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REVIEW ARTICLE

New plant pathogens reported in Greece, 1990-2007

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Summary A summary of new plant pathogen records in Greece during the period 1990-2007 is presented based on data published in national and international literature. Plant pathogenic fungi, bacteria, viruses and viroids are considered.

A total of 47 new plant pathogenic fungi have been reported, most of which cause damaging plant diseases in Greece and only few seem to have a negligible effect. The new pathogens were found on a variety of host plants, annual or perennial, cultivated or forest species, vegetables or ornamentals etc. Based on available information up to now, the introduction of *Ceratocystis platani,* the young-grapevine decline and esca disease pathogens and the new vascular wilts caused by *Fusarium oxysporum* are among the most alarming cases. Additionally, Alternaria brown spot on Minneola mandarins in Northwestern Greece and *Phytophthora boehmeriae* in irrigated cotton crops create severe problems.

Five new records of plant pathogenic bacteria have been reported. All five pathogens were found on horticultural crops and the disease symptoms caused to them were described. The pathogens which were detected and identified were *Pantoea ananas* on watermelon, *Pseudomonas syringae* pv. *apii* on celery, *Pseudomonas syringae* pv. *porri* on leek, *Xanthomonas campestris* pv. *vitians* on lettuce and *Xanthomonas cynarae* on artichoke. The pathogens, although not yet widespread in the country, may have a serious economic impact since they can cause damage by reducing the market value or rendering harvest of the affected crops unmarketable.

Fifty-two new viruses and viroids have been reported and characterized. Of these, nine viruses represent the most serious threat, since they are transmitted by insect vectors able to disseminate them further and they affect important crops such as citrus, tomato, cucumber and potato. These viruses are: *Citrus tristeza virus* (CTV), *Tomato yellow leaf curl virus* (TYLCV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV), *Tomato chlorosis virus* (ToCV), *Tomato infectious chlorosis virus* (TICV), *Beet pseudo-yellows virus* (BPYV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Cucumber aphid borne yellows virus* (CABYV) and *Potato virus Y*^{NTN} (PVY^{NTN}). Eradication, application of strict quarantine procedures and use of virus-free propagation material are some of the most important measures for their control. Almost half of these reports derived from survey work aiming at estimating the sanitary situation of vegetatively propagated crops as a first step towards the production of healthy propagation material. Molecular methods developed during last years have greatly contributed to the identification and characterization of the viruses and viroids reported.

Introduction

New records following accidental importation of non-native plant pathogens, pests and weeds into a country has been greatly facilitated by the icreasing free trade of plants, plant propagation material and plant products and the improving long-distance transport possibilities. In addition, new records often arise from pre-existing

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plant pathogens, pests and weeds whose recognition is becoming possible with the improving diagnostic techniques and the revised identification procedures. Both cases create the need for a closer watch if the real risk to plant production is to be assessed. Furthermore, a more regular revision of national checklists of pathogens, pests and weeds is becoming necessary.

Comprehensive lists of plant pathogens and pests and weed species occurring in Greece have been published up to 1990. For the period 1990-2007 an attempt has been initiated by scientific staff of the Benaki Phytopathological Institute to elaborate on data published in national and international literarure and present a summary of the new records during this period. The attempt is finally aiming at obtaining a reliable update of the respective national checklists.

New records of plant pathogens, including fungi, bacteria, viruses and viroids, are presented in this article. New records of plant pests and weed species for the same period will be presented in a second article that is to be published in the next issue of this journal.

1. Plant pathogenic fungi

Plant pathogenic fungi recorded in Greece during the period 1990–2007 are presented below in an order related to the importance of the host they were found on and the disease they cause. In cases that a number of species from the same genus are involved, they are presented under the name of the genus. The names of all new pathogens are listed in alphabetical order in Table 1.

◆ ***Ceratocystis platani***

Ceratocystis platani (J.M. Walter) Engelbr. and T.C. Harr. [syn. *C. fimbriata* (Ellis and Halsted) Davidson f. sp. *platani* Walter] was reported to cause canker stain disease of plane tree, in natural populations of the important riparian species oriental plane tree (*Platanus orientalis* L.), in a small area of Southwestern Peloponnese (Southwestern Greece). Cankers were found on both trunks and branches. The inner bark and the cambial region of the cankered area were discoloured bluish-black and the underlying wood stained dark reddish-brown to bluish-black. In cross section, the stained wood formed characteristic radial patterns.

C. platani is considered to be indigenous to the USA, while in Europe the pathogen has caused severe attacks in Italy, France and Switzerland. Genetic analyses of Greek isolates, using nuclear and mitochondrial DNA fingerprints, showed the fungus to be identical to the genotype reported from Italy, France and Switzerland. Earlier studies indicated that the most common European genotype had been introduced from eastern North America to Italy during World War II. The recent introduction into Greece appears to have originated from Italy, France, or Switzerland, rather than from Northeastern America.

The pathogen is an EPPO A2 quarantine organism having a dramatic impact on the natural population of *P. orientalis* in Southwestern Greece and containment measures should be imposed before it spreads throughout the natural range of this ecologically and historically important host (Ocasio-Morales *et al.*, 2007; Tsopelas and Angelopoulos, 2004).

◆ ***Phaeomoniella chlamydospora, Cylindrocarpon destructans, Phaeoacremonium* sp.**

Phaeomoniella chlamydospora (W. Gams, Crous M.J. Wingfield and L. Mugnai) Crous and W. Gams and other anamorphic fungi such as *Cylindrocarpon destructans* (Zins.) Scholten and *Phaeoacremonium* sp. were involved in the young-grapevine (*Vitis vinifera* L.) decline and have been isolated from the wood of rooted vine cuttings which show brown to black streaks in longitudinal or vertical sections countrywide. *P. chlamydospora* together with *Fomitiporia medi-*

terranea M. Fischer also cause esca disease complex of grapevine.

A dramatic upsurge of esca disease occurrs the last years, not only in old but also in young vineyards and also in other hosts (olive, citrus, kiwifruit) in Greece (Elena *et al*., 2003; Elena *et al.,* 2006; Elena and Paplomatas, 2002; Paplomatas *et al.,* 2006; Rumbos, 2001; Rumbos *et al.,* 2006).

◆ ***Fusarium* spp.**

◊ ***Fusarium oxysporum***

New formae speciales of *Fusarium oxysporum* Schlechtend.: Fr caused severe diseases to several crops.

Root-stem rot and wilt of cucumber (*Cucumis sativus* L.) caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* Vakal. were reported for the first time. The disease caused devastating losses in greenhouse cucumber crops and within few years it gradually spread in the major cucumber producing areas of Crete and the remaining Greece (Vakalounakis, 1996).

Fusarium oxysporum f. sp. *cumini* Prasad and Patel is responsible for early pre-harvest plant death which has resulted in severe losses in cumin (*Cuminum cyminum* L.) yield in the island of Chios. The use of seeds from healthy crops and the avoidance of cultivation of cumin in severely infested sites were recommended (Pappas and Elena, 1997).

Mimosa [*Albizia julibrissin* (Willd.) Durazz.] was found with symptoms of a wilt disease caused by *Fusarium oxysporum* f. sp. *perniciosum* Hepting and many trees were dying or dead in Thessaloniki (Northern Greece). In the more advanced stages of the disease, dark sap exudations flowed out of bark cracks (Skarmoutsou and Skarmoutsos, 1999).

Fusarium wilt of sweet basil (*Ocimum basilicum* L.) caused by *Fusarium oxysporum* f. sp. *basilici* (Dridrariya) Armst. is now a serious problem for the commercial crops in Greece. Of 14 tested commercial basil cultivars 6 out of 8 large-leaved cultivars were resistant, while all 6 small-leaved cultivars were susceptible (Biris *et al.,* 2004).

A severe disease was observed on Canary Island palm (*Phoenix canariensis* Hort. Ex Chab.) caused by *Fusarium oxysporum* f. sp. *canariensis* first in Attica (Southeastern Greece) and then in other counties. First symptoms began on the mature pinnae at the base of the plant, which became dry. Initially the leaflets on one side of the rachis died but eventually the entire plant died (Elena, 2005).

Leaf yellowing and brown discoloration caused by *Fusarium oxysporum* f. sp. *nicotianae* (J. Johnson) Snyder and Hansen, was observed in tobacco (*Nicotiana tabacum* L.) plants cv. Burley TN97 in Central Greece. The pathogen was present in tobacco seed batches imported in 2000 and 2001, which indicates that the infected seed was most probably the primary source of the disease in Greece (Tjamos *et al.,* 2006).

◊ ***Fusarium compactum***

Fusarium compactum (Wollenw.) Gordon caused foot rot of banana (*Musa* sp.), grown under glass in Crete (Frisullo *et al.,* 1994).

◆ ***Alternaria* spp.**

Different new species or pathotypes of the genus *Alternaria* were found to cause diseases in Greek crops.

Leaf spot disease of cucumber and melon (*Cucumis melo* L.) caused by *Alternaria alternata* (Fr.: Fr.) Keissler f. sp. *cucurbitae* Vakal. was recorded for the first time in Crete (Vakalounakis, 1990a, b).

In the area of Marathon (Southeastern Greece) many chicory (*Chichorium* sp.) crops were infected by *Alternaria sonchi* J.J. Davis (Grigoriou, 1992b) and severe infection of carnation (*Dianthus caryophyllus* L.) petals by *Alternaria dianthicola* Neerg. was observed in open-field crops of carnation (Grigoriou, 1996b).

A new proposed pathotype *Alternaria alternata* f. sp. *helianthina* caused leaf spot disease of sunflower (*Helianthus annuus* L.) in Northern Greece (Lagopodi and Thanassoulopoulos, 1996).

Serious leaf spotting was observed on *Dichondra repens* L. caused by the fungus *Alternaria dichondrae* Gambogi, Vannacci & Triolo in Attica (Southeastern Greece) (Vloutoglou and Lascaris, 2000).

Severe leaf spotting on poinsettia (*Euphorbia pulcherrima* Willd. ex. Klotzsch) leaves occurred in red bract glasshouse culture in Thessaloniki (Northern Greece), caused by *Alternaria euphorbiicola* E.G. Simmons & Engelhard (Eleftheriadou and Tahmatsidou, 2001).

Alternaria brown spot on Minneola mandarins (*Citrus reticulata* Blanco x *Citrus paradisi* Macf.) caused by *Alternaria alternata* pv. *citri,* was observed in Northwestern Greece causing drop of leaves and fruits. Mandarins Minneola, Nova and Page were very susceptible to Greek *Alternaria* isolates tested while Clementine SRA and Poros Clementine were resistant (Elena, 2006a).

◆ ***Phytophthora* spp.**

Two new *Phytophthora* species were identified during last years.

A boll rot of cotton (*Gossypium hirsutum* L.) caused by *Phytophthora boehmeriae* Sawada was observed first in the Larissa and Volos areas (Central Greece) and later became a severe problem in all cotton growing areas. When the soil was irrigated the pathogen formed its sporangia which disseminated by the rain or sprinkler irrigated drops (splash-dispersed) to the lower half to two-thirds of cotton plants. Usually fields with drip irrigation were not affected (Elena and Paplomatas, 1998).

Root rot of parsley [*Petroselinum crispum* (Mill.) Nym. Ex A.W. Hill] caused by *Phytophthora primulae* Tomlinson caused yield losses in commercial fields during the winter at the area of Marathon (Elena and Grigoriou, 2007).

◆ ***Armillaria* spp.** and ***Heterobasidion* spp.**

New *Armillaria* species have been reported to cause root diseases of woody agricultural plants and forest trees.

Armillaria tabescens (Scopoli) Emel was detected in almond [*Prunus dulcis* (Miller) D.A. Webb] orchards in Central Greece causing considerable damage (Tsopelas and Tjamos, 1997).

Armillaria gallica Marxm. and Romagn. was common in coniferous and broadleaved forest trees in the high altitudes of Central and Northern Greece, predominating in beech (*Fagus* sp.) forests (Tsopelas, 1999).

Armillaria ostoyae (Rom.) Herink has a wide distribution in the mountain forests of Northern Greece and causes significant damage on fir (*Abies borisii-regis* Mattf.), black pine (*Pinus nigra* Arnold), Scots pine (*Pinus sylvestris* L.) and spruce [*Picea abies* (L.) Karst.] (Tsopelas, 1999).

Armillaria cepistipes Velen. was recorded at high altitudes on mountains of Northern Greece (Tsopelas, 1999).

Three other basidiomycetes: *Heterobasidion annosum* (Fr.) Bref., *Heterobasidion parviporum* Niemela and Korhonen and *Heterobasidion abietinum* Niemela and Korhonen caused root and butt rot on coniferous forest trees in the highlands of Greece (Tsopelas and Korhonen, 1996; Niemelä and Korhonen, 1998).

◆ ***Phomopsis* spp.**

Phomopsis mali Roberge causing fruit decay of apple (*Malus domestica* Borkh. cv. Red Chief) was observed in the region of Imathia (Northern Greece) (Karaoglanidis and Bardas, 2006). The pathogen has previously been reported on pear (*Pyrus communis* L.) (Holevas *et al.,* 2000).

In the same area, the fungus *Phomopsis amygdali* (Del.) Tuset & Portilla was frequently isolated from decayed peaches [*Prunus persica* (L.) Batsch. cv. Andross) (Michailides and Thomidis, 2006).

The species *Phomopsis obscurans* (Ellis & Everh.) Sutton caused leaf spot on strawberry (*Fragaria vesca* Coville), in the orchard of the Agricultural University of Athens (Grigoriou, 1992a).

Stem blight of asparagus (*Asparagus officinalis* L.) caused by *Phomopsis asparagi* (Sacc.) Grove was found in Western Greece. Elongated, oval-shaped, lesions were formed on the stems (Elena, 2006b).

Table 1. New plant pathogenic fungi in Greece, 1990-2007.

Pathogen	Host	Diseases caused	Reference
Alternaria alternata pv. *citri*	Minneola mandarin	leaf & fruit necrotic spot	Elena, 2006
Alternaria alternata f. sp. *cucurbitae*	cucumber, melon	leaf spot	Vakalounakis, 1990a,b
Alternaria alternata f. sp. *helianthina*	sunflower	leaf spot	Lagopodi & Thanassoulopoulos, 1996
Alternaria dianthicola	carnation	petal necrotic spot	Grigoriou 1996b
Alternaria dichondrae	dichondra	leaf spot	Vloutoglou & Lascaris, 2000
Alternaria euphorbiicola	poinsettia	leaf spot	Eleftheriadou & Tahmatsidou, 2001
Alternaria sonchi	chicory	necrotic flecks of the leaves	Grigoriou 1992b
Aphanomyces cochlioides	sugar beet	damping-off	Lascaris & Doulias, 2000
Armillaria cepistipes	forest trees	root rot	Tsopelas, 1999
Armillaria gallica	forest trees	root rot	Tsopelas, 1999
Armillaria ostoyae	fir, black pine, Scots pine, spruce	root rot	Tsopelas, 1999
Armillaria tabescens	almond	root rot	Tsopelas & Tjamos, 1997
Bipolaris spicifera	kiwi	fruit rot	Bourbos & Skoudridakis, 1992
Botryosphaeria parva	kiwi, English holly, American elm	dieback	Rumbos, 2006a, b
Botrytis elliptica	lilium	necrotic leaf spot	Elena *et al.* 1996
Ceratocystis platani	oriental plane tree	canker stain	Ocasio–Morales *et al.,* 2007; Tsopelas & Angelopoulos, 2004
Cercosporidium punctum	parsley	leaf spot	Grigoriou, 1996
Cryptosporella umbrina	rose	cane death	Paplomatas *et al.,* 2001
Cylindrocarpon destructans	grapevine	young grapevine decline	Elena *et al.,* 2003; Rumbos, 2001, Rumbos *et al.,* 2006
Diplodia cupressi	cypress	canker	Alves *et al.,* 2006; Xenopoulos and Tsopelas, 2000
Fusarium compactum	banana	foot rot	Frisullo *et al.,* 1994
Fusarium oxysporum f. sp. *basilici*	basil	wilt	Biris *et al.,* 2004
Fusarium oxysporum f. sp. *canariensis*	Canary island palm	wilt	Elena, 2005
Fusarium oxysporum f. sp. *cumini*	cumin	wilt & damping-off	Pappas & Elena, 1997
Fusarium oxysporum f. sp. *nicotianae*	tobacco	wilt	Tjamos *et al.,* 2006

Table 1 (continued)

Pathogen	Host	Diseases caused	Reference
Fusarium oxysporum f. sp. *perniciosum*	mimosa	wilt	Skarmoutsou & Skarmoutsos, 1999
Fusarium oxysporum f. sp. *radicis-cucumerinum* f. sp nov	cucumber	wilt & root-stem rot,	Vakalounakis, 1996
Gnomonia comari	strawberry	leaf spot	Grigoriou, 1992a
Hendersonula toruloidea	strawberry-tree	leaf spot	Tsahouridou & Thanassoulopoulos, 2000
Heterobasidion abietinum	forest trees	root & butt rot	Tsopelas & Korhonen, 1996; Niemelä & Korhonen, 1998
Heterobasidion annosum	forest trees	root & butt rot	Tsopelas & Korhonen, 1996; Niemelä & Korhonen, 1998
Heterobasidion parviporum	forest trees	root & butt rot	Tsopelas & Korhonen, 1996; Niemelä & Korhonen, 1998
Marssonina salicicola	weeping willow	leaf spot & shoot cankers	Tzavella-Klonari *et al.,* 1997
Oidium arachidis	peanut	powdery mildew	Bourbos & Skoudridakis, 1996
Oidium magniferae	mango	powdery mildew	Bourbos & Skoudridakis, 1995
Phaeoacremonium sp.	grapevine	young grapevine decline	Elena *et al.,* 2003; Rumbos, 2001; Rumbos *et al.,* 2006
Phaeomoniella chlamydospora	grapevine	young grapevine decline & esca	Elena *et al.,* 2003; Rumbos, 2001; Rumbos *et al.,* 2006
Phomopsis asparagi	asparagus	stem blight	Elena, 2006
Phomopsis amygdali	peach	fruit decay	Michailides & Thomidis, 2006
Phomopsis mali	apple, pear	fruit decay	Holevas *et al.,* 2000; Karaoglanidis & Bardas, 2006
Phomopsis obscurans	strawberry	leaf spot	Grigoriou, 1992b
Phytophthora boehmeriae	cotton	boll rot	Elena & Paplomatas, 1998
Phytophthora primulae	parsley	root rot	Elena & Grigoriou, 2007
Plasmopara halstedii	sunflower	downy mildew	Thanassoulopoulos & Mappas, 1991
Puccinia horiana	chrysanthemum	rust	Vakalounakis, 1997
Verticillium dahliae (defoliating pathotype)	cotton	wilt	Elena & Paplomatas, 2001
Verticillium tricorpus	potato	tuber rots	Thanassoulopoulos & Giapanoglou, 1994

◆ ***Other fungi***

Plasmopara halstedii (Farl.) Berl. de Toni, causing downy mildew of sunflower, was found in Thrace (Northern Greece) (Thanassoulopoulos and Mappas, 1991).

The fungus *Bipolaris spicifera* (Bain) Subram. was found to cause soft rot of kiwifruit (*Actinidia deliciosa* C.F. Liang & A.R. Fergu-

son). The fact that it was also isolated from mycoflora of healthy fruits in the field but becomed active later in the marketplace indicated that the fungus was inactive during cool storage (Bourbos and Skoudridakis, 1992).

The species *Gnomonia comari* P. Karst. caused leaf spot on strawberry in the area of Marathon (Southeastern Greece) (Grigoriou, 1992a).

Potato (*Solanum tuberosum* L.) tuber rot caused by the fungus *Verticillium tricorpus* I. Isaak, as a not seriously damaging case, was reported in Northern Greece (Thanassoulopoulos and Giapanoglou, 1994).

Immature leaves of mango (*Mangifera indica* L.) trees, imported from Israel and growing in a greenhouse in Crete, were found to be infected by the fungus *Oidium mangiferae* Berthet, while old leaves were not infected (Bourbos and Skoudridakis, 1995).

Oidium arachidis Chorin, was observed in irrigated peanut (*Arachis hypogaea* L.) crops in Crete, attacking leaves and petioles (Bourbos and Skoudridakis, 1996).

In the Marathon area *Botrytis elliptica* (Berk.) Cooke totally destroyed all the lilium (*Lilium michiganense* L.) plants in a greenhouse (Elena *et al,* 1996) while parsley crops were infected by *Cercosporidium punctum* (Lacroix) Deighton which causes leaf spot (Grigoriou, 1996a).

Chrysanthemum (*Denranthema x morifolium*) cv. Reagan in Crete was affected by the rust *Puccinia horiana* P. Hennings. The pathogen attacked young leaves and symptoms appeared as light-green sunken spots on the upper side of the leaves and bulbous spore pustules on the reverse side (Vakalounakis, 1997).

A serious disease of weeping willow (*Salix babylonica* L.) trees was observed, caused by *Marssonina salicicola* (Bres.) Magnus in Northern Greece. Leaves became distorted and fell. Small, light, sunken cankers, elongated in shape, appeared on the young shoots and on the leaf petioles. Severely affected shoots lost their weeping habit on which the trees ornamental value is based (Tzavella-Klonari *et al.*, 1997).

Aphanomyces cochlioides Drechs. caused damping-off and stunting of sugar beet (*Beta vulgaris* L.) seedlings in Northern Greece. It seems that the fungus is widespread and probably has been present in Greek soils for several years (Lascaris and Doulias, 2000).

Leaves of the wild strawberry-tree (*Arbutus unedo* L.) were heavily spotted with small, necrotic brown spots caused by *Hendersonula toruloidea* Nattrass. in Northern Greece. When intense spotting was present, strong defoliation was observed (Tsahouridou and Thanassoulopoulos, 2000**)**.

The fungus *Sphaeropsis* sp. [identical to *Diplodia pinea* f.sp. *cupressi* (Desmar.) J. Kickx fil., described in Israel], was reported to cause cankers on cypress (*Cupressus sempervirens* L.] in an experimental plot in Western Peloponnese. Later the *Diplodia* of cypress was characterized as new species *Diplodia cupressi* (Alves *et al.,* 2006; Xenopoulos and Tsopelas, 2000).

Brown canker of rose (*Rosa* sp.) caused by the fungus *Cryptosporella umbrina* (Jenkins) Jenkins and Wehm was recorded in Northern Greece, resulted in the death of the main canes or the whole plant (Paplomatas *et al.,* 2001).

The cotton defoliating pathotype of *Verticillium dahliae* Kleb. was first reported in Central Greece during a study of pathogenicity, vegetative compatibility and RAPDs analysis. Moreover the defoliating pathotype has not spread in Greece yet (Elena and Paplomatas, 2001).

Botryosphaeria parva Pennycook and Samuels caused severe dieback symptoms on commercial kiwi plantations cv. Hayward in the areas of Larissa and Volos (Central Greece) (Rumbos, 2006a). The same fungus was also found later to infect the forest species *Ilex aquifolium* Withering (English holly) and *Ulmus montana* L. (American elm). *Ilex aquifolium* is of economic importance and is cultivated in the

mountain of Pelion for its young shoots which are used for decoration during the Christmas days (Rumbos, 2006b).

1.1. Concluding remarks

A total of 47 new plant pathogenic fungi have been reported, most of which cause damaging plant diseases in Greece and only few seem to have a negligible effect. The new pathogens were found on a variety of host plants, annual or perennial, cultivated or forest species, vegetables or ornamentals etc.

Based on available information up to now, the following are among the most alarming cases:

- The introduction of *Ceratocystis platani* to Greece is a threat for the native population of *Platanus orientalis*. It is a recent introduction and infestation is still in a restricted area. An aggressive sanitation program is urgently required to prevent spread of the pathogen to other areas.
- Young-grapevine decline and esca have already become serious problems for Greek viticulture, causing difficulties in replanting programs and yield reductions in established vineyards. Production and use of certified healthy propagation material is the basis to manage these problems.
- Of the new vascular wilts caused by *Fusarium oxysporum,* the most important are the root-stem rot and wilt of cucumber with devastating losses in greenhouse cucumber crops and Fusarium wilt of sweet basil that is now a serious problem for the commercial crops in Greece.
- Alternaria brown spot on Minneola mandarins is a severe problenm in Northwestern Greece.
- *Phytophthora boehmeriae* became a severe problem in irrigated cotton crops. Crops with drip irrigation are usually not affected.

2. Plant pathogenic bacteria

Five new records of plant pathogenic procaryotes were reported in Greece since 1990, which were all bacteria. All pathogens were found on horticultural cultivated plants, such as watermelon, celery, leek, lettuce and artichoke. The bacteria belonged to the genera *Pantoea, Pseudomonas* and *Xanthomonas*. The methods used to detect and identify the pathogens included cultural, physiological, biochemical, pathogenicity, serological, protein electrophoresis and molecular tests.

The new plant pathogenic bacteria are discussed in the text below and summarized in Table 2.

◆ ***Pantoea ananas*** (Serrano) Kergaert, Verdonk & Kersters [syn. ***Erwinia ananas*** Serano; ***Pantoea ananatis*** (Serrano) Kergaert, Verdonk & Kersters]

The pathogen was reported for the first time in Greece, in 1992, to infect watermelon (*Citrullus lanatus* (Thunb.) Mansf.) fruits in the area of Perama, Rethymnon prefecture, Crete (Goumas and Hatzaki 1998). Later the pathogen was found several times in the same area and in the area of Messara, Heraklion prefecture, Crete.

The symptoms of the disease which appeared in the fruit only and not on any other part of the plants, were similar to those of the disease known as "watermelon rind necrosis". No symptoms were seen externally on the fruits during the harvest period. The symptoms however were restricted to the rind of the fruits. Cut fruits showed numerous water soaked, yellowish, brown necrotic spots, which formed necrotic corky areas in the rind. In severely infected fruits the brown necrotic corky areas were extended in the rind, but not in the flesh of the fruit. As a result of the infection the disease, easily visible in cut fruits, severely reduced the market value of the harvest.

The pathogen was detected in plants by direct isolation and identification using morphological, physiological, biochemical and pathogenicity tests (Goumas and

Hatzaki, 1998).

Measures of integrated control (destroy infected plant debris and fruits, crop rotation with resistant hosts, sprays with copper compounds [at least after petal drop]) are recommended to eliminate the disease incidence.

◆ ***Pseudomonas syringae* pv. *apii***
(Jagger) Young, Dye & Wilkie

The pathogen was found in 2003 for the first time in Greece, to infect cultivated celery (*Apium graveolens* L.) plants in the area of Heraklion prefecture and other places of Crete (Goumas and Lalla, 2004).

The symptoms caused by the pathogen, were initially small water soaked spots, circular or angular, usually on old leaves, surrounded by a chlorotic halo. Gradually the spots became yellow, necrotic and brown. Under favorable conditions they enlarged, coalesced and resulted in the necrosis of whole leaves. The pathogen *P. s*. pv. *apii* was reported to be transmitted with celery seeds (Little *et al*., 1997; Goumas and Lalla, 2004) and hot water treatment of seeds was reported (Bant and Storey, 1952), as a measure against the pathogen.

The pathogen was detected in plants by direct isolation, and identification, using morphological, physiological, biochemical and pathogenicity tests.

Since the pathogen affects leaves the main marketable part of the plant, when the infection is spread in the culture, there is a considerable economic loss.

Measures of integrated control (hot water treatment of seeds at 50°C for 25 min., destruction of infected plants and plant debris, use of drip instead of overhead irrigation, sprays with copper componds) are recommended to diminish the disease incidence.

◆ ***Pseudomonas syringae* pv. *porri***
Samson, Shafik, Benjama, Gardan

The pathogen was found in 2000 for the first time in Greece, to infect leek plants (*Allium porrum* L.) a vegetable crop of minor uses cultivated in the area of Megara, Attiki (Glynos and Alivizatos, 2006).

The symptoms appeared on leaves of almost all plants, as small, oily, longitudinal or irregular spots without halo. Later the spots enlarged, coalesced and spread across the leaf blades. Subsequently the leaves showed a chlorosis at the tips, wilted, dehydrated and finally they collapsed. The spread of the infection to the base of the leaves caused their shrinkage and necrosis. Severely diseased plants remained small and unsuitable for the market.

The detection of the pathogen in infected leek plants was easily performed by direct isolation, morphological, biochemical, serological, pathogenicity tests and by analysis of the whole cell protein profile on PAGE.

Measures of integrated control (destroy infected plants and plant debris, crop rotation, avoid overhead irrigation, sprays with copper compounds) of *P.s*. pv. *porri* are recommended.

◆ ***Xanthomonas campestris* pv. *vitians***
(Brown) Dye

The pathogen was found for the first time in Greece in 1992 to cause a serious disease of lettuce (*Lactuca sativa* L.) in Attica (Alivizatos and Glynos, 1992).

The symptoms produced by *X. c*. pv. *vitians* on lettuce leaves were initially small water-soaked oily, circular or angular translucent spots (0.5-1 mm in diameter) which progressively became larger (5-6 mm in diameter) and darkbrown. The symptoms were more advanced in old leaves and resulted in a serious economic loss, since lettuce heads lost their market value.

The detection of the pathogen in infected plants was easily performed by direct isolation and identification using morphological, physiological, biochemical, pathogenicity tests and the profile of cell proteins on PAGE (Alivizatos and Glynos, 1992). A semiselective medium was also reported (Toussaint *et al*., 2001) for the isolation of *X. c.* pv. *vitians* from soil and plant debris. Susceptibility of lettuce to the pathogen varied among culti-

Table 2. New plant pathogenic bacteria in Greece, 1990-2007.

No	Name of the pathogen	Host plant	Reference
1.	*Pantoea ananas*	watermelon	Goumas & Hatzaki, 1998
2.	*Pseudomonas syringae* pv. *apii*	celery	Goumas & Lalla, 2004
3.	*Pseudomonas syringae* pv. *porri*	leek	Glynos & Alivizatos, 2006
4.	*Xanthomonas campestris* pv. *vitians*	lettuce	Alivizatos & Glynos, 1992
5.	*Xanthomonas cynarae*	artichoke	Goumas *et al*., 2002

vars, which were grouped to highly resistant, partially resistant and susceptible ones (Sahin and Miller, 1997; Carisse *et al*., 2000).

Besides lettuce, other natural hosts of the pathogen are orchids, ornamental plants (*Syngonium* sp.), asparagus lettuce and wild lettuce (Bradbury, 1986). Symptoms were also produced by the pathogen on tomato and pepper after artificial inoculation (Sahin and Miller, 1998).

The pathogen is transmitted with contaminated seed (Sahin and Miller, 1997; Carisse *et al*., 2000). The primary sources of inoculum include the infected plant debris in the field and the contaminated seeds for long distance transmission. It is interesting for the epidemiology of the disease that the pathogen was found to survive on leaves of weeds (Barak *et al*., 2001).

Measures of integrated control (use of healthy seed or seed heat treated in hot water at 50°C/20 min, destroy infected plant debris, use drip irrigation, crop rotation, resistant varieties, sprays with copper compounds) are recommended to eliminate the pathogen and the disease.

◆ ***Xanthomonas cynarae*** Trebaol, Gardan, Manceau, Tanguy, Tirily, Boury

The pathogen was found for the first time in Greece, to infect artichoke (*Cynara scolymus* L.) cultivations in the prefecture of Heraklion, Crete (Goumas *et al.,* 2002).

The symptoms of the disease were watersoaked spots on the bract leaves of the artichoke heads. The spots gradually coalesced to form larger brown necrotic areas. No symptoms were observed on other parts of the plants. Among varieties cv. Lardati was more susceptible.

The detection of the pathogen in infected artichokes was easily performed by direct isolation and identification using morphological, biochemical tests and the polymerase chain reaction (PCR).

As a result of the infection of the bracts the quality and the market value of the harvested artichoke heads are depreciated.

Integrated control measures (destroy infected heads, avoid overhead irrigation, sprays with copper compounds) are recommended to diminish the infection.

2.1. Concluding remarks

The five plant pathogenic bacteria reported for the first time in Greece, since 1990, are responsible for serious diseases and considerable economic losses, in many countries. However, they do not currently seem to constitute a serious threat in Greece, since there are not reports of considerable damages and wide spreading of the pathogens in the country yet. It is possible that the integrated control measures recommended after the first detection-identification of the pathogens, were applied successfully to a great extend. An assessment of the cultivar resistance to the pathogens will be useful in future crop rotation schemes.

3. Plant viruses and viroids

The last twenty years research on plant viruses has been extensively carried out by

different research groups in Plant Pathology/Virology laboratories both in Academic and Research Institutions in Greece. These laboratories have adopted the modern molecular detection developed worldwide and also contributed to the development of new ones. As a result, since 1990 a significant number of new viruses and viroids has been identified and characterized as causal agents of new diseases or as the actual causal agents of known and established ones. This increased appearance of new virus/viroid pathogens is mainly due to the extensive international trade of plant propagation material. The risk that these agents represent after their introduction in the country greatly depends upon the exclusion measures undertaken by the farmers and the Ministry of Agriculture (Crop protection division), the availability of efficient virus vectors assuring their further spread and establishment either in the native flora and/or cultivated susceptible crop plants and finally upon the agricultural practices adopted. Also, following the above work on characterization of new viruses/viroids a number of research projects related to the development of reliable detection molecular methods, the production of virus-free propagative material in fruit trees and grapevine and the control of virus-vectors are in progress.

This review paper describes epidemiological data on newly characterized viruses and viroids and attempts to highlight the risks they represent for the crops concerned. Viruses are described and listed according to their mode of transmission.

3.1. Graft transmitted viruses and viroids

- ***Cherry leaf roll virus*** (CLRV, genus *Nepovirus*)

The virus was first reported in walnut (*Juglans regia* L.) trees in Peloponnese (Ilia, Argolida) and Central Greece (Evritania) in 2006 (Sclavounos *et al*., 2006). Affected trees produced fewer fruits exhibiting external necrosis whereas their leaves were showing chlorotic mosaic and ring patterns. CLRV is an important pathogen of walnut where is both seed and pollen transmitted, thus aggravating its potential risk for the crop.

The virus was also detected in asymptomatic olive trees (*Olea europea* L.) (Kaponi and Kyriakopoulou, 2006).

- ***Cherry necrotic rusty mottle virus*** (CNRMV, genus *Foveavirus*) *and* ***Little cherry virus I*** (LChVI, genus *Closterovirus*)

In 2003, a limited scale survey of cherry trees (*Prunus avium* L.) in three counties of Northern Greece (Pieria, Pella, Imathia) revealed a high incidence of CNRMV (36%) and LChVI (32%). Both are perpetuated and spread by infected vegetative propagation material (Maliogka *et al*., 2006a). Further investigations are needed to estimate their presence and effects on production.

- ***Grapevine angular mosaic virus*** (GAMV, genus *Ilarvirus*)

In 1994, characteristic virus-like symptoms were observed on grapevine hybrid *Baresana* × *Baresana* in the collection of the Grapevine Institute in Athens, Greece. In 2000, a new grapevine ilarvirus was isolated from affected plants, characterized and identified as the causal agent of the disease by fulfilment of Koch's postulates (Girgis *et al*., 2000). In addition, the virus is pollen transmitted, but its overall economic importance remains unknown.

- ***Grapevine leafroll-associated viruses***

Grapevine leafroll disease has been known to occur in all grapevine regions in Greece. A number of filamentous viruses are associated with the disease: *Grapevine leafroll-associated virus 1*, *3*, *5* (GLRaV-1, GLRaV-3, GLRaV-5, genus *Ampelovirus*), *Grapevine leaf roll-associated virus 4*, *6, 8*, 9 (GLRaV-4, GLRaV-6 GLRaV-8, GLRaV-9, unclassified genus *Ampelovirus*), *Grapevine leafroll-associated virus 2* (GLRaV-2, genus *Closterovirus)*, *Grapevine leafroll-associated virus 7* (GLRaV-7, un-

classified family *Closteroviridae*). Surveys in different regions of the country and subsequent serological and/or molecular analysis revealed high incidence of GLRaV-1 and -3 (12-42.4%, 21-47.8%) (Avgelis *et al*., 1997; Dovas *et al*., 2003a, b; Maliogka *et al*., 2006b), important incidence of GLRaV-2 in Northern Greece (22%) (Dovas *et al*., 2003a) and lower in Southern Greece (9.3%) (Maliogka *et al*., 2006b), occasionally of GLRaV-5 (8.67-42%) (Dovas *et al*., 2003b; Maliogka *et al*., 2006b) and GLRaV-4 (12.67%) (Maliogka *et al*., 2006b) and also presence of GLRaV-6 and GLRaV-7 (Dovas *et al*., 2003b; Maliogka *et al*., 2006b). Furthermore, two putatively new ampeloviruses that were recently isolated from Greek grapevine varieties Debina and Prevezaniko respectively were detected in a survey (4-6.66%) (Maliogka *et al*., 2006b). Most of the *Grapevine leafroll-associated viruses* are spread primarily through infected propagating material and grafting. However increasing evidence suggests that GLRaV-1 and GLRaV-3 are also transmitted by soft scale insects and mealybugs (Coccoidea) respectively. Recently, it was also shown that, under lab conditions, GLRaV-5 and GLRaV-9 are also transmitted by mealybugs.

◆ ***Grapevine rugose wood-associated viruses***

Rupestris stem pitting appears to be the most widespread disease of the rugose wood complex of grapevines. *Rupestris stem pitting associated virus-1* (RSPaV-1, genus *Foveavirus*), a recently characterized virus, is closely associated with the disease and was detected in high incidence (>80%) in grapevines of Northern Greece (Dovas *et al*., 2001c).

Other diseases are Kober stem grooving and Grapevine corky bark which are caused by *Grapevine virus A* (GVA, genus *Vitivirus*) and *B* (GVB, genus *Vitivirus*), respectively, are also present in Greece. GVA is the most widespread with an overall incidence of 29.5% (Avgelis and Rumbos, 2000) whereas in Crete it was detected in 54.8% of mother plants of local wine varieties (Avgelis and Grammatikaki, 2006). Rugose wood is distributed over distances by infected propagation material, whereas spread of GVA and GVB in the vineyards is mediated by pseudococcid mealybugs. The poor sanitary status of grapevines especially those grafted on American rootstocks has been demonstrated together with the need of organization of a virus certification scheme in the country.

◆ ***Viroids***

Viroids have been only recently identified by molecular methods first in pome and then in stone fruits in Greece. *Apple scar skin viroid* (ASSVd, genus *Apscaviroid*), *Pear blister canker viroid* (PBCVd, genus *Apscaviroid*) and *Peach latent mosaic viroid* (PLMVd, genus *Avsunviroid*) were reported to infect naturally wild (*Pyrus amygdaliformis* Vil.) and cultivated pear (*Pyrus communis* L.) in Greece in 1998 (Kyriakopoulou and Hadidi, 1998). Among those, ASSVd on apple (*Malus domestica* Borkh.) and PLMVd on peach (*Prunus persica* (L.) Batsch) are known to induce the most serious diseases. ASSVd causes severe scar skin, dappling or cracking on the surface of apple fruit and the affected trees of susceptible cultivars produce unmarketable fruits. PLMVd is responsible for rapid ageing of the trees, which produce irregularly shaped, flattened, colourless fruits with cracked sutures. Scar skin disease, originally observed in a severely damaged commercial pear orchard, was later found widespread in cultivated and wild pear in Northern Peloponnese. In Greece, wild pear has traditionally been used as rootstock of pear and apple, and pear infection has obviously been taking place by grafting on infected wild pear rootstock or using infected budwood.

During 2003-2004, ASSVd was in addition detected in apple (Peloponnese, Etoloakarnania, Magnesia), PLMVd in apricot (*Prunus armeniaca* L.) (Peloponnese,

Etoloakarnania) and PBCVd in quince (*Cydonia oblonga* Mill.) (Peloponnese) (Boubourakas *et al*., 2006b). In this study, the wild species *Crataegus* was for the first time reported as a host of PBCVd and PLMVd.

In 2000, *Hop stunt viroid* (HSVd, genus *Hostuviroid*) was reported on apricot with an incidence of 5% and although latent on this host, it could potentially be transmitted to other susceptible crops including stone fruits (Amari *et al*., 2000).

Recently citrus mother trees of the collection of the Agricultural Station of Poros were found to be highly infected by *Citrus bent leaf viroid* [CBLVd, formerly called citrus viroid (CVd)-I, genus *Apscaviroid*] and *Citrus viroid III* (CVd-III, genus *Apscaviroid*). They were often found in mixed infections with HSVd [formerly called citrus viroid (CVd)-II], causal agent of citrus cachexia disease and *Citrus exocortis viroid* (CEVd, genus *Pospiviroid)*, causal agent of exocortis disease (Vidalakis *et al*., 2006). The need to establish viroid-free mother plantations through a national certification program seems to be the best way to combat these serious diseases.

3.2. Insect and mite transmitted viruses

3.2.1.Transmitted by aphids and mites

◆ ***Apium virus Y*** (ApVY, genus *Potyvirus*) *and* ***Celery mosaic virus*** (CeMV, genus *Potyvirus*)

During the years 2002-2004, a survey was carried out to determine virus incidence in celery (*Apium graveolens L*.) and parsley [*Petroselinum crispum* (Mill.) Nyman ex A.W. Hill] crops showing virus-like symptoms, such as mosaic, yellowing, stunting and leaf distortion. A total of 2094 celery samples and 221 parsley samples from various parts of Greece were examined (Houliara *et al*., 2006). In celery crops, incidence of non-persistently transmitted aphid-borne viruses CeMV and ApVY was 60.6% and 8.1%, respectively. Parsley samples also were found infected by ApVY (64.3%) and CeMV (3.6%).

◆ ***Beet western yellows virus***
(BWYV, genus *Luteovirus*)

BWYV is a persistently transmitted aphid-borne virus producing yellowing symptoms on a wide range of natural hosts including vegetables, ornamentals and arable weeds. In 2000, a survey in spinach (*Spinacea oleracea* L.) crops revealed a high incidence (18.7%) of the virus in Northern Greece, Attica and Evia (Dovas *et al*., 2001a). Moreover, the virus was also widespread in other cultivated plants such as chickpea (*Cicer arientinum* L.), *Sinapis alba* L., broad bean (*Vicia faba* L.) and in various annual and perennial arable weeds (*Flomis fruticosa*, *Reseda alba*, *R. lutea* and *Malva sylvestris*) which consist important sources of virus inoculum (Dovas *et al*., 2001e).

◆ ***Citrus tristeza virus***
(CTV, genus *Closterovirus*)

Citrus tristeza virus causes the most destructive virus disease of *Citrus* spp. grafted on sour orange rootstock and is one of the most important quarantine pests in the country. In 2000, the first CTV-infected sweet orange cv. Lane Late tree grafted on CTV-tolerant Carrizo citrange was found in Argolis county, Peloponnese (Dimou *et al*., 2002). This tree belonged to a batch of CAC propagation material (20 trees) illegally introduced from Spain in 1994. Eradication measures were undertaken and the disease was probably ruled out in Argolis. However, in Crete, the second region having accepted the same infected material from Spain, the situation is more critical, since the virus has been identified not only in the Chania region, where the material was initially introduced, but also in Rethymnon and Heraklion prefectures. Over 4000 trees have been eradicated up to now. The virus was recently detected in orange trees of the Arta valley (Northwestern Greece) (Barbarossa *et al*., 2007) and in Clemenpons trees in Skala Lako-

nias (Southern Peloponnese) imported from Spain five years ago (Dimou, personal communication), so that special actions have to be taken in these regions too.

CTV is transmitted semi persistently by aphids and *Aphis gossypii* (Glover), although not very efficient, is considered as the most important vector in Greece. The arrival of *Toxoptera citricida* (Kirkaldy), the most efficient vector of CTV, in 2003 in Europe (Portugal and Spain) renders the whole situation even more critical, imposing the establishment of rigorous quarantine and phytosanitary measures.

- ***Cucumber aphid borne yellows virus*** (CABYV, genus *Polerovirus*)

CABYV is responsible for yellowing symptoms, in cucurbits especially in open fields grown melon (*Cucumis melo* L.) and cucumber (*C. sativus* L.), causing serious crop losses. This virus is phloem restricted, aphid transmitted in a persistent manner, was first world characterized in France in 1992 and was first reported in Greece the same year (Katis *et al*., 1992). In 2000, a survey in cucurbit crops showed that its incidence in zucchini (*Cucurbita pepo* L.), in melon and cucumber crops was 40%, 20% and 20% respectively (Papavassiliou *et al*., 2006).

- ***Onion yellow dwarf virus*** (OYDV, genus *Potyvirus*), ***Leek yellow stripe virus*** (LYSV genus *Potyvirus*), ***Garlic common latent virus*** (GCLV, genus *Carlavirus*), ***Shallot latent virus*** (SLV, genus *Carlavirus*), ***Garlic virus B*** (GarV-B, genus *Allexivirus*), ***Garlic virus C*** (GarV-C, genus *Allexivirus*), ***Garlic virus D*** (GarV-D, genus *Allexivirus*)

A large scale survey conducted a few years ago on garlic (*A. sativum* L.), leek (*A. porrum* L.), onion (*A. cepa* L.) crops and wild *Allium* species in Greece revealed serious virological problems, especially on garlic, as a result of Greek growers' practice to produce their own garlic propagation material (Dovas *et al*., 2001b). The potyviruses, OYDV and LYSV, which are transmitted by aphids in a non persistent manner, are the most important viruses of garlic, resulting in high yield reduction. These viruses were found to be the most abundant and widespread in Greece (up to 100%).

Significant differences were found regarding the frequency and the distribution of the garlic carlaviruses, which are also transmitted non-persistently by aphids, but cause only latent infections. GCLV was restricted to Southern Greece (in Arcadia 97.6%) and SLV was only detected in low incidence in areas, where propagation material was imported from China and Iran. Since aphids transmit carlaviruses less effectively than potyviruses, it seems that carlaviruses are mainly transmitted by vegetative propagation

Allexiviruses are mite transmitted and induce mild or no symptoms in *Allium* spp. GarV-B, GarV-C and GarV-D were found only in garlic and their regional distribution was irregular. Their incidence was very high (up to 100%) in three out of five regions. The spread of allexiviruses takes place primarily during bulb storage, where their eriophyid mite vector *Aceria tulipae* (Keifer) can easily spread, and rather scarcely in the field. When farmers use their own propagative material as it happens in Greece, 100% total crop infection is most likely to occur.

The incidence of virus-like symptoms in leek crops ranged from 10% to 90% in different regions and fields and all symptomatic plants were found to be infected by LYSV. All-year-around cultivation of leek is a common practice and it seems to be the main reason for the high incidence of the virus in the country.

Virus incidence in onion was low and OYDV was the only virus found in few samples from Southern Greece. *A. ampeloprasum* L. spp. *ampeloprasum* (L.) Breistr. and *A. Flavum* L., were the only wild *Allium* species found to be infected with LYSV.

The poor phytosanitary condition mainly of garlic crop, as shown above, can be only overcome through the use of virus-free

Table 3. New plant viruses and viroids in Greece, 1990-2007.

Pathogen	Host	Transmission	Reference
Apium virus Y	Parsley, celery	Aphids-non persistent	Houliara *et al*., 2006
Apple scar skin viroid	Pear	Grafting, mechanically, pollen	Kyriakopoulou & Hadidi, 1998
Bean leaf roll virus	Pea	Aphids-persistent	Chatzivassiliou *et al*., 2006
Beet mosaic virus	Pea	Aphids-non persistent	Chatzivassiliou *et al*., 2006
Beet pseudo-yellows virus	Cucumber	*Trialeurodes vaporariorum*- semi persistent	Livieratos *et al*., 1998
Beet western yellows virus	Spinach, chickpea, broad bean,	Aphids-persistent	Dovas *et al*., 2001
Celery mosaic virus	Parsley, celery	Aphids-non persistent	Houliara *et al*., 2006
Cherry leaf roll virus	Walnut, olive	Grafting, seed, pollen	Sclavounos *et al*., 2006; Kaponi & Kyriakopoulou, 2006
Cherry necrotic rusty mottle virus	Cherry	Grafting	Malioga *et al*., 2006
Citrus tristeza virus	Orange, mandarin	Grafting, aphids-semi persistent	Dimou *et al*., 2002
Citrus bent leaf viroid	Citrus	Grafting, mechanically	Vidalakis *et al*., 2006
Citrus viroid III	Citrus	Grafting, mechanically	Vidalakis *et al*., 2006
Cucumber aphid borne yellows virus	Zucchini, cucumber, melon	Aphids-persistent	Katis *et al*., 1992
Cucumber toad-skin virus	Cucumber	Hoppers-persistent	Katis *et al*., 1995
Cucurbit yellow stunting disorder virus	Cucumber, melon	*Bemisia tabaci*- semi persistent	Boubourakas *et al*., 2006
Cymbidium mosaic virus	Orchid	Mechanically	Dovas *et al*., 2001
Eggplant mottled dwarf virus	Tomato	*Agallia vorobjevi*- persistent	Kyriakopoulou, 1995
Garlic common latent virus	Garlic	Aphids-non persistent	Dovas *et al*., 2001b
Garlic viruses B, C and *D*	Garlic	*Aceria tulipae*-semi persistent	Dovas *et al*., 2001b
Grapevine angular mosaic virus	Grapevine	Grafting, pollen	Girgis *et al*., 2000
Grapevine leafroll-associated virus 1	Grapevine	Grafting, scale insects	Avgelis *et al*., 1997
Grapevine leafroll-associated virus 3	Grapevine	Grafting, mealybugs	Avgelis *et al*., 1997
Grapevine leafroll-associated viruses 2, 5, 6, 7	Grapevine	Grafting	Dovas *et al*., 2003a; Dovas *et al*., 2003b
Grapevine viruses A and *B*	Grapevine	Grafting, mealybugs	Avgelis & Roumbos, 2000
Hop stunt viroid	Apricot	Grafting, mechanically	Amari *et al*., 2000
Leek yellow stripe virus	Garlic, leek	Aphids–non persistent	Dovas *et al*., 2001b

Table 3 (continued)

Pathogen	Host	Transmission	Reference
Little cherry virus I	Cherry	Grafting	Maliogka *et al*., 2006
Maize rough dwarf virus	Maize	Planthoppers-persistent	Dovas *et al*., 2004
Odontoglossum ringspot virus	Orchid	Mechanically	Dovas *et al*., 2001d
Olive mild mosaic virus	Spinach	?	Gratsia *et al*., 2006
Onion yellow dwarf virus	Garlic, onion	Aphids–non persistent	Dovas *et al*., 2001b
Parietaria mottle virus	Tomato	?	Roggero *et al*., 2000
Pea enation mosaic virus	Pea	Aphids- persistent	Chatzivassiliou *et al*., 2006
Pea seed-borne mosaic virus	Pea	Aphids– non persistent	Chatzivassiliou *et al*., 2006
Peach latent mosaic viroid	Pear	Grafting, mechanically	Kyriakopoulou & Hadidi, 1998
Pear blister canker viroid	Pear	Grafting, mechanically	Kyriakopoulou & Hadidi, 1998
Potato virus Y ^{NTN} *Potato virus Y* ^{N}W	Potato	Aphids–non persistent	Bem *et al*., 1999 Varveri, 2006
Rupestris stem pitting associated virus-1	Grapevine	Grafting	Dovas *et al*., 2001c
Shallot latent virus	Garlic	Aphids–non persistent	Dovas *et al*., 2001b
Sowbane mosaic virus	Spinach	Pollen, seed	Gratsia *et al*., 2006
Tomato chlorosis virus	Tomato	Aleurodes-semi persistent	Dovas *et al*., 2002
Tomato infectious chlorosis virus	Tomato	*T. vaporariorum*-semi persistent	Dovas *et al*., 2002
Tomato mild green mosaic virus	Tree tobacco	Mechanically	Mathioudakis *et al*., 2006
Tomato yellow leaf curl virus	Tomato	*B. tabaci-persistent*	Avgelis *et al*., 2001
Tomato yellow leaf curl Sardinia virus	Tomato	*B. tabaci-persistent*	Papagiannis *et al*., 2007a

propagating material.

- ***Pea enation mosaic virus*** (PEMV, genus *Enamovirus*), ***Bean leaf roll virus*** (BLRV, genus *Luteovirus*), ***Pea seed-borne mosaic virus*** (PSbMV, genus *Potyvirus*), ***Beet mosaic virus*** (BtMV, genus *Potyvirus*)

During 2000, a survey was conducted in pea (*Pisum sativum* L.) crops in Thessaloniki and Thessaly and symptomatic samples were collected. Persistently (PEMV, BLRV) and non persistently (PSbMV, BtMV) aphid transmitted viruses were identified by serological methods: PEMV (44%), BLRV (40%), PSbMV (12%) and BtMV (4%) (Chatzivassiliou *et al*., 2006).

- ***Potato virus Y*** ^{NTN}***, Potato virus Y*** ^{N}**Wilga** (PVY^{NTN}, PVY^{N}W, genus *Potyvirus*)

Potato tuber necrotic ringspot disease (PTNRD) was first observed in commercial potato (*Solanum tuberosum* L.) fields of the Nevrokopi region in Northern Greece in 1994. Measures taken at that time halted further disease spread. PTNRD reappeared in the same area in 1998 in a more aggressive manner (Bem *et al*., 1999). Severe symptoms appeared in 80% of the tubers of the cultivar Hermes imported from Scotland whereas other cultivars were affected at a lower rate. Symp-

toms appeared in early September, 40 days after defoliation of the plants and became more serious after storage. The virus variant responsible for PTNRD was called PVY^{NTN} and formed a distinct PVY^{N} subgroup, in which high variability was observed. Most recent molecular detection methods of NTN isolates rely on the recombination event between PVY^{O} and PVY^{N} groups, usually (but not always) occurring on the coat protein gene. Lately, it was further shown that probably all PVY^{N} isolates could produce some necrotic symptoms on tubers of sensitive varieties and that it may be necessary to set a cultivar-specific Disease Index threshold to separate PVY^{N} from PVY^{NTN}. It is also noteworthy that all recently obtained potato isolates were of PVY^{NTN}-like type. This virus type was isolated from most areas of the country, showing a tendency to predominate (Varveri, 2006).

Another type of new virus variant isolates is the $PVY^{N}W$ type recently recorded in Greece. These isolates are also recombinant, biologically resembling PVY^{N} isolates but serologically the PVY^{O} ones, and are considered to be particularly infectious in potato (Varveri, 2006).

3.2.2. Transmitted by whiteflies

- ***Beet pseudo-yellows virus*** (BPYV, genus *Crinivirus*), ***Cucurbit yellow stunting disorder virus*** (CYSDV, genus *Crinivirus*)

The last fifteen years, two whitefly-transmitted criniviruses that elicit identical symptoms of interveinal chlorosis (yellowing) were identified in greenhouse- and field-grown cucurbits: BPYV which is transmitted by *Trialeurodes vaporariorum* (Westwood) and CYSDV transmitted by *Bemisia tabaci* (Gennadius) (Livieratos *et al*., 1998). In 2000-2003 a survey was carried out in glasshouse and occasionally open field cucumber and melon crops all over the country. In most cases disease incidence ranged from 50 to 80%. Application of molecular diagnostic methods showed that BPYV was predominant in cucumber (68%) and melon (80%) crops, whereas CYSDV, reported for the first time in Greece, was isolated only in three regions of Southern Greece (Rhodes, Crete, Arkadia) at a lower incidence (cucumber 32%; melon 20%) (Boubourakas *et al*., 2006a, Katis *et al*., 2006).

- ***Tomato infectious chlorosis virus*** (TICV, genus *Crinivirus*), ***Tomato chlorosis virus*** (ToCV, genus *Crinivirus*)

Since 1997, a yellowing disease has been observed in greenhouse tomato (*Lycopersicon esculentum* Mill.) in Southern Greece and the island of Crete. By 2001, the disease was widespread including also open field tomato crops and in most cases the incidence was 80 to 90% or even 100%. Epidemics in glasshouses were always associated with high populations of the whiteflies *T. vaporariorum* and *B. tabaci*, the major whitefly pests in vegetable crops in Greece. Affected plants exhibited a generalized interveinal bright yellowing of leaves, were less vigorous and underwent severe yield losses due to reduced fruit size and delayed ripening. Molecular analysis revealed infection with the phloem restricted criniviruses TICV (87%) and ToCV (16%). TICV is transmitted by *T. vaporariorum* and ToCV by *B. tabaci*, *T. vaporariorum* and *T. abutilonea* (Haldeman) (Dovas *et al*., 2002) in a semi-persistent manner.

- ***Tomato yellow leaf curl virus*** (TYLCV, genus *Begomovirus*), ***Tomato yellow leaf curl Sardinia virus*** (TYLCSV, genus *Begomovirus*)

In 2000, tomato crops grown under greenhouses in Crete (Ierapetra, Tympaki) and Southern Peloponnese (Lakonia) showed severe symptoms of Tomato yellow leaf curl disease (TYLCD), one of the most devastating tomato diseases in the Mediterranean basin. The disease is caused by a complex of closely related whitefly persistently transmitted begomoviruses. Immunological, molecular and biological techniques confirmed TYLCV

presence for the first time in the country and in particular the Israeli virus species (TYLCV-Is) (Avgelis *et al*., 2001). More than 30 ha of tomato greenhouses were affected and the disease incidence ranged from 15 to 60%. All affected greenhouses were infested by dense populations of the virus vector *B. tabaci*, also observed outside the greenhouses on several weeds. Since then the virus has become endemic in the above mentioned regions and has been reported in others as well (Preveza, Karditsa). In 2005 a new epidemic occurred in Crete, Southern Peloponnese and Rhodes island. Molecular analysis revealed recently the presence of a second species namely TYLCSV in Peloponnese and Crete (Papagiannis *et al*., 2007a). Both species are commonly found in mixed infections and are spread by the vector and the exchange of commercial products from one country to another. Lately, the virus was found on bean (*Phaseolus vulgaris* L.) plants (1-5%) co-cultivated with infected tomatoes and showing typical symptoms caused by Begomoviruses. Since bean is often used as an intercrop between tomato crops, infected plants may serve as a potential reservoir for virus survival and spread (Papagiannis *et al*., 2007b).

3.2.3. Transmitted by hoppers

- ***Cucumber toad-skin virus***

(CTSV, genus *Nucleorhabdovirus*)

In 1993-1994, virus-like symptoms were observed in glasshouse growing cucumbers in Thessaloniki and Preveza at a very low incidence (1-2%). Affected plants showed severe stunting, leaf crinkling, vein clearing and fruit deformation. Biological indexing and electron microscopy suggested infection by CTSV (Katis *et al*., 1995) and recent research based on serology and RT-PCR revealed that CTSV is a variant of *Eggplant mottled dwarf virus* (EMDV, genus *Nucleorhabdovirus*) (Katis, personal communication), transmitted persistently by a leafhopper.

- ***Eggplant mottled dwarf virus***

(EMDV, genus *Nucleorhabdovirus*)

EMDV was reported to occur in tomato (syn. *Tomato vein yellowing virus*) (Kyriakopoulou, 1995) and tobacco crops (Chatzivassiliou *et al*., 2001; 2004). Tobacco producing areas in Greece were surveyed for virus presence, from 1997 to 2000, and EMDV was detected in several areas (Lamia, Kilkis, Drama, Karditsa and Komotini) but always its incidence was very low (<0.01%). The virus is transmitted by the leafhopper *Agallia vorobjevi* (Dlabola) in a persistent manner.

- ***Maize rough dwarf virus***

(MRDV, genus *Fijivirus*)

In 2002, maize (*Zea mays* L.) crops grown in Northern Greece (Macedonia), showed severe dwarfing, reduced cob size and in some cases leaf reddening. The dwarfing disease was epidemic and in some regions (Imathia and Serres) crop losses over 70% were estimated. Symptoms were different from those caused by *Maize dwarf mosaic virus,* which is endemic in maize crops in Macedonia. In 2003, only a few cases were recorded. Serological and molecular analysis identified MRDV as the causal organism (Dovas *et al*., 2004). The virus is transmitted by planthoppers of the family Delphacidae in a persistent manner.

3.3. Mechanically transmitted viruses

- ***Cymbidium mosaic virus*** (CymMV, genus *Potexvirus*)***, Odontoglossum ringspot virus*** (ORSV, genus *Tobamovirus*)

In 1998, orchid plants belonging to the genera *Cymbidium* and *Phalenopsis* and showing chlorotic and necrotic lesions and stripes were found in a greenhouse near Patras (Achaia). Mechanically transmitted CymMV and ORSV were diagnosed by serology (ELISA), electron microscopy, artificial inoculation of indicator plants and polymerase chain reaction (PCR) (Dovas *et al*., 2001d).

◆ ***Tomato mild green mosaic virus*** (TMGMV, genus *Tobamovirus*)

During 2002-2003, a survey was conducted to estimate the incidence of viruses infecting tobacco tree (*Nicotiana glauca* Graham). A total of 125 samples originating from six areas were tested serologically (ELISA) and molecularly (nested RT-PCR and RFLP analysis). The results showed relatively high incidence of TMGMV (19.2%), a mechanically transmitted virus (Mathioudakis *et al*., 2006).

3.4. Viruses transmitted by other means

◆ ***Parietaria mottle virus*** (PMoV, genus *Ilarvirus*)

A necrotic disease of tomato causing apical necrosis, necrotic spots on the leaves, and corky rings and brown patches on the fruit surface was occasionally observed in Thessaloniki and Chalkidiki in 1997. An unusual ilarvirus was isolated from tomato plants, biologically and serologically related to PMoV, now considered as a tomato strain of PMoV (PMoV-T) (Roggero *et al*., 2000; Katis *et al*., 2001). Disease symptoms are generally observed in June to July in young tomato plants and at the early stages of infection are very similar to those caused by *Tomato spotted wilt virus* (TSWV) or *Cucumber mosaic virus* (CMV) carrying the necrotic CARNA 5 satellite. Since then the disease appears sporadically in the country without causing serious problems. No vector of the virus is known.

◆ ***Sowbane mosaic virus*** (SoMV, genus *Sobemovirus*), ***Olive mild mosaic virus*** (OMMV, genus *Necrovirus*)

In 2004, a new virus disease inducing mosaic and deformation in spinach occurred in Marathon (Attica). Two viruses were isolated, SoMV, which is transmitted by seeds (up to 60% in *Chenopodium quinoa* Willd.) and probably by a vector, and a virus recently isolated from olive with the provisional name Olive mild mosaic virus, for which till then the only known host was olive (Gratsia *et al*., 2006).

3.5. Concluding remarks

A total of 52 new viruses and viroids have been reported and characterized in Greece since 1990. Twenty-six were identified on vegetable crops, 22 on fruit trees and grapevine, two on the ornamental orchid, one on maize and one on a perennial weed (*N. glauca*). Among these newly reported viruses and viroids those that represent serious threat for the country are the following: CTV in citrus, TYLCV, TYLCSV, ToCV, TICV in tomato, BPYV, CYSDV, CABYV in cucurbits and PVY^{NTN} in potato. These viruses are either aphid (CTV, CABYV, PVY^{NTN}) or whitefly transmitted (Begomo- and Criniviruses). Aphid-borne viruses consist a major threat for open field crops whereas the whitefly-borne viruses for the protected ones. Whitefly transmitted viruses were considered problematic principally in tropical and subtropical countries but recently have become real threats in the countries of the Mediterannean basin. Production of virus-free seedlings produced in large nursery enterprises is very important for the control of whitefly transmitted viruses.

Among the above mentioned viruses, two, namely CTV and PVY^{NTN}, were clearly introduced through importation of infected plant material from Spain and Scotland, respectively. Although eradication measures were undertaken against CTV, the virus remains a serious threat especially in Crete and possibly in Southern of Peloponnese (Skala Lakonias) where recently detected. The recent introduction of its most efficient vector *T. citricida* in Europe (Portugal and Spain) makes imperative the application of strict quarantine procedures and the continuation of eradication measures. Vigilance is also required as far as health certificates are concerned because it was revealed that they do not always guarantee the total absence of serious pathogens. For this reason control tests are important

for all types of imported propagation material, essentially when the country of origin is hosting the pathogen in concern. Although efforts are continuing for the eradication of CTV from the country, the situation regarding PVY^{NTN} is quite different, as the virus is already widespread and its control is only based on the use of relatively resistant potato cultivars.

An important number (22) of the pathogens described in this review paper results from survey work aiming mostly to estimate the sanitary situation of vegetatively propagated crops like fruit trees, grapevine, garlic, onion, orchids, etc. The poor sanitary situation of these crops was demonstrated. Production of healthy propagation material remains the only method to control viruses and viroids perpetuated and spread through infected plant material.

Last, it should be also pointed out that the molecular techniques developed these past twenty years and now applied in virology labs greatly facilitate the identification and characterization of viroids and viruses. More than one third of the above described pathogens can be detected in the laboratory only by using molecular methods.

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ΑΡΘΡΟ ΕΠΙΣΚΟΠΗΣΗΣ

Νέες αναφορές παθογόνων των φυτών στην Ελλάδα, 1990-2007

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Περίληψη Τα νέα παθογόνα των φυτών που αναφέρθηκαν στη χώρα μας κατά την περίοδο 1990-2007 συνοψίζονται με βάση δεδομένα που δημοσιεύθηκαν στην ελληνική και στη διεθνή βιβλιογραφία. Περιλαμβάνονται φυτοπαθογόνοι μύκητες, βακτήρια και ιοί ή ιοειδή.

'Οσον αφορά στους φυτοπαθογόνους μύκητες, συνολικά 47 νέα παθογόνα αναφέρθηκαν στην Ελλάδα από το 1990, από τα οποία τα περισσότερα προκαλούν σοβαρές ζημιές σε καλλιέργειες ή σε

δασικά είδη και μόνο λίγα φαίνεται να έχουν αμελητέες επιπτώσεις. Οι σοβαρότερες περιπτώσεις, με βάση τα μέχρι σήμερα δεδομένα, είναι οι εξής:

- Η είσοδος του *Ceratocystis platani* στη χώρα μας και η εμφάνιση της ασθένειας «έλκος του πλατάνου», η οποία έγινε σχετικά πρόσφατα και βρίσκεται ακόμη σε περιορισμένη έκταση στη Νοτιοδυτική Πελοπόννησο. Λόγω της σοβαρότητας της ασθένειας θα πρέπει να ληφθούν επειγόντως μέτρα για τον περιορισμό της ασθένειας.
- Η ίσκα των αμπελώνων, με νέα παθογόνα που απομονώθηκαν από νεαρά πρέμνα, αποτελεί ήδη σοβαρό πρόβλημα τόσο στην εγκατάσταση των νέων αμπελώνων όσο και μετέπειτα. Η χρησιμοποίηση πολλαπλασιαστικού υλικού απαλλαγμένου από την ασθένεια είναι πρωταρχικό μέτρο που πρέπει να ληφθεί για την αντιμετώπιση της ασθένειας.
- Η νέα ειδική μορφή του *Fusarium oxysporum* f. sp. *radicis-cucumerinum* προκαλεί σήψη της βάσης και αδρομύκωση της αγγουριάς με σοβαρές ζημιές στις θερμοκηπιακές καλλιέργειες της χώρας.
- Η κηλίδωση των μανταρινιών Minneola από τον *Alternaria alternata* pv. *citri* είναι ένα σοβαρό πρόβλημα στη Βορειοδυτική Ελλάδα.
- Οι προσβολές των καψών του βαμβακιού από τον *Phytophthora boehmeriae* είναι ένα σοβαρό πρόβλημα σε φυτείες που συνήθως ποτίζονται διαφορετικά από το σύστημα «στάγδην».

΄Οσον αφορά στα φυτοπαθογόνα βακτήρια, υπήρξαν πέντε νέες αναφορές από το 1990 μέχρι το 2007 στην Ελλάδα. Όλα τα παθογόνα διαπιστώθηκαν επί καλλιεργούμενων λαχανοκομικών φυτών και περιγράφηκαν τα συμπτώματα. Τα βακτήρια που ανιχνεύθηκαν και προσδιορίσθηκαν ήταν: *Pantoea ananas* επί καρπουζιού, *Pseudomonas syringae* pv. *apii* επί σέλινου, *Pseudomonas syringae* pv. *porri* επί πράσου, *Xanthomonas campestris* pv. *vitians* επί μαρουλιού και *Xanthomonas cynarae* επί αγκινάρας. Σην Ελλάδα δεν έχει διαπιστωθεί ακόμα ευρεία εξάπλωση των βακτηρίων αυτών αλλά, με βάση στοιχεία από άλλες χώρες, η προκαλούμενη από αυτά ζημιά μπορεί να έχει σοβαρή οικονομική επίπτωση γιατί μειώνει την εμπορική αξία ή καθιστά μη εμπορεύσιμη τη συγκομιδή των προσβεβλημένων φυτών.

Όσον αφορά στους ιούς και τα ιοειδή, έγιναν 52 συνολικά νέες αναφορές στη χώρα μας στο ίδιο διάστημα. Από αυτές, τη σοβαρότερη νέα απειλή για σημαντικές καλλιέργειες όπως τα εσπεριδοειδή, η τομάτα, το αγγούρι και η πατάτα αποτελούν εννέα ιοί οι οποίοι διαθέτουν έντομα-φορείς ικανούς να διασφαλίσουν την περαιτέρω μετάδοσή τους. Αυτοί είναι: ο ιός της τριστέτσας των εσπεριδοειδών (*Citrus tristeza virus*, CTV), ο ιός του κίτρινου καρουλιάσματος των φύλλων της τομάτας (*Tomato yellow leaf curl virus,* TYLCV), ο ιός του κίτρινου καρουλιάσματος των φύλλων της τομάτας της Σαρδηνίας (*Tomato yellow leaf curl Sardinia virus,* TYLCSV), ο ιός της χλώρωσης της τομάτας (*Tomato chlorosis virus*, ToCV), ο ιός της μολυσματικής χλώρωσης της τομάτας (*Tomato infectious chlorosis virus*, TICV), ο ιός του ψευδο-ίκτερου των τεύτλων (*Beet pseudo-yellows virus*, BPYV), ο ιός του κίτρινου παραμορφωτικού νανισμού των κολοκυνθοειδών (*Cucurbit yellow stunting disorder virus*, CYSDV), ο ιός του αφιδομεταδιδόμενου κίτρινου ίκτερου των κολοκυνθοειδών (*Cucumber aphid borne yellows virus,* CABYV) και ο ιός Υ της πατάτας στέλεχος NTN (*Potato virus* Y^{NTN}, PVY^{NTN}). Η εκρίζωση ασθενών φυτών, η εφαρμογή αυστηρών διαδικασιών φυτοκαραντίνας και η χρησιμοποίηση πολλαπλασιαστικού υλικού απαλλαγμένου ιών είναι κάποια από τα σημαντικότερα μέτρα για την αντιμετώπισή τους. Τα μισά σχεδόν από αυτά τα περιγραφέντα παθογόνα προέκυψαν από επισκοπήσεις που είχαν σκοπό τη διερεύνηση της φυτοϋγιεινής κατάστασης αγενώς πολλαπλασιαζόμενων καλλιεργειών, σαν πρώτο βήμα για την παραγωγή υγιούς πολλαπλασιαστικού υλικού. Οι μοριακές μέθοδοι που αναπτύχθηκαν τα τελευταία χρόνια έχουν συμβάλει σημαντικά στην ταυτοποίηση και τον χαρακτηρισμό των αναφερόμενων ιών και ιοειδών.

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Effect of different rates of nitrogen application on the concentration of micronutrients in lettuce and spinach plants

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Summary The effects of several nitrogen application rates on Fe, Mn, Zn and Cu concentration in lettuce and spinach plants were studied in three experiments. The applied nitrogen treatments were: seven concentrations of nitrate nitrogen (N_1:0.1, N_2:0.5, N_3:1.0, N_4:2.0, N_5:4.0, N_6:7.0, N_7:14.0 mM N) for Butterhead lettuce, six (N_1:1.0, N_2:3.0, N_3:6.0, N_4:10.0, N_5:16.0, N_6:22.0 mM N) for spinach, in hydroponics, and six nitrogen fertilization rates (N_1:0, N_2:45, N_3:90, N_4:135, N_5:180, N_6:225 kg N/ha) for Romaine lettuce in the field. The growth of Butterhead lettuce and spinach plants in hydroponics was increased by increasing nitrogen supply; foliage Mn, Zn and Cu concentration was higher at the low nitrogen levels whereas at high N supply a gradual reduction in the relevant micronutrient concentration occurred. In the field, there was a significant increase on lettuce plant fresh weight up to the level N_5 with increasing the quantity of N fertilizer whereas at the highest nitrogen level N_6, a significant reduction of plant growth occurred; a significant positive correlation was found between the foliage fresh weight and Zn and Cu concentration. In all the experiments, no correlation was found between N application and foliage Fe concentration.

Additional keywords: N fertilization, N supply, growth, Fe, Mn, Zn, Cu, nutrient interactions

Introduction

The contents of nutrient elements in leafy vegetables are important to human health, mainly for people in developing world. Besides, lettuce and spinach as dietetic food has long been the object of many investigations. It is well known that fertilization practices can have significant effects on the accumulation of micronutrients on the edible plant products (15). Furthermore, the interactions among nutrients and/or growth effects caused by increased supply of a nutrient could affect accumulation of micronutrients in plants.

The interactions among nutrients are often complex and occur when the uptake of one element affects the uptake and assimilation of another and they are expressed in different ways, including uptake phenomena and biochemical reactions. A nutrient may interact simultaneously with more than one nutrient; this may induce deficiencies, toxicities and/or modified nutrient composition. In crop plants, the nutrient interactions are generally measured in terms of uptake and "concentration effects" due to modified plant growth. Better understanding of nutrient interactions may be useful in understanding the importance of balanced supply of nutrients and consequently the improvement in plant growth and yields (1).

Nitrogen may interact simultaneously with more than one nutrient. It is of fundamental importance in order to improve plant growth and development, to understand the interactions of nitrogen with other nutrients. The quantity and the

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form of nitrogen supply play a pivotal role in the mineral nutrition of plants; e.g. the form of N supply controls the uptake ratio of cations and anions and influences dry matter production (5, 6).

The aim of the current work was to study the effect of several nitrogen application rates on concentration of Fe, Mn, Zn and Cu in lettuce and spinach in order to obtain some information, which could improve their nutritional status and productivity.

Materials and Methods

1. Plant culture and treatments

Three separate experiments (A, B and C) using lettuce and spinach plants were carried out successfully.

Experiment A was conducted in a glasshouse, from March to May 2001, in hydroponics. Seedlings of Butterhead lettuce (cv. Divina) were grown in sand culture without supplementary heating and lighting; the day and night temperature were 20-25^{0}C and 10-15^{0}C, respectively. The pots were arranged in a complete randomized block design with 4 replicates and 7 nitrate nitrogen levels (N_1:0.1, N_2:0.5, N_3:1.0, N_4:2.0, N_5:4.0, N_6:7.0, N_7:14.0 mM NO_3-N).

Experiment B was conducted in a commercial vegetable field from March to April 2002; data concerning soil analysis of the experimental field are shown in *Troyanos* et al. 2004a (12). Lettuce seedlings Romaine (cv. Toledo) at 3-4 leaves growth stage were transplanted in the field. One week after transplanting, six nitrogen fertilization rates (N_1:0, N_2:45, N_3:90, N_4:135, N_5:180, N_6:225 kg N/ ha) were applied by using ammonium nitrate fertilizer (33.5% N). The experimental design was Latin square with 6 rows and 6 columns along the longest direction of the plot area (30 x 6 m) which was divided into 36 plots. More details about plant culture, experimental designs and data analysis of the effects of nitrogen application rate on the growth characteristics and nitrogen study in both lettuce experiments, are presented elsewhere (12, 13).

In experiment C, spinach seedlings of the curly leaved variety Viroflay were grown in sand culture, in the aforementioned glasshouse, from December 2001 to February 2002. The mean monthly air temperatures were 10^{0}C, 13^{0}C and 16^{0}C, respectively. The pots were also arranged in a complete randomized block design with 4 replicates and six nitrate nitrogen concentrations (N_1:1, N_2:3, N_3:6, N_4:10, N_5:16 and N_6:22 mM NO_3-N).

Nutrient solutions in experiments A and C were applied manually using 250 ml of modified Long Ashton nutrient solution per plant per day (2). Nitrogen was applied as calcium nitrate and the differences in the concentration of Ca in the nutrient solution resulting from the different nitrogen regime were compensated by the addition of $CaCl_2$.

In all experiments different harvests were performed during the growth of plants but the data from the fifth harvest are presented here; the total number of plants analyzed was 28, 144 and 24 for experiments A, B and C, respectively.

2. Determination of Fe, Mn, Zn and Cu concentration in the foliage and roots

In experiments A and C, after the removal of the plants from the pots, they were divided into foliage and roots and they were carefully washed three times with deionised water. In the field experiment only the heads were cut and their fresh weight was taken. The plant material was dried to constant weight in a forced draught air oven at 80^{0}C, weighed and dry-ashed in a furnace at 500^{0}C. The dry digest was extracted in 1N HCL, and the concentration of Fe, Mn, Zn and Cu was determined by using a Varian A220 atomic absorption spectrometer.

3. Statistical analysis

The statistical analysis was carried out

using the Statistica package (Statsoft, Inc). Analysis of variance (ANOVA) was applied to different plant variables (Fe, Mn, Zn and Cu concentration of foliage and root, fresh and dry weight of foliage etc), the significance effects of nitrogen treatments were tested by F-test and significant differences in mean values between nitrogen treatments were evaluated by LSD test.

Results

Experiment A

The Fprs of the ANOVA for fresh, dry weight and Mn, Zn, Cu concentration in the plant foliage were found statistically different ($P<0.05$) whereas the Fpr for Fe concentration was not. There was an increase of foliage fresh and dry weight up to level N_7 with increasing nitrogen supply in the growth medium; the maximum RGR of plants was achieved at external supply of nitrogen ≥ 4 mM; the relevant data are shown in Troyanos *et al.* 2004b (13). The concentrations of Fe, Mn, Zn and Cu at N_1 level were found higher than those at higher nitrogen levels. In the case of Zn and Cu, these differences were significant, the concentration of Mn was statistically significant higher at N_1, N_2 levels whereas the concentration of Fe was independent of the nitrogen supply (Table 1). Besides, the negative correlations between the foliage fresh weight and the concentrations of Mn, Zn and Cu were found statistically significant; Mn ($r=-0.85^{***}$), Zn ($r=-0.74^{***}$) and Cu ($r=-0.44^{*}$).

Experiment B

The fresh weight of the plants increased significantly up to N_5 fertilization rate and decreased thereafter. The decrease in plant fresh weight at N_6 fertilization rate was probably due to negative effect of excess fertilizer applied to the soil.

The Fprs of the ANOVA for the concentration of Zn and Cu in the plant foliage were found statistically different ($P<0.05$) whereas the relevant ones for Fe and Mn were not. Foliage Zn concentration at N_5 fertilization rate was found significantly highest compared to N_1, N_2, N_3, N_4, N_6 ones whereas the relevant Cu concentrations at N_4, N_5 rates were found significantly higher compared to N_1, N_2, N_3 ones (Table 2). At N_6 rate the foliage micronutrient concentrations presented a similar to fresh weight trend; they were found decreased as compared to the relevant ones at N_5 rate . Furthermore, a statistically significant positive correlation was found between the foliage fresh weight and the concentra-

Table 1. Effect of nitrogen concentration in the nutrient solution on foliage fresh and dry weights and Fe, Mn, Zn and Cu concentration in lettuce Butterhead (cv. Divina) (means, n=4). The values followed by different letters within a column are significantly different at $P<0.05$.

N in the nutr. solution	Foliage f. w.	Foliage d. w.	Foliage Fe	Foliage Mn	Foliage Zn	Foliage Cu
(mM)	(g)		(mg kg^{-1} d.w.)			
N_1 – (0.1)	5.8 a	0.71 a	175.3 a	103.2 a	167.3 a	20.5 a
N_2 – (0.5)	23.2 a	2.08 ab	99.3 e	86.8 ab	112.0 b	11.2 b
N_3 – (1.0)	54.6 b	4.02 b	120.0 a-e	73.8 b	117.5 b	9.7 b
N_4 – (2.0)	97.2 c	6.54 c	145.8 a-e	36.0 c	97.8 bd	10.1 b
N_5 – (4.0)	153.2 d	8.96 c	171.8 ab	14.3 c	63.0 e	10.0 b
N_6 – (7.0)	198.5 e	10.78 d	160.3 a-d	19.8 c	76.3 de	10.6 b
N_7 – (14.0)	232.7 f	14.21 de	160.3 abc	12.3 c	65.7 de	10.3 b

Table 2. Effect of six different nitrogen fertilization rates on foliage fresh and dry weights and Fe, Mn, Zn and Cu concentration in Romaine (cv. Toledo) lettuce grown in the field (means, n=36). The values followed by different letters within a column are significantly different at $P<0.05$.

N Fertilization rate	Foliage f. w.	Foliage d. w.	Foliage Fe	Foliage Mn	Foliage Zn	Foliage Cu
(Kg N/ha)	(g)		(mg kg^{-1} d.w.)			
N_1 – (0.0)	124.8 d	7.72 d	183.9 ab	35.8 a	30.8 c	9.2 bc
N_2 – (45.0)	257.7 c	13.72 bc	171.5 b	34.5 a	30.8 c	8.9 c
N_3 – (90.0)	275.5 c	14.54 abc	177.4 ab	30.4 b	32.9 c	9.0 c
N_4 – (135.0)	314.8 b	15.51 ab	172.0 b	34.0 a	40.4 b	11.8 a
N_5 – (180.0)	363.0 a	15.73 a	189.2 a	34.9 a	47.6 a	11.6 a
N_6 – (225.0)	263.6 c	13.32 c	174.5 ab	30.3 b	42.1 b	10.6 ab

Table 3. Effect of six different nitrogen concentrations in the nutrient solution on the growth and concentration of Fe, Mn, Zn and Cu in spinach (cv. Viroflay) foliage and root (means, n=4). The values followed by different letters within a column are significantly different at $P<0.05$.

N conc.	Foliage f.w.	Root f.w.	Foliage Fe	Root Fe	Foliage Mn	Root Mn	Foliage Zn	Root Zn	Foliage Cu	Root Cu
(mM)	(g)		(mg kg^{-1} d.w.)							
N_1 - (1)	10.8 a	1.14 a	114.8 a	182.3 ab	159.8 a	116.4 b	355.5 a	247.0 a	13.3 a	21.7 ab
N_2 - (3)	18.7 ab	1.90 ab	105.0 a	190.1 a	141.0 a	256.3 a	234.5 b	378.8 a	10.5 b	24.2 a
N_3 - (6)	20.7 ab	2.25 b	107.0 a	134.6 c	87.0 b	202.7 ab	165.0 b	250.1 a	10.3 bc	19.5 ab
N_4-(10)	19.6 ab	1.93 ab	105.8 a	133.5 c	96.3 b	205.0 ab	174.5 b	336.8 a	9.8 bcd	19.0 ab
N_5-(16)	23.2 b	2.31 b	114.5 a	155.7 bc	71.0 b	192.2 ab	159.5 b	307.8 a	7.8 cd	19.1 ab
N_6-(22)	24.1 b	2.47 b	114.3 a	137.7 c	66.8 b	146.8 b	188.0 b	340.4 a	7.5 d	16.2 b

tions of Zn ($r=0.37^{***}$) and Cu ($r=0.18^{*}$).

Experiment C

The concentrations of Mn, Zn and Cu in the foliage of spinach plants were found significantly different among the nitrogen treatments whereas the foliage fresh weight and Fe concentration were not significantly affected. At N_1 level, the foliage Mn, Zn and Cu concentrations were found higher than those in the other treatments; at this nitrogen level the foliage fresh weight was found reduced (Table 3). The correlations between nitrogen supply and the foliage Mn, Zn and Cu concentrations were found significantly negative; Mn ($r=-0.77^{***}$), Zn ($r=-0.60^{**}$) and Cu ($r=-0.74^{***}$) whereas the concentration of Fe was found to be independent of nitrogen supply. Besides, the concentration of Fe, Mn, Zn and Cu was greater in the roots than in the foliage (Table 3).

Discussion

At the low concentrations of nitrogen in the nutrient solution, the growth of Butterhead lettuce and spinach plants (experiments A and C) was reduced due to nitrogen deficiency; characteristic symptoms of nitrogen deficiency like senescence of older leaves, as well as, decline in expansion rate of both existing and newly developed leaves appeared on Butterhead lettuce plants grown with 0.1-0.5 mM N (13) and on spinach plants grown with 1 mM N; besides, the total nitrogen concentra-

tion in foliage of lettuce Butterhead and the concentration of nitrates in foliage of spinach plants were found lower than the relevant critical ones (10, 13). At those nitrogen supplies the concentration of Mn, Zn and Cu in the foliage was increased (Tables 1, 3). The observed accumulation of these micronutrients was probably due to the "concentration effect" (increase in concentration caused by reduced plant size) and/or due to specific changes in the absorption, transport and distribution of the micronutrients in the plants (7). In the same experiments, the observed reduction in the concentration of Mn, Zn and Cu at high concentrations of nitrogen in the nutrient solution could be explained by the increased plant size due to increased nitrogen supply and/or changes in the uptake rate of micronutrients. The increased nitrogen supply caused an increased demand for micronutrients, which could not be satisfied by an enhanced uptake of micronutrients since their concentrations were stable in the supplied nutrient solution of the different treatments. Furthermore, it is well known that the form of nitrogen added (ammonium or nitrate) causes changes in the pH at root surface and root apoplast. These changes influence the uptake, translocation, remobilization and utilization of several micronutrients (1, 14, 16). Therefore, it is probable that the reduction of Mn, Zn and Cu concentration in the plants could be attributed, as well, to pH increase at root surface and root apoplast because of nitrate nitrogen added in the nutrient solution. Many plants species grown on a complete medium with nitrate-N usually excrete OH^- or HCO_3^- ions into the nutrient solution causing pH increase in the rhizosphere (5, 6, 4, 3, 1).

Regarding lettuce plants grown in the field (experiment B), an increase was found in foliage Zn and Cu concentration with increasing nitrogen supply up to the level N_5. These results are different from those in lettuce and spinach plants grown in the nutrient solution. Comparisons of these experiments could not be made since into the soil, greater reserves of the micronutrients could be mobilized and satisfy the increased demand of the plants; besides, soil analysis of the experimental field (12) showed that the concentrations of Mn, Zn and Cu in the soil were found between adequate and high levels.

In all experiments, leaf Fe concentration of plants grown either in hydroponics or in the field was not significantly differentiated among the nitrogen treatments, as well as, leaf Fe concentration was not significantly correlated with plant growth. This discrepancy could be explained by the assumption that only a portion of Fe in plants participates in metabolic reactions or is incorporated into molecular structures (8) and leaf analysis does not always reflect plant Fe status (9).

Furthermore, in experiment C, comparing the concentration determined in the root system to that of foliage, a higher accumulation of micronutrients was found in roots (Table 3). Similar results have been reported by other researchers (10, 16); according to them, more Fe was immobilized in the root, due to high pH (plants fed with 100% NO_3-N).

We hope that this preliminary study of the impact of several nitrogen levels on plant micronutrient concentrations will contribute to better understanding of nutrient balance and can lead to more efficient crop production.

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Επίδραση διαφόρων επιπέδων αζωτούχου λίπανσης στη συγκέντρωση ορισμένων ιχνοστοιχείων στο μαρούλι και σπανάκι

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Περίληψη Σε τρία διαφορετικά πειράματα, υδροπονίας και αγρού, μελετήθηκε η επίδραση του χορηγούμενου αζώτου στη συγκέντρωση των ιχνοστοιχείων Fe, Mn, Zn και Cu στο μαρούλι και το σπανάκι. Οι επεμβάσεις που εφαρμόστηκαν στην υδροπονία αφορούσαν επτά συγκεντρώσεις νιτρικού αζώτου (N_1:0.1, N_2:0.5, N_3:1.0, N_4:2.0, N_5:4.0, N_6:7.0, N_7:14.0 mM N) σε μαρούλι τύπου Butterhead και έξι (N_1:1.0, N_2:3.0, N_3:6.0, N_4:10.0, N_5:16.0, N_6:22.0 mM N) σε σπανάκι ποικ. Viroflay ενώ στον αγρό εφαρμόστηκαν έξι επίπεδα αζωτούχου λίπανσης (N_1:0, N_2:45, N_3:90, N_4:135, N_5:180, N_6:225 kg N/ ha) σε μαρούλι τύπου Romaine. Στα πειράματα στην υδροπονία, αυξανομένου του χορηγουμένου αζώτου αυξανόταν το νωπό βάρος των φυτών μαρουλιού και σπανακιού αλλά μειώνονταν οι συγκεντρώσεις Mn, Zn και Cu στο φύλλωμα. Στο μαρούλι στον αγρό, αυξανομένου του χορηγουμένου αζώτου μέχρι και του επιπέδου N_5, αυξανόταν τόσο το νωπό βάρος των φυτών όσο και η συγκέντρωση των ιχνοστοιχείων Mn, Zn και Cu στο φύλλωμα ενώ στο επίπεδο N_6, πιθανόν λόγω υπερβολικής ποσότητας αζωτούχου λίπανσης, μειώθηκε τόσο η αύξηση των φυτών όσο και η συγκέντρωση των προαναφερόμενων ιχνοστοιχείων. Όσον αφορά τη συγκέντρωση του Fe, σε κανένα από τα τρία πειράματα δε διαπιστώθηκε σημαντική διαφοροποίηση του στοιχείου στο υπέργειο τμήμα των φυτών λόγω της ποσότητας του χορηγουμένου αζώτου.

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New data on the scale insects (Homoptera: Coccoidea) of the Greek Entomofauna

P.G. Milonas[1], F. Kozár[2] and D.C. Kontodimas[1]

Summary Ornamental, greenhouse, cultivated and wild plants were surveyed in 2005 in a study of Coccoidea fauna in Greece. In this study, thirteen species of scale insects belonging to 12 genera of 6 families were identified. Four of them, *Newsteadia sussannae*, *Gueriniella serratulae*, *Antoninella inaudita*, and *Chionaspis lepineyi* are new records for the scale insect fauna of Greece.

Additional keywords: insect fauna, Coccidae, Diaspididae, Kermesidae, Margarodidae, Ortheziidae, Pseudococcidae

Introduction

Scale insects are important pests of many crops and are an important group for biodiversity studies as well. The scale insect fauna of Greece is poorly investigated. According to Lindinger (11), Koroneos (6), Borchsenius (5), Paloukis (12), Argyriou (1), Kozar (7), and Kozar et al. (9) and the Scale net, 143 species have been identified. However, certain of these species cited in the literature, require additional study for confirmation. The present study aimed at a further investigation of the scale insect fauna in Greece.

Materials and Methods

The collection surveys were made during 2005 in the area of Attica. Microscope slides were prepared following the method described by Kosztarab and Kozar (10). The dry material and the microscope slides were deposited in the Collection of the Plant Protection Institute in Budapest, Hungary and in the Laboratory of Biological Control at the Benaki Phytopathological Institute in Athens, Greece. During collection, data such as stage of insect development, sex, and host plant were recorded.

Results and Discussion

In this collection survey, the following scale insect species, arranged by family, were identified:

ORTHEZIIDAE
Newsteadia sussannae Kozar & Foldi, Parnitha Mount Athens, 28.10.2005 Gramineae roots, female: It is a new species to the Greek fauna. It has been earlier reported only from France - Corsica on forest litter (8). The genus *Newsteadia* belongs to the subfamily Newsteadiinae. Fifty-seven species are known at this time from this genus, of which only four from Europe (8).

MARGARODIDAE
Gueriniella serratulae (F.), Maroussi Athens, 27.07.2005, *Pinus halepensis*, female: It is a new species to the Greek fauna. It is a common species in the Mediterranean region on pine trees (2).

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Icerya purchasi Maskell, Athens, 16.11.2005, *Citrus* sp., female: It has been reported by Koroneos (6).

PSEUDOCOCCIDAE

Planococcus vovae (Nassonov), Kifisia, Athens, 09.09.2004. *Cupressus leylandii*, female: It has been reported by Cox (3).

Planococcus citri (Risso), Kifisia, Athens, 09.09.2005, *Citrus* sp., female: It has been reported by Argyriou (1).

Pseudococcus afinis (Maskell), Kifisia, Athens, 10.10.2005, Water melon, female: It has been reported for the first time by Kozar *et al.* (9).

Trionymus aberrans Goux, Maroussi Athens, 31.10.2005, Gramineae in leaves sheaths, female: It has been reported for the first time by Kozar *et al.* (9).

Antoninella inaudita Kiritchenko, Maroussi, Athens, 31.10.2005, Gramineae roots, female: It is a new species to the Greek fauna. It is known to occur in central Europe (Germany) and in France, Italy and Ukraine. Its hosts are mainly species of *Festuca* and other Gramineae (9).

COCCIDAE

Philippia follicularis Targioni-Tozzetti, Varibobi, Athens, 01.11.2005, *Olea europea* stems and leaves, larvae: It has been reported by Argyriou (1).

DIASPIDIDAE

Chionaspis lepineyi Balachowsky, Parnitha mount, Athens, 03.11.2005, *Quercus ilex* on stems, female & male: It is a new species to the Greek fauna. This species is known to occur in the Mediterranean region and the Middle East. It is a pest of *Quercus* species and has also been found on *Castanea sativa* (9).

Aonidiella aurantii (Maskell), Athens, 10.09.2005, *Citrus* sp., female: It has been reported by Argyriou (1).

Diaspidiotus perniciosus (Comstock), Athens, 20.09.2005, *Malus* sp., female: It has been reported by Argyriou (1).

KERMESIDAE

Kermes vermilio Planchon, Gargalianoi Pelloponesos, 08.08.2005, *Quercus* sp. Female, first instar larvae: It has been reported by Argyriou (1).

A total of 13 species were identified in the present study and 4 of them are new records to the Greek fauna. The limited sampling, conducted in this study mainly in one region of Greece (Attica), came up with 30% new species. This implies that the database on scale insect fauna in Greece is yet incomplete and extensive sampling is required to locate and identify the present species.

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Νέα είδη κοκκοειδών εντόμων (Homoptera: Coccoidea) για την ελληνική εντομοπανίδα

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Περίληψη Η γνώση της πανίδας των κοκκοειδών εντόμων της Ελλάδος παραμένει περιορισμένη. Για το λόγο αυτό έγινε προσπάθεια συλλογής και αναγνώρισης τέτοιων εντόμων από περιοχές της Ελλάδος. Κατά τη συλλογή συλλέχθησαν 13 είδη που ανήκουν σε 6 οικογένειες, συγκεκριμένα Ortheziidae, Margarodidae, Pseudococcidae, Kermesidae, Diaspididae, Coccidae. Από αυτά, 4 είδη και συγκεκριμένα τα *Newsteadia sussannae*, *Gueriniella serratulae*, *Antoninella inaudita* και *Chionaspis lepineyi*, ήταν νέα για την ελληνική πανίδα.

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Check list of mealybugs (Homoptera: Pseudococcidae) in Greece: three new records

P.G. Milonas[1] and F. Kozár[2]

Summary An updated list of the pseudococcid species observed in Greece is presented in this study. Wild plants and cultivated crops from different habitats were surveyed and mealybug samples were collected from 2005 to 2006. Three mealybug (Homoptera: Pseudococcidae) plant pest species, *Heliococcus bohemicus, Phenacoccus hordei*, and *Heterococcus nudus*, are recorded for the first time in Greece. The species *Planococcus citri,* a common pest of citrus, *P. ficus* a major pest of vineyards, *P. vovae,* a common pest of cypress trees, and *Puto tauricus* from Gramineae were also encountered.

Additional keywords: Coccoidea, insect fauna, plant pests, scale insects

Introduction

Mealybugs are important plant pests worldwide (20,21). Their feeding may cause leaf yellowing, defoliation, reduced plant growth, and in some cases plant death. They may also cause indirect damage to plants by serving as vectors of plant diseases (13). In addition, production of honeydew contributes to the development of sooty mould that decreases photosynthesis and may reduce the marketability of plant products such as fruits. Besides direct and indirect damage to crops, mealybugs are also of quarantine concern, adding to costs of production to prevent or eliminate their presence on plants and plant products.

Information on the occurrence and distribution of mealybug species in Greece is limited. Eleven species have been recorded by ScaleNet in Greece and a few additional by Kozar *et al*. (19) and Milonas *et al*. (22). In this study we present an updated list of mealybugs recorded from Greece and we document new mealybug records from agricultural and wild plants in Greece.

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Materials and Methods

A thorough literature review was conducted to locate any publication, including the database ScaleNet, that refers to mealybugs recorded in Greece. In addition to the literature review, mealybugs were collected during 2005-2006 from various plants in Greece to identify the species present. Mealybug specimens were collected when detected on plants during fieldwork or other activities of the first author and from plant samples infested by scale insects that were brought to the Benaki Phytopathological Institute (BPI) for identification.

All mealybug specimens were slide-mounted for identification using the method outlined in Kosztarab and Kozar (15). Voucher specimens of these insects are deposited in the arthropod collections of the Plant Protection Institute in Budapest (Hungary) and the BPI (Greece).

Results and Discussion

The literature review and the ScaleNet data provided a list of Pseudococcidae species that have been recorded in Greece. In total, 32 species have been reported to be pres-

ent in Greece up to date (Table 1) (1, 2, 8, 7, 10, 11, 14, 16, 17, 18, 19, 22, 24, 25). Amongst these species, *Eumyrmococcus corinthiacus* Williams, found in the jaws of queen ants of the genus *Plagiolepis,* is the only record of a myrmecophilous species in Europe (25). Another species, *Ripersiella palestinae,* recorded by Hambleton (14) in 1979 from lily bulbs exported from Greece to the USA, has not been found in Greece since then.

In the present study, three new species, recorded for the first time in Greece, as well as some other species that had been previously recorded in the country were collected and identified (Table 2). *Heliococcus bohemicus* Sulc. was collected from grapes of a heavily infested vineyard in Korinthos. In the same vineyard, *Planococcus citri* (Risso) and *P. ficus* (Signoret) were collected from the trunks and grapes. *Heterococcus nudus* (Green) and *Phenacoccus hordei* (Reuter) were found on various Gramineae plants. Finally, *Pseudococcus longispinus* (Targioni Tozzetti) was found on leaves of *Cycas* sp. in an open nursery in Athens, *Puto tauricus* (Leonardi) on Gramineae leaves and *Plano-*

Table 1. Species of the family Pseudococcidae recorded in Greece.

	Species	Author	Reference
1	*Antoninella inaudita*	Kiritchenko	Milonas *et al*. (in press)
2	*Atrococcus arakalianae*	(Maskell)	Kozár & Nagy 1998
3	*Balanococcus orientalis*	Danzig & Ivanova	Kozár *et al*. 1991
4	*Chaetococcus phragmitis*	(Marchal)	Kozár 1985
5	*Eumyrmococcus corinthiacus*	Williams	Williams 1993
6	*Heliococcus bohemicus*	Sulc	present study
7	*Heterococcus nudus*	(Green)	present study
8	*Hypogeococcus pungens*	Granara de Willink	Ben-Dov *et al.* 2002
9	*Mirococcopsis elongatus*	Borchsenius	Kozár *et al*. 1991
10	*Mirococcus inermis*	(Hall)	Kozár 1985
11	*Peliococcopsis priesneri*	Borchsenius	Kozár 1985
12	*Peliococcus kimmericus*	Kiritchenko	Kozár *et al*. 1991
13	*Peliococcus turanicus*	(Kiritchenko)	Kozár 1985
14	*Pellizzaricoccus gabrielis*	Kozár	Kozár 1991
15	*Phenacoccus bicerarius*	Borchsenius	Kozár *et al*. 1991
16	*Phenacoccus hordei*	(Lindeman)	present study
17	*Phenacoccus interruptus*	Green	Kozár 1985
18	*Phenacoccus yerushalmi*	Ben-Dov	Ben-Dov *et al.* 2006
19	*Planococcus citri*	(Risso)	Argyriou 1983, Kozár 1985,
20	*Planococcus ficus*	(Signoret)	Ezzat & McConnell 1956
21	*Planococcus vovae*	(Nasonov)	Cox 1989
22	*Pseudococcus calceolariae*	(Maskell)	Kozár *et al*. 1991
23	*Pseudococcus longispinus*	(Targioni Tozzetti)	Ben-Dov, Y. 1994
24	*Pseudococcus viburni*	Signoret	Kozár *et al.* 1991
25	*Puto tauricus*	(Leonardi)	Danzig 1999
26	*Rhizoecus albidus*	Goux	Kozár 1985
27	*Ripersiella palestinae*	Hambleton	Hambleton 1979
28	*Ritsemia pupifera*	Lichtenstein	Savopoulou *et al*. 1995
29	*Spilococcus halli*	(McKenzie Williams)	Kozár 1985
30	*Trionymus aberrans*	Goux	Kozár *et al.* 1991
31	*Trionymus cynodontis*	(Kiritshenko)	Kozár 1985
32	*Vryburgia amarylldis*	(Bouche)	ScaleNet

Table 2. Mealybug (Homoptera: Pseudococcidae) species collected in the present study.

Mealybug species	Collection location	Collection date	Host plant
Heliococcus bohemicus Sulc	Korinthos	2004	*Vitis vinifera*
Heterococcus nudus (Green)	Chalkidiki	10 April 06	Gramineae
Phenacoccus hordei (Lindeman)	Magnesia, Pelion	25 April 06	*Thymus vulgaris*
Planococcus citri (Risso)	Korinthos Attika	2004 11 July 06	*Vitis vinifera* *Citrus sinensis*
Planococcus ficus (Signoret)	Korinthos	29 June 06	*Vitis vinifera*
Planococcus vovae (Nasonov)	Kalamata Athens	6 June 06 2 June 06	*Juniperus* sp. *Cypressus sempervirens*
Pseudococcus longispinus (Targioni Tozzetti)	Athens, Elliniko	22 July 06	*Cycas* sp.
Puto tauricus (Leonardi)	Athens, N. Ionia	27 April 06	Gramineae

coccus vovae (Nasonov) on various *Cypressus* species. Although *P. citri*, *P. ficus*, *P. tauricus* and *P. longispinus* have been previously found in Greece, *H. bohemicus*, *H. nudus*, and *P. hordei* are less well known and this is the first record of their presence in Greece.

H. bohemicus has a palaearctic distribution and is present in central Europe and China (3). It is known as a pest of grapevines in central Europe, but its hosts include many plant species belonging to 12 families. Its biology has been studied in northern Italy, where it develops two generations per year (9). Although *H. bohemicus* is uniformly distributed in the vineyards along with *P. ficus,* in contrast to the latter, it does not pose a threat to viticulture, due to its ecological habits that constantly expose the species to the environmental conditions. Parasitization in some years might reach 60% (23).

H. nudus is a cosmopolitan species occurring in the leaf sheath and crown of various Gramineae plant species (4). It may cause browning and stunting of grasses and is closely related to *H. abludens* Borchsenius but the latter has only 6-segmented antennae.

Phenacoccus hordei has a palaearctic distribution and is fairly common, occurring on the roots of plants, especially grasses (5). It has also been reported on Leguminoseae and Umbellifereae plants. It is capable of producing three generations per year. It overwinters as second, rarely as third instars and its adults develop by the end of April.

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Κατάλογος ειδών ψευδοκόκκων (Homoptera: Pseudococcidae) στην Ελλάδα: τρία νέα είδη

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Περίληψη Παρουσιάζεται ένας πλήρης κατάλογος των κοκκοειδών εντόμων της οικογένειας Pseudococcidae που έχουν αναφερθεί στην Ελλάδα. Επιπλέον, από δειγματοληψίες σε καλλιεργούμενα και αυτοφυή φυτά από διάφορες περιοχές βρέθηκαν και τρία νέα είδη για την ελληνική εντομοπανίδα. Πρόκειται για τα είδη *Heliococcus bohemicus, Phenacoccus hordei* και *Heterococcus nudus*. Τα είδη *Planococcus citri,* κοινός εχθρός στα εσπεριδοειδή, *P. ficus* σημαντικός εχθρός της αμπέλου, *P. vovae,* κοινός εχθρός σε είδη κυπαρισσιού, και *Puto tauricus* σε αγρωστώδη, εντοπίστηκαν και στην παρούσα μελέτη.

Hellenic Plant Protection Journal **1:** 39-41, 2008

SHORT COMMUNICATION

Host plants of the planthopper *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae) and observations on its phenology in Greece

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Summary A survey conducted in areas of western and northern Greece from October 2005 to October 2006 indicated that *Metcalfa pruinosa* (Say) is hosted on a large number of ornamentals, fruit crops and weed species and on two cultivated vegetables (tomato, eggplant).
Year-round sampling from olive and citrus orchards in western Greece indicated that *M. pruinosa* develops one generation per year with adult activity spanning from late June to late September.

The planthopper *Metcalfa pruinosa* (Say) (Hemiptera, Flatidae) has its origin in North America (1). In Europe it was first reported from eastern North Italy in 1979 (5) and subsequently distributed in several European countries (3, 4). In Greece it was first recorded in May 2002 (2). All larval stages of *M. pruinosa* produce wax and honeydew. Honeydew is often a good substrate for the development of sooty moulds from several taxa, especially the family Capnodiaceae.

A survey of the plant species hosting *M. pruinosa* was conducted from October 2005 to October 2006 in areas of western (Preveza, Parga, Lefkada, Corfu) and northern (Serres) Greece.

To study the phenology of *M. pruinosa,* a tangerine orchard and an olive orchard were selected in the area of Preveza. Twenty samples, consisting of tree twigs (about 25 cm in length) were randomly taken every 15 days, placed in plastic bags and transferred to the laboratory to identify immature life stages and adults of *M. pruinosa* as they developed during the year.

Results of the survey indicated that *M. pruinosa* in the above locations is hosted on a large number of ornamental plants, fruit crops and weed species, as well as on two cultivated vegetables (Tables 1, 2 and 3) confirming its polyphagous behaviour.

From the phenology study it was concluded that *M. pruinosa* in western Greece (an area characterized by its rather mild climate) completes one generation per year. First instar larvae emerge from over-

Fig. 1. *Nerium oleander* infested by *M. pruinosa.*

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winting eggs in late May and after passing through five stages they transform into adults. Adult activity started late June and continued up to late September.

Table 1. Ornamental and vegetable plant species recorded to host *M. pruinosa* in the surveyed areas of Greece.

Ornamentals
Azalea indica L. (Ericaceae)
Aucuba japonica Thunb. (Cornaceae)
Begonia rex Putz.(Begoniaceae)
Cedrus libani A. Rich. (Pinaceae)
Chrysanthemum indicum L. (Compositae)
Cydonia japonica Thunb. (Rosaceae)
Ficus elastica Roxb. (Moraceae)
Gardenia jasminoides Ellis (Rubiaceae)
Hydrangea macrophylla Thunb. (Hydrangeaceae)
Ilex aquifolium L. (Aquifoliaceae)
Jasminum fruticans L. (Oleaceae)
Laurus nobilis L. (Lauraceae)
Ligustrum vulgare L. (Oleaceae)
Magnolia grandiflora L. (Magnoliaceae)
Nerium oleander L. (Apocynaceae) (Fig. 1)
Ocymum basilicum L. (Labiateae)
Platanus orientalis L. (Patanaceae)
Punica granatum L. (Punicaceae)
Ricinus communis L. (Euphorbiaceae)
Rhododendron arboreum Sm. Ericaceae)
Rosa sp. (Rosaceae)
Tagetes erecta L. (Compositae)
Thuja orientalis L. (Cupressaceae)
Viburnum opulus L. (Caprifoliaceae)
Viola odorata L. (Violaceae)
Washingtonia sp. (Palmaceae)
Vegetables
Lycopersicum esculentum Mill. (Solanaceae)*
Solanum melongena L. (Solanaceae)*

* Only adults of *M. pruinosa* were found on the two vegetable species.

Table 2. Fruit crop species recorded to host *M. pruinosa* in the surveyed areas of Greece.

Actinidia chinensis Plan. (Actinidiaceae)
Citrus sinensis L. (Rutaceae)
Citrus limon Burn (Rutaceae)
Citrus reticulata Blanco (Rutaceae)
Citrus aurantium L. (Rutaceae)
Corylus avellana L. (Betulaceae)
Ficus carica L. (Moraceae)
Juglans redia L. (Juglandaceae)
Olea europaea L. (Oleaceae) (Fig. 3)
Persea americana Mill. (Lauraceae)
Pyrus communis L. (Rosaceae)
Prunus americana Marsh. (Rosaceae)
Prunus domestica L. (Rosaceae)
Prunus persica L. (Rosaceae)
Vitis vinifera L. (Vitaceae)

Table 3. Weed species recorded to host *M. pruinosa* in the surveyed areas of Greece.

Amaranthus blitoides Watson, S. (Amaranthaceae)
Amaranthus retroflexus L. (Amaranthaceae)
Bromus sp. (Gramineae)
Chenopodium album L. (Chenopodiaceae)
Conyza canadensis (L.) Cronq. (Compositae)
Digitaria sanguinalis (L.) Scop. (Gramineae)
Hedera helix L. (Araliaceae)
Hypericum sp. (Hypericaceae)
Melissa officinalis L. (Labiateae)
Menta aquatica L. (Labiatae)
Phytolacca americana L. (Phytolaccaceae)
Plantago sp. (Plantaginaceae)
Pteridium aquilinum L. (Polypodiaceae)
Setaria sp. (Gramineae)
Solanum nigrum L. (Solanaceae)
Rubus fruticosus L. (Rosaceae) (Fig. 2)
Urtica dioica L. (Urticaceae)
Verbascum sp. (Scrophulariaceae)
Xanthium strumarium L. (Compositae)

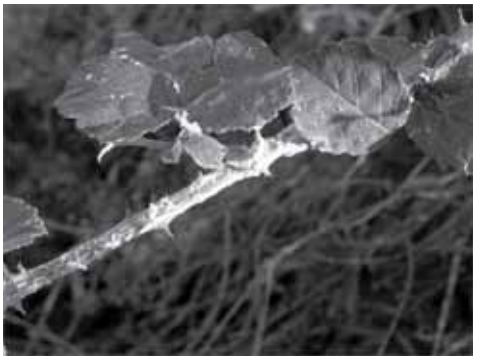

Fig. 2. *Rubus fruticosus*, hosting *M. pruinosa*.

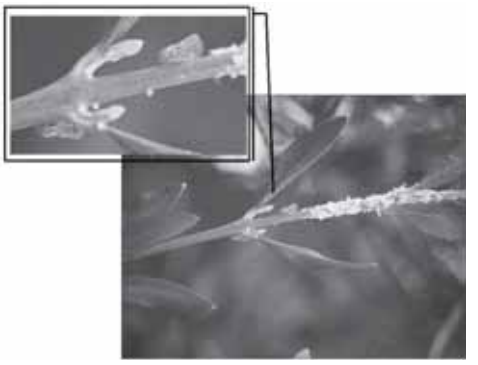

Fig. 3. Adults of *M. pruinosa* on olive tree.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Ξενιστές του εντόμου *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae) και παρατηρήσεις επί της φαινολογίας του στην Ελλάδα

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Περίληψη Επισκόπηση που πραγματοποιήθηκε σε περιοχές της δυτικής (Πρέβεζα, Πάργα, Λευκάδα, Κέρκυρα) και της βόρειας (Σέρρες) Ελλάδας από τον Οκτώβριο του 2005 μέχρι τον Οκτώβριο του 2006 έδειξε ότι το έντομο *Metcalfa pruinosa* (Say) φιλοξενείται σε ένα μεγάλο αριθμό καλλωπιστικών και καλλιεργούμενων καρποφόρων φυτών, σε πολλά αυτοφυή ζιζάνια, καθώς και σε δύο καλλιεργούμενα λαχανικά (τομάτα, μελιτζάνα).
Με διαδοχικές δειγματοληψίες στη διάρκεια ενός έτους φάνηκε ότι το έντομο στη δυτική Ελλάδα συμπληρώνει μια γενεά το χρόνο, με τα ενήλικα άτομα να δραστηριοποιούνται στην περίοδο από τα τέλη Ιουνίου μέχρι τα τέλη Σεπτεμβρίου.

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SHORT COMMUNICATION

First record of *Acalles barbarus* (Lucas) (Coleoptera: Curculionidae) in Greece. A serious pest of caper in the island of Ios

C. Souliotis[1]

Summary *Acalles barbarus* (Lucas) (Col.: Curculionidae) was found in 2005 on caper (*Capparis spinosa*) plants of an organic crop in the island of Ios. It is a new species to the Greek entomological fauna. The insect attacks main shoots and twigs of the caper plants with the larvae boring galleries on them. Damaged twigs are short, rather thin and scrubby with small chlorotic leaves. First observations on biology and ethology of the insect in the area indicated that it completes one generation per year, hibernates at the larval stage inside capper twigs and adult appearance starts in mid-June to beginning of July.

The caper (*Capparis spinosa*, of the family Capparidaceae) thrives in temperate, tropical and subtropical climate zones and is a common species of the Mediterranean coastal flora. It grows wild as a shrub of up to 1 m height and 1.5 m width (or as a creeper) on stony wasteland along the coast, in rock crevices, in the mountains and even on ruins.

In addition to its pharmaceutical use, the caper is also known for its nutritive qualities. Pickled young shoots, flower buds and young leaves may be consumed as appetizer or used in cookery.

Lately there has been a growing interest by inhabitants of Ios and other Cycladic islands in culturing the caper. It provides an additional source of family income in particular to the inhabitants of those islands where farming conditions are adverse and jobs are hard to find. The promotion of organic farming has evoked an additional interest in the commercial exploitation of the wild growing caper and has prompted its systematic culture in the field or under simple greenhouses, in small family farms, as is the case in the island of Ios. The organic caper crop in the field and under greenhouses in Ios, from where the first sample of damaged caper originated, is a typical example of such a family run exploitation of culturing caper.

The identification of the insect was made by using the adult beetles collected from the first samples (twigs and shoots) that were sent to the Laboratory in September 2005. Identification to the genus level was based on Brisout de Barneville (1), Caillol (2), Kocher (5), Liotta (6), Solari (10), Meyer (9) Hoffmann (3), and Vitale (11). Professor Liotta (Facoltà di Agraria dell' Università di Palermo, Italy), provided information about the species *Acalles barbarus* (7). This species (Fig. 1) is reported for the first time from Greece as it is a new species of the Greek entomological fauna.

Species of the genus *Acalles* Schh. are

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Fig. 1. *Acalles barbarus,* female adult.

widespread in countries of the Palaearctic regions, as well as in the parts of America and Australia that are washed by the Pacific Ocean. They occur on many plant species (herbs, shrubs and trees), of various families (Leguminosae, Compositae, Euphorbiacae, Crassulaceae and others) and feed on scrubby and weakish plants rather than on healthy ones (6, 8, 11) .

Available literature on *A. barbarus* L. from the Mediterranean basin reports its presence on caper in Pantelleria, south of Sicily (Italy), which is the dominant island of caper culture and trade in the Mediterranean (6, 8, 11). *Acalles* species are not generally characterized as pests (4, 6, 8) and therefore the damage they can cause to various plants they feed on has not been assessed.

A. barbarus feeds on plants weakened by various biotic and abiotic factors (pests, diseases, frost etc). Only the larvae of the insect are harmful to the plants. The slight damage caused by the adults is considered non economically important. On vigorous and healthy plants damage is negligible while on plants parasitized at the same time by other insect pests, in particular by species of Diaspididae and Pseudococcidae, damage may be considerable (6, 8).

Preliminary observations on the biology and ethology of A. barbarus: As the species is new to the insect fauna of Greece, some preliminary observations are presented on the biology and ethology of the insect. In addition, for the first time the developmental stages of the insect and the symptoms on caper are recorded (Fig. 2, 3, 4).

A. barbarus has one generation per year. It hibernates at the larval stage inside attacked twigs. Adults start appearing from mid-June till beginning of July. During the day they are difficult to be found as they are hiding in the 5 cm upper layer of the soil, while at night they leave their shelter and clamber up the caper plants moving slowly and gnawing at the margin of the leaves as all *Acalles* species do.

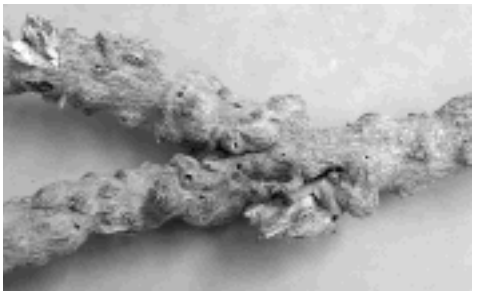

Fig. 2. Exit holes of the adults from infested caper shoots.

A. barbarus displays a curious behaviour when disturbed by conspicuous sounds. It then shams death: it retracts its legs, remains motionless and immediately lets itself drop to the ground where it is difficult to spot because of the colour it is taking. In this pose it may remain for a considerable time. The male is smaller than the female. There are records in the literature of the male following a female around for many days without copulation being observed (6). The adult lives for a

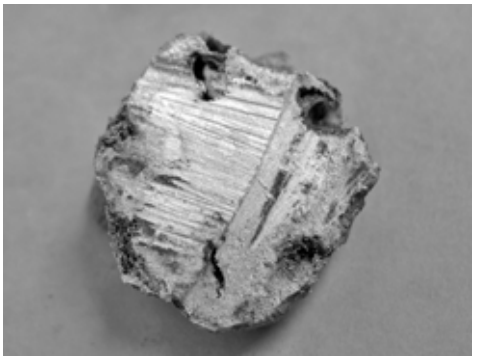

Fig. 3. Feeding galleries of the larvae on a caper shoot (cross section).

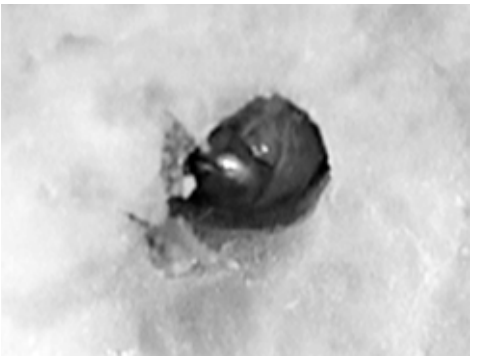

Fig. 4. The head of a larva in a gallery.

long time. Adults that emerged from the cage during the last ten days of June were found to be still alive up to the first ten days of September. In field crops, the first larvae can be found under the bark of the trunk and branches about one month after appearance of the adults. The larvae start boring galleries between the bark and the wood and rarely between the wood and the pith. No larvae were observed in dead plants. Many larvae may be found at the same time in attacked plants, but galleries seldom cross each other.

Over summer, autumn and winter the larvae feed and grow till they pupate the next spring after having prepared a cavity a little wider than the gallery for the adult to emerge.

Damage: Attacked plants have short, rather thin and weak shoots, carrying small, chlorotic leaves and a reduced number of flower-buds, while the plant in general looks weakish and scrubby. They recover slowly unless there are annular galleries on the main stem. In the latter case the plant dies off in a short time.

Based on observations on the organic caper crop in Ios, the *A. barbarus* seems to be the main cause for a poor growth of the plants, a decline in production and a general weakness of the cultured and wild plants in the area.

This species, therefore, may, under Greek conditions, cause severe damage in caper. Very limited data is available on the biology of the pest and its control, exclusively from international sources. It is worthy, therefore, to undertake a systematic study of the biology and ethology of the insect. A recording of its natural enemies and their activity in the area may assist in developing a method for its control, as there is no appropriate insecticide authorized for use on caper.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του *Acalles barbarus* (Lucas) (Coleoptera: Curculionidae) στην Ελλάδα. Ένας σοβαρός εχθρός της κάππαρης στη νήσο Ίο

Κ. Σουλιώτης

Περίληψη Το *Acalles barbarus* (Lucas) (Coleoptera: Curculionidae), που διαπιστώθηκε σε βιολογική καλλιέργεια κάππαρης (*Capparis spinosa*) στη νήσο Ίο το 2005, αποτελεί νέο είδος για την ελληνική εντομολογική πανίδα και πρώτη καταγραφή για τη χώρα μας. Το έντομο προσβάλλει τα κεντρικά στελέχη και τους βλαστούς των φυτών, μέσα στους οποίους οι προνύμφες ορύσσουν στοές. Οι προσβεβλημένοι βλαστοί είναι βραχείς, αρκετά λεπτοί και ασθενικοί με φύλλα μικρά και χλωρωτικά. Η προσβολή καταλήγει σε μείωση των μπουμπουκιών και κατά συνέπεια της παραγωγής. Προκαταρκτικές παρατηρήσεις της βιολογίας του εντόμου στην περιοχή έδειξαν ότι αυτό συμπληρώνει μία γενεά το χρόνο, διαχειμάζει ως προνύμφη μέσα στους προσβεβλημένους βλαστούς και η εμφάνιση των πρώτων τέλειων ατόμων γίνεται από τα μέσα Ιουνίου μέχρι τις αρχές Ιουλίου.

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First report of *Phytophthora primulae* in Greece: identification based on morphology and DNA analysis and determination of its host range

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Summary A severe disease of parsley was observed in Southeastern Greece during the winter 2002 that reappeared the following years. Symptoms of the disease included root and stem rot, chlorosis and wilting of the leaves. Isolates that belonged to the genus *Phytophthora* were obtained from diseased plants. The morphological and physiological characters were close to these of *Phytophthora primulae*. PCR-RFLPs patterns of the ITS region were generated from isolates of *P. porri, P. primulae* and *P. syringae* by digestion with the restriction endonucleases *Alu*I, *Msp*I and *Taq*I. *P. syringae* showed different PCR-RFLPs patterns from those of *P. porri* and *P. primulae,* which were identical. After amplification of the ITS region the sequence of the parsley isolate was found to share high homology with the CBS620.97 (AF266802) isolate of *P. primulae*. Pathogenicity of *P. primulae* isolates was tested on artificially inoculated parsley plants as well as on primula plants and various vegetable species including tomato, and the winter crops: lettuce, cauliflower, broccoli, red cabbage, white cabbage, leek, Brussels sprout, and carrot. *P. primulae* isolates were pathogenic to parsley, but not to any other plant tested. All parsley plants developed symptoms similar to those observed in the field and died three to five weeks after inoculation. Control (non inoculated) plants and all the other plant species tested remained healthy. Isolates of *P. cryptogea, P. citrophthora P. porri* and *P. nicotianae,* were not pathogenic, when tested on parsley plants. Finally, when apple fruits, potato tubers and onion bulbs were inoculated with *P. primulae*, only apple fruits were infected. To our knowledge, this is the first report of *Phytophthora primulae* in Greece and the second on parsley wordwide.

Additional keywords: Pathogenicity, *Petroselinum crispum,* primula, ITS, PCR-RFLP

Introduction

A root rot of parsley [*Petroselinum crispum* (Mill.) Nym. Ex A.W. Hill] occurred for the first time in commercial fields at Marathon, Attica County during the winter 2002. The disease reappeared the following years, causing significant yield losses. Symptoms included root and stem rot, chlorosis and wilting of the leaves. When soil moisture levels were high, plants were destroyed. Initial observations placed the pathogen in *Phytophthora* sp. Species of the genus *Phytophthora* are destructive pathogens, attacking a broad range of economically important woody perennials or annual crops, including vegetables, in Greece. Among vegetables they cause heavy losses to tomatoes (*Lycopersicon esculentum* Miller) potatoes (*Solanum tuberosum* L.), peppers (*Capsicum anuum* L.), *lettuce (Lactuca sativa* L.) etc (5, 8). Additionally, many species of *Phytophthora* from several other hosts such as citrus, deciduous and nut

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trees, cotton and ornamentals have been identified, causing serious soilborne diseases in Greece (6, 7, 10, 11, 12, 13).

The objectives of this study were to identify the pathogen causing root rot of parsley, using morphological and molecular features, and to determine its host range.

Materials and Methods

Pathogen morphology and physiology

Cornmeal agar (CMA) was used to isolate the pathogen from diseased parsley plants, to maintain the cultures and to detect the maximum growth temperature. Morphological characteristics of the isolates were observed on mounts made after growth on CMA, pea-broth (200 g frozen pea in 500 mL H_2O mashed for 5 min in a blender, the mixture was centrifuged for 10 min at 3000 rpm and the supernatant was collected and made up to 1L with distilled water; the latter was diluted with distilled water in a ratio 1 to 8 and then autoclaved at 121^{0}C for 20 min) and soil extract (1g of soil was mixed in 100 mL of distilled water, left for 24 h, allowed to sediment and the supernatant was filtered to obtain the extract). For sporangia production the isolates from CMA were transferred to Petri plates 9 cm in diameter, containing pea-broth medium, incubated for 24 h and then transferred to Petri dishes containing a shallow layer of soil extract.

The species identification was based on the morphological and physiological characteristics using the revised tabular key for the genus *Phytophthora* and C.M.I. descriptions (16, 17).

RFLP analysis and PCR sequencing reaction

For sequence analysis of ITS region the protocol of Cooke and Duncan (2) was followed. For DNA extraction, five parsley isolates (P1, P2, P3, P4, P5) were grown at 20^{0}C, each in two Erlenmeyer flasks containing 100 ml pea-broth. Seven days later and after vacuum filtration the mycelium was freeze-dried and kept at -20^{0}C. For DNA extraction 100 mg mycelium from each isolate was ground in plastic Eppendorf tubes with 50 mg sterile sand and 750 µL extraction buffer [200 mM 1M Tris-HCl pH 7.5, 250 mM 5M NaCl, 25 mM 0.5M EDTA pH 8 and 0.5% from 10% SDS (sodium dodecyl sulfate)]. The samples were centrifuged for 5 min at 13.000 rpm; the upper phase was extracted with 250 µL phenol and 250 µL iso-amyl alchohol. After centrifigation for 5 min the upper phase was transferred to new tubes adding 0.54 volumes isopropanol and was centrifuged for 10 min. The DNA pellet was washed with 1000 µL frozen ethanol, air dried and resuspended in 100 µL SDW (sterile distilled water) with RNAse (5 mg/ml) for extended storage at -20^{0}C. DNA concentrations were determined both spectrophotometrically and on agarose gels. The PCR amplification was conducted on 50 µL mixture that was overlaid with 30 µL of sterile mineral oil and subjected to thermal cycling. A single round PCR using the universal primers ITS6 (GAAGGTGAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC) was applied (5 µL buffer, 6 µL $MgCl_2$ 25 mM, 5 µL dNTPs 2 mM, 1 µL of each primer 30 pM, 1 µL *Taq* DNA polymerase, 2 µL sample and 29 µL SDW). PCR conditions were a single denaturation step at 94^{0}C for 3 min, followed by 35 cycles of annealing at 55^{0}C for 30 s, extension at 72^{0}C for 60 s, and denaturation at 94^{0}C for 30 s, with a final extension step at 72^{0}C for 10 min. The reaction mixture was run on 2% agarose gels then stained with ethidium bromide and visualized under UV illumination to test the yield and size of the product.

The PCR products from the isolate P1 in addition to BPIC1989 isolate of *P. porri* and BPIC2514 isolate of *P. syringae*, with morphological and physiological similarities

were digested with the restriction enzymes *Alu*I, *Msp*I and *Taq*I to generate characteristic banding patterns (Restriction Fragment Length Polymorphism, RFLP analysis).

Macrogen DNA Sequencing Service (Macrogen Korea, 10F Meridian Center, 60-24 Kasan-dong, Kumchun-ku Seoul, Korea 153-023) carried out direct sequencing of PCR products for the isolate P1 using ITS6 and ITS4 primers.

Pathogenicity tests

To complete Koch's postulates, 35-day-old parsley plants were artificially inoculated with the tested fungus. To determine the host range of the pathogen additional 35-day-old plants of other vegetable species, cultivated in the Marathon area, such as tomato (*Lycopersicon esculentum* L.) hybrid Belladona, and the winter crops: lettuce (*Lactuca sativa* L.) cultivar Paris Island Cos, cauliflower (*Brassica oleracea* var. *botrytis* L.*)* cultivar Alpha, broccoli (*Brassica oleracea* var. *asparagoides* L.) cultivar Ramoso Calabrese, red cabbage (*Brassica oleracea* var. *capitata* L. f. *alba*) cultivar Langedijk autumn, white cabbage (*Brassica oleracea* var. *capitata* L. f. *tubra*) cultivar Merveille d' Octobre, leek (*Allium porrum* L.) cultivar Blue solaise, Brussels sprout [*Brassica oleracea* L. var. *gemmifera* (DC) Thell] cultivar Groninger, carrot (*Daucus carota* L.) cultivar Nantes, and primula [*Primula acaulis* (L.) Hill, syn. *P. vulgaris* Hudson] were inoculated with P1, P2, P3 isolates of *P. primulae*.

Parsley plants were also inoculated with two isolates of *P. cryptogea* (BPIC1189 and BPIC1191) and *P. citrophthora* (BPIC1133 and BPIC1185), one isolate of *P. porri* (BPIC1985) and one isolate of *P. nicotianae* (BPIC2000), derived from the Benaki Phytopathological Institute Culture Collection (BPIC).

All the plants were grown separately in 10 cm pots filled with moist compost (Klassman Potground). Three mycelial discs 10 mm in diameter taken from the edge of a 12–day-old colony on lima-bean agar were inserted around the crown and roots of the plants, 1 cm under the moist soil surface and wounded prior to inoculation, according to Sitepu and Bumbieris (15). The control plants were inoculated with Lima-bean-agar discs. Soil moisture was maintained by placing each pot in a plastic container filled continually with distilled water. For each plant species and *Phytophthora* strain fifteen replications were used. The air temperature in the greenhouse fluctuated 15-20^0 C with a 12 h photoperiod.

Apple fruits (*Malus domestica* Borkh.), potato tubers (*Solanum tuberosum* L) and onion bulbs (*Allium cepa* L.) were artificially inoculated with *P. primulae* isolate P1 according to Tomlinson method (18). Three mycelial discs 10 mm in diameter taken from the edge of a 12–days-old colony on lima-bean agar were inserted in three points on each fruit under the rind. Ten replications of fruits, tubers or bulbs were used and in respective controls water-agar plugs were inserted.

The experiments were repeated once.

Results

Pathogen morphology and physiology

The causal organism, isolated from diseased parsley plants, was a slow-growing species of the genus *Phytophthora*. The pathogen developed on CMA sparse aerial mycelium. Microscopic examination revealed coiled hyphae (Fig. 1), and hyphal swellings.

On soil extract medium, at laboratory temperature (18-22^{0}C), abundant number of sporangia were formed within 24 h. Persistent semipapillate sporangia, simple and compound, variable in shape and size were formed on undifferentiated sporangiophores. Simple sporangia were ovoid or ellipsoid, limoniform, very often elongated with several constrictions (Fig. 2B). The dimensions of these sporangia were 42.5-75 x 22.5-43.75 (average 63 x 31) µm.

The length-breadth ratio varied from 1.3 to 2.4:1 (average 1.8:1). Sporangia consist-

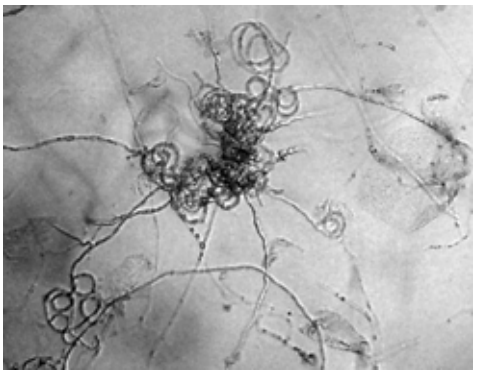


Fig. 1. Coiled hyphae of *Phytophthora primulae* from parsley.

ed of spherical or irregularly shaped segments with structures in chain fashion, following the formation of septa at the constrictions or delimitation of the sporangiophore, were also abundant in the cultures (Fig. 2A, B, C). The protoplasmic contents were often passed to the terminal development, while the other segments were empty (Fig. 2B, C). They measured 85-390 x 7-45 µm. Tomlinson (18) called these segmented bodies "compound sporangia". The isolates were omothallic, forming oogonia abundantly on CMA, terminally or laterally, 27-48 (average 37.5) µm in diameter. Antheridia were mostly paragynous, diclinous, often two per oogonium and occasionally amphigynous, while the young oospores were hyaline, average 32 µm in diameter (Fig.3). The maximum temperature for mycelial growth was 26^{0}C.

The morphological and physiological characteristics of the causal microorganism were consistent with the published descriptions of *Phytophthora primulae* (16, 18).

RFLP analysis and PCR sequencing

All isolates yielded an equal size fragment (~900 bp) when amplified with primers ITS6 and ITS4. Identical digestion patterns were generated by BPIC1989 (*P. porri*) and P1 (*P. primulae*) isolates which had been

Fig. 2. Sporangia of *Phytophthora primulae* from parsley on soil extract A: compound sporangium with pronounced constrictions B: sporangia and compound sporangium. C: compound sporangium, the protoplasmic contents were passed to the terminal development of the sporangium.

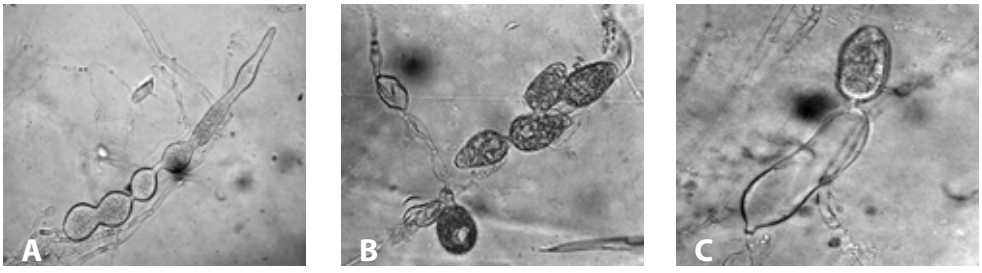
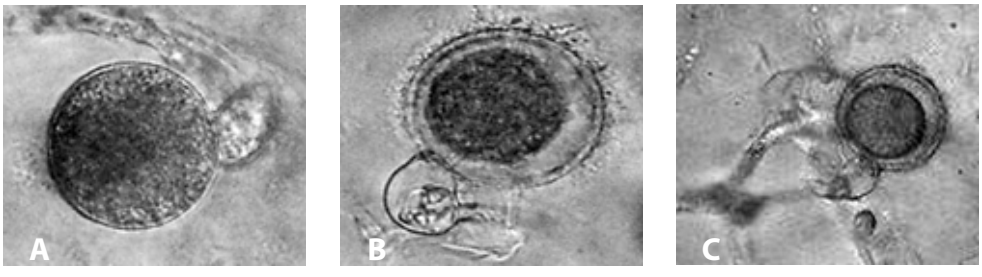


Fig. 3. Oogonium and oospores with paragynous antheridia, of *Phytophthora primulae,* from parsley on corn meal agar A: immature oogonium B: mature oospore. C: mature oospore with two antheridia.



digested with the enzymes *Alu*I, *Msp*I and *Taq*I, while the BPIC2514 isolate of *P. syringae* showed a different band pattern (Fig. 5).

Employing the BLAST procedure in NCBI, the ITS sequence of P1 isolate was found to share high homology with *P. primulae* strain CBS620.97 (AF266802.1), isolated from *Primula acaulis* with identities 790/791. According to a phylogenetic tree based on neighbour-joining analysis [program NCBI/BLAST/blastin (nucleotide databases)], the species with the highest degree of similarity to *P. primulae* was *P. porri* (Fig. 4). After sequencing, the P1 isolate was deposited to Benaki Phytopathological Institute Collection (BPIC) with culture number BPIC2584.

Pathogenicity tests

The isolates of the fungus from parsley were pathogenic to parsley, but not to other plant species. Parsley pants developed symptoms similar to those observed in the field and most of them died three to five weeks after inoculation, while control plants (non-inoculated) and the other plant species remained healthy (Table 1). *P. primulae* was reisolated from the infected crowns and roots of the artificially inoculated plants. The other *Phytophthora* species tested did not infect the parsley plants (Table 2).

Apple fruits developed a brown rot from the wound to the center, while the controls, the inoculated potato tubers and the onion bulbs remained healthy (Table 3) (18).

The results of the the repeated experiments and the tests with the other parsley isolates were similar.

Discussion

Pathogenicity of *P. primulae* was confirmed on primula plants, since this plant species is the main host of *P. primulae* (18). Tomlinson has identified for the first time

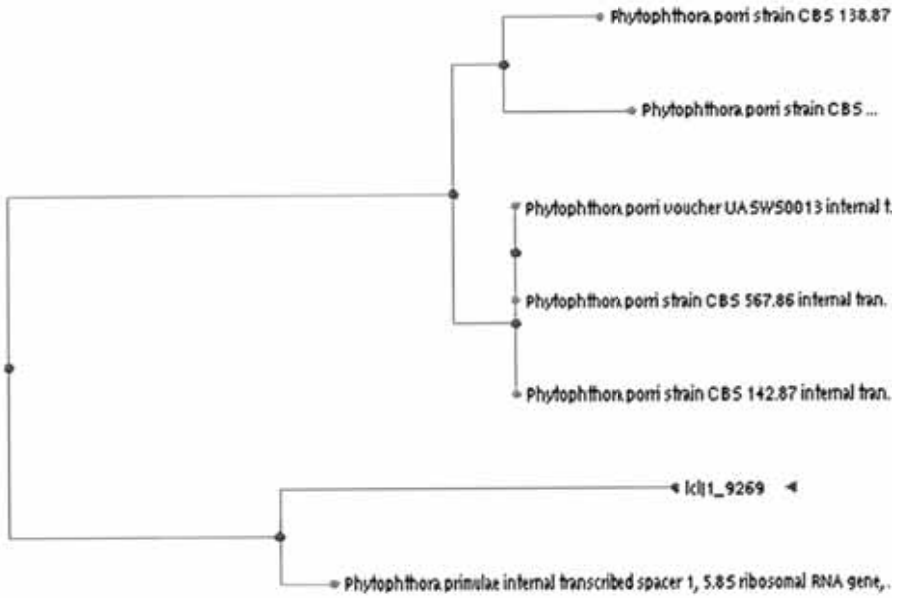


Fig. 4. Phylogenetic tree for *Phytophthora* species, from PCR amplified products of ribosomal DNA, using the ITS6 and ITS4 primers, based on neighbour-joining analysis by "Macrogen". The ITS sequence of parsley isolate BPIC2584 (lc‖1_9269) was close to strain CBS620.97 (AF266802) of *P. primulae*. The nearest species, exhibiting the highest degree of similarity to *P. primulae* is *P. porri.*

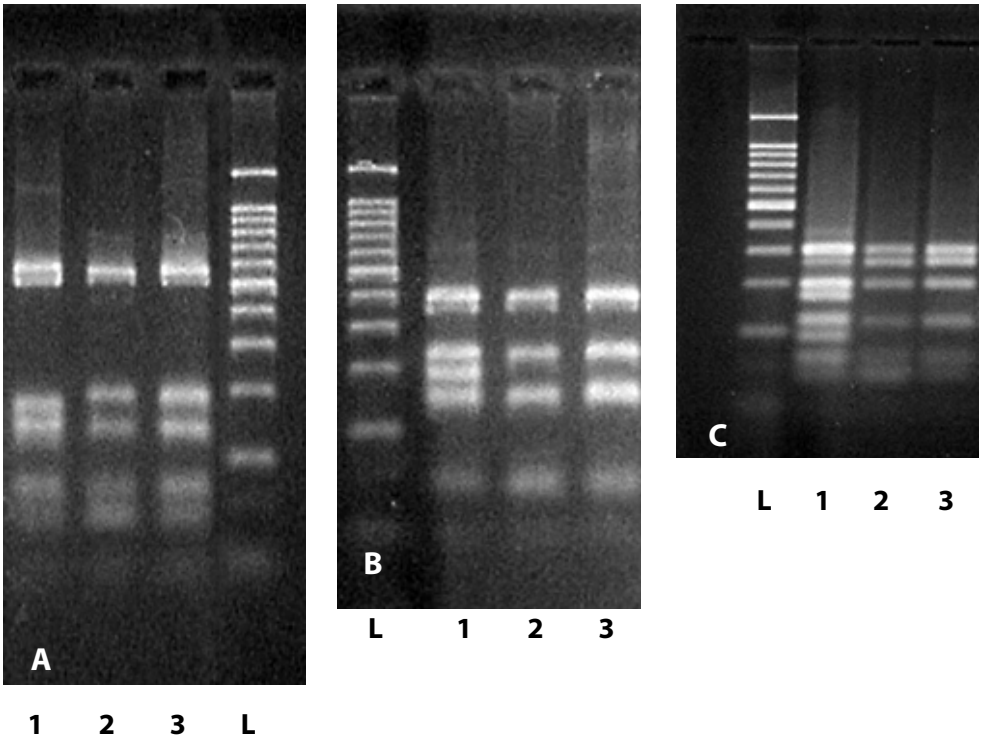


Fig. 5. PCR amplified products from ribosomal DNA, using the ITS6 and ITS4 primers, were digested with the restriction enzymes *Alu*I (A), *Msp*I (B) and *Taq*I (C) for RFLP (Restriction Fragment Length Polymorphism) patterns, obtained by gel electrophoresis. The same digest patterns were generated by BPIC2584 isolate of *P. primulae* (2) and BPIC1989 of *P. porri* (3) while the BPIC2514 isolate of *P. syringae* (1) showed a different distinctive pattern, L: the size marker is 100 bp ladder.

Table 1. Susceptibility of different plant species to *Phytophthora primulae*.

Plant species	*Phytophthora primulae* BPIC2584 Disease index
Petroselinum crispum	3.93
Lycopersicon esculentum hybrid Belladona	1
Lactuca sativa cv. Paris Island Cos	1
Brassica oleracea var. *botrytis* cv. Alpha	1
Brassica oleracea var. *asparagoides* cv. Ramoso Calabrese	1
Brassica oleracea var. *capitata* cv. Langedijk autumn	1
Brassica oleracea var. *capitata* cv. Merveille d' Octobre	1
Allium porrum cv. Blue solaise	1
Brassica oleracea var. *gemmifera* cv. Groninger	1
Daucus carota cv. Nantes	1
Primula acaulis	1

Disease index (average of 15 replications), 1: no infection, 5: 100% plants dead or almost dead.

Table 2. Susceptibility of *Petroselinum crispum* plants to different *Phytophthora* species.

Phytophthora species	*Petroselinum crispum* plants Disease index
P. cryptogea BPIC1189	1
P. cryptogea BPIC1191	1
P. citrophthora BPIC1133	1
P. citrophthora BPIC1185	1
P. nicotianae BPIC2000	1
P. porri BPIC1985	1

Disease index (average of 15 replications), 1: no infection, 5: 100% infection.

Table 3. Susceptibility of different species to *Phytophthora primulae*.

Plant species	*Phytophthora primulae* BPIC2584 Disease index
Malus domestica fruits	5
Solanum tuberosum tubers	1
Allium cepa bulbs	1

Disease index (average of ten replications), 1: no infection, 5: 100% infection.

P. primulae causing brown core and root rot of *Primula polyantha*. Tomlinson's isolates were also virulent to *P. vulgaris*, but not to *P. veris* L. The new *Phytophthora* species was very close taxonomically to *P. syringae*, but Tomlinson has distinguished *P. primulae* by the formation of large sporangia and mainly from the compound sporangia. Also *P. primulae* has been recorded in New Zealand and Denmark, causing brown core of *P. polyantha* (1). In our pathogenicity tests, strains of *P. primulae* from parsley were not pathogenic to primula plants. There is only one report of *P. primulae*, causing root and crown rot of parsley in UK (16).

Pathogenicity of *P. cryptogea, P. citrophthora*, *P. porri* and *P. nicotianae* strains isolated from other than parsley hosts was tested on parsley since these species are common to Greek crops. *P. nicotianae* and *P. cryptogea* are described as parasites of parsley (3, 9). There was a *P. citrophthora* isolate that caused root rot of parsley in Greece; however this isolate has been lost (K. Elena 1993, unpublished data).

Phytophthora species identification is often difficult since morphology and growth of isolates can be variable. Kouyeas and Chitzanidis (13) examined Greek isolates from citrus peach and apricot trees, having a low maximum growth temperature and characterized these as *Phytophthora syringae* (Klebahn) Klebahn, based on morphological characters. The isolates formed hyphal swellings in a chain-like fashion reminding the figures and descriptions given by Tomlinson (18) for the compound sporangia of *P. primulae*. Additionally, in the above isolates the chain of swellings ended occasionally to a sporangium, a character that brought them even closer to *P. primulae*. These characters are obvious in Fig. 2 of the respective article (13). However these isolates did not form oogonia except the citrus isolate, which produced abundantly oogonia with paragynous antheridia only the first period after their isolation. According to Kouyeas and Chitzanidis the characters that Tomlinson used to distinguish *P. primulae* from *P. syringae* did not seem strong enough. We were not able to compare our

isolates with those of Kouyeas and Chitzanidis, since the last have been lost. Noviello and Snyder (14) described, for some isolates, the compound sporangia of Tomlinson; however the writers considered finally all isolates as *P.* syringae, based on Tucker taxonomy (19) in spite of Tomlinson's (18) description of the new species *P. primulae*. Our isolates from parsley were confirmed as *P. primulae,* since the classical characterization and sequence analysis were totally consistent. According the two identification methods used in our study, the most similar to *P. primulae*, was *P. porri*, isolated from lettuce (8). BPIC1989 (*P. porri*) and BPIC2584 (*P. primulae*) isolates yielded identical PCR-RFLP patterns, although they had been characterized as different species by classical phytopathological methods and the ITS sequencing. They also had different pathogenicity in cross inoculation tests using the hosts from where they were isolated (unpublished data). The BPIC2514 isolate of *P. syringae* showed distinctive patterns in the same test, separated from *P. primulae* and *P. porri* isolates. Employing restriction enzymes, to generate PCR-RFLP patterns, is a simple and rapid method, useful to distinguish *Phytophthora* species; however, morphological characteristics should also be taken into account (4), specifically for species with small differences in the DNA band sizes that are difficult to estimate by the common laboratory techniques.

The results of this study indicated that in artificial inoculations only parsley was susceptible to *P. primulae*, while all the other tested vegetables were resistant; parsley was resistant to other *Phytophthora* species tested. This disease caused by *P. primulae* could be of importance for parsley in areas where low soil temperatures occur during winter.

To the best of our knowledge this is the first report of *P. primulae* in Greece and the second causing parsley disease worldwide.

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Πρώτη αναφορά του *Phytophthora primulae* στην Ελλάδα: ταξινόμηση με βάση τους μορφολογικούς χαρακτήρες και την rDNA ανάλυση και καθορισμός των ξενιστών του παθογόνου

Κ. Ελένα και Α. Γρηγορίου

Περίληψη Προσδιορίστηκε ένα νέο είδος του γένους *Phytophthora* για την Ελλάδα και μελετήθηκε μια σοβαρή ασθένεια που προκαλεί στο μαϊντανό, η οποία διαπιστώθηκε για πρώτη φορά το 2002. Τα συμπτώματα στα φυτά ήταν σήψη των ριζών και του στελέχους με αποτέλεσμα το κιτρίνισμα και τη μάρανση των φύλλων. Όταν οι συνθήκες του περιβάλλοντος, κυρίως της εδαφικής υγρασίας, ήταν ευνοϊκές για την ασθένεια ακολουθούσε η καταστροφή των φυτών με αποτέλεσμα να καταστρέφεται μέχρι και το 50% αυτών στους αγρούς. Από τους προσβεβλημένους ιστούς απομονώθηκε στέλεχος του γένους *Phytophthora με* βραδεία αύξηση και χαμηλό μέγιστο θερμοκρασίας ανάπτυξης. Για το χαρακτηρισμό του είδους χρησιμοποιήθηκε ένα ολοκληρωμένο σύστημα προσδιορισμού με κλασσικές και μοριακές τεχνικές. Η μελέτη των μορφολογικών (μορφή αποικίας και μυκηλίου καθώς και μεγέθη σποριαγγείων και ωοσπορίων) και των φυσιολογικών (μέγιστο θερμοκρασίας ανάπτυξης) χαρακτήρων των απομονώσεων που χρησιμοποιήθηκαν τις κατέταξε στο είδος *P. primulae*. Επιπλέον εξετάστηκε ο πολυμορφισμός της περιοχής ITS του ριβοσωμικού DNA. Μετά την εξαγωγή του DNA έγινε ενίσχυση με PCR με τη χρησιμοποίηση των γενικής χρήσης εκκινητών ITS6 και ITS4. Στο προϊόν της PCR για τις απομονώσεις BPIC1989 (*P. porri*), BPIC2514 (*P. syringae*) και BPIC2584 (*P. primulae*), με ομοιότητες ως προς τους μορφολογικούς και φυσιολογικούς χαρακτήρες, έγινε πέψη με τα περιοριστικά ένζυμα *Alu*I, *Msp*I και *Taq*I για τη δημιουργία RFLPs (Restriction Fragment Length Polymorphisms). Διαπιστώθηκε ότι οι απομονώσεις BPIC1989 (*P. porri*) και BPIC2584 (*P. primulae*) είχαν όμοιες ηλεκτροφορητικές κατατομές και με τα τρία ένζυμα, αν και είχαν χαρακτηριστεί ως διαφορετικά είδη με τη κλασσική μέθοδο προσδιορισμού ενώ η απομόνωση BPIC2514 (*P. syringae)* είχε διαφορετική κατατομή. Επιπλέον τα αποτελέσματα της αλληλούχησης ξεχώρισαν και τα τρία είδη. Δοκιμές παθογένειας των απομονώσεων του *P. primulae* έγιναν σε φυτά μαϊντανού, πρίμουλας, τομάτας και μιας σειράς χειμερινών κηπευτικών δηλαδή μαρουλιού, κουνουπιδιού, μπρόκολου, λάχανου, κόκκινου λάχανου, πράσου, λάχανου Βρυξελλών και καρώτου. Επιπλέον μήλα, κόνδυλοι πατάτας και βολβοί κρεμμυδιού μολύνθηκαν με απομονώσεις του είδους. Στα φυτά μαϊντανού δοκιμάστηκε και η παθογένεια απομονώσεων των ειδών *P. cryptogea, P. citrophthora, P. porri* και *P. nicotianae.* Οι απομονώσεις του *P. primulae* από το μαϊντανό αποδείχθηκαν παθογόνες στο μαϊντανό και στα μήλα αλλά όχι στα άλλα είδη φυτών. Τα άλλα είδη *Phytophthora* που δοκιμάστηκαν δεν ήταν παθογόνα στο μαϊντανό. Από όσο γνωρίζουμε αυτή είναι η πρώτη αναφορά του *P. primulae* στην Ελλάδα και η δεύτερη αναφορά του να προκαλεί ασθένεια στο μαϊντανό παγκόσμια.

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Reference styles for Literature cited

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