six-month to one-year-old Washingctonia seedlings, after injection of a bacterial suspension (10^6 cfu ml^{-1}) into the crown area of the seedlings.

Characterization of *Agrobacterium tumefaciens* isolates from pepper. D.E. Goumas[1] and E. Prousanidou[2]. *[1]National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, P.O. Box 2228, 71003 Heraklion, Crete, Greece. [2]Technological Education Institute, P.O. Box 1939, 71004 Heraklion, Crete, Greece.*

During the last two years, important and unusual infections by *Agrobacterium tumefaciens* have been observed on pepper growing in different parts of Crete (Ierapetra, Arvi and Tympaki). Infection is manifested by gall formation mainly on the stem, with a disease incidence ranging from 20 to 100%. Galls develop at the level of the crown and in injured parts of the plant stem. Initially tumorous tissues are formed at pruning wounds, reaching a size bigger than the stem diameter. Pepper plants with many galls are stunted and eventually die. Infected plants show microelement deficiencies. The crop period was reduced to one to two months, and consequently yield losses were estimated at 10–30%. Bacteria isolated from the galls were identified by standard morphological, biochemical and physiological tests as tumorigenic strains of *Agrobacterium tumefaciens*. The high disease incidence is possibly correlated with the extensive use of commercially available pepper transplants.

Leek rot caused by the bacterium *Pseudomonas syringae* pv. *porri*. P.E. Glynos and A.S. Alivizatos. *Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

Small, oily irregular, isolated spots without a halo were observed on leek (*Allium porrum* L.) leaves early in August 2000, in the area of Megara, Attica, Greece. These spots later coalesced to form larger spots and to spread to the whole leaf area. Fall of the affected leaves was observed at an advanced stage of the disease, such fall was favoured by the overhead irrigation. Observation under the microscope of oily spot tissues taken from diseased samples of leek plants, showed large numbers of bacterial cells exuding from the diseased tissues. Spread of the bacterial suspension onto culture media NA and NAS, caused one type of colonies to form, which were identified as colonies of the bacterial genus *Pseudomonas*. Based on the morphological, cultural, biochemical, immunological, pathogenicity characteristics and the electrophoretic pattern of whole cell proteins of four isolates of the bacterium, these colonies were identified as members of the Ia group of the LOPAT tests and as *Pseudomonas syringae* pv. *porri*. This is the first report of this pathovar for Greece.

Macromycetes associated with *Alnus glutinosa* in Greece. D.M. Dimou[1], E. Polemis[1,2] and G.I. Zervakis[2]. *[1]Agricultural University of Athens, Laboratory of General and Agricultural Microbiology, 75 Iera Odos str., 11855 Athens, Greece. [2]National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute of Kalamata, 85 Lakonikis str., 24100 Kalamata, Greece.*

The genus *Alnus* in Greece is almost exclusively represented by *Alnus glutinosa* (L.) Gaertner. This tree species is distributed throughout northern and central Greece, in central and western Peloponnese and in some Aegean islands (Andros, Naxos, Ikaria). It grows in damp regions, often by lakes or rivers, solitary or in stands. Although these are habitats that favor the occurrence of macrofungi, the reports from *Alnus* in Greece are extremely few. During the last six years *Alnus* stands have been investigated in only two regions: near the mountainous village of Gardiki (Fthiotida) by the Nisvaris torrent and in the Vori valley of Andros island by the seaside. Approximately 50 macromycetes species on *Alnus* have been recorded on *Alnus*, and almost all of them constitute new records for Greece or they were reported on/under *Alnus* for the first time. *Paxillus rubicundulus* is the only species found which is known to be associated exclusively with *Alnus*, while all the others occur in diverse habitats. Some of the wood-destroying species recorded were: *Bjerkandera adusta, Botryobasidium candidans, Coniophora puteana, Crepidotus cinnabarinus, Cyathus striatus, Exidia thuretiana, Phellinus ferruginosus, Hyphoderma setigerum, Scutellinia scutellata,* and *Tomentella stuposa*. In addition, *Conocybe siennophylla, Entoloma ameides, Hemimycena cuculata, H. epichloë, Hypholoma polytrichi, Limacella guttata, Omphalina philonotis, Russula subfoetens* var. *grata, Tubaria conspersa,* and *Xerocomus truncatus* were found growing near *A. glutinosa* (on the soil, on plant remains or among grasses and moss). Of particular interest is the finding of *Galeropsis angusticeps*, which is the first representative of the family Galeropsidaceae and the second secotioid species ever reported in Greece.

Susceptibility of melon cv. Golden Head to races of *Fusarium oxysporum* f. sp. *melonis*. K. Elena[1], A.C. Pappas[2] and V. Papavassiliou[1]. *[1]Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece. [2]University of Thessaly, School of Agriculture, Plant Production and Agricultural Environment, 38446, N. Ionia, Volos, Greece.*

The susceptibility of melon cv. Golden Head to *Fusarium oxysporum* Schlecht. f. sp. *melonis* (Leach and Currence) Snyder and Hansen isolates of the four known races was evaluated. Eight isolates from Israel, representing the four races (0, 1, 2, and 1,2), 10 isolates from Greece, originating from various commercial melon cultivars, and belonging to races 0, 2, and 1,2, and 9 Greek isolates of undetermined race, were tested. Pathogenicity was tested by dipping the roots of 12-day-old seedlings in a conidial suspension of the fungal isolates (10^7 conidia ml^{-1}). Disease severity was assessed 30 days after inoculation. Melon cv. Golden Head was susceptible to four races of *F. oxysporum* f. sp. *melonis* and to all isolates with undetermined race. The isolates of races 1, 2, and 1,2 were highly virulent. Most of the inoculated plants showed apoplexy. The fungus also had a negative affect on the plant's vigor. By the end of the experiment the diseased plants had significantly less height and dry weight compared with the uninoculated controls. Fusarium wilt is the main limiting factor for the reduction of melon cultivation in some areas of northern Greece.

Pathogenicity of *Phytophthora* species to tomato hybrids and a tomato variety. K. Elena and I. Spyropoulou. *Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

The virulence of five isolates of the genus *Phytophthora* was tested on hybrids or varieties of tomato. The isolates tested were the BPIC1131 of *Phytophthora capsici* Leonian, BPIC1184 of *P. cryptogea* Pethybridge and Lafferty, BPIC1197 of *P. erythroseptica* Pethybridge, and BPIC1132 and BPIC1133 of *P. nicotianae* Breda de Haan. Thirty-day-old tomato seedlings cv. Rio Bojo and the hybrids Belladona F_1, Electra F_1, Polo F_1, tomato 1028, tomato 1410, tomato 1415 and tomato 1418 were used.

Isolates of the fungus were subcultured on Petri dishes with corn meal agar (CMA) for 15 days. Inoculum was prepared by blending the contents of 14 inoculated dishes in a litre of deionized water. Twenty ml of inoculum was poured into the soil around the stem of each plant. Seven plants from each variety were inoculated with each *Phytophthora* strain. The isolates most virulent to all the tomato plants were these of *P. nicotianae,* and specifically *P. nicotianae* isolate BPIC1133. The tomato plants most resistant to this virulent isolate were the hybrids Belladona F_1 and Polo F_1. The isolates of *P. capsici* and *P. erythroseptica* were not very virulent. The isolate of *P. cryptogea was* medium virulent. The study showed that tomato was primarily attacked by *P. nicotianae*.

First report of *Alternaria* spp. as a foliar pathogen of lettuce in Greece. I.A. Laidou and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 26, 54006 Thessaloniki, Greece.*

Heavily spotted leaves of lettuce were found in the winter of 1999, 2000 and 2001 in parts of northern Greece, Thessaloniki and Halkidiki. The brown necrotic spots were initially rather small, with concentric cycles, and later becamne confluent, creating extensive infected areas. Intense spotting occurred mainly on older leaves. From the leaves, eight isolates of fungi belonging to the genus *Alternaria* were isolated on potato dextrose agar. Pathogenicity tests were performed on healthy, four-week-old plants inoculated with a sterile water suspension containing 5×10^3 conidia ml^{-1} plus 0.05% Tween 20. Control plants received only sterile distilled water plus 0.05% Tween 20. After incubation in plastic bags for 24 h, the plants were replanted near the greenhouse. Symptoms identical to the original ones were observed ten days after inoculation in all plants except the control plants. From the eight isolates, three belonged to group 1; one to group 2; three to group 6 (according to Simmons' taxonomy) and one did not sporulate. This is the first report of *Alternaria* spp. causing leaf spotting of lettuce in Greece.

First report of *Alternaria alternata* as a foliar pathogen of a *Magnolia* sp. in Europe. I.A. Laidou and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 26, 54006 Thessaloniki, Greece.*

Leaves of Magnolia plants were found covered with small, brown to necrotic spots during a disease survey of Magnolia *(Magnolia* sp.*)* in the spring of 1999, 2000 and 2001 in northern Greece. Intense spotting was associated with defoliation as well as impairment of the ornamental value of the plants. Three fungal isolates from symptomatic leaves incubated on PDA belonged to the genus *Alternaria*. Pathogenicity tests, according to Koch's postulates, were conducted by wounding leaves with a sterilized needle and inoculating two plants per isolate with 5 ml of a sterile water suspension of 5×10^3 conidia ml^{-1}. Two control plants received only sterile distilled water. Plants were placed in a growth chamber at 25°C. A week later, necrotic lesions developed on the leaves similar to those originally observed and the fungi were reisolated from all except the control plants. Of these three isolates, one belonged to group 1 and the rest belonged to group 4 according to Simmons's taxonomy. One of those isolates that belonged to group 4 was identified as *Alternaria alternata* (Nees: Fries) Keissler, by its conidial and morphological characteristics. This is the first report of *Alternaria alternata* causing leaf spotting and defoliation of Magnolia plants in Europe.

Hosts of *Verticillium dahliae* race 2 in Greece and worldwide. E.K. Ligoxigakis[1], D.J. Vakalounakis[1] and C.C. Thanassoulopoulos[2]. [1]*National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, 71003 Heraklion, Crete, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 54006 Thessaloniki, Greece.*

To determine new hosts of *Verticillium dahliae* race 2 in Greece, the pathogenicity of 105 isolates, collected in several parts of Crete in 1997–2002 from 25 cultivated and 12 weed species, was checked in artificial inoculation tests (root-dip technique) on the differential tomato cultivars Earlypak No 7 (lacking the *Ve* gene) and ACE 55 VF (possessing the *Ve* gene). Of the 105 isolates: 50, obtained from 21 cultivated species: *Brassica oleracea* var. *botrytis*, *B. oleracea* var. *capitata*, *B. oleracea* var. *italica, CaΠcum annuum, Cicer arietinum, Cichorium endivia, C. intybus, Citrullus vulgaris, Cucumis melo*, *C. sativus, Cucurbita pepo, Lactuca sativa var. longifolia, Lathyrus ochrus, Lycopersicon esculentum, Olea europea, Phaseolus vulgaris, Raphanus sativus, Solanum melongena, S. tuberosum, Tagetes erecta* and *Vicia sativa,* and 17, obtained from nine weed species: *Capsella bursa-pastoris*, *Cardaria draba, Convolvulus arvensis, Erodium* sp., *Raphanus raphanistrum, Senecio vulgaris, Sinapis alba, Solanum nigrum* and *Trifolium* sp., belonged to race 2. Of the remaining 38 isolates, 12, obtained from nine cultivated and one weed species, belonged to race 1; whereas 26, obtained from ten cultivated and six weeds species were not pathogenic to tomato. Of the 30 hosts of *V. dahliae* race 2, nine: *C. arietinum*, *C. melo, C. sativus, C. pepo, L. sativa* var. *longifolia*, *L. esculentum*, *O. europea, S. melongena* and *S. nigrum,* belonging to five botanical families, are known; while the remaining 21, belonging to seven families, are newly reported worldwide.

Pathotypes of *Verticillium dahliae* in Greece. E.K. Ligoxigakis[1], D.J. Vakalounakis[1] and C.C. Thanassoulopoulos[2]. [1]*National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, 710 03, Heraklion, Crete, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

To determine the pathotypes of *Verticillium dahliae* in Greece, 105 isolates of the fungus, collected from several parts of Crete in 1997–2002 from 25 cultivated and 12 weed plant species, were checked for pathogenicity in artificial inoculation tests using the root-dip technique on four differential plant species: *Brassica rapa* var. *rapifera*, *Capsicum annuum*, *Lycopersicon esculentum* and *Solanum melongena*. Seventy-one of the 105 isolates, from 21 cultivated and 10 weed species belonging to ten families, were pathogenic to tomato, eggplant and turnip, and classified as pathotype I; 26 isolates, from ten cultivated and six weed species, belonging to seven families, were pathogenic to eggplant and turnip, and classified as pathotype II; and eight isolates, from two cultivated species belonging to one family, were pathogenic to all four differential plant species and classified as pathotype III. Pathotypes I, II and III are widely, moderately and sparsely distributed respectively.

***In vitro* and *in planta* screening of a non–pathogenic isolate of *Fusarium oxysporum* and two *Pseudomonas* strains as biocontrol agents against *Verticillium dahliae* on eggplant.** G.A. Bardas, C.C. Thanassoulopoulos and A.L. Lagopodi. *Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Lab., 54006 Thessaloniki, Greece.*

The non-pathogenic isolate Fo47 of *Fusarium oxysporum* and the bacterial strains *Pseudomonas fluorescens* WCS365 and *P. chlororaphis* PCL1391 were tested *in planta* and *in vitro* for their potential as biocontrol agents against the fungus *Verticillium dahliae* on eggplant cv. Tsakoniki. *In vitro* tests were carried out on the interactions between the bacterial strains and the pathogenic fungus in dual and triple cultures, on King's broth medium+2% bacteriological agar. The growth of *V. dahliae* was not inhibited by *P. fluorescens* WCS365. In contrast, *P. chlororaphis* PCL1391 caused complete inhibition of *V. dahliae* growth. In the *in planta* tests, test plants were grown under the simultaneous effect of *V. dahliae* and one of the biocontrol agents. The pathogenic fungus and the non-pathogenic Fo47 were mixed thoroughly in the plant growth medium in a ratio of 300 microslerotia of *V. dahliae* and 3000 conidia^{-1} of Fo47 per g of soil. Bacteria were applied by a seed coating with methyl cellulose containing 2×10^9 cfu ml^{-1}. Only *P. chlororaphis* PCL1391 reduced disease incidence significantly. This bacterial strain did not cause significant differences of measurable plant growth characteristics in comparison to plants that were grown with *V. dahliae* alone.

Incidence of insect-borne cucurbit viruses in Greece. C. Papavassiliou[1], C.I. Dovas[1], L.C. Papayiannis[1], A.D. Avgelis[2], P.E. Kyriakopoulou[3], K. Doulias[4] and N.I. Katis[1]. [1]*Aristotle University of Thessaloniki, Plant Pathology Laboratory, 54124 Thessaloniki, Greece.* [2]*Crop Protection Institute, Plant Virus Laboratory, Katsambas, 71100 Heraklion, Greece.* [3]*Agricultural University of Athens, Faculty of Crop Sciences, Department of Plant Pathology, 75 Iera Odos str., 11855 Athens, Greece.* [4]*Hellenic Sugar Industry, Orestias Factory, Plant Pathology Department, 68200 Orestiada, Greece.*

In 1999 and 2000, a survey was carried out to determine the incidence of insect-borne viruses in cucurbit crops in Greece. A total of 1453 and 4995 leaf samples were collected in 1999 and 2000 respectively, from symptomatic plants in 32 parts of Greece. Tests were performed by ELISA using antibodies against: *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus-2* (WMV-2), *Papaya ringspot virus*, (PRSV) *Zucchini yellow fleck virus* (ZYFV), *Squash mosaic virus* (SqMV) and *Cucurbit aphid-borne yellows virus* (CABYV). A total of 1951 arable weeds (38 species) were also tested by mechanical inoculation onto indicator plants, while 834 samples were tested for CABYV by ELISA. Watermelon samples were predominantly infected with WMV-2 (49%) and ZYMV (43%), and with PRSV (7%) in a limited number of samples. Melon samples were infected predominantly with WMV-2 (76%), followed by CMV (50%) and CABYV (20%). A limited number of melon samples was infected with ZYMV (7%), SqMV (6%) and PRSV (1%). Zucchini samples were infected with WMV-2, (78%), CABYV (40%), ZYMV (31%), CMV (11%), ZYFV (3%), PRSV (2%) and SqMV (0.15%). Cucumber samples were infected with CMV (53%), WMV-2 (20%), CABYV (20%), ZYMV (5%) and PRSV (1%). Arable weed plants (4%) of the families Amaranthaceae, Caryophyllaceae, Compositae, Cruciferae, Cucurbitaceae, Labiatae, Malvaceae, Portulacaceae, Rubiaceae, Solanaceae, Umbelliferae, Urticaceae and Zygophylaceae were infected with CMV, ZYMV, ZYFV and WMV-2, whereas CABYV was detected on *Amaranthus retroflexus* and *Abutilon theophrasti*.

Study of potential aerial infection of wounded stems of cucumber plants grafted onto rootstock resistant to *Fusarium oxysporum* f. sp. *radicis-cucumerinum*. G.C. Pavlou[1] and D.J. Vakalounakis[2]. [1]*N.AG.RE.F, Olive and Horticultural Crops Institute, 85 Lakonikis str., 24100 Kalamata, Greece.* [2]*National Agricultural Research Foundation (N.AG.RE.F), Plant Pro-*

tection Institute, P.O. Box 2228, 71003 Heraklion, Crete, Greece.

The capacity of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* to infect aerially wounded stems of cucumber plants (Brunex F_1) grafted onto resistant rootstock TZ-148 F_1 (*Cucurbita maxima*×*C. moschata*) was investigated in an experimental unheated (Ierapetra's type) greenhouse. Two sets of artificial-inoculation experiments were carried out on wounded stems and cross-sectioned petioles of adult plants, 36 and 125 days old, in autumn (Nov 27) and winter (Feb 4), respectively. The following inoculation treatments were given in five replicates arranged in a complete randomized block design: (A) In autumn: (i) through incisions, 2–3 cm long and 2–3 mm deep, using a surgical blade. (ii) through incisions tangentially on the stems, using a surgical blade, where a piece of tissue1.5–2 cm long, 0.5 cm wide and 2–3 mm thick was removed. Incisions were swabbed with a spore suspension of the pathogen adjusted to 7×10^6 spores ml^{-1}. (B) In winter: (i) through incisions and swabbings made as in summer treatments (i) and (ii). (ii) through incisions made as in summer treatment (i) and as well spraying with the same spore suspension. (iii) through cross-sections of petioles on the nodes, using a surgical blade and spraying them with the same spore suspension. (iv) through cross-sections of petioles as in (iii) and then dusted with a soil/powder mixture containing 1.4×10^7 spores ml^{-1}. All treatments in both seasons were applied at three heights on the stem: 20, 100 and 180 cm from the ground. Wounded stems of the cucumber plants could be infected with the pathogen at any height. Since heavy masses of pathogenic conidia are produced on the stems of diseased plants in commercial greenhouse cucumber crops, and since these conidia can be aerially transmitted to neighbouring plants, it is concluded that *F. oxysporum* f. sp. *radicis-cucumerinum* can cause secondary infections on the above-ground parts of cucumber, like a polycyclic foliar pathogen.

Preliminary results from the inventory of macrofungi in mountainous areas of Naupactia (Aitoloakarnania, central Greece). E. Polemis[1,2], D.M. Dimou[2], L. Pountzas[3,4], D. Tzanoudakis[3] and G.I. Zervakis[1]. [1]*National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute of Kalamata, 85 Lakonikis str., 24100 Kalamata, Greece.* [2]*Agricultural University of Athens, Laboratory of General and Agricultural Microbiology, 75 Iera Odos str., 11855 Athens, Greece.* [3]*University of Patras, Department of Biology, Panepistimioupoli, 26500 Rion, Greece.* [4]*Technological Education Institute of Mesologgi, Nea Ktiria, 30200 Mesologgi, Greece.*

A mycofloristic study of the Naupactia region was recently undertaken as a part of a project on the inventory and mapping of Greek macromycetes. The field study started in autumn 2001, within the greater area of the Kryoneria, Ambelakiotissa, Ano Chora and Limnitsa villages, at an altitude of 600–1200 m. The examined sites consisted mainly of *Quercus pubescens* and *Abies cephalonica* forests, and mixed stands of *A. cephalonica* with *Castanea sativa* or *Carpinus orientalis*, Mediterranean evergreen shrubs, streams, mountain meadows and pastures, etc. From the specimens collected so far, 83 species of macrofungi have been identified, which belong to 61 genera. Of these, 17 are reported for the first time in Greece: *Phaeomarasmius erinaceus* (first record of the genus in Greece); *Gleocystidiellum luridum* and *Hypoxylon fuscum* on *C. orientalis; Hemimycena crispula; Tomentella badia; T. fibrosa* and *Tubulicrinis sororius* on *C. sativa; Hyphoderma argillaceum; Panaeolus guttulatus* and *Pluteus plautus* on *Junglans regia;* and *Tubulicrinis calothrix* on *A. cephalonica*. Furthermore, *Cheilymenia stercorea*, *Conocybe coprophila*, and *Coprinus schroeteri* were found on cow manure, and *Entoloma nitens*, *Omphalina grisella* and *Russula alutacea* in mixed stands of *A. cephalonica* and *C. sativa*. The mycorrhizal taxa *Boletus calopus*, *B. queletii*, *B. reticulatus*, *Lactarius azonites*, *L. piperatus*, *Russula acrifolia* and *R. nigricans* are reported for the first time in association with *C. sativa*, while 17 wood-rot fungi are first host-records in Greece.

Nitrate critical concentrations in lettuce plants. Y. Troyanos and A. Assimakopoulou. *Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Athens, Greece.*

In lettuce growing, it is important to apply nitrogen fertilizer according to the nitrogen requirements of the crop, so as to produce plants with a nitrate content low enough for safe human consumption. Current nitrogen requirements are assessed by means of plant-tissue analysis. This technique is a useful guide to achieve high nitrogen use efficiency and minimize environmental pollution. However, the optimization of nitrogen nutrition in the field by applying diagnostic tissue analysis is time-consuming and sometimes not easy to apply in fast growing plants like lettuce. For this reason a semi-quantitative method of nitrate-nitrogen determination, known as Merckoquant strips, was used in this study, which was carried out on butterhead lettuce grown in sand culture with different concentrations of nitrate. The nitrate-nitrogen content of petioles of young, fully expanded leaves, as determined by Merckoquant strips, correlated well with the growth rate of the plants. These results along with the additional information obtained from field trials showed that the Merckoquant strips could be used reliably to check the nitrogen nutrition of lettuce plants.

Molecular diagnosis of the cucumber pathogens *Fusarium oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *radicis-cucumerinum*. G.A. Fragkiadakis and D.J. Vakalounakis. *National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, P.O. Box 2228, 71003 Heraklion, Crete, Greece.*

In a previous study (Vakalounakis and. Fragkiadakis, 1999. *Phytopathology* 89, 161–168) we used random amplification of polymorphic DNA (RAPD) to assess the genetic variability of 106 pathogenic isolates of *Fusarium oxysporum* obtained from cucumber and classified by pathogenicity tests as *formae speciales* of either *cucumerinum* (Fusarium wilt) or *radicis-cucumerinum* (root and stem rot). For diagnostic purposes, twelve polymorphic RAPD-bands that distinguished the isolates of the two *formae speciales* were purified from 2% agarose gels, cloned and sequenced in both DNA strands. Sequences were used to design specific primer pairs of 18–20 nucleotides in length. The specificity and sensitivity of the primer pairs in the detection/identification of each pathogen was tested using the polymerase chain reaction. Five pairs of primers that distinguished the two *formae speciales* using the polymerase chain reaction with an annealing temperature of 54°C were detected.

Viruses infecting pea (*Pisum sativum* L.) crops in Greece. E.K. Chatzivassiliou[1], H. Boubourakas[1], S. Winter[2], D.E. Lesemann[2], A.D. Avgelis[3] and N.I. Katis[1]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 54124 Thessaloniki, Greece. [2]Department of Plant Virology, Microbiology and Biosafety, Messeweg 11-12, D-38104 Braunschweig, Germany. [3]National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute, Plant Virology Laboratory, 71110 Heraklion, Crete, Greece.*

During the spring of 2000, pea crops in the Thessaly and Thessaloniki prefectures showed severe virus-like symptoms consisting of vein-clearing, leaf and pod distortion, stem necrosis, and stunting. Samples from symptomatic plants were collected and tested with electron microscopy (EM), as well as serologically with DAS and TAS ELISA. In the EM preparations, filamentous virus particles were observed in most cases, while serological methods identified seven viruses either in single or mixed infections. *Pea enation mosaic virus* (PEMV) (44%), *Bean leaf roll virus* (BLRV) (40%) and *Pea seedborne mosaic virus* (PSbMV) (12%) were found in the majority of the samples. A lower incidence was recorded for *Cucumber mosaic virus* (CMV) (4%), *Beet mosaic virus* (BtMV) (4%) and *Beet western yellows virus* (BWYV) (4%). A limited number of samples collected in Thessaloniki were infected with *Tomato spotted wilt virus* (TSWV). None of the samples were infected with *Potato virus Y* (PVY), *Broad bean wilt virus* (BBWV), *Turnip mosaic virus* (TuMV), *Watermelon mosaic virus 2* (WMV-2), *Lettuce mosaic virus* (LMV), *Bean common mosaic virus* (BCMV), *Alfalfa mosaic virus* (AMV) or *Tobacco mosaic virus* (TMV). Additionally, a primer set was designed for the detection of RNA-1 of PEMV, and polymerase chain reaction (PCR) tests showed the presence of both RNAs of the PEMV complex. The occurrence of other viruses as well as the incidence of seed-borne viruses in the seed lots that were used for the establishment of the affected crops are under study.

Chemical control

Oral and poster presentations

The control of late blight of potato and tomato and grape downy mildew in southern Europe with a Famoxate® based mixture. C. Theocharis[1], Y. Stamatas[1], P. Cagnieul[2]. *[1]Du Pont Agro Hellas S.A., 12 Solomou str. and Vas. Georgiou 15232 Athens, Greece. [2]Du Pont de Nemours France S.A., C.P.P., 137 rue de l'Université, F-75334 Paris Cédex 07, France.*

Famoxadone (Famoxate®) is a new active ingredient from DuPont with outstanding fungicidal properties. On grape downy mildew *(Plasmopara viticola)* and late blight of potato and tomato (*Phytophthora infestans)* it primarily exerts a potent preventive activity by inhibiting spore survival and germination even at very low concentrations. Mixtures of famoxadone (Famoxate®) and cymoxanil (Curzate,) were tested on potatoes, tomatoes and grapes for combined protectant and curative activity. The results of field experiments indicate that in southern Europe this combination can provide better control of *P. infestans* than the standard mixtures with penetrant properties, resulting in parallel in a reduction of the total fungicide rate of more than 80%.

Control of Cercospora leaf-spot disease of sugarbeets (*Cercospora beticola*) with specific spraying programs using fewer fungicide applications. C. Doulias[1], A. Gkizelis[2], P.M. Ioannidis[3]. K. Pashalidis[4] and X. Nerantzis[5]. *[1]H.S.I. Orestias Sugar Factory, Plant Protection Service, 68200 Orestiada. [2]H.S.I. Thesaloniki Head Quarter, Plant Protection Service, 54000 Thesaloniki. [3]H.S.I. Platy Sugar Factory, Plant Protection Service, 59032 Platy Hemathias. [4]H.S.I. Seres Sugar Factory, Plant Protection Service, 62100 Seres. [5]H.S.I. Xanthi Sugar Factory, Plant Protection Service, 67100 Xanthi, Greece.*

The leaf-spot disease *Cercospora beticola* is the most important disease of sugar beet, and under Greek climatic conditions can cause yield losses of up to 30–35%. Control of this disease with chemical fungicides (6–8 treat-

ments) is very expensive and has a high risk of development of resistance to fungicides and of environmental concerns. Proper resistant sugar beet varieties for definite solutions have not yet been developed. A study of the application of specific fungicide programs gives promising results. An integrated pest management system for the control of *C. beticola* is being developed step-by-step based on the use of resistant varieties and specific fungicide programs. This system has already improved control of leaf spot and reduced the cost. A wide range of spraying programs was evaluated in field trials in the framework of a project lasting several years. Climatic conditions (e.g. temperature, relative humidity) and agronomic parameters (sowing date, row closure, irrigation), which are very important factors in determining the timing and intervals of fungicide spraying, have been determined. The succession of fungicides applied as stand-alone treatments, in mixtures or in alternation, has been defined. A warning system for sprays based on prevailing temperatures was also studied. High daily temperatures (more than 32–35°C), which are very common during July, inhibit the germination and growth of *C. beticola* conidia, leading to suppression of the disease. As long as these conditions are met the interval between applications can be prolonged to as much as 30 days, according to the susceptibility of the cv., resulting in reduced cost of control to farmers and a lower impact on the environment.

Effect of phenylpyrrole-resistant mutations on the phytopathogenic fitness of *Botrytis cinerea*. B.N. Ziogas, A.N. Markoglou and V. Spyropoulou. *Agricultural University of Athens, Laboratory of Pesticide Science, 75 Iera Odos str., 11855 Athens, Greece.*

Mutants of *Botrytis cinerea* highly resistant to fludioxonil (Rf: 5.000, based on EC_{50} values) were isolated from a wild-type strain, with a high mutation frequency of 10^{-3}, after chemical mutagenesis with *N*-methyl-*N*-nitrosoguanidine (MNNG). Approximately 95% of fludioxonil-resistant mutants were found to be more sensitive to high osmotic pressure than the wild-type strain of *B. cinerea*. Cross-resistance studies with other fungicides showed that the mutations for resistance to phenylpyrroles affect the sensitivity of mutant strains to the aromatic hydrocarbons dicloran (Rf: 100), chloroneb (Rf: 130) tecnazene (Rf: 70) and tolclofos-methyl (Rf: 750) and to the dicarboximides iprodione (Rf: 100) and chlozolinate (Rf: 40), but not to benzimidazole benomyl, the mixture of benzimidazole-phenylcarbamate (carbendazim+diethofencarb), to the anilinopyrimidine cyprodinil, to the phenylpyridinamine fluazinam, to the hydroxyanilidine fenhexamid, to chlorothalonil and to the sterol biosynthesis inhibitors triadimenol and fenpropimorph. A study of fitness parameters in the wild-type and representative fludioxonil-resistant mutants of *B. cinerea* showed that the osmotic-sensitive isolates showed a significant reduction in characteristics determining phytopathogenic fitness, such as mycelial growth, sporulation, conidial germination, sclerotia production and pathogenicity on cucumber seedlings. By contrast, in the case of the osmotic-resistant isolates the mutations for phenylpyrrole-resistance may or may not affect phytopathogenic fitness. *In planta* experiments on cucumber seedlings under greenhouse conditions showed that, with the exception of the osmotic-resistant mutants, all the isolates of the osmotic-sensitive phenotypic class had a decreased infection ability compared with the wild-type. Preventive applications of the commercial formulation fludioxonil (Saphire 50 WP) were effective against lesion development on cotyledons by the wild-type, but were ineffective even in high concentrations against disease caused by both phenotypic classes of fludioxonil-resistant isolates. Experiments on the stability of the fludioxonil-resistant phenotype showed a significant reduction of resistance when the mutant isolates were grown on an inhibitor-free medium. A recovery of the high resistance level was observed after they were returned to the selection medium. Studies on the competitive ability of resistant isolates against the wild-type strain of *B. cinerea* by applications of a mixed population showed a significant reduction of resistant isolates with an increase in the population of the wild-type strain.

Fungicidal action and the resistance risk of fluazinam. B.N. Ziogas, A.G. Vitoratos, A. Kyriakou and S. Georgoudeli. *Laboratory of Pesticide Science, Agricultural University of Athens, 75 Iera Odos str., 18855 Athens, Greece.*

Fluazinam is a new protective fungicide against *Botrytis cinerea*. It belongs to the chemical group of phenylpyridinamines with a broad spectrum of activity against phycomycetes, ascomycetes, basidiomycetes and adelomycetes. *Ustilago maydis* strains, with a low-to-moderate resistance to fluazinam (Rf: 5-50), were isolated with a mutation frequency of 7×10^{-6} after chemical mutagenesis with N-methyl-N-nitrosoguanidine (MNNG). Genetic analysis resulted in the identification of two chromosomal genes. A study of the effect of mutant genes on the phytopathogenic fitness of *Ustilago maydis*, showed that the resistance mutations had no apparent effect on the mycelial growth rate and on *U. maydis* pathogenicity on young corn plants. Cross-resistance studies showed that the mutations for resistance to fluazinam also caused resistance to oligomycin but not to dinitrophenol. A dose-dependent inhibition of glycose oxidation in whole cells was observed by both fluazinam and oligomycin, and a complete inhibition was found at 40 μg ml^{-1}. In contrast,

substrate oxidation was uninhibited by dinitrophenol at concentration up to 50 μg ml^{-1}. These results suggest that fluazinam has a fungicidal action on ATP-synthetase than that it is an uncoupler of the oxidative phosphorylation from mitochondrial electron transport. Study of resistant risk to fluazinam in *Botrytis cinerea* showed the possibility of appearance of only low-resistant mutants (Rf: 5). Furthermore, study of phytopathogenic fitness parameters of both wild and mutant strains showed that the mutations for resistance to fluazinam may or may not affect growth rate, conidial production and germination, and pathogenicity. Preventive applications of commercial formulation of fluazinam IKF-1216 SC (500 g l^{-1} fluazinam) were effective against lesion development on cotyledons by both wild and mutant strains.

Study of the inherent resistance risk to fenhexamid in *Botrytis cinerea*. B.N. Ziogas, A.N. Markoglou and A.A. Malandrakis. *Laboratory of Pesticide Science, Agricultural University of Athens, 75 Iera Odos str., 18855 Athens, Greece.*

After chemical mutagenesis of a wild-type strain of *Botrytis cinerea* with *N*-methyl-*N*-nitrosoguanidine (MNNG), two phenotypes that are respectively highly and moderately resistant to fenhexamid were isolated with a high mutation frequency of 0.9×10^{-5}. Resistance factors, based on EC_{50} values, were 460–570 and 10–15 respectively. The mutation(s) for resistance to fenhexamid did not affect the sensitivity of mutant strains to the benzimidazole benomyl, the phenylpyridinamine fluazinam, the anilinopyrimidine cyprodinil, the guanidine iminoctadine or the sterol-biosynthesis-inhibiting fungicides fenarimol, fenpropimorph and tridemorph, but there was an increased sensitivity (EC_{50} ratios 0.2 to 0.6) of fenhexamid-resistant strains to the phenylpyrrole fludioxonil and the dicarboximide iprodione. Fitness parameters of fenhexamid-resistant isolates of both phenotypic classes showed that these mutation(s) had no apparent effect on mycelial growth and on sensitivity to high osmolarity, but did affect one or more other characteristics, such as sporulation, conidial germination and sclerotium production. In tests on cucumber seedlings under greenhouse conditions, all highly fenhexamid-resistant isolates tested had lower infection ability than the wild-type. Preventive applications of a commercial formulation of fenhexamid, Teldor 50 WP (fenhexamid 500 g kg^{-1}), were effective against lesion development on cotyledons by the wild-type, but ineffective, even in concentrations higher than the manufacturers' recommended dose, against disease caused by the fenhexamid-resistant isolates. The risk of resistance to fenhexamid arising during commercial use of fenhexamid is discussed in the light of these results.

Protective and curative activity of the fungicide azoxystrobin against *Cercospora beticola*. T. Anesiadis, G.S. Karaoglanidis and K. Tzavella-Klonari. *Faculty of Agriculture, Plant Pathology Laboratory, Aristotelian University of Thessaloniki, P.O. Box 269, 54006, Thessaloniki, Greece.*

Strobilurin fungicides constitute a new group of chemical compounds with a novel mode of action and showing fungicidal activity against several fungal species. In order to evaluate the fungicidal activity of azoxystrobin against *Cercospora beticola*, a set of *in planta* experiments was carried out. Fungicides used in this study were azoxystrobin (Quadris 25 SC) at 8 and 16 μg a.i. ml^{-1}, difenoconazole (Score 25EC) at 8 μg a.i. ml^{-1} and chlorothalonil (Daconil 75WP) at 118.75 μg a.i. ml^{-1}. For the purposes of the study, 4-5-week-old sugarbeet plants (cv. Rizor) were artificially inoculated with a conidial suspension ($11–20\times10^4$ spores ml^{-1}) of a fungal isolate obtained from the field. After inoculation plants were maintained in a growth-chamber at 25°C, RH 95% and a 16-h photoperiod. Fungicides were applied prior (preventive treatments) and after (curative treatments) inoculation at 24-, 48- and 96-hour-intervals respectively. Disease incidence was measured 20 days after inoculation using the 9-scale disease index of KWS. Results showed that azoxystrobin provided mainly a preventive action, particularly when applied 24 h before artificial inoculation of the plants, with an efficacy level of 90%. Significantly lower, at 70%, was the control effectiveness provided by chlorothalonil when applied 24 h before inoculation. Azoxystrobin also showed curative activity, particularly when applied 24 h after inoculation of the plants, with a control effectiveness of 85%; this was similar to that obtained by difenoconazole (82%) 24 h after inoculation. The control effectiveness of curative applications of chlorothalonil, at 60%, was significantly lower .

Effectiveness of the fungicide ORTIVA 25 SC (azoxystrobin 25%) on asparagus rust (*Puccinia asparagi*). A. Kazantzidou, I. Papageorgiou and A.Tsingas. *Syngenta Hellas A.E.B.E., Leoforos Anthousas, 15349 Anthousa, Attiki, Greece.*

Ortiva is a broad-spectrum fungicide belonging to the group of strobilurins and it is used on a great number of crops. In 1999–2001 in 5 field trials carried out in the area of Giannitsa, the effectiveness of ORTIVA against asparagus rust was evaluated. Ortiva was applied at the rates of 75, 80, 100 cc hl^{-1}, and the reference product was hexaconazole. Applications started preventively in June, since ORTIVA is mainly a protective product, due to its mode of action. Sprays were applied at 10–16-day intervals. After the beginning of infection, 4–5 assessments were made in each trial. The infection appeared in July, and incidence became high (up to 74%) in the

control plots. Statistical analysis showed that treatment differences were highly significant, and that ORTIVA gave excellent (in some cases 100%) control. Phytotoxicity symptoms did not appear in any of the trials.

Cross-resistance patterns among Ergosterol biosynthesis inhibiting fungicides (EBIs) in *Cercospora beticola*. G.S. KARAOGLANIDIS and C.C. THANASSOULOPOULOS. *Aristotelian University of Thessaloniki, Faculty of agriculture, Plant Pathology Laboratory P.O. Box 269, 54006 Thessaloniki, Greece.*

Thirty single-spore isolates of *Cercospora beticola*, collected from several fields in northern Greece and representing a broad-spectrum sensitivity to the demethylation-inhibiting fungicide flutriafol, were tested for sensitivity to eleven other ergosterol biuosynthesis inhibiting fungicides (EBIs) and to the guanidine fungicide dodine. Sensitivity was measured in terms of EC_{50} values for each fungicide, log-transformed EC_{50} values were pairwise correlated and the correlation coefficient was estimated. These pairwise comparisons showed high correlation coefficients between DMIs, indicating the existence of cross-resistance relationships among them. However, the degree of cross-resistance between DMIs varied greatly. Non-significant correlation coefficients were measured for morpholine fungicide fenpropimorph and each DMI, suggesting the lack of cross-resistance between morpholines and DMIs in *C. beticola*. Similarly, low and statistically not different from 0 were the correlation coefficients measured with the pairs of the guanidine fungicide dodine with all the other fungicides tested, indicating that there is no negative cross-resistance between dodine and EBIs in *C. beticola*.

Sterol analysis in DMIs-resistant and -sensitive strains of *Cercospora beticola*. G. S. KARAOGLANIDIS[1], O. MENKISSOGLOU-SPIROUDI[2] and C.C. THANASSOULOPOULOS[1]. *[1]Plant Pathology Laboratory. [2]Pesticide Laboratoy, Faculty of Agriculture, Aristotelian University of Thessaloniki, P.O. Box 269, 54006 Thessaloniki, Greece.*

The effect of the sterol-demethylation-inhibiting fungicide flutriafol on the sterol composition of DMI-sensitive and resistant strains of *C. beticola*, was studied. In the absence of flutriafol these strains had the same sterol composition regardless of their sensitivity to DMIs. Ergosterol was the major sterol of fungal membranes. Following treatment with flutriafol, 4-desmethyl sterol levels were lower and eburicol, obtusifoliol and 14α-methylergosta-8,14,24(28)-trienol were higher, indicating an inhibition of 14α-demethylase in all the *C. beticola* strains tested. The accumulation of methyl-sterols in the resistant strains occurred after their exposure to concentrations of flutriafol higher than those in the case of the sensitive strains. Therefore, DMI-resistance cannot be associated with a deficiency of the C-14 demethylation enzyme in the ergosterol biosynthetic pathway. The reason for the different effects of flutriafol on the sterol composition of the various *C. beticola* isolates may be due to lower levels of the fungicide in the mycelium and to other, not yet identified mechanisms of resistance.

Pyrenophorin and pyrenophorol production by *Drechslera avenae* f. sp. *sterilis*. M.A. KASTANIAS and M. CHRYSAYI. *Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos str., 11855 Athens, Greece.*

Drechslera avenae f. sp. *sterilis* is a *forma specialis* with a host-specificity for *Avena sterilis*. The fungus produces the phytotoxic macrodiolides pyrenophorin and pyrenophorol. In the present study, the production of these secondary metabolites in culture medium was studied over time. Quantitative determinations were made by gas chromatography-mass spectrometry (GC-MS). Pyrenophorin was at detectable levels in cultures incubated for three days (5 $\mu g\ g^{-1}$ of culture medium), reached a maximum yield in 10 days (364 $\mu g\ g^{-1}$ of culture medium) and dropped to concentrations below the limit of detection after 25–27 days. Pyrenophorol was at detectable levels in cultures incubated for five days (2 $\mu g\ g^{-1}$ of culture medium), reached a maximum production in 35 days (240 $\mu g\ g^{-1}$ of culture medium) and then the yield declined, but remained at detectable quantities (87 $\mu g\ g^{-1}$ of culture medium) even after 90 days. The production pattern of the two macrodiolides indicates that pyrenophorin may be a precursor in the pathway of pyrenophorol biosynthesis.

Development and implementation of a new dynamic and electrodynamic technique within the framework of electrostatic spraying. S. KOTSOPOULOS[1], R. CRAMARIUC[2], L. PANAGIOTOPOULOS[3], N. YIOLDASIS[1], S. FOTOPOULOS[4], D. ZEVGOLIS[4] and A. KOTSOPOULOS[3]. *[1]Wireless Telecommunications Laboratory, Department of Electrical and Computer Engineering, School of Engineering, University of Patras, Patra, Greece. [2]National Institute for Scientific Research in Electrostatics and Electro technologies, Bucharest, Romania. [3]Department of Agricultural Machinery and Irrigation, Technological Educational Institute of Mesologgi, Greece. [4]Department of Physics, University of Patras, Greece.*

Within the framework of the upgrading of the Agricultural Sector, advanced machinery plays an important role, giving new directions to both the performed cultivation procedures and the existing antagonistic market environment. In the present work, the technical parameters involved are investigated, in order to apply new pesticide techniques. Moreover, a new method is proposed that is based on a technique of spraying in an electrostatic field. Using the proposed

technique, the minimum quantity of pesticide liquid is required. Moreover, for the improvement of the proposed technique, the following are examined: (a) The process theoretical and experimental research for the improvement of the dynamic systems of electrostatic spraying, (b) the process theoretical and experimental research for the improvement of the electrodynamic systems of electrostatic spraying; and (c) the process theoretical and experimental research in the fields of scanning and image processing by means of a computer, for the analysis of the jet of spraying globules, via: (i) the design and the development of algorithms for 3-D image-scanning, (ii) the design and the development of algorithms for image-analysis and the recognition of specific patterns, (iii) the scanning experiments and the image processing of the electrostatic field of the dynamic and electrodynamic systems for electrostatic spraying. The present work is part of the Research Project "DEMETRA", sponsored by the Hellenic General Secretariat of Research and Technology of the Ministry of Development. This project is within the framework of Bilateral Cooperation between Greece and Romania.

Evaluation of the fungicide MAXIM 035 FS applied as seed treatment for the control of soil-borne diseases in Maize. I. Papageorgiou, A. Tsingas and A. Kazantzidou. *Syngenta Hellas A.E.B.E., Leoforos Anthousas, 15349 Anthousa Attiki, Greece.*

In field trials the effectiveness of the fungicide MAXIM 035 FS on soil-borne diseases caused by *Pythium* spp. and *Fusarium* spp. and affecting seeds and young plants of maize was evaluated. MAXIM 035 FS contains 2.5 g l^{-1} fludioxonil + 1 g l^{-1} metalaxyl-m. Fludioxonil is a non-systemic active substance belonging to the group of phenylpyrroles and is effective against a number of fungi such as *Botrytis cinerea, Sclerotinia* spp., *Monilinia* spp., *Rhizoctonia solani* and *Fusarium* spp. Metalaxyl-m, which belongs to the group of phenylamides, is a systemic fungicide and is biologically active on fungi such as *Phytopthora, Pythium, Plasmopara, Peronospora*, *Pseudoperonospora*. In 2000–2001, 4 field trials were carried out in Greece. MAXIM was applied at two rates (70 and 100 cc 100 kg^{-1} seed) and was compared to the reference product APRON 35 WS (35% metalaxyl). After treatment, maize seeds were drilled in the field and emerged plants were counted 21 and 45 days after drilling. In plots where MAXIM was applied, seed-emergence 21 days after drilling was 93%, while in the control plots it was 83% (mean from 4 trials). In addition, 45 days after drilling the percentages were 89% in the treated plots and 80% in the control plots. Statistical analysis detected that differences between treated and untreated plots were significant. Phytotoxicity symptoms were not observed.

***In vitro* evaluation of antifungal activity of saponins from the annual medic *Medicago arabica*.** E.J. Paplomatas[1], A. Tzima[1], Z. Bialy[2] and M. Jurzysta[2]. *[1]Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos, 11855 Athens, Greece. [2]Department of Biochemistry and Plant Quality, Institute of Soil Science and Plant Cultivation, Czartoryskich 8, 24 – 100 Pulawy, Poland.*

Saponins, glycosylated triterpenoid or steroid compounds, are widely found in higher plants. In many cases, the antifungal activity of saponins has been shown *in vitro* and *in vivo*. Saponins that have been isolated from cultivated medic (alfalfa) *Medicago sativa* (L.) are usually a composite blend of triterpenoid glycosides. Depending on the structure of the aglycones, they can be separated into several groups: derivatives of medicagenic acid, oleanolic acid, zanhic acid, hederagenin and soyasapogenols. In contrast with alfalfa, in the annual herb medic *Medicago arabica* (L.), Huds. medicagenic or zanhic acid glycosides have never been found. The aim of the present study was to evaluate *in vitro* the antifungal activity of eight saponin samples from *M. arabica* that had never been reported from a *Medicago* species before. The chemical structure of these saponis had been defined by FAB-MS and NMR to be glycosides of hederagenin, bayogenin and 2-hydroxyoleanolic acid. The biological activity of the samples in the study was tested against the plant pathogenic fungi *Pythium ultimum* and *Rhizoctonia solani* (AG4). The saponins were added to the culture medium (PDA) at concentrations of 10, 25, 50 and 100 μg ml^{-1}. Their antifungal activity was estimated as percent inhibition of the linear growth of the fungal mycelium compared with the untreated control. It was found that inhibition of growth with *P. ultimum* was 25.5%, while with *R. solani* it was even greater: 38.8%. The biological activity of the most effective *in vitro* saponins samples will be evaluated *in planta* on host plants of the above phytopathogenic fungi.

Comparative toxicity of fungicides on *Verticillium fungicola* and *Agaricus bisporus*. M. Chrysayi[1], M.A. Kastanias[1], P. Diamantopoulou[2] and A. Filippoussis[2]. *[1]Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos str., 11855 Athens, Greece. [2]National Agricultural Research Foundation (N.AG.RE.F), Institute of Agricultural Engineering, Edible Fungi Laboratory, 61 Democracies str., 13561 Athens, Greece.*

Production of cultivated mushrooms can be very greatly affected by fungi. The chemical control of fungi that infect edible mushrooms requires highly selective fungicides. The aim of this work was to evaluate *in vitro* the effectiveness of the new fungicides famoxadone, trifloxystrobin and tebuconazole against *Verticillium fungicola*, which causes dry bubble disease on the white mushroom *Agaricus bisporus*. Prochloraz and carben-

dazim were used as reference fungicides. The ED_{50} values of famoxadone, trifloxystrobin, tebuconazole, prochloraz and carbendazim were 0.5, 0.12, 0.01, 0.002 and 0.07 μg ml^{-1} respectively. The effect of these fungicides was also evaluated on the *in vitro* growth of *A. bisporus* on agar culture and, in an attempt to simulate commercial mushroom cultivation, on a casing layer in glass tubes. All fungicides at concentrations which gave satisfactory mycelial inhibition of *V. fungicola* were without effect on the mycelial growth of *A. bisporus*. Based on the observed *in vitro* selectivity of the above fungicides, further evaluation of their formulated products at commercial production scale seems of interest.

Effectiveness of BION (50% benzothiadiazole) in the control of fire blight. J. Tsiantos[1] and P.G. Psallidas[2]. [1]*National Agricultural Research Foundation (N.AG.RE.F), Plant Protection Institute of Volos, 38001 Volos, Greece.* [2]*Benaki Phytopathological Institute, 8 St. Delta str., 14561 Kifissia, Greece.*

The effectiveness of BION (50% benzothiadiazole) at doses of 20 g and 10 g 100 l^{-1} H_2O, which induces systemic acquired resistance for controlling fire blight (*Erwinia amylovora*), was tested in the years 2000 and 2001. BION was compared with Agrept (20% streptomycin sulphate) at a dose of 50 g 100 l^{-1} H_2O in 2000 and 2001, and Aliette (80% phosetyl-Al) at a dose of 250 g 100 l^{-1} H_2O in 2001, and Kocide (50% copper-hydroxide) at a dose of 90 g 100 l^{-1} H_2O also in 2001. Whole pear trees (cultivar Krystalli) were sprayed with the chemicals two and four days before artificial inoculation of preselected flowers with a water suspension of the pathogen at concentrations of 10^5 and 10^7 cfu ml^{-1}. Kocide was applied once two days before inoculation. The evaluation of the infection (percentage of infected flowers) was made 15 days after the treatment. The results indicated that BION at a dose of 20 g, and Aliette provided significant protection, but were not as effective as Agrept. The chemical BION at 10 g and Kocide did not show any significant protection.