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ABSTRACTS

Summaries of invited lectures, oral and poster presentations given at the Thirteenth Hellenic Phytopathological Congress, Athens, Greece, 16–19 October 2006

The 13th National Phytopathological Congress, organized every two years by the Hellenic Phytopathological Society (HPS), was held in Athens, Greece, on October 16–19, 2006. The meeting was attended by more than 400 participants, and 49 oral presentations, 65 posters and 4 invited lectures were presented dealing with plant diseases caused by fungi, bacteria and viruses, non-parasitic disorders and disease control. In addition, one round table discussion was held on "The contribution of Phytopathology in the production of safe and high-quality agricultural products". Abstracts of the invited lectures, oral presentations and posters of the congress are presented below.

Fungal diseases

Forest tree diseases in Greece by alien invasive fungal species. P. TSOPELAS. *NAGREF-Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 115 28 Athens, Greece.*
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During the 20th century, certain plant diseases, caused by alien invasive fungal species, had major impacts on forest ecosystems in many areas of the world. Chestnut blight, caused by the fungus *Cryphonectria parasitica*, is a characteristic example of mass destruction of indigenous forests due to an invasive pathogen. *C. parasitica* is considered native to East Asia and was introduced from Japan into North America during the late 19th century; in about 50 years the pathogen had eliminated the American chestnut, *Castanea dentata,* within its natural range. From N. America the fungus was introduced into Europe; it was recorded in Italy in 1938 and in 1963 was found in Greece. The pathogen spread gradually all over Greece in natural stands and chestnut plantations causing extensive tree mortality. Two pandemics of Dutch elm disease occurred during the 20th century in Europe, N. America and SW Asia by species of the genus *Ophiostoma*. The first pandemic, caused by *Ophiostoma ulmi*, a fungus indigenous in East Asia, appeared in NW Europe around 1910 and was spread all over Europe, Asia and N. America. The second pandemic started in the 1940's in Europe and N. America by two new more virulent strains: *Ophiostoma novo-ulmi* ssp. *novo-ulmi* in Europe and Asia and *O. novo-ulmi* ssp. *americana* in N. America, which was also introduced into UK in the 1960's. The second pandemic of Dutch elm disease was more destructive than the first one in both Europe and N. America. In Greece, the disease was recorded for first time in 1968 in some localities of Macedonia; since then, the pathogen has spread all over the country eliminating the elm trees growing in natural conditions as well as those that had been planted as ornamentals. Another destructive disease, similar to Dutch elm disease, is the canker stain of plane trees, caused by the fungus *Ceratocystis platani*. The fungus is of American origin and was introduced into Europe from the United States during World War II. It has caused severe attacks in Italy and France and has also been reported in Switzerland. The pathogen was found in Greece in 2003; up to the present it has been detected in SW Peloponnese. In natural stands of *Platanus orientalis* in Greece there is widespread mortality, with thousands of dead and

dying trees. This is mainly because *P. orientalis* seems to be a highly susceptible host. The pathogen has the potential to spread into natural ecosystems of *P. orientalis* from Peloponnese to the rest of the mainland and to completely eliminate the indigenous host. The invasion in Europe of the fungus *Seiridium cardinale*, that causes the cypress canker disease, is a threat to cypresses and other species of the Cupressaceae family, especially in some Mediterranean areas where the disease has taken epidemic proportions. The fungus, also of American origin, was possibly introduced into Europe in the 1940's. In Greece, the disease was originally recorded in 1961. Since then, the pathogen has spread all over the country killing thousands of cypress trees. The disease is more severe in areas with wet climatic conditions.

Effects of *Drechslera avenae* f. sp. *sterilis* metabolites on leaf ultrastructure of *Avena sterilis*. K.A. ALIFERIS[1], M. CHRYSAYI[1] and K. FASSEAS[2]. [1]*Agricultural University of Athens, Department of Crop Production, Pesticide Science Laboratory, 75 Iera Odos, 11855 Athens, Greece.* [2]*Agricultural University of Athens, Department of Agricultural Biotechnology, Laboratory of Electron Microscopy, 75 Iera Odos, 11855 Athens, Greece.*
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Drechslera avenae f. sp. *sterilis,* a host-specific pathogen of *Avena sterilis,* produces the phytotoxic metabolites pyrenophorin and pyrenophorol. Under a source of illumination, a loss of phototosynthetic pigments was observed in the leaf tissues of *A. sterilis* treated with the above phytotoxins, whereas in the absence of light the tissues retained their photosynthetic pigments and leaf senescence was delayed. Transmission electron microscope (TEM) examination of *A. sterilis* leaves treated with pyrenophorin revealed cytoplasm and membrane disorganization and degenerated chloroplasts containing osmiophilic globuli. These effects were apparent in the presence but also in the absence of light. By contrast, TEM examination of tissues treated with pyrenophorol showed that cytoplasm and cell membrane disorganization took place only in the light whereas in the dark the cell ultrastructure was preserved. Taking into consideration that pyrenophorin is produced in cultures of *D. avenae* f. sp. *sterilis* prior to pyrenophorol and that in *A. sterilis* tissues it causes oxidative stress due to the induction of a reactive oxygen species, it is possible that pyrenophorin is employed by the fungus for the cell disintegration essential during host penetration. On the contrary, pyrenophorol which is produced later and is more systemic than pyrenophorin seems not to be a determining chemical for the initial of host infection, but is so for a subsequent stage.

Oxidative stress to *Lemna minor* caused by the phytotoxin pyrenophorin. K.A. ALIFERIS, I. TSOUTSANIS, S. MATERZOK and M. CHRYSAYI. *Agricultural University of Athens, Department of Crop Production, Pesticide Science Laboratory, 75 Iera Odos, 11855 Athens, Greece.*
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Pyrenophorin is a non-selective phytotoxin produced by *Drechslera avenae* f. sp. *sterilis*. It causes oxidative stress in *Avena sterilis* tissues and its mechanism of action is associated with the production of a reactive oxygen species. The objective of the work was to study the response of *Lemna minor,* a sensitive plant frequently employed in studies of bioactivity of various xenobiotics, to pyrenophorin. *L. minor* was exposed to pyrenophorin solutions and the phytotoxicity was evaluated by estimating the chlorophyll content of the colonies 96 hours after treatment. It was found that pyrenophorin was phytotoxic to *L. minor,* and that when it was applied at 70 and 140 μM it caused a 47.28 and 57.63% reduction in chlorophyll respectively. The addition of hydroquinone (250 and 500 μM) to the pyrenophorin solution diminished the effect of the phytotoxin. Since hydroquinone is an electron scavenger, such interference indicated that a reactive oxygen species was involved in the expression of pyrenophorin phytotoxicity. When the photosystem II inhibitor diuron (100 μM) was applied together with pyrenophorin (70 or 140 μM), on the other hand, the effect was similar to that of pyrenophorin applied alone.

Race characterization and molecular diversity of 35 Greek *Colletotrichum lindemuthianum* isolates. G.A. BARDAS[1], O. KOUTITA[2], T. VELOUKAS[1] and K. TZAVELLA-KLONARI[1]. [1]*Laboratory of Plant Pathology, School of Agriculture, Aristotle University of Thessaloniki, Greece.* [2]*Plant Breeding Department of Hellenic Sugar Industry, S.A. 57400 Sindos, Thessaloniki, Greece.*
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Colletotrichum lindemuthianum (Sacc. & Magnus) Briosi & Cavara is the causal agent of anthracnose in common bean. Anthracnose is one of the most important diseases of bean in the world. In order to produce effective breeding programmes for anthracnose control, a knowledge of pathogen variability and distribution in a given area is necessary. In this study 35 isolates of *C. lindemuthianum* were collected from different regions of Greece, and three isolates, UPS2 (Africa), UPS36S (South America) and UPS9 (Europe), were used as references. The variability of the isolates was examined in pathogenicity tests on a set of 12 differential cultivars as proposed by Pastor-Corrales and Tu. The results showed the existence of three races: race 2, race 6 and race 22. Molecular characterization of *C. lindemuthianum* isolates was made using RAPD and RFLP analysis. The dendrogram from RAPD analysis grouped the tested samples into seven distinct clusters. There was no similarity between the isolates of race 22 and the isolates of the two other races. The Greek isolates were more similar to the European reference

strain UPS9 than to the reference strains from South America or Africa. RFLP analysis showed that according to rDNA region variability, the majority of Greek isolates (33 out of the 35 tested) and the European reference strain belonged to Group II polymorphism, while only two isolates showed Group I polymorphism, like the African and the Southern American reference strains. This is the first race and molecular characterization of *C. lindemuthianum* isolates in Greece, and also the first report of race 22 in Europe.

Development of molecular techniques for the detection and identification of pathogens associated with esca and young grapevine decline. M. CHRISTOPOULOU and E.J. PAPLOMATAS. *Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.*
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During the last years outbreaks of esca in young grapevines have been associated with using phylloxera resistant rootstocks for replanting. Even though propagation material is suspected to be the main means of dissemination of the pathogens implicated in young grapevine decline, this assumption has not been verified experimentally. The newly identified esca of young grapevines, and also the black streaking of the wood around white soft rot (esca proper) that is observed in adult vines, is ascribed to the fungus *Phaeomoniella chlamydospora*. Other pathogens also implicated in young grapevine decline are various species of the genus *Phaeoacremonium*, especially *Phaeoacremonium aleophilum* along with *Cylindrocarpon destructans*. Unlike young vine decline, the soft rot of hardwood seen mostly in adult vines is attributed to species of the genus *Fomitiporia*. The aim of this study was to investigate the molecular differentiation of the *Fomitiporia* species involved in the disease, and using DNA technology to detect and identify the pathogens that cause decline of young vines in propagation material. To separate *Fomitiporia mediterranea* from *F. punctata*, species-specific primers were designed and evaluated on 48 isolates from ten areas of Greece, two isolates from Italy, three from Sweden and two from Finland, originating in six hosts. *F. mediterranea* was the only species found in Greece irrespective of host or geographic origin, while *F. punctata* infected forest trees in northern Europe. Subsequently, grapevine propagation material at various stages of production was examined for *P. chlamydospora* and *C. destructans,* using both classical and molecular means. Total genomic DNA isolation from vine wood was then carried out using a protocol modified for this purpose, while nested PCR was used for pathogen detection and identification. A total of 80 rooted or unrooted cuttings, 100 grafted cuttings six weeks after being transplanted in the field, 15 one-year old grafted vines, and 15 two-year old grafted vines were tested by pathogen isolations. From the above categories, 43, 20, 6 and 15 plants respectively were tested with molecular techniques. Unlike classical detection, the molecular method revealed high percentages of pathogens in vine tissues with intense discoloration, and in asymptomatic plants. *Cylindrocarpon destructans* was detected in vine cuttings for the first time, while the high frequency of the pathogens involved in the decline of young vines and found in propagation material underscored the importance of such material in the dissemination of the disease. The method of molecular detection of esca pathogens that has been developed could be applied in large-scale phytosanitary inspections of grapevine grafting cuttings and also for the certification of healthy propagation material.

Comparison of classical and molecular methods to identify species of the genus *Phytophthora*. K. ELENA. *Benaki Phytopathological Institute, 8 S. Delta St. 145 61 Kifissia, Attica, Greece.*
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Species of the genus *Phytophthora* cause some of the most devastating plant diseases; some of these species are host-specific, while others have a broad host range. Identification of *Phytophthora* species by morphological and physiological means was always difficult and often unreliable, but it is very important in order to control the diseases that the *Phytophthora* cause. In this study, an integrated system of classical and molecular methods was used to identify eight isolates of the genus *Phytophthora*. Besides morphological and physiological techniques we also used ribosomal DNA (rDNA) internal transcribed spacer (ITS) variation for molecular identification, in order to compare the two types of technique. For DNA extraction, freeze-dried mycelium from every isolate, (kept at –20°C) was used. Then a single round of PCR with the universal primers ITS6 and ITS4 was applied. Using this protocol, sufficient DNA was present in the PCR products, ~900 base pairs in length for each sample, as was expected for species of *Phytophthora*. The results of the ITS sequences and the identification by classical means of the isolates were completely correlated. The PCR products for isolates BPIC1989, BPIC2514 and BPIC2584, with the morphological and physiological similarities, were digested with the restriction enzymes *Alu*I, *Msp*I and *Taq*I for RFLP (Restriction Fragment Length Polymorphism) analysis. The same digest patterns were generated by BPIC1989 (*P. porri*) and BPIC2584 (*P. primulae*) isolates with the three enzymes, whereas they were characterized as different species by the classical methods and by the ITS sequence; they also showed a different pathogenicity in cross-inoculation tests using the hosts from which they were derived. The method using restriction enzymes is simple and rapid; however, morphological characteristics should also be taken into account. After identification, the isolates were deposited in the fungal culture collection of the Benaki

Phytopathological Institute as follows: *P. nicotianae* Breda de Haan BPIC1922; *P. boehmeriae* Sawada BPIC1923; *P. nicotianae* BPIC1936; *P. cryptogea* Pethybridge & Lafferty BPIC1962; *P. citrophthora* (R. & Sm.) Leonian BPIC1967; *P. porri* Foister BPIC1989; *P. syringae* Klebahn BPIC2514; and *P. primulae* Tomlinson BPIC2584.

The fungus *Fusarium proliferatum* is transmitted to asparagus by the insects *Hexomyza* (*Ophiomyia) simplex* and *Delia (Hylemyia) platura*. K. ELENA, M. ANAGNOU-VERONIKI and D.C. KONTODIMAS. *Benaki Phytopathological Institute, 8 S. Delta St. 145 61 Kifissia, Attica, Greece.*
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The fungus *Fusarium proliferatum* (Matsushima) Nirenberg is an important pathogen of asparagus, causing crown and root rot. The fungus is one of the main factors responsible for the decline of asparagus cultivation in Greece and worldwide. *Fusarium proliferatum* was isolated on a V8 Fusarium-selective medium, from sections of the dipteran insects *Hexomyza* (*Ophiomyia) simplex* (Loew), Agromyzidae, and *Delia (Hylemyia) platura* (Meigen), Anthomyidae, both of which attack asparagus. These insects attack the stems of asparagus and were collected using yellow sticky traps that were placed in several experimental asparagus plantations in Orestias-Evros and Chryssoupolis-Kavala counties. The pathogenicity of three of the *F. proliferatum* isolates, F644, F652 and F654, was tested on seedlings of the asparagus hybrid Steline. Three mycelial discs, 5 mm in diameter, from seven-day-old cultures, were transferred to test tubes containing solid Hoagland medium and incubated at 27°C. Tubes with sterile agar discs were used as controls. Three days later, a germinated seed was aseptically transferred from Petri dishes with wet paper at the bottom (used as small germinators) to the culture tubes (one seedling per tube). The asparagus seedlings started to grow and became infected with the pathogen. All three isolates were pathogenic to asparagus seedlings, whereas the control plants remained healthy. This is the first report of *Delia platura* as a vector of pathogenic strains of *F. proliferatum* to asparagus.

***Inonotus andersonii*: a rare parasitic basidiomycete in Europe.** D. FLOUDAS and Z. GONOU-ZAGOU. *University of Athens, Faculty of Biology, Department of Ecology and Systematics, Panepistimiopolis, 157 84 Athens, Greece.*
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Inonotus andersonii is a basidiomycete (Hymenochaetales, Hymenochaetaceae) mainly infecting species of *Quercus*. Its behavior is parasitic and it has an interesting life cycle. Its mycelium grows on living trees and can survive for many decades, its presence almost impossible to detect. The fungus during its lifetime reproduces asexually and sexually. Both stages are difficult to find in nature. The asexual stage, although not unusual, is hard to discern and is produced only in living trees. The sexually formed basidiocarps are uncommon and develop under the bark of recently dead trees for only one season. The species is not widespread. It is known from N. America (USA), Asia (Korea and China) and Europe (Czech Republic, Germany, Poland, Russia and Italy), where references are sporadic. Basidiocarps of *Inonotus andersonii* have recently been collected from the lowland mixed oak forest of Kouri in Magnisia and are deposited in the Mycological Herbarium of the University of Athens (ATHU–M). This is the first report of *Inonotus andersonii* from Greece.

***Drechslera avenae* f. sp. *sterilis*: a new pathogen of *Avena sterilis*.** M. KASTANIAS and M. CHRYSAYI. *Agricultural University of Athens, Department of Crop Science, Pesticide Science Laboratory, 75 Iera Odos, 11855 Athens, Greece.*
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A fungus was isolated from infected leaves of *Avena sterilis* and was grown on oat meal agar. The fungus formed grey colonies with characteristic buff tufts. On infected leaves the fungus caused a brown-red streaking that eventually became necrotic. Brown conidiophores bearing conidia on the apex developed on infected plant tissues and on oat meal agar. The conidia were cylindrical with blunt ends, borne singly or rarely in chains, multicellular and 1–5-distoseptate. The conidiogenous nodes as well as the conidiogenous walls were verruculose. Conidia formed on host tissues were of the same dimensions (40–65 μm long and 10–14 μm wide). Conidia formed on nutrient medium were of 30–64 μm long and 9–14 μm wide. The fungus was identified as the anamorph *Drechslera avenae* (Eidam) Scharif belonging to the Moniliales (Dematiaceae family). *D. avenae* has been isolated from species of Avena (*A. sativa*, *A. fatua*, *A. bysantina*) and Trisetum. It has also been found on *Triticum aestivum* and *Hordeum vulgare* in several parts of the world. The pathogen has however not been found on any of these crops in Greece. This is the first report of *D. avenae* isolated from *A. sterilis*. Pathogenicity tests showed that the fungus was pathogenic to *A. sterilis* but not to *A. fatua*, *A. sativa*, T. *aestivum*, *H. vulgare*, *Sorghum bicolor*, *Zea mays*, *Lens culinaris*, *Lycopersicon esculentum* or *Phaseolus vulgaris*. Therefore, it can be characterized as a host-selective pathogen and named *Drechslera avenae* f. sp. *sterilis*.

Phytotoxic metabolites of the fungus *Alternaria alternata* from persimmon. I.A. LAIDOU and C.C. THANASSOULOPOULOS. *Plant Pathology Laboratory, School of Agriculture, Aristotelian University, P.O.B. 269, Thessaloniki, 540 06, Greece.*
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Fungi of the genus *Alternaria* produce secondary metabolites toxic to plants and animals. *Alternaria alternata* causes leaf spots on persimmon. The purpose of this work was to study phytotoxin metabolites of culture filtrates of *A. alternata* and the range of their biological activity in several plant species. Analysis of thin layer chromatography TLC) of the aqueous and organic layer (of ethyl acetate) showed one spot that was absent in the control. One major peak was detected by HPLC at 300 nm with Rt=9 min, and two major peaks at 265 nm with Rt=8.5 min (peak 3) and Rt=9.5 min (peak 4) that were absent in the control. One peak corresponded to tentoxin as identified by LC/MS. Twenty-two of the 38 plant species tested were sensitive to the aqueous layer as well as to the organic layer of the fungus. The other plant species were not sensitive to either the aqueous or the organic layer. Only eight of the 22 species were sensitive to the isolated fractions from the organic layer (peak 3 and 4). This is first report of the toxicity of secondary metabolites such as tentoxin from *A. alternata* to plants other than persimmon.

First report of a fruit rot disease of peaches caused by a *Fusicoccum* sp. in Imathia, Greece. T.J. MICHAILIDES[1], T. THOMIDIS[2] and C. TSIPOURIDIS[2]. [1]*University of California, Davis, Department of Plant Pathology, Kearney Agriculture Center, 9240 South Riverbend Ave., Parlier 93648,CA, USA.* [2]*Pomology Institute Naoussa (NAGREF), R. S. Naoussas 38, P.C. 59200 Imathia, Greece.*
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Preharvest fruit rots constitute a serious problem in peach fruit production worldwide. Brown rot, caused by *Monilinia laxa*, is considered to be the main cause of fruit rots of peaches in Greece. Immature peach fruits of the cultivar Catherine with symptoms of rot were collected from a peach orchard located in Mesi, Veria, and transferred to the plant pathology laboratory of the Pomology Institute, Naoussa. Dark, flask-shaped pycnidia of a fungus were found partially embedded in the infected tissues of the fruit. Isolation of the putative pathogen was made on potato dextrose agar acidified (2.5 ml of an 85% lactic acid solution per liter; pH 3.5). To fulfil Koch's postulates in the laboratory, immature and mature peach fruits of the cultivar Catherine were mechanically inoculated by wounding the fruit surface and placing a drop of a 10^5 conidial suspension of the isolated fungus on the fruits. Inoculated fruits were enclosed in plastic containers and placed in an incubator at 23–25°C for 7 days. Decay symptoms and production of pycnidia were similar to those in naturally infected fruit. Reisolation of the fungus indicated that it was similar to that initially used for fruit inoculation, fulfilling Koch's postulates. Based on colony morphology, and the size, shape, and color of the pycnidiospores, the fungus was identified as a *Fusicoccum* sp. To determine the distribution of this *Fusicoccum* sp. in Imathia County, Greece, 500 rotted peach fruits of the cultivars Andross, RedHaven, Sun Crest and Sun Cloud were collected from various peach orchards and transferred to the laboratory. Isolation of the casual agents was again made on acidified potato dextrose agar (2.5 ml of an 85% lactic acid solution per liter), and the incidence of fruit rotted by the *Fusicoccum* sp. was recorded. The pathogen was isolated at 30% incidence of total mature rotted peaches only in the location Ammos-Mesi-Meliki, Veria, Imathia County. In addition, of 30 isolations of blighted annual peach shoots collected from orchards in Ammos-Mesi-Meliki, 2 were infected with *Fusicoccum* sp. Thus far, only the pycnidial stage of the pathogen has been found in peaches in Imathia County. Although *Botryosphaeria dothidea*, the sexual stage of *Fusicoccum* sp., has been reported as a pathogen of peaches in other countries, this is the first report of a *Fusicoccum* sp. causing fruit rot of peaches in Greece.

Screening of local phytopathogenic fungi for the detection of dsRNA-hosting isolates. C. PAPACHRISTOS, A. PAPAVLASOPOULOS, C. KOLLA, P. MAGLARAS, A. ALEXANDRATOS, V. TSILIMPARI, A. VARELI, I. SAINIS and E. HATZILOUKAS. *University of Ioannina, Department of Biological Applications and Technologies, Laboratory of Molecular Biology, 45110 Ioannina, Greece.*
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Mycoviruses are widespread in nature, but in most cases their occurrence remains undetected because they do not generate any visible alterations in their hosts. In some instances, however, a mycovirus infecting a fungus causes considerable changes to various functions of the plant host, including the reduction or alleviation of the pathogenicity of the mycovirus-infected fungus, a phenomenon called hypovirulence. This last property of mycoviruses has already been exploited for biological control purposes and it is currently attempted to further expand its use. Within this framework, samples from fungus-infected parts of various crop plants were collected from different areas of the Epirus region. Fungal isolates were grown and screened for double-stranded RNA (dsRNA), which constitutes significant evidence for the occurrence of a viral infection. A total of 257 isolates were examined, and dsRNA molecules were confirmed in nine (3.5%), while another eight suspected isolates are currently under examination. Confirmation was based on resistance to the action of the enzymes DNase I and RNase A. On the basis of their electrophoretic patterns, the drRNA molecules detected belonged to different mycoviral families, such as the Totiviridae, Partiviridae and Chrysoviridae. Some of the isolated dsRNA species are currently being cloned.

Incidence of phytopathogenic fungal isolates hosting dsRNA in the region of Epirus. A. PAPAVLASOPOULOS, C. PAPACHRISTOS, C. KOLLA, P.

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Fungal viruses or mycoviruses are widespread in nature but in most cases they do not cause symptoms. Occasionally however some mycoviruses kill their hosts, while others induce hypovirulence or hypervirulence in the host fungi. This study reports on the incidence of double-stranded RNA (dsRNA) in the region of Epirus and discusses the extraction technique used. Two hundred and fifty seven fungal isolates collected from different locations were used. Solid and liquid cultures were prepared (potato dextrose agar and potato dextrose medium). CF-11 cellulose column chromatography using buffers was conducted for dsRNA extraction. The sample was analyzed by agarose gel electrophoresis and visualized by ethidium-bromide. RNase A and DNase I digestion of extracted nucleic acids was carried out to confirm the presence of dsRNA. All the fungal isolates were screened for dsRNA. Nine of the isolates (3.5%) contained dsRNA of various sizes.

Dynamics of black aspergilli and ochratoxin contamination of Sultanina grapes and raisins in Crete. M. PATERAKI[1,2], D. LYDAKIS[1], I. FYSARAKIS[1] and N. MAGAN[2]. *[1] Technological Educational Institute (TEI) of Crete, Greece. [2]Applied Mycology Group, Cranfield Health, Cranfield University, Silsoe, MK45 4DT UK.*
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Black aspergilli generally, and *Aspergillus carbonarius* and the *A. niger* aggregate in particular, are key fungi causing ochratoxin A (OTA) contamination of grapes and dried vine fruits from the Mediterranean basin. In this study the populations of black aspergilli and specifically the mycotoxigenic species *A. carbonarius* were recorded in vineyards and during the drying of grapes originating at three altitudes. Data were collected in two seasons (2004 and 2005). In harvested grapes the dominant species were *A. niger* aggregate, *Penicillum* spp. and *yeasts* spp. They were isolated in about 3.9, 3.6 and 3.3 $\log_{10}$ CFUs gr^{-1} grapes respectively. Altitude seemed to have an influence on population structure. Both *A. niger* aggregate (4.43 $\log_{10}$ CFUs gr^{-1}) and *A. carbonarius* (0.5 $\log_{10}$ CFUs gr^{-1}) were favoured at low and medium altitudes. The same pattern was found during drying. The *A. niger* aggregate was the dominant fungi isolated present in 6.3 $\log_{10}$ CFUs gr^{-1} sultanas (up to 70% of the total fungal population) while *A. carbonarius* increased to 1.7 $\log_{10}$ CFUs gr^{-1} (18%) at sea level at the end of drying. Climatic conditions dramatically influenced population diversity. A high altitude correlated with the duration of drying procedure may increase total fungal diversity (6 genera, on the 10th day of drying).

Hypovirulent strains of *Cryphonectria parasitica* in Greece. C. PERLEROU and S. DIAMANDIS. *National Agricultural Research Foundation, Forest Research Institute, 57006 Vassilika, Thessaloniki, Greece.*
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The ascomycete *Cryphonectria parasitica*, the causal agent of chestnut blight, may be infected by unencapsitated dsRNA viruses called *Cryphonectria* hypoviruses (CHVs). This infection reduces fungal virulence to levels ranging from avirulence to near-virulence. Such infected strains with a proven reduction in their virulence are termed hypovirulent and can be used in the biological control of the blight. The virulence of five Greek strains of *C. parasitica* infected with European CHV1 was tested on chestnut trees. For comparison, five virus-free strains were tested along with one Italian strain infected with CHV1. Seven trees were inoculated with each strain. The inoculum was produced on PDA. It was then deposited in holes 5 mm in diameter which were punched into the tree bark. The resulting cankers were measured □ver a 17-month-period, every 15 days for the first six months, and once monthly for the remaining 11 months. All the strains that contained CHV1 were less virulent than the virus-free strains, and the extent of their cankers was stopped by the creation of callous tissue. Significant differences were also found among the virus-free strains, the cankers of which, however continued to grow with no sign of callous tissue formation. Differences between the infected and the virus-free strains were also observed in pycnidia production and in the appearance of adventitious shoots. The results show that there are Greek strains of *Cryphonectria parasitica* infected with CHV1 having reduced virulence, and that these strains can be used in the biological control of chestnut blight.

New hosts of the fungus *Botryosphaeria parva*. I.C. RUMBOS. *NAGREF, Plant Protection Institute of Volos, Volos 380 01, Greece.*
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The fungus *Botryosphaeria parva* Pennycook & Samuels was found to infect walnut and kiwifruit in Greece causing dieback of twigs and branches. Over the last 5 years similar symptoms have been observed on the forest trees *Ilex aquifolium* and *Ulmus montana*. *I. aquifolium* is an economically important tree and is cultivated in Mount Pelion for its young shoots, which are used for decoration during the Christmas season. Pycnidia of the anamorph stage of the fungus *Fusicoccum parvum* Pennycook & Samuels were easily found in infected tissues of this tree. Conidia were hyaline, thin-walled and aseptate, becoming olivaceous with 1–2 septa with age. They measured 15–22×4–7.5 μm. Pathogenicity tests on young shoots of *I. aquifolium* using isolates of *B. parva* from *I. aquifolium*, walnut and kiwifruit, as well as isolates of *B. dothidea* from pistachio, olive and

grape, were all positive. Similar studies with isolates of *I. aquifolium* on pistachio, walnut, olive, almond, pear, quince and cherry demonstrated the high pathogenicity of these isolates. It is concluded that forest trees represent a considerable inoculum reservoir of *B. parva* which may be a threat to fruit trees.

Incidence of black foot disease of grapevine in grape propagative material in Greece. I.C. RUMBOS and A. CHATZAKI. *NAGREF, Plant Protection Institute of Volos, Volos 380 01, Greece.*
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Black foot disease of grapevine caused by *Cylindrocarpon* spp. is a serious problem in some grape-growing countries. The disease is relatively unknown in Greece and mainly affects young grapevines. *Cylindrocarpon destructans* is the main pathogen isolated from vine cuttings and young vineyards. An extensive study was carried out during 2002 and 2003 covering all grape propagative material marketed in Greece in those years. The study examined unrooted rootstock cuttings from governmental and private mother nurseries, rooted rootstock plants from Greek nurseries and other European countries, scion cuttings and bench-grafted rooted vines from Greece and other European countries. Over 20,000 cuttings or grafted rooted vines were examined. The fungus was not isolated from unrooted rootstock or scion cuttings. In rooted rootstock cuttings it was isolated at an average of 9% in the first year of the study. However, in some nurseries the percentage was much higher (20–28%). In the second year of sampling the isolation percentage was very low, due probably to appropriate measures having been taken by nurserymen. In grafted rooted vines the fungus was isolated in a low percentage (0–1%) in Greek nurseries, although in some cases the percentage was higher (4–16%). In the material from other European countries the percentage was also higher (average 6.7%). The fact that the pathogen infects vine cuttings in nursery soils signifies that appropriate measures must be taken to prevent or eradicate these infections. Since no effective curative measures exist and none of the chemical and biological treatments prevents infection of the basal ends of cuttings in the nursery, producers have to direct their efforts to improving cultural conditions, as for example by avoiding soil compaction and poor drainage.

Black dead arm: a new grapevine disease caused by *Botryosphaeria dothidea*. I.C. RUMBOS and A.I. RUMBOU. *NAGREF, Plant Protection Institute of Volos, Volos 380 01, Greece.*
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Botryosphaeria species cause cankers and dieback in several woody hosts including fruit trees. The importance of these species on grapevine has been underestimated, although they are often isolated from discolored woody vine tissues and cancers, sometimes together with fungi causing esca or Eutypa dieback. The asexual form of the fungus belongs to *Fusicoccum aesculi* and forms hyaline, unicellular conidia, 24–29×4–5 μm in size, not becoming darker or septate with age. Isolations from diseased vines over the last 10 years have shown that *Botryosphaeria* spp. occur in different grapevine growing areas of Greece and affecting several cultivars. The main symptoms associated with the fungus were: dead canes, observed in early spring during pruning; dead buds which do not open in spring; brown discoloration of older wood; formation of cankers; interveinal and marginal leaf discolorations resembling esca; grape bunches that dried as a result of the fungus attacking the main stem of a cluster; dried berries and brown wood-discolorations of grafted cuttings at the basal end of the rootstock and at the graft union. In this last case the infection very likely comes from the nurseries and is associated with problems that have been affecting young vineyards for the last four years. Some of these symptoms are traditionally associated with other diseases such as cane and leaf spot (*Phomopsis viticola*) or Eutypa dieback (*Eutypa lata*), making field diagnosis very difficult. Different isolates of *B. dothidea* collected from affected stems, clusters, berries and grafted cuttings were used for pathogenicity tests on different grape cultivars and rootstocks in the greenhouse and in the field. All isolates tested were virulent. Further pathogenicity studies revealed that the fungus infects both wounded and unwounded berries.

Fruit core rots of the peach variety Fayette caused by *Alternaria alternata* in Greece. T. THOMIDIS[1], T.J. MICHAILIDES[2] and C. TSIPOURIDIS[1]. *[1]Pomology Institute Naoussa (NAGREF), R. S. Naoussas 38, P.C. 59200 Imathia, Greece. [2]University of California, Davis, Department of Plant Pathology, Kearney Agriculture Center, 9240 South Riverbend Ave., Parlier, CA 93648, USA.*
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The peach variety Fayette is one of the most important late varieties of peach cultivated in northern Greece. In the last few years, however, problems with this variety have arisen because of a core rot, even though growers have applied the spray programme recommended against this type of rot. The aim of this study was to investigate the causal agent of a common fruit core rot of the peach variety Fayette. Mature 'Fayette' peaches with core rot symptoms were collected and transferred to the plant pathology laboratory of the Pomology Institute in Naoussa, Imathia County. The putative pathogen was isolated on potato dextrose agar and identified as *Alternaria alternata* by its morphological characteristics (brown conidiophores, 50×3–6 μm in size, and brown conidia, 20–60×9–18 μm, with a short pedicel

formed on long chains). Immature and mature Fayette peaches were inoculated in the laboratory with 0.1 ml of a 10^5 conidial suspension into the pulp. The fruits were incubated at 24–26°C for 7 days and any decay was recorded. Koch's postulates were satisfied by reisolating the fungus from the decayed fruits. One hundred fruits were randomly collected from each of five commercial 'Fayette' peach orchards located in Imathia and Pella counties, Greece, at harvest time in the second part of August. All fruits were cut open and checked for core rot. Only mature fruits with brown, soft and wet decay moving into the flesh around the core were considered, and isolations were made on acidified potato dextrose agar. *A. alternata* was isolated from approximately 5% of decayed fruits per orchard. Although *A. alternata* has been reported in other countries as causing core rot of apples, this is the first report of this pathogen causing core rot of peaches worldwide.

Sensitivity of *Monilinia* spp. to the fungicides thiophanate methyl, carbendazim, and tebuconazole. T. THOMIDIS[1], T.J. MICHAILIDES[2] and C. TSIPOURIDIS[1]. *[1]Pomology Institute Naoussa (NAGREF), R. S. Naoussas 38, P.C. 59200 Imathia, Greece. [2]University of California, Davis, Department of Plant Pathology, Kearney Agriculture Center, 9240 South Riverbend Ave., Parlier, CA 93648, USA.*
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The systemic fungicides carbendazim, thiophanate methyl and tebuconazole are widely used to control brown rot caused by *Monilinia* spp. Repeated use of these fungicides, however, can lead to fungicide-resistant populations of *Monilinia* spp. The main aim of this study was to investigate 194 isolates of *Monilinia* spp., collected from peach, cherry and plum fruit with brown rot and growing in commercial orchards of Imathia County, for their sensitivity to these fungicides. A 6-mm mycelial plug from cultures of *Monilinia* isolates was transferred to the center of a Petri dish (9 mm diameter) amended with 900 μg/ml^{-1} thiophanate methyl, 750 μg/ml^{-1} carbendazim, or 750 μg ml^{-1} tebuconazole. The fungicide concentrations chosen were based on those recommended by the manufacturers. Colony growth at 25°C was recorded after 4 days and compared with that of the isolates on dishes containing potato dextrose agar without fungicide. Mycelial growth revealed that 15% of the *Monilinia* isolates were resistant to carbendazim and thiophanate methyl. Most (14%) of the resistant isolates were from the Mesi-Ammos-Meliki area, Imathias. In contrast, all the isolates were sensitive to tebuconazole. Based on these results, to successfully control brown rot, growers of the Mesi-Ammos-Meliki Imathias area should use fungicides exhibiting other modes of action against the brown rot pathogen. In addition, the use of carbendazim and thiophanate methyl in commercial stone fruit orchards in the area Mesi-Ammos-Meliki, Imathias should be limited for at least 3 years. However, growers can continue using tebuconazole against brown rot of stone fruit.

The role of the G protein β subunit in *Verticillium dahliae* pathogenesis through RNA-mediated gene silencing or gene replacement. A. TZIMA[1], E.J. PAPLOMATAS[1] and S. KANG[2]. *[1]Agricultural University of Athens, Laboratory of Plant Pathology, Iera Odos 75, 118 55 Athens, Greece. [2]Pennsylvania State University Department of Plant Pathology, University Park, PA 168 02, USA.*
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G proteins are thought to be involved in transducing stimulants from the environment to the interior of eukaryotic cells. The inactivation of one or more subunits of G proteins in several plant pathogenic fungi indicates that they are implicated in the infection process of these fungi. To explore the early stages of the interaction between the soilborne fungus *V. dahliae* and the host, which are critical for the disease, the role of the G protein in *V. dahliae* pathogenesis was examined. Part of the G protein β subunit gene was amplified in *V. dahliae* using degenerate primers, and two strategies were adopted to study this G protein subunit. The first strategy reduced the expression of the gene by activating the RNA-mediated gene silencing mechanism due to the presence of dsRNA of the gene. The second completely inactivated the gene by replacing it with a mutant allele of the gene under study, disrupted by an antibiotic resistance gene cassette. To silence the G protein β subunit gene in *V. dahliae,* a 460 bp hairpin of the gene was constructed and integrated into the fungal genome under the control of appropriate promoter and terminator sequences, via *Agrobacterium tumefaciens* transformation. *V. dahliae* isolates genetically modified to express green fluorescent protein (GFP) were transformed with the hairpin construct so that the infection process of the transformants could be examined histopathologically. Preliminary assays of Southern hybridization of four transformants confirmed the presence of the hairpin in two to three copies. However, Northern hybridization in two of these transformants revealed that the absence of silencing absence of silencing as an expression of the G protein β subunit gene was similar to both the transformants and the wild-type strains. Currently, more transformants are being examined so that the percentage and level of silencing can be specified. With the more common technique of gene replacement, the geneticin resistance cassette was inserted in part of the G protein β subunit gene. This mutant allele of the gene will be inserted in the *V. dahliae* genome through Agrobacterium in order to replace the endogenous gene by homologous recombination. These two approaches will give a complete view of the role of this gene not only in pathogenesis, but also in the physiology of *V. dahliae*. In parallel, the two techniques will be evaluated

for their efficiency and velocity in studying pathogenicity genes of *V. dahliae*.

Morphology and mycelial compatibility of *Sclerotium cepivorum* Berk. isolates in Greece. I. VLOUTOGLOU[1], D. LASCARIS[1], J. ASPROMOUGOS[1] and N. RESKOU[2]. [1]*Benaki Phytopathological Institute, Plant Pathology Department, Laboratory of Mycology, 8, S. Delta St., 145 61 Kifissia, Athens, Greece.* [2]*Technological Educational Institute of Kalamata, Greece.*
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One hundred and sixteen isolates of *Sclerotium cepivorum* Berk. from various parts of Greece were studied *in vitro* for their morphology and their mycelial (or vegetative) compatibility. The isolates fell into four morphological groups, based on their colony morphology and the formation of sclerotia on PDA dishes. The presence or absence of a barrage zone at the contact of mycelia of two isolates grown on the same dish was taken to indicate the vegetative incompatibility or compatibility of the isolates. Four mycelial compatibility groups (MCG-A, MCG-B, MCG-C and MCG-D) were identified among the *S. cepivorum* isolates. Most of the isolates (78%) belonged to MCG-A, 15% to MCG-D, 5% to MCG-C and 1% to MCG-B; one isolate (1%) was compatible with isolates from three groups: MCG-A, MCG-B and MCG-C. Pairings of 56 Greek isolates with nine isolates from England, Australia, Canada, The Netherlands, Switzerland and Spain, each belonging to a different mycelial compatibility group (MCG-1 to MCG-9) and kindly offered by Dr G.J. Boland, University of Guelph, Ontario, Canada, showed that 68% of the Greek isolates were compatible with MCG-8, 5% with MCG-2, 2% with MCG-1 and 2% with MCG-3, while 23% of the isolates were not compatible with any of these MCG groups.

The *Pleurotus cystidiosus* and *P. eryngii* species-complexes: ecology, systematics and molecular phylogeny of populations with a world-wide distribution. G. ZERVAKIS[1], M. BESSI[1], R. VILGALYS[2], J-M. MONCALVO[2], G. VENTURELLA[3] and Y-J. YAO[4]. [1]*N.AG.RE.F., Institute of Kalamata, Kalamata, Greece.* [2]*Duke University, Department of Botany, Durham, NC, USA.* [3]*University of Palermo, Department of Botany, Via Archirafi 38, Palermo, Italy.* [4]*Chinese Academy of Sciences, Institute of Microbiology, Beijing, China.*
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Fungi of the genus *Pleurotus* produce edible mushrooms of excellent organoleptic qualities, while they are also have several other agro-industrial and environmental applications of economic importance. The *P. eryngii* and the *P. cystidiosus* species-complexes are of particular interest since the former develops biotrophic associations with host plants and the latter forms asexual synnematoid fructifications. Forty *P. eryngii* isolates, representative of the geographical distribution of this species-complex (Mediterranean Europe, Middle East, Asia), were studied by examining their ecomorphological characters and by molecular techniques, i.e. by sequencing the internally transcribed spacers (ITS 1 and ITS 2), the intergenic spacers (IGS) and the 5.8S rDNA gene. The results demonstrated that there are two distinct species: *P. eryngii* (growing in association with a wide range of Apiaceae host-plants, and consisting of several ecotypes-varieties), and *P. nebrodensis* (growing particularly on *Cachrys ferulacea*), which evolve through a sympatric speciation process. From the group of the coremia-forming *Pleurotus*, 41 strains from five continents were examined by mating compatibility experiments and ITS 1 - ITS 2 and 5.8S rDNA gene sequencing. Application of the phylogenetic species-concept resulted in the delimitation of *P. cystidiosus*, *P. smithii*, *P. fuscosquamulosus* and *P. australis*. Those taxa presented characteristics of genetic isolation at the level of individual populations, and an allopatric speciation process.

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Bacterial diseases

Recent progress in phytoplasma research. X. FOISSAC. *UMR-1090 Génomique Diversité et Pouvoir Pathogène, INRA et Université Victor Ségalen Bordeaux 2, BP 81, 33883 Villenave d'Ornon, France.*
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Among the phloem-restricted bacteria, phytoplasmas are the most damaging plant pathogens with an important economical impact on perennial crops. They are naturally transmitted by leafhoppers, planthoppers and psyllids which are hemipteran phloem feeders, but also spread through nurseries when infected planting material is propagated. Very few genetic resistance to phytoplasma infection have been reported and disease control rely on prophylactic sanitary measures consisting in the destruction of infected crop, the chemical control of insects and protection of nurseries. Many yellowing diseases involve more than one phytoplasma or phloem restricted bacterium and relative incidence of each causal agent has to be determined. In Europe, this is notably the case for yellows of grapevine and strawberry plants. To achieve phytoplasma identification, many polyvalent and species-specific DNA methods for diagnosis have been developed last fifteen years. As phytoplasma cannot be grown in artificial medium, reference strains had been propagated *in planta* by grafting or continuous insect transmission. The taxonomic classification of phytoplasma is mainly based

on 16SrDNA phylogeny and ecological properties such as plant host range and nature of insect vectors. This lead to the description of 23 *Candidatus* Phytoplasma species. Phytoplasma classification is still evolving, and emerging or re-emerging plant diseases involving uncharacterised phytoplasmas are regularly described. Prospective in phytoplasma epidemiology therefore necessitates a more intensive characterization of phytoplasma diversity in wild plant and insect reservoirs, which in many cases have a great impact on disease emergence and control.
A potential driving force for phytoplasma evolution is the necessity to adapt to new plant or insect hosts after invasion of new ecological niches. This can happen when new insect vectors or new plants are introduced. However, phytoplasmas like the other members of the bacterial class *Mollicutes*, have limited genome, with size ranging from 530 kbp to 1,350 kbp. The first two phytoplasma genomes have recently been sequenced and analysis of their 671 to 754 genes confirmed that phytoplasma went through extreme reductive evolution but still maintain an important genome plasticity. Phytoplasma genes encoding surface proteins involved in the interaction with the insect vector vary much more rapidly than the rest of the genome. This diversifying effect is seen as a consequence of a strong positive selection, resulting of the necessary adaptation of phytoplasmas to their complex and changing environment. Such a species-specific, variable gene constitute an interesting marker to target in molecular epidemiology and has been recently identified for stolbur phytoplasma, an endemic phytoplasma in Europe. Comparative genomics will facilitate the identification of the phytoplasma genetic determinants governing plant pathogenicity. Plant genes, which expression is affected by phytoplasma infection can be identified using a candidate gene strategy. However, to identify the mechanisms allowing phytoplasmas to disturb plant physiology and organogenesis, original approaches are needed to overcome the difficulty of dealing with non cultivable bacteria.

Susceptibility of 34 cherry genotypes to *Pseudomonas syringae* pv. *syringae*. D. GALAITSIS, A. GALIATSATOU, T. THOMIDIS and I. CHATZICHARISIS. *Pomology Institute Naoussa (NAGREF), R. S. Naoussas 38, P.C. 59200 Imathia, Greece.*
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The bacterium *Pseudomonas syringae* pv. s*yringae* is a serious problem for cherry cultivation because it can cause the death of stems, shoots, and even entire trees. The use of resistant cultivar/rootstock combinations is the most effective way to control the disease. The main aim of this study was to investigate the susceptibility of 34 new cherry genotypes, established at the experimental field of the Pomology Institute Naoussa, to *P.s.* pv. *syringae*. Experiments in the laboratory consisted of an excised twig assay and the excised shoot method and experiments in the field involving the inoculation of excised shoots. Both bacterium strains used in this study (from pear and citrus trees) were pathogenic to all cherry genotypes, which showed high susceptibility. The results of the laboratory assays were generally in agreement with the field experiments. The results indicate that caution must be taken when it is decided to use any of these genotypes in areas where *P.s.* pv. s*yringae* is a threat.

***Pseudomonas viridiflava* is the causal agent of a new bacterial disease of artichoke.** D.E. GOUMAS, C.X. GATZILAKIS and S.E. GOUMA. *Technological Educational Institute of Crete, P.O.B.* 1939 *71004, Heraklion, Crete, Greece.*
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In March 2006, an unusual bacterial outbreak was observed on artichoke (*Cynara scolymnus* L. cv. Lardati) crops (1 ha) cultivated near Heraklion, Crete and the same symptoms were seen two months latter at the Lassithi plateau. Symptoms of the disease were not restricted to the external bracts of the capitulum, as occurs with infection caused by the bacterium *Xanthomonas cynarae*, but were also observed on the internal bracts. Infection began as water-soaked and dark-green spots on the capitulum bracts. Diseased bract leaves had many sunken, elongated necrotic lesions with a brown to black centre surrounded by thin water-soaked halos and with large dark red-brown margins. Lesions expanded to form dark green, water-soaked streaks and patches in the internal bract leaves of the capitulum. They turned darker with age until they were almost black. Disease incidence reached on average 70% of the capitula and may have been promoted by prevailing conditions, which were unfavorable for artichoke growth. Specifically, plants were stressed by repeated spraying with growth regulators and by rainy and windy weather. No boron deficiency was detected in soil or plant samples. The disease reduced the marketability of the artichokes. Bacteria were consistently isolated from diseased bract leaves and were identified as strains of *Pseudomonas viridiflava* on the basis of morphological, physiological, biochemical and pathological characters. To our knowledge this is the first record of *P.viridiflava* as a pathogen on artichoke in Europe.

***Acanthus mollis*: a new host of *Pseudomonas viridiflava*.** D.E. GOUMAS, C.X. GATZILAKIS and K.H. NAVROUZOGLOU. *Technological Educational Institute of Crete, P.O.B.* 1939, *71004 Heraklion, Crete, Greece.*
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Acanthus mollis (**Bear's Breech),** belonging to the Acanthaceae family, is a perennial foliage plant, cultivated for landscape and the shrub border decoration. It is native to southern Europe and the Mediterranean countries. In the spring of 2006, a leaf blight was observed on *A. mollis*

plants grown in the botanical garden of the Technological Educational Institute of Crete at Heraklion. Typical symptoms were tan to dark brown leaf spots and rapid blighting of foliage under shady and moist conditions. Leaf spots often appeared as concentric circles and when the affected tissues dropped, the leaves had a ragged appearance. Chlorotic halos were very common around the necrotic lesions and there was considerable defoliation and stunting of whole plants. Similar lesions were also observed on the flower spikes. Affected plants rarely died but the presence of lesions and the blighting of leaves reduced aesthetic quality and growth. No fungal growth was observed on the lesions themselves. The bacterium *Pseudomonas viridiflava* (Burkholder) Dowson was identified as the causal agent of the blight on the basis of morphological, biochemical and pathological characters. This is the first report worldwide of *P. viridiflava* as a pathogen on *Acanthus mollis*.

Detection and identification of bacterial diseases infecting tomato crops in Cyprus. L.C. PAPAYIANNIS. *Agricultural Research Institute, P.O.B. 22016, Nicosia 1516, Cyprus.*
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In greenhouse tomato crops of the Pyrgos and Parekklisia areas (Limassol district) and in open field tomatoes of the Odou, Melini and Vavatsinia areas (Larnaca district), symptoms of black necrotic lesions with yellow haloes, black necrotic patches on the leaves and petioles, yellow-brown discoloration of the vascular tissue, stem and pith necrosis, dark necrotic lesions on the leaf surface and petioles, and severe wilt were observed in the spring of 2005. Forty-two symptomatic plants were collected and infected tissues were inspected under the microscope for bacterial cells. Pure cultures from all plants were obtained on nutrient agar (NA), sucrose nutrient agar (SNA), King's medium B (KB) and yeast-peptone-glucose agar (YPGA). Three different colonies were isolated from infected tissues, one of which gave a Gram-positive reaction. DNA was extracted from all colonies and amplified by Polymerase chain reaction (PCR) using primers for *Pseudomonas syringae* pv. *tomato*, *Pseudomonas corrugata*, *Clavibacter michiganensis* subsp. *michiganensis, Ralstonia solanacearum* and *Xanthomonas vesicatoria.* The PCR results revealed three bacterial micro-organisms. *Pseudomonas syringae* pv. *tomato* (causing bacterial speck disease of tomato), *P. corrugata* (tomato pith necrosis) and *Clavibacter michiganensis* subsp. *michiganensis* (bacterial canker of tomato) were identified as the causal agents of the symptoms observed in the Pyrgos and Parekklisia areas, whereas *C.m*. subsp. *michiganensis* was the only pathogen identified in wilted plants from the Odou, Melini and Vavatsinia areas.

Further studies on the etiology of small apple fruit disorder occurring in some orchards of Mount Pelion. A. RUMBOU[1], L. CARRARO[2], I. BOUTLA[3], M. SAMARA[4] and I.X. RUMBOS[1]. [1]*NAGREF, Plant Protection Institute of Volos, Volos 380 01, Greece.* [2]*Dipartimento Biologia Applicata Difesa Piante, University of Udine, Italy.* [3]*Agricultural Cooperative of Zagora, Greece.* [4]*Prefectoral Self-government of Magnesia, Volos, Greece.*
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Extensive investigations in 2004–2005 on the etiology of small apple fruit disorder in some apple orchards of Mount Pelion showed that there are no scientific findings to support the belief that the disorder has a nutritional or physiological cause. The electromagnetic radiation measured by the Polytechnic School of the Aristotelian University of Thessaloniki was very low and below the legal limit for emissions of radio, TV or mobile phone stations. Results on tropospheric O_3 concentrations were also negative. From September 2005, the study focused on identifying the phytoplasmas of apple proliferation disease. For this purpose the following steps were planned: a) transmission of the pathogen by grafting, b) remission of the symptoms after injection with suitable chemicals and c) detection of phytoplasmas using molecular techniques. The PCR assay detected the pathogen *Candidatus* Phytoplasma mali. The pathogen was identified in the leaves and/or roots in 112 samples (41%) in the Zagora area , in 36 samples (39%) in Pouri, and in 7 samples (43%) in Makryrachi. Phytoplasmas were detected in 158 samples (34%) of the cv. Starking Delicious, the main cultivar grown at Mount Pelion, in 6 samples (83%) of the cv. Red Chief, and also in the cv. Royal Gala (both trees of this cv. examined) and the cv. Golden Delicious. In addition, the pathogen was detected in all five apple rootstock shoots which developed near the soil surface as well as in the cv. Firiki which was grafted one year before on affected 'Starking Delicious' trees. The high percentage of trees infected with *Candidatus* Phytoplasma mali gave a strong correlation between small apple fruit disorder and apple proliferation disease. The evaluation, to be carried out in 2006, of the injections made on 120 affected trees during September–October 2005, will be a strong indication of the role that *Candidatus* Phytoplasma mali plays in small apple fruit disorder.

Development of nested PCR using generic and specific primers for the detection of phytoplasmas in various hosts in Greece. P.A. SAINIS[1], C.I. DOVAS[2] and N.I. KATIS[1]. [1]*Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, 54 124 Thessaloniki, Greece.* [2] *National Agricultural Research Foundation (NAGREF), Plant Protection Institute of Thessaloniki, Thermi 570 01, Thessaloniki, Greece.*
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Nested PCR assays were developed for the generic detection of phytoplasmas using degenerate primers for the amplification of the 16S rRNA gene and the 16S-23S

intergenic spacer region. Their efficiency was evaluated using 12 phytoplasma-isolates from different host species belonging to 7 phylogenetic groups: 16SrI (Aster yellows group), 16SrII (Peanut WB group), 16SrIII (X-disease group), 16SrV (Elm yellows group), 16SrVII (Ash yellows group), 16SrX (Apple proliferation group) and 16SrXII (Stolbur group). Additionally, 5 nested primers were designed and evaluated for the specific detection of Aster yellows (AY), Stolbur (STOL), Apple proliferation (AP) and European stone fruit yellows (ESFY). For phytoplasma identification in Greece, 299 samples were collected during 2004–2005 from different crop plants showing symptoms typical of phytoplasma infection. Also, 437 weed samples (with or without symptoms) were collected from affected crops. Some isolates were identified by sequencing the PCR products or/and by digestion with restriction enzymes. Stolbur was detected in tomato, pepper, tobacco and grapevine samples, and in the arable weeds *Cirsium arvense*, *Xanthium strumarium*, *Convolvulus arvensis* and *Solanum nigrum*. AY was detected in 3.43% of the weeds collected. ESFY is an important plum pathogen that in the orchards tested had an incidence ranging from 10–50%, and was responsible for serious disease symptoms. ESFY was also detected, though at a lower frequency, in plum, peach, apricot and almond orchards. Finally, AP was detected in Zagora, Pilio area, in 7.8% of apple samples that had little fruit symptoms.

Detection and characterization of the phytoplasma that causes stolbur disease of tomato in Greece. E.K. VELLIOS[1], F. LIOLIOPOULOU[1], A. KASSAVETI[1] and P.E. KYRIAKOPOULOU[2]. *[1]Department of Crop Production and Agricultural Environment, University of Thessaly, Greece. [2] Department of Crop Production, Agricultural University of Athens, Greece*.
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During the 2005 and 2006 growing seasons, surveys were carried out in tomato fields in different parts of Greece. Fields of tomato with typical stolbur symptoms were found in many areas with a disease incidence usually ranging between 1–2%, although in some cases it went up to 15%. The occurrence of phytoplasmas was verified by PCR analysis using primers for the 16S-23S rRNA ITS region. These phytoplasmas were classified in the XII-group (Stolbur group) with the use of group specific primers and RFLP analysis.

VIRUS DISEASES

RNA silencing: mechanism and its application in phytopathology. K. KALANTIDIS. *Institute of Molecular Biology and Biotechnology, Heraklion, Greece, and Department of Biology, University of Crete, Heraklion, Greece.*
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RNA silencing is a conserved mechanism of RNA-mediated regulation, defence and genomic stability. It is found in almost all eucaryotes, - with the notable exception of *Saccharomyces cerevisiae* -, from *S. pombe,* through fungi, plants and flies, and to mammals. Over the last decade an impressive number of reports has been published on this important mechanism of RNA-dependent phenomena, and the number is still growing. RNA silencing is highly sequence-specific; its specificity is achieved through the activity of characteristic small (from -21 to -23 nt long) RNA molecules. Phenomena related to RNA silencing include: degradation of aberrant RNA, sequence-specific transcriptional gene silencing through DNA methylation – often of transposable elements, – and the post-transcriptional regulation of gene expression through the activity of a subgroup of small RNA molecules called micro-RNAs.
In plants, RNA silencing is an RNA-mediated immune mechanism with a very important role in plant defence against viral infection. It has been shown that provided the silencing mechanism against a specific virus is turned on early enough (as for example by appropriate molecular constructs), plants will show tolerance or even immunity to the 'silenced' virus. In addition, RNA silencing can be used in functional studies of many organisms, including plants and fungi. This application of RNA silencing has gained popularity of late due to some important advantages it has over traditional genetic approaches. For example, no labour-intensive and/or biohazardous mutagenesis is required, suppression can be highly sequence-specific, but at the same time appropriately designed constructs can target multiple gene family members simultaneously, and it has other advantages.

Study of Greek isolates of *Turnip mosaic potyvirus* (TuMV). M.G. BERBATI[1], O.G. MELITA[1], I.E. TZANETAKIS[2], I.N. BOUBOURAKAS[1], M.E. GRATSIA[1], M.S. KAPONI[1], A.E. VOLOUDAKIS[3], M-S GIRGIS and P.E. KYRIAKOPOULOU[1]. *[1]Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Pathology, Iera Odos 75, 118 55 Athens, Greece. [2]Naval Hospital of Salamina, 18 900 Salamina, Greece. [3]Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Breeding and Biometry, Iera Odos 75, 118 55 Athens, Greece.*
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Four hundred and fifteen samples of cultivated and wild herbaceous plants, showing mosaic, mottling, necrotic lesions and striping, were collected from eighteen prefectures of Greece in 2003–2006, and tested for *Turnip mosaic potyvirus* (TuMV). One hundred and eighteen samples were found positive by DAS-ELISA and they were indexed on plant-indicators. Twenty-one of these samples, representative of all eighteen prefectures,

were chosen to determine the host range and symptomatology of the virus and tested by IC-RT-PCR with specific primers amplifying a genome fraction inluding the coat protein (CP). A fraction of 600 bases of the CP deriving from two isolates, 1R-SP6 (from *Sinapis alba,* Amarousion, showing mosaic, and reacting to *Chenopodium quinoa* by causing leaf curling) and 4F-T7 (from *Eruca sativa,* Marathon, showing mosaic, and reacting to *Petunia hybrida* by causing gradual necrosis) were cloned on a pCR®4-TOPO plasmid and sequenced. The results indicated that the two isolates were TuMV. Sequence comparison gave the isolates a nucleotide homology of 87% and an aminoacid homology of 92%. A phylogenetic comparison with other isolates from the NCBI database indicated that isolate 1R-SP6 belonged to the World B group of TuMV, and isolate 4F-T7 to the Basal B group.

Incidence of *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV) and *Apple chlorotic leafspot virus* (ACLSV) in almond orchards of Greece and Cyprus. A.TH. CHAROU[1], V.I. MALIOGKA[1], M.M. MATHIOUDAKIS [1], K.E. EFTHIMIOU [1], L.C. PAPAYIANNIS[2] and N.I. KATIS [1]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O.B. 269, 54006, Thessaloniki, Greece.* [2]*Agricultural Research Institute, P.O.Box 22016, Nicosia 1516, Cyprus.*
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The incidence of the ilarviruses *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV) and *Apple chlorotic leafspot virus* (ACLSV) was studied in almond orchards of Greece and Cyprus. For this purpose 136 samples were randomly collected from the prefectures of Larissa, Thessaloniki, Magnisia, Ioannina and Serres and 52 samples from Cyprus. The samples were initially tested with ELISA using polyclonal antibodies against PNRSV and PDV. Samples that were serologically negative were further tested with a generic RT-PCR to detect the Ilarviruses, followed by two nested PCR assays in which specific downstream primers were used to detect PNRSV and PDV. The results showed high infection rates of PNRSV in Cyprus (84.6%), the prefecture of Larissa (77.7%), Serres (78.9%), Magnisia (45.2%) and Ioannina (20%), while PNRSV was not detected in Thessaloniki. The incidence of PDV was lower in both Greece and Cyprus, ranging from 10.0 to 48%. Specifically, PDV was detected in Cyprus (48%) and in the prefectures of Larissa (37.7%), Magnisia (33.3%), Serres (31.5%) and Ioannina (10%). Mixed infections of the two viruses were also observed in a number of samples ranging from 16.6 % (Magnisia) to 37.7% (Larissa). Molecular detection of the Ilarviruses by RT-PCR was also carried out in samples from Cyprus (18/32, 56.25%) and Greece (15/54, 27.77%).

Virus incidence in certified and non-certified seed-potatoes from Greece. E.K. CHATZIVASSILIOU[1], E. MOSCHOS[2], S. GAZI[2], P. KOUTRETSIS[2] and M. TSOUKAKI[2]. [1]*Democritus University of Thrace, Department of Agricultural Development, Plant Pathology Laboratory, Pantazidou 193, 682 00 N. Orestiada, Greece.* [2]*Hellenic Ministry of Rural Development and Food, Control Station for Vegetative Propagating Material, 19300 Aspropyrgos, Greece.*
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Having virus-free plant material is of major importance for the control of virus diseases, especially diseases caused by vegetatively propagating viral species. In this study, the occurrence of viruses in certified (categories basic and certified) and non-certified seed-potatoes was studied in several areas of Greece. One hundred potato tubers were sampled from each lot and sprouts were tested for *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV) using ELISA. Basic-category seed-potatoes were also tested for *Potato virus X* (PVX). In 2005 and 2006, 96 and 84 lots of basic-category seed potatoes were tested respectively, and infections were detected in 39 lots in 2005 and 38 lots in 2006. PVY was detected in 25 and 33 lots, and PLRV in 12 and three lots, in the two years respectively. In 2005, the infection rate was a maximum of 11% for PVY (cv. Spunta in Tripoli) and of 7% for PLRV (cv. Hermes in Serres). In 2006, the maximum infection rate was 7% for PVY and 2% for PLRV (cv. Liseta in Pyrgos). PVX infections were recorded in nine lots (max. 10%, cv. Spunta, in Thessaloniki) and seven lots (max. 7%, cv. Spunta, in Athens), in 2005 and 2006 respectively. In certified-category seed-potatoes, of the 302 and 336 lots tested in 2005 and 2006 respectively, 105 contained infected tubers in 2005, and 167 in 2006. PVY was detected in 86 and 145 of these lots respectively, and PLRV in 31 and 55 lots. In 2005, the highest rate of infection was 20% for PVY and likewise 20% for PLRV (cv. Agria, in Amynteo), while in 2006, infection rates were 32% for PVY and 65% for PLRV (cv. Agria, in Drama). In the non-certified seed-potatoes, a failure rate in germination of up to 30% was recorded. All lots were infected with PVY in especially high percentages ranging from 8% (cv Liseta, Orestiada) to 100% (cv. Marfona, in Tripoli). PLRV was detected in six lots and rates reached 29% (cv. Agria, Aridaia). The importance of these results on the epidemiology of these viruses in potato crops is discussed.

Two viruses associated with a new disease of spinach in Greece. M.E. GRATSIA[1], I.E. TZANETAKIS[2], K.P. FASSEAS[3], M-S GIRGIS and P.E. KYRIAKOPOULOU[1]. [1]*Laboratory of Plant Pathology, Department of Crop Science, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece.* [2]*Salamine Naval Hospital, 18 900 Salamine, Greece.* [3]*Laboratory of Electronic*

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In January 2004, a spinach crop in Marathon presented some uncommon symptoms of a viral disease: yellow or chlorotic mosaic/spots/patterns and deformation. Spinach seedlings of the same batch of seeds (variety, Spinaker, procured from the Dutch seed company Bejo Zaden b.v.), showed the same symptoms when grown in the laboratory greenhouse, indicating that the disease was transmitted by the seed. Mechanical transmission to a number of potential hosts including the spinach variety Spinaker gave two distinct types of symptoms: a) chlorotic/yellow spots/mosaic/mottling and deformation, and b) necrotic spots 1.5–3 mm. Molecular (RT-PCR, IC-PCR) and serological examination detected two viruses: *Sowbane mosaic virus (*SoMV) in indicator plants with chlorotic/yellow symptoms and Olive mild mosaic virus (OMMV) in indicator plants with necrotic spots. Electron microscopy with negative staining of pure preparations from chlorotic/yellow tissues showed virions of SoMV. This is the first report worldwide on the molecular analysis of SoMV, and on its phylogenetic relationship with Sesbania mosaic virus and Southern bean mosaic virus. The nucleotide OMMV-spinach was completed, revealing a roughly 90% similarity to OMMV-olive at the nucleotide level. This is the first record worldwide of the natural transmission of OMMV in plants other than olive, showing that OMMV is an independent species, and not an accidental recombination occurring in olive between *Tobacco necrosis virus* A (TNV-A) and TNV-D.

Induction of resistance to *Cucumber mosaic virus* (CMV) in tobacco and tomato by application of dsRNA produced *in vitro* or *in vivo*. M.C. HOLEVA[1], A.P. SCLAVOUNOS[2], P.E. KYRIAKOPOULOU[3] and A.E. VOLOUDAKIS[1]. *[1]Laboratory of Plant Physiology, Department of Agricultural Biotechnology, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece. [2]ELGA, Km 2 of New National Rd, Lamia-Athens, Lamia 35100, Greece. [3]Laboratory of Plant Pathology, Department of Crop Sciences, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece.*
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Cucumber mosaic virus (CMV) infects a wide range of host-plants, including tomato, in which it causes yellow mosaic patterns, fern-leaf shoestring, leaf/fruit necrosis and/or plant shrinkage. As only few sources of natural resistance for this pathogen have been reported, this study focused on exploiting pathogen-derived resistance in order to protect plants. This involved employing the dsRNA of the capsid protein (CP) gene of CMV, produced *in vitro* or *in vivo* in bacterial cells, to silence the expression of the CP gene at the post-transcriptional level inducing resistance in tobacco and tomato plants against CMV. Up to 40% of tobacco and tomato plants co-inoculated with a mixture of the highly virulent hellenic CMV-G isolate plus dsRNA produced as above remained asymptomatic; the remaining plants exhibited delayed and milder symptoms: light mosaic without any leaf necrosis in tobacco, upward leaf curling and stem purpling in tomato. These results are promising and warrant further research, mainly on optimizing the application of the dsRNA to make plants immune to CMV. Such an approach to inducing plant resistance to CMV can take advantage of modern bombardment techniques to introduce dsRNA into young plants without any need for genetic engineering of these plants.

Real-time PCR detection and quantification of vector trichodorid nematodes and *Tobacco rattle virus* (TRV). R.C. HOLEVA[1], M.S. PHILLIPS[1], R. NEILSON[1], D.J.F. BROWN[2], V. YOUNG[1] and V.C. BLOK[1]. *[1]Scottish Crop Research Institute, Invergowrie, Dundee, Scotland DD2 5DA, UK. [2]Central Laboratory of General Ecology, Gagarin Street 2, 1113 Sofia, Bulgaria.*
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This report describes a novel diagnostic method for virus-vector trichodorid nematodes and the associated *Tobacco rattle virus* (TRV) based on a real-time fluorogenic 5' nuclease PCR assay (TaqMan). Two independent primer/probe sets were designed targeting the 18S gene of the ribosomal cistron for the trichodorid species *Paratrichodorus pachydermus* and *Trichodorus similis*. Assays using purified plasmid DNA containing clones of the 18S region and genomic DNA extracted from individuals from both these nematode species were highly specific as no cross-reaction was observed either between species, or with two non-target trichodorid species, *Paratrichodorus anemones* and *Trichodorus primitivus*. Target DNA present in unknown samples was quantified by comparing the fluorescence signals of the samples with those obtained from standard plasmid dilutions. Three primer/probe sets were also used to target TRV; one set for RNA1 and the other two sets for the RNA2 of specific isolates (TRV-PpK20 and TRV-TpO1). Both trichodorid species and TRV RNA1 and RNA2 were detced in a single sample, and field samples were used to demonstrate the potential of this assay to provide rapid, accurate and sensitive molecular information needed for risk assessment in the field.

Transgenic plants expressing dsRNA derived from viral sequences: a method to induce a natural defence mechanism leading to virus resistant plants.
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Until very recently, virus diseases of plants could not be cured. Once infected with a virus strain, the course of the disease and its symptoms was inevitable. Since plants lack an immune defence system, it was also not possible to immunize them against virus infections. In recent years, an ancient mechanism regulating defence and gene expression has been elucidated in plants and other organisms, in which long (100 nucleotides and more) and/or short (21–27 nucleotides) double-stranded RNA plays an important role. Long dsRNAs are degraded in plant cells by dsRNA-specific ribonucleases, called dicers. Short dsRNAs, defined as siRNAs (small interfering RNAs), are produced by dicers. They are incorporated into a ribonucleoprotein complex that has a specific ribonuclease activity, defined by the short RNA segment. This mechanism of specific RNA degradation and interference is used by many organisms a) to control expression of repetitive genomic sequences like retroelements or retroviruses, b) to protect invading genomes of viruses, and c) to control gene expression and the development of the organism itself. We used the expression of dsRNAs derived from different viruses, to enhance this natural defence mechanism and to create virus-resistant transgenic plants. Potato virus Y and cucumber mosaic virus are two viruses which infect a large number of vegetables, ornamental plants, and industrial plants such as tobacco. Tobacco was used as a model to test the effectiveness of the method for enhancing resistance. Transgenic tobacco plants of the oriental variety Basmas and the variety Virginia were created, which expressed the dsRNA segments of a) CMV, and b) PVY. A number of transgenic lines were generated for each virus, by agrobacterium transformation, which could be categorized into three groups: susceptible, recovered and resistant. Resistant plants could not be infected at all with the viruses. Recovered plants were initially infected, but recovered fully in the following weeks. In conclusion, expression of dsRNAs was a very effective method for the generation of virus-resistant transgenic plants. Tobacco plants of the 5th generation of the CMV lines were still fully resistant. The method was also used to generate potato plants resistant to PVY. With this tetraploid, vegetatively propagated crop plant it was possible to generate potato lines fully resistant to the virus. The dsRNA method is presented as a very effective new biotechnological method for the creation of virus-resistant crops. Transgenic plants expressing non-translated segments of the viral genome in the form of dsRNA may be suitable for use in agriculture for another reason as well: since no functional viral proteins are expressed, there is less risk of side effects in the food chain, or of an unexpected recombination event between the transgene and the virus.

Virp1 is a necessary host factor for infectivity of PSTVd and CEVd in *Nicotiana benthamiana* and *Nicotiana tabacum* plants. K. KALANTIDIS[1], M.A. DENTI[1], S. TZORTZAKAKI[1], E. MARINOU[2], M. TABLER[1] and M. TSAGRIS[1,2]. [1]*Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology, P.O. Box 1385, 71110 Heraklion, Crete Greece.* [2]*Department of Biology, University of Crete, P.O. Box 2208, 71409 Heraklion, Greece.*
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Viroids are the smallest pathogens known, consisting of a small circular RNA which does not code for proteins, is infectious and replicates on several host plants. Viroids are separated in two families, the Avsunviroidae, which replicate in the chloroplast of the infected cells, and the Pospiviroidae, which replicate in the nucleus. Besides this difference in the location of their replication, viroids of both families are able to spread from cell to cell, and to infect their hosts systemically, moving from the epidermal/mesophyll/bundle sheath boundary to the phloem companion cells and the vascular tissue, and from there to new developing tissue. Viroids of the Pospiviroidae family [the potato spindle tuber viroid (PSTVd) and the citrus exocortis viroid (CEVd)] are replicated in the nucleus, most probably by the DNA-dependent RNA polymerase II of the cell. This has been shown a) by *in vitro* studies, b) by studies with alpha amanitine as an inhibitor in isolated protoplasts, and c) by co-localization studies of the viroid RNA and DNA-dependent RNA polymerase II. Besides the possible involvement of PolII in the replication, no other proteins of the cell had so far been shown to have a role. We have now isolated a tomato protein, Virp1, which in an *in vitro* assay specifically bound to PSTVd (and to other members of the PSTVd group). Virp1 is in a complex with PSTVd in the tomato cells (Martinez). Virp1 binds specifically to the right terminal part of the viroid PSTVd, recognizing there a sequence- and structure-specific motif which we denominated the RY motif (Maniataki, Gozmanova). *Nicotiana tabacum* (a non host for specific PSTVd strains) and *N. benthamiana* (a non-symptomatic host for PSTVd) are suitable and easy to transform plant species that can be used for replication and pathogenicity studies. We isolated and characterized the Virp1 genes (at the cDNA level) in these two plants. Unlike the tomato cv. Rentita, which has only one gene, both *Nicotiana* species contain two homologue cDNAs of Virp1, sharing 88–89% identity with the corresponding tomato gene at the nucleotide level. To elucidate the role of Virp1 in viroid replication, we generated a) transgenic *N. tabacum* and *N. benthamiana* plants that overexpressed the Virp1 of tomato, and b) transgenic plants that suppressed the endogenous genes of Nt and Nb via RNAi. We used these plants to study the role of Virp1 in viroid replication. The results showed that none of the *Nicotiana* plants of either species overexpressing Virp1 of tomato changed

their respective behaviour towards PSTVd infectivity and pathogenicity: *N. benthamiana* overexpressing Virp1 remained a symptomless host, while *N. tabacum* overexpressing Virp1 remained a non-host for PSTVd strain KF440–2. This strongly indicated that the sequence differences between the Virp1 genes in these plants and tomato did not account for the differences in replicability or pathogenicity between tomato and the *Nicotiana* species. Further, and most importantly, it was shown that *N. benthamiana* and *N. tabacum* plants, in which the endogenous Virp1 genes are suppressed to a very low level by RNAi, do not support PSTVd and CEVd viroid replication, thus proving that Virp1 is a necessary factor for the replication of the PSTVd group of viroids. If it can be shown that the suppressed plant lines are not different from wild-type plants in their phenotypic characteristics, Virp1 will be a potential source for a resistance gene, the first resistance gene isolated for viroids. This work may have an impact on protecting crops from viroid diseases, a group of diseases that adversely affect the yield of many important crops, such as potato, tomato, citrus, ornamentals, and others.

Indexing for viruses in seed potatoes produced by *in vitro* culture. T. KAPARI-ISAIA, S. GREGORIOU, G. MINAS, L. PAPAYIANNIS and N. SERAPHIDES. *Agricultural Research Institute, P.O. Box 22016, 1516, Nicosia, Cyprus.*
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To produce seed potatoes of the varieties Spunta, Nicola and Cara, potato micro-plants were produced *in vitro,* which after hardening, were planted in the screen house to produce mini tubers. Other micro-plants were planted in the soil in another screen house. Pre-sprouted potato tubers of the three varieties, which were kept under thermotherapy for forty days at 36°C and 95% humidity, were used as mother propagating material. All tubers were indexed by ELISA for the viruses PLRV, PVA, PVS, PVX, PVY and PVM. One tuber was infected with PVY and was discarded. During potato cultivation in the screen houses, all plants were indexed for all the above viruses by ELISA and by macroscopic examination. One plant was found infected by PVY and was discarded. Six plants growing adjacent to the PVC-infected plant were also discarded. All other plants were free of the examined viruses. For the control of aphids, which are virus-vectors, four yellow sticking traps were placed in the screen house and twelve indicator plants were marked for observation of the aphids.

Sanitation of Frappa by shoot-tip grafting *in vitro*. T. KAPARI-ISAIA, I. IOANIDES, M. PANAYIOTOU, D. POLYCARPOU, CH. MICELLIDOU and A. KYRIAKOU. *Agricultural Research Institute, P.O. Box 22016, 1516, Nicosia, Cyprus.*
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The technique of shoot-tip grafting *in vitro* was used for the sanitation of Frappa (*Citrus maxima* [Burm.] Merrill). Five mature Frappa trees were selected as mother propagating material. All mother trees were indexed for virus and virus-like diseases by biological and laboratory methods. All mother plants were free of citrus tristeza virus (CTV), citrus psorosis virus (CPsV), and citrus infectious variegation virus (CIVV). Three trees were infected with CEVd and CVdII, one tree with CVdII and one tree with CVdII, CVdIII and CVdIV. Rootstocks of Carrizo citrange, Swingle citrumelo and Troyer citrange, produced *in vitro*, were micrografted with apical meristems from the mother Frappa trees. Of the forty-five micrografts, nineteen were successful (average success rate 42%) and were grafted onto sour orange rootstocks *in vivo*. All the new plants are being indexed for the viroids that were present on the mother trees. Two new Frappa plants derived from two mother trees have so far been found free of viroids.

Effect of Citrus exocortis and related viroids on eleven citrus rootstocks. T. KAPARI-ISAIA, A. KYRIAKOU, A. GEORGIOU and C. GREGORIOU. *Agricultural Research Institute, P.O. Box 22016, 1516, Nicosia, Cyprus.*
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Eleven citrus rootstocks were evaluated for their sensitivity to exocortis, a disease caused by Citrus exocortis viroid (CEVd). The variety Ortanique tangor was used and trees were planted in 1980 at the Experimental Station of the Institute in Acheleia, Greece. Since 1996 the rootstocks Sour orange, Palestine sweet lime, Rough lemon, Red rough lemon, Estes rough lemon, Rangpur lime, Troyer citrange, Carrizo citrange, Swingle citrumelo, Volkameriana and Amplycarpa have been evaluated on the basis of symptom expression. Severe bark scaling was seen on sensitive rootstocks such as Palestine sweet and Rangpur lime. Rootstocks that were hybrids of *Poncirus trifoliata*, including Swingle citrumelo, Troyer citrange, and Carrizo citrange, exhibited mild bark scaling. The rootstocks Sour orange, Estes rough lemon, Volkameriana and Amplycarpa were free of bark scaling. However, some of the trees that budded on the rootstocks Estes rough lemon, Volkameriana, Rough lemon and Rangpur lime were infected with *Phoma tracheiphila,* the causal agent of mal secco disease, and died.

Olive viruses in Greece. M.S. KAPONI and P.E. KYRIAKOPOULOU. *Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Pathology, Iera Odos 75, 118 55 Athens, Greece.*
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Samples from one hundred and fifteen olive trees from different regions of Greece, most of them showing pox

and hump symptoms of the fruits, and yellowing and malformations of the leaves (mainly sickle-leaves) were tested for 11 known viruses infecting this species, as well as for unknown viruses. The test methods used included biological indexing with herbaceous species, DAS-ELISA, RT-PCR and dsRNA analysis, as well as eleven different nucleic acid extraction protocols. The results so far provide evidence for the occurrence of *Arabis mosaic nepovirus* (ArMV), *Cherry leaf roll nepovirus* (CLRV), *Cucumber mosaic cucumovirus* (CMV) and *Tobacco mosaic tobamovirus* (TMV) in a limited number of samples, deriving mainly from the Peloponnese and Attica. In addition, dsRNA analysis exhibited one electrophoretic band of more than 10,000 bases, and at least two bands of about 10.000 and 6.000 bases, in samples showing fruit pox and sickle leaf respectively. The improvement in molecular methods will facilitate further surveys and lead to more effective procedures to detect and control viruses that affect olive, which is the most important arboreal crop in Greece.

Epidemics of whitefly-borne viruses in tomato and cucurbit crops in Greece: their spread and strategies of control. N.I. KATIS[1], K.E. EFTHIMIOU[1], C.I. DOVAS[2], V.I. MALIOGKA[1], L.C. PAPAYIANNIS[3], A.D. AVGELIS[4], E. KIARAS[5] and A. PARASKEYOPOULOS[6]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, Thessaloniki, Greece. [2]National Agricultural Research Foundation (NAGREF), Crop Protection Institute of Thessaloniki, Thermi 570 01 Thessaloniki, Greece. [3]Agricultural Research Institute, P.O.Box 22016, Nicosia 1516, Cyprus. [4]National Agricultural Research Foundation (NAGREF), Agricultural Research Station of Ierapetra, Crete, Greece. [5]Elanco Hellas S.A.C.I., 335 Messogion St., 152 31 Chalandri, Athens, Greece. [6]Directorate of Agricultural Development and Animal Husbandry, Prefecture of Kyparissia, 245 00 Kyparissia, Greece.*
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Over the last 20 years there have been several serious epidemics of whitefly-borne viruses belonging to the families Geminiviridae (genus Begomovirus) and Closteroviridae (genus Crinivirus) and affecting tomato and cucurbit (cucumber, melon) crops. All diseased crops were also heavily infested by the whitefly. In tomato crops, *Tomato yellow leaf curl virus* (TYLCV) which is transmitted by *Bemisia tabaci* (Gennadius) in a persistent manner, and the semi-persistent viruses *Tomato infectious chlorosis virus* (TICV) and *Tomato chlorosis virus* (ToCV), transmitted respectively by the glasshouse whitefly *Trialeurodes vaporariorum* (Westwood) and *B. tabaci* were detected. An epidemic of TYLCV was first reported in Crete in 2001 (Ierapetra, Tympaki, Chania) and (with a low incidence) in Marathon (Attica). Later, epidemics have occurred with high crop losses in Rhodes (2004), Lakonia (2005), and Kyparissia (2006). In tomato crops with typical yellowing of the plants, TICV and ToCV were detected. TICV was detected in 195 out of 233 (83.6%) samples with yellowing, whereas the incidence of ToCV was relatively low (34/221, 15.38%). In cucurbit crops with yellowing, the semi-persistent viruses *Beet pseudo-yellows virus* (BPYV) and *Cucurbit stunting disorder virus* (CYSDV), vectored by *T. vaporariorum* and *B. tabaci* respectively, were detected. BPYV was detected in the majority of cucumber (65/95, 68.42%) and melon (8/10, 80%) crops, whereas the incidence of CYSDV was lower (cucumber: 30/95, 31.57%; melon: 2/10, 20%). In this work, measures to restrict epidemics of these viruses are also discussed, including the use of virus-free planting material, the establishment of insect proof nets in glasshouse openings and doors, the use of UV-absorbing polyethylene films for glasshouse covering, the use of tolerant cultivars (mainly against TYLCV), the eradication of infected plants, the control of weeds and, lastly, the control of whiteflies.

Transgenic *Nicotiana benthamiana* plants resistant to *Plum Pox Virus*. D. KOTSIS[1], K. KALANTIDIS[2], R. HOLEVA[1], S. TZORTZAKAKI[2], M. TABLER[2] and M. TSAGRIS[1,2]. *[1]Department of Biology, University of Crete, P.O. Box 2208, 71409 Heraklion, Crete, Greece. [2]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, P.O. Box 1385, 71110 Heraklion, Crete, Greece.*
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Plum Pox Virus (PPV) is a serious, insect-borne plant pathogen that affects Prunus species such as peaches, plums and apricots, drastically reducing marketability and yield. Here, we report on an application of a hairpin-mediated RNA silencing technique to obtain resistance to PPV infection in the herbaceous model species *Nicotiana benthamiana*. A synthetic gene producing dsRNA and derived from the virus genome, and a viral-sense RNA-producing gene were constructed and transferred to Agrobacterium binary vector pART27 for plant transformation. For the synthetic hairpin gene, a ca 500 bp region, spanning the conserved 3' prime end of the RNA-dependent RNA polymerase (RDRP) of a Greek PPV isolate, was combined in an antisense orientation with a 1444 bp spacer DNA and a ca. 1500–bp-long PPV cDNA in sense orientation, under the transcriptional control of the Cauliflower Mosaic Virus 35S promoter. Following transcription, a double-stranded RNA molecule with regions specific to PPV and ca. 500 bp long was formed, in order to trigger downregulation of the NIb gene via post-transcriptional gene silencing (PTGS). For the sense gene, the same region of ca. 1500 bp spanning the Nib gene was also inserted under the control of CaMV35S. *N. benthamiana* plants were transformed with *Agrobacterium* harbouring the hairpin and the sense PPV genes. Several independent transformant lines for both

plasmids (sense gene and hairpin gene) were isolated and tested for resistance to PPV first at the T0 generation. Selected T1 transgenic lines were also evaluated for PPV resistance by monitoring the symptoms, or by carrying out DAS-ELISA or PCR. For the sense PPV RNA gene, plants from 4 transformant lines showed no or only very mild symptoms at the T0 generation. This phenotype was verified by an ELISA assay. However, when plants of the same resistant lines were tested at the T1 generation, none of them had remained resistant. From the lines transformed with the hairpin construct, 23 lines (T0) contained plants that did not have symptoms two to six months after infection with PPV. Some of these resistant lines were selected for analysis at the T1 generation (16 resistant, plus 1 susceptible as a control). The segregation ratio of the transgenes in these lines was first evaluated after germination on kanamycin containing agar. From these, seven lines (6 multiple-locus plus one single-locus), were randomly selected for further analysis of the T1 generation. From each line, five plants of the T1 generation were tested which could possibly show segregation of the phenotype. Four of these seven lines showed complete resistance at the T1 generation, as judged by the lack of symptoms and DAS-ELISA assays. Symptoms on susceptible T1 plants appeared 8 days after infection, as in wild-type plants, while resistant plants remained symptomless throughout the testing period. Persistence of resistance is currentlybeing evaluated on the T2 generation in conjunction with challenging the plants with different PPV virus strains.The results obtained so far indicate that RNA silencing hairpin technology in *Prunus* species can provide a novel and agriculturally sustainable approach to obtaining PPV-resistant plants.

Virus, virus-like and other diseases of citrus in Cyprus. A. KYRIAKOU, T. KAPARI–ISAIA, L. PAPAYIANNIS, A. HADJINICOLIS, D. POLYCARPOU, A. HADJINICOLI and N. IOANNOU. *Agricultural Research Institute, P.O. Box 22016, 1516 Lefcosia, Cyprus.*
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Citrus has been cultivated in Cyprus for centuries and virus, virus-like and other diseases are associated with the history of the crop on the island. There are indications that citron was introduced not later than the first century B.C. and lemon, sour orange and sweet orange were certainly cultivated on the island in the 15th century. Jaffa oranges and mandarins were introduced in the 19th century. All these species came from the neighbouring countries of Palestine and Asia Minor, while in the early 20th century several citrus varieties were introduced from South Africa and Israel, at a time when virus diseases were not well known. After the 1960s, virus-free propagating material of several varieties was introduced from California and Florida. As a result, citrus grown in Cyprus is affected by the same diseases that affect citrus in neighbouring countries. One of the viruses affecting citrus is *Citrus tristeza virus* (CTV), which was initially introduced with South African material. A 20-year (1985–2005) survey has shown that *Citrus psorosis virus* and *Citrus impietratura virus* are frequent only in old plantings established using material introduced before the 1960s, and that *Citrus infectious variegation virus* is rare. Stubborn, which is caused by *Spiroplasma citri*, affects about 5% of Valencia and navel oranges, whereas viroids, including *Citrus exocortis, cachexia* or *hop stunt*, bent leaf and viroids III and IV, are widespread, but most trees are symptomless, as sour orange, the main rootstock used, is tolerant to viroids. CTV, the most serious virus, affects about 4% of citrus on the island, and since 1992 it has been controlled by systematic surveys and the uprooting of infected trees wherever feasible. The establishment of a compulsory citrus certification programme during the last decade aims at preventing the reinfection of areas cleared from the disease, as well as the introduction and spread of severe exotic CTV strains and other serious citrus diseases, such as greening and citrus canker. A serious fungal disease, apart from *Phytophthora*, is mal secco, caused by *Phoma tracheiphila*, which infects a number of varieties not only from the tops, but also from the roots of trees, causing quick decline and death of trees, and inflicting heavy losses to citriculture. Lemon and Ortanique tangor are the most sensitive varieties, followed by Minneola tangelo and grapefruit. However, if inoculum pressure is high in an area, all trees become susceptible to the form of the fungus that causes root infection. All lemon rootstocks appear more sensitive to this infection than sour orange.

Analysis of *Potato yellow vein virus* (PYVV) dsRNA extracted from potato plants. I.C. LIVIERATOS[1], E. ELIASCO[2], E.G. MÜLLER[3], R.C.L. OLSTHOORN[4], R.F. SALAZAR[3], C.W.A. PLEIJ[4] and R.H.A. COUTTS[2].
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Potato yellow vein virus (PYVV) is a whitefly (*Trialeurodes vaporariorum*)-transmitted member of the genus *Crinivirus*, infecting potato plants in Peru. Double-stranded RNA from PYVV-infected potato plants was purified and used for cloning and sequencing the genome of PYVV. Nucleotide sequence analysis and hybridization indicated that PYVV possesses a tri-partite genome. The 3'-untranslated regions of all sequenced crinivirus genomes were aligned to show the presence of conserved stem loop and pseudo-knot

structures, for which a role in virus replication has been hypothesized. The nucleotide sequence of two defective dsRNAs was determined to show the arrangement of all three dsRNA genomic fragments.

Detection of viruses belonging to the genera *Closterovirus, Foveavirus* and *Ilarvirus* in cherry orchards in Greece. V.I. MALIOGKA[1], C.I. DOVAS[2], E.V. DROUGKAS[1], K.E. EFTHIMIOU[1] and N.I. KATIS[1]. *[1]Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, P.O.B. 269, 54 124 Thessaloniki, Greece. [2]National Agricultural Research Foundation (NAGREF), Plant Protection Institute of Thessaloniki, P.O.B. 324, Thermi 570 01, Thessaloniki, Greece.*
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Cherry is susceptible to a number of viruses belonging mainly to the genera *Ilarvirus, Nepovirus, Closterovirus, Trichovirus* and *Foveavirus*. To study virus incidence in cherries and to evaluate molecular detection methods, 59 samples were randomly collected from five major cherry-cultivating areas. Samples were initially tested using generic nested RT-PCR assays for the detection of viruses belonging to the genera *Closterovirus, Foveavirus* and *Ilarvirus*. Some of the samples that were positive with the generic RT-PCR assays were sequenced and after comparison with already published homologous sequences they were identified as *Little cherry virus* 1 (LChV-1), *Cherry necrotic rusty mottle virus* (CNRMV) and *Prune dwarf virus* (PDV). For their specific detection, and for the additional detection of *Prunus necrotic ringspot virus* (PNRSV) and *Apple mosaic virus* (ApMV), specific downstream primers were designed that were used in the respective nested PCR assays. The results showed a high incidence of PDV (46%), followed by CNRMV (36%), LChV-1 (32%) and PNRSV (10%), while ApMV was not detected. Interestingly, no LChV-1 or CNRMV infection was found in the 9 clostero- or foveavirus-positive samples. These isolates are currently being characterized.

Ampeloviruses phylogenetically related to GLRaV-5: genetic relationship and detection. V.I. MALIOGKA[1], C.I. DOVAS[2] and N.I. KATIS[1]. *[1]Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, P.O.B. 269, 54 124 Thessaloniki, Greece. [2]National Agricultural Research Foundation (NAGREF), Plant Protection Institute of Thessaloniki, P.O.B. 324, Thermi 570 01, Thessaloniki, Greece.*
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Grapevine leafroll disease (GLD) is one of the most important graft-transmissible diseases of grapevine: At least 9 serologically different viruses of the family *Closteroviridae* (GLRaV-1, to -9) are associated with this disease. The genus *Ampelovirus* of this family includes GLRaV-1,-3 and -5, while GLRaV-4 and -6 are tentative members. In this study, partial nucleotide sequences of the HSP70 coding gene of already known (GLRaV-4,-5,-6) and two putative new viruses, (GLRaV-10Gr and GLRaV-11Gr, Greek isolates), members of the *Closteroviridae,* were determined. Phylogenetic analysis clustered GLRaV-4,-5,-6,-9, -10Gr and -11Gr into one subgroup. These viruses had a significantly lower genetic distance among them than the other ampeloviruses (GLRaV-1 and -3). Members of this subgroup seem to share a common evolutionary history which is probably related to a) the genetic diversity observed in a population of variants of an RNA virus (quasispecies) existing in a perennial host, b) the efficient transmission of all the virus variants through the vegetative propagation of grapevine, and c) the lack of a strong selective agent e.g. an effective insect-vector. Interestingly, this subgroup includes all the recently characterised *Closteroviridae* species identified in grapevine. To detect and further study the genetic relationship between known and unknown viruses belonging to this subgroup, a molecular method was developed that included an RT-PCR for the generic detection of *Closteroviridae* species using primers that target the HSP70 coding gene, followed by nested PCR for the generic detection of all members of the GLRaV-5 phylogenetically related subgroup. This method was also applied to generate data of homologous nucleotide sequences from different variants of this subgroup. These new sequences were used to design new nested primers for the specific detection of GLRaV-4, -5, -6, -10Gr and -11Gr. These primers were successfully applied in the respective nested PCR assays.

Generic detection, species differentiation and genus demarcation of Ilarviruses using a generic ramped-annealing nested RT-PCR. V.I. MALIOGKA[1], C.I. DOVAS[2] and N.I. KATIS[1]. *[1]Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, P.O.B. 269, 54 124 Thessaloniki, Greece. [2]National Agricultural Research Foundation (NAGREF), Plant Protection Institute of Thessaloniki, P.O.B. 324, Thermi 570 01, Thessaloniki, Greece.*
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In this study, a generic ramped-annealing nested RT-PCR was developed, that permitted the simultaneous detection and rapid characterization of members of the *Ilarvirus* genus. The method involves a one-step RT-PCR in which a pair of degenerate primers amplifies a 381 bp part from the polymerase gene of the ilarviruses, followed by a nested PCR amplification that increases detection sensitivity. The sensitivity of the method was further enhanced by applying a ramped-annealing thermocycling step in both the first RT-PCR and the subsequent nested PCR. The 371 bp nested amplicons can be sequenced directly,

without cloning, to obtain initial sequence information on *Ilarvirus* genomes or they can undergo restriction-enzyme analysis for fast identification of already known virus species. Neighbour–joining and maximum parsimony trees were calculated using published homologous partial amino acid sequences from different species that corresponded to the 371 bp nested amplicon, and taxonomic relationships between members belonging to *Ilarvirus* and the other genera of the *Bromoviridae* family were successfully established. The specific partial polymerase sequence is phylogenetically informative, discriminating the different genera within the *Bromoviridae* family. The method was successfully applied to eight different *Ilarvirus* species that occur in Europe.

Inspection of the phytosanitary status of vineyards in the VQPRD region of Korinthia and Argolida. V.I. MALIOGKA[1], P.A. SAINIS[1], A.TH. CHAROU[1], L. LOTOS[1], D. DIMOU[3], C.I. DOVAS[2] and N.I. KATIS[1]. *[1]Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, P.O.B. 269, 54 124 Thessaloniki, Greece. [2]National Agricultural Research Foundation (NAGREF), Plant Protection Institute of Thessaloniki, P.O.B. 324, Thermi, 570 01 Thessaloniki, Greece. [3]Directorate of Agricultural Development, Prefecture of Argolis, 21 100 Nafplion, Greece.*
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Grapevine is infected by more than 55 viruses belonging to 20 genera with the most important belonging to the genera *Nepovirus*, *Closterovirus*, *Foveavirus* and *Vitivirus*. In Greece and more specifically in the VQPRD (Vin de qualité produit dans une région déterminée – Quality wine produced in a well-defined region) region of Korinthia (this zone includes 12 areas in Nemea) and Argolida (2 areas) which is of great viticultural importance, information on the phytosanitary status of vineyards is limited. For this reason an extensive sampling was carried out in vineyards of the region during the autumn of 2005. A total of 150 samples were collected from 13 cultivars (44 vineyards), showing characteristic virus-like symptoms such as leafroll, reddening and/or yellowing of the leaves, double node fasciations, shortening of the internodes and abnormal branching. The samples were tested either serologically (ELISA) or by molecular assays (RT-PCR) for already known viruses (GVA, GVB, GFLV, GFKV, GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-5, GLRaV-6, GLRaV-7) as well as for two putatively new viruses (GLRaV-10Gr, GLRaV-11Gr) recently isolated from Greek grapevine varieties and which seem to be related to the grapevine leafroll virus. The results showed high infection rates for GVA (88.6%), GFKV (53.3%), and GLRaV-3 (40.6%), followed by infections with GLRaV-1 (16.6%), GLRaV-4 (12.7%), GLRaV-2 (9.3%), GLRaV-5 (8.7%), GLRaV-7 (8%), GLRaV-11Gr (67%), GLRaV-10Gr (4%), GLRaV-6 (2%), and GFLV (1.4%), while GVB was not detected. In most cases mixed infections of grapevines were found, which included from two (35.4% of the total number of samples) to as many as eight (2%) different viruses in the same vine sample.

Phylogeny and genetic variability of *Apple stem pitting virus* (ASPV) in Greece. M.M. MATHIOUDAKIS[1], C.I. DOVAS[2] and N.I. KATIS[1]. *[1]Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, 54 124 Thessaloniki, Greece. [2]National Agricultural Research Foundation (NAGREF), Plant Protection Institute, Thermi 57 001, Thessaloniki, Greece.*
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The genetic variability of the capsid protein (CP) encoding gene from ASPV isolates obtained during a survey of the sanitary status of pome fruits in Greece was studied. For this purpose, different specific primers were designed to amplify the CP gene and part of its 3' untranslated genomic region from various ASPV isolates. The specific PCR products ranged from 1214–1374 bp, depending on the isolate and the primer pair used. A preliminary test for genetic variability and to select diverse isolates was performed by RFLP analysis using three restriction enzymes, whereas for phylogenetic analysis the amplicons were cloned and sequenced. The CP gene was amplified in 104 of the 164 isolates tested, and 14 isolates from different hosts and geographical regions, and showing different digestion profiles in the RFLP analysis, were selected for sequencing. Alignment of the amino acid sequences showed a high percentage of variability in the CP gene. Phylogenetic analysis generated using the CP gene nucleotide sequences and based on the Maximum likehood method revealed that apple isolates were clustered in separate monophyletic groups, whereas pear and quince isolates were clustered in one big mixed group. The close phylogenetic relationship of pear and quince isolates is probably due to the fact that quince is used as a pear rootstock and thus transmission of ASPV isolates between the two hosts can easily occur. There was no relationship between the geographical origin of the isolates and their phylogenetic clustering.

Incidence of viruses in tree tobacco (*Nicotiana glauca*) in Greece. M.M. MATHIOUDAKIS, K. EFTHIMIOU and N.I. KATIS. *Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, 54 124 Thessaloniki, Greece.*
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In 2002–2003 a survey was conducted to determine the incidence of viruses infecting tree tobacco (*Nicotiana glauca*). A total of 125 samples from six areas were tested serologically (ELISA) for already known viruses of *N. glauca* (CMV, TMGMV, TMV) and other viruses (AMV, PaMMV, PMMoV, PVY, RMV, ToMV, TSWV) that infect Solanaceae species

but not specifically tree tobacco. The results showed high incidences of CMV (48%), TMGMV (19.2%) and TMV (4.8%). The percentages of mixed infections were 11.2% (CMV, TMGMV), 2.4% (TMGMV, TMV) and 0.8% (TMGMV, TMV, CMV). The serological tests for the two tobamoviruses were confirmed by mechanical inoculations on *N. glutinosa* and with a nested RT-PCR, after spotting of plant sap on nylon membranes, followed by RFLP analysis to differentiate the *Tobamovirus* species. An IC-RT-PCR application confirmed the results of the serological tests for CMV detection, and in 53.5% of the CMV-infected samples, satellite RNA (CARNA-5) was also detected. RFLP analysis found that 42 isolates belonged to the CMV-I subgroup and one to the CMV-II subgroup. This is the first report of TMV, CMV-I and CARNA-5 in tree tobacco in Greece.

Phylogenetic analysis of *Apple chlorotic leaf spot virus* (ACLSV) and the host effect in the population structure of the virus. M.M. MATHIOUDAKIS and N.I. KATIS. *Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, 54 124 Thessaloniki, Greece.*
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Apple chlorotic leaf spot virus (ACLSV) has a wide host range that includes a number of pome and stone fruit species. It has biological variability, and it causes various diseases with an economic impact, such as cherry fruit necrosis, plum bark split and plum pseudopox. Many studies have shown that the coat protein (CP) encoding gene has high genetic variability. To study the genetic variability of the virus in Greece, 9 apple and pear isolates, obtained during a survey of the health status of pome fruits, were used. A one-tube RT-PCR assay using specific primers which amplified a 670 bp product covering 85% of the CP gene was used. This region is phylogenetically informative for the study of relationships among ACLSV isolates from various hosts. Nucleotide sequence comparison showed the existence of high genetic variability in the 3' non coding region, whereas phylogenetic analysis based on the neighbour–joining method clustered the isolates into 4 groups. Two groups included stone fruit isolates, whereas the other two groups included mainly pome fruit isolates. The results showed that the population structure of ACLSV in cultivated hosts was influenced by the host genotype (Maloidea vs Prunoidea), but that occasional virus transmission between heterogeneous groups may also exist. In contrast, geographical origin does not seem to play any role in the genetic variability of ACLSV.

Development of a nested RT-PCR assay for the detection of ASPV and its association with quince fruit deformation disease. M.M. MATHIOUDAKIS[1], V.I. MALIOGKA[1], CH.I. DOVAS[2], N. BARBAYIANNIS[3] and N.I. KATIS[1]. *[1]Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, 54 124 Thessaloniki, Greece. [2]National Agricultural Research Foundation (NAGREF), Plant Protection Institute, Thermi, 57 001 Thessaloniki, Greece. [3]Aristotle University of Thessaloniki, School of Agriculture, Soil Science Laboratory, 54 124 Thessaloniki, Greece.*
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In 2004 symptoms of quince fruit deformation (QFD) disease were found in several quince orchards of central and northern Greece. To investigate the causal agent of the disease, 22 samples were collected from trees showing typical QFD symptoms and they were tested for ACLSV, ASPV and boron deficiency. Due to the high genetic variability of ASPV, the molecular detection of this virus is unreliable. For the specific detection of all ASPV isolates/strains, a nested RT-PCR using degenerate primers targeting domains of the RdRp encoding gene was therefore developed. For primer selection, a multiple alignment of homologous published amino acid sequences of the RdRp of different foveaviruses, including ASPV strains, was carried out. In the first RT-PCR, degenerate primers for the generic detection of foveaviruses were used, whereas in the nested PCR, primers specific for ASPV detection were used. It was found that simple sample preparation by spotting plant sap on nylon membranes was just as reliable as when total RNA was used as a template. To detect ACLSV, a one-tube RT-PCR was performed using specific primers. The results showed that neither ACLSV nor boron was related to QFD, whereas ASPV always occurred in quince plants with typical QFD symptoms but was absent in apparently healthy trees. The molecular technique developed can be used as a rapid and reliable diagnostic tool for ASPV with a wide detection range for different strains.

Occurrence of *Apple chlorotic leaf spot virus* (ACLSV) and *Apple stem pitting virus* (ASPV) in cultivated, ornamental and wild *Rosaceae* species in Greece. M.M. MATHIOUDAKIS, V.I. MALIOGKA and N.I. KATIS. *Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, 54 124 Thessaloniki, Greece.*
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During 2005, a survey was conducted in Greece to determine the occurrence of *Apple chlorotic leaf spot virus* (ACLSV) and *Apple stem pitting virus* (ASPV) in apple and pear orchards, as well as in samples from ornamental and wild species of the *Rosaceae*. A total of 256 samples (96 from pear, 110 from apple, 40 *Cydonia japonica*, 7 *Pyrus amygdaliformis*, 1 *Pyrus calleryana* and 2 apple rootstocks [EM9, MM106]) was randomly collected from 19 areas of Greece. ACLSV and ASPV were detected with already known molecular diagnostic techniques using specific and degenerate primers. The results showed a high incidence of ACLSV (pear, 16%; apple 74%) and ASPV (pear, 50%; apple 98%) in cultivated species, and

the viruses were also detected in the ornamental and wild species. Mixed infections with both viruses occurred in 9.4% of pear samples and 62.7% of apple samples . The wide dissemination of ACLSV and ASPV in pome fruit plantations, suggests that certification schemes should be set up, as this is the most efficient method to combat viral diseases in fruit trees. Interestingly, both viruses occurred in ornamental and wild species, and their possible role as virus reservoirs in nature should be investigated in future. This is the first relatively large-scale survey of the incidence of ASPV in various host species in Greece.

Tomato yellow leaf curl disease in Greece and Cyprus. L.C. PAPAYIANNIS[1], A.D. AVGELIS[2], N. IOANNOU[1] and N.I. KATIS[3]. *[1]Agricultural Research Institute, P.O.Box 22016, Nicosia 1516, Cyprus. [2]Plant Protection Institute, National Agricultural Research Foundation (NAGREF), 71003 Heraklion, Crete, Greece. [3]Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 54 124 Thessaloniki, Greece.*
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Tomato yellow leaf curl virus (TYLCV) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV) are two *Begomovirus* species (family Geminiviridae) causing tomato yellow leaf curl disease (TYLCD) on tomato (*Lycopersicon esculentum*) in the Mediterranean basin. In Cyprus, TYLCV was first reported in 1985 and has become the most important limiting factor in greenhouse and open field tomato production. In Greece, TYLCV was initially reported in Crete and later in Lakonia (southern Peloponnese). During the summer of 2005, an epidemic broke out in greenhouse tomato crops in the southern Peloponnese and Crete, with plants showing severe stunting, reduced leaf size and curling, yellowing, shortened internodes and a bushy appearance, symptoms that could easily be attributed to tomato yellow leaf curl disease. High populations of the whitefly vector *Bemisia tabaci* (Gennadius) also occurred in both greenhouse and field tomatoes. Symptomatic plants were collected from Greece (areas in the southern Peloponnese, Glykovrysi, Agios Ioannis, Neapoli), Crete (Mires, Tympaki, Ierapetra), Rhodes (Kamiros, Mantriko, Skala) and Cyprus (Nicosia, Limassol, Famagusta, Larnaca and Paphos). DNA was extracted from the leaves of 236 symptomatic plants and a capsid protein (CP) gene 580 bp in size was amplified by polymerase chain reaction (PCR). Restriction fragment length polymorphism (RFLP) analysis (Ava II) of the PCR product, produced a 360, 150 and 68 bp pattern from 26 isolates collected from the Peloponnese, and from Mires and Tympaki (Crete) indicating the existence of TYLCSV. All isolates from Ierapetra (Crete) and from Cyprus produced a TYLCV pattern (302 and 277 bp). Amplified DNA from several TYLCSV isolates was cloned and sequenced. All sequences were identical (EMBL no. AM 259652) and showed 100% nucleotide identity to a TYLCSV isolate from Sicily (EMBL no. Z28390). These results show that species of both TYLCV and TYLCSV coexist on Crete, whereas only TYLCSV occurs in the Peloponnese. To our knowledge this is the first report of TYLCSV in Greece. In Cyprus TYLCV is the only *Begomovirus* species infecting tomato crops.

Characterization of Citrus tristeza virus (CTV) isolates from Cyprus. L. PAPAYIANNIS, T. KAPARISAIA and A. KYRIAKOU. *Agricultural Research Institute, 1516, Nicosia, Cyprus.*
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Citrus tristeza virus (CTV) was first reported in Cyprus in 1968. In cooperation with the Greek Department of Agriculture, a project was undertaken in 1992 to control the disease caused by this virus. The project mainly involved the systematic survey of all citrus trees and the removal of infected trees wherever feasible. So far more than 600,000 trees have been inspected using serological techniques. Of the trees, 4% were found to be infected. In the light of these findings, it was decided to carry out the molecular characterization and strain differentiation of several CTV isolates, selected from the main citrus-growing areas of the island on the basis of a different symptomatology on the host plants. Twenty-five isolates, originating in different parts of the island and producing a wide diversity of symptoms, were tested. Symptoms on field trees ranged from inconspicuous to twig die back, as well as the decline and death of sweet orange or grapefruit trees on sour orange rootstock. Similarly, on Mexican lime, symptoms ranged from barely noticeable leaf vein clearing to vein corking, stem pitting and plant stunting. The coat protein (CP) gene of the virus isolates was analyzed and characterized. The techniques used were single-strand conformation polymorphism (SSCP) to differentiate the isolates prior to cloning and sequencing and Restriction Fragment Length Polymorphism (RFLP) to compare the local CTV isolates with known isolates/strains. In addition, amplicons of RT-PCR were cloned in a plasmid vector and sequenced. Results showed that four of the analyzed isolates had a high homology with isolate B249 from Africa. Five isolates showed a high similarity with the Portuguese isolate 28C. Two CTV isolates causing decline were similar to a quick-decline strain from Florida. Finally, the other CPG sequences from Cyprus were very similar to published isolates from Africa and Portugal. These results provide molecular information about the occurrence of mild, stem-pitting and quick-decline inducing isolates of CTV in Cyprus. Quick-decline isolates are of special concern because of the wide use of the susceptible sour orange rootstock on the island.

Occurrence of grapevine viruses in Cyprus. L.C. PAPAYIANNIS[1], T. KAPARI[1], G. MINAS[1], S. SAVVIDES[1], N. IOANNOU[1], C.I. DOVAS[2] and N.I. KATIS[3]. *[1]Agricultural Research Institute, P.O.B. 22016, Nicosia 1516, Cyprus.*

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Grapevine is a very important crop in Cyprus, covering more than 19000 ha, 90% of which yield wine grapes and 10% table grapes. In Cyprus several viral diseases of grapevine have been recorded and such diseases are one of the main threats to the quality and yield of grapevines. Recently, a survey was conducted by the Agricultural Research Institute of Cyprus to study the incidence and distribution of grapevine viruses in basic and other grapevine plantations of Cyprus. A total of 420 randomly selected grapevine plants were collected from plantations in the areas of Orites, Achelia and Saittas during 2005–2006. Results showed that GLRV-3 was the most widespread virus in grapevines in Cyprus, and occurred in almost all introduced and local varieties with a high incidence. GLRV-3 incidence was very high in the Akhelia area (52%) whereas its incidence in Saittas and Orites was low (18 and 9% respectively). By contrast, GLRV-6 occurred only in the Akhelia area but was very rare (only 3/175 plants). Generic RT-PCR revealed virus species belonging to the genus *Viti-* and/or *Foveavirus* in 46 samples. GLRV-1, GFLV, GFkV and ArMV were not detected in the samples tested.

Incidence of viruses infecting tomato crops in Cyprus. L.C. PAPAYIANNIS[1], A. SAVVIDES[2], K. CHATZIAFKSENTIS[2], T. KAPARI[1], C. DOVAS[3] and N.I. KATIS[2]. [1]*Agricultural Research Institute, P.O. Box 22016, Nicosia 1516, Cyprus.* [2]*Aristotle University of Thessaloniki Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, Thessaloniki 54006, Greece.* [3]*National Agricultural Research Foundation (NAGREF), Plant Protection Institute of Thessaloniki, P.O.B 324, Thermi 570 01, Thessaloniki, Greece.*
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A survey was carried out during 2005–2006 to determine the identity and prevalence of viruses infecting tomato crops in Cyprus. A total of 1380 samples of greenhouse and open-field tomatoes were collected from many areas of 5 districts all over the island. Virus identification was by Enzyme linked immuno-sorbent assay, (ELISA) for the detection of *Tomato mosaic virus*, (ToMV), *Cucumber mosaic virus*, (CMV), *Potato virus Y*, (PVY), *Pepino mosaic virus*, (PepMV), *Tomato spotted wilt virus*, (TSWV) and *Tomato ring spot virus*, (ToRSV). *Tomato yellow leaf curl virus*, (TYLCV) was detected by Polymerase Chain Reaction (PCR). Tomato plants with yellowing symptoms were subjected to a Reverse Transcription (RT) PCR assay for the multiplex detection of *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV). TYLCV was the most common virus, with an incidence of 52% in all samples. PVY, TSWV and ToMV were detected in 22%, 15% and 1% of samples respectively. ToCV was detected for the first time on the island, on 25 tomato plants. CMV ToRSV and PepMV were not detected in any solanaceous crop during this survey.

Detection of *Cherry leafroll virus* (CLRV) from walnut (*Juglans regia* L.) in Greece. A.T.P. SCLAVOUNOS[1,3,4], P.E. KYRIAKOPOULOU[2], M.C. HOLEVA[1], P. KOSTAS[3] and A.E. VOLOUDAKIS[1]. [1]*Laboratory of Plant Physiology, Department of Agricultural Biotechnology, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece.* [2]*Laboratory of Plant Pathology, Department of Crop Sciences, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece.* [3]*ELGA, Km 2 New National Rd, Lamia, Athens, Lamia 35100, Greece.* [4]*ELGA, Australias 88 and Kanellopoulou, Patra 26442, Greece.*
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Over the last six years, severe ringlike mosaic symptoms have been found in adult walnut trees (*Juglans regia* L.) in Epidaurus and in some Eleia regions (Peloponnese), and in the summer of 2005, in walnuts of the Timfristos area (central Greece), and in 2006 in the Mycenae area (Peloponnese). The symptoms indicated that the infection was caused by *Cherry leafroll virus* (CLRV). To detect the virus, samples from symptomatic trees suspected to harbour the virus were collected from the areas of Timfristos and Mycenae, and used as starting material in the beginning of May and of July 2006 respectively. The presence of CLRV was confirmed by ELISA and with RT-PCR and IC-RT-PCR using two sets of primers (a set reported in the literature and a set designed for the present study). The PCR products obtained were the expected ones of 350 bp and 700 bp for the two primer sets, respectively. It is important to note that the walnut trees in the area of Timfristos are grown from seed and are found in a forest-like area, isolated from any other cultivated plant species. These trees are approximately 50 years old, they show a reduction in yield, necrosis of the pericarp and sometimes shrinkage and blackening of the endocarp. It also needs to be mentioned that repeated attempts to isolate the virus using RT-PCR, with samples obtained during March-April 2006, failed, indicating that this period was unsuited to detect CLRV. This virus is economically important in walnut plantations of America and Europe. As far as we know, this is the first report of CLRV in walnut in Greece.

The nucleotide sequence of *Strawberry chlorotic fleck associated virus*, a novel *Closterovirus*. I.E. TZANETAKIS[1] and R.R. MARTIN[2]. [1]*Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA.* [2]*Horticultural Crops Research Laboratory, USDA-ARS, Corvallis, OR 97330, USA.*
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Chlorotic fleck, a strawberry disease caused by a graft-

and aphid-transmissible agent, was identified in the early 1960s. The mode of transmission suggested that a virus was associated with the symptoms. Double-stranded RNA was extracted from a chlorotic fleck disease positive clone, and a novel closterovirus was identified in the plant, as well as other, already known strawberry viruses. Sequence analysis of the virus revealed a close relationship between the novel virus and members of the *Closterovirus* genus, the aphid-transmitted closteroviruses, in line with the aphid transmissibility of the chlorotic fleck agent. The complete nucleotide sequence of the virus was determined. The genome organization is similar to that of *Beet yellows virus* but its sequence is most closely related to *Citrus tristeza virus*. Several hundred plants have been tested for this virus in areas of high aphid incidence, with only one plant securely infected with it. We are currently testing several aphid species for their ability to transmit the virus.

How many viruses are involved in *Rubus* mosaic disease? Three novel viruses isolated from raspberry. I.E. TZANETAKIS[1] and R.R. MARTIN[2]. [1]*Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA.* [2]*Horticultural Crops Research Laboratory, USDA-ARS, Corvallis, OR 97330, USA.*
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One raspberry accession that originated in Scotland caused severe symptoms including leaf mottling, epinasty and apical necrosis when grafted onto black raspberry (*Rubus occidentalis*) cv. Munger indicators. The symptoms classified the plant as a *Rubus* mosaic disease positive. *Rubus* mosaic disease was first recognized in the 1920s and is believed to be caused by various combinations of four or more viruses some of which are still understudied in the molecular level. Double-stranded RNA (dsRNA) was extracted and cloned, disclosing the presence of three novel viruses. Phylogenetic analysis indicated that the first virus clusters with the *Closterovirus* genus, which represents the aphid-transmitted members of the *Closteroviridae* family. The second virus is related to several members of the *Flexiviridae* that infect rosaceous hosts (*Cherry green ring mottle virus* and *Cherry necrotic rusty mottle virus*). The third virus is related to a group of insect-infecting picorna-like viruses, similar to the recently identified Strawberry latent virus. Detection protocols have been developed for all three viruses. The closterovirus is the most widespread of the three and the complete nucleotide sequence of the virus has been acquired. We are presently working with scientists at SCRI, Scotland, to determine if the closterovirus is one of the previously identified virus-like agents in *Rubus*.

Molecular characterization and epidemiology of Mint virus 2, a new member of the genus *Vitivirus*. I.E. TZANETAKIS[1], J.D. POSTMAN[2] and R.R. MARTIN[3]. [1]*Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA.* [2]*National Clonal Germplasm Repository, USDA-ARS, Corvallis, OR 97333, USA.* [3]*Horticultural Crops Research Laboratory, USDA-ARS, Corvallis, OR 97330, USA.*
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Three viruses have been identified in the ornamental mint clone *Mentha* × *gentilis* L. 'Variegata'. Several 'Variegata' clones were ordered from nurseries in the United States to identify a virus consistently associated with symptoms. One clone did not show the typical symptoms, and steps were taken to identify the agents that may be involved in the novel symptomatology. This communication focuses in one of the three viruses found in the off-type clone, named hereafter as Mint virus 2 (MV2). The majority of the genome has been sequenced and phylogenetic analysis revealed the close relationship of the virus to members of the genus *Vitivirus*. Purification of the virus proved recalcitrant and only a few virions were visible under the electron microscope. MV2 shares many features with Grapevine viruses A and B, including several epitopes in the coat protein genes, but antibodies against the two grapevine viruses did not cross-react with MV2 in ELISA assays. The virus can be transmitted with the mint aphid (*Ovatus crataegarius*) in the presence of the closterovirus Mint virus 1, while transmissions assays from MV2 single infected plants are under way.

Blackberry virus Y: a putative member of a new genus in the *Potyviridae*. I.E. TZANETAKIS[1], J. SUSAIMUTHU[2], R.C. GERGERICH[2] and R.R. MARTIN[3]. [1]*Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA.* [2]*Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA.* [3]*Horticultural Crops Research Laboratory, USDA- ARS, Corvallis, OR 97330, USA.*
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Blackberry yellow vein disease causes devastating symptoms and losses in blackberry in the south and southeastern United States. Blackberry yellow vein associated virus (BYVaV) has been identified as the putative agent of the disease but the identification of latent infections of BYVaV has led to the investigation of additional agents involved in symptomatology. A potyvirus, here named Blackberry virus Y (BVY) has been identified in blackberry plants also infected with BYVaV. The complete nucleotide sequence revealed that BVY is the largest potyvirus sequenced to date. BVY is the first potyvirus to encode an AlkB domain. The virus has minimal sequence similarities with known members of the family and should be considered a member of a novel genus in the *Potyviridae*. Detection protocols developed verified the presence of the virus in several blackberry plants, but it is not the causal agent of blackberry yellow vein disease since several symptomatic plants were found that were not infected with the virus.

Study of a *Plum pox virus* (PPV) rendered mild by low temperature treatment. N. VASSILAKOS, C. VARVERI and A. TZIMA. *Benaki Phytopathological Institute, 8 Stefanou Delta St., 145 61 Kifissia, Attica, Greece.*
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Plum pox virus (PPV), the causal agent of sharka disease in stonefruits, is one of the main diseases of stonefruits worldwide. Variations in PPV (PPV-D-GR) virulence were naturally induced in *Nicotiana benthamiana* plants by keeping the plants at 15°C for at least 15 days. A total of 802 single-lesion isolates were screened for mildness and 20 mild isolates (2.5% variation) were assayed for cross-protection efficiency towards the severe PPV-M isolate occurring in Greece. The mild isolate PPV-B2 was selected for further study as it caused only mild symptoms in *N. benthamiana*, no symptoms in *N. clevelandii*, and was 100% effective in cross-protection against the severe PPV-M-GR. PPV-B2 multiplied less than PPV-D-GR in both *N. clevelandii* and *N. benthamiana* and it was not transmitted by aphids. Sequence comparison between PPV-B2, the precursor isolate in HC-Pro, P3-6K$_1$ and the coat protein genes revealed two substitutions in the HC-pro and two in the P3 protein. The role these substitutions in virus pathogenicity must be further examined in both herbaceous and woody hosts.

Pilot survey of citrus mother trees in Greece to detect viruses and viroids. G.E. VIDALAKIS[1], I.N. BOUBOURAKAS[2], A.E. VOLOUDAKIS[3], T. AGORASTOU[4], G. MAGRIPIS[4] and P.E. KYRIAKOPOULOU[2]. [1]*University of California Riverside, Department of Plant Pathology, Riverside, CA 92521 USA.* [2] *Agricultural University of Athens, Department of Plant Production, Laboratory of Plant Pathology, 11855 Athens, Greece.* [3]*Agricultural University of Athens, Department of Agricultural Biotechnology, Laboratory of Plant Physiology, 11855 Athens, Greece.* [4]*Poros Arboricultural Station, 18020, Poros, Troizinia, Greece.*
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Since the beginning of 2005 and under the research program Pythagoras II (2005–2006) "Survey, detection and study of citrus viruses and viroids in Greece", a preliminary study of citrus mother trees in the Poros Arboricultural Station (PAS) was undertaken for the detection of citrus viruses and viroids. We selected twelve orange, ten mandarin, six lemon and two grapefruit varieties from the mother-tree collection of PAS to be checked for the viruses *Citrus psorosis ophiovirus* (CPsV), *Citrus tatter leaf capillovirus* (CTLV), *Citrus variegation ilarvirus* (CVV), *Citrus crinkly leaf ilarvirus* (CCLV) and *Citrus leaf rugose ilarvirus* (CLRuV) and the viroids *Citrus viroid* I (CVd-I), *Citrus viroid* II (CVd-II), *Citrus viroid III* (CVd-III), *Citrus viroid IV* (CVd-IV) and *Citrus exocortis viroid* (CEVd), using a combination of biological indexing and laboratory diagnostic techniques (ELISA, RT-PCR, imprint hybridization) with tissue from the grafted indicators. The bio-indexing trials did not record any symptoms in Dweet tangor, sour orange or Carrizo citrange, but only leaf epinasty and vein corking in Etrong citron. No virus was detected in any of the varieties tested, but some viroids were identified. CEVd was the most frequently found (16/22), followed by CVd-I and CVd-III (11/22), while CVd-II was detected in only 4 out of 12 orange varieties. CVd-IV was not detected. Eight out of ten orange varieties tested were found mix-infected with CEVd, CVd-I, CVd-II and CVd-III, while all lemon and grapefruit varieties were mix-infected with CEVd, CVd-I and CVd-III. The mandarin varieties were usually infected with CVd-I and CEVd. The orange varieties Tarocco and Lane late and the mandarin varieties Nova, Clementine sra-63 and Clementine of Poros were found free from viruses and viroids. These results show the need to expand the program to additional mother trees of PAS and the need for sanitation of the elite citrus varieties in order to obtain healthy citrus propagation material in Greece.

Non-parasitic diseases

Further investigation of rind-spotting of 'Clementine' mandarin in maturity. A. ASSIMAKOPOULOU[1], C. FASSEAS[2], K. ELENA[3] and D. DIMOU[4].[1]*Ministry of Agriculture, District Laboratory of Agricultural Advisory Service and Fertilizer Analysis, 204 00 Xylokastro, Greece.* [2]*Agricultural University of Athens, Laboratory of Electron Microscopy, Iera Odos 75, 118 55 Botanikos, Athens, Greece.* [3]*Benaki Phytopathological Institute, Laboratory of Mycology, 8 S. Delta St., 145 61 Kifissia, Greece.* [4]*Ministry of Agriculture, Direction of Agricultural Development, 211 00 Nafplion, Argolida, Greece.*
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Severe damage to 'Clementine' mandarins in the area of Assini in Argolis was investigated for two successive years. Irregularly-shaped necrotic spots on the rinds of mandarins, mainly at the fruit stylar-ends, were observed from color-break till harvest time. In the first year of study, in order to investigate whether there was any correlation between the rind-spotting and the inorganic nutrition of the fruits, concentrations of N, P, K, Ca, Mg, Fe, Mn, Zn, Cu and B were determined in mandarin rinds with and without symptoms in several orchards of the Assini area. Significant differences were found in the concentration of some nutrients between damaged and healthy rinds, but these differences varied from orchard to orchard. Significant differences were also found between trees in any given orchard, regardless of the presence of symptoms. As regards relevant results in the second year, after the sampling

of mandarins was modified, no significant differences were found in the concentration of the majority of the nutrient elements tested. The inorganic nutrition of fruits from both years of study did not have a constant effect on symptom performance. Using an electron scanning microscope we found cracks in the cuticle and the external strata of the rind of injured fruits; these cracks were not visible macroscopically, and the oil glands remained intact. On the spots of the diseased fruits there were also secondary infections caused by fungi, mainly of the genera *Alternaria, Cladosporium* and *Penicillium*. These fungi caused rot, followed by decay of the whole fruit. Consequently, the results of the chemical analyses, microscopic inspection, as well as information from growers, showed that this disorder is due to sudden changes of climatic conditions such as temperature and relative humidity, and not to a nutritional imbalance.

Nutritional status of the grape wine variety Agiorgitiko in relation to the rootstock and the trellis system. A. ASSIMAKOPOULOU and CH. TSOUGRIANIS. *Ministry of Agriculture, District Laboratory of Agricultural Advisory Service and Fertilizer Analysis, 204 00 Xylokastro, Greece.*
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The effect of two rootstocks (41B and 110R) and two vertical shoot-positioned trellis systems on the nutritional status of the grape wine variety Agiorgitiko was studied; the wine 'Nemea' (V.Q.P.R.D.) is produced from that variety. The first trellis system consisted of three single wires, and the second of one single plus two pairs of foliage wires, resulting in two different leaf areas. At full bloom, leaf and soil samples were collected from several vineyards in the area of Nemea. The leaf blades were separated from the petioles just after the leaves were collected and they were analyzed separately to determine their concentrations of N, P, K, Ca, Mg, Fe, Mn, Zn, Cu and B. According to ANOVA, the main effect of the rootstock was that vineyards grafted on 41B, as compared with those on 110R, presented: a) significantly higher K concentrations in the petioles, b) significantly lower levels of N, also in the petioles, and c) significantly lower levels of B in the leaf blades. The main effect of the trellis system was that vineyards with the two pairs of foliage wires, as compared with the single wires, had: a) significantly higher N concentrations in the petioles, b) significantly lower levels of P, also in the petioles, and c) significantly lower Mn, Zn and B concentrations in the leaf blades. The correlations between the concentrations of the majority of nutrient elements in the blades and the relevant nutrients in the petioles were significant, except with Zn and B. The correlation coefficients were for N (r=0.78***), P (r=053**), K (r=0.48*), Ca (r=0.64**), Mg (r=0.64**), Fe (r=0.66**), Mn (r=0.64**) and Cu (r=0.94***).

Ozone measurements and its effects on bioindicators and chlorophyll content of Greek fir on Mount Taygetos. D. VELISSARIOU and I. SALMAS. *Technological Educational Institute of Kalamata, 241 00 Antikalamos, Kalamata, Greece.*
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Over the last 15 years there has been a noticeable decline in the Greek fir (*Abies cephalonica*) ecosystem of Mount Taygetos (Peloponnese, southern Greece), predominantly in its northern part, which is directly exposed to a nearby point source of primary air pollution, the coal power plant at Megalopolis. For this reason, and also since ozone is known as the most phytotoxic secondary long-range air pollutant, and is deeply involved to the forest decline phenomenon, an ozone monitoring campaign was conducted in the Mount Taygetos fir ecosystem from April to September 2005, using passive samplers and bioindicator plants (tobacco BelW-3). At the same time, the chlorophyll content of the fir needles was measured, since one serious chronic ozone effect on sensitive plants is to disrupt the chloroplast thylacoid membranes, where chlorophyll is located. Six ozone-monitoring and needle-sampling sites were set up on Mount Taygetos, at altitudes from 860 to 1330 m, three in the northern part and three in the southern part, in order to detect differences between the north and south. Six exposures of passive samplers and bioindicators, as well as needle samplings, were carried out at two or three-week intervals. Passive sampler ozone monitoring showed a) mean daily ozone concentrations above the critical threshold for plants (40 ppb or 80$\mu g/m^3$) in most cases, and b) higher ozone concentrations at the northern part of the ecosystem than at the southern The bioindicators showed a) phytotoxic ozone levels along the fir ecosystem of Mount Taygetos, and b) more severe damage of the bioindicators at the northern sites than at the southern. Lastly, the chlorophyll content was significantly lower in the fir needles from the northern sites than those from the southern. If these results are co-assessed, it appears that the higher phytotoxic ozone levels at the northern part of the ecosystem account for the differences in chlorophyll content between north and south. Further experimental work is needed to confirm this effect on Greek fir chlorophyll.

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Biological and non-chemical control

Effect of two *Pseudomonas* strains on bean growth and on the disease severity caused by three races of *Colletotrichum lindemuthianum*. G.A. BARDAS and K. TZAVELLA–KLONARI. *Laboratory of Plant*

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Anthracnose is one of the major bean diseases worldwide. *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi and Cavara, the causal agent of this disease, can be controlled by using disease-free seed, by seed coating, by foliar applications of fungicides and by agricultural practices. The aim of this research was to test *in vitro* and *in planta* the effect of two bacterial strains, *Pseudomonas fluorescens* WCS365 and *P. chlororaphis* PCL1391, in controlling anthracnose of the bean cultivar Zargana Hrisoupolis, with a view to using these bacteria in an integrated system of pest control. In *in vitro* experiments, dual cultures on PDA showed that *P. chlororaphis* PCL1391 reduced growth and sporulation of all three races of anthracnose. A combination of the bacterial strains in triple cultures with races of *C. lindemuthianum* also achieved a significant reduction in growth and sporulation. Plants that emerged from seeds coated by and continuously irrigated with bacterial suspensions of *P. chlororaphis* PCL1391 alone and in combination with *P. fluorescens* WCS365, likewise showed a lower disease severity from the *C. lindemuthianum* races and enhanced plant-growth characteristics. These results are promising for the further development of an integrated control system of bean pathogens.

Control of apple and peach diseases by lime-sulfur in organic agriculture. G.A. BARDAS[1], C. VASILIKIOTIS[2], P. PADOPOULOS[2] and K. TZAVELLA - KLONARI[1]. [1]*Laboratory of Plant Pathology, School of Agriculture, Aristotle University of Thessaloniki, Greece.* [2]*Geotechnical Laboratory S.A., Paleochori, Plati, Imathia, Greece.*
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The control of apple scab and peach leaf curl by lime-sulfur alone, or in combination with Bordeaux mixture, was studied in commercial orchards of the Pella and Kozani areas. The orchards were cultivated organically. Experiments were carried out during the winter and summer of 2005. Three treatments were applied. Each consisted of two winter sprays of high-density lime-sulfur, alone or together with copper. All treatments were followed by low-density sprays at fortnightly intervals till July. The control of apple scab, varying with the inoculum pressure from area to area, reached a maximum of 97.8% of infected leaves (i.e. a 3% infection rate compared with 100% for the untreated control). The most effective treatment was 8.5% lime-sulfur combined with Bordeaux mixture, in winter sprays. In the case of peach leaf curl, all treatments decreased the percentage of infected leaves by 5.5 to 15%, while infection of the untreated control varied from 36.3% to 95.8%. In order to study the effect of limesulfur on disease control, yield and other characteristics of apple and peach trees, this program has been extended into 2006 and 2007.

The effect of essential oils on the transmission of *Tomato spotted wilt virus* (TSWV) by *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). E.K. CHATZIVASSILIOU. *Democritus University of Thrace, Department of Agricultural Development, Plant Pathology Laboratory, Pantazidou 193, 68 200 N. Orestiada, Greece.*
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The effect of essential oil of oregano (*Origanum vulgare*) and lavender (*Levandulla officinalis*) on the transmission of *Tomato spotted wilt virus* (TSWV) by *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) was studied in laboratory tests. Thrips oviposition, as well as acquisition and transmission of TSWV, were recorded at four concentrations per oil (0.01–1%). In the oviposition experiments, *T. tabaci* females were confined to tobacco (*Nicotiana tabacum* cv. Basmas) leaves and the number of larvae that emerged was recorded. The oviposition rate was recorded in no-choice tests, and oviposition preference under choice conditions. Significantly fewer larvae emerged on the oil-treated leaves, while the effect on the concentration depended on the oil. In the choice experiments, both oils had a deterring effect on oviposition at the lower concentration, but not at the higher (here it was 1%). In the acquisition experiments, 0–24 h old larvae were placed on TSWV-infected leaves. Their survival rate decreased as the oil concentration increased, with a maximum of 27% for oregano and 42% for lavender oil at a 1% concentration. Among the adult thrips produced, the number of viruliferous individuals was similar in the different concentrations (max. 57%). Viruliferous individuals were also confined to tobacco leaf discs treated with the oils, and their survival and transmission rates were recorded. Transmission rates were 65–72% with oregano and 60–85% with lavender oil, depending on the concentration. No significant thrips mortality was recorded. In choice tests, no oil application fully deterred TSWV transmission to *Petunia hybrida* leaves.

Integration of fertility management, hybrid selection and alternative spray treatments for the control of powdery mildew (*Leveillula taurica*) in organic greenhouse tomato crops. N.G. DAFERMOS[2], A.M KASSELAKI[1], N.E. MALATHRAKIS[1] and C. LEIFERT[2]. [1]*Technological Educational Institute of Crete, Stavromenos 71004, Heraklion, Crete, Greece,* [2]*Department of Agriculture, The University of Newcastle, Newcastle Upon Tyne, NE 1 7RU, UK.*
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Powdery mildew caused by *Leveillula taurica* (Lev.) Arn. is one of the most serious foliar diseases of greenhouse and open-field tomato. The disease is currently controlled with the use of organic fungicides and sulphur, the latter being the only product permitted in organic crops. The aim of

this study was to investigate the potential of controlling the disease by integrating: a) hybrids of low susceptibility to the disease, b) organic fertilisers (chitin) and c) alternative spray treatments. The triple interaction of the above factors proved statistically significant (ANOVA) and some of their combinations were highly effective in decreasing the percentage of disease severity. Specifically the combination of the hybrid of low susceptibility with the addition of chitin to the substrate and the spray treatment Milsana®+chitosan was just as effective as sulphur. These results indicate that the combination of the above factors could probably be used as an alternative to sulphur in controlling *L. taurica* in the greenhouse.

Control of seedborne *Clavibacter michiganensis* subsp. *michiganensis* by applying alternative treatments to tomato seed. D.E. GOUMAS[1], A.M KASSELAKI[1], N.E. MALATHRAKIS[1] and C. LEIFERT[2]. *[1]Technological Educational Institute of Crete, Stavromenos 71004, Heraklion, Crete, Greece. [2]Department of Agriculture, The University of Newcastle, Newcastle Upon Tyne NE1 7RU, UK.*
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The bacterium *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) survives on seed and is spreading to new regions. Naturally infected seed (1:10,000) can lead to an epidemic outbreak with up to 80% yield loss. The aim of this study was to investigate whether alternative treatments of the seed could control seedborne Cmm. All experiments were done *in vitro*. Mechanically inoculated seeds were soaked in the following treatments: a) various concentrations of sodium nitrite ($NaNO_2$), and b) suspensions of antagonistic bacteria, mostly *Bacillus* spp. Three of the 21 antagonistic strains completely inhibited disease incidence in all experiments for at least 21 days after treatment application. In contrast the performance of the sodium nitrite solutions was inconsistent, although they were significantly better than the untreated control in both experiments performed. Specifically, disease incidence was decreased to 0–4% compared to 35% (control, exp. 1) and to 4–14% compared to 41% (control, exp. 2), regardless of the combination of nitrite concentration and the duration of application. The fungicide copper hydroxide was highly effective in all the experiments. Results of *in vivo* experiments are in progress and may be quite different.

The effect of neem (azadirachtin) in the immobilisation of second stage juveniles of the root-knot nematodes (*Meloidogyne* spp.). F.T. GRAVANIS, I.K. VAGELAS, D.G. NATSIOPOULOS and S.V. LEONTOPOULOS. *Technological Educational Institute of Larissa, Department of Plant Production, 41110 Larissa, Greece.*
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Meloidogyne spp. root-knot nematodes (RKNs) significantly affect glasshouse tomatoes in central Greece. Eliminating or inhibiting second stage juveniles (J2s) could be a way to control the RKNs. Recently, it was reported that a commercial product of Neem (*Azadirachta indica*) seed extract (Azadirachtin 1% EC) significantly reduced the number of galls on tomato roots and egg masses, compared to the untreated control. In the present study neem was found to have a direct immobilising effect on RKN J2s. Different concentrations (0, 0.05, 0.1, 0.25, 0.5, 1, 5, 10, 25 and 50%) of the neem product were added to Petri dishes containing water and fresh J2s, and to soil naturally infected with RKNs. Immobilised J2s were counted after 10 min, 1 h, 6 h, 24 h and 72 h of neem application in water, and after six days of neem application in the soil. Neem concentrations of 5% and 10%, resulted in 85% (±3.2) and 100% of immobilised J2s respectively, with a significant concentration effect ($P<0.001$). When the same concentrations of neem were added to the water to extract the nematodes from the soil, 14.25% (±4.01) and 1.7%, of the J2s extracted from the soil were immobilised. Here too there was a significant concentration effect ($P=0.008$), compared with the untreated control.

Biological control of *Pythium* damping-off in cotton, tomato and radish by means of *Pseudomonas oryzihabitans*. A.V. KAPSALIS[1], N. EFSTATHIOU[1], S.R. GOWEN[2] and F.T. GRAVANIS[1]. *[1]Technological Educational Institute of Larissa, Department of Plant Production, 41110 Larissa, Greece. [2]The University of Reading, School of Agriculture Policy and Development, P.O. Box 236, Reading, RG6 6AT, UK.*
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The biological effect of the entomopathogenic bacterium *Pseudomonas oryzihabitans* on damping-off caused by *Pythium* spp. on cotton, tomato and radish (*Raphanus sativus*) was investigated. *Pythium* spp. and *P. oryzihabitans* in dual cultures on Petri dishes containing PDA completely inhibited mycelial growth of *Pythium* spp., possibly on account of metabolites produced by *P. oryzihabitans*. In an *in planta* experiment, seeds of cotton, tomato, and radish were dipped in bacterial cell suspensions of *P. oryzihabitans* at concentrations of 10^3, 10^4, 10^5 and 10^6 cells ml^{-1} for 10 minutes. The seeds were then seeded in pots containing peat that had been inoculated with *Pythium* spp. (10^9 oospores g^{-1}) and incubated for 4 weeks at 26°C, with a 12-h day. There were 10 replicates per treatment; 10 plants per species were kept as controls. High concentrations (10^6 cells ml^{-1}) of *P. oryzihabitans* cells resulted in better growth of both young cotton and tomato plants. The treated plants were significantly higher in fresh weight and height compared with the other treatments with and the untreated control in both the cotton cultivars (Coker and Aria) as well as in both the tomato cultivars (Viomichaniki

and Karampola) tested. In cotton plants there was no significant difference between bacterial cell concentrations of 10^5 and 10^6 cell ml^{-1}. Radish plants were well protected from Pythium damping-off when the seeds were treated with 10^4 and 10^5 bacterial cells ml^{-1}, and exhibited a better growth rate than was achieved with the other treatments and with the untreated control. In radish the 10^6 ml^{-1} bacterial cell concentration produced toxic symptoms after the first two weeks of incubation.

Nematocidal effects of the nematode-trapping fungi *Arthrobotrys dactyloides* and *A. oligospora*, and nematostatic activity of the plant-derivative azadirachtin against the plant parasitic nematode *Meloidogyne* sp. E. KARANASTASI[1], M. KORBI[2] and D. LASKARIS[1]. [1]*Benaki Phytopathological Institute, 8 S. Delta St., 14561 Kifissia, Greece.* [2] *Technological Educational Institute (TEI), Kalamatas, Antikalamos, 24100 Kalamata, Greece.*
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Interest in the biomanagement of pests and diseases, stimulated by the increasing number of restrictions on chemical means of control, has also extended to plant nematology. Several nematode control methods are currently being developed; but most are still at the experimental stage since for field application rapid action, low cost and absence of adverse environmental impact are all prerequisites and must be satisfied. The present work reports on a comparative study of the nematocidal effects of *Arthobotrys oligospora* and *A. dactyloides*, two fungi that have previously yielded promising results. These fungi were tested at two rates, and compared with two widely used chemical nematocides, Nemacur and Nemathorin 10G, and with azadirachtin, a compound derived from the Neem-tree, which is an insecticide but which also exhibits nematostatic activity. All experiments were performed in natural soil, where numerous factors always concur. On several occasions the results were very promising though they occasionally contradicted previous findings. As regards azadirachtin, only one of two tested products containing this ingredient (Oikos, Neemazal), significantly reduced the nematodes. The nematocide Nemathorin (a.i. fosthiazate) was more effective than Nemacur (a.i. fenamiphos), although the Nemathorin-tested plants developed toxicity symptoms at the application rates used in the experiment and the effectiveness of this product may be lower at lower rates. Of the eight different treatments, the most effective against *Meloidogyne* was Nemathorin, followed by Neemazal.

Control of seedborne *Didymella lycopersici* with alternative treatments of tomato seed. A.M KASSELAKI[1], D.E. GOUMAS[1], N.E. MALATHRAKIS[1] and C. LEIFERT[2]. [1]*Technological Educational Institute of Crete, Stavromenos 71004, Heraklion, Crete, Greece.* [2]*Department of Agriculture, The University of Newcastle, Newcastle upon Tyne NE 1 7RU, UK.*
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The fungus *Didymella lycopersici* infects tomato seed and causes substantial losses before germination, as well as contamination of the crops. To control the disease, seed companies use thiram as a preventive agent, although human allergy problems have been reported with this product. For this reason, as well as to address the needs of organic agriculture, this study focused on alternative methods of control. Nitrite solutions and inducers of resistance were tested in the growth chamber. Results showed that soaking the seed in a nitrite concentration of 300 mM (in citric acid buffer, pH 2) for 10 minutes completely inhibited losses from low seed germination and disease in the germinated seedlings. When applied for longer periods, sodium nitrite was toxic, and for shorter periods it was not as effective. The inducers Tillecur (mustard seed extract) at 0.5 g 10 ml^{-1}, and chitosan (85% deacetylated chitin) at 0.05 g ml^{-1}, were just as effective as sodium nitrite in inhibiting disease in germinated seedlings. None of the above treatments was significantly different from thiram in effectiveness and they can be used instead of the fungicide to control seedborne *D. lycopersici* in tomato.

Induction of systemic resistance and colonization by an endophytic *Fusarium solani* strain in tomato plants. N. KAVROULAKIS[1], K.K. PAPADOPOULOU[3], S. NTOUGIAS[1], C. EHALIOTIS[2] and G.I. ZERVAKIS[1]. [1]*National Agricultural Research Foundation, Lakonikis 87, 24 100 Kalamata, Greece.* [2]*Agricultural University of Athens, Department of Natural Resources and Agricultural Engineering, Iera Odos 75 118 55 Athens, Greece.* [3]*Department of Biochemistry and Biotechnology, University of Thessaly, Ploutonos 26 and Aiolou, 412 21 Larissa, Greece.*
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An endophytic root fungal strain (npFs) was isolated from a compost that suppressed soil and foliar pathogens of tomato. The strain, which was identified as *Fusarium solani* by the sequence of its 18S and IGS regions, inhibited the growth of the pathogens. Inhibition was confirmed by *in vitro* tests. Colonization of the tomato roots by this non pathogenic fungus was examined under the microscope. Interestingly, the roots of tomato inoculated and colonized by the *Fusarium solani* strain had lower levels of expression of certain pathogenesis-related genes, such as *PR5* and *P69*, but in the leaves the expression pattern was not affected. The npFs strain protected tomato plants from the root pathogen *Fusarium oxysporum* f. sp. *radicis lycopersici,* if certain application practices were observed. On the contrary, extract from the fungal isolate seemed actually

to be conducive to disease development. The fungal isolate also elicited induced systemic resistance in tomato plants, as was shown in a different pathosystem, using the tomato foliar pathogen *Septoria lycopercici*. Although the ethylene biosynthesis level was not affected by npFs, tomato plants of the mutant line *Nr (Never ripe)* inoculated with *Fusarium oxysporum* f. sp. *radicis lycopersici* were not protected by the antagonistic fungal strain. This indicates that the ethylene perception pathway is involved in the establishment of endogenous plant defense.

Alternative methods to control garlic white rot (*Sclerotium cepivorum* Berk.). D. LASCARIS[1], J. ASPROMOUGOS[1], I. VLOUTOGLOU[1], A. KOUTOUKIDIS[2] and D. GILPATHI[3]. *[1]Benaki Phytopathological Institute, Plant Pathology Department, Laboratory of Mycology, 8 S. Delta St., 145 61 Kifissia, Athens, Greece. [2]Union of Agricultural Cooperatives of Orestiada, 19 Skra St., 68 200 Orestiada, Greece. [3]Ministry of Rural Development and Food, General Directorate of Plant Produce, Directorate of Plant Produce Protection, 150 Syggrou Av., 176 71 Kallithea, Athens, Greece.*
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The effectiveness of some alternative methods to control garlic white rot (*Sclerotium cepivorum* Berk.), as compared with conventional fungicides, was evaluated in a naturally infected field in the area of Orestiada in 2004–2005. Treatments included: soil solarization (SS), incorporation of chicken manure into the soil (CM, 2 kg m^{-2}), dimethyl disulfide (DMDS, 80 g m^{-2}), diallyl disulfide (80% DADS, 1 ml m^{-2}), calcium cyanamide (CC, 100 g m^{-2}) or a combination of SS plus one of the last four treatments. Control plots did not receive any treatment. An experimental design of randomized complete blocks with six replicate plots (each 3×6 m) for each treatment was used. Experimental plots receiving soil solarization remained covered with a transparent polyethylene sheet of low permeability to gases from mid-July to mid-September 2004. The field was planted with garlic gloves of a local variety in mid October 2004. The severity of the disease was assessed in May and June (harvest) 2005. The yield (weight of commercially accepted bulbs) was also assessed. Results showed that soil solalization alone or in combination with chicken manure, DMDS, DADS or calcium cyanamide was very effective in controlling garlic white rot, reducing disease severity by 66–86%, increasing yield and improving the quality of the bulbs, as compared with the untreated control.

Control of *Verticillium dahliae* using compost amendment or biocontrol agents. I. MALANDRAKI, S. TJAMOS, I. PANTELIDIS and E. PAPLOMATAS. *Department of Plant Pathology, Agricultural University of Athens, Greece.*
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Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae,* is a devastating disease affecting a wide range of herbaceous plant hosts. Since there are no chemical treatments to control this fungus (except by soil fumigation, which normally is applied only to high-value crops, usually in the greenhouse), management strategies are focused on preventive measures. Compost soil amendments and biocontrol agents should therefore be evaluated as an alternative means to control *V. dahliae*, especially now that methyl bromide, which was a very effective soil fumigant, has been banned. In this study the effectiveness of compost amendment GR6 (consisting of horse manure and wood chips) with a known activity against *V. dahliae* was investigated. Eggplants grown in sterilised or non- sterilised compost were transplanted at the 4-leaf stage to plastic pots containing 45 *V. dahliae* microsclerotia g^{-1} of soil amended or not with 20% of sterilised or non- sterilised compost. The percentage of diseased leaves was most strongly reduced when eggplants were grown in compost and transplanted to infected soil amended with the compost. The reduction in wilt with this compost may have had a microbial cause since if the compost was sterilised it did not reduce the symptoms more than the control. Several microbes were isolated from the rhizosphere of eggplants grown in GR6 compost and tested *in vitro* against *V. dahliae*. Two bacterial strains that showed a zone of inhibition against *V. dahliae,* and two fungal strains, F2 and F4, which were the most frequently isolated, were selected for further evaluation under glasshouse conditions. The two fungal strains F2 and F4, and one of the two bacterial strains, B-6, reduced the percentage of wilted leaves as compared with the control treatment. The fungal strains F2 and F4 were found to belong to *Fusarium oxysporum,* and bacterial strain B-6 to the *Pseudomonas fluorescens* complex. These biocontrol agents are being further evaluated under field conditions.

Evaluation of some compost amendments against soilborne pathogens. E. MARKAKIS[1], S. TJAMOS[1], I. CHATZIPAVLIDIS[2], P. ANTONIOU[1], E. PAPLOMATAS[1] and E. TJAMOS[1]. *[1]Department of Plant Pathology, Agricultural University of Athens, Greece, [2]Department of Microbiology, Agricultural University of Athens, Greece.*
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Verticillium dahliae and *Fusarium oxysporum* f. sp. *melonis* are two devastating soilborne fungi that cause serious economic losses. Strategies to control them mainly focus on preventive measures. These include, among others, compost soil amendments and biocontrol agents. Seven different composts and *Paenibacillus alvei* strain K165 were evaluated for their effectiveness against *V. dahliae* and *F. oxysporum* f. sp. *melonis* under greenhouse conditions. Eggplants at the 4-leaf stage were transplanted to plastic pots containing 40 *V. dahliae* microsclerotia g^{-1} of soil

amended with 20% compost or 10 ml 10^8 CFU ml^{-1} K165. The composts GR6 (consisting of horse manure and wood chips) and K165 were the most effective against *V.dahliae*. They were therefore selected for further evaluation under field conditions. In the field a statistically significant difference in disease severity was observed between the control treatment and K165. However, neither compost GR6 nor K165 produced a yield higher than that achieved with the control. As regards *F. oxysporum* f. sp. *melonis,* watermelons at the 2-leaf stage were transplanted to plastic pots containing sterile soil amended with 20% compost or 10 ml 10^8 CFU ml^{-1} K165 and inoculated by drenching with 40 ml 10^7 ml^{-1} conidia of *F. oxysporum* f. sp. *melonis*. Composts GR8 (cotton cake), GR10 (peach cake) and K165 were the most effective against *F. oxysporum* f. sp. *melonis* and they will therefore be further evaluated under field conditions. We also investigated whether the mode of action of any of the composts was based on inducing systemic resistance in the plants. In a novel *Arabidopsis thaliana/V.dahliae* biocontrol agent system we observed that 3 of the composts, GR5 (leonardite), GR8 and GR3 (spent mushroom) induced systemic resistance in *A. thaliana* against *V. dahliae;* however to a less exten than did the K-165 strain.

Isolation of natural plant antioxidant substances from olive and katsigaros and their exploitation in plant protection. T. MAURAKIS[1], M. TRANTAS[1,2], A. AGALIAS[3], L. SKALTSOUNIS[3] and F. VERVERIDIS[1]. *[1]Department of Crop Science, Technological and Educational Institute of Crete, Heraklion, Greece. [2]Department of Biology, University of Crete, Heraklion, Greece. [3]Department of Pharmacognosy and Natural Products Chemistry, University of Athens, Greece.*
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Phenolic compounds in *Olea europaea* fruits/tissues have pharmacological properties and are natural antioxidants, thus inhibiting the Gram-positive micro-organisms involved in olive fruit fermentation. Oleuropein, the main phenolic compound present in *O. europaea* fruits and leaves (up to 14% of the dry weight in unripe olives), is a 3,4-dihydroxyphenylethanol (hydroxytyrosol) ester with a β-glucosylated elenolic acid and produces the bitter taste of unripe olives. The aglycon, which is obtained from oleuropein hydrolysis, is well-known as a pharmacologically active molecule with a potential for application as an antimicrobial agent in some fairly common olive tree diseases. Moreover, oleuropein (from olive tissues) and its derivatives (derived from katsigaros, or olive mill waste waters) have a variety of biochemical roles, including anti-inflammatory and antithrombotic activity. Our aim was to exploit these natural antioxidants by using them as phytoprotective agents against various economically important pathogens in Greece. The results of the study showed that oleuropein inhibited the growth of various economically important pathogens such as the Gram-positive *Clavibacter michiganensis* at 0.5% w:v, and the Gram-negative *Pseudomonas syringae* pv. *tomato* at a concentration of 0.1% w:v. An extension of the study to greenhouse experiments is being discussed.

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Heterologous biosynthesis of resveratrol from *Saccharomyces cerevisiae* and its application against *Botrytis*. D. MILIOS [1], M. TRANTAS[1,2], A. TAMPAKAKI[1,2], N. PANOPOULOS[2] and F. VERVERIDIS[1]. *[1]Department of Crop Science, Technological and Educational Institute of Crete, Heraklion, Greece. [2]Department of Biology, University of Crete, Heraklion, Greece.*
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Resveratrol, a trans-3,5,4'-trihydroxystilbene, is produced in various plants, including grape leaves (*Vitis vinifera*), where concentrations are particularly high, reaching 400μg g^{-1} fresh tissue. Resveratrol is a phytoestrogen with very interesting properties . It has been associated with the prevention of cardiovascular diseases and atherosclerosis and its chemopreventive activity has been associated with tumor initiation and progression. It has also been shown that it can act like the phytoalexins, serving as an antimicrobial agent following attack by a pathogen. The aim of our work was to genetically engineer new strains of *Saccharomyces cerevisiae* by genetically reconstituting its plant biosynthetic pathway. Yeast cannot naturally form resveratrol, and thus the enzymatic system that exists in plants converting resveratrol to its biologically inactive derivatives is beneficially absent in yeast. As a result resveratrol can potentially be overproduced in yeast cells, so that it can be used for plant health. All the four implicated genes of the resveratrol pathway were cloned from grapevine, soy and tobacco and the pathway was reconstructed by subcloning the genes to appropriate vectors designed for the expression and functional analysis of eukaryotic genes in yeast. Resveratrol levels were analysed and detected by a capillary electrophoresis system, after first concentrating the polyphenol. The plant-protective action of resveratrol appears to be promising and it could form an alternative (non-chemical) means to fight *Botrytis*.

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Freeze drying and shelf-life studies of the biocontrol yeast *Pichia anomala*. S. MOKIOU[1] and N. MAGAN[2]. *[1]National Agricultural Research Foundation, Plant Protection Institute of Thessaloniki, 570 01 Thermi, Greece. [2]Applied Mycology Group, Biotechnology Centre, Cranfield University, Silsoe, Bedford MK45 4DT, UK.*
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Nowadays, there is increasing interest in the use of fungal biological control agents (BCAs) as an alternative

method to chemical pesticides. However, practical aspects of their development, such as the ecological competence and shelf-life of such agents must be ensured. *P. anomala* is being considered for commercial treatment of moist cereals to control *Penicillium roqueforti* and the mycotoxigenic *P. verrucosum*. This study examined the impact that protective additives, rehydration media, *P. anomala* cell modification, and cell post-harvest washing with isotonic solutions have on yeast viability and storage stability after freeze drying. In the absence of protective and rehydration media, the survival rate of freeze dried *P. anomala* cells decreased dramatically (<20%). However, a protective solution of 10% skimmed milk + 10% sucrose resulted in a high survival rate (94%) and gave the final product a porous structure that made rehydration easier. An isotonic post-harvest washing treatment resulted in modified *P. anomala* cells with increased freeze-drying tolerance, particularly in the absence of a protective medium. An increased intracellular accumulation of trehalose (which has been shown to occur in *P. anomala* cells under a low water activity regime when proline and NaCl are added to molasses media (Mokiou & Magan, 2002), might account for this phenomenon. When a protective medium was used the effect of isotonic washing was masked by the latter. Storage stability of freeze dried *P. anomala* cells at 4°C was particularly high (>86%) over a period of 150 days, whereas storage of such cells at 22°C led to a more rapid decreased in their viability (<35%) over a period of 30 days. Osmoprotection using a post-harvest isotonic washing treatment had no effect on storage stability.

Biological control of *Verticillium dahliae* wilt in eggplants by means of the mild pathogen *V. nigrescens*. D.G. NATSIOPOULOS[1], I.K. VAGELAS[1], I.D. SALIGKARIAS[2] and F.T. GRAVANIS[1]. [1]*Technological Educational Institute of Larissa, Department of Plant Production, 41110 Larissa, Greece.* [2]*Prefecture of Ilia, Direction of Agricultural Development, Dioikitirio, 27100 Pyrgos, Greece.*
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Verticillium dahliae and *V. nigrescens*, isolated from greenhouse soil, are repectively aggressive and mild agents of wilt on eggplant. Pathogenicity tests of these agents were carried out on eggplants grown in pots. Eggplants at the two-leaf stage were uprooted, inoculated and then repotted. Plants were inoculated with both fungi on the same day, and with a 4-day interval between them, in all combinations. Ten untreated plants were kept as controls. All treatments were replicated 10 times. Wilt severity, plant height, stem diameter, fresh weight and dry weight of eggplants were assessed after incubation in a glasshouse for 60 days. All plants that were inoculated with *V. nigrescens* before, or even on the same day, as *V. dahliae,* were significantly taller and heavier, and had fewer wilt symptoms than eggplants receiving any of the other treatments, but they were not, however, significantly different from the control.

Insights into the role of ethylene during infection of plants by *Verticillium dahliae*. I. PANTELIDIS, S. TJAMOS and E. PAPLOMATAS. *Department of Plant Pathology, Agricultural University of Athens, Greece.*
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Ethylene plays a major role in many physiological mechanisms in plants, including dormancy, senescence and necrosis, and is also implicated in symptom development when *Verticillium dahliae* infects the plants. The role of ethylene *in V. dahliae* infection of *Arabidopsis thaliana* and tomato was studied. Twenty-day-old *A. thaliana* wild-type and *etr1-1, ein1-1* mutants (ethylene insensitive) were inoculated with 2 ml 10^7 conidia/ml. *Etr1-1* plants were less stongly infected than wt and *ein* plants. In addition, tomato plants cv. Ailsa Craig, wt and *Never ripe* mutants were inoculated by dipping their root system in 10^7 conidia/ml. *Never ripe* tomato plants were less stongly infected than wt. The ethylene-insensitive plants therefore showed less symptom development than the wt plants. Current studies are examining whether the reduced symptom development is correlated with the activation of plant defense mechanisms or with physiological aspects of plant metabolism.

Researching the capacity of composts to suppress *Sclerotium cepivorum*, the causal agent of white rot of bulbous vegetables of *Allium* species. E.J. PAPLOMATAS and O.G. KYRIAKOPOULOU. *Agricultural University of Athens, Laboratory of Plant Pathology, Iera Odos 75, 118 55 Athens, Greece.*
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White rot of *Allium* spp. is a disease caused by the soilborne fungus *Sclerotium cepivorum*. This fungus attacks species of the genus *Allium,* and white rot is the most important limiting factor in the cultivation, of onion, and mainly that of garlic. The aim of this work was to investigate whether selected composts could control white rot of *Allium* spp. Ten composts from Greece and other European countries were evaluated against white rot of onion (*Allium cepa*) and garlic (*Allium sativum*). Composts of Greek origin that were tested were: GR3, GR5, GR6 and GR7, derived respectively from spent mushroom substrate, leonardite (a type of lignite), horse manure and oil mill waste. The foreign composts were: CO2 (France) from composted horse manure and green wastes, CO4 (France) from urban biowastes, DECO1 (Netherlands) from yard waste composted with wood chips and grass clippings, BOM3 (Netherlands) from yard residues, 8.1S (Netherlands) from wood chips, manure and clay, and

ISBS (Israel) containing urban biosolids. Pathogenicity tests were performed under controlled greenhouse conditions using an isolate of the pathogen. Composts used as soil amendments tended to reduce the white rot. However, control effectiveness for both garlic and onion varied with the compost. The greatest control of white rot of garlic was achieved with BOM3 and GR5, with 19% and 15% of white rot reduction respectively, compared with the untreated control. Composts 8.1S, DECO1, GR7, CO4, GR3, CO2, and ISBS reduced the disease but the results they produced did not differ statistically from the control, while GR6 was no better than the control. Against white rot of onion the most effective compost was CO4, which was significantly better than the control (14%). GR6, GR3 and GR7 reduced white rot but with no statistical difference from the control, and composts GR5, CO2, DECO1, ISBS, BOM3 and 8.1S acually resulted in a higher percentage of infection than the control. When comparing the effect of composts against both hosts, CO4 was the most effective (11%), differing significantly from the control; composts GR5, GR3, GR7, GR6, BOM3 and CO2 limited the disease, but not significantly, and composts DECO1, 8.1S and ISBS produced the same percentage of infection as was found in the control. These results indicate the need for further research that should be focused on the mechanism by which the composts suppressed *Sclerotium cepivorum,* so as to make the control of white rot more effective.

Preliminary study of the possible biological control of root and stem rot of cucumber (*Fusarium oxysporum* f. sp. *radicis-cucumerinum)* by means of a *Fusarium solani* strain. G.C. PAVLOU, N.I. KAVROULAKIS and G.I. ZERVAKIS. *National Agricultural Research Foundation, Olive and Horticultural Crops Institute of Kalamata, Lakonikis 87, 24100 Kalamata, Greece.*
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A *Fusarium solani* (FS) strain isolated from the composted organic mixtures of agricultural by-products (grape marc wastes plus extracted olive press cake) has been found to be a non-pathogenic strain effectively protecting tomato plants from the root pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici.* The possibility of controlling root and stem rot of cucumber caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC), using the same FS strain, was investigated in the present preliminary study with two successive experiments of 14 and 13 weeks duration, conducted in a growth chamber at 17–26°C with a 12–13-h photoperiod under artificial lighting. Cucumber seedlings cv. Sandra F1 were grown in 300 cm^3 plastic pots (one seedling per pot) containing 250 cm^3 of soil consisting of peat moss and sandy loam 2:1 v:v, pasteurised at 74°C for 7 h. Two days after seed germination the soil in the pots was inoculated with a spore suspension of FS to a final concentration of 10^4 and 10^6 spores/cm^3 in the first experiment, and 10^3, 10^5 and 10^7 spores/cm^3 in the second experiment. Ten and 20 days later the soil in the pots was inoculated with a spore suspension of FORC to a final concentration of 10^5 spores/cm^3 in the first experiment and 10^6 spores/cm^3 in the second experiment. Controls with and without FORC and FS inocula were included in both experiments. Macroscopic inspection of each plant included the following: size and colour of leaves; colour, swelling and rot of hypocotyls; small dead fruits or developed edible fruits. The inspection showed that FS did not protect cucumber plants from FORC, probably because of the high inoculum concentration of FORC in the soil. However, a slight suppressive effect of FS on disease development during the early stages of plant growth was seen. This was probably due to the endophytic character of FS, whose presence was traced from the root system up to the crown.

This study was performed in the framework of a research project entitled: 'Biological treatment and valorization of olive mill wastewaters: mechanisms and integrated application. (GCRT, Action 4.5.1 – FP66).

Ecology of fungi associated with the oak powdery mildew agent *Microsphaera alphitoides*. E. TOPALIDOU and M.W. SHAW. *School of Biological Sciences, The University of Reading, Whiteknights, Reading RG6 6AU, UK.*
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Powdery mildew (*Microsphaera alphitoides)* on oak (*Quercus robur*) is very often intimately associated with other fungi. Some of these fungi were isolated on artificial media and were organised in groups, since their identification based on morphological characteristics was not possible. Three of the isolated symbionts (under the group names: Group I, Group IV and Group V) were tested in a glasshouse experiment. Symbionts were applied singly and in combinations of two to oak leaves that had been previously inoculated with powdery mildew. The treated oak leaves were covered with permeable-transparent bags to reduce entry of external micro-organisms. Assessments of powdery mildew severity were made twice weekly. When the leaf area was about 80–100% covered with powdery mildew, the leaves were carried to the laboratory and a final assessment was made macroscopically and microscopically. The relationship of the symbionts to the powdery mildew varied. The symbionts of group IV inhibited powdery mildew; those of group V exacerbated powdery mildew severity; and the powdery mildew severity of the leaves treated with group I symbionts was similar to that of the control leaves. The relationship between the population densities of powdery mildew and its symbionts is being investigated, as well as the ecology of these microorganisms in relation to and independently of powdery mildew. Molecular techniques are being used to identify the micro-organisms applied.

The effectiveness of laminarin in inducing systemic acquired resistance (SAR) of cucumber and tomato plants to *Botrytis cinerea*. G. TSIKRITEAS, A.N. MARKOGLOU and B.N. ZIOGAS. *Agricultural University of Athens, Laboratory of Pesticide Science, 75 Iera Odos, Votanikos, 118 55 Athens, Greece.*
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A relatively novel approach to control plant diseases is to use natural or synthetic chemical compounds interfering with the host-pathogen interaction. The effectiveness of laminarin, a metabolite of marine brown algae, against grey mould caused by *Botrytis cinerea* was investigated in the present study. *In vitro* fungitoxicity tests did not show any effect of laminarin on mycelial growth, sporulation or the conidial germination of *B. cinerea*, even at concentrations up to 10,000 $\mu g/ml^{-1}$ laminarin. *In planta* experiments showed that lesions of *B. cinerea* on cucumber plants were strongly inhibited (up to 75%) by preventive applications of laminarin at a concentration of 20 μg/ml, and plant inoculation 5 to 20 days after laminarin application. Concentrations of 5 to 10 $\mu g/ml^{-1}$ laminarin reduced plant infection by only a maximum of 30%. In tomato plants on the other hand, the pathogenicity of *B. cinerea* was only slightly reduced (10%) even at a lamarin concentration of 40 $\mu g/ml^{-1}$. These results indicate that a few days interval between the application of laminarin on cucumber and inoculation with *B. cinerea*, is critical for effective induction of systemic acquired resistance (SAR) to *B. cinerea* in cucumber plants.

Effect of essential oils on the growth of *Botrytis cinerea in vitro*. G.T. TZIROS and A.L. LAGOPODI. *Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Plant Pathology, P.O. 269, 54124 Thessaloniki, Greece.*
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Sixteen commercial essential oils were tested to determine their effect on the growth of *Botrytis cinerea,* which is an important pathogen of a large number of cultivated plants in the field and also a significant causal agent of postharvest diseases. Essential oils from thymus (*Thymus vulgaris*), banana (*Musa acuminata*), mastic (*Pistacia lentiscus*), black pepper (*Piper nigrum*), mandarin (*Citrus reticulata*), bergamot (*Citrus bergamia*), lemon (*Citrus limonum*), sweet orange (*Citrus sinensis*), oregano (*Boswellia thurifera*), spearmint (*Mentha viridis*), anise (*Illicium verum*), bay laurel (*Laurus nobilis*), rosemary (*Rosmarinus officinalis*), basil (*Ocimum basilicum*), fennel (*Foeniculum vulgare*) and parsley (*Petroselinum sativum*) were tested. In the *in vitro* tests the essential oils were incorporated into the nutrient medium (PDA) and afterwards mycelial growth was estimated. All the essential oils exhibited antifungal activity to various degrees. The most effective were the essential oils from thymus which inhibited mycelial growth at a concentration of 200 ppm, oregano, at 300 ppm and bay laurel, at 500 ppm, followed by fennel (1000 ppm), anise (1000 ppm), bergamot, sweet orange, basil and spearmint (2000 ppm) and parsley (3000 ppm). From the other essential oils, higher concentrations were required to inhibit mycelial growth completely. From the above results it is clear that some essential oils are effective against *B. cinerea*. These oils should now be tested *in vivo*, and their biocontrol potential should be investigated in a program of integrated management of postharvest diseases caused by *B. cinerea*.

***Pasteuria penetrans:* a promising bio-nematicide.** I.K. VAGELAS, D.G. NATSIOPOULOS and F.T. GRAVANIS. *Technological Educational Institute of Larissa, Department of Plant Production, 41110 Larissa, Greece.*
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Pasteuria penetrans is a bacterial parasite of *Meloidogyne* spp. root-knot nematodes (RKNs). This parasite shows great potential as a biocontrol agent. *P. penetrans* produces spores that adhere to the cuticle of the RKNs. A commercial product that consists of a strain of the bacterium with good attachment to *Meloidogyne* spp. was used to control a tomato greenhouse mixed population of RKN consisting of *Meloidogyne incognita*, *M. javanica* and *M. hapla*. Treatment with a commercial product diluted to 5000 bacterial endospores per ml resulted in an attachment rate of 10–15 endospores per freshly hatched juvenile 24 h after application. It was observed that juveniles to which 5–15 endospores per juvenile were attached managed to enter the roots but they were parasitized by the bacteria. When higher densities of endospores were attached to the nematodes (more than 30 bacterial spores per juvenile) the rate of nematode root infection was greatly reduced.

INTEGRATED AND CHEMICAL CONTROL

Is there such a thing as a fungicide resistance strategy? A modeller's perspective. M.W. SHAW. *School of Biological Sciences, University of Reading, Lyle Tower, Whiteknights, Reading RG6 6AJ, UK.*
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The three key suggestions for strategies to manage fungicide resistance are dose modification, alternation or restriction of use, and mixture with another active ingredient. I argue on empirical and theoretical grounds that, per dose, an increase in resistance frequency is usually minimised by minimising the dose; that the substantial selective disadvantages to resistant types required to make alternation or regional marketing useful are rarely taken into consideration; and that mixtures are in general not helpful, but that they are so only under specific circumstances dependent on the interactions of the particular components used. I conclude that an effective

fungicide should be regarded as a finite resource which will always sooner or later fall to resistance; in specific cases there are tactics that may be useful to extend their life, but there is no general pre-existing strategy.

Metabonomics: a new method for the study of the mechanisms of action of phytotoxic compounds. K.A. ALIFERIS and M. CHRYSAYI. *Agricultural University of Athens, Department of Crop Production, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Athens, Greece.*
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About thirty different mechanisms of action of phytotoxic compounds have so far been recognized. In most cases identification of subcellular targets is based on biochemical methods that are rather time-consuming. Metabonomics, a new method using ^{1}H NMR fingerprinting, aims to facilitate investigation of the mode of action of phytotoxic compounds. The principle of this method is that the metabolic profile of a plant tissue at any given time can be expressed as a unique ^{1}H NMR spectrum of crude plant extracts. Consequently, substantial changes caused by a phytotoxic substance in the metabolic profile can be detected and recognized in the ^{1}H NMR spectrum of the plant. Prerequisites for the reliability and successful application of the method are: fully controlled plant-growth conditions, careful handling of the samples, and suitable methods for data analysis. ^{1}H NMR spectra of plant extracts after treatment with phytotoxic compounds were compared by multivariate analysis and analyzed statistically with principal components analysis (PCA), partial least squares-discriminant analysis (PLS-DA) and soft independent modelling of class analogy (SIMCA). So far, the metabonomics approach has given encouraging results for investigating the mechanisms of action of synthetic and natural phytotoxic compounds.

Control of pear leaf fleck caused by benzimidazole-sensitive and benzimidazole-resistant strains of *Septoria pyricola*. M. CHATZIDIMOPOULOS and A.C. PAPPAS. *University of Thessaly, Department of Agriculture Plant Production and Rural Environment, Laboratory of Plant pathology, Fitokou St., 384 46 N. Ionia, Magnesia, Greece.*
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The effectiveness of various fungicides against pear leaf fleck disease, caused by artificially introduced inocula, was investigated. Five fungicide spays were applied, commencing at the 3–4 expanded leaf stage (30/3/06, 13/4/06, 27/4/06, 25/5/06, 8/7/06). Following the primary three fungicide applications, pear leaves were sprayed with a spore suspension (10^5 spores ml^{-1}) of a mixture (1:1) of a benzimidazole-sensitive and a benzimidazole-resistant isolate of *S. pyricola*. Disease severity was evaluated at two dates (11/5/06 and 15/6/06), by counting the number of spots on a randomly collected sample of 35 leaves per treatment/replicate, using a scale going from 1 (no leaf spotting) to 5 (over 10 spots per leaf). The sterol demethylation inhibitors (DMIs) bitertanol (Baycor 25 WP, 0.8 g l^{-1}) and flusilazole (Punch 40 EC, 0.065 ml l^{-1}) and the strobilurin fungicides (QoIs) azoxystrobin (Ortiva 25 SC, 1 ml l^{-1}), kresoxim-methyl (Stroby 50 WG, 0.2 g l^{-1}), trifloxystrobin (Flint 50 WG, 0.1 g l^{-1}) and pyraclostrobin in mixture with boscalid (anilides), applied as Bellis 38 WG, 0.8 g l^{-1}, gave satisfactory control (disease severity under 2). With carbendazim (Carbendazim 50 WP, 1 g l^{-1}), disease severity was almost 5, not significantly different from the untreated control. From these results it can be assumed that QoI fungicides are a good alternative to the DMI for the control of pear leaf fleck when benzimidazole-resistant strains are present.

Effect of fungicides on the bacterium *Agaricus bisporus*. M. CHRYSAYI[1], F. FLOURI[1], M. KASTANIAS[1], P. DIAMANTOPOULOU[2] and A. PHILIPPOUSSIS[2]. *[1]Agricultural University of Athens, Department of Crop Science, Pesticide Science Laboratory, 75 Iera Odos, 11855 Athens. [2]National Agricultural Research Foundation, Institute of Agricultural Engineering, Laboratory of Edible Fungi, 61 Democratias St., 135 61 Athens, Greece.*
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The cultivated white mushroom *Agaricus bisporus* is an intensive crop grown in special growing rooms and the crop needs careful post-harvest handling due to the perishable nature of the basidiocarps. Not only the quality characteristics of the mushrooms, such as whiteness and nutritional value, but also sporophore maintenance can be affected by microflora. In a project to evaluate the effectiveness of some fungicides against dry bubble disease of *A. bisporus* caused by *Verticillium fungicola*, the effect of tebuconazole, trifloxystrobin, carbendazim, prochloraz, mancozeb, and famoxadone mixtures with cymoxanil or mancozeb, on the total aerobic bacterial population and the whiteness of the sporophores was examined. The fungicide formulations were drenched on mushroom beds 5 days after casing at rates ranging from 0.5 to 1.2 g of active ingredient per m^2 of culture. Famoxadone+cymoxanil, tebuconazole and trifloxystrobin did not reduce the total microbial load of the sporophores, but there was a statistically significant reduction in bacterial forming units on sporophores of *Agaricus bisporus* when carbendazim, prochloraz, mancozeb and famoxadone+mancozeb were used. As regards sporophore whiteness, there was no correlation between any particular bacteria and color deviation during storage.

Effect of triazole-resistance mutations on ochratoxin production of *Aspergillus ochraceus* Wihl. E.G. DOUKAS, A.N. MARKOGLOU and B.N. ZIOGAS. *Laboratory of Pesticide Science, Agricultural University of*

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The effect of triazole-resistance mutation(s) on the mycotoxigenic ability of laboratory mutant strains of *Aspergillus ochraceus* was investigated. Mutants of *A. ochraceus* with moderate resistance (Rf 5–25) to the triazole fungicides were readily isolated (mutation frequency 10^{-5}) from a wild-type strain after UV mutagenesis and selection on a medium containing epoxiconazole. Cross-resistance studies with other fungicides showed that the mutation(s) for resistance to epoxiconazole also reduced the sensitivity of the mutant strains to other C-14 demethylase inhibitors (DMIs), such as flusilazole, difenoconazole (triazoles) and imazalil (imidazole). However, almost none of the mutant strains became more sensitive to morpholine fenpropimorph, phenylpyrrole fludioxonil, anilinopyrimidine cyprodinil, phenylpyridinamide fluazinam, carboxamide boscalid or benzimidazole carbendazim. Interestingly, with iprodione (dicarboximide) and pyraclostrobin (QoI) one half of the mutant isolates became less sensitive. Furthermore, epoxiconazole-resistant strains became increasingly sensitive to chlorothalonil. A study of the fitness parameters of the wild-type and epoxiconazole-resistant mutants of *A. ochraceus* showed that the triazole-resistance mutation(s) may or may not affect fitness characteristics. Chromatographic techniques (TLC, HPLC-FLD), showed that one of the effects of mutation(s) on ochratoxin A (OTA) and B (OTB) production was to reduce fungal ochratoxigenic capacity. These results indicate that the risk that triazole-resistant strains with a high ochratoxin production will appear is low. Apparently, triazoles do not increase the risk that ochratoxin will contaminate agricultural products.

The effect of the variables of a deterministic model on the estimation of the potential short-term intake of pesticide residues. M. KASTANIAS[1], K. KOKKINAKI[1] and K. MACHERA[2]. *[1]Ministry of Rural Development and Food, General Directorate for Plant Protection, Directorate for Plant Produce Protection, Department for Pesticides, 150 Sygrou Ave., 17671 Athens, Greece. [2]Benaki Phytopathological Institute, Department of Pesticides Control, Laboratory of Pesticides Toxicology, 7 Ekalis St., 145 61 Kifissia, Greece.*
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When maximum residue levels (MRLs) are to be set, short-term as well as chronic intake is estimated, to prove that the MRLs are safe for the consumer. In this way it is ensured that the potential intake of pesticide residues through the diet does not exceed the toxicological limits of the acute reference dose (ARfD) and the acceptable daily intake (ADI). The estimate of consumer short-term intake within the EU but also within the countries adhering to the Codex Alimentarius is based on a deterministic model. The variables that affect the equation of this model are: LP (highest large portion), HR (highest residue in a mixed sample), HR-P (highest residue for processed food commodity), bw (body weight of consumers), U (unit weight) and v (variability factor). This work on the one hand studied the approach already followed in the EU Member States and other International Organizations (Codex Alimentarius), and on the other hand examined how any changes in the variables affect short-term intake-estimates, and hence, the MRLs that are set.

Pesticide residues in and on food: Comparison of control procedures before and after authorization is granted. M. KASTANIAS and D. VLACHOS. *Ministry of Rural Development and Food, General Directorate of Plant Produce, Directorate of Plant Produce Protection, Department for Pesticides, 150 Sygrou Ave., 17671 Athens, Greece.*
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According to Council Directive 91/414/EEC, a plant protection product (PPP) in the EU is authorized only if the product is recognized as effective and is not directly or indirectly harmful to human or animal health or to the environment. In Greece the competent authority for PPPs is the Ministry of Rural Development and Food. One of the requirements for granting a PPP is that it any residues must be controlled, i.e. maximum residue levels (MRLs) must be set and adhered to. The MRL determines the PPP residue levels in food and feed that are toxicologically safe when the PPP is used as authorized. To set MRLs, the following is required: a) determination of metabolism in plants, b) determination of the metabolism and feeding of the livestock, c) supervised residue trials in crops, food and feedstuffs, and determination of the effect of industrial processing on the nature and magnitude of the residues that will be created, d) the residues in subsequent crops are also evaluated and an assessment of acute and chronic dietary risk is conducted in order to ensure food safety. Pesticide residues are controlled in the market once the MRLs have been set. Such control is exercised in domestic and imported agricultural products of plant origin. PPPs are authorized on the understanding that good agricultural practice must be followed and that food safety is paramount. PPPs are controlled in conformity with an annual programme and control procedures are defined in EU Directives.

Effect of fungicide residues on the growth of *Saccharomyces cerevisiae* ph.v. *bayanus* (Fermol Blanc®). G. KATSIKOGIANNIS[1], M. LIOUNI[2] and F. FLOURI[3]. *[1]Directorate of Rural Development of Samos, Crop Protection Department, Vathi Samou, 831 00 Samos, Greece. [2]National and Kapodistrian University of Athens, Faculty of Chemistry, Industrial Chemistry Laboratory, Panep/poli, 157 01 Ilissia, Athens, Greece. [3]Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos St., 118 55 Athens, Greece.*
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Fungicides used to control *Plasmopara viticola* and *Uncinula necator* in vineyards may inhibit alcoholic fermentation. This work examined the effect of the residues of eleven widely used fungicides (azoxystrobin, captan, fenarimol, folpet, metalaxyl-M, penconazole, propineb, spiroxamine, tebuconazole, triadimefon and triadimenol) on the growth of the commercial wine dry yeast strain *Saccharomyces cerevisiae* ph.v. *bayanus* - Fermol Blanc®). Seven concentrations of each active substance were applied. An inoculum level of 10^5 cells l^{-1} culture was used (the recommended dose). Yeast growth was monitored at several time points by measuring absorbance in a microplate reader. The time point of 21 h (late exponential phase) was selected to evaluate the inhibition rates. Captan, folpet and tebuconazole totally inhibited yeast growth at concentrations substantially below or around the maximum residue levels (MRLs). In contrast, azoxystrobin, fenarimol and metalaxyl-M had no effect on growth even at 100× the MRL concentrations. Variable inhibition rates (6.5–76% of the control) were found when penconazole, propineb, spiroxamine, triadimefon and triadimenol were applied at 5x the MRL concentration. In most cases, a still higher inoculum level (10× the recommended dose) lowered the inhibition rate achieved with the fungicide residues.

Study of the inherent resistance risk to the benzamide fungicide zoxamide in *Phytophthora infestans*. Ch. KRITIKOS, A.N. MARKOGLOU and B.N. ZIOGAS.
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Mutants of *Phytophthora infestans* with high resistance (Rf:>1.000) to the benzamide fungicide zoxamide were isolated from a wild-type strain, at a low mutation frequency of 2.7×10^{-11}, after UV-mutagenesis and selection on a medium containing 1 μg/ml zoxamide. Cross-resistance studies with other oomycete fungicides from different chemical groups showed that the mutation(s) for resistance to zoxamide also greatly reduced the sensitivity of the mutant strains to the amidocarbamates iprovalicarb and benthiavalicarb, the cyanoimidazole cyazofamid, the phenylamide metalaxyl-m, the acetamide cymoxanil, the morpholine dimethomorph and to chlorothalonil. Mutant strains were also less sensitive to the Qo inhibitors of the cytochrome bc_1 complex of the mitochondrial respiratory chain azoxystrobin and pyraclostrobin (strobilurins) and famoxadone (azolone). A resistance correlation was not apparent for the dithiocarbamate propineb and the phenylpyridinamine fluazinam. A study of the fitness parameters of the wild-type and mutant strains of *P. infestans* showed that most resistant isolates had significantly lower sporulation and sporangial germination, but that there was no reduction in the mycelial growth rate or in the differentiation of sporangia into zoospores. Pathogenicity tests on tomato seedlings showed that most resistant isolates were as pathogenic as the wild-type parent strain. This is the first report on the isolation of mutant strains of *P. infestans* highly resistant to the novel site-specific oomycete fungicide zoxamide, and to chemically unrelated fungicides inhibiting different sites of the cellular pathway.

Phytopathological and molecular characterization of laboratory mutants of *Cercospora beticola* resistant to Qo inhibitors. A.A. MALANDRAKIS, A.N. MARKOGLOU, D.C. NIKOU, J.G. VONTAS and B.N. ZIOGAS. *Laboratory of Pesticide Science, Agricultural University of Athens, 75 Iera Odos, Votanikos, 188 55 Athens, Greece.*
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Mutants of *Cercospora beticola* with high (Rf: 80 to more than 200, based on EC_{50}), moderate (Rf: 50–70) or low (Rf: 3–5) resistance to pyraclostrobin were isolated from a wild-type strain after UV-mutagenesis and selection on a medium containing pyraclostrobin. Fungitoxicity tests on the response of resistant isolates to a medium containing pyraclostrobin and salicylhydroxamate (SHAM), a specific inhibitor of cyanide-resistant (alternative) respiration, indicated that the biochemical mechanism of alternative oxidase did not lower the sensitivity to pyraclostrobin that was seen in half the mutants. Cross-resistance studies with other fungicides showed that the mutation(s) for resistance to pyraclostrobin also reduced the sensitivity of the mutant strains to other QoIs, such as azoxystrobin and fenamidone, but not to the QoI cyazofamid, the carboxamide boscalid, the triazoles epoxiconazole and flutriafol, or the benzimidazole benomyl, which affect other steps of the cellular pathway. A study of the fitness parameters showed that most mutants significantly reduced sporulation and pathogenicity compared with the wild-type parental isolate. However, experiments on the stability of the resistant phenotype did not show a significant reduction of resistance when it was grown for at least four generations on a pyraclostrobin-free medium. Molecular analysis of cytochrome *b* cDNA, isolated from the wild-type and the pyraclostrobin-resistant mutant isolates, revealed two novel amino acid replacements at positions involved in Qo resistance in other species. The replacement of glycine (GGT) by serine (AGT) at position 143 (G143S) was found in two isolates of the highly resistant phenotype, whereas a second replacement, of phenylalanine (TTC) by valine (GTC) at position 129 (F129V), occurred in a mutant strain of the moderately resistant phenotype. Four additional mutations located in conserved regions of the mitochondrial cytochrome *b* gene (I154L, N250D, E256G and V261D) were detected in some mutant isolates of *C. beticola,* but their possible role in Qo-resistance needs to be further investigated. This is

the first report on *C. beticola* strains becoming resistant to QoI fungicides due to the biochemical mechanism of target-site modification resulting from amino acid changes in the mitochondrial cytochrome b gene.

The sensitivity of wine-making dry yeasts to fungicide residues. N. MARGARITI[1], M. LIOUNI[2] and F. FLOURI[3]. [1]*Department of Agricultural Development of Naxos, 843 00 Naxos, Greece.* [2]*National and Kapodistrian University of Athens, Department of Chemistry, Faculty of Industrial Chemistry, Ilisia, 15701 Athens, Greece.* [3]*Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos St., 118 55 Athens, Greece.*
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The plant-protection products currently used in viticulture may adversely affect alcoholic fermentation. In the present work, some commercial wine-making dry yeasts were examined to determine their sensitivity to residues of nine widely used fungicides. Initially, two active substances, folpet and tebuconazole, were tested against 43 different yeast strains. A varied response was observed from yeast strains when a low concentration (0.6 μg ml^{-1}) of folpet was used, with growth rates varying from 17 to 98% compared to the control. In contrast, tebuconazole at the same concentration permitted a growth of more than 65% of all strains compared to control. Based on this initial screening and their share in the Greek market, 11 dry yeasts were selected for further testing. The active substances, captan, tebuconazole, chlorothalonil, cyprodinil, fludioxonil, fluazinam and vinclozolin were applied at concentrations equal to their maximum residue limit (MRL). Captan and chlorothalonil, which have a similar mode of action, strongly inhibited (>80%) all yeast strains. Inhibition caused by tebuconazole ranged from 13 to 54%, while inhibition due to fluazinam was from 5 to 95%. The active substances vinclozolin, cyprodinil and fludioxonil, as well as a combination of the last two, showed very weak or no inhibition.

Effect of anilinopyrimidine-resistance mutations on the aflatoxigenic ability of Aspergillus parasiticus Speare. A.N. MARKOGLOU, E.G. DOUKAS and B.N. ZIOGAS. *Laboratory of Pesticide Science, Agricultural University of Athens,75 Iera Odos, Votanikos, 188 55 Athens, Greece.*
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Mutants of Aspergillus parasiticus highly resistant (Rf: >100) to the anilinopyrimidines were isolated with a high mutation frequency (1.3×10^{-5}) after UV mutagenesis and selection in a medium containing cyprodinil. Cross-resistance studies with other fungicides showed that the mutation(s) for resistance to cyprodinil also reduced the sensitivity of the mutant strains to other anilinopyrimidines, such as pyrimethanil and mepanipyrim. In addition, a slightly lower sensitivity to chemically unrelated fungicides was observed in a few mutant strains. A study of the fitness parameters in the wild-type and cyprodinil-resistant mutants of *A. parasiticus* showed that the mutation(s) for resistance to anilinopyrimidines did not affect the saprophytic fitness characteristics. Studies on the effect of the mutation(s) on aflatoxin production (AFB_1, AFB_2, AFG_1 and AFG_2), using chromatographic techniques (TLC, HPLC-FLD, LC/APCI-MS), showed that the resistance mutation(s) did not affect fungal aflatoxigenic capacity. These results indicate that there is a risk that *A. parasiticus* alfatoxigenic strains highly resistant to anilinopyrimidines may arise. The incorrect use of anilinopirimides therefore includes an increased risk that aflatoxin will contaminate agricultural products.

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A high multi-drug resistance to chemically unrelated oomycete fungicides in *Phytophthora parasitica* var. *nicotianae*. A.N. MARKOGLOU, J. KALAMPOKIS, E.G. DOUKAS and B.N. ZIOGAS. *Laboratory of Pesticide Science, Agricultural University of Athens, 75 Iera Odos, Votanikos, 188 55 Athens, Greece.*
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Mutants of *Phytophthora parasitica* var. *nicotianae* highly resistant (Rf:>1000) to the amidocarbamate fungicide benthiavalicarb were isolated from a wild-type strain at a low mutation frequency of 6×10^{-10}, after UV-mutagenesis and selection on medium containing 1 μg ml^{-1} benthiavalicarb. *In vitro* fungitoxicity tests showed that all resistant strains were much less sensitive to iprovalicarb. Cross-resistance studies with other oomycete fungicides from different chemical groups showed that the mutation(s) for resistance to the amidocarbamates also greatly reduced the sensitivity of mutant strains to the phenylamide metalaxyl-m, the acetamide cymoxanil, the benzamide zoxamide and the morpholine dimethomorph, but not to the dithiocarbamates propineb and maneb. There was an interesting increase in the sensitivity of mutant isolates to chlorothalonil and to phenylpyridinamine fluazinam. The fitness parameters of mutant isolates showed that the mutation(s) for resistance to amidocarbamates might or might not affect saprophytic fitness determining characteristics such as mycelial growth, sporulation, chlamydospore production, sporiangial germination and pathogenicity on tomato seedlings. This is the first report of a high level of multi-drug resistance to novel oomycete fungicides in *P. parasitica* var. *nicotianae*.

Control of major grape pathogens with a terpene-based new formulation under commercial conditions. V. I. MAVROEIDI[1], E. MARKELLOU[2], I. VLOUTOGLOU[2], A. KALAMARAKIS[2] and K. BLOUKIDIS[1].

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Six field trials were conducted in commercial vineyards in southern and northern Greece to investigate the effectiveness of a new formulation, based on a mixture of terpenes, against grey mould (*Botrytis cinerea*), downy mildew (*Plasmopara viticola*) and powdery mildew (*Uncinula necator*) in wine-producing grape cultivars (*Vitis vinifera*). The terpene formulation was tested against powdery mildew by applying 4 and 8 ml l^{-1} of the formulation at weekly intervals in three vineyards, starting before symptoms of any diseases appeared. The terpene mix was 42–70% effective on the vine leaves, and 26–73% on the bunches, On the bunches there was no significant difference ($P<0.05$) in effectiveness between the terpene mixture and wettable sulphur (the reference product) (35–84%). However, on the leaves the terpene mixture was less effective than wettable sulphur (77–95%). When the terpene mixture was applied curatively at 4, 8 and 12 ml l^{-1}, against grey mould just before harvest (disease severity in controls: 7% of the infected bunch surface area), effectiveness at 8 and 12 ml l^{-1} ranged from 39 to 43% respectively and the terpenes at these rates were significantly more effective ($P<0.05$) than the reference fungicide ROVRAL® 50 WP, iprodione 50% w:w (with 24–25%). *In vitro* tests showed that *B. cinerea* mycelium growth was inhibited by 95% at a concentration of 0.14 μg ml^{-1} of the terpene mixture (EC_{95}). Field trials showed that the terpene mixture had no effect on downy mildew. Preliminary micro-vinification studies in which disease-free bunches were sprayed with the terpene mixture showed that the mixture did not have an adverse impact on the quality parameters of the must or the wine. Experiments are in progress to define the most effective rates and times of application of the terpene mixture.

Effect of fungicide mixtures and dose on selection for triazole resistance in leaf blotch fungus of wheat (*Mycosphaerella graminicola)* under field conditions. V.I. MAVROEIDI[1] and M. W. SHAW[2]. [1]*Ecogen S.A., 1 Karori St, GR-105 51, Athens, Greece.* [2]*School of Plant Sciences, 2 Earley Gate, University of Reading, Reading RG6 6AU, UK.*
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Since systemic site-specific fungicides came into common use in the early 1970s, the problem of fungicide resistance in fungal pathogens of plants has become increasingly important. The evolution of fungicide resistance in field populations is in outline a simple process. The existence and the amount of selection for resistance after pesticides are adopted for use against a population depends on many factors. These may be either pathogen-dependent (biology, genetics, and epidemiology), fungicide-dependent (type of compound, dose, frequency of application), or host-dependent (architecture, host resistance). In the study reported here we focused on the selection pressure caused by the fungicides used and their doses. The effects of varying doses of fungicides, alone or in mixture, on selection for triazole resistance were examined under field conditions. Two experiments were conducted using the triazole fungicide fluquinconazole with the strobilurin fungicide azoxystrobin as a mixture partner. Inoculated wheat plots with a known ratio of more sensitive to less sensitive isolates of the leaf blotch fungus *Mycosphaerella graminicola* were sprayed with this fungicide and sampled once symptoms had appeared. Selection for fluquinconazole resistance increased in proportion to the dose, up to one-half of the full dose (the maximum tested) in both experiments. At doses of fluquinconazole higher than one half the full dose, the addition of azoxystrobin was associated with a decrease in selection (not significant in the first experiment) for triazole resistance. Control by low doses of fluquinconazole was increased by the mixture with azoxystrobin, but at higher doses a mixture with azoxystrobin sometimes decreased control, so that a reduced selection was obtained at the cost of some reduction in control. A mixing of fungicides does not invariably lead to greater resistance, so that the properties of any specific mixture need to be demonstrated experimentally. Selection was inversely related to control in the unmixed treatments in both experiments, but the relationship was weaker in the mixtures with azoxystrobin.

Monitoring the sensitivity of *Botrytis cinerea* isolates to the fungicides anilinopyrimidine, phenylpyrrole, hydroxyanilide, benzimidazole and dicarboximide. C.K. MYRESIOTIS[1], G.S. KARAOGLANIDIS[2] and K. TZAVELLA-KLONARI[1]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O.B 269, 54124 Thessaloniki, Greece.* [2]*Hellenic Sugar Industry S.A., Plant Protection Department, Sugar Factory of Platy, 59132 Platy Imathias, Greece.*
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In a study conducted in 2004–2006, 55 single-spore isolates of *Botrytis cinerea*, were tested for sensitivity to five groups of fungicides: the anilinopyrimidines (cyprodinil and pyrimethanil), the hydroxyanilides (fenhexamid), the phenylpyrroles (fludioxonil), the dicarboximides (iprodione) and the benzimidazoles (carbendazim). The isolates were collected from vegetable crops (cucumber, eggplant, tomato) grown in greenhouses in Crete, Greece. Some 61.81% of the isolates were resistant to carbendazim, and 18.18% were resistant to iprodione. For the first time, a strong resistance to the anilinopyrimidine fungicides was detected in greenhouse vegetable crops on Crete, with resistance frequencies of 57.40 for cyprodinil and 48.14% for pyrimethanil. In addition, one isolate was resistant to fenhexamid, while no strains resistant to fludioxonil were

detected. Of the 55 isolates tested, four were resistant to both carbendazim and iprodione, 13 were resistant to both carbendazim and anilinopyrimidines, six were resistant to both iprodione and anilinopyrimidines, one isolate was resistant to carbendazim, iprodione and cyprodinil, one isolate was resistant to both anilinopyrimidines and fenhexamid, and eight isolates were sensitive to all the fungicides tested. Monitoring for cross-resistance showed that there was a strong relationship between the two anilinopyrimidine fungicides cyprodinil and pyrimethanil (r=0.71). Despite the detection of several phenotypes with simultaneous resistance to chemically unrelated active ingredients, none of the remaining possible fungicide pairs showed any kind of cross-resistance.

Molecular characterization of DMI-resistance in *Cercospora beticola*. D.C. NIKOU, A.A. MALANDRAKIS, J.G. VONTAS, A.N. MARKOGLOU and B.N. ZIOGAS. *Laboratory of Pesticide Science, Agricultural University of Athens, 75 Iera Odos, Votanikos, 188 55 Athens, Greece*.
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Cercospora beticola mutants with high and moderate resistance to epoxiconazole were isolated from sugarbeet fields in Serres, Veria, Xanthi and Orestiada, Greece, that had been heavily sprayed with triazoles for a number of years. Cross-resistance studies showed that epoxiconazole-resistant strains were less sensitive to the triazole flutriafol but not to the benzimidazole benomyl or the carboxamide boscalid. A slight reduction in sensitivity to the QoIs pyraclostrobin, azoxystrobin and fenamidone was observed in a few mutant strains. Resistance mutations did not have any adverse effect on phytopathogenic fitness. Most mutants retained their resistance even after four generations on fungicide-free medium. Using primers designed on conserved sequences and RACE techniques, the c14 demethylase (CYP51) gene – the target site of the DMI fungicides - was isolated from mutant and wild type strains. Transcription levels of the C-14 demethylase gene are also being compared between resistant and wild type strains using real-time PCR.

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The possible anti-viral activity of the strobilurin pyraclostrobin against *Cucumber mosaic virus*. C. VARVERI[1], M CHRISTOPOULOU[1], E. MARKELLOU[1], N. VASSILAKOS[1], A. TZIMA[1] and D. SERVIS[2]. *[1]Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Greece. [2]BASF Agro Hellas S.A., Aigialeias 48, 151 25 Paradeisos, Amarousiou, Greece.*
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Cucumber mosaic virus (CMV) is one of the most serious and destructive viruses in tomatoes; and one of the most difficult to control. The possible anti-viral activity of the strobilurin fungicide pyraclostrobin, of which there are indications that it elicits plant defense mechanisms, was investigated in greenhouse experiments. Potted tomato plants were sprayed with pyraclostrobin (formulation F 500 containing 25% a.i. w:v, application rate 0.8 ml formulation l^{-1} water), 24 or 48 hours before mechanical inoculation with the virus. Plants were monitored for virus infections at regular intervals by ELISA. Fungicide-treated plants were more resistant to the virus than untreated plants. The fungicide treated plants showed a lower disease incidence and slower development of infection when the virus was inoculated 24 hours after treatment. Overall results of three experiments with different levels of inoculum pressure showed that one application of the fungicide on average increased the number of healthy plants by 10–15% compared to the controls 20 days after inoculation. The greatest difference between treatments in terms of percentage of healthy plants was recorded one week after inoculation. Specifically, it was found that i) with high inoculum pressure, F 500 increased healthy plants by 40%, ii) with medium pressure, it increased healthy plants by 35%, and iii) with low pressure, healthy plants were increased by 20% compared with the controls. When a second application of F 500 was carried out one week after the first, the difference in percentage of healthy plants between the fungicide and the control was 70% 20 days after inoculation with the virus. The results indicated that pyraclostrobin induced defense mechanisms in tomatoes against CMV when the plants were challenged with the virus shortly after fungicide application. When inoculations were carried out 48 hours after fungicide treatment, on the other hand, differences between treatments were no longer significant. This is the first study of the possible anti-viral activity of a strobilurin fungicide under greenhouse conditions.

Disease management in a controlled plant production system aiming at certification. Correlation with legal demands and controls of plant protection products. D. VLACHOS. *Hellenic Ministry of Rural Development and Food, General Directorate of Plant Produce, Directorate of Plant Produce Protection, Department of Pesticides, Sygrou 150, 17671 Athens, Greece.*
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The need to apply integrated plant protection methods to control plant diseases, in a context of an internal and external market demand for high-quality agricultural products, and particularly the need to comply with relevant legal requirements, is leading more and more producers/farmers to adhere to farm certification standards, such as EUREPGAP, AGRO 2.1. and AGRO 2.2. The control points and conformity criteria of the certification standards and the relevant checklists for plant protection products cover the legal requirements governing the use of PPPs. The need to implement continually updated

certification systems keeps the producer/farmer aware of any changes in PPPs legislation so that they can apply them to their agricultural practice. Disease management in a controlled plant production system aiming at certification not only improves the quality of the products but also enables producers/farmers to comply in advance with any legal demands that may be made during an official inspection.

The legal framework and procedures relating to the control of plant protection products in the market: a contribution to the production of quality agricultural products. D. VLACHOS and M. KASTANIAS. *Ministry of Rural Development and Food, General Directorate for Plant Protection, Directorate for Plant Produce Protection, Department for Pesticides, 150 Sygrou Ave., 17671 Athens, Greece.*
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The production of quality agricultural products, competitive in the domestic and the international market, depends on various parameters and factors. Some of these parameters concern crop protection techniques. Plant protection products (PPPs) for use against diseases and pests in cultivations play a significant role in ensuring the quality of agricultural commodities. The Ministry of Rural Development and Food, as the competent authority for PPPs in Greece, plans, coordinates and conducts control operations in cooperation with the Regional Centers of Plant Protection and Quality Control, the Rural Development Directorates and the Benaki Phytopathological Institute. Controls cover the various phases of the production, sale and use of PPPs. Controls in PPP production include controls of industrial production procedures, the composition and physicochemical properties of the preparations sold by retailers, as well as the label. Control operations of PPPs on the market deal with the function parameters of the sale points in which the PPPs are sold to the users. At user level, controls are targeted to the application and use of PPPs by the farmers, and the monitoring of pesticide residues in products of plant origin. The legal framework of the control system is based on the relevant EC directives. The continuing intensification and increasing frequency of controls in combination with the prohibitive sanctions imposed in cases of any violation of the rules contribute to the production of high-quality agricultural products.

The Safe Use Initiative Project - Experimental Part. F. YDRAIOU[1], M. GASPARI[1], I. SKLAVOS[1], H. FELBER[2], K. MACHERA[3], A. TSAKIRAKIS[3] and D. FAMELIARIS[3]. *[1]Hellenic Crop Protection Association, 53 Patission St., 104 33 Athens, Greece. [2]European Crop Protection Association, Avenue E. Van Nieuwenhuyse 6 1160 Brussels, Belgium. [3]Benaki Phytopathological Institute, 7 Ekalis St., 145 61Kifissia, Greece.*
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The Hellenic Crop Protection Association (H.C.P.A.) in co-operation with the Laboratory of Pesticide Toxicology, Benaki Phytopathological Institute (BPI), undertook a three-year pilot project under the title '*Safe Use Initiative*'. The project, funded by the European Crop Protection Association (E.C.P.A.), was initiated on the 1st of January 2005 and its activities are carried out in Crete. The H.C.P.A. conducted wearer comfort trials using different types of cotton coveralls. Each type of coveralls was tested by farmers who wore them while they sprayed greenhouse crops under realistic conditions. The 2 most ergonomic coveralls were a) cotton with a repellent finish (Nano-Pel), 215 g/m^2, and b) cotton, absorbent, 287 g/m^2. The coveralls were also tested in terms of wearer protection by the Laboratory of Pesticide Toxicology of the BPI by measuring accidental body-penetration (penetration trials). Draft results of the penetration trials indicated that both coveralls provide satisfactory protection. The least penetration was measured with Nano-Pel coveralls, because of their repellent finish based on nano-technology. Apart from the comfort and penetration trials, a new spraying device called *Fumicar* was tested by farmers. *Fumicar* encountered an enthusiastic reception because of its many advantages. One of these is to significantly lower operator exposure during spraying operations compared with ordinary spray applicators. Operator exposure was measured during greenhouse applications in Crete by the Research Agricultural Center of Ghent. The same equipment was also tested by the crop protection industry for its biological effectiveness.

Safe Use Initiative Project - Training of Farmers – Communication. F. YDRAIOU[1], M. GASPARI[1], I. SKLAVOS[1], H. FELBER[2], K. MACHERA[3], A. TSAKIRAKIS[3] and D. FAMELIARIS[3]. *[1]Hellenic Crop Protection Association, 53 Patission St. 104 33 Athens, Greece. [2]European Crop Protection Association, Avenue E. Van Nieuwenhuyse 6 1160 Brussels, Belgium. [3]Benaki Phytopathological Institute, 7 Ekalis St., 145 61 Kifissia, Greece.*
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The Safe Use Initiative project is a three-year pilot project initiated in 2005 and supervised by the Hellenic Crop Protection Association (H.C.P.A.). One of the aims of the project is to provide training and information on the safe and responsible use of plant protection products, on personal protection equipment and on environmental matters. In this context, the H.C.P.A. has prepared educational material (a booklet and a DVD) which will be used by OGEEKA-Dimitra to train farmers. The H.C.P.A. along with the Laboratory of Pesticide Toxicology of the Benaki Phytopathological Institute will themselves provide training for these official trainers. Training will start in Crete and the intention is to extend it to at least the main agricultural areas. Moreover, demo-farms will be established for practical applications.

For the communication campaign, radio and TV messages, posters and newsletters have been prepared. The radio messages have been transmitted by radio stations in Crete, while the TV messages have been accepted by the Greek National Council for Radio and Television as communications of social importance and have been broadcast by national and local TV. The 'Safe Use Initiative' project has been presented to the mass media, conferences, exhibitions, farmers, educational institutes, universities and officials such as those in the Ministry of Rural Development and Food. Finally, a website (www.safeuse.gr) has been set up which is regularly updated and which provides the latest results and developments.

Molecular analysis and the detection of fungicide resistance in plant-pathogenic fungi: the example of benzimidazole-resistance in *Botrytis cinerea*.
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Molecular techniques have recently made it possible to explore the resistance mechanisms of fungi down to the DNA level, allowing us to develop novel strategies with which we can truly manage resistance. Laboratory and field mutant isolates of *Botrytis cinerea* resistant to the benzimidazole fungicides, and the molecular basis of their resistance was investigated. Mutants of *B. cinerea* moderately (Rf: 10–20) resistant to the benzimidazoles, and having wild-type sensitivity to diethofencarb, were isolated at low mutation frequency (6.7×10^{-8}) from a medium containing carbendazim and diethofencarb. Strains highly resistant to the benzimidazoles (Rf >100) but sensitive to diethofencarb were isolated with a high mutation frequency (5.3×10^{-4}) from a medium containing carbendazim. All field isolates were benzimidazole-resistant and sensitive to dienthofencarb. A study of the fitness parameters of the diethofencarb-sensitive isolates showed that the mutation(s) for resistance to benzimidazoles had no apparent effect on the fitness-determining parameters. By contrast, the benzimidazole-resistant strains that were not more sensitive to diethofencarb had a significantly lower pathogenic fitness. Analysis of the sequence of b-tubulin, the target site of the benzimidazole fungicides, in resistant strains revealed two amino acid replacements as compared with the wild-type sequence. One was the replacement of glutamic acid with alanine at position 198 (E198A), which was identified in the highly resistant strains, a mutation previously implicated in benzimidazole resistance; the other was a novel resistant mutation, a replacement of alanine with glycine at the same position (E198G), which was found in strains with a moderate resistance level. This second mutation is reported for the first time in *B. cinerea*. The current diagnostic molecular assay used to detect altered-tubulin based resistance in field isolates of *B. cinerea* does not detect this latter resistant allele, and hence this assay may cause the prevalence of resistance to be underestimated. A novel diagnostic PCR-RFLP assay was developed to detect both resistant alleles simultaneously.

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