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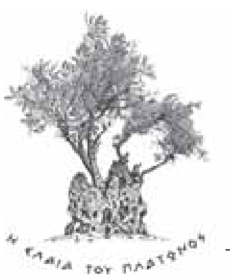

The olive tree of Plato in Athens is the emblem of the Benaki Phytopathological Institute

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REVIEW ARTICLE

New records of plant pests and weeds in Greece, 1990-2007

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Summary More than 70 new insect records have been reported from Greece during the period 1990-2007. The woolly whitefly *Aleurothrixus floccosus* Maskell and the citrus leaf-miner *Phyllocnistis citrella* Stainton, which are among the most important new records, have already spread throughout the country but severe damage has been avoided with the introduction of effective exotic parasitoids. Other species of the newly reported pests that are considered important are: the thrips *Pezothrips kellyanus* (Bagnall) and *Frankliniella occidentalis* (Pergande), the red palm weevil *Rhynchophorus ferrugineus* (Olivier), the gall inducing wasps *Ophelimus maskeli* (Ashmead) and *Leptocybe invasa* Fisher & LaSalle and the asparagus pests *Parahypopta caestrum* (Hübner) and *Hexomyza simplex* (Loew).

Nine new records of phytophagous mites have been reported from Greece during the period 1990-2007. Of these species, *Eutetranychus orientalis* (Klein) and *Tetranychus evansi* Baker & Pritchard are quarantine mites. Especially *T. evansi* although not yet widespread in the country may have a serious economic impact since it can cause damage by reducing market value of the affected crops.

A total of 30 plant parasitic nematode species have been reported from Greece, during the period 1990-2007, most of which are known to be important pests worldwide. From these, only one species, *Globodera pallida* Stone on potato, appears to be of special interest.

Three weed species known to be very troublesome and widespread in the United States have recently appeared in Greece and are causing serious problems in irrigated summer crops, particularly in maize. They include *Ipomoea hederacea* (L.) Jacquin (Convolvulaceae) which has already spread in the western part of the country, *Sicyos angulatus* L. (Cucurbitaceae) which is spreading in the northern part and *Panicum dichotomiflorum* Michaux (Gramineae) which is currently restricted in a small area in the central part of the country. The appearance of these weeds in Greece demonstrates the need for measures to prevent invasion and spread of aggressive alien species.

Introduction

An attempt has been initiated by scientists at the Benaki Phytopathological Institute to elaborate on data published in national and international literature and summarize new records reported for Greece during the period 1990-2007. This will provide a reliable update of existing up to 1990 national checklists for plant pathogens, pests and weed species occurring in Greece.

The plant pest records, including insects, mites and nematodes as well as the weed species records for the period 1990-

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2007 are summarized in this article. The plant pathogen records, including fungi, bacteria, viruses and viroids, for the same period, were recently published by Elena *et al*. (2008).

1. Insect pests

The data presented here are mostly based on records reported in the literature and only a small number comes from samples examined at this laboratory and registered either in the Annual Reports or the Archive material of the Benaki Phytopathological Institute. New aphid species, coccids and Agromyzidae are not included here as they have been recently recorded and summarized (Katsoyannos, 1994; Kozár e*t al., 1*994, Kavallieratos *et al.,* 2001, 2004b, 2004c, Souliotis & Süss, 2004).

The species are discussed in the text arranged by the order and the family and listed in Table 1 alphabetically.

1.1. Hemiptera - Homoptera

The woolly whitefly ***Aleurothrixus floccosus*** Maskell (Aleyrodidae), one of the most important pests of citrus, was first recorded in Attica by Katsoyannos, (1991). Currently it is present in all citrus growing areas of Greece and causes economic damage when at high population densities. The introduced parasitoid *Cales noacki* Howard has managed to control the whitefly where insecticide spraying against other citrus pests was avoided. The present pest status of the woolly whitefly is considered to be "under control" as it shows periodic increases at certain areas that are however controlled by the parasitoid.

Two aphid species are included here as they have become very important recently, namely ***Aphis citricola*** van der Goot (Aphididae) on citrus (Lycouressis, 1990a) and ***Macrosiphum euphorbiae*** (Thomas) (Aphididae) on cotton (Lycouressis, 1990b). We should mention, however, that in Greece there are many species of aphids that can be important pests. All these species have already been presented by other authors (Katsoyannos, 1994, Kavallieratos *et al*., 2001, 2004b, 2004c).

The grapevine aphid ***Aphis illinoisensis*** Shimer (Aphididae), a native to America, was reported for the first time from Greece (and generally from Europe) in 2005 to feed on grapevine plants in Crete (Aggelakis *et al*., 2005). The aphid is already widespread in Crete. It has been found on almost all grapevine varieties grown in the area and seems to prefer the vigorous ones.

The soft scale ***Parthenolecanium persicae*** (Fabricius) (Coccidae) was found on grapevines in Messinia (Peloponnese) in October 2000 and on *Viburnum tinus* in Central Greece in June of the same year (Stathas, 2003c). The insect was first recorded in Greece (July 1983) on *Morus* sp. in Northern Greece (Epanomi) (Kozár, 1985).

Nemolecanium graniformis (Wünn) (Coccidae) is a pest of Grecian fir (*Abies cephalonica*) that was found in 1996 in the mountain of Parnitha. The insect produces honeydew on which sooty mould develops (Stathas, 1997).

The pyriform scale insect, ***Protopulvinaria pyriformis*** (Cockerell) (Coccidae) was found on bay plants (*Laurus nobilis*) in Kalamata (Peloponesse) in October 2003. The insect was also found in many other countries on more than 100 plant species (Ben-Dov *et al.,* 2003).

The elm bark scale ***Ritsemia pupifera*** Lichtenstein (Pseudococcidae) was found on elm trees (*Ulmus* sp.). It develops on the bark of the tree and is covered with dense white waxy secretions (Savopoulou *et al.,* 1997)

Lepidosaphes pistaciae Archangelskaya (Diaspididae) was observed in 1990 on pistachio and terebinth (*Pistacia terebinthus*). (Katsoyannos & Stathas, 1995). It establishes on young stems, on the fruit and the leaves of the trees and can cause deformities and drying of the plants.

In June 2001 the scale insect ***Lepidosaphes gloverii*** (Packard) (Diaspididae) was observed on orange trees in the area of Gastouni (Peloponnisos). The insect infests mainly the upper surface of the leaves, the fruit and to a lesser extent the stems (Stathas, 2003a).

The species ***Eriococcus coccineus*** Cockerell (Eriococcidae) was first recorded to infest *Echinocactus grusonii* plants in glasshouses in the area of Acharnai (Attica) in October 2000. Young nymphs of this insect are found on the fleshy part of the cactus (Stathas, 2003b).

In addition, a list of the scale insects recorded in Greece until 2006 has been presented by Milonas *et al.* in 2007. Currently, the number of known scale insects has reached 168. Six new records were included: ***Heterococcus nudus*** (Green) Pseudococcidae, ***Asterodiaspis variolosa*** (Ratzeburg) Asterolecanidae, ***Lecanopsis turcica*** (Bodenheimer) Coccidae, ***Phenacoccus hordei*** (Lindeman) Pseudococcidae, ***Poliaspis cycadis*** Comstock Diaspididae and ***Orthesia yasushi*** Kuwana Ortheziidae.

Insects of the family Psyllidae are considered at present as the most important pests of pistachio. They are new pests of pistachio in Greece causing the characteristic symptoms of Psyllidae: abnormal development of buds and shoots, premature leaf drop, formation of honeydew and very weak appearance of the plants. In Greece, four species of this family are found to infest pistachios: ***Agonoscena pistaciae*** Burckhardt et Lauterer (Lauterer *et al*., 1998), ***A. targionii*** (Lichtenstein) (Zartaloudis *et al*., 1996), ***A. cisti*** Puton (B. Polymerou, Personal communication) and ***Megagonoscena gallicola*** Burckhardt et Lauterer (Souliotis & Tsourgianni, 1999). *A. pistaciae* was recorded in West Korinthia and is the dominant species in the areas of Fthiotis, Boeotia, Euboea and Attica. *A. targionii* was found in Halkidiki in locations where pistachio is one of the main cultivations. The remaining species (*A. cisti* and *M. gallicola*) are of no economic importance as their population and activity in pistachio orchards are low Souliotis & Tsourgianni, 2000).

Trioza alacris Flor (Triozidae) was found on Appolo bay plants (*Laurus nobilis)* in 2004 in Attica to cause a serious infestation of the leaves (Tsagarakis & Papadoulis, 2004).

Metcalfa pruinosa (Say) (Flatidae) was first observed in Greece in 2001 in the area of Preveza (Drosopoulos *et al.,* 2004) on citrus and olive trees (first record on olive). Currently, the insect has spread to other areas infesting fruit trees, grapevines, ornamental plants and wild plants (Souliotis *et al.,* 2008). The insect sucks the plant sap and excretes plenty of honeydew where sooty mold develops. As a result the plants weaken and eventually die. Chemical control of this pest is difficult. Its control currently relies on the use of parasitoid natural enemies.

Pemphigus fuscicornis (Koch) (Pemphigidae) is a root living aphid that was found on sugarbeet for the first time in 1985 in Imathia (Ioannidis, 1997) causing severe damage to plants.

1.2. Thysanoptera

The banded greenhouse thrip, ***Hercinothrips femoralis*** (Reuter) (Thripidae), was recorded for the first time in 2004 in greenhouse-grown organic banana in the area of Sitia (northeastern Crete). Banana fruits were severely damaged by the thrips and a typical smoky-red discoloration of the fruit was observed. During 2005 *H. femoralis* was also found causing severe damage in conventional banana plantations in Arvi, the main banana-growing area of Crete (Roditakis *et al,*. 2006).

Pezothrips kellyanus (Bagnall) (Thripidae), a new serious citrus pest, was recently (2003) recorded in Crete. Its presence was noticed during flowering when population densities were high especially on lemon flowers (Varikou *et al.,* 2003).

The species ***Taeniothrips inconsequens*** (Uzel) (Thripidae), was found in 1999 to cause a severe infestation on pear trees var. Kontoula (Papadopoulos *et al.,* 2002). The symptoms were obvious on infested buds, which do not develop normally or do not develop at all. Also, there were stems with few or no leaves and fruits with surface scarring.

Frankliniella occidentalis (Pergande) (Thripidae) has been recorded in Crete (Roditakis 1994) and in Kavala (Katsoyannos, 1992; Paloukis *et al.,* 1995). It causes primary damage during the flowering period and later it causes fruit scarring. Important damage (20-40% of grapes) was observed in Korinthia in 1996 (Tsitsipis *et al.* 1997). *F. occidentalis* is a polyphagous species of glasshouse crops (with more than 200 host species), it causes spots (with small black specks), scarring and the drying of the plant tissues. On cotton *F. occidentalis* was observed in July 1993 at the perfectures of Imathia and Pella (Kyparissoudas & Alexandrakis, 1993). The infestation was evident at the shoot tips while development of the whole plant was slower. It infests the flowers especially the yellow ones that remain closed without forming any fruits ("bolls") or forming small atrophic "bolls". As a pest it is regarded as one of great economic importance.

The species ***Thrips parvispinus*** (Karny) (Thripidae), a native to South-East Asia, was recorded for the first time in Europe (2000), including Greece, to infest gardenia plants (Mound & Collins, 2000).

The species ***Tenothrips frici*** (Uzel) (Thripidae) was recorded in 1992 for the first time in Greece on *Lycopersicon esculentum* in the area of Thiva (Deligeorgidis, 2002).

Since 2000 a severe infestation of the outter parts of the canopy of *Ficus microcarpa* has been observed in several areas of Greece (Attica, Messinia, Kyparissia). The damage is due to ***Gynaikothrips ficorum*** (Marshal) (Phlocothripidae) that infests the young leaves of *F. microcarpa*. It also attacks *F. elastika* and *F. benjamina* (Papadoulis & Emmanouel, 2001).

1.3. Coleoptera

The red palm weevil, ***Rhynchophorus ferrugineus*** (Olivier) (Curculionidae), the most important pest of palm trees, causing severe damage, has been found for the first time in Hersonissos (Heraklion, Crete), infesting *Phoenix canariensis, during* 2005 and for the first time in Cyprus, infesting also *P. canariensis,* during 2006. During 2007, the weevil was found in the island of Rhodes and in Attica infesting mainly *P. canariensis* (Kontodimas *et al.,* 2007).

The weevil ***Mesagroicus pilifer*** (Boheman) (Curculionidae) has been observed to cause a serious infestation of sugarbeet seedlings in Melia (Thessalia, Central Greece) during 2000-2005, particularly in 2003 and 2004. The infestation, manifested around the roots of the seedlings where ball-like soil accumulations were observed, was more evident on the leaves which were peripherally eaten (Papadopoulou, 2005).

High numbers of ***Tychius quinquepunctatus*** (Linneaus) (Curculionidae) were found on *Vicia sativa* (garden vetch), in Larissa (Central Greece) during 2003. Extensive infestations by this insect seem to happen occasionally. Larvae, which destroy the seeds within the pods, are the most damaging stage (Koveos *et al*., 2003).

The species ***Otiorrhynchus aurifer*** Boheman (Curculionidae), was observed in Attica (1991) to cause damage on leaves of various ornamental plants (Bouchelos, 1991).

The tansy leaf beetle ***Galeruca tanaceti*** (Linneaus) (Chrysomelidae**)** was recorded in May 2005 as a pest of *Origanum vulgare* L. growing in the wild in Crete. At the same time the insect was observed to be a pest of origanum plants in a commercially grown organic crop, in the area of Meronas (Perfecture of Rethymno) (Roditakis & Roditakis, 2006).

The rose curculio ***Homalorhynchites hungaricus*** (Herbst) (Rhynchitidae**)** has been recorded in Attica (May 2004), infesting blooms of ornamental roses (*Rosa* sp.) (Kontodimas & Kavallieratos, 2004).

The wood eating beetle ***Coroebus rubi*** (Linneaus) **(**Buprestidae**)** was observed in 2004 in the area of Korinthia to cause an infestation on blackberries *Rubus* spp. (Bouchelos & Chalkia, 2004).

Diaperis boleti Linneaus (Tenebrionidae**)** was recorded in 1994 in peach orchards. The adults open shallow burrows on branches where they hibernate. The larvae develop in mines on the branches and appear around mid June. Infested branches break and the trees as well as the production are severely affected (Savopoulou-Soultani & Chatzivassiliadis, 1999).

Sinoxylon sexdentatum (Olivier) (Bostrychidae) was recorded as a pest of walnuts in the area of Larissa and Magnesia in May 1996. The insect in other countries has a wide range of tree species as hosts. It is a wood-eating insect attacking the stems of young vigorous trees (Gravanis *et al.,* 1998)

Cassida seraphina Menetries (Chrysomelidae**)** was found on sugarbeet in the Region of Evros (Northern Greece) in 1992 (Doulias & Ioannidis, 1995). It feeds on the leaves and can cause severe damage by sceletonizing the leaves.

Attagenus unicolor (Brahm) (Dermestidae) was recorded in 1999 as a pest of stored wheat products in Farsala and Lakonia (Bouchelos & Athanassiou, 1999).

Pseudopachymerina lallemantii (Marseul) (Bruchidae) was recorded on the ornamental tree *Acacia farnesiana*. It infests the pods causing a significant damage on it (Bouchelos & Chalkia*,* 2003).

The species ***Prionus coriarius*** Linnaeus, ***Cerambyx velutinus*** Brulle, ***Cerambyx dux*** Faldermann and ***Rhopalopus clavipes*** (Fabricius) of the family Cerambycidae were recorded in 1994 as pests of the almond tree in the area of Magnesia (Koutroubas, 1994). They are all wood-eating insects.

1.4. Diptera

Hexomyza (Ophyiomya) simplex (Loew) (Agromyzidae) is a stem miner that was recorded for the first time in 2002 on asparagus plants in the areas of Aitoloakarnania (Central-Western Greece) and Orestias (North-Eastern Greece) (Anagnou-Veroniki *et al.* 2004). Damage is caused to the hypodermal cells of the stem. In cases of a large infestation the epidermis dries out and tears apart. The most serious damage is caused to young asparagus plantations. Damaged plant tissue favours fungal infestation. The pest is currently spreading in Greece while its control is difficult.

Two new leaf-miners, originating from the United States, ***Liriomyza huidobrensis*** (Blanchard) and ***L. trifolii*** (Burgess) (Agromyzidae), were found in 1993 in Crete (Roditakis 1993) while soon later they were found in cultivations all over Greece. *L. huidobrensis* Blanchard, that is considered as the tomato leaf-miner is of the greatest economic importance while it has the most hosts of all the other leaf-miners and it is found in great numbers on mainland Greece as well as in the Greek islands. *L. trifolii* prefers warmer areas and this is why it appears especially in Southern Greece and in Crete.

Delia (Phorbia) platura (Meigen) (Anthomyiidae) was reported to cause significant damage to asparagus plantations in asparagus-growing locations of the Imathia and Pella perfectures (Stamopoulos *et al.* 1994). The pest has probably been present in the area since the end of 1980s. The larvae burrow into the soft stems and around the neck of the plant causing anomalous development and curling of the main stems. The most important damage is on young shoots before they are harvested, thus seriously reducing the marketable yield.

The species ***Plioreocepta (Platyparea) poeciloptera*** Schrank (Tephritidae**)** was found in 1992 in asparagus plantations at Platy (Imathia) damaging the stems of young plants (Chlapoutakis, 1999).

A population of ***Diopsis* sp**. (Diopsidae**)** was found in 2002 and 2003 in rice pads at the area of Chalastra (Thessaloniki), causing the named "green sickness" (chlorosis) to the rice plants. The characteristic symptom is the development of chlorotic leaves and later the drying of the plants creating gaps in the crop. The insect is regarded as an important pest of rice in Africa (Bouras *et al.,* 2003).

The gall inducing insect ***Monarthropalpus buxi*** (Laboulbene) (Cecidomyiidae**)** was found in Attica in infested leaves on a species of *Buxus* (Vamvakas *et al*., 2007).

The dipterous insect ***Lasioptera* sp.** (Cecidomyidae) was first recorded in 2001 on cucumber plants in the area of Trifilia in Western Peloponnese. Since then infestations are observed in this area mainly from September to December on tomato and from March to June and from September to December on cucumber. In 2004 and 2005 the insect was also recorded from greenhouse tomato and cucumber in the area of Marathon (Attica). The insect infests the peduncles at the point of their attachement to the fruit which turns to black. In tomato, upon infestation, plant growth is retarded and number and size of fruit is reduced., while the presence of larvae on the fruit, at the point of their attachment to peduncles, renders them unmarketable. In cucumber, the effect of insect infestation becomes evident mainly on the apical part of the plant (Perdikis *et al.*, 2006).

1.5. Lepidoptera

The citrus leaf-miner ***Phyllocnistis citrella*** Stainton (Gracillariidae) was first recorded in Rhodes (Anagnou-Veroniki, 1995), most probably introduced into Greece from eastern countries of the Mediterranean basin. It has already spread throughout the country and is currently found on most citrus species. For the control of this pest, parasitoids were introduced and established so that to supplement its indigenous natural enemies.

The leafminer ***Cameraria ohridella*** Deschka & Dimic (Gracillariidae) was found in 1999 to cause a serious infestation on leaves of horse chestnut (*Aesculus hippocastaneum* L.) in areas of the North-Western and Central Greece. The infestation was known to exist since 1984 at the area of Ohrid (Emmanouel & Broumas, 2000; Diamantis *et al*., 2001).

The spotted tentiform leaf-miner ***Phyllonorycter blancardella*** (Fabricius) (Gracillariidae), that is known to feed on apple trees, was found in 1997 to attack also cherry trees of the varieties "Tragana Edessis" and "Bourla" in the area of Pella. Damaged cherry leaves had the characteristic whitish elliptical mines on their underside and corresponded to palegreen spots on the upper surface (Kyparissoudas, 1997).

Loxostege stictialis (Linnaeus) (Pyralidae), which is considered to be a pest specific to cotton, was recorded in 1989 to feed on sugar beet, alfalfa and maize. This infestation is caused by its larvae that eat the lower epidermis and the leaf parenchyma (Evangelopoulos, 1994).

Parahypopta* (*Hypopta*) *caestrum (Hübner) (Cossidae) was first recorded in

Table 1. New records of insect plant pests from Greece during 1990-2007.

Insect species	Order/Family	Host	Reference
Agonoscena cisti	Hemiptera: Psyllidae	pistachio	Polymerou (Personal communication)
Agonoscena pistaciae	Hemiptera: Psyllidae	pistachio	Lauterer *et al.,* 1998
Agonoscena targionii	Hemiptera: Psyllidae	pistachio	Zartaloudis *et al.,* 1996
Aleurothrixus floccosus	Hemiptera: Aleyrodidae	citrus	Katsoyannos, 1991
Allantus cinctus	Hymenoptera: Tenthredinidae	ornamental roses	Simoglou, 2004
Aphis citricola	Hemiptera: Aphididae	citrus	Lycouressis, 1990a
Aphis illinoisensis	Hemiptera: Aphididae	grapevine	Aggelakis *et al*., 2005
Arge ochropus	Hymenoptera: Argidae	ornamental roses	Simoglou, 2004
Asterodiaspis variolosa	Hemiptera: Asterolecanidae	*Quercus coccifera*	Milonas *et al.,* 2007
Attagenus unicolor	Coleoptera: Dermestidae	wheat stored products	Bouchelos & Athanassiou, 1999
Cameraria ohridella	Lepidoptera: Gracillariidae	*Aesculus hippocastaneum*	Emmanouel & Broumas, 2000; Diamantis *et al.,* 2001
Cassida seraphina	Coleoptera: Chrysomelidae	sugar beet	Doulias & Ioannidis, 1995
Cerambyx dux	Coleoptera: Cerambycidae	almond tree wood	Koutroubas, 1994
Cerambyx velutinus	Coleoptera: Cerambycidae	almond tree wood	Koutroubas, 1994
Cladius difformis	Hymenoptera: Tenthredinidae	ornamental roses	Simoglou, 2004
Coroebus rubi	Coleoptera: Buprestidae	blackberries (*Rubus* spp.)	Bouchelos & Chalkia, 2004
Delia (Phorbia) platura	Diptera: Anthomyiidae	asparagus	Stamopoulos *et al.,* 1994
Diaperis boleti	Coleoptera: Tenebrionidae	peach	Savopoulou & Chatzivassiliadis, 1999
Diopsis sp.	Diptera: Diopsidae	rice	Bouras *et al*., 2003
Eriococcus coccineus	Hemiptera: Eriococcidae	*Echinocactus grusonii*	Stathas, 2003b
Eurytoma schrenerii	Hymenoptera: Eurytomidae	apricots	Koveos *et al.* 2000
Frankliniella occidentalis	Thysanoptera: Thripidae	glasshouse crops, grapes, cotton	Roditakis, 1991; Katsoyannos, 1992

Table 1 (continued)

Insect species	Order/Family	Host	Reference
Galeruca tanaceti	Coleoptera: Chrysomelidae	*Origanum vulgare*	Roditakis & Roditakis, 2006
Gynaikothrips ficorum	Thysanoptera: Thripidae	*Ficus microcarpa* *Ficus* sp.	Papadoulis & Emmanouel, 2001
Hellula undalis	Lepidoptera: Pyralidae	Cruciferous crops	Simoglou *et al.,* 2007
Hercinothrips femoralis	Thysanoptera: Thripidae	banana	Roditakis *et al.,* 2006
Heterococcus nudus	Hemiptera: Pseudococcidae	Gramineae	Milonas *et al.,* 2007
Hexomyza (Ophyiomya) simplex	Diptera: Agromyzidae	asparagus	Anagnou-Veroniki *et al.,* 2004
Homalorhynchites hungaricus	Coleoptera: Rhynchitidae	ornamental roses	Kontodimas & Kavallieratos, 2004
Lasioptera sp.	Diptera: Cecidomyidae	tomato and cucumber	Perdikis *et al.,* 2006
Lecanopsis turcica	Hemiptera: Coccidae	Gramineae	Milonas *et al.,* 2007
Lepidosaphes gloverii	Hemiptera: Diaspididae	orange trees	Stathas, 2003
Lepidosaphes pistaciae	Hemiptera: Diaspididae	pistachio and terebinth (*Pistacia terebinthus*)	Katsoyannos & Stathas, 1995
Leptocybe invasa	Hymenoptera: Eulophidae	eucalyptus trees	Potasov *et al.,* 2007
Liriomyza huidobrensis	Diptera: Agromyzidae	vegetable crops, ornamental plants	Roditakis, 1993
Liriomyza trifoliii	Diptera: Agromyzidae	vegetable crops, ornamental plants	Roditakis, 1993
Loxostege stictialis	Lepidoptera: Pyralidae	sugar beet, alfalfa, maize	Evangelopoulos, 1994
Macrosiphum euphorbiae	Hemiptera: Aphididae	cotton	Lycouressis, 1990b
Megagonoscena gallicola	Hemiptera: Psyllidae	pistachio	Souliotis & Tsourgianni, 1999
Mesagroicus pilifer	Coleoptera: Curculionidae	sugarbeet seedlings	Papadopoulou, 2005
Metcalfa pruinosa	Hemiptera: Flatidae	citrus and olive trees	Drosopoulos *et al.,* 2004
Monarthropalpus buxi	Diptera: Cecidomyiidae	*Buxus* sp.	Vamvakas *et al.,* 2007
Nemolecanium graniformis	Hemiptera: Coccidae	Grecian fir (*Abies cephalonica*)	Stathas, 1997
Ophelimus eucalypti	Hymenoptera: Eulophidae	eucalyptus trees	Potasov *et al.,* 2007

Table 1 (continued)

Insect species	Order/Family	Host	Reference
Ophelimus maskeli	Hymenoptera: Eulophidae	eucalyptus trees	Potasov *et al.,* 2007
Orthesia yasushi	Hemiptera: Ortheziidae	*Erica arborea*	Milonas *et al.,* 2007
Otiorrhynchus aurifer	Coleoptera: Curculionidae	ornamental plants	Bouchelos, 1991
Parahypopta (*Hypopta*) *caestrum*	Lepidoptera: Cossidae	asparagus	Kyparissoudas & Chlapoutakis, 1992
Parthenolecanium persicae	Hemiptera: Coccidae	grapevines, *Viburnum tinus*	Stathas, 2003c
Paysandisia archon	(Lepidoptera: Castniidae)	Palm trees	Vassarmidaki *et al.,* 2006
Pemphigus fuscicornis	Hemiptera: Pemphigidae	sugarbeet	Ioannidis, 1997
Pezothrips kellyanus	Thysanoptera: Thripidae	citrus	Varikou *et al.,* 2003
Phenacoccus hordei	Hemiptera: Pseudococcidae	*Thymus vulgaris*	Milonas *et al.,* 2007
Phyllocnistis citrella	Lepidoptera: Gracillariidae	citrus	Anagnou-Veroniki, 1995
Phyllonorycter blancardella	Lepidoptera: Gracillariidae	cherry (var. Tragana Edessis)	Kyparissoudas, 1997
Plioreocepta (Platyparea) poeciloptera	Diptera: Tephritidae	asparagus	Chlapoutakis, 1999
Poecilimon cretensis	Orthoptera: Phaneropteridae	olive	Polyrakis, 1991
Poliaspis cycadis	Hemiptera: Diaspididae	*Cycas* sp.	Milonas *et al.,* 2007
Prionus coriarius	Coleoptera: Cerambycidae	almond tree wood	Koutroubas, 1994
Protopulvinaria pyriformis	Hemiptera: Coccidae	Appolo bay plants (*Laurus nobilis*)	Ben-Dov *et al.,* 2003
Pseudopachymerina lallemantii	Coleoptera: Bruchidae	sweet acacia trees (*Acacia farnesiana*)	Bouchelos & Chalkia, 2003
Rhopalopus clavipes	Coleoptera: Cerambycidae	almond tree wood	Koutroubas, 1994
Rhynchophorus ferrugineus	Coleoptera: Curculionidae	*Phoenix canariensis*	Kontodimas *et al.,* 2007
Ritsemia pupifera	Hemiptera: Pseudococcidae	elm trees (*Ulmus* sp.)	Savopoulou *et al.,* 1997
Sinoxylon sexdentatum	Coleoptera: Bostrychidae	walnuts	Gravanis *et al.,* 1998
Taeniothrips inconsequens	Thysanoptera: Thripidae	pear trees (var. Kontoula)	Papadopoulos *et al.,* 2002

Table 1 (continued)

Insect species	Order/Family	Host	Reference
Teleiodes decorella	Lepidoptera: Gelechidae	pistachio	Tsourgianni, 1994
Tenothrips frici	Thysanoptera: Thripidae	*Lycopersicon esculentum*	Deligeorgidis, 2002
Thrips parvispinus	Thysanoptera: Thripidae	gardenia	Mound & Collins, 2000
Trioza alacris	Hemiptera: Triozidae	Appolo bay plants (*Laurus nobilis)*	Tsagarakis & Papadoulis, 2004
Tychius quinquepunctatus	Coleoptera: Curculionidae	*Vicia sativa* (garden vetch)	Koveos *et al.,* 2003

1992 in Northern Greece and is now considered as one of the most economically important pests of asparagus (Kyparissoudas & Chlapoutakis, 1992). The larvae infest the shoots and the roots of the plant and can completely destroy them when at high densities.

The small moth ***Teleiodes decorella*** (Haworth) (Gelechidae) was recorded in 1994 on pistachio trees. It commonly coexists with *Archips rosanus* on young leaves while it can also damage the developing shoots (Tsourgianni *et al.*, 1994).

The species ***Paysandisia archon*** Burmeister (Castniidae) native to South America, was recovered from infested palm trees in Greece in 2005. This species, along with the red palm weevil, is a major threat to the palm nursery industry and to parks and recreation areas in coastal regions (Vassarmidaki *et al.*, 2006).

The species ***Hellula undalis*** (Fabricius) (Pyralidae), an important pest of cruciferous crops in tropical and subtropical regions, was for the first time reported in Greece in 2007 (Simoglou *et al.*, 2007). The larvae of this species were found in August to cause severe damage to cabbages, cauliflowers and brocoli grown in several locations in the north of the country.

1.6. Hymenoptera

Eurytoma schreinerii Schreiner (Eurytomidae) has been recorded on apricot fruits in the area of Nafplion (Koveos *et al.*, 2000; 2002). Affected fruits were smaller and had a smaller endocarp. The presence of the white legless larva in the endocarp is of diagnostic value. The species is known as a pest of plums in South Russia.

The species ***Ophelimus eucalypti*** (Gahan), ***O. maskeli*** (Ashmead) and ***Leptocybe invasa*** Fisher & LaSalle (Eulophidae) were recently recorded as pests of *Eucalyptus* trees in the Mediterranean basin including Greece. In the report *O. eucalypti* is probably a misidentification of *O. maskeli* (Kavallieratos *et al.*, 2004a; P. Milonas personal observations; Potasov *et al.* 2007).

Three hymenopterous species, namely ***Cladius difformis*** (Panzer) (Tenthredinidae), ***Allantus cinctus*** (Linnaeus) (Tenthredinidae) and ***Arge ochropus*** (Gmelin) (Argidae) have been found since 1995 to feed on rose plants growing in parks and gardens in the town of Rodolivos in the prefecture of Serres (Simoglou, 2004). The young stages of these insects first feed on the underside of the leaves and later destroy the leaf blades reducing the ornamental value of the rose plants.

1.7. Orthoptera

The wingless locust ***Poecilimon cretensis*** Werner (Phaneropteridae) was observed in 1991 to cause a serious problem on trees of the olive variety “Koroneiki” in Crete (Polyrakis, 1991). The problem had first appeared in 1983. The insect develops at high numbers especially on young trees on which it may feed on the leaves causing a complete defoliation.

1.8. Concluding remarks

More than 70 insect species, mostly Hemiptera-Homoptera, have been recorded for the first time in Greece since 1990. The woolly whitefly *Aleurothrixus floccosus* and the citrus leaf-miner *Phyllocnistis citrella*, both serious citrus pests of worldwide importance, are invasive species and their control relied on clasical biological control programmes by introducing exotic parasitoids that were proved to be highly effective. Other species of the newly reported pests that are considered important are: the thrips *Pezothrips kellyanus* and *Frankliniella occidentalis*, the red palm weevil *Rhynchophorus ferrugineus,* the gall inducing wasps *Ophelimus maskeli* and *Leptocybe invasa* and the asparagus pests *Parahypopta caestrum* and *Hexomyza simplex*. Several of the new recorded species are of palearctic origin but their presence in Greece had not been documented so far. It is important in concluding to note that, because of the serious threats imposed by the invasive species, there is always the need for a thorough survey to early detect potential new invaders and for an organized rapid reaction upon their detection.

2. Phytophagous mites

New records of phytophagous mites belonging to the families of Tetranychidae, Eriophyidae and Tenuipalpidae have been reported from Greece, during the period 1990-2007, and are summarized in this article. Three species, namely *Aculus olearius* Castagnoli (Papaioannou-Souliotis, 1982), *Eriophyes* (*Aceria*) *paradianthi* (Keifer) (Papaioannou-Souliotis, 1987) and *Phytocoptella yuccae* (Keifer) (Papaioannou-Souliotis, 1993), although recorded before 1990, are also included here because an outbreak of their population has been recently observed and they are now considered to cause high economic damage. The reported species are discussed arranged by family in the text and listed alphabetically with their hosts in Table 2.

2.1. Tetranychidae

The citrus brown mite ***Eutetranychus orientalis*** (Klein) was first recorded in Greece, in the area of Helleniko (Prefecture of Attica), on lemon and orange trees, in autumn 2001 (Papaioannou-Souliotis & Markoyiannaki-Printziou, 2002). *E. orientalis* is a serious pest of citrus, more detrimental than *Tetranychus urticae* Koch and *Panonychus citri* (Mc Gregor). It mainly affects lemon and orange trees and to a lesser extent mandarins. Feeding generally starts on the upper side of the leaf along the midrib and then moves to the lateral veins. As a result, leaves become chlorotic and yellow streaks develop along the veins. Heavy infestation may cause leaf fall, die-back of branches and defoliation. Until now there are no data on chemical control or other kind of management measures.

The red spider mite ***Tetranychus evansi*** Baker and Pritchard is of South American origin and has been introduced into other parts of the world (Northern and Southern Africa, Spain, Portugal). *T. evansi* tends to prefer solanaceous crops: tomato, aubergine (eggplant), potato, tobacco, but it can be found on several other crops (e.g. beans, citrus, cotton, castor bean) and ornamental plants (e.g. *Rosa* sp.), as well as on many weed species. It was first recorded in Greece, in the area of Tympaki (South-Central Crete) on *Solanum nigrum* in September 2006 (Tsagkarakou *et al.*, 2007). Damage is similar to that caused by other spider mites. Feeding punctures lead to whitening or yellowing of leaves, followed by desiccation and eventual-

Table 2. New records of phytophagous mites from Greece during 1990-2007.

Mite species	Host	Reference
Aculus olearius (Eriophyidae)	olive trees	Papaioannou-Souliotis, 1982
Eutetranychus orientalis (Tetranychidae)	citrus trees	Papaioannou-Souliotis & Markoyiannaki-Printziou, 2002
Eriophyes cynodoniensis (Eriophyidae)	Bermuda grass	Kapaxidi *et al.,* 2008
Eriophyes (*Aceria*) *granati* (Eriophyidae)	pomegranate	Papaioannou-Souliotis, 1994
Eriophyes (*Aceria*) *paradianthi* (Eriophyidae)	carnation	Papaioannou-Souliotis, 1987
Eriophyes peucedani (Eriophyidae)	carrot	Papaioannou-Souliotis, 1993
Phytocoptella yuccae (Eriophyidae)	yucca	Papaioannou-Souliotis, 1991
Raoiella macfarlanei (Tenuipalpidae)	olive trees	Papadoulis & Emmanouel, 2002
Tetranychus evansi (Tetranychidae)	Solanaceous plants	Tsagkarakou *et al*., 2007

ly defoliation. In case of severe attacks, plants may die. *T. evansi* is morphologically similar to the other spider mite species of the genus *Tetranychus* already present in Europe, it can be easily confused with them and thus escape detection. For the control of this mite, classical chemical methods are recommended.

2.2. Eriophyidae

In the group of four olive mites, *Eriophyes oleae* (Nalepa), *Oxycenus maxwelli* (Keifer), *Dytrimacus athiasellus* (Keifer) and *Aceria cretica* (Hatzinikolis), which cause damage of high economic impact in several olive growing areas in Greece, one more species was added, ***Aculus olearius*** Castagnoli (Castagnoli, 1977). This species was first recorded in Greece in 1982, in olive groves of Lefkada and Zakynthos (Papaioannou-Souliotis, 1982). These mites can cause brown patches to young shoots and subcircular, irregular greenish patches that turn chlorotic on leaves. Large mite populations on the inflorescences and young fruits turn them brown and make them fall.

Eriophyes granati (Canestrini and Massalongo) was initially regarded as a Mediterranean species but today it is considered cosmopolitan. This species was first recorded in Greece on nursery trees of ornamental pomegranate, which is its only reported host (Papaioannou-Souliotis, 1994). The infested leaves become twisted.

The carrot bud mite ***Eriophyes peucedani*** (Canestrini) is a pest of the umbeliferous crops and is a common species in Europe and North-Western America. It was reported as a new record for Greece, in the area of Kopaida (Central Greece) on carrot plants, in 1993 (Papaioannou-Souliotis, 1993). During 1995 it was also found in the island of Lesvos on the same crop plant. This mite can cause discoloration of leaves, while in heavy infestations it extends its feeding to the leaf margins resulting in deformation. The plants grow abnormally, bearing few or no flowers at all. Infestation possibly starts from wild plants of the family Umbeliferae while large populations may develop due to the intensive cultivation.

Eriophyes* (*Aceria*) *paradianthi Keifer is a very common species in Argentina and California (U.S.A.) on *Dianthus caryophyllus* L. It was first recorded in Greece, in glasshouses in the area of Attica and in Crete during 1987 (Papaioannou-Souliotis, 1987). Since then, it has also been recorded in the regions of Galata and Poros. It is supposed to have been introduced in Greece through the large and frequent imports of ornamental plants. This mite usually reduces growth and causes a pale yellow discoloration of the carnation plants. Appropriate control method is the chemical one.

Phytocoptella yuccae (Keifer) is a pest of yucca plants. It was recorded for the first time in Greece in the area of Lehonia (Volos, Magnisia) in 1988 (Koutroubas & Bakogiannis, 1989). In the region of Attica it was first observed in the early 90's in many glasshouses (Papaioannou-Souliotis, 1991). As a result of mite infestation the upper surface of leaves exhibits pale white fluffy patterns that later turn brown.

The Bermuda grass mite ***Eriophyes cynodoniensis*** Sayed can be a serious pest wherever Bermuda grass (*Cynodon dactylon* L.) is grown as a turf grass. It is considered to be of African origin. In the United States, the mite may be found from Florida to Arizona. Its only host plant is Bermuda grass. It was oobserved for the first time in Greece, in Aliartos (Viotia), in September 2007 (Kapaxidi *et al*., 2008). Initial damage is observed in spring when turf grass fails to begin normal growth and shows yellow or brown patches in the lawn. Shortening of the stem internodes gives the plants a stunted, rosette appearance. Under a heavy infestation the grass turns brown and dies.

2.3. Tenuipalpidae

Raoiella macfarlanei Pritchard and Baker was first recorded in Greece in olive groves of the island of Corfu in 2002 (Papadoulis & Emmanouel, 2002). This mite can cause discoloration and deformation of leaves.

2.4. Concluding remarks

Two species of the family Tetranychidae, six of the family Eriophyidae and one of the family Tenuipalpidae have been reported for the first time in Greece since 1990. The tetranychid species *E. orientalis* and *T. evansi* constitute the most alarming cases not only because they affect important crops such as citrus and vegetables, respectively, but also because the climatic conditions in southern Greece are appropriate for their population establishment. *E. orientalis* and *T. evansi* can develop rapidly in favorable conditions, while data on their ability to develop pesticide resistance are lacking.

3. Plant parasitic nematodes

Several species of plant parasitic nematodes can be found in cultivated and non-cultivated soils in Greece. Nematode extractions from soil samples deriving from nurseries and other plantations frequently yield moderate to very low populations of *Criconemoides, Helicotylenchus, Longidorus, Paratylenchus, Pratylenchus*, *(Para) Trichodorus, Tylenchorhynchus, Tylenchus* and *Xiphinema* species, however these do not appear to cause serious damage to plants and in most instances are not treated. Thirty species, most of which are worldwide considered important pests, have been reported from Greek soils since 1990. All were found inhabiting cultivated plants and are discussed in the text below, while being summarised alphabetically in Table 3. Amongst the above, reference is made to ten species that have occasionally been found inhabiting plant roots, but are not considered to have a serious economic impact.

Globodera pallida Stone 1973 is one of the two potato cyst nematode (PCN) species. A first indication of its presence was reported by Vovlas & Grammatikaki in 1989 from the island of Crete but this was not confirmed until 2007. A second study performed in Crete showed the presence of only *G. rostochiensis* (Tzortzakakis *et al.*, 2004) and only very recently, Karanastasi & Manduric (unpublished data) confirmed the presence of *G. pallida* in Messinia, while investigating the distribution of the two PCN species in Greece.

***Bursaphelenchus* spp.** To date, approximately 50 *Bursaphelenchus* species have been described, of which about one third inhabit coniferous trees (pine, spruce, fir, larch). These nematodes are known to be vectored by insects of the Cerambycidae and Scolytidae families and are transferred from place to place by timber. The most important species, *B. xylophilus*, has caused rapid decline and subsequent death in many pine forests in East Asia and is of quarantine significance in Europe. A widespread epidemic of pine trees dying in South Eastern Europe triggered an extended survey for the presence of the species in Greece, however only six other *Bursaphelenchus* species were reported: *B. hellenicus* n. sp. from *Pinus brutia*, *B. leoni* from *P. brutia, P. nigra, P. pinaster* and *P. radiata, B. eggersi* from *P. pinaster*, *B. sexdentati* from *P. brutia, P. halepensis, P. nigra* and *P. pinaster* (Skarmoutsos *et al.*, 1996; 1998; Skarmoutsos & Skarmoutsou, 1999), *B. mucronatus* and *B. teratospicularis, B. mucronatus* from *P. brutia* and *B. teratospicularis* from *P. brutia, P. pinaster, P. halepensis* and *P. maritima* (Michalopoulos-Skarmoutsos *et al.*, 2003). All these species may cause wilting of different species of pine. Another species of the family, *Ektaphelenchoides pini,* has also been extracted from wilting *P. brutia, P. nigra* and *P. maritima*, but it was not confirmed whether the nematode was the primary cause of wilt (Skarmoutsos *et al.*, 1996).

Longidoridae. The genera *Longidorus, Paralongidorus* and *Xiphinema* are important plant pests and besides the direct damage they cause to plant roots, several species are natural vectors of nepoviruses (Taylor & Brown, 1997). Until today, a few records on longidorids in Greece have been published. These nematodes do not appear to be serious pests in Greece, except where *Xiphinema index* is found in association with vineyards and *Grapevine fanleaf virus* (GFLV).

Populations of *Longidorus fasciatus* were recovered from fields of artichoke (*Cynara scolymus* L.), in the areas of Iria and Kandia, Argolis, Greece (Brown *et al.*, 1997). The artichoke plants exhibited patchy chlorotic stunting, which is caused by the *Artichoke Italian latent virus* (AILV), a virus naturally transmitted by the nematode. As was recently reported (Karanastasi *et al.*, 2006a) and lately confirmed by subsequent field samples in the area (Karanastasi & Kyriakopoulou, unpublished data), the nematode appears moderately distributed in the above area, which is a traditional artichoke-growing region. No control measures are taken since no yield loss has been reported.

Longidorus cretensis was first described by Tzotzakakis *et al.* (2001), when found in association with grapevine and one olive tree in Crete, however the two plant species where not confirmed to be hosts of the nematode. The species occurred in an area where the leaves of vines exhibited virus-like symptoms, but these were later attributed to GFLV and *X. index*.

Longidorus closelongatus Sturhan & Argo 1983 (Stoyanov 1964), an uncommon species, was only once reported being present in the rhizosphere of olive in Chania, Crete, but was not considered important (Lamberti *et al.*, 1996). Also, *L. pisi* was found on tobacco without mention on locality (Robbins *et al.*, 1995).

Trichodoridae. The family Trichodoridae comprises soil-inhabiting ectoparasitic nematodes that belong to five genera: *Trichodorus* Cobb, 1913*, Paratrichodorus* Siddiqi, 1974*, Allotrichodorus* Rodriguez-M *et al.*, 1978*, Monotrichodorus* Andrássy, 1976 and *Ecuadorus* Siddiqi, 2002. Of these, 54 putative *Trichodorus* species and 33 *Paratrichodorus* species have a worldwide distribution, a very broad host range and can cause severe direct damage to their hosts via root feeding. Moreover, these

Table 3. New plant parasitic nematode records from Greece during 1990-2007.

Nematode species	Host	Region	Reference
Bursaphelenchus eggersi	*Pinus pinaster*	-	Skarmoutsos & Skarmoutsou, 1999
Bursaphelenchus hellenicus	*P. brutia*	Thessaloniki	Skarmoutsos *et al.*, 1998
Bursaphelenchus leoni	*P. nigra,* *P. maritima* *P. pinaster,* *P. radiata,* *P. brutia,* *P. nigra*	Chalkidiki Chalkidiki - - - -	Skarmoutsos *et al.*, 1996 Skarmoutsos & Skarmoutsou, 1999
Bursaphelenchus mucronatus	*P. brutia*	-	Michalopoulos-Skarmoutsos *et al.*, 2003
Bursaphelenchus sexdentatii	*P. brutia,* *P. nigra,* *P. pinaster,* *P. halepensis*	- - - -	Michalopoulos-Skarmoutsos *et al.*, 2003
Bursaphelenchus teratospicularis	*P. brutia,* *P. pinaster,* *P. halepensis,* *P. maritima*	- - - -	Skarmoutsos *et al.*, 1996; Michalopoulos-Skarmoutsos *et al.*, 2003 Skarmoutsos *et al.*, 1996
Criconemoides xenoplax	grapevine, *Viburnum* sp.	Samos, Crete, Attiki (Kifissia)	Karanastasi *et al.*, 2008
Ektaphelenchoides pini	*Pinus brutia,* *P. nigra,* *P. maritima*	Thessaloniki, Kilkis, Chalkidiki, Chalkidiki	Skarmoutsos *et al.*, 1996
Globodera pallida	potato	Crete, Messinia	Vovlas & Grammatikaki, 1989 Karanastasi & Manduric, unpublished
Helicotylenchus multicinctus	banana	Crete	Vovlas *et al.,* 1994
Helicotylenchus pseudorobustus			Vovlas *et al.,* 1993
Hemicycliophora hellenica	*Arundo donax*	Filippias, Epirus	Vovlas, 2000
Heterodera avenae	cereals	-	Vlachopoulos, 1994
Heterodera schachtii	sugarbeet	Imathia (Stavros)	Kyrou, 1993
Longidorus cretensis	grapevine, olive	Crete	Tzortzakakis *et al.*, 2001
Longidorus closelongatus	olive trees	Crete (Chania)	Lamberti *et al.*, 1996
Longidorus fasciatus	artichoke	Argolis (Iria, Kandia)	Brown *et al.*, 1997
Longidorus pisi	tobacco	-	Robbins *et al.*, 1995
Paratrichodorus minor	potato, melon	Amaliada	Karanastasi *et al.*, 2006b
Paratrichodorus teres	artichoke	Argolis (Iria)	Karanastasi *et al.*, 2006a
Pratylenchus goodeyi	banana	Crete	Vovlas *et al.,* 1994
Pratylenchus penetrans	artichoke		Greco *et al.,* 2005
Rotylenchus campensis			Vovlas *et al.,* 1993
Rotylenchus graecus	*Hedera helix,* *Arundo donax*	Filippias, Epirus	Vovlas & Troccoli, 1996

Table 3 (continued)

Nematodes species	Host	Region	Reference
Rotylenchulus macrodoratus	grapevine (cv. Sultanina)	Crete	Vovlas & Vlachopoulos, 1991
Trichodorus similis	tobacco	N. Greece	Brown *et al.*, 1996
Trichodorus sparsus		Patra	Karanastasi *et al.*, 2006b
Tylenchus davainei	citrus, olive olive, English walnut, chestnut, apricot, almond, peach	Korinthos, Messolongi, Larissa	Vlachopoulos, 1991

nematodes may induce severe indirect damage to economically important plant species such as potato, tobacco, bulbous and ornamental plants, by transmission of tobraviruses (Taylor & Brown, 1997).

The first trichodorid reported from Greece was *T. similis*, a vector of *Tobacco rattle virus* (TRV), which was found in 1996 in a tobacco plantation in Northern Greece (Brown, *et al.*, 1996). Later, during a comprehensive study on the distribution of the species in Greece, 14 trichodorid populations were recovered, among which, *P. minor*, *P. teres* and *T. sparsus* were reported for the first time from Greece (Karanastasi *et al.*, 2006a, b).

Besides the above, Roca (1998) isolated a new species in the area of Kato Souli, Marathon, Attica, which he named *Trichodorus variabilis* but was not considered an important pest.

Heterodera schachtii was first recorded from sugar beet in Greece (Kyrou, 1993) and *H. avenae* from cereals (Vlachopoulos, 1994), but although the species are considered important pests, no more information is available in national level.

Criconemoides xenoplax (Raski, 1952) Loof & De Grisse, 1989 of the family Criconematidae was initially extracted from the rhizosphere of grapevines in Samos and Crete but was not reported at that time. A few years later, the same species was also collected from the rhizosphere of dead *Viburnum* sp. plants from the back yard of a house in Kifissia, Attica, Greece, but it was not confirmed whether the nematodes or a fungus found associated with the plants was the primary cause of death (Karanastasi *et al.*, 2008).

Other species. A few other plant parasitic nematode species have occasionally been reported from Greece, but these do not have a serious economic impact. These are: *Helicotylenchus pseudorobustus* found by Vovlas *et al.* in 1993 and one year later *H. multicinctus* on banana (Musa AAA Cavendish subgroup: Dwarf Cavendish) in Crete (Vovlas *et al.*, 1994). *Hemicycliophora hellenica* Vovlas, 2000, a new species was found in Filippias, Epirus, Greece, inhabiting the roots of *Arundo donax* (giant reed) and some unidentified aquatic species, along the line of irrigation canals (Vovlas, 2000); *Hoplotylus femina* in Tirnavos, Larissa (Vlachopoulos, 1991); *Pratylenchus goodeyi* on banana (Musa AAA Cavendish subgroup: Dwarf Cavendish) in Crete (Vovlas *et al.*, 1994); *P. penetrans* on artichoke (Greco *et al.*, 2005); *Rotylenchulus macrodoratus* found widespread in vineyards (cv. Sultanina) in Crete, causing histological damage to the root cells, but no substantial effects on yield (Vovlas & Vlachopoulos, 1991); *Rotylenchus graecus* n. sp. (Nematoda: Hoplolaimidae) in Filippias, Epirus, Greece, inhabiting the roots of *Hedera helix* (ivy) and *A. donax* along the line of irrigation canals

(Vovlas & Troccoli, 1996); *R. campensis* (Vovlas *et al.*, 1993); and *Tylenchus davainei* on citrus and olive trees in Korinthos, olive trees in Messolongi and English walnut, chestnut, apricot, almond and peach trees in Larissa (Vlachopoulos, 1991).

3.1 Concluding remarks

A total of 30 plant parasitic nematode species have been reported from Greece since 1990, most of which are known to be important pests worldwide. However, most cases reported herein were sporadic incidents and cannot be considered a threat to their hosts. Based on available information and experience, the most important nematode pest that growers should be concerned about is *Globodera pallida*. This is an EPPO A2 quarantine organism and legislation regarding the presence of this species, as also *G. rostochiensis*, is very strict, while they both have a dramatic impact on potato. In seed potato growing areas, when a field is found infested, it is legally forbidden to grow potatoes therein and producers should follow a three-year crop rotation scheme alternating cereals and legumes, while concurrently using chemical nematicides. Prior to replanting potatoes, the field has to be re-examined for the presence of cysts.

4. Weed species

There is no systematic monitoring in Greece for introduced plant species and particularly for their spread as weeds in cultivated land of major agricultural areas. Thus, although quite often new records of plant species are presented in a number of papers exploring Greek flora, data on their acclimatization and potential weed-status is seldom provided.

The plant species presented in this article are mostly cases of recently introduced plant species that became noticeable because of some reported problems to growers after their invasion and establishment as weeds in an agricultural area. New plant species for which there is no documentation of having attained a weed status are not included. Since the presentation is not based on a systematic survey, the list of new weed species may not be complete.

The species are discussed in the text according to the chronological order of their observation and listed alphabetically in Table 4.

4.1. Species already acclimatized

In 1990, Giannitsaros presented some non indigenous grass species which appeared to be in a stage of acclimatization and with the potential of becoming weed species in Greece. They were *Bromus catharticus* Vahl., *Digitaria ciliaris* (Retz.) Koeler, *Digitaria ischaemum* (Schreber) Muhl, *Echinochloa colona* (L.) Link, *Eleusine indica* (L.) Gaertner, *Paspalum dilatatum* Poiret, *Paspalum distichum* L. and *Setaria adhaerens* (Forskal) Chiov. (Giannitsaros, 1990). Three of these species, namely *Eleusine indica*, *Paspalum dilatatum* and *Paspalum distichum* have already spread and are now regarded as important weeds in some irrigated crops (particularly lucerne) and in landscape turf throughout the country. *Echinochloa colona* has developed into a minor weed occurring locally in some areas. *Setaria adhaerens* probably occurs as a variant of *Setaria verticillata* (L.) Beauv. (Clayton, 1980) which during recent years has become a very common weed in many crops throughout the country.

4.2. Species in a stage of acclimatization

In 1994, growers in the Louros village near Preveza (western Greece) observed for the first time a new weed that grew throughout summer in irrigated fields of maize, cotton and other crops, causing severe problems by climbing on the crop plants, tying many of them together and

Table 4. New weed species records in Greece, 1990-2007

Weed species	Family	Distribution: Areas (crops)	Reference
Echinochloa colona	Gramineae	Locally, minor weed	Giannitsaros, 1990
Eleusine indica	Gramineae	Throughout (lucerne, turf)	Giannitsaros, 1990
Ipomoea hederacea	Convolvulaceae	Western Greece (maize, cotton, lucerne)	Giannopolitis & Papachristos, 1997
Panicum dichotomiflorum	Gramineae	Kopaida (maize, cotton, turf)	Giannopolitis, 2003a
Paspalum dilatatum	Gramineae	Throughout (lucerne, turf)	Giannitsaros, 1990
Paspalum distichum	Gramineae	Throughout (lucerne, turf)	Giannitsaros, 1990
Sicyos angulatus	Cucurbitaceae	Kavala, Orestiada (maize)	Giannopolitis, 2003b

impeding movement of workers and machinery. The plant was identified as ***Ipomoea hederacea*** (L.) Jacquin and was a new species for the Greek flora (Giannopolitis & Papachristos, 1997). A survey conducted in the area during the summer of 2003 revealed that *I. hederacea* had already evolved into a serious weed for all spring crops throughout the valley of the Louros river. It had also spread well beyond the place of its first appearance being present at high densities in maize fields throughout another valley (Acheron river) of the Preveza prefecture as well as to many fields in the area of Philipiada in the neighboring Arta prefecture (Giannopolitis *et al.,* 2004). There was unverified information that the weed had also spread to areas near Agrinion in the neighboring Aitoloakarnania prefecture. *I. hederacea* is an annual plant species reproducing by seed but it also has the potential to root and regenerate easily from stem cuttings (Kati & Giannopolitis, 2006). It seems to be favoured at the above river valley conditions where it emerges from mid-spring to late summer, grows vigorously, produces many seeds and generally attains all the characteristics of a very competitive, extremely troublesome and difficult to control weed. At present, its distribution seems to be confined to the western part of the country.

In 2002, maize growers from Kavala (Northern Greece), who complained about having problems with a difficult to control weed, were asked to send a specimen for identification. The plant was identified as ***Sicyos angulatus*** L. and its presence in Greece was reported for the first time (Giannopolitis, 2003b). Specimens of the same species were also sent from the area of Orestiada (Thrace), later in the same year. It seems that *S. angulatus* has recently come to Greece and has been present only in the northern part of the country, not yet spread to the south. It is an annual weed reproducing by seed which becomes particularly troublesome in irrigated summer crops by growing vigorously and climbing on the plants. It has a prolonged emergence period, from mid-spring to mid-summer, and is difficult to control without the use of residual herbicides. In the area of Kavala, where the weed was first noticed, soils are organic and effectiveness of residual herbicides is drastically reduced.

In summer 2003, during a weed survey in onion crops of the Kopaida area (Central Greece), a new grass species competing with the onion plants was observed in a field near Orchomenos (Viotia prefecture). The plant was identified to be ***Panicum dichotomiflorum*** Michaux, a species new for the Greek flora (Giannopolitis, 2003a). The next summer, a survey for the detailed assessment of the presence of this species in the wider Viotia area was conducted (Giannopolitis & Efthimiadis, 2004). Plants of *P. dichotomiflorum* were only found along the route from Orchomenos to Aliartos and not along other main routes within the agricultural area of Viotia. The plant was mostly found in fields along the route where maize was grown and to a lesser extent in fields grown with cotton, but it was not found in fields grown with lucerne. Particularly in maize it formed very dense and highly competitive stands. Plants of *P. dichotomiflorum* were also present in nearby cultures of turf grown for the production of ready-to-use turf carpets. It seems possible, therefore, that *P. dichotomiflorum* was introduced in the area through imported turf seed. This must have happened recently, because the plant has not spread in the area yet. *P. dichotomiflorum* is an annual plant reproducing by seed. A striking characteristic of this plant, as it grows in the area, is its ability to form many tillers with branching stems and many panicles per tiller, thus producing an enormous amount of seeds per plant.

4.3. Concluding remarks

The three species *Ipomoea hederacea*, *Sicyos angulatus* and *Panicum dichotomiflorum*, that are currently under acclimatization in Greece, are believed to be natives to Americas and known as very important weeds in the United States (USDA, 2008). The two of them, *S. angulatus* and *P. dichotomiflorun*, have been present in Europe, eg. in Italy (Pignatti, 1982), but not widespread yet.

All three species have already demonstrated their ability of evolving into a serious weed threat for irrigated summer crops grown in Greece. Measures to effectively prevent their further spread should therefore been taken the soonest.

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ΑΡΘΡΟ ΕΠΙΣΚΟΠΗΣΗΣ

Νέες καταγραφές εχθρών των φυτών και ζιζανίων στην Ελλάδα, 1990-2007

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Περίληψη Πάνω από 70 νέες καταγραφές εντόμων έγιναν στην Ελλάδα την περίοδο 1990-2007. Ο εριώδης αλευρώδης (*Aleurothrixus floccosus* Maskell) και ο φυλλοκνίστης (*Phyllocnistis citrella* Stainton), που είναι από τις σπουδαιότερες νέες καταγραφές, έχουν ήδη επεκταθεί σ' όλες τις περιοχές της χώρας όπου καλλιεργούνται εσπεριδοειδή, αλλά σοβαρές ζημιές έχουν αποφευχθεί με την εισαγωγή και εξαπόλυση αποτελεσματικών παρασιτοειδών. Άλλα νεοεισελθόντα είδη που θεωρούνται σημαντικά είναι οι θρίπες *Pezothrips kellyanus* (Bagnall) και *Frankliniella occidentalis* (Pergande), το σκαθάρι των φοινικοειδών *Rhynchophorus ferrugineus* (Olivier), οι κηκιδόμυες *Ophelimus maskeli* (Ashmead) και *Leptocybe invasa* Fisher & LaSalle και οι δύο εχθροί των σπαραγγιών *Parahypopta caestrum* (Hubner) και *Hexomyza simplex* (Loew).
Για τα φυτοφάγα ακάρεα έγιναν 9 νέες καταγραφές στην Ελλάδα κατά τη συγκεκριμένη περίοδο. Από αυτές, οι τετράνυχοι *Eutetranychus orientalis* (Klein) και *Tetranychus evansi* Baker & Pritchard είναι είδη καραντίνας. Ιδιαίτερα ο *T. evansi*, ο οποίος δεν έχει ακόμα εξαπλωθεί ευρέως στη χώρα μας, μπορεί να έχει σοβαρές οικονομικές επιπτώσεις γιατί είναι σε θέση να κάνει ζημιές και να μειώσει την εμπορική αξία των προϊόντων των καλλιεργειών που προσβάλλει.
Συνολικά αναφέρθηκαν στη χώρα μας 30 είδη φυτοπαρασιτικών νηματωδών κατά την περίοδο 1990-2007, τα περισσότερα των οποίων είναι γνωστά παγκοσμίως ως σημαντικοί εχθροί καλλιεργειών. Από τα είδη αυτά, όμως, μόνο το είδος *Globodera pallida* Stone, που είναι ένας από τους κυστονηματώδεις της πατάτας, παρουσιάζει ιδιαίτερο ενδιαφέρον για την Ελλάδα.
Τρία είδη ζιζανίων, τα οποία είναι γνωστά ως ευρύτατα διαδεδομένα και επιζήμια στις ΗΠΑ, εμφανίσθηκαν τα τελευταία χρόνια στην Ελλάδα και προκαλούν σοβαρά προβλήματα σε αρδευόμενες θερινές καλλιέργειες και ιδιαίτερα στον αραβόσιτο. Πρόκειται για το *Ipomoea hederacea* (L.) Jacquin (Convolvulaceae) το οποίο έχει ήδη εξαπλωθεί στο δυτικό τμήμα της χώρας, το *Sicyos angulatus* L. (Cucurbitaceae) το οποίο εξαπλώνεται στο βόρειο τμήμα της χώρας και το *Panicum dichotomiflorum* Michaux (Gramineae) το οποίο προς το παρόν απαντάται σε μια περιορισμένη έκταση στην Κωπαϊδα στο κεντρικό τμήμα της χώρας. Η εμφάνιση των ζιζανίων αυτών στην Ελλάδα δείχνει την ανάγκη λήψης μέτρων για την παρεμπόδιση της εισόδου και της εξάπλωσης επικίνδυνων ξενόφερτων ειδών φυτών στη χώρα μας.

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Effects of Mg supply on the growth and mineral composition of pre-rooted hardwood cuttings of "Colt" (*P. avium* L. x *P. pseudocerasus* L.)

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Summary The effects of Mg supply on growth and mineral composition of pre-rooted hardwood cuttings of "Colt" (*P. avium* L. x *P. pseudocerasus* L.) were investigated in a sand culture experiment. The results showed that the growth of "Colt" was increased with increasing the external supply of Mg from 0 to 1500 μmol l^{-1}. Mg deficiency symptoms occurred on the lower leaves and progressed towards the apical leaves of the new shoots of the plants supplied with 0 and 150 μmol Mg l^{-1}. When the concentration of Mg in leaves was less than 2 mg g^{-1} dry weight, deficiency symptoms appeared on the basal leaves of the plants. Mg-deficient plants had a smaller weight of plant organs, smaller leaf area, stem diameter and new shoot extension growth than Mg-sufficient plants. The root ÷ shoot dry weight ratio was reduced with decreasing external supply of Mg. The concentrations of K, Ca, N and P in leaves were unaffected by changes in the external supply of Mg. However, the unit absorption rates of K, Ca, N and P was increased with increasing the external supply of Mg.

Additional keywords: Cherry, magnesium, Mg deficiency, nutrient composition, shoot growth

Introduction

P. avium L. is susceptible to Mg deficiency (18) which causes chlorosis of the leaf margins, necrosis and subsequent leaf abscission. Differences in the susceptibility to Mg deficiency between cherry varieties have been found by Hipps *et al.* (14). These authors working with *P. avium* L. seedling and hardwood cuttings of "Colt" (*P. avium* L. x *P. pseudocerasus* L.) grown in the same soil found that the former showed Mg deficiency symptoms whereas the latter did not. Further experiments in a flowing solution culture system showed that "F. 12/1" (*P. avium* L.) cherry variety was more susceptible to Mg deficiency than "Colt" (21) since it needed greater concentration of Mg, [Mg] in the nutrient solution to achieve its maximum growth.

In the present experiment, the effects of Mg deficiency on the growth and mineral composition of one-year-old plants of "Colt" were investigated. The aim of this experiment was to define the [Mg] in the leaves at which the plants started showing symptoms of deficiency and to investigate the effect of the deficiency on the growth and nutrient composition of the plants.

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Abbreviations: [Mg]; Mg concentration, [K]; K concentration, [Ca]; Ca concentration, [N]; N concentration, [P]; P concentration, DAP; days after transplanting planting, DW; dry weight, L_A; leaf area, UAR; unit absorption rate, RUR; relative uptake rate and W_r; dry weight of roots.

Materials and Methods

Culture of the plants

One-year-old, pre-rooted hardwood cuttings of "Colt" taken off hedges were grown in black plastic pots (26 x 10 x 10 cm) filled with medium grade quartz sand. The sand had been washed with 3% v/v of concentrated HCl and leached with de-ionized water (Conductivity ≤3 μS cm^{-1}) (10) until the pH of nutrient solution draining from the pots was approx. 5.5. The analyses of sand samples that taken from the pots and extracted with 1 M ammonium acetate at pH = 7 showed that the sand had a residual concentration of Mg of approx. 30 μmol l^{-1}. When the sand leached, the plants were weighted and planted. During their establishment, they were watered on alternate days with 600 ml of half strength of the nutrient solution presented in Table 1. After two weeks, when the plants started growing, 600 ml of the full strength nutrient solution (Table 1) were applied each day. Three different concentrations of Mg: 0, 150 and 1500 μmol l^{-1} using $MgCl_2.6H_20$ were applied to the plants.

Table 1. Composition of the nutrient solution.

Macronutrients	μmol l^{-1}
$Ca(NO_3)_2.4H_2O$	4000
KNO_3	4000
$NaH_2PO_4.2H_2O$	1330
$MgCl_2.2H_2O$	0, 150, 1500
$CaSO_4.H_2O$	500
Micronutrients	As in Long-Ashton nutrient solution
FeEDTA	100
pH	5.5

Growth and mineral analyses

During the experiment, four destructive harvests were done at 30, 50, 71 and 92 days after transplanting (DAP) by removing the plants from the pots. The plants were divided into leaves, stems, new shoots and roots and were rinsed three times with de-ionized water. The fresh weights of the leaves, stems and roots were measured. Afterwards, the plant material was dried to constant weight in a forced air oven at 85^{0}C for determination of dry weight and ground for determination of the nutrient content. The leaf area (L_A) was measured with a Delta-T leaf area meter (Burwell, Cambridge, England) and the diameter of the stems was measured at 10 cm above the root collar.

One hundred milligrams of the dried ground plant material was digested with 2 ml 18 M H_2SO_4 containing 1 g l^{-1} Se and 1 ml hydrogen peroxide. After cooling, the digests were made up to 20 ml with distilled water. Ca and Mg were determined by atomic absorption spectrophotometer on the digests after dilution with 20 volumes of a 1 g l^{-1} solution of $LaCl_3$ as a releasing agent. K was determined on the same solution by atomic emission photometer. Total nitrogen was determined using an auto- analyzer by indophenol blue method (23) and phosphorus determined by the molybdenum blue method (6).

Experimental design

A complete randomized block design was used with three [Mg] : 0, 150 and 1500 μmol l^{-1} as the treatments allocated to four blocks and four harvest dates. To take account of the variation due to initial plant weight, the plants were put into 4 groups of 12 plants depending on their initial fresh weight as follows: the largest being in the 1st group, and the smallest in the 4th group. The 12 plants in each group were then assigned at random to each block and each plant was assigned at random to each Mg and harvest combination. By using this randomization the difference in the initial plant weight was included into the block effect (2**)**. At each harvest, one plant was removed from each pot thus, 12 plants were removed per harvest.

Statistical analyses

The statistical analyses were performed with Genstat (9) (Genstat 5 Committee, 1987). Plant growth data were analysed using polynomial regression models fitted to the data which were logarithmically transformed (log_e) to stabilize the variance over time. F-tests were used to determine whether differences were statistically significant. Analysis of variance (ANOVA) was used also to assess the effect of the treatment factor at the different harvest. The derivation of the mean unit absorption rates was based on the dynamic growth analysis (15) and was carried out as follows:

The unit absorption rate (UAR, nmol $mg^{-1}day^{-1}$) i.e. the rate of the mineral nutrient (nmol) absorbed per day and unit root weight (mg), was estimated using the following equation:

$$UAR = RUR\frac{M}{W_r} \qquad (1)$$

Where:

1. RUR = relative nutrient uptake rate (µmol $µmol^{-1}day^{-1}$) i.e. the rate of nutrient content (µmol) increase per nutrient (µmol) per day.
2. M ÷ W_r = the ratio between the plant nutrient content, (M in µmol) and dry weight of roots, (W_r, in mg).

The RUR was estimated after fitting an equation between the logarithmically transformed nutrient content of the plants (log_eM) over time in days (t) after transplanting as follows:

$$log_e(M) = a + bt + ct^2 \qquad (2)$$

The RUR could be estimated after differentiation of the equation (2) with respect to time as follows:

$$RUR = \frac{d(log_e M)}{dt} = \frac{1}{M}\frac{dM}{dt} = b + 2ct \qquad (3)$$

The M ÷ W_r was estimated after fitting an equation between the ratios of the nutrient content of the plants ÷ dry weight of roots (W_r) over time in days (t) after transplanting as follows:

$$\frac{M}{W_r} = a_1 + b_1 t + c_1 t^2 \qquad (4)$$

By multiplication of equations (3) and (4) the UAR could be derived as follows:

$$UAR = RUR\frac{M}{W_r} = \frac{1}{M}\frac{dM}{dt}\frac{M}{W_r} = \frac{1}{W_r}\frac{dM}{dt} = \qquad (5)$$

$$= (b + 2ct)(a_1 + b_1 t + c_1 t^2) = a_3 + b_3 t + c_3 t^2 + d_3 t^3$$

For derivation of the unit absorption rates UAR the dry weight of the roots with approximate diameter <2 mm were used.

Results

Growth

Based on regression analyses, quadratic equations were found to be the most appro-

Table 2. The effect of Mg external supply on the relationship between log_e-transformed total plant (W), leaf (WL), stem (WS), new shoot (WNSH) and root (WR) dry weight, leaf area (LA), shoot diameter (SD) and new shoot length (LNSH) of `Colt' and time. The equations fitted were quadratics (a+ bt+ ct^2) where a, b, c were the parameters estimated by the statistical analysis and t time in days. R^2 coefficients of determination, SED standard error of differences.

			0 µmol Mg l^{-1}			150 µmol Mg l^{-1}			1500 µmol Mg l^{-1}		
	R^2	SED	a	b	c	a	b	c	a	b	c
W	0.84	0.0001	8.636	0.0039	0.00024	8.636	0.0039	0.00012	8.636	0.0039	0.000006
W_L	0.82	0.0001	6.738	0.061	0.00041	6.738	0.061	0.00027	6.738	0.061	0.00021
W_S	0.74	0.0009	8.768	0.016	0.00085	8.768	0.016	0.000007	8.768	0.016	0.000038
W_{NSH}	0.84	0.0002	3.599	0.106	0.00066	3.599	0.106	0.00048	3.599	0.106	0.00040
W_R	0.86	0.0001	4.891	0.080	0.00054	4.891	0.080	0.00036	4.891	0.080	0.00029
S_D	0.72	0.00005	1.464	0.019	0.00014	1.464	0.019	0.000098	1.464	0.019	0.000073
L_{NSH}	0.78	0.0001	8.495	0.076	0.00046	8.495	0.076	0.00039	8.495	0.076	0.00033
L_A	0.88	0.0002	7.879	0.103	0.00040	7.879	0.103	0.00025	7.879	0.103	0.00017

priate (Table 2) to explain the increase over time in the leaf, stem, new shoot (shoots produced during the course of the experiment) and root dry weight (mg), new shoot length (cm), stem diameter (mm) and L_A (cm^2). There was a significant ($P<0.001$) increase in the growth of all the plant organs with increasing external supply of Mg. The diameter of the stem and new shoot extension growth also increased significantly ($P<0.05$) with increasing external supply of Mg. The root ÷ shoot dry weight ratio (shoot = stems + leaves + new shoots) increased with increasing external supply of Mg (Figure 1). However, the differences between 150 and 1500 µmol Mg l^{-1} were significant ($P<0.05$) on day 92. Furthermore, the root ÷ shoot dry weight ratio was increased at all treatments ($P<0.05$).

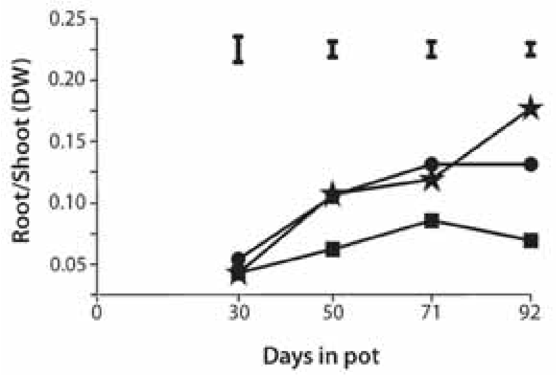

Figure 1. The effect of Mg external supply on the root ÷ shoot dry weight ratio of "Colt". ■ - ■ 0, ● - ● 150 and ★-★ 1500 µmol Mg l^{-1}. The vertical bars are the SEDs.

Mineral nutrients contents

Magnesium

The [Mg] in leaves, stems, new shoots

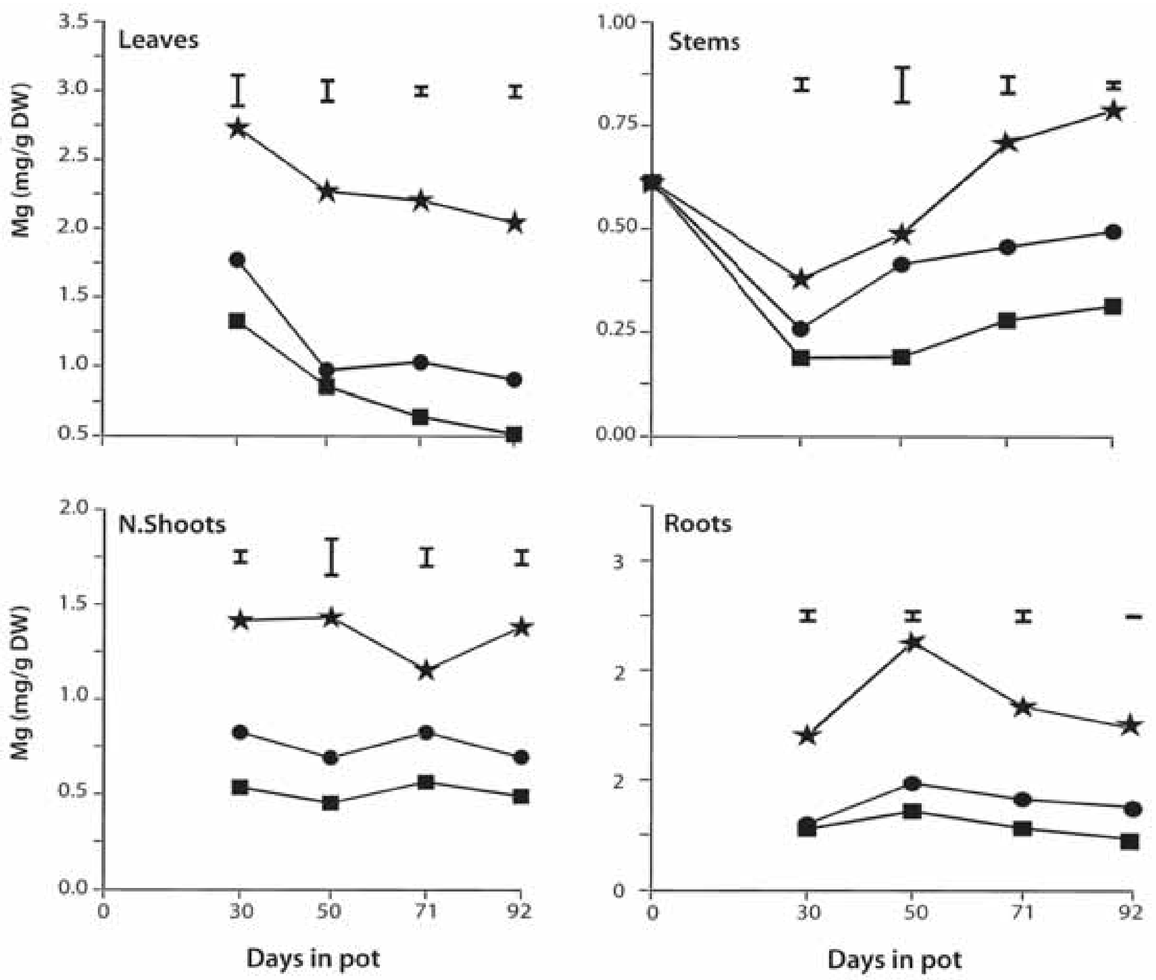

Figure 2. The effect of Mg external supplies on the [Mg] in leaves, stems, new shoots and roots of "Colt". ■ - ■ 0, ● - ● 150 and ★-★ 1500 µmol Mg l^{-1}. The vertical bars are the SEDs.

and roots increased significantly (P<0.001) with increasing external supply of Mg (Figure 2). There was a continuous decrease in the [Mg] in leaves, whereas, the [Mg] in shoots and roots were stable. At all levels of Mg supply, there were a decline in [Mg] in stems from transplanting until 30 DAP and a continuous increase thereafter.

Symptoms of Mg deficiency occurred on the lower leaves of the new shoots and progressed towards the apical leaves of the plants supplied with 0 and 150 µmol Mg l^{-1}. At the last harvest (92 DAP), Mg deficiency symptoms appeared also on the basal leaves of the plants supplied with 1500 µmol Mg l^{-1}. The symptoms included an interveinal or marginal orange or yellow brown necrosis (often surrounded by a thin band of purple or pale green tissue) which spread over most of the leaf blade; all the leaves became tattered and curled upwards along the midrib, and abscised soon after (Figure 3).

Figure 3. Symptoms of Mg deficiency in “Colt” leaves.

Potassium, Calcium, Nitrogen and Phosphorus

The [K] in leaves, stem, new shoots and roots were not affected (P>0.05) by the external supply of Mg (Figure 4) and, therefore, only the means of

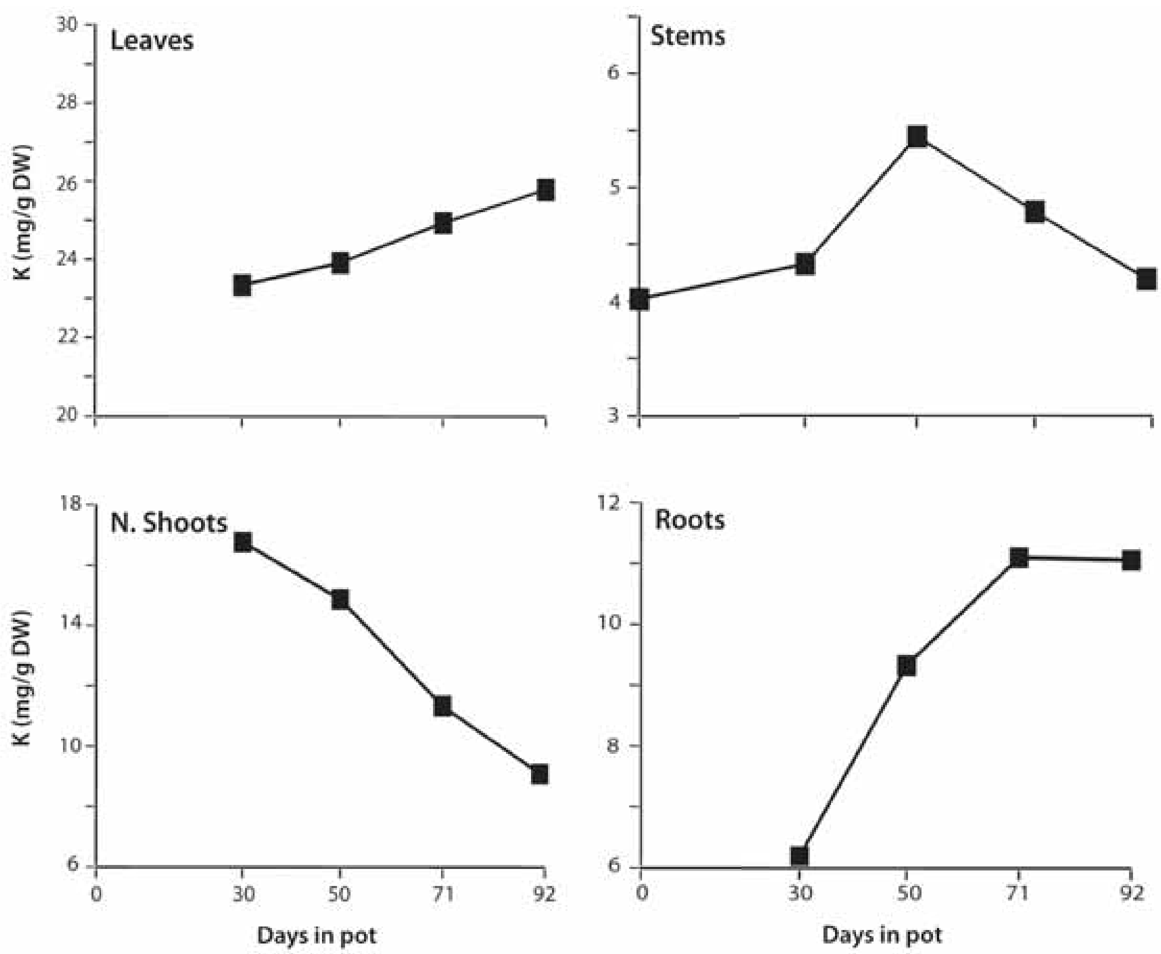

Figure 4. The effect of Mg external supply on the [K] in leaves, stems, new shoots and roots of ”Colt”.

the [K] at each harvest are presented in figure. The [K] in leaves and roots increased with time, whereas, there was a continuous decrease in new shoots. The [K] in stems increased from transplanting until 50 DAP and decreased thereafter.

The [Ca] in leaves, stems and new shoots were not affected by the external supply of Mg (Figure 5) and therefore, only the mean [Ca] at each harvest are presented. The [Ca] in stems and new shoots increased with time, whereas, there was an initial increase in leaves and roots until 50 DAP and a continuous decrease thereafter. In the roots, there was a significant ($P<0.001$) decrease in [Ca] with increasing external supply of Mg.

The [N] in leaves, stems and new shoots were unaffected by the external supply of Mg (Figure 6). The [N] in leaves was within the range 31-33 mg g^{-1} DW and the [N] in new shoots and roots decreased with increasing external supply of Mg. The [P] in leaves, stem, roots and new shoots were not affected by the increased external supply of Mg (Figure 7) and generally increased during the course of the experiment.

Total contents of mineral nutrients

Based on regression analyses, quadratic equations were found to be the most appropriate (Table 3) to explain the increase over time in Mg, K, Ca, N, and P content of the plants. There was a significant increase ($P<0.001$) in the RUR with increasing external supply of Mg. This resulted in a greater con-

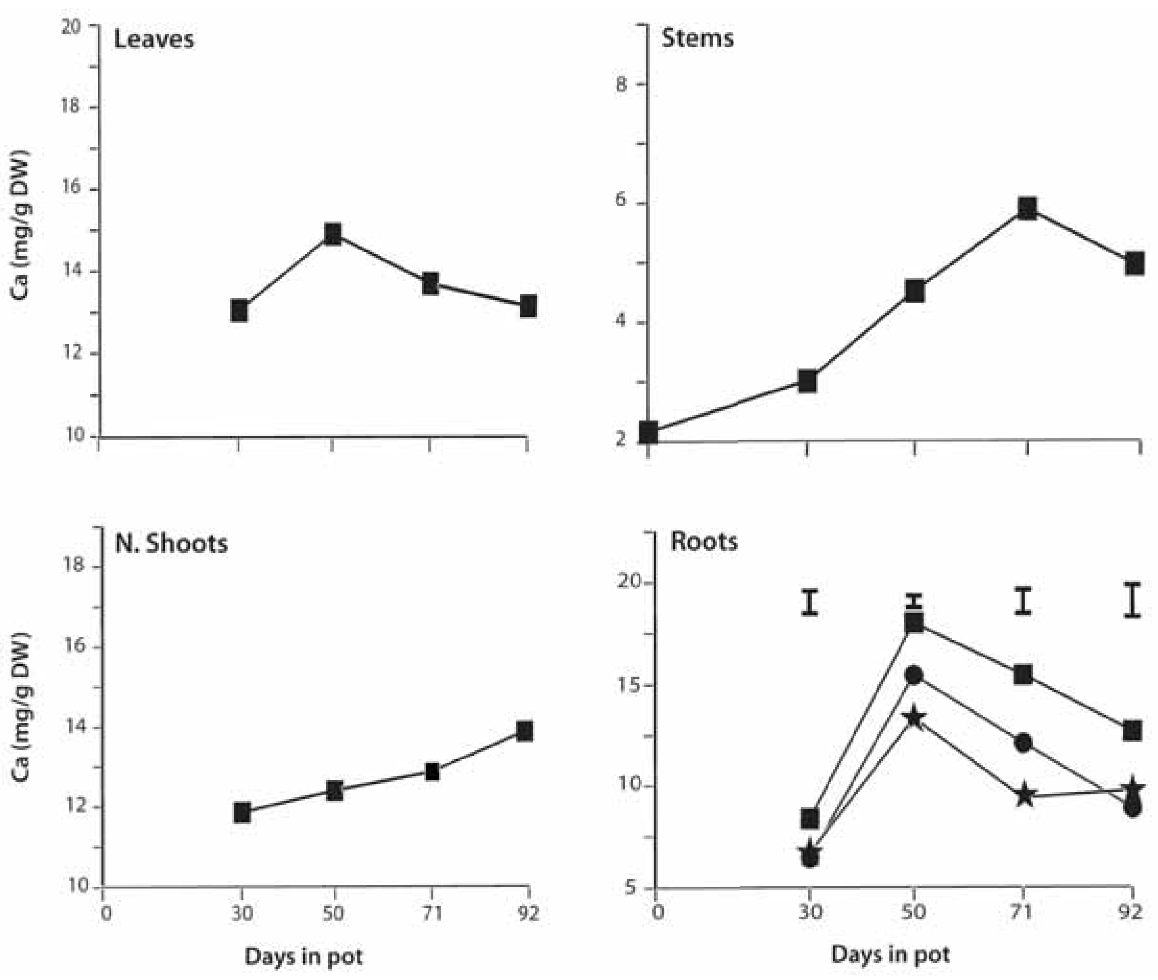

Figure 5. The effect of Mg external supply on the [Ca] in leaves, stems, new shoots and roots of "Colt". ■-■ 0, ●-● 150 and ★-★ 1500 µmol Mg l^{-1}. The vertical bars are the SEDs.

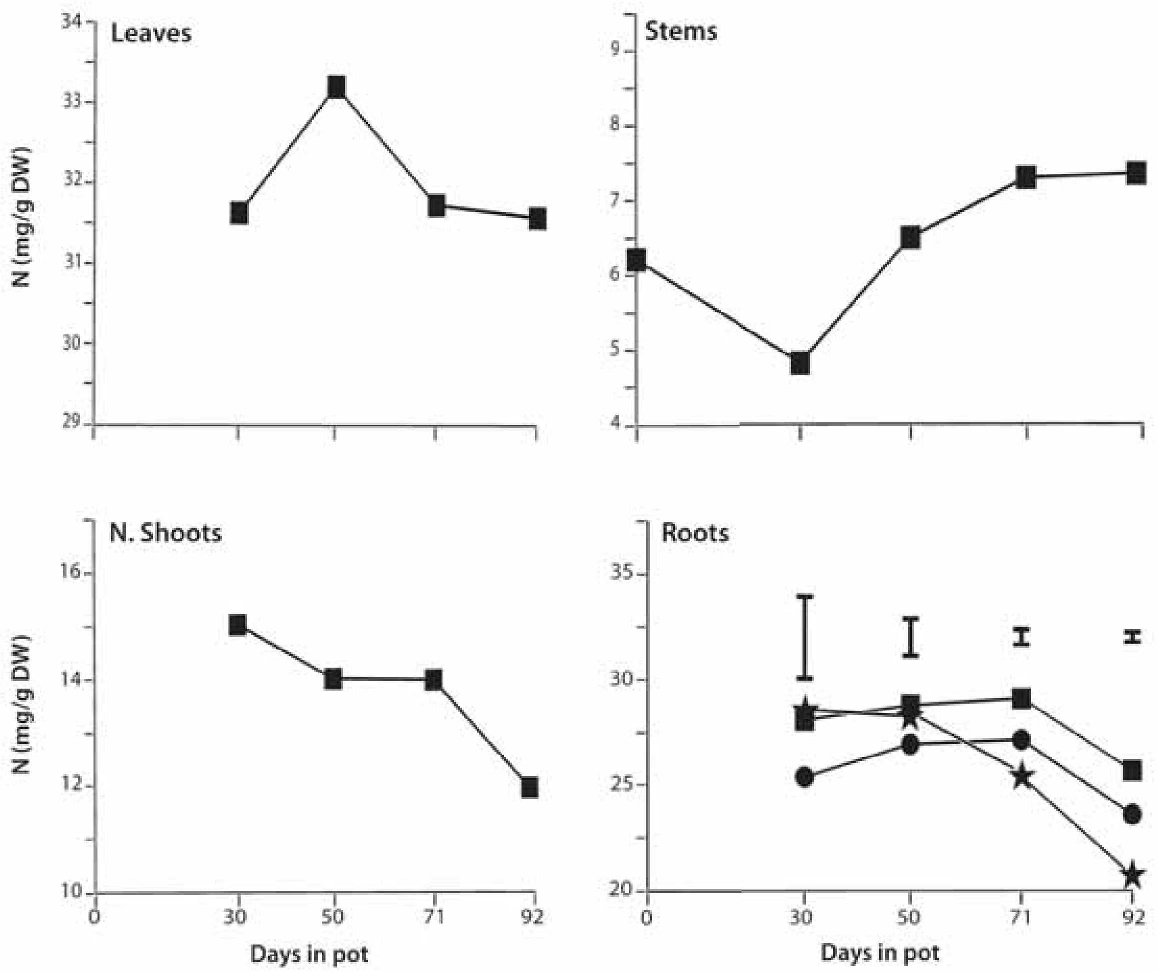

Figure 6. The effect of Mg external concentration on the [N] in leaves, stems, new shoots and roots of "Colt". ■-■ 0, ●-● 150 and ★-★ 1500 µmol Mg l^{-1}. The vertical bars are the SEDs.

Table 3. The effect of Mg external supply on the relationship between log_e- transformed total amount of Mg, Ca, K, N and P (µmol) in plants and time (days). The equation fitted was ($a + bt + ct^2$) where a, b and c were the parameters estimated by the statistical analysis and t time. R^2 coefficients of determination, SED standard error of differences.

			0 µmol Mg l^{-1}			150 µmol Mg l^{-1}			1500 µmol Mg l^{-1}		
	R^2	SED	a	b	c	a	b	c	a	b	c
Mg	0.85	0.00013	5.417	0.031	-0.00027	5.417	0.031	-0.00007	5.417	0.031	+0.00016
Ca	0.81	0.00013	6.303	0.066	-0.00044	6.303	0.066	-0.00030	6.303	0.066	-0.00026
K	0.82	0.00012	7.191	0.048	-0.00031	7.191	0.048	-0.00017	7.191	0.048	-0.00011
N	0.82	0.00012	8.332	0.056	-0.00037	8.332	0.056	-0.00023	8.332	0.056	-0.00017
P	0.85	0.00012	4.896	0.069	-0.00043	4.896	0.069	-0.00029	4.896	0.069	-0.00024

tent of Mg in the plants supplied with 1500 µmol Mg l^{-1} than in the plants supplied with 150 and 0 µmol Mg l^{-1}. The contents of the other mineral nutrients also increased with increasing external supply of Mg (Table 3).

Unit absorption rates

The UAR of Mg followed a cubic equa-

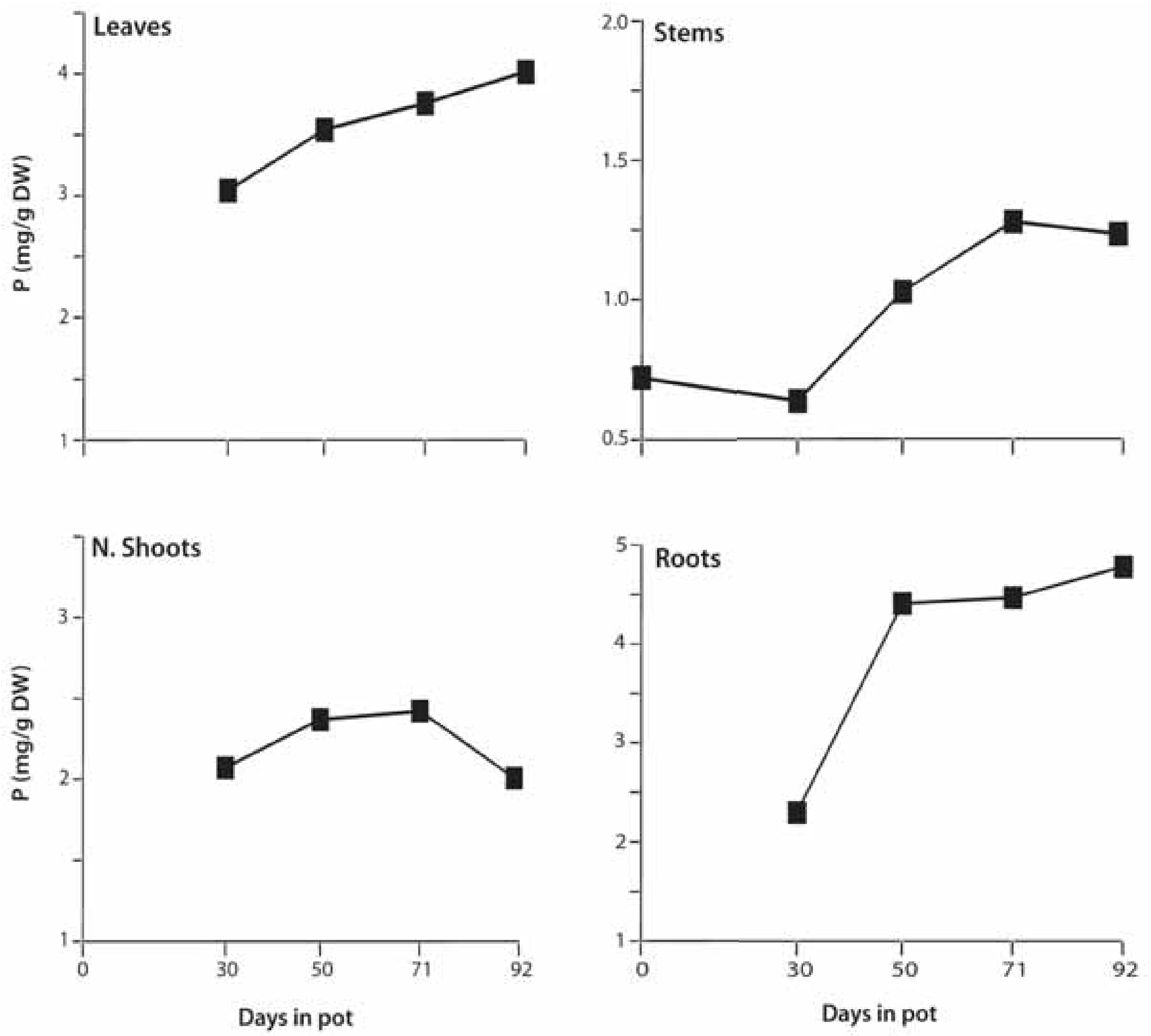

Figure 7. The effect of Mg external supply on the [P] in leaves, stems, new shoots and roots of "Colt".

Table 4. The effect of Mg external supply on the relationship unit absorption rate of Mg, Ca, K, N and P (nmol $mg^{-1}day^{-1}$) and time (days).

	0 µmol Mg l^{-1}				150 µmol Mg l^{-1}				1500 µmol Mg l^{-1}			
	a	b	c	d	a	b	c	d	a	b	c	d
Mg	29.729	-1.06	0.0124	-0.00005	29.729	-0.667	0.0056	-0.00001	29.729	-0.351	0.0013	0.000031
Ca	200.508	-3.168	0.0066	-	200.508	-2.318	0.0045	-	200.508	-2.075	-2.075	-
K	348.000	-9.967	0.108	-0.00049	348.000	-7.937	0.077	-0.0027	348.000	-7.067	0.063	-0.00017
N	1382.08	-36.183	0.356	-0.00016	1382.08	-29.273	0.266	-0.00096	1382.08	-26.311	0.228	-0.00072
P	53.613	-0.609	-0.609	-0.00073	53.613	-0.392	-0.392	-0.00050	53.613	-0.314	-0.314	-0.00041

tion over time and increased by increasing external supply of Mg (Table 4). At 0 µmol Mg l^{-1} the UAR of Mg declined over time with a greater rate that that at 150 µmol Mg l^{-1} whereas, at 1500 µmol Mg l^{-1} Mg there was an increase in the RUR over time. The UAR of K and N followed a cubic equation over time and there was an increase with increasing external supply of Mg (Table 4). The UAR of Ca and P an increased with increasing Mg supply (Table 4). At 0 µmol Mg l^{-1} the UAR of K, Ca, N and P approached zero from 71 DAP and thereafter. At the other external Mg supplies the UAR of K, Ca, N and P decreased over time.

Discussion

The symptoms of Mg deficiency (Figure 3) were first appeared on the basal mature leaves of the "Colt". This re-distribution of Mg was expected since it is generally regarded as an easily redistributed nutrient (20), although there is some conflicting evidence (1). The results of the current experiment showed that when the [Mg] in leaves were less than 2 mg g^{-1} DW the plants showed Mg deficiency symptoms.

Mg-deficient "Colt" plants had a decreased stem diameter. Similar results were found by Ford (7) in Mg-deficient MM-102 apple rootstock. The L_A was also reduced by decreasing the external Mg supply (Table 2). This reduction probably caused a reduction in the assimilate production from the leaves that could have affected the growth of the stem since Harper (10) showed that the increase in the diameter of a stem, especially at its base, depended upon assimilates from active leaves and Proebsting (19) found a cessation of the radial increase of wood in defoliated apple trees.

In the current experiment the length of new shoots of cherry increased with increasing external supply of Mg. However, in apple the extension growth of the new shoots and leaf production, as well as internode length, were unaffected by Mg deficiency (8).

The severe Mg deficiency (0 µmol Mg l^{-1}) probably altered the distribution of the assimilates between the shoot and root of "Colt". The root ÷ shoot ratio decreased with a decreasing external supply of Mg implying a relatively greater shoot growth in respect to root growth in the Mg-deficient plants. This greater shoot than root growth of the Mg-deficient plants was not found by Troyanos *et al* (22) in cherries grown in a flowing solution culture system, Hermans and Overburden (12) in *Arabidopsis thaliana* and Hermans *et al.* (11) in spinach. However, Cakmak *et al.* (3, 4) found a similar increase in the growth of shoots of Mg-deficient plants of *Phaseolus vulgaris* L. and Ericsson and Kahr (5) in birch.

The concentrations of K (22-26 mg g^{-1} DW), Ca (12-15 mg g^{-1} DW), N (31 -33 mg g^{-1} DW) and P (3-4 mg g^{-1} DW) were within the range considered to be *optimum* for the growth of cherries (16). The unit absorption rates of K, Ca, N and P increased with increasing the external supply of Mg whereas their concentrations were not affected significantly by the Mg external supply. This probably suggests that the growth made in response to an increased external supply of Mg determined the uptake of K, Ca, N and P.

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Επίδραση της τροφοδοσίας με Mg στην αύξηση και στη συγκέντρωση θρεπτικών στοιχείων σε ριζοβολημένα μοσχεύματα "Colt" (*P. avium* L. x *P. pseudocerasus* L.)

Γ.Ε. Τρωγιάνος και N.A. Hipps

Περίληψη Σε ένα πείραμα υδροπονίας μελετήθηκε η επίδραση του Mg στην αύξηση και συγκέντρωση του Mg, K, Ca, N και P σε φυτά κερασιάς "Colt" (*P. avium* L. x *P. pseudocerasus* L.). Τα αποτελέσματα του πειράματος έδειξαν ότι ο ρυθμός αύξησης των φυτών ήταν μεγαλύτερος όσο η συγκέντρωση του Mg στο θρεπτικό διάλυμα αυξανόταν από 0 σε 1500 μmol l^{-1}. Τα συμπτώματα της έλλειψης Mg παρατηρήθηκαν πρώτα στα ώριμα φύλλα της βάσης των νέων βλαστών σε φυτά που αναπτύσσονταν με 0 και 150 μmol Mg l^{-1}. Στη τελευταία συγκομιδή των φυτών (ημέρα 92) όταν η συγκέντρωση του Mg στα φύλλα ήταν μικρότερη από 2 mg g^{-1} βάρος ξηρής ουσίας, συμπτώματα της έλλειψης του Mg εμφανίστηκαν επίσης και σε ώριμα φύλλα των φυτών που αναπτύσσονταν με 1500 μmol Mg l^{-1}. Ο λόγος του ξηρού βάρους της ρίζας ÷ ξηρό βάρος του υπέργειου τμήματος του φυτού μειώθηκε με την μείωση τη συγκέντρωσης του Mg στο θρεπτικό διάλυμα. Επιπλέον, φυτά τα οποία παρουσίαζαν συμπτώματα έλλειψης Mg είχαν μικρή διάμετρο κορμού και μικρό μήκος νεαρής βλάστησης. Η συγκέντρωση του K, Ca, N και P δεν επηρεάστηκε από την συγκέντρωση του Mg στο θρεπτικό διάλυμα. Ο μοναδιαίος ρυθμός της πρόσληψης του K, Ca, N και P αυξήθηκε με την αύξηση της συγκέντρωσης του Mg στο θρεπτικό διάλυμα.

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SHORT COMMUNICATION

First record of *Aceria cynodoniensis* feeding on Bermuda grass in Greece

E.V. Kapaxidi[1], D. Markoyiannaki-Printziou[1] and P. Papaioanou-Souliotis[1]

Summary *Aceria cynodoniensis* Sayed was found feeding on Bermuda grass plants in a commercial turfgrass nursery in Aliartos (Viotia) and is reported for the first time in Greece. Information on its morphology and the symptoms of damage is also provided.

Additional keywords: Acari, *Aceria neocynodonis*, *Cynodon dactylon,* Eriophyidae, turfgrass

Aceria cynodoniensis Sayed (=*Aceria neocynodonis* Keifer) (Acari: Eriophyidae) is a mite infesting Bermuda grass [*Cynodon dactylon* (L.) Pers.], causing a rosette symptom to the plants. It was first recorded in Egypt on grass (9). Since then it has been reported from the U.S.A. (Arizona, Georgia, Florida and California) as *A. neocynodonis* Keifer (2, 3, 4, 13), South Africa (7, 11) and Australlia (8). In all cases it was found infesting grass species of the genus *Cynodon*.

In September 2007, symptoms of abnormal Bermuda grass growth were observed in a turfgrass nursery near Aliartos (region of Viotia). From plants brought to the laboratory twelve female and one male mites were collected and identified as *A. cynodoniensis*. To our knowledge this is the first time that this mite is reported from Greece.

Mites were observed using a stereoscopic microscope (Olympus SZ61) and then carefully collected from the infested leaves (rosettes) of Bermuda grass. For clearing and mounting the individuals, Hoyer's medium was used (6). The identification was made by using the keys of Amrine *et al*. (1) and Keifer's description of *A. neocynodonis* (4).

A. cynodoniensis (Figures 1 and 2) is a slender, wormlike, yellowish mite, 170-210 μm long. The female has 6– and the male 5- rayed featherclaws; the dorsal shield is marked with a distinctive design of 2 scalloped, parallel ridges running the full length of the shield, with a straight, short ridge in between. The microtubercules are elliptical; the coverflap of the female genitalia has 11-12 ribs, with the median ribs longer than the lateral ones (5).

The mites feed and seek shelter in the leaf sheaths. Mite feeding apparently inhibits plant growth, thus the leaf sheaths become swollen, closely packed, thickened, and bunched at the stem node, and the leaf blades become stunted. Affected stems have greatly deformed and enlarged nodes and shortened internodes. The infestation showing the characteristic injury becomes evident in the spring on Bermuda grass grown in lawns; and then browning and thinning out of the grass follow.

Data on the biology of *A. cynodonien-*

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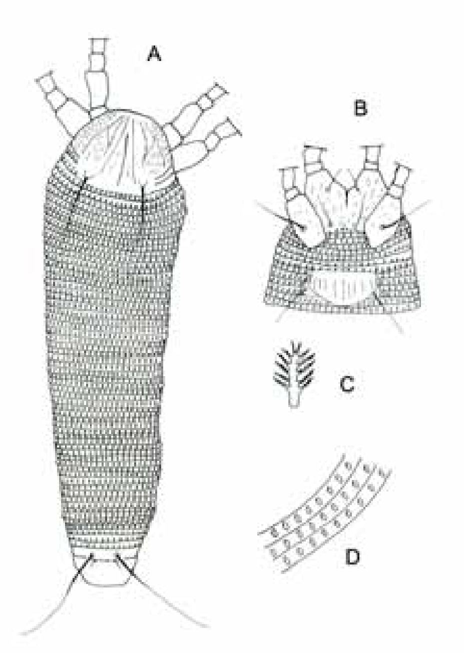

Figure 1. *Aceria cynodoniensis* Sayed: A. adult female, B. coxal region and genital shield, C. empodium raylets, D. Microtubercules.

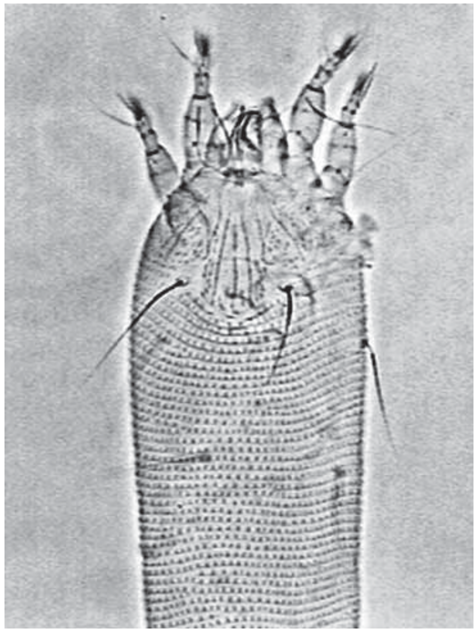

Figure 2. Bermuda grass mite, *A. cynodoniensis*: microscope photograph of a female propodosoma.

sis is limited. Short and Buss (10) reported that one generation can develop in 7 to 10 days and individuals may be active most of the year in Florida (U.S.A). All life stages (eggs, nymphs, adults) live under the leaf sheath. The population grows during late spring and summer. Mites may disperse by the help of the wind, or on other insects and by grass clippings. Infestations usually develop in the taller grass (rough areas, around sand traps, along canals, fence rows etc.). The population of mites varies from a few to a hundred or more individuals in a single sheath. They are less abundant in lawns where flood irrigation is used. The variety of grass seems to be an important factor in susceptibility to attack, thus *A. cynodoniensis* is characterized as an extremely specific species (12). Soil fertilization seems also to increase the infestation (13).

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή στην Ελλάδα του *Aceria cynodoniensis* που προσβάλλει αγρωστώδη είδη γκαζόν του γένους *Cynodon*

Ε.Β. Καπαξίδη, Δ. Μαρκογιαννάκη-Πρίντζιου και Π. Παπαϊωάνου-Σουλιώτη

Περίληψη Στην παρούσα εργασία καταγράφεται για πρώτη φορά στην Ελλάδα το *Aceria cynodoniensis* (Acari: Eriophyidae), το οποίο προσβάλλει αγρωστώδη φυτά του γένους *Cynodon* (γκαζόν, ποικιλίες Bermuda grass). Δίνονται επίσης πληροφορίες για τη μορφολογία του ακάρεος και τη συμπτωματολογία της προσβολής του.

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SHORT COMMUNICATION

Strong sorption of glyphosate and aminomethylphosphonic acid from methanolic solutions on glassware surfaces

C.N. Giannopolitis[1] and V. Kati[1]

Summary In HPLC analyses with direct injection of aqueous and methanolic solutions of glyphosate and its metabolite aminomethylphosphonic acid (AMPA), it was observed that apparent recovery of the two analytes is significantly lower from the methanolic (containing or not water up to 10%) than from the aqueous solutions. The reduced recovery is associated with a stronger adsorption of glyphosate and AMPA on the glassware surfaces in the methanolic solutions. The reduction correlates to the ratio of the solution volume against the total container volume and is increased when shaking the solutions. Determination of the two compounds, therefore, requires special attention if methanol is used for sample spiking or extraction.

Additional keywords: AMPA, adsorption, recovery, quantitative determination, HPLC analysis

Glyphosate [N-(phosphonomethyl) glycine] has been one of the most applied herbicides in the world since it came on the market in 1974 and its use is expanding dramatically in recent years with the incorporation of resistance genes into several crops grown in large acreage. Monitoring for residues of glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) in soils, natural waters and agricultural commodities is therefore of increasing importance.

During a procedure of analytical performance optimization and method validation in this laboratory, a methanolic solution of glyphosate and AMPA was received as an external quality control material (LEAP Scheme of FAPAS). Quantitative determination of the analytes in this material resulted in unexpected low apparent recoveries for both compounds and this prompted an investigation for the causes. The term "apparent recovery" is used in these studies as more appropriate to the term "recovery" (1).

Glyphosate is insoluble in methanol and other organic solvents (2) and the same is true for AMPA. Therefore, for the purpose of this investigation, the two compounds were first dissolved in water and then transferred to methanol by adding small volumes of the water solution to it.

Aqueous stock solutions containing 50 or 100 μg/ml of each, glyphosate and AMPA, were prepared using analytical reference standards (Monsanto, certified as 99.8 and 99.5% respectively) of the two compounds and HPLC grade water (Fisher Scientific). Working methanolic solutions were prepared by adding a volume of 25, 50 or 100 μl of one of the above aqueous stock solutions to methanol (same grade and origin as water) to obtain three concentration levels for gly-

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phosate and AMPA (125, 500 and 1000 ng/ml) and supplemental pure water to obtain varying water percentages (0.5, 1, 2, 3 or 4%) in a final volume of 10 ml. A reference solution in pure water was prepared for each concentration level. Mixing and preservation of these solutions was in 20-ml screw-capped clear glass bottles, unless described differently.

Glyphosate and AMPA in the methanolic and the respective reference (aqueous) solutions were quantitatively determined soon after preparation and the next day. Analysis was performed using cation exchange HPLC and fluorescence detection following post-column derivatization with hypochloride and o-phthalaldehyde (OPA), which is an improved version of the US EPA method 547 (5, 8).

The instrumentation consisted of a solvent delivery system (LC-10ADVP, Shimadzu), an auto-injector (SIL-10ADVP, Shimadzu), a cation-exchange column 4x150 mm in the K^+ form connected with a guard column 3x20 mm K^+ form (both from Pickering Laboratories), a post-column derivatizer (PCX5200, Pickering Laboratories) and a fluorescence detector (RF-10AXL, Shimadzu). Each solution was first filtered through a 0.22 μm disposable syringe filter with a PTFE membrane, into a 2 ml amber borosilicate glass vial and then directly injected into the HPLC system at 10 μl.

All conducted tests were repeated three times and results from a typical run of each test are presented here.

Apparent recovery of glyphosate and AMPA at the three concentration levels, from methanolic solutions containing up to 4% water, are summarized in Figure 1. Data indicate that there is a significant reduction in the apparent recovery of both compounds from the methanolic solutions, compared to that from the respective aqueous solutions. Depending on the analyte, the concentration level and the water content of the methanolic solutions, apparent recovery was reduced to a level varying between about 20% and 70%. The reduction did not seem to correlate to the concentration level of glyphosate while it correlated inversely to the concentration of AMPA, suggesting that solubility alterations in the methanolic solutions cannot account for the associated reductions in apparent recovery.

Many researchers involved in trace analysis of glyphosate and AMPA in wa-

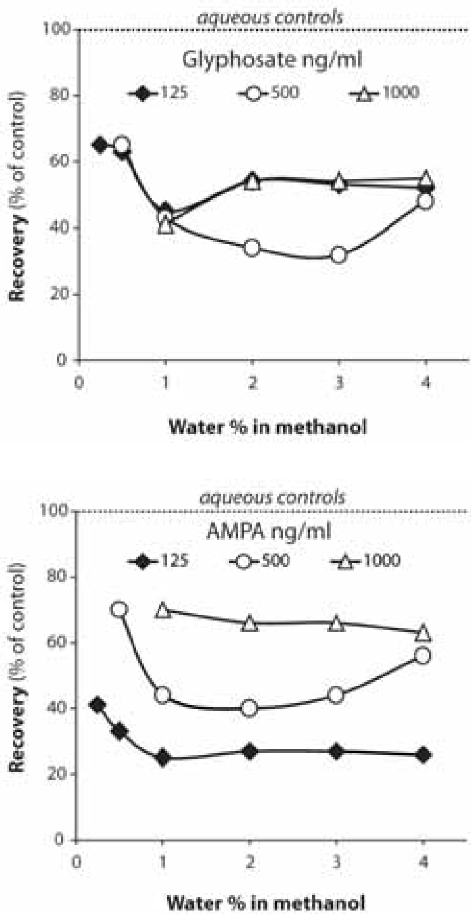

Figure 1. Apparent recovery of glyphosate and AMPA from methanol mixed with aqueous stock solutions at three analyte concentration levels. The methanolic solutions (containing up to 4% water) were analyzed by directly injecting 10 μl and compared to similarly treated pure water solutions of the same concentrations (taken as 100% recovery).

ters have suspected a tendency of these compounds to adsorb on glass surfaces and have used polypropylene containers during sample preparation and preservation (3, 4, 6). It is reasonable, therefore, to assume that an increased sorption onto the glass surfaces may account for the reduced apparent recovery associated with the methanolic solutions. A first evidence for this assumption was obtained by comparing the recovery reductions from methanolic solutions prepared in the same glass bottles as above and in polypropelene tubes (Figure 2). The apparent recovery reduction was significantly higher in the glass bottles than in the propylene tubes.

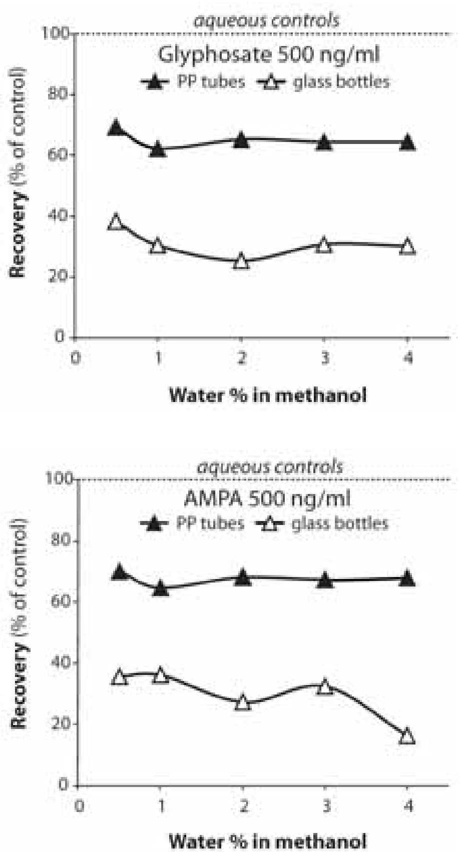

Figure 2. Apparent recovery of glyphosate and AMPA from methanolic solutions prepared and kept for 24 h either in polypropelene tubes or in glass bottles. Similarly prepared pure water solutions served as controls (100% recovery).

Results from a series of supplementary tests further verified the apparent recovery reduction and provided additional evidence that a stronger sorption of glyphosate and AMPA on glassware surfaces, when they are in methanolic solutions, is responsible for the reduced apparent recovery from such solutions:

1. When apparent recovery of the two compounds was examined with solutions prepared and kept in other types of clear glass containers (e.g. 25-ml glass volumetric flasks, 20-ml screw-capped glass tubes, 4-ml crimp-capped glass bottles), it was in all cases found to be significantly lower from the methanolic solutions compared to that from aqueous solutions of the same concentration in the same type of container. Differences in apparent recovery reduction were observed among the various types of glassware suggesting that some may be more adsorptive than others. Polypropelene containers (a 250-ml polypropelene centrifuge bottle was used in these tests) do cause a recovery reduction with the methanolic solutions but generally seem to be less adsorptive than glass containers. Differences were also observed within a type of glass container depending on the ratio of the solution volume to the total volume of the container (results from a typical experiment in Table 1). This indicates a positive correlation of the apparent recovery reduction to the area of available glassware surfaces and provides further support to the adsorption explanation.
2. When apparent recovery of the two compounds was examined at various time intervals after preparation of the methanolic solution in a glass container, it was realized that recovery de-

creased with time for an equilibration period of about 2-3 hours and in any case it had reached the lowest level by the next day (at room temperature). Shaking the solution always increased or accelerated the apparent recovery reduction (results from a typical experiment in Table 2) and this provides further support for the adsorption explanation.

3. When recovery of missing quantities of both compounds was attempted, it was succeeded by a washing of the container with water. In Figure 3, the typical chromatogram obtained with the first water wash of a glass bottle that contained a methanolic solution (apparent recovery about 50%) is shown against the same chromatogram from the reference glass bottle that contained a similar aqueous solution (apparent recovery 100%). Solutions from both bottles had been as thoroughly as possible removed and the bottles left to dry of methanol before an equal volume of deionized water was added. The water was analyzed after a vigorous hand shaking

Table 1. Apparent recovery of glyphosate and AMPA from an aqueous and a methanolic solution prepared and kept in two types of containers at three volume ratios.

Solution[1]	Container[2]	Volume ratio[3]	Recovery %	
			Glyphosate	AMPA
Aqueous	PP bottle	0.25	102	101
	Glass test tube	1.00	103	102
		0.50	103	102
		0.25	102	101
Methanolic	PP bottle	0.25	98	88
	Glass test tube	1.00	66	64
		0.50	58	56
		0.25	55	41

[1] The solutions contained 500 ng/ml of glyphosate and AMPA in pure water (aqueous) or in methanol + 1% water (methanolic).
[2] Results presented here are with a 250-ml, all-polypropylene, screw-cap, centrifuge bottle and a 20-ml, glass, screw cap, test tube.
[3] The ratio of the solution volume to the total container volume.

Table 2. Effect of shaking on glyphosate and AMPA apparent recovery from a methanolic solution.

Treatment	Recovery (%)	
	Glyphosate	AMPA
No shaking	41	46
Hand shaking, 5 min	30	40
Ultra sonic, 5 min	31	40
Ultra sonic, 10 min	28	28

The methanolic solution (500 ng/ml each of glyphosate and AMPA and 1% water) was prepared in the 20-ml screw-capped glass bottles (5 ml of solution/bottle) and kept at room temperature until analyzed the next day. Shaking was done just before analysis. A similar solution in pure water was used as reference (100% recovery).

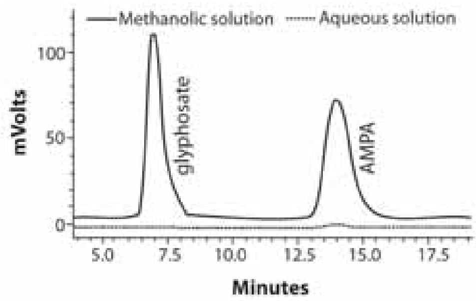

Figure 3. Chromatograms of the first water wash of two 20-ml glass bottles after removal of the solutions they contained. The methanolic solution consisted of 500 ng/ml glyphosate, 500 ng/ml of AMPA and 1% water in methanol while the aqueous solution consisted of the same concentrations of glyphosate and AMPA in pure water. Bottles had been filled with 5 ml of the respective solution and stood at room temperature for about 16 hrs, before the solutions were removed and the bottles were washed with 5 ml of deionized water.

of the bottles. The first water washing yielded about 80% of the missing glyphosate and AMPA from the methanolic solution. A second water washing with half the volume was needed for an almost complete recovery.

All the above results clearly demonstrate the reduced apparent recovery of glyphosate and AMPA from methanolic solutions prepared by mixing methanol with an aqueous solution of the two compounds, thus containing a low percentage of water (from 0.25 to 4%). In other tests, using solutions of the two compounds in pure methanol, it was shown that the presence of water is not necessary for the recovery reduction to occur. In these tests, a low concentration solution of glyphosate and AMPA in methanol was prepared by directly dissolving small quantities of the two compounds in pure methanol with repeated stirring and light heating in a polypropylene bottle. When this solution was transferred to glass bottles and analyzed as in the previous tests, a significant reduction of apparent recovery (to levels about 50%) was observed. Adding water to methanol at up to 5% had little effect on the apparent recovery, while at 10% or more had an improving effect on it and resulted in a complete recovery at water percentages of 50% in the methanol.

Other researchers have used methanol as a leaf wash for removing unabsorbed ^{14}C-labeled glyphosate, in studies of herbicide penetration into leaves and, as shown later, recovery is much better with water (7).

In analytical work with glyphosate and AMPA, therefore, methanol is not a good solvent to be used either in the preparation of spiking stock solutions for calibration purposes or as an extractant for residue determination in various matrices. Strong adsorprion on surfaces of glass containers and to a less extent to plastic containers should be expected with methanolic solutions regardless if they contain water (up to 50%). This certainly leads to low apparent recoveries for both compounds and may lead to erratic results if measures are not taken. This adsorption and reduced recovery problem associated with the use of methanol is expected to become even more important in cases where the determination of the two compounds is at trace concentrations.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Ισχυρή προσρόφηση του glyphosate και του AMPA, από μεθανολικά διαλύματα, στις επιφάνειες των υαλικών

Κ.Ν. Γιαννοπολίτης και Β. Κατή

Περίληψη Σε αναλύσεις με υγρή χρωματογραφία υψηλής πίεσης (HPLC), με απευθείας έγχυση υδατικών ή μεθανολικών διαλυμάτων του ζιζανιοκτόνου glyphosate και του μεταβολίτου του AMPA, διαπιστώθηκε σημαντικά μειωμένη ανάκτηση των δύο ενώσεων από τα διαλύματα που περιείχαν μεθανόλη είτε καθαρή είτε με ένα χαμηλό ποσοστό (μέχρι 10%) ύδατος. Η μειωμένη αυτή ανάκτηση οφείλεται στην ισχυρή προσρόφηση του glyphosate και του AMPA στις επιφάνειες των ύαλινων δοχείων η οποία συμβαίνει στα μεθανολικά και όχι στα υδατικά διαλύματα. Η μείωση της ανάκτησης συσχετίζεται με την αναλογία του όγκου διαλύματος προς το συνολικό όγκο του δοχείου και επιτείνεται με την ανάδευση των διαλυμάτων. Τα αποτελέσματα δείχνουν ότι για την ακριβή μέτρηση των δύο ενώσεων, όταν έχει χρησιμοποιηθεί μεθανόλη για την προετοιμασία των δειγμάτων ή την εκχύλιση, χρειάζονται να παρθούν ιδιαίτερα μέτρα για αποφυγή απωλειών λόγω προσρόφησης.

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Determination of polycyclic aromatic hydrocarbons in water by GC/MS/MS

K. Liapis[1], E. Bempelou[1] and P. Aplada-Sarlis[1]

Summary In the present study the development and validation of an analytical method for the determination of 8 compounds of the polycyclic aromatic hydrocarbons group [naphthalene, fluoranthene, anthracene, benzo [b] fluoranthene, benzo [a] pyrene, benzo [k] fluoranthene, benzo [g,h,i] perylene and indeno [1,2,3-cd] pyrene] by GC/MS/MS was carried out in water samples. The method was applied to ground waters from Greece. The method was validated in three fortification levels and the observed evaluation parameters were found to be acceptable. The limit of quantification was equal to the lowest level of fortification (LOQ: 0.05 μg/l) indicating that the method has adequate sensitivity for monitoring purposes.

Additional keywords: analytical method, gas-chromatography, PAHs, tandem mass spectrometry

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are organic compounds with 2-10 aromatic rings, readily formed during many pyrolysis and incomplete combustion processes of organic materials. Potential sources of PAHs are vehicle exhaust emissions from gasoline and diesel engines, combustions of timber, coal, oil, gas and wood, industrial activity etc. (2). Some PAHs have been classified as priority pollutants (4). This classification has been set by both the Environmental Protection Agency (EPA) of U.S.A. and the European Union. Sixteen of these substances are of interest for their action in the environment, while 6 of them have been characterized as potentially carcinogenic compounds: benzo [a] anthracene, benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene, dibenzo [ah] anthracene and indeno [1,2,3-cd] pyrene (1). This fact makes necessary the development of analytical methods for the determination of these pollutants in environmental substrates as well as in products of plant and animal origin involved in human diet.

In the environment, PAHs can be detected in air, water and soil, while they have also been found in areas far away from their possible source of emission (1, 6, 7, 9). In natural waters they are often detected in sediment and biota, because of their low polarity, where carciginogenesis or metallaxigenesis have been observed. PAHs are rapidly metabolized in fish but not in invertebrates (1).

Published results of PAHs have been mainly determined by gas chromatography coupled with mass spectrometry. According to the official method of the Environmental Protection Agency, PAHs are determined in potable water by GC/MS using internal standard, the limit of quantification being equal to 0.2 μg/l (5).

Shackelford and McGuire have also used the GC/MS technique for the determination of priority pollutants, including

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PAHs (11). Volkers and Pouwels, on the other hand, have presented a method for determination of PAHs in water samples by high performance liquid chromatography with a fluorescence detector (HPLC/FLD) after an optimization of the solid phase extraction procedures (12).

The European Union has established parametric values (maximum acceptable limits) only for 5 of the PAHs, according to the directive 98/83/EC. For benzo [a] pyrene this value is 0.01 μg/l, while for the sum of benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [g,h,i] perylene and indeno [1,2,3-cd] pyrene the respective parametric value is 0.1 μg/l.

The aim of this study was to develop and validate a robust method for the determination of 8 PAHs (naphthalene, fluoranthene, anthracene, benzo [b] fluoranthene, benzo [a] pyrene, benzo [k] fluoranthene, benzo [g,h,i] perylene and indeno [1,2,3-cd] pyrene) in water samples with GC/MS/MS, using 2 transitions for each compound. It has to be mentioned that fluoranthene functions as an indicator of the existence of PAHs in water (3). The method has been used to monitor presence of PAHs in ground and surface water samples.

Materials and Methods

1. Chemicals and reagents

Analytical standards of benzo [b] fluoranthene (99%), benzo [k] fluoranthene (98%), benzo [g,h,i] perylene (99,4%) and indeno [1,2,3-cd] pyrene (99,5%) were purchased from ChemService (West Chester, UK). Naphthalene (99,5%), fluoranthene (99%), benzo [a] pyrene (98,5%) and anthracene (99,5%) were obtained from Dr Ehrenstofer (Augsburg, Germany). Ethyl acetate, methanol and acetone were of pesticide residues grade and water of HPLC grade, all obtained from Lab Scan (Dublin, Ireland).

2. Standard Solutions

Analytical standard stock solutions of each compound at 1000 μg/l were made in acetone and working standard solutions were obtained at various concentrations by dilution of the stock solution in ethyl acetate. All these solutions were stored at -20°C. Three standard spiking solutions containing all the compounds at the concentration of 0.05, 0.1 and 0.2 μg/ml were used for the fortification purposes.

3. Sample procedure

The extraction procedure applied was as previously described by Miliadis *et al.* (10) for the determination of pesticide residues in water. Sample preparation was performed by solid phase extraction with pre-packed reversed-phase octadecyl (C-18) bonded silica contained in cartridges. Isolute cartridges (International Sorbent Technology), containing 500 mg of packing material were conditioned prior to loading the sample, by passing successively 1ml/100 mg sorbent (5 ml) ethyl acetate, 5 ml methanol and 10 ml of organic free water, with a glass syringe. Volumes of 500 ml of water sample were cleaned by removal of any floating or insoluble material and were passed through the cartridge. A SPE large volume sampler was connected to a vacuum source equipped with a gauge and a balast valve to adjust the vacuum, for the extraction of the samples in order to obtain a sampling rate of 15 ml water/ml. Teflon tubes were inserted to the sample containers and adapters to the cartridges connected the other ends. In this way, up to eight water samples could pass through cartridges simultaneously. After passing the sample, suction continued for 30 min to dry out the packing material. The cartridges were disconnected and the adsorbed compounds were eluted with ethyl acetate into 1 mL volumetric flasks. The extract was then injected to the GC/MS/MS system.

4. Instrumentation

A Varian CP-3800 gas chromatography system, interfaced to a triple quadrupole mass spectrometer (Varian 1200 L) was used for the residues identification and quantification. The identification and quantification of the studied analytes was carried out, based on the technique of MS/MS using the transitions (precursor ion – product ion, Multiple Reaction Monitoring – MRMs), which are presented in Table 1. The GC was equipped with a split/splitless injector operating in the splitless mode and an autosampler CP-8400. The analytical capillary column used was a 30 m x 0.25 mm I.D., coated with 0.25 μm film thickness, VF-5 ms (Varian 25200 Commercentre Drive, Lake Forest, CA, U.S.A.). The oven temperature program consisted of 2 min hold at 70°C, ramp at 30°C/min to 180°C, 1.8°C/min to 230°C, 30°C/min to 280°C and a final hold for 30min. The injector was operated at 300°C, pulse pressure 10 psi and pulse duration 0.25 min. The carrier gas was He at 1ml/min. The injection volume was 1 μl. The temperature of the source was 200°C, the interface temperature at 290°C and the solvent delay 5 min.

Quantification in this method was made by comparing the peak areas of the sample solutions to the peak areas of two analytical standard solutions bracketing the area of the sample and not differing in concentrations more than 20% from each other, thus not using a calibration curve.

Results and Discussion

The substances benzo [a] anthracene, benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene and indeno [1,2,3-cd] pyrene have been classified as priority pollutants (4), a fact that makes their detection and determination in water necessary. Furthermore fluoranthene is an indicator substance and its presence in a water sample is taken as evidence for a PAHs contamination of the water.

In the present study an effort has been made to adjust an already existing method, routinely used in our laboratory for

Table 1. Retention times (min) of the PAHs, transitions (the quantitative ion is marked in bold) and collision energy (V).

Analyte	Retention time (min)	MRMs	Collision Energy (V)
Naphthalene	5.5	**128 > 128**	10
		128 > 102	30
Anthracene	11.8	**178 > 178**	10
		178 > 152	30
Fluoranthene	18.2	**202 > 202**	10
		202 > 200	30
Benzo [b] fluoranthene	36.5	**252 > 252**	10
		252 > 250	30
Benzo [k] fluoranthene	37.1	**252 > 252**	10
		252 > 250	30
Benzo [a] pyrene	38.2	**252 > 252**	10
		252 > 250	30
Indeno [1,2,3-cd] pyrene	43.9	**276 > 276**	10
		276 > 274	30
Benzo [g,h,i] perylene	45.5	**276 > 276**	10
		276 > 274	30

pesticide residue analysis, to the GC/MS/MS determination of PAHs in water. This would allow determination of pesticides as well as PAHs using one single extraction and thus reducing the sample preparation time.

Performance of the method was evaluated by determining the basic validation parameters, i.e. accuracy, precision and sensitivity. The accuracy was assessed from the attained recoveries, the precision from the relative standard deviation (RSD) values, and the sensitivity by estimating the limits of quantification (LOQs).

The method did not provide satisfactory resolution between benzo [b] fluoranthene and benzo [k] fluoranthene and for this reason they were treated as one peak in the calculation of the obtained recoveries (Figure 1). Since the maximum acceptable limit set for these compounds is expressed as the sum of the four compounds (benzo [b] fluoranthene, benzo [k] fluoranthene, indeno [1,2,3-cd] pyrene and benzo [a] anthracene (3), actually there is no problem from the poor resolution of the two.

Recoveries (Table 2) for anthracene were high, 146-160%, but with an acceptable repeatability (relative standard deviations ranged from 10.3 to 17.8%). For the other PAHs, as shown in Table 2, the average recovery values were 50.7%-84% in the lower level of fortification (0.05 μg/l), 58.4-81.7% in the second fortification lev-

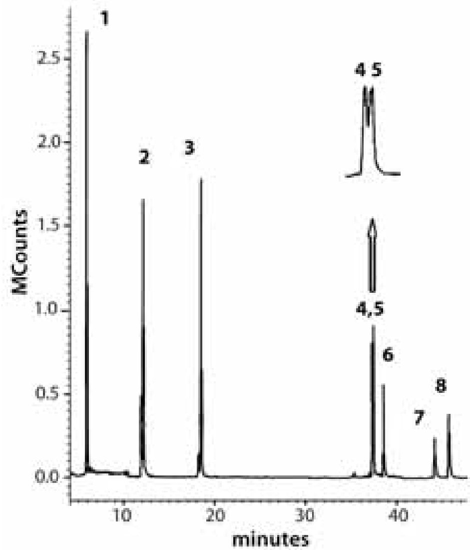

Figure 1. Chromatogram of a fortified water sample at 0.05μg/L with (1) naphthalene, (2) anthracene, (3) fluoranthene, (4) benzo [b] fluoranthene, (5) benzo [k] fluoranthene, (6) benzo [a] pyrene, (7) indeno [1,2,3-cd] pyrene and (8) benzo [g,h,i] perylene.

Table 2. Mean recoveries (%) and relative standard deviation values (RSD, %) after fortification of water samples in 3 fortification levels with 6 replicates/level.

Compound	1st level (n=6)			2nd level (n=6)			3rd level (n=6)		
	C (μg/l)	Recovery (%)	RSD (%)	C (μg/l)	Recovery (%)	RSD (%)	C (μg/l)	Recovery (%)	RSD (%)
Naphthalene	0.05	84	9.8	0.1	81.7	12.1	0.2	86	13.7
Anthracene	0.05	146	13.5	0.1	160	10.3	0.2	148	17.8
Fluoranthene	0.05	82.4	11.8	0.1	80.1	13.6	0.2	80.6	12.4
Benzo [b] fluoranthene	0.05			0.1			0.2		
Benzo [k] fluoranthene	0.05	62.1	7.9	0.1	73.6	13.6	0.2	51.9	20.2
Benzo [a] pyrene	0.05	58.4	16.8	0.1	59.4	14.9	0.2	52.4	21.8
Indeno [1,2,3-cd] pyrene	0.05	61.7	18.6	0.1	58.4	16.8	0.2	60.7	23.4
Benzo [g,h,i] perylene	0.05	50.7	18.4	0.1	67.8	15.5	0.2	52.94	14.5

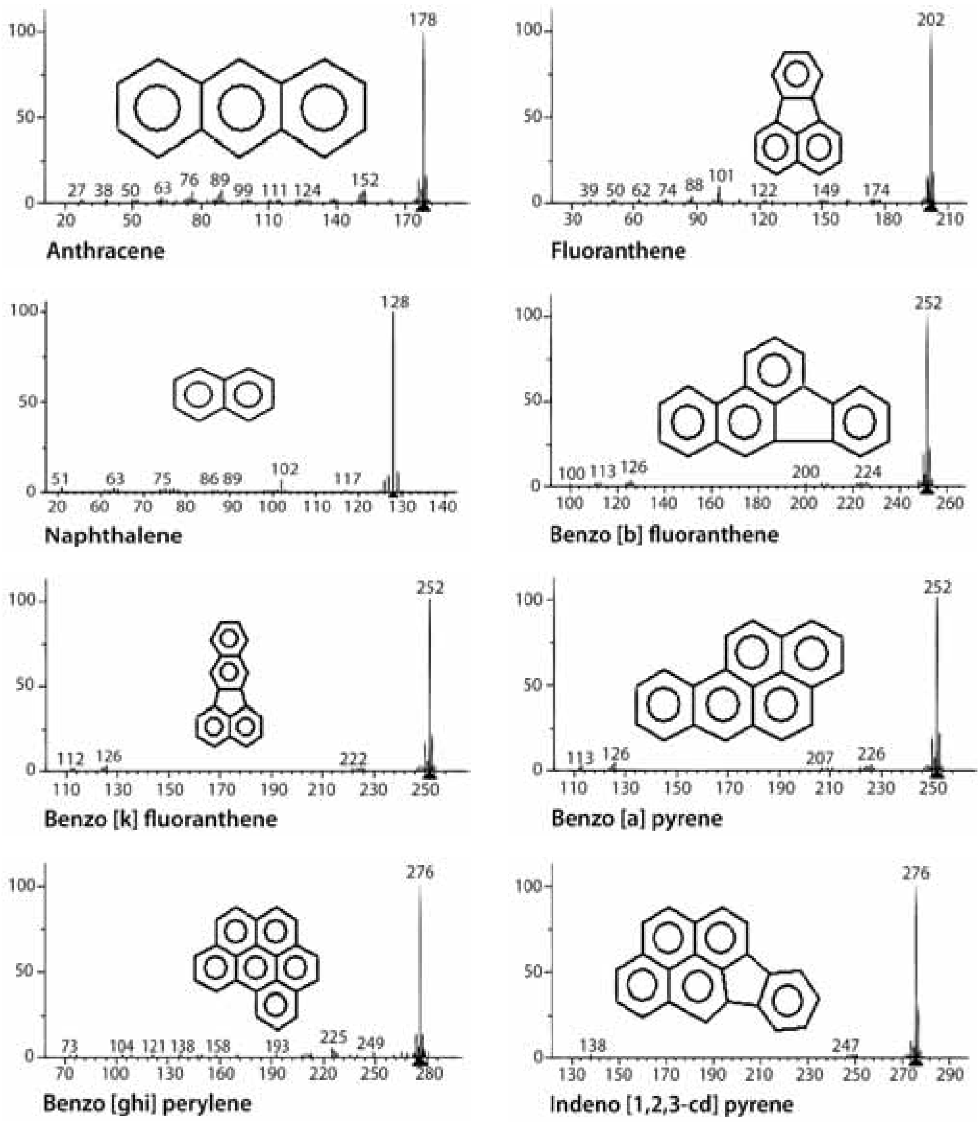

Figure 2. Chemical structures and mass spectra of 8 PAHs after injection in the GC/MS/MS system.

el (0.1 µg/l) and 51.9-86% in the third fortification level (0.2 µg/l). The relatively low recovery values for some of the examined PAHs may be due to their lower solubility in water (8), a fact that has also been observed with the official EPA method (5).

Relative standard deviation values were 7.9-18.6%, 10.3-16.8% and 12.4-23.4%, in the first, second and third fortification level respectively (Table 1), indicating a good precision of the method. The limit of quantification (LOQ) is equal to the lowest fortification level (LOQ: 0.05 µg/l). At this level accuracy and precision were acceptable.

During the quantitative analysis 2 multiple reaction monitoring (MRM) of 2 ions from separate transitions of the molecular ion were used. The 8 tested compounds did not present intense fragmentation, as it can be seen in Figure 2, and therefore the molecular ion was the one used for quantification purposes. These quantification ions are presented in Table 1 in **bold**. In this Table, the retention time of each analyte is also provided, as well as the collision energy used for the fragmentation of the precursor ions.

Quantification and confirmation of results by monitoring further reaction products of selected ions by tandem mass spectrometry (MS/MS using triple quadropole systems) offers a greater confindence of results, compared to MS in a single stage, according to principles on analytical chemistry by adding one more step in the total analytical procedure.

As far as benzo [a] pyrene is concerned, the analytical method was validated in the fortification level of 0.05 μg/l, while the maximum acceptable limit is 5 times lower. In our next research work, we are going to focus to this compound in order to achieve a LOQ at the required maximum acceptable limit.

The suitability of the present analytical method for the determination of PAHs in ground water samples was proved from the successful participation of our laboratory in the proficiency test "IMEP-23: the eight WFD PAHs in water in presence of humic acid", organized by the Institute for Reference Materials and Measurements (IRMM) within the Scheme of International Measurement Evaluation Programme (IMEP). The test material of this interlaboratory comparison was ground water.

The described method was further used for the analysis of ground water samples from the regions of Volos (Magnesia) and Rodos in Greece. A total of 15 samples were analysed and no PAHs were detected in any sample.

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Προσδιορισμός πολυκυκλικών αρωματικών υδρογονανθράκων στο νερό με GC/MS/MS

Κ. Λιαπής, Ε. Μπεμπέλου και Π. Απλαδά-Σαρλή

Περίληψη Στην παρούσα μελέτη πραγματοποιήθηκε η ανάπτυξη και επικύρωση της αναλυτικής μεθόδου προσδιορισμού 8 ουσιών PAHs, των naphthalene, fluoranthene, benzo [a] pyrene, anthracene, benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [g,h,i] perylene και indeno [1,2,3-cd] pyrene σε δείγματα νερού με την τεχνική GC/MS/MS και η εφαρμογή της σε δείγματα ελληνικών υπογείων υδάτων. Η μέθοδος επικυρώθηκε σε τρία επίπεδα εμβολιασμού, το ένα εκ των οποίων αντιστοιχεί στο θεσπισθέν από την Ευρωπαϊκή Ένωση για τις ουσίες αυτές ανώτατο αποδεκτό όριο (0,1 μg/l) και παρατηρήθηκαν αποδεκτές τιμές των παραμέτρων αξιοπιστίας της μεθόδου. Το όριο αναλυτικού προσδιορισμού της μεθόδου ισούται με το κατώτατο επίπεδο φόρτισης, LOQ=0,05 μg/l, βεβαιώνοντας πως η μέθοδος διαθέτει την απαιτούμενη ευαισθησία για μετρήσεις δειγμάτων ως προς τη συμμόρφωσή τους με τα απαιτούμενα όρια.

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Wild oat variability in wheat fields of Viotia in Central Greece

I.S. Travlos[1], C.N. Giannopolitis[2] and E.A. Paspatis[1]

Summary Wild oat variability in wheat fields of Greece was examined by conducting a two-year field survey in Viotia, a typical wheat producing region in the central part of the country, and greenhouse experiments to compare seedlings grown from wild oat accessions of this region under the same conditions.

The field survey indicated that, despite the widely adopted practice of using herbicides, wild oats were found at the time of maturation to be present in most of the wheat fields (83-91%). The species present in all cases was *Avena sterilis* while *A. fatua* was found to coexist in small patches and at lower densities only in 11-15% of the surveyed fields. For the safe *in situ* recognition of the species, a set of selected characters based on the mature spikelets is proposed.

In the greenhouse experiments great variability was observed with regards to growth habit and tillering in seedlings of the *A. sterilis* but not of the *A. fatua* accessions. Seedlings of the *A. sterilis* accessions exhibited an erect, a semi-erect or a prostrate growth habit, the latter being associated with a larger number of tillers per plant.

Additional keywords: *Avena sterilis*, *Avena fatua*, biotypes, ecotypes, growth pattern, phenotypic variation, weeds

Introduction

Of the various *Avena* species (wild oats) known to occur in Europe (15), two are commonly found in crops and are regarded as important weeds, *Avena fatua* L. and *Avena sterilis* L. (5, 10, 14). These two species are particularly competitive in cereal crops, A. *fatua* being widespread in North West Europe or the cooler regions of Europe and *A. sterilis* usually found in warmer regions of Southern Europe and the Mediterranean basin (9, 13).

Wild oat variability in a region is an important parameter which must be considered in evaluating efficacy of control strategies. This variability is firstly dependent on the species present and secondly on morphological and physiological variation within each species. There are reports in the literature describing *A. fatua* as a highly polymorphic species exhibiting wide variability (11, 12). In the case of *A. sterilis*, there have also been reports of wide variability for several traits, including number of spikelets per panicle and seed traits (8, 12).

In Greece, much of the area under cereals is invariably infested with wild oats. The main species is said to be *A. sterilis* while *A. fatua* is thought to be of minor importance and confined to the north of the country (13). In a survey conducted by Damanakis in 1982 in the wheat fields of Central Greece, there was no presence of *A. fatua* recorded while *A. sterilis* was so abundant to rank as the most widespread and troublesome weed in cereals of the area (1). Morphological and physiological variation within *A. sterilis* in Greece has already been documented by Efthimiadis *et al* (3).

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The purpose of this study was to obtain a better picture of the currently existing wild oat variation, both intra- and inter-specific, in wheat fields of Greece. This information was thought as exceptionally useful on undertaking studies for the evaluation of actual control efficiency and herbicide resistance problems in cereal crops of the country. Therefore, crops in a selected typical wheat producing region of Greece, namely Viotia, were surveyed for two growing seasons with the main objectives to determine the wild oat species competing with the crop, their relative frequency of occurrence and the main variations in seedling morphology and growth habit.

Materials and Methods

Field survey and seed collection

The region of Viotia, which is a typical wheat producing area in Greece, was selected for the wild oat survey and the collection of seeds. To facilitate recording and for a better distribution of the sampling sites, the region was divided into 8 distinct subregions: (A) Thiva to Chalkida, (B) Thiva to Mouriki, (C) Schimatari, (D) Thiva, (E) Agios Thomas, (F) Asopia, (G) Thespies-Elopia and (H) Orchomenos (Figure 1). A number of wheat fields, corresponding to about 1% of the total wheat area in each subregion, were randomly surveyed. The survey was conducted during a two week period at the beginning of maturity, in the first weeks of June 2007 and the last weeks of May 2008. Each surveyed field was walked through by the two diagonals and records were kept of the wild oat species present and of their density. Furthermore, a representative sample of panicles and seeds were collected and transferred to the laboratory.

The collected wild oat panicles and seeds were further examined in the laboratory to verify species identification; the seeds were separated, air-dried and stored in paper bags at room temperature until used.

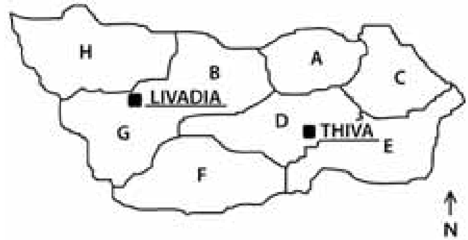

Figure 1. Locations of the wheat fields surveyed in Viotia region, Greece: (A) Thiva to Chalkida, (B) Thiva to Mouriki, (C) Schimatari, (D) Thiva, (E) Agios Thomas, (F) Asopia, (G) Thespies-Elopia and (H) Orchomenos.

Plant growth experiments

To examine variability within the species, the collected wild oat accessions were grown under the same conditions, in pot experiments conducted in the greenhouse in December of 2007. Five seeds of each accession collected during the first year of the survey were sown in a 9-cm pot. Each accession was sown in ten replicates (pots). Following germination, seedlings were thinned to three per pot. In the greenhouse, the minimum and the maximum temperature were 15 and 37° C, respectively. Throughout the experiment, the pots were watered as needed and supplied with 50 ml/pot of modified Hoagland's solution (0.25 strength) every 10 days (4).

The following characteristics were recorded for each accession: growth habit, plant height, tillering and first panicle emergence. Statistical analyses were performed using the Statistica software package (StatSoft Inc. 1999). Differences between means were compared using Fisher's least significant difference (LSD) test at $p < 0.05$.

Results and Discussion

The results of the 2-year survey (Table 1) indicate presence of wild oats in 91% (2007) and 83% (2008) of the wheat fields in Viotia. Not shown in the table but important to note is that the density of wild oats in these fields, although usually moderate to light, was at times very high. The high frequency of occurrence (83-91%), even in moderate or light densities at a time near harvest, shows that wild oats are an important weed for wheat crops in Viotia taking into account that the results of this survey reflect plants having escaped the use of herbicides which is a widely adopted practice in the region.

The results of the survey also show that *A. sterilis* is the species occurring in all wheat fields in which presence of wild oats was recorded (83-91% of the surveyed fields) while *A. fatua* was found only in 11-15% of the surveyed fields, always coexisting with *A. sterilis* (Table 1). In fact, *A. fatua,* whenever found, was in small patches and low plant densities within a more spread and dense population of *A. sterilis*.

Our results on the relative abundance of the two wild oat species are consistent with results from similar surveys conducted several years ago in Spain which indicated frequencies of 32 and 8%, for *A. sterilis* and *A. fatua*, respectively (7), while in another survey in Andalusia, *A. sterilis* was the most frequent grassy species (being present in 65% of the surveyed fields) and *A. fatua* was hardly found at all (9).

The presence of *A. fatua* in cereal crops in Greece has for long been questionable. Thus, in previous weed surveys conducted in Central Greece it was not found at all (1) while in other publications its presence in the country was reported to be rather limited (2, 3) or doubtful (15). The results of this survey prove the actual presence of *A. fatua* in cereal crops of Greece at low, but probably increasing, frequencies and densities.

The recognition of the *Avena* species, especially the distinction of *A. fatua* from *A. sterilis*, is not easy and seems rather impossible at growth stages before maturation. A detailed study of the mature spikelet characters as they appear at the time of shedding indicated that the two species have some clear differences (Table 2) which can be used for the *in situ* recognition of the species.

The spikelet characters used for the *in situ* species recognition (Table 2) were selected after many appropriate comparisons of mature spikelets from the surveyed wheat fields and standard samples of the two species kept in the Herbarium and the weed seed collection of the Weed

Table 1. Frequency of presence of *Avena* species in wheat fields in the Viotia region (8 subregions, A-H) during 2007 and 2008.

Year	*Avena* species	Number of wheat fields surveyed in each subregion									Frequency (%)
		A	B	C	D	E	F	G	H	Total	
2007	Surveyed fields	8	6	5	3	6	6	4	7	45	
	Avena-free fields	1	1	0	0	0	1	0	1	4	8.9
	Avena sterilis	7	5	5	3	6	5	4	6	41	91.1
	Avena fatua	1	1	0	1	0	0	0	2	5	11.1
2008	Surveyed fields	5	8	7	5	5	6	4	8	48	
	Avena- free fields	1	0	1	1	1	2	0	2	8	16.7
	Avena sterilis	4	8	6	4	4	4	4	6	40	83.3
	Avena fatua	1	1	2	0	0	0	0	3	7	14.6

Table 2. Mature seed characters discriminating *A. fatua* from *A. sterilis* in the field**.**

Character	*Avena sterilis*	*Avena fatua*
Maturity	Earlier maturing	Later maturing
Awn length (cm)	3-7	3-4
Awn attachment	Below middle of floret	Above middle of floret
Floret length (cm)	1.7-3.0	1.4-2.1
Basal scar shape	Large, elongated	Absent or small round
Hair covering	50-80% of floret surface	30-60% of floret surface

Science Department at the Benaki Phytopathological Institute. These characters can provide a safe recognition of the species. In actual measurements of spikelets in the field the mean length of the awns, for example, was 5.514 cm for *A. sterilis* and 3.625 cm for *A. fatua*. Also, the mean length of the florets was 2.392 and 1.85 cm for *A. sterilis* and *A. fatua*, respectively. Moreover, a difference was observed in the time of maturation, *A. fatua* maturing about 10-15 days later than *A. sterilis,* and this is likely associated with different temperature requirements for germination; optimum germination temperatures for *A. sterilis* are reported to be lower than those for *A. fatua* (6, 17).

It is worthy to mention that the above characters can provide a safe recognition of the species if used on the seeds as they are shedding. However, processing of the seeds after shedding, which may cause breaking of awns, rubbing off the hairs, breaking of florets or bases etc. is expected to reduce the diagnostic value of these characters.

The wild oat accessions from the Viotia region, when grown under the same conditions in pots in the greenhouse, exhibited a significant variation in growth habit and other parameters of seedling development (Table 3). Variability was wide in the *A. sterilis* accessions but very limited in the *A. fatua* accessions.

The most important variation found was related to the growth habit of the *A. sterilis* accessions, in some of which the seedlings had an erect growth pattern, in some others a semi-erect pattern and in others a prostrate growth pattern (Figure 2). In all *A. fatua* accessions from Viotia the seedlings exhibited only the erect growth habit. The peculiar prostrate pattern of growth for some *A. sterilis* accessions has also been observed by other researchers in Greece (3) as well as in other countries (12, 16).

Significant differences among wild oat accessions were also observed when plant height and tillering were compared (Table 3). Tillering, measured as the number of tillers per plant, seems to correlate negatively with plant height and positively with the growth habit, being higher in plants of semi-erect and highest in plants of the prostrate growth pattern, in the *A. sterilis* accessions. In the *A. fatua* accessions, in which only the erect growth pattern was observed, tillering was mini-

Figure 2. Growth habit of *A. sterilis* accessions from the Viotia region in Greece. Erect (A), Semi-erect (B) and Prostrate (C) seedling growth pattern .

Table 3. Vegetative growth parameters of *A. sterilis* and *A. fatua* accessions from Viotia, grown in pots for 40 days after seedling emergence.

Species	Accessions	Plant height (cm)	Tillering No/plant	Growth habit	First panicle emergence
A. sterilis	A6	25 b	1.3 d	Erect	No
	B4	12 cd	3.3 c	Semi-erect	No
	C3	19 b	1.6 d	Erect	Yes
	D2	21 b	1.5 d	Erect	No
	E6	5 e	5.1 a	Prostrate	No
	F2	6.5 de	4.2 b	Semi-erect	No
	G3	18 bc	1.4 d	Erect	Yes
	H4	17 bc	2.0 cd	Erect	Yes
A. fatua	A1	36 a	0 e	Erect	No
	B1	39 a	0 e	Erect	No
	D1	34 a	0 e	Erect	No
	H2	37 a	0 e	Erect	No

mal (0 tillers/plant) under the conditions of the experiments.

Regarding the time from seedling emergence to the emergence of the first panicle, there seems to be a clear tendency of the *A. sterilis* accessions to reach the flowering stages earlier than the *A. fatua* accessions (Table 3).

The intraspecific variability regarding the two species of wild oats is quite interesting. Previous reports describe *A. fatua* as a highly polymorphic species exhibiting wide variability (12, 16) but in our study there was no significant phenotypic variation among the Greek *A. fatua* accessions. On the other hand, significant variation was found within *A. sterilis* when the Greek accessions from Viotia were examined and this is in agreement with previous reports describing a wide variability also within this species (3, 12, 16).

The results of these studies show that wild oat variability in the wheat fields of Viotia is significant as expected. The presence of *A. fatua*, which was questionable so far, is now verified and part of the variability derives from the coexistence of the two species. Most of the variability, however, derives from morphological and physiological variations which seem to be quite evident within *A. sterilis* and not within *A. fatua*.

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Παραλλακτικότητα αγριοβρώμης σε σιταγρούς της Βοιωτίας στην Κεντρική Ελλάδα

Η.Σ. Τραυλός, Κ.Ν. Γιαννοπολίτης και Ε.Α. Πασπάτης

Περίληψη Η παραλλακτικότητα της αγριοβρώμης σε σιταγρούς της Ελλάδας εξετάσθηκε με επισκόπηση του ζιζανίου για δύο χρόνια σε αγρούς της Βοιωτίας, η οποία είναι μια τυπική σιτοπαραγωγική περιοχή στο κεντρικό τμήμα της χώρας, και με πειράματα θερμοκηπίου στα οποία συγκρίθηκε η ανάπτυξη των σποροφύτων βιοτύπων αγριοβρώμης που συλλέχθηκαν από την περιοχή αυτή.

Η επισκόπηση των σιταγρών έδειξε ότι, παρά την ευρέως διαδεδομένη πρακτική της χρήσης ζιζανιοκτόνων, αγριοβρώμη βρέθηκε κατά το χρόνο της ωρίμασης να υπάρχει στους περισσότερους αγρούς (83-91%). Το είδος αγριοβρώμης που υπήρχε ήταν σ' όλες τις περιπτώσεις *Avena sterilis* και μόνο στο 11-15% των αγρών συνυπήρχε και η *A. fatua* κατά μικρές κηλίδες και σε χαμηλότερη πυκνότητα. Για την ασφαλή «επί τόπου» αναγνώριση του είδους προτείνεται ένας επιλεγμένος αριθμός χαρακτήρων των ώριμων σταχυδίων.

Στα πειράματα θερμοκηπίου βρέθηκε μεγάλη παραλλακτικότητα όσον αφορά τον τύπο ανάπτυξης και το βαθμό αδελφώματος στα σπορόφυτα των βιοτύπων της *A. sterilis* αλλά όχι εκείνων της *A. fatua*. Τα σπορόφυτα των βιοτύπων της *A. sterilis* είχαν ανάπτυξη όρθια, ημι-όρθια ή πλάγια (έρπουσα), με τον τελευταίο τύπο να παρουσιάζει και το μεγαλύτερο αριθμό αδελφιών ανά φυτό.

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