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REVIEW ARTICLE

Eriophyoid mites (Acari: Eriophyoidea) in Greek orchards and grapevine: A review

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Summary Eriophyoid mites (Acari: Prostigmata: Eriophyoidea), one of the most diverse group of mites, are plant feeder specialists, causing various symptoms on plants, and many of them are economically important pests on orchards and grapevines. They are commonly known as gall, rust, bud, and blister mites. Up to date approximately a hundred species of eriophyoid mites have been reported in Greece, thirty three of them, belonging to the families Eriophyidae, Diptilomiopidae and Phytoptidae, in agricultural orchards and vineyards. Information about their hosts, damage and natural enemies is presented. Also, the subjects of monitoring and chemical control of eriophyoid mites are discussed.

Additional keywords: blister mites, bud mites, control, natural enemies, rust mites

Introduction

Among phytophagous mites, the eriophyoid mites (Acari: Prostigmata: Eriophyoidea) are the most diverse group and many of eriophyoid species are economically important pests (Van Leeuwen *et al.*, 2010). Around 3,700 species are currently recognized (Amrine *et al.*, 2003) on angiosperms, coniferous plants and ferns throughout the world. They are commonly known as gall, rust, bud, and blister mites.

Members of the Eriophyoidea are soft bodied, wormlike, or spindle shaped, unique among the mites because they have only two pairs of legs and size so small that they are almost invisible to the unaided eye. Eriophyoids are one of the most specialized groups of plant feeders. They are characterized by the intimate relationships they have with their hosts and the restricted range of plants upon which they can reproduce. Eighty per cent of eriophyoids have been reported on only one host species, 95% on one host genus, and 99% on one host family (Skoracka *et al.*, 2010).

Symptoms of their feeding are varying from simple russeting to complex gall formation and may appear on buds, shoots, stems, twigs, flowers and leaves of the plants. Gall formation occurs as a result of mite attack on individual plant cells; it is a localized growth reaction of the host plant to the attack. Common examples are leaf galls, bud galls, and erineum. In some plants, elongation of flower stems and lateral branches is inhibited, causing the development of contorted foliage, flowers, and branches. One of the most conspicuous examples is witches'-broom, which is a cluster of brush like growth of stunted twigs or branches on trees. Some eriophyid species arrest shoot development, causing leaf sheaths to become enlarged, closely packed, and bunched at stem nodes. Others cause the well-known "big bud", which consists of an aggregation of swollen, thickened scale leaves. Eriophyids also cause an array of nongall abnormalities, such as leaf folding and twisting, blisters, and discoloration. Russeting and silvering or bronzing of leaves are also induced by eriophyid feeding. As a result of infestation flowers or young fruits maybe falling off (Keifer *et al.*, 1982).

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Eriophyoid mites (Acari: Eriophyoidea) have been recognized as important pests in agriculture and forestry all over the world (Lindquist *et al.*, 1996). A number of eriophyoid species are considered to be main pests on some crops, while others are known to be a quarantine threat for several countries. Nevertheless, several eriophyoid species seldom attain high population levels and thus their economic importance is a matter of discussion (Duso *et al.*, 2010).

There are quite a few difficulties evaluating eriophyoid population densities and related yield losses in most cases of eriophyoid infestations. Their minute size and concealed way of life (several species live and reproduce well hidden in buds or in induced plant structures, like galls, erinea and blisters) represent an obstacle for detailed studies aiming at determining their impact on agricultural crops. The small size and the behavior of eriophyoids are frequently implied in misdiagnoses with implications for yield losses (Duso *et al.*, 2010). In contrast, their symptoms are sometimes spectacular but not of economic impact. Knowledge of the economic impact of eriophyoids on crop yields and threshold levels is a fundamental requirement for the improvement of IPM.

Up to date approximately 100 species of eriophyoid mites have been reported from Greece (Malandraki, 2012), thirty three of them in agricultural orchards and grapevines. In Table 1, the name of the mite species and the host plant species as well as the corresponding references are summarised. Most of the papers are dealing with the taxonomic status, distribution records and control. A few studies deal with their biology and control.

Eriophyoid species in Greek orchards

Eriophyoids of stone fruits

***Acalitus phloeocoptes* (Nalepa) [*Phytoptus phloeocoptes* Nalepa, *Eriophyes phloeocoptes* (Nalepa)]**

Common name: plum blister mite

Damage: The plum blister mite infests mainly almond and plum. On almond, it causes permanent irregular galls of various sizes around the buds and deforms the fruit. Infested trees fail to form fruit buds and lose vigor. On plum, the mites form small, irregular, subsphaerical galls, 1.3-1.8 mm in diameter surrounding the buds and also deforming the fruit. The galls may appear singly or clustered around the buds and become woody.

On almond the damage appears to be progressive and irreversible resulting in death of the tree in 3-6 years. Unlike almond, infested plum trees often recover from mite attack and do not show permanent injury (Keifer *et al.*, 1982).

In the area of Magnesia (Central Greece) on "Skopelos" plum tree the mite starts to migrate from the galls to new buds in mid-April and the migration phase lasts 50 days. Population builts up until December with successive generations, not damaging the production or the tree (Papanikolaou and Bakoyiannis, 1991).

***Aculus fockeui* (Nalepa and Trouessart) [*Phyllocoptes fockeui* Nalepa and Trouessart, *Vasates fockeui* (Nalepa and Trouessart)]**

Syn: *Phyllocoptes cornutus* Banks [*Aculus cornutus* (Banks); *Vasates cornutus* (Banks)]
Phyllocoptes paracornutus Keifer
Common name: plum rust mite

Damage: This is a pest mainly of plums, peaches/nectarines and cherries in orchards and nurseries. This mite produces asteroid chlorotic spots on leaves. When populations are high the leaf is wavy or slightly twisted about its longitudinal axis. Heavy infestations of *A. fockeui* may form rosette shoots and keep leaves from expanding to normal size. Deutogynes hibernate in niches or near the current season growth. They crawl into bark crevices, especially around injuries, but are also behind potentially active buds, or under available loose bud scales. As the buds expand in spring the deutogynes crawl to, and feed upon, emerging embryonic leaves and lay eggs. Trees are losing

Table 1. List of known Eriophyoidea in Greek orchards and grapevine.

Species	Hosts	References
Eriophyidae		
Acalitus phloecoptes (Nalepa)	*Prunus domestica* L. *Prunus dulcis* (Mill.) D.A.Webb	Hatzinikolis, 1969b; Hatzinikolis, 1970a; Hatzinikolis, 1970b; Papanikolaou and Bakoyiannis, 1991; Papaioannou-Souliotis *et al.*, 1994
Aceria cretica Hatzinikolis	*Olea europaea* L.	Hatzinikolis, 1989; Papaioannou-Souliotis *et al.*, 1994
Aceria erineus (Nalepa)	*Juglans regia* L.	Issakides, 1936; Hatzinikolis, 1967; Hatzinikolis, 1969a; Hatzinikolis, 1969b; Hatzinikolis, 1970b; Papaioannou-Souliotis *et al.*, 1994
Aceria ficus (Cotte)	*Ficus carica* L.	Hatzinikolis, 1969b; Hatzinikolis, 1970a; Papaioannou-Souliotis *et al.*, 1994; Malandraki, 2012.
Aceria granati (Canestrini and Massalongo)	*Punica granatum* L.	Hatzinikolis, 1967; Hatzinikolis, 1969a; Hatzinikolis, 1969b; Hatzinikolis, 1970b; Koveos *et al.*, 2010; Malandraki, 2012.
Aceria oleae (Nalepa)	*Olea europaea* L.	Kavadas, 1927; Koroveos, 1939; Pelekassis, 1962; Bouchelos *et al.*, 1963; Hatzinikolis, 1967; Hatzinikolis, 1969a; Hatzinikolis, 1969b; Hatzinikolis, 1969c; Hatzinikolis, 1970b; Hatzinikolis,1970d; Hatzinikolis, 1971; Mourikis and Vassilaina – Alexopoulou, 1975; Emmanouel, 1981; Hatzinikolis, 1984; Hatzinikolis and Kolovos,1985; Hatzinikolis, 1986; Hatzinikolis, 1989; Papaioannou-Souliotis *et al.*, 1994; Papaioannou-Souliotis and Markoyiannaki, 2003; Tzanakakis, 2003; Malandraki, 2012.
*Aceria olivi (*Zacher and Abou-Awad)	*Olea europaea* L.	Hatzinikolis and Kolovos,1985
Aceria pistaciae (Nalepa)	*Pistacia terebinthus* L. *Pistacia vera* L.	Hatzinikolis, 1970c; Papaioannou-Souliotis *et al.*, 1994; Malandraki, 2012.
Aceria sheldoni (Ewing)	*Citrus limon* (L.) Burm, *Citrus sinensis* (L.) Osbeck	Soueref and Komblas, 1961; Pelekassis, 1962; Bouchelos *et al.*, 1963; Hatzinikolis, 1967; Hatzinikolis, 1969a; Hatzinikolis, 1969b; Hatzinikolis, 1970b; Mourikis and Vassilaina – Alexopoulou, 1975; Papaioannou-Souliotis *et al.*, 1994; Malandraki, 2012.
Aceria tristriatus (Nalepa)	*Juglans regia* L.	Pelekassis, 1962; Hatzinikolis, 1967; Hatzinikolis, 1969a; Hatzinikolis, 1969b; Hatzinikolis, 1970b; Papaioannou-Souliotis *et al.*, 1994; Malandraki, 2012.
Aculops benakii (Hatzinikolis)	*Olea europaea* L.	Hatzinikolis, 1968; Hatzinikolis, 1969c; Hatzinikolis, 1970b; Hatzinikolis, 1974; Emmanouel, 1981; Hatzinikolis and Kolovos,1985; Papaioannou-Souliotis *et al.*, 1994; Tzanakakis, 2003

Species	Hosts	References
Aculops pelekassi (Keifer)	*Citrus limon* (L.) Burm, *Citrus deliciosa* Ten. *Citrus sinensis* (L.) Osbeck *Citrus* spp.	Keifer, 1959; Pelekassis, 1962; Hatzinikolis, 1967; Hatzinikolis, 1969a; Hatzinikolis, 1969b; Hatzinikolis, 1970b; Mourikis and Vassilaina – Alexopoulou, 1975; Papaioannou-Souliotis *et al.*, 1994; Malandraki, 2012.
Aculus fockeui (Nalepa and Trouessart)	*Prunus persica* (L.) Batsh, *Prunus dulcis* Miller D.A. Webb *Prunus avium* L., *Malus domestica* Borkh	Hatzinikolis, 1969b; Hatzinikolis, 1970a; Hatzinikolis, 1978; Papaioannou-Souliotis *et al.*, 1994; Savopoulou-Soultani and Koveos, 1993; Malandraki, 2012.
Aculus olearius Castanogli	*Olea europaea* L.	Hatzinikolis and Kolovos,1985; Papaioannou-Souliotis *et al.*, 1994; Papaioannou-Souliotis and Markoyiannaki, 2003; Anagnou-Veroniki *et al.*, 2008
Aculus schlechtentali (Nalepa)	*Malus domestica* Borkh *Malus sylvestris* Mill.	Hatzinikolis, 1978; Papaioannou-Souliotis *et al.*, 1994; Savopoulou-Soultani and Koveos, 1993.
Calepitrimerus baileyi Keifer	*Malus domestica* Borkh, *Pyrus communis* L.	Hatzinikolis, 1978; Papaioannou-Souliotis *et al.*, 1994; Malandraki, 2012.
Calepitrimerus vitis (Nalepa)		Pelekassis, 1962; Bouchelos *et al.*, 1963; Hatzinikolis, 1969b; Hatzinikolis, 1970b; Hatzinikolis, 1970c; Papaioannou-Souliotis *et al.*, 1994
Cecidophyopsis vermiformis (Nalepa)	*Corylus avellana L.*	Koutroubas and Bakoyiannis, 1990
Colomerus vitis (Pagenstecher)	*Vitis vinifera* L.	Issakides, 1935; Pelekassis *et al.*, 1960; Pelekassis, 1962; Bouchelos *et al.*, 1963; Bouchelos *et al.*, 1965; Hatzinikolis, 1967; Hatzinikolis, 1969a; Hatzinikolis, 1969b; Hatzinikolis, 1970b; Mourikis and Vassilaina-Alexopoulou, 1975; Katsoyannos, 1992; Papaioannou-Souliotis *et al.*, 1994; Malandraki, 2012.
Coptophylla lamimani (Keifer)	*Corylus avellana* L.	Hatzinikolis, 1969b; Hatzinikolis, 1970c; Papaioannou-Souliotis *et al.*, 1994
Ditrymacus athiasella Keifer	*Olea europaea* L.	Hatzinikolis, 1969b; Hatzinikolis, 1969c; Emmanouel, 1981; Hatzinikolis, 1984; Hatzinikolis and Kolovos,1985; Katsoyannos, 1992; Papaioannou-Souliotis *et al.*, 1994; Papaioannou-Souliotis and Markoyiannaki, 2003; Tzanakakis, 2003; Malandraki, 2012.
Epitrimerus pyri (Nalepa)	*Pyrus communis* L.	Hatzinikolis, 1978; Papaioannou-Souliotis *et al.*, 1994; Malandraki, 2012.
Eriophyes padi (Nalepa)	*Prunus dulcis* Miller D.A. Webb	Hatzinikolis, 1967; Hatzinikolis, 1969a; Hatzinikolis, 1969b

Species	Hosts	References
Eriophyes pyri (Pagenstecher)	*Pyrus communis* L. *Malus domestica* Borkh	Issakides, 1935; Pelekassis, 1962; Bouchelos *et al*., 1963; Hatzinikolis, 1967; Hatzinikolis, 1969a; Hatzinikolis, 1969b; Hatzinikolis, 1970b; Mourikis and Vassilaina – Alexopoulou, 1975; Papaioannou-Souliotis *et al*., 1994;Malandraki, 2012.
Oxycenus maxwelli (Keifer)	*Olea europaea* L.	Hatzinikolis, 1967; Hatzinikolis, 1969a; Hatzinikolis, 1969b; Hatzinikolis, 1969c; Emmanouel, 1981; Hatzinikolis and Kolovos,1985; Papaioannou-Souliotis *et al*., 1994; Papaioannou-Souliotis and Markoyiannaki, 2003.
Oxycenus niloticus Zacher and Abou-Awad	*Olea europaea* L.	Hatzinikolis and Kolovos,1985
Phyllocoptes abaenus Keifer	*Prunus domestica* L., *Prunus domestica* ssp. *insititia* (L.) C.K.Schneid.	Hatzinikolis, 1978; Papaioannou-Souliotis *et al*., 1994; Malandraki, 2012.
Phyllocoptruta oleivora (Ashmead)	*Citrus limon* (L.) Burm, *Citrus medica* L. *Citrus sinensis* (L.) Obseck	Hatzinikolis, 1970c; Papaioannou-Souliotis *et al*., 1994
Shevtchenkella oleae (Natcheff)	*Olea europaea* L.	Hatzinikolis, 1969b; Hatzinikolis, 1969c; Hatzinikolis and Kolovos,1985.
Tegolophus hassani (Keifer)	*Olea europaea* L.	Hatzinikolis, 1969b; Hatzinikolis, 1969c; Hatzinikolis, 1970d; Papaioannou-Souliotis and Markoyiannaki, 2003.
Diptilomiopidae		
Diptacus gigantorhynchus (Nalepa)	*Prunus domestica* L. *Prunus persica* (L.) Batsh	Hatzinikolis, 1983; Papaioannou-Souliotis *et al*., 1994; Malandraki, 2012.
Rhycaphytoptus ficifoliae Keifer	*Ficus carica* L.	Hatzinikolis, 1982; Papaioannou-Souliotis *et al*., 1994; Malandraki, 2012.
Phytoptidae		
Phytoptus avellanae Nalepa	*Corylus avellana* L.	Pelekassis, 1962; Hatzinikolis, 1967; Hatzinikolis, 1969a; Hatzinikolis, 1969b; Hatzinikolis, 1970b; Papaioannou-Souliotis *et al*., 1994; Malandraki, 2012.

their vigor, sometimes they produce small-sized fruits that drop off (Keifer *et al*., 1982).

The plum rust mite is a frequent pest on peaches in northern Greece and in some cases develops high populations in summer (July to August) mainly in upper young leaves (Savopoulou-Soultani and Koveos, 1993).

***Diptacus gigantorhynchus* (Nalepa) [*Phyllocoptes gigantorhynchus* Nalepa, *Epitrimerus gigantorhynchus* (Nalepa), *Rhyncaphytoptus gigantorynchus* (Nalepa), *Diptilomiopus gigantorhynchus* (Nalepa)]**
Syn: *Diptacus prunorum* (Keifer) [*Diptilomiopus prunorum* Keifer]
Common name: big-beaked plum mite
Damage: Mites are vagrants on under leaf surface causing no apparent symptoms. In some cases leaf discoloration or even defoliation (Carmona, 1973; Bayan, 1988; Schliesske, 1992) may occur. Alford (2007) reported similar leaf symptoms *to A. fockeui*. It is considered of not economic importance.

***Eriophyes padi* (Nalepa) [*Phytoptus padi* Nalepa]**
Syn: *Eriophyes eupadi* (Newkirk) [*Phytoptus eupadi* Newkirk]
Common name: plum leaf gall mite
Damage: It causes prominent, finger shaped, red or dark red galls on the upper surface of plum foliage. The galls are often clustered closely together but cause little or no distortion of leaves (Alford, 2007). It is considered of not economic importance.

***Phyllocoptes abaenus* Keifer**
Damage: It lives on undersides of plum leaves, including ornamentals and frequents basal hairs along midribs, causing not apparent symptoms. It is considered of not economic importance.

Eriophyoids of pome fruits

***Aculus schlechtendali* (Nalepa) [*Phyllocoptes schlechtendali* Nalepa, *Vasates schlechtendali* (Nalepa)]**
Common name: apple rust mite
Damage: This species is a widespread pest of apple worldwide. It causes patchy felt-like malformation and a yellowing of hairs below the leaves, the upper surface of foliage appearing speckly, dull and faded. In heavy infestations, it damages terminal growth; the leaves curl lengthwise and become rusty brown, which gives the tree the appearance of being affected by drought. It also produces fruit russeting. The deutogynes find resting places under lateral buds not far below terminals, or in crevices on old wood. In the spring they move to opening buds, especially during the bloom period (Jeppson *et al*., 1975).

***Calepitrimerus baileyi* Keifer**
Syn: *Calepitrimerus aphrastus* (Keifer) [*Phyllocoptes aphrastus* Keifer]
Damage: This mite causes browning on the underside of apple leaves. It is considered to be of no importance.

***Diptacus gigantorynchus* (Nalepa) [see stone fruits]**
Damage: It is considered to be of no importance.

***Epitrimerus pyri* (Nalepa) [*Tegonotus pyri* Nalepa]**
Syn: *Epitrimerus pirifoliae* Keifer
Common name: pear rust mite
Damage: This mite attacks both pear leaves and fruit in the spring. High infestations cause severe browning of leaves and russeting of fruit. Lower populations may injure only the calyx end of fruit.

Clear-skinned fruit varieties show the most injury. In Greece the pear varieties "Kontoula" and "Krystalli" show great susceptibility to the infestation causing reduction of the product quality with economic impact (Papaioannou-Souliotis, 2001).

***Eriophyes pyri* (Pagenstecher) [*Phytoptus pyri* Pagenstecher]**
Common name: pear leaf blister mite
Damage: It causes small galls, green at the beginning of the infestation, that gradually turn to reddish and finally to dark brown. At first, the galls are along sides of the main

vein but as attack develops they cover most of leaf surface. Badly infested leaves die and fall off. It also damages the fruitlets and fruit stalks, which may drop prematurely.

Eriophyoids of Citrus

Aceria sheldoni (Ewing) [*Eriophyes sheldoni* Ewing]

Common name: citrus bud mite

Damage: The citrus bud mite feeds within the buds causing variable symptoms such as distortion of shoot growth, excessive and grotesque deformation of fruit, foliage, and blossoms, discoloration of fruit, and more commonly the production of numerous buds. The last may develop abortive twigs in tight clusters resembling "witches broom" and bunged terminal growth of distorted stems and leaves. Most malformed fruits drop prematurely. Mature lemon fruits show blackened areas on the rind beneath the sepals (buttons), where large colonies of mites are concealed. Deformed leaves and blossoms have various shapes; the leaf plates are constricted at their middle, curled, twisted, divided and divergent at the tips; the blossoms are stunted and abnormal. The symptoms of injury on oranges are similar to those on lemons except fruit deformation is not so grotesque. Affected oranges usually develop to maturity, but they are commonly flattened, resembling the shape of tomatoes; or they are skinfolds, seams and ridges, or small apertures in the stylar end.

Aceria sheldoni has been found in all Greek citrus-growing regions, causing damage mainly in lemons, which can be significant only during years with high population levels (Papaioannou-Souliotis, 1985; Papaioannou-Souliotis *et al*., 1999).

Aculops pelekassi (Keifer) [*Aculus pelekassi* Keifer, *Vasates pelekassi* (Keifer)]

Common name: pink citrus rust mite

Damage: Pink rust mites not only cause russeting of fruit and leaves, but also mild to severe distortion of new growth, brown lesions on lower surfaces and along midribs of immature leaves, and may produce mesophyll collapse, chlorosis, and leaf drop. It is potentially capable of causing more damage to its host than citrus rust mite *Phyllocoptruta oleivora* (Jeppson *et al*., 1975).

Aculops pelekassi was first found in Greece in 1958 and since then its presence is frequent all over the country (Papaioannou-Souliotis, 1985; Papaioannou-Souliotis *et al*., 1994). It is active during mild winters and can develop more than five generations per year. In population outbreaks it can cause up to 60% loss of yield (Papaioannou-Souliotis, 1985).

Phyllocoptruta oleivora (Ashmead)

Common name: citrus rust mite

Damage: Its feeding destroys the epidermal cells of the rind, producing silvering or russet effects. The ring of the affected fruit is thicker than normal, and the fruit tends to be smaller. Another result of the infestations on lemons and grapefruit is a condition known as "shark skin", in which the outer layer of the skin can be peeled. Heavy populations of mites feeding on leaves and twigs can also cause bronzing.

Greek citrus orchards are mainly infested by *A. pelekassi* and *A. sheldoni*, which can cause serious damage on fruit production when outbreaks of their population occur (Papaioannou-Souliotis, 1985, 1991, 1996; Papaioannou-Souliotis *et al*., 1992). *P. oleivora* only occur in limited part of orchards (Papaioannou-Souliotis *et al*., 1994).

Eriophyoids of Nut trees

Aceria erineus (Nalepa) [*Phytoptus tristriatus* var. *erineus* Nalepa, *Eriophyes tristriatus* var. *erineus* (Nalepa), *Eriophyes erineus* (Nalepa)]

Common name: persian walnut erineum mite

Damage: The infestation caused by this mite is most noticeable as shiny convex swellings on the upper surface of the leaf blade and on the underside as patches of shallow, large, solitary concavities lined with felty, yellowish hairs, among which the mites are found. These patches have well defined edges and lie to the side of the midrib between the lateral veins; the erineum growths miss the small secondary veins and appear as thickened partitions. The erineum is particularly characteristic in that each structure is covered with short, minute, unicellular hairs. Although erineum patches are fewer on the leaves, they are easily recognized because of their size and color.

***Aceria tristriatus* (Nalepa) [*Phytoptus tristriatus* Nalepa, *Eriophyes tristriatus* (Nalepa)]**

Common name: persian walnut leaf gall/ blister mite

Damage: It infests leaves, preferably young and produces small, brown, hard pustules that are about 1¼ mm in diameter. The mites place these galls along midribs and larger lateral veins, but in heavy infestations blisters occur elsewhere. Badly galled leaves are twisted and misshapen (Castagnioli and Oldfield, 1996).

***Cecidophyopsis vermiformis* (Nalepa) [*Phytoptus vermiformis* Nalepa, *Cecidophyes vermiformis* (Nalepa), *Eriophyes vermiformis* (Nalepa)]**

Damage: It is found on common hazel tree usually in association with *Phytoptus avellanae*. It is considered of no economic importance.

***Coptophylla lamimani* (Keifer) [*Phyllocoptes lamimani* Keifer]**

Damage: It lives as leaf vagrant on underside of hazel nut leaves causing no evident symptoms. It is considered of no economic importance.

***Phytoptus avellanae* Nalepa [*Phytocoptella avellanae* (Nalepa)]**

Syn: *Acarus pseudogalarum* Vallot [*Phytoptus pseudogalarum* (Vallot)]
Phytoptus coryli Frauenfeld
Phytoptus coryligallorum Targioti-Tozzeti [*Eriophyes coryligallorum* (Targioti-Tozzeti)]

Common name: filbert bud mite

Damage: This species is considered as a minor pest of cultivated hazelnut. It produces bud galls, known as big buds, consisting of an aggregation of swollen, thickened scale leaves, often containing hundreds of mites. Their feeding activities suppress the developing young leaves or inflorescences closed within the scales. Eventually the enlarged buds become dark and reddish brown as the immature.

***Aceria pistaciae* (Nalepa) [*Eriophyes pistaciae* Nalepa]**

Common name: pistachio bud mite

Damage: Lives on pistachio and turpentine trees causing flower stalk brooming and some leaf deformation on certain pistachio species. The brooms are reddish and noticeable.

Eriophyoids of Olive tree

***Aceria cretica* Hantzinikolis**

Damage: The mite is found on the under leaf surfaces causing subcircular patches (Hatzinikolis, 1989). It is a species that is reported only from Crete Island.

***Aceria olea* (Nalepa) [*Eriophyes oleae* Nalepa, *Phytoptus oleae* (Nalepa)]**

Common name: olive bud mite

Damage: This mite is a pest of all varieties of olive in the Mediterranean area and is especially injurious to young trees or the trees that have pollarded. It causes leaf and fruit deformation, and seriously reduces the amount and quality of olives available for pickling. As the result of mite infestations and due to the distraction of the normal silvery stellate hairs, mature leaves may show subcircular, irregular greenish patches that turn brown as necrosis progresses. These patches may bulge out as small chlorotic areas above the general leaf surface, giving the leaf an embossed appearance. In heavy infestations, the mites extend their feeding to leaf margins, which results in deformations. Damage on young fruit first appears as silvering, then browning and ends in fruit deformation. Mites congregate in large numbers at the stem end of the fruit, and find shelter under sepal rudiments. Most damage to fruit is confined to the stem.

Fruits that are fully developed before the mites become abundant do not show much damage. The species overwinters under the stellate hairs of the leaves and migrates early in spring in the flowers where it stays until the fruit is formed.

In Greece, this mite appears very frequently in high population densities mainly in regions with mild winters and humid summers. In many cases it is found associated with *A. benaki*, *D. athiasella*, *T. hassani* (Hatzinikolis and Kolovos, 1985), and it is considered an occasionally serious pest.

Aceria olivi **(Zaher and Abou-Awad) [*Eriophyes olivi* Zaher and Abou-Awad]**
Damage: The mite forms characteristic concave patches on the undersides of the leaves, and may cause malformation to the succulent terminal leaves (Zaher and Abou-Awad, 1979). It is not considered of importance in Greece as its distribution is quite limited (Hatzinikolis and Kolovos, 1985).

Aculops benakii **(Hatzinikolis) [*Aculus benakii* Hatzinikolis]**
Common name: olive yellow spot mite
Damage: It lives on the underside of olive leaves under the stellate hairs. As a result the stellate structures drop off, making yellow leaf spots.

In Greece it has been found mainly in coastal areas, with mild winters and relatively cool and humid summers. It attacks leaf and flower buds, flower and young fruits. It is of great economic importance in olive growing locations in Western Greece, Crete and Lesvos (Hatzinikolis and Kolovos, 1985).

Aculus olearius **Castagnoli**
Damage: It is found only in the inflorescences from the emergence of the flower buds to the setting of the fruit. It causes the browning and withering of the flower and small fruits. It is not considered of importance in Greece as its distribution is quite limited (Papaioannou-Souliotis *et al*., 1994).

Ditrymacus athiasella **Keifer**
Damage: It produces some leaf pitting, deformation and discoloration. It is usually found on the upper surface of young leaves, the flowering buds and small fruits (Hatzinikolis, 1982) and for a short period of time on flowers (Castagnoli and Papaioannou-Souliotis, 1982) and older leaves (Castagnoli and Pegazzano, 1986). It usually coexists with the other eriophyids and it is difficult to estimate the damage caused by this single species. Hatzinikolis (1982) reported deformation of leaves and flower and young fruit dropping due to the bud infestations.

Ditrymacus athiasella attacks on the buds causing malformed leaves and inflorescences that fall off before full development. On the leaves, mite attack is evident from the appearance of yellow-white spots on their upper surface which correspond with swellings on the lower surface. Attacks on the flowers result in drying and fall of flowers together with the secondary axes of inflorescences. Infestation of the fruits takes place only during the first stages of development, leading to premature drop of the fruits (Hantzinikolis, 1984). It has caused economic problems in Argolis and Arcadia (Hatzinikolis, 1991) and reported to occur in great population densities causing economic damage in the olive oil producing areas of Peloponnese and central Greece.

Oxycenus maxwelli **(Keifer) [*Oxypleurites maxwelli* Keifer]**
Common name: olive leaf and flower mite
Damage: *O. maxwelli* feeds preferentially on the upper surface of terminal leaves, but in high infestations it also feeds on the lower leaf surface, buds, new shoots, flowers and stems (Jeppson *et al*., 1975). Heavy infestations may cause premature flower drop as well as leaf spotted discoloration and distortion. High infestation of the mite on young leaves can cause silvering and distortion, which reduces light absorption and decreases photosynthesis. Another problem attributed to infestations by *O. maxwelli* is the reduction in internodal length, leading to the formation of overbudding (bunchtop). In young plants, bud infestation can lead to deficient plant growth (Castagno-

li and Oldfield, 1996). Although *O. maxwelli* is frequently present in Greek olive groves, Hatzinikolis and Kolovos (1985) reported that it was found in small populations and that is not a pest of economic importance for Greece.

***Oxycenus niloticus* Zaher and Abou-Awad**
Damage: The mite infests leaves preferring the upper surface around mid-vein (Zaher and Abou-Awad, 1979). It forms characteristic concave patches on the underside of leaf and may cause deformation to the succulent terminal leaves. Its distribution in Greece is limited (Hatzinikolis and Kolovos, 1985).

***Shevtchenkella oleae* (Natcheff) [*Tegonotus oleae* Natcheff, *Lovonotus oleae* (Natcheff)]**
Damage: It attacks leaves, stems, buds and inflorescences. It has been found in small populations, and it is considered of no economic importance in Greece.

***Tegolophus hassani* (Keifer) [*Tegonotus hassani* Keifer]**
Common name: olive rust mite
Damage: It lives on both surfaces of olive leaves and apparently causes russeting and some form of leaf deformation or defoliation.

It reaches high population levels in many regions of the country where it is found in association with the other eriophyid species (Hatzinikolis and Kolovos, 1985) and may cause loss of production (Hatzinikolis, 1972).

Eriophyids of fig tree

***Aceria ficus* (Cotte) [*Eriophyes ficus* Cotte]**
Syn: *Eriophyes fici* Ewing
Common name: fig bud mite
Damage: This mite not only injures fig buds, but it also transmits fig mosaic virus, a disease that is present in Greece (Martelli *et al.*, 1993). Feeding by mites that carry no virus produces variable symptoms such as russeting or surface browning, bud blasting, impedance of new growth, bad distortion, leaf chlorosis, and in severe cases the result can be defoliation of branches or of whole trees. These mites make no galls (Keifer *et al.*, 1982).

***Rhyncaphytoptus ficifoliae* Keifer**
Common name: fig leaf mite
Damage: It lives as a vagrant among the under surface leaf hairs, causing no apparent symptoms.

Eriophyoids of pomegranate

***Aceria granati* (Canestrini and Massalongo) [*Phytoptus granati* Canestrini and Massalongo, *Eriophyes granati* (Canestrini and Massalongo)]**
Common name: pomegranate leaf curl mite
Damage: Pomegranate leaf curl mite occurs throughout Mediterranean region. The mite tightly rolls the leaves from the sides down onto the undersurface; these leaves maybe so tightly rolled as to produce a nearly leafless appearance to the twig but the twigs continue to elongate, indicating the twig terminal is not damaged.

Pomegranate culture has become popular during the last decade in Greece. *Aceria granati* was reported to infest the orchards of northern Greece (Drama) (Koveos *et al.*, 2010), however its status as a pest is not yet determined, as the extent of the infested orchards and damages has not been studied.

Eriophyoids of grapevine

***Calepitrimerus vitis* (Nalepa) [*Phytoptes vitis* Nalepa, *Epitrimerus vitis* (Nalepa)]**
Common name: grapevine rust mite
Damage: Heavy infestations of this species prevent vines from growing normally during the earlier parts of the season. Internodes are shortened, foliage becomes bunched, which interferes with proper pruning; grape production is reduced. Damage to grape clusters occurs either because flowers are injured or because development is delayed. The foliage has a browning and russeting aspect. The leaves present malformation followed by a premature dropping. As a result of the shortened internodes and the devel-

opment of additional shoots after the death of the main bud, the vine presents "witches broom" appearance.

***Colomerus vitis* (Pagenstecher) [*Phytoptus vitis* Pagenstecher, *Eriophyes vitis* (Pagenstecher)]**

Syn: *Eriophyes vitis* (Landois) [*Phytoptus vitis* Landois]

Common name: grape bud mite or grape erineum mite

Damage: Three forms of *C. vitis* have been reported to cause different types of injury to grape vines. One form feeds on the leaves and causes the appearance of patches of felty erineum on the lower surface, followed by blister-like swellings on the upper surface. The erineum patches are whitish at first, then yellow and finally reddish brown. At times they are abundant in early spring in commercial vineyards or throughout the season on abandoned and backyard vines. Another form of *C. vitis* attacks grape buds, causing deformation of the primordial bud clusters, distortion of the basal leaves, stunting of the main growing point, and often death of the overwintering buds. This form does not produce erineum on the leaves. The third form produces leaf curl and abnormal plant hairs at the colonies sites.

Natural enemies

Much of the ongoing research aiming at controlling eriophyoid mites in the last decade has been focused on biological control with the use or conservation of predatory mites (van Leeuwen *et al.*, 2010).

Predators of the eriophyoid mites include insects (Chalcidoidea, Thysanoptera) and predaceous mites of Phytoseiidae, Stigmaeidae and Anystidae (Jeppson *et al.*, 1975; Sabelis, 1996). The importance of the predatory phytoseiid and stigmaeid mites for the control of eriophyoid mite populations has been well documented by several authors (Abou-Awad and El-Banhawy 1986; Amano and Chant 1986; Abou-Awad *et al.*, 1998; Abou-Awad *et al.*, 2005).

Among the phytoseiid species that use mostly eriophyoid mites as a food source are *Iphiseius degenerans* (Berlese), *Euseius finlandicus* (Oudemans), *Euseius stipulatus* (Athias-Henriot), *Kampimodromus aberrans* (Oudemans), *Amblyseius andersoni* (Chant), *Typhlodromus* (*Typhlodromus*) *pyri* Scheuten, *Typhlodromus* (*Typhlodromus*) *exhilaratus* Ragusa, *Typhlodromus* (*Typhlodromus*) *athiasae* Porath and Swirski, *Paraseiulus talbii* (Athias-Henriot) and species of the genus *Neoseiulus* (Sabelis, 1996; McMurtry and Croft, 1997; Kreiter and Tixier, 2010). Also, stigmaeid mites, *Zetzellia mali* Ewing and *Agistemus* spp. are well-known predators of eriophyoid mites (Abou-Awad *et al.*, 1998; Childers *et al.*, 2001; Gerson *et al.*, 2003; Duso *et al.*, 2008).

In Greece, many phytoseiid species are found in fruit orchards and vineyards. Among the phytoseids recorded in stone fruits, *E. finlandicus*, *E. stipulatus*, *A. andersoni* and *K. aberrans* are the more frequent and abundant. The stigmaeid predator *Z. mali* is also very frequent (Papaioannou-Souliotis *et al.*, 1994). Papanikolaou and Bakoyiannis (1991) reported a hymenopteran larva (unidentified species, probably belonging to the family Eulophidae) associated with galls of *A. phloeocoptes*, which showed very low predation.

Typhlodromus pyri, *A. andersoni* and *E. finlandicus* are frequent in apple orchards (Papaioannou-Souliotis *et al.*, 1994; Markoyiannaki-Printziou *et al.*, 2000; Papadoulis *et al.*, 2009) and may play a major role in keeping apple rust mite populations below economic damage levels (Easterbrook 1996; Duso and Pasini 2003; Fitzgerald *et al.*, 2003).

Phytoseiid predatory species found in citrus orchards in Greece include *E. stipulatus*, *Euseius scutalis* (Athias-Henriot), *Typhlodromus* (*Anthoseius*) *athenas* Swirski and Ragusa, *T.* (*T.*) *athiasae*, *P. talbii*, *A. andersoni* and *I. degenerans* (Papadoulis *et al.*, 2009). *Euseius stipulatus* is the main phytoseiid predator holding 80% of the phytoseiid population in citrus groves (Papaioannou-Souliotis, 1991). Generalist predators such as *E. stipulatus* can control the phytophagous mite populations at low densities (McMurtry *et al.*, 1992).

Ozman Sullivan (2006) evaluated the biology of the phytoseiid *K. aberrans*, a possible predator of the big bud mite *P. avellanae*, which is a common pest in Greek hazelnut orchards, and concluded that *K. aberrans* can play an important role in IPM programmes to control *P. avellanae* when it is released in early spring to boost the population before *P. avellanae* migration.

In olive groves, predatory species of Phytoseiidae comprise *A. andersoni*, *T.* (*A.*) *athenas*, *Typhlodromus* (*Anthoseius*) *foenilis* Oudemans, *Typhlodromus* (*Typhlodromus*) *cotoneastri* Wainstein and *K. aberrans* (Papaioannou-Souliotis *et al.*, 1994; Papadoulis *et al.*, 2009). *Euseius stipulatus*, *K. aberrans*, *E. finlandicus* and *Phytoseius plumifer* (Canestrini and Fanzago) are the most common species found on fig trees (Papaioannou-Souliotis *et al.*, 1994).

On pomegranate, the following phytoseiid species have been reported *T.* (*A.*) *athenas*, *Typhlodromus* (*Anthoseius*) *psyllakisi* Swirski and Ragusa, *T.* (*T.*) *athiasae* (Papadoulis *et al.*, 2009). Koveos *et al.* (2010) found an unidentified stigmaeid mite in association with *A. granati* on pomegranate.

On grapevine, the most common and abundant species of Phytoseiidae are *K. aberrans*, *E. finlandicus*, *P. plumifer*, *T.* (*A.*) *athenas*, *T.* (*T.*) *exhilaratus* and *P. talbii*, (Soulioti *et al.*, 1998; Papadoulis *et al.*, 2009). The stigmaeid mites *Zetzellia graeciana* Gonzalez and *Z. mali* have also been reported (Papaioannou-Souliotis *et al.*, 1994).

Monitoring

Monitoring eriophioid mites is a difficult task due to their minute size (average 100 μ) and concealed way of life. The presence of eriophyoids is usually transpicuous when the symptoms become apparent. In the case of rust mites, monitoring involves collection of leaves or fruits and counting the number of mites. To assess populations, leaves should be examined with a hand lens with at least 10x magnification. Although it is impractical to obtain accurate population counts with this method, if many individuals are noticed, more intensive sampling should be considered, ideally with a dissecting microscope. Hall *et al.* (2005, 2007) investigated the effects of reducing the sample size on the accuracy of estimation of citrus rust mite densities in oranges and proposed a binomial sampling based on the proportion of eriophyoid infested samples.

Chemical Control

In general, eriophyoid mites prove to be fairly susceptible to the most commonly used acaricides, as was demonstrated by Childers *et al.* (1996) who made a thorough review of the chemical control of eriophyoids. Since then there have been changes to registered acaricides mostly in Europe, and most of the substances tested are no longer in use. However, a rather limited amount of reports has investigated the suitability of modern crop protection compounds for controlling rust, gall, blister and bud mites. Moreover, these reports are mainly restricted to a number of major crops like citrus and apple orchards and major pests as *P. oleivora* and *A. schlechtendali*, respectively. The main reason for the lack of information on the toxicity and other aspects of new compounds can be probably brought back to the lower economic importance of these mites, in comparison to other mite pests such as the spider mites (Acari: Tetranychidae).

In Greece, there are no thresholds for the damage caused by eriophyoid mites. The usual practice is acaricide or sulfur treatment when the infestation is evident to affect the trees' vigor or yield. Registered acaricides (active substances) for control of mites in orchards and grapevine are given in Table 2.

The usual practice for the management of *A. phloeocoptes* and *A. fokeui* in stone fruit orchards is application of selective acaricides. For *A. phloeocoptes* application time is in early spring (March) at the opening of the buds when the mites are migrating to new buds while for *A. fokeui* it is in summer (Pa-

Table 2. Registered acaricides of tree orchards and grapevine in Greece (Authorized Plant Protection Products Data Base, Hellenic Ministry of Rural Development and Food, 2012) (http://wwww.minagric.gr/syspest/syspest_ENEMY_crops.aspx).

Crops	Active substance of registered acaricides
Stone fruits	
Plum	paraffin oil, clofentezine
	fatty acid potassium salt, paraffin oil, etoxazole and clofentezine
Almond	fatty acid potassium salt, paraffin oil, acequinocil, clofentezine, etoxazole, hexythiazox, spirodiclofen and pyridaben
Peach	paraffin oil
Cherry	paraffin oil, fatty acid potassium salt, clofentezine ,hexythiazox, tebufenpyrad, etoxazole, acequinocyl
***Citrus* spp.**	paraffin oil, fatty acid potassium salt, clofentezine, diflubenzuron, etoxazole, fenpyroximate, tebufenpyrad, pyridaben, milbemectin, acequinocyl
Olive	paraffin oil
Nuts	
Walnut	fatty acid potassium salt
Pistachio	none
Hazelnut	fatty acid potassium salt
Fig tree	none
Pomegranate	none
Grapevine	paraffin oil, fatty acid potassium salt, sulphur, copper oxychloride, hexythiazox

paioannou-Souliotis, 2001).

The recommended practice for infestations of apple rust mite is pre-blossom treatment with paraffin oil and/or summer spraying with acaricides (Papaioanou-Soulioti, 2001). No threshold limit is established in Greece for *A. schlechtendali*. Ontario Ministry of Agriculture, Food and Rural Affairs reports a threshold on apple rust mite population, which is 200-500 mites per leaf (Solymar and Walker, 2011). In lower population, application must be avoided because the apple rust mite provides valuable prey for predatory mites.

In citrus groves in Greece, *A. pelekassi* and *A. sheldoni* may develop high populations when weather conditions are favorable. In the case of high infestation of *A. pelekassi* and *A. sheldoni* in citrus orchards, selective acaricides should be applied against *A. pelekassi* in summer (beginning of June) and autumn (mid-September to mid-November) and against *A. sheldoni* in spring and at the beginning of June (Papaioannou-Souliotis, 1985).

In olive groves when outbreaks of eriophyoids occur under favorable weather conditions, two applications with sulfur or paraffin oil are recommended, the first early in spring before flowering and the second 10-15 days later (Broumas and Katsoyiannos, 2009).

In grapevine, it is well demonstrated that sulfur sprayings applied early in the spring can control mite populations e.g. sulfur sprayings applied at the onset of the wooly bud stage when mites become active, followed by a second spraying approximately 10 days later when late-developing buds open. Papaioannou-Souliotis *et al.* (1998) investigated the effect of some commonly used fungicides and insecticides on phytoseiid populations in vineyards in four regions of Greece.

There are no registered acaricides in Greece for fig and pomegranate cultivations. The cultivation of fig tree is traditional in southern parts of mainland Greece and the Aegean islands, yet yield loss due eriophyoid infestation has been reported (Vachamidis and Vemmos, 2010). Pomegranate culture has become popular during the last decade in Greece. *Aceria granati* has been reported to infest cultivations in northern Greece recently (Koveos *et al.*, 2010) but the possible effect on yield is not yet determined.

Discussion

The economic importance of eriophyoid mites has been estimated in some countries but the variability of environmental conditions, cultural practices, cultivar features and market standards make a generalisation based on these studies difficult. A number of eriophyoid species emerged as economically important and their pest status has been reconfirmed recently, mainly in crops like citrus, apples, grapes, hazelnuts, coconuts and tomatoes (Van Leeuwen *et al.*, 2010). In Greece, the economic importance of eriophyoid mites as pests of orchards and grapevine has not been much exploited. Most of them are considered occasional pests whereas outbreaks often occur after warm winters and springs with high rainfalls. The change in control strategies mainly towards the use of fungicides lacking acaricidal activity and of insecticides having a detrimental effect on predatory mites might cause outbreaks of rust mites and result in the permanent pest status of the species (Easterbrook, 1996; Croft and Slone 1998). In addition, the climate change might be important of future outbreaks of the eriophyoid mite pests.

In general, eriophyoid mites are susceptible to most commonly used acaricides, some insecticides and fungicides (especially sulphur). The European Union review programme of the existing active substances under the Directive 91/414/EEC, resulted in the reduction of the available substances with acaricidal effect. The lack of registered acaricides for some cultivations such as pomegranate in Greece, may cause a problem in the future.

The main problem with the control of eriophyoids is getting the compounds in contact with the mites due to hidden lifestyle of a number of important species. Mites hiding in galls, blisters and buds are not easily accessible. In these cases, an accurate timing of the applications is crucial, in order to reach the life stages that (temporarily) leave the hiding places, and can, only at those times, be reached with pesticides. That is why, in most cases, control is directed against the adults which are searching for spots to induce their hiding places for the immature life stages (gall mites) or for existing shelters (bud mites) and for a limited time, few days or weeks. Hence, control is best succeeded with acaricides providing long residual activity. On the other hand, rust mites have a more superficial lifestyle on the underside of leaves, leaving them exposed throughout their life cycle and resulting in easier control. In cropping systems where eriophyoid mites cause economic damage, such as apple and citrus orchards, and Tetranychidae are also main pests, application timing and product choice should reflect concerns on the economic damage of both species. (Van Leeuwen *et al.*, 2010).

The conservation of indigenous natural enemies for controlling eriophyoid mites is gaining more attention in the last decade (Smith and Papacek, 1991). Morover, the search for exotic natural enemies and release remains an option, in cases that local predators fail or are less successful in controlling these mite pests (Argov *et al.*, 2002).

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ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

Ακάρεα της υπεροικογένειας Eriophyoidea σε δενδρώδεις καλλιέργειες και αμπέλι στην Ελλάδα

Ε.Β. Καπαξίδη

Περίληψη Τα ακάρεα της υπεροικογένειας Eriophyoidea απαντώνται σε όλες τις δενδρώδεις καλλιέργειες και αμπελώνες της Ελλάδας. Είναι εξειδικευμένα παράσιτα φυτών και κάποια είδη μπορεί να προκαλέσουν ζημιές στην παραγωγή. Τα συμπτώματα που προκαλούν ποικίλουν από παραμορφώσεις φύλλων, βλαστών ή καρπών μέχρι και μεταχρωματισμούς. Μέχρι σήμερα τριάντα τρία είδη έχουν αναφερθεί στις δενδρώδεις καλλιέργειες και το αμπέλι στην Ελλάδα, τα οποία ανήκουν στις οικογένειες Eriophyidae, Diptilomiopidae και Phytoptidae. Στην παρούσα εργασία δίδονται στοιχεία για τους ξενιστές, την συμπτωματολογία, τους φυσικούς εχθρούς και την αντιμετώπισή τους ως εχθρούς των Ελληνικών οπωρώνων και αμπέλου.

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Ability of nitrogen containing salts to control the root-knot nematode (*Meloidogyne javanica*) on tomato

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Summary The influence of different nitrogen containing salts and sodium chloride at gradual electrical conductivity levels (ECs 2, 4, 6 and 8 mS/ cm) on the root-knot nematode (*Meloidogyne javanica*) and their interaction with tomato was evaluated under growth chamber and greenhouse conditions. Both ammonium chloride (NH_4Cl) and ammonium sulfate ($(NH_4)_2SO_4$) were more effective than ammonium nitrate (NH_4NO_3) which was more effective than potassium nitrate (KNO_3) and sodium chloride (NaCl) in suppressing *M. javanica* by reducing root galling and nematode reproduction on tomato cv. GS12. Under greenhouse conditions, the minimum significant galling index values assessed for NH_4Cl, $(NH_4)_2SO_4$, NH_4NO_3 and KNO_3 were 1.60, 2.04, 2.30 and 3.30, respectively whereas the maximum value (4.01) corresponded to NaCl and was not statistically different from the control (4.92). A significant increase in tomato growth and protein content for $(NH_4)_2SO_4$ and NH_4NO_3 was observed. On the other hand, in NaCl treatment, there was a decrease in dry weights and protein content due to salinity compared with the control. The higher salt ECs did not affect the pH of the rhizospheric soil but slightly increased its measured EC and salinity. Hence, $(NH_4)_2SO_4$ is a more suitable candidate than NH_4Cl for the effective control of the root-knot nematode when irrigation water is a limiting factor with high salinity level similar to NaCl. Therefore, the use of ammonium containing salts especially $(NH_4)_2SO_4$ and NH_4Cl alone or in combination with other control measures may result in controlling *M. javanica*.

Additional keywords: fertilizers, management, *Solanum lycopersicum*, suppression

Introduction

Root-knot nematodes (*Meloidogyne* Goldi 1892 - RKN) are obligate parasites of higher plants distributed worldwide and considered major nematode pests, causing great crop losses annually (Sasser, 1987; Sasser and Freckman, 1987; Nickle, 1991) and reduction of product quality on almost every plant species (Anastasiadis *et al*., 2011).

Tenuta and Ferris (2004) reported that *Meloidogyne javanica* (Treub) Chitw. reared on solid medium and in hydroponic culture, were slightly more sensitive to specific ion and osmotic effects than nematodes of similar colonizer-persister groups obtained from soil. Also, gradients of salts of the specific ion repellents (NH_4^+, K^+, Cl^-, and NO_3^-) for *M. incognita* have been demonstrated to shield tomato (*Solanum lycopersicum* Mill.) roots from root knot nematode infection in soil (Marks and Sayre, 1964; Edongali and Ferris, 1982; Ismail and Saxena, 1977; Castro *et al*., 1991), furthermore, NH_4^+, K^+ and NO_3^- have been recognized as beneficial for plant growth (Castro *et al*., 1991). Root galling and *M. incognita* reproduction efficiency increased in tomato plants exposed to ammonia at 76μg/m^3, while treatments with higher concentration (152μg/m^3) caused nematode suppression (Khan and Khan, 1995). Additionally, urea and ammonium sulfate at rates of more than 250mg N/kg soil resulted in suppression of *M. incognita* population on tomato plants (D'Addabbo *et al*., 1996).

Stimulation or depression effects of some salts on various plant physiological functions are already known, especially when combined with other stressful agents such as nematodes (Edongali and Ferris, 1982).

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The objectives of this study were to investigate the effects of five salts at four electrical conductivity (EC) levels (2, 4, 6 and 8 mS/ cm) on the control of the root nematode *M. javanica*. The test salts comprised of four nitrogen containing salts; ammonium chloride (NH_4Cl), ammonium nitrate (NH_4NO_3), potassium nitrate (KNO_3) and ammonium sulfate ($(NH_4)_2SO_4$), and sodium chloride (NaCl). The interaction of the effects of the salts, the EC levels and the root-knot nematode on the susceptibility and growth of tomato were also assessed under growth chamber and greenhouse conditions.

Materials and Methods

Analytical reagent grade of five salts, NH_4Cl, NH_4NO_3, KNO_3, NaCl and $(NH_4)_2SO_4$, was used. Electrical conductivity for each salt solution used was 2 (EC2), 4 (EC4), 6 (EC6) and 8 (EC8) mS/ cm and was achieved by dissolving 1.07, 2.14, 3.21 and 4.28 g/l NH_4Cl, 1.60, 3.20, 4.80 and 6.39 g/l NH_4NO_3, 2.02, 4.04, 6.06 and 8.08 g/l KNO_3, 1.17, 2.34, 3.50 and 4.67 g/l NaCl or 2.63, 5.28, 7.92 and 10.55 g/l $(NH_4)_2SO_4$, respectively, in water.

A field population of *M. javanica* previously collected from a cucumber field at Ein-Sarah region in Karak Province of Jordan and extracted in the laboratory was used for this study. The nematode population was morphologically identified and molecularly characterized using the sequence characterized amplified region-polymerase chain reaction (SCAR-PCR) test (Karajeh, 2004).

Infectivity test

Two-week-old seedlings (ca. 5 cm tall) of *M. javanica*-susceptible tomato cv. GS12 were transplanted into 100 ml plastic pots (one seedling/ pot), filled with a sterilized mixture of 1:1:1 peat: sand: perlite. For each treatment, one thousand 2nd stage juveniles (J2s) *of M. javanica* were picked and transferred into a small Petri dish containing 10 ml of each solution at each EC or sterile tap water (served as control). The juveniles were two-days-old and hatched from eggs which were surface-sterilized with 0.5% NaOCl.

All Petri dishes were kept at room temperature for one hour before inoculating the tomato seedlings through pouring the solutions into the rhizospheric region of each seedling one week after transplanting. Each treatment was replicated five times. The plants were regularly irrigated with water. The treated and control plants were arranged according to a completely randomized design (CRD) and maintained in a growth chamber at 25°C and 16/8 hour light/dark regime.

Six weeks after inoculation, the plants were up-rooted and the galling index evaluated according to the 0-5 scale: 0=no galling, 1=1-2 galls, 2=3-10, 3=11-30, 4=31-100 and 5=over 100 galls (Taylor and Sasser, 1978). Egg-masses were picked from the roots, extracted with a 0.5% NaOCl solution for 30 seconds (Hussey and Barker, 1973) and quantified under a compound microscope at 10X magnification level. Nematode reproduction factor (RF) was calculated as the number of eggs per plant (Pf) divided by the initial J2 inoculum number (Pi).

Greenhouse experiment

To determine the effects and interactions of *M. javanica* with variable levels of electrical conductivity of the five salts on the growth of tomato in greenhouse conditions, one-month old seedlings (about 15cm tall) of tomato cv. GS12 were tested. The seedlings were planted in 5dm^3 pots filled with 1.5 kg of a 1:2 mixture of water-washed sand and a non-sodic, non-saline sandy loam soil (EC 1.3 mS/ cm, pH 7.1, 0.6% organic matter, 1.1mg/g total nitrogen, and 43% $CaCO_3$) that had been previously sterilized at 85ºC for 5 days. One week after transplanting, the desired level of electrical conductivity (EC2, EC4 and EC8) was achieved and maintained by irrigating the soil with saline solutions to field capacity level for three consecutive weeks. For the control treatments, the same procedure was followed except that tap water replaced the salt solution.

Each treatment was replicated six times (one plant per pot) and each seedling was inoculated with 3000 nematode eggs. Inoculation was performed one day after salt treatment by pouring the egg suspension of the nematode into three holes made in the rhizospheric soil. Non-inoculated plants were used as controls. The plants were transferred into a greenhouse (25 ±5 °C air temperature and 12/12 hour light/dark regime) and maintained without fertilization. All treatments were arranged in a randomized complete block design (RCBD).

At the end of the experiment, sixty days post-inoculation, plants were removed from the pots and the roots were carefully washed to remove soil particles. Fresh and dry weights of plant shoots and roots were recorded. The galling index and nematode reproduction factor were evaluated as previously described. After sieving and grounding of 10g of the rhizospheric soil (oven dried) and dissolving it in distilled water, soil pH, EC and salinity were measured using PCSTestr35 (Eutech Instruments, USA). Representative random samples of oven dried plants were finely grounded and analyzed for protein, potassium and phosphorus content (Lowry *et al*., 1951; Meiwes *et al*., 1984).

Statistical analysis

Data were analyzed statistically using general linear model (GLM) procedure (SPSS software version 11.5; SPSS Inc., Chicago, USA). Significance of main factors and interactions was tested at the 0.05 probability level. Least significance difference (LSD) test was used for mean separation at the 0.05 probability level.

Results

Under controlled growth conditions, all nitrogen containing salts were able to reduce the extent of root galling over the control, with the exception of NaCl with EC levels at 2, 4 and 6 mS/ cm. Both NH_4Cl and $(NH_4)_2SO_4$ were relatively more effective in reducing root galling index to less than 2 as compared to KNO_3 and NH_4NO_3 (Figure 1a) and this reduction was accompanied by a significant reduction in nematode reproduction expressed as lower RF values (Figure 1b).

Results from the greenhouse experiment revealed that nitrogen containing salts caused significant reduction in tomato root galling index, compared to the NaCl treatment and control, and this reduction was clearer at higher EC levels (Table 1). The minimum significant galling index value was observed for the NH_4Cl treatment (1.60), regardless the EC level, followed by the $(NH_4)_2SO_4$ (2.04), NH_4NO_3 (2.30), KNO_3 (3.30), NaCl treatments (4.01) and the control (4.92) (Table 1). A similar pattern was observed in the case of RF values since KNO_3, NaCl and control caused profound nematode reproduction, while the other salts showed a significantly reduced reproduction (Table 1).

There was a significant increase in tomato plant and root dry weights and protein content in the treatments $(NH_4)_2SO_4$ and NH_4NO_3 over the control whereas the treatments NH_4Cl and KNO_3 were not significantly different from the control. Nevertheless, a decrease in dry weight and protein content was observed in the NaCl treatment compared to the control (Table 1). In general, there was a significant reduction in phosphorus plant content in the salt treatments over the control. The highest and most significant potassium content was observed for the KNO_3 treatment (ca. 40g/kg) compared to the other salts and the control. There were no significant pH differences among the treatments. As the EC level increased, there was a gradual increase of soil EC. The highest measured EC was recorded for the NaCl treatment (3.33 mS/ cm) at the highest EC (EC 8) and the lowest for the control (1.84mS/cm). Measured salinity was significantly higher for NaCl and NH_4Cl treatments than for the other treatments. Potassium nitrate did not cause a significant increase in the level of measured salinity over the control (Table 1).

Root galling of tomato plants was affected mainly by the nematode, salt type and the interaction between them and by their interaction with EC (Table 2). The salt effect was significant on plant and root fresh

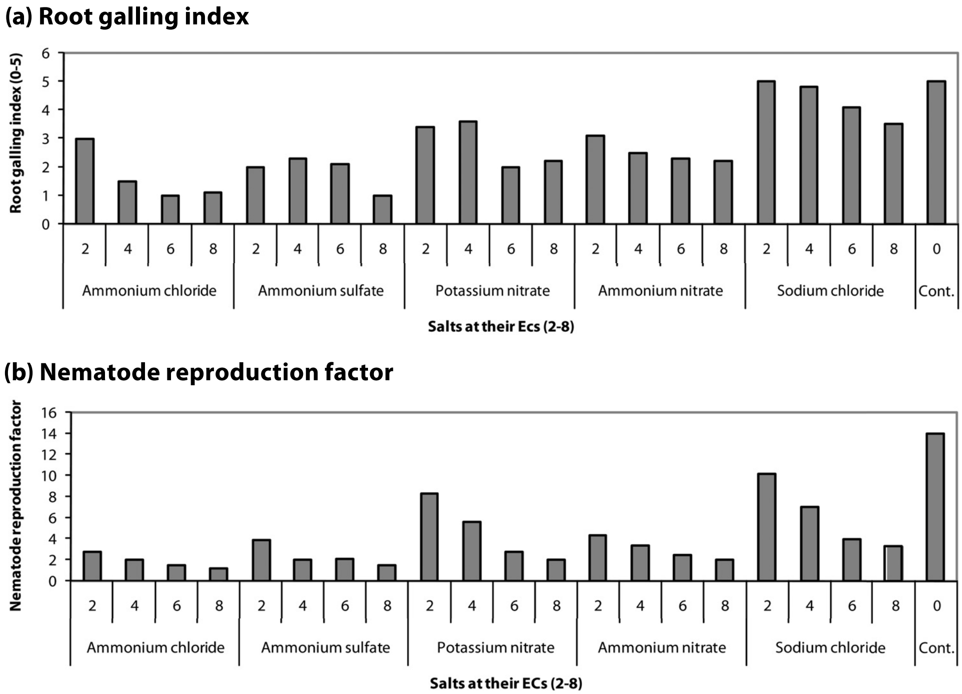

Figure 1. Effect of five Nitrogen containing salts at four levels of electrical conductivity (EC) on (a) root-galling (LSD0.05= 1.58 at P= 0.05) and (b) reproduction factor (LSD0.05= 2.41) of the root-knot nematode, *Meloidogyne javanica*, in tomato under growth incubator conditions.

and dry weights and on the measured EC ($P \leq 0.05$). The presence or absence of nematode was important for root dry weight. Electrical conductivity level, as a main factor, had no significance on the tested parameters (Table 2).

Discussion

Tomato is a high fertilizer-input crop in which the form of nitrogen is particularly important because it influences plant growth (Atherton *et al.*, 1986) and plant response to a range of diseases (Hendrix and Toussoun, 1964; Huber and Watson, 1974). Ammonium chloride was previously reported to be more effective than KNO_3 on tomato (Karajeh and Al-Nasir, 2008) and this result was confirmed in this study where another two ammonium-containing salts ($(NH_4)_2SO_4$ and NH_4NO_3) were additionally tested. Ammonium sulfate was as effective as NH_4Cl but NH_4NO_3 was less effective than both of these salts and more effective than KNO_3 and NaCl in suppressing *M. javanica* by reducing the rate of root galling and nematode reproduction on tomato under laboratory and greenhouse conditions. Akhtar and Mahmood (1994) reported that the addition of $(NH_4)_2SO_4$ (110kg N/ha) reduced the total population of plant-parasitic nematodes as well as root-galling induction by *M. incognita* on tomato, whereas it increased the number of free-living nematodes. Other ammonia-releasing compounds; NH_4OH, $(NH_4)_2HPO_4$ and NH_4HCO_3, were also found effective and showed the greatest nematicidal activity on *M. javanica* at concentrations of 300mgN/kg soil. In a field experiment, the nematicidal efficacy of NH_4OH on tomato plants at doses of 1000 and 2000kgN/ha was equivalent to those of metham sodium in combination with cadusafos (Oka and Pivonia, 2002). Furthermore, the application of ammonium salts had a nematicidal effect against *Pratylenchus penetrans* (Cobb) Filipjev *et* Schuurmans Stekhoven (Walker,

Table 1. Effect of four nitrogen containing salts and NaCl at four levels of electrical conductivity (EC) on the root galling index and reproduction factor of the root-knot nematode, *Meloidogyne javanica*, in tomato pot plants and on plant and soil properties.

Salt		Root-Knot nematode		Plant properties					Soil properties		
Type	EC[1] (mS/ cm)	GI (0-5)	RF	PDW (g)	RDW (g)	P Content (g/kg)	K Content (g/kg)	Protein content (mg/g)	pH (0-14)	EC (mS/ cm)	Salinity (ppt)
NH_4Cl	2	2.31[2] cd[3]	2.21 d	9.14 c	2.19 bc	1.56 d	34.31 c	165.23 a	6.58 a	2.11 d	1.04 c
	4	1.68 d	1.35 d	10.29 b	1.92 bc	1.52 d	34.52 c	56.10 d	6.70 a	2.92 b	1.50 ab
	8	1.60 d	1.31 d	8.29 cd	1.90 bc	1.99 b	37.50 b	91.33 c	6.42 a	3.14 a	1.65 a
$(NH_4)_2SO_4$	2	2.89 cd	2.31 d	11.40 b	2.66 ab	1.86 bc	31.00 d	108.51 bc	6.60 a	1.88 e	0.94 c
	4	2.33 cd	1.84 d	12.61 ab	2.35 b	1.41 d	34.44 c	135.40 b	6.51 a	2.20 d	1.12 c
	8	2.04 d	1.67 d	12.59 ab	1.67 bc	1.24 e	31.63 d	150.33 a	6.48 a	2.63 bc	1.30 b
KNO_3	2	3.85 b	8.40 b	10.97 ab	1.50 a	1.75 c	37.51 b	91.13 c	6.55 a	2.54 c	1.22 bc
	4	3.30 bc	4.35 c	9.74 c	1.73 bc	1.40 d	40.65 a	55.64 d	6.50 a	2.60 bc	1.28 bc
	8	3.45 bc	3.60 cd	9.18 c	1.74 bc	1.88 bc	40.50 a	92.75 c	6.55 a	2.70 bc	1.20 bc
NH_4NO_3	2	3.44 b	4.69 c	12.81 ab	2.21 bc	1.88 bc	30.10 d	134.50 b	6.63 a	2.11 d	1.08 c
	4	2.30 cd	3.58 cd	13.01 a	2.28 b	1.75 c	31.23 d	82.34 cd	6.58 a	2.20 d	1.23 bc
	8	2.70 cd	3.90 c	14.88 a	1.75 bc	1.88 bc	31.14 d	109.47 bc	6.55 a	2.71 bc	1.42 b
NaCl	2	4.86 a	12.71 a	6.05 e	1.58 cd	1.45 d	34.33 c	78.10 c	6.62 a	2.48 c	1.25 b
	4	4.53 a	7.97 b	7.95 de	1.51 cd	1.44 d	37.56 b	91.89 c	6.64 a	2.75 bc	1.54 ab
	8	4.01 ab	4.17 c	9.58 c	1.74 bc	1.56 d	31.22 d	60.13 d	6.53 a	3.33 a	1.77 a
Control	0	4.92 a	13.34 a	8.66 cd	1.54 cd	2.17 b	31.24 d	106.25 bc	6.55 a	1.84 e	0.91 c

[1] EC: electrical conductivity, GI: root galling index, RF= Reproduction factor, PDW: plant dry weight, RDW: root dry weight, P: phosphorus, K: potassium.
[2] Average of 6 replicates per each treatment.
[3] Means within columns followed by the same letters are not significantly different at 0.05 probability level using LSD.

1971). A higher ammonium level in nutrient medium, in the presence or absence of excised roots, decreased the total number of J2s that hatched from dispersed eggs and egg-masses (Sudirman and Webster, 1995). Increasing of ammonium levels reduced the percentage of J2s, which penetrated the roots over time as compared to the control (Sudirman, 1992).

The mode of action of ammonium on the root-knot nematode can be explained by the assumption that higher concentrations of ammonium may have been sufficient to modify malate dehydrogenase activity (Viglierchio, 1979). This would have subsequently a) decreased the energy available for egg hatching and plant invasion processes and/or b) significantly affected the electrical potential around the root tip area where J2s penetrated and thus influenced nematode penetration of the roots through diminished attractiveness of the root tips (Scott and Martin, 1962) and/or c) significantly inhibited giant cell formation and nematode development without affecting root growth (Orion *et al*., 1980; Orion *et al*., 1995).

Increasing the salt EC level from EC2 up to EC8 resulted in decreasing the development of the root-knot nematode on tomato and increasing plant growth. The higher salt EC did not affect the pH of the rhizospheric soil but slightly increased its measured EC and salinity. Hence, $(NH_4)_2SO_4$ is a more suitable candidate for the control of the nematode than NH_4Cl, which showed similar salinity level to NaCl.

In a previous report where the effect of concentration gradient of KNO_3 on horizontal migration of *M. javanica* J2s was studied, the juveniles moved preferentially toward the lower mineral salt concentration region (Prot, 1979). However, in our experiments, high concentrations of nitrate as KNO_3 did not considerably affect nematode infection or reproduction on the host. This is in an agreement with the results of other studies (Marks and Sayre, 1964; Ismail and Saxena, 1977).

Generally, ammonium was more effi-

Table 2.: Main and interaction effects (probability values) of the nematode *Meloidogyne javanica*, salt and electrical conductivity (EC) on root galling, plant and root fresh and dry weights, plant contents of proteins, phosphorus and potassium of tomato and on measured pH, EC and salinity of rhizospheric soil at a pot experiment.

Source	GI[1]	PFW	RFW	PDW	RDW	Protein	P	K	pH	EC	Salinity
Salt	0.028[2]	0.002	0.031	0.003	0.032	0.320	0.123	0.799	0.115	0.001	0.629
Nematode	0.002	0.353	0.718	0.726	0.011	0.084	0.415	0.310	0.062	0.659	0.577
EC	0.171	0.320	0.320	0.295	0.715	0.210	0.251	0.766	0.061	0.276	0.322
Salt x Nematode	0.030	0.284	0.803	0.609	0.081	0.224	0.124	0.104	0.048	0.046	0.892
Salt x EC	0.111	0.310	0.722	0.797	0.894	0.198	0.078	0.233	0.592	0.158	0.530
EC x Nematode	0.245	0.110	0.967	0.706	0.583	0.145	0.141	0.257	0.764	0.057	0.540
Salt x Nematode x EC	0.039	0.317	0.612	0.478	0.101	0.157	0.400	0.147	0.806	0.097	0.288

Experimental analysis based on 6 replicates for NH_4Cl, NH_4NO_3, KNO_3, NaCl, and $(NH_4)_2SO_4$ salts at EC 2, 4 and 8 mS/cm.
[1] GI: root galling index, PDW: plant dry weight, RDW: root dry weight, P: phosphorus, K: potassium.
[2] Probability values ≤ 0.05 are significant.

cient than nitrate. *Meloidogyne incognita* resistant tomato cultivar 'VFN-8' and the moderately susceptible one 'Rutgers' grew better when ammonium nitrate was compared to the control. Ammonium could reduce nematode numbers less than nitrate in the resistant cultivar (Melakeberhan, 1998). The orientation of juveniles of *M. incognita* was induced by the constitutive salt cation e.g. calcium salts had no effect while other salts, especially those with ammonium were strongly nematode repellent (Le Saux and Queneherve, 2002).

Compared with the nitrogen containing salts, NaCl affected tomato growth due its salinity effect, therefore it cannot be used for controlling the disease when irrigation water is a limiting factor.

No phytotoxicity was observed when nitrogen containing salts were applied at all EC levels which complies with previous reports that indicate induced phytotoxicity by some nitrogen containing salts (Rodríguez-Kabana, 1986), although this was considered a dose-dependent issue.

The combination of nitrogen containing salts with other control measures e.g. soil-solarization may increase their efficacy on the control of root-knot nematode (McSorley and McGovern, 2000). The management of *M. incognita* was improved when soil-solarization was combined with ammonium phosphate or composted chicken litter (Gamliel and Stapleton, 1993) while the effect of solarization was not enhanced by the combination with ammonium amendments, except for one instance where application of ammonium bicarbonate or $(NH_4)_2SO_4$ resulted in lower numbers of *Belonolaimus longicaudatus* than in the unamended control (McSorley and McGovern, 2000). Moreover, Oka *et al.*, (2007) reported that soil application of ammonium sulfate in combination with Neem extract significantly reduced root galling caused by *M. javanica*. Therefore, the use of $(NH_4)_2SO_4$ and NH_4Cl alone or in combination with other control measures e.g. organic amendments and/or soil solarization could improve the management of *M. javanica*.

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Αποτελεσματικότητα των ανόργανων αλάτων αζώτου στην αντιμετώπιση του νηματώδη *Meloidogyne javanica* στην τομάτα

M.R. Karajeh και F.M. Al-Nasir

Περίληψη Στην παρούσα εργασία μελετήθηκε η επίδραση διαφόρων ανόργανων αλάτων αζώτου και του χλωριούχου νατρίου σε συνδυασμό με αυξανόμενα επίπεδα ηλεκτρικής αγωγιμότητας (ECs 2, 4, 6 and 8 mmhos/cm) στον νηματώδη *Meloidogyne javanica* και η αλληλεπίδραση αυτών των παραγόντων σε φυτά τομάτας cv. GS12, σε συνθήκες εργαστηρίου και στο θερμοκήπιο. Το χλωριούχο αμμώνιο (NH_4Cl) και το θειικό αμμώνιο [$(NH_4)_2SO_4$] ήταν περισσότερο αποτελεσματικά από το νιτρικό αμμώνιο (NH_4NO_3), το οποίο ήταν πιο δραστικό από το νιτρικό κάλιο (NH_4NO_3) και το χλωριούχο νάτριο (NaCl) στην καταστολή του *M. javanica* προκαλώντας μείωση των όγκων στις ρίζες και στην αναπαραγωγή των νηματωδών στα φυτά της τομάτας. Σε συνθήκες θερμοκηπίου, οι ελάχιστες σημαντικές τιμές του δείκτη παρουσίας όγκων στις ρίζες ήταν 1,60, 2,04, 2,30 και 3,30, στις επεμβάσεις με NH_4Cl, $(NH_4)_2SO_4$, NH_4NO_3 και KNO_3 αντίστοιχα, ενώ η μέγιστη τιμή (4,01) παρατηρήθηκε στο NaCl και δεν διέφερε στατιστικά σημαντικά από το μάρτυρα (4,92). Επιπλέον, παρατηρήθηκε σημαντική αύξηση στην ανάπτυξη των φυτών τομάτας και στην περιεκτικότητα τους σε πρωτεΐνη στις επεμβάσεις με $(NH_4)_2SO_4$ και NH_4NO_3. Αντίθετα, στην επέμβαση με NaCl, το ξηρό βάρος των φυτών και η περιεκτικότητα σε πρωτεΐνη ήταν μειωμένη λόγω της αλατότητας σε σχέση με τον μάρτυρα. Οι υψηλότερες τιμές ηλεκτρικής αγωγιμότητας στα διαλύματα των αλάτων αζώτου δεν επηρέασαν το pH του εδάφους της ριζόσφαιρας αλλά αύξησαν ελαφρώς την ηλεκτρική αγωγιμότητα και την αλατότητά του. Συνεπώς, το $(NH_4)_2SO_4$ είναι καταλληλότερο άλας ανόργανου αζώτου από το NH_4Cl για την αποτελεσματική αντιμετώπιση του νηματώδη *M. javanica* όταν το νερό άρδευσης είναι περιοριστικός παράγοντας με επίπεδα αλατότητας ανάλογα με αυτά του NaCl. Η χρήση αμμωνιακών αλάτων, ειδικά $(NH_4)_2SO_4$ και NH_4Cl, από μόνη της ή σε συνδυασμό με άλλα μέτρα αντιμετώπισης μπορεί να είναι αποτελεσματική στην αντιμετώπιση του νηματώδη *M. javanica*.

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Fumigant toxicity of six essential oils to the immature stages and adults of *Tribolium confusum*

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Summary Six essential oils derived from *Lavandula hybrida*, *Laurus nobilis*, *Thuja orientalis*, *Citrus sinensis*, *Citrus limon*, and *Origanum vulgare* were evaluated in a preliminary fumigation screening test on 10-, 25- and 31-days-old larvae, 2-days-old pupae and 10- and 60-days-old adults of *Tribolium confusum*. Beetles were exposed to essential oils' vapors at a series of concentrations ranging from 0.27 to 165 µl/l of air depending on the essential oil, insect developmental stage, age and sex. All but *O. vulgare* essential oils exhibited strong fumigant toxicity to all developmental stages of *T. confusum*. In general, 10 day-old larvae were the most susceptible and the 25 and 31 day-old larvae were the most tolerant. LC_{50} values ranging between 1.8 and 109 µl/l air depending on the essential oil and insect developmental stage, age and sex. Furthermore, pupae exposed to these essential oil vapours showed various degrees of inhibition of morphogenesis.

Additional keywords: Adultoids, developmental stage, inhibition of morphogenesis, mortality, larvae, pupae

Introduction

The measures used worldwide to control stored product insect infestations rely mainly on the use of fumigants such as methyl bromide and phosphine. However, their usefulness is severely limited by their adverse effects on the environment and non-target organisms (Dansi *et al*., 1984; Fields and White, 2002) and by the development of resistance (Boyer *et al*., 2012). This situation led researchers to develop safe, low-cost alternatives that are convenient to use and environmentally friendly. Among the most promising of these agents are essential oils directly toxic to bacteria, fungi and insects (Isman, 2000; Isman and Machial, 2006). Equally important, essential oils have low mammalian toxicity (Hall and Oser, 1965; Isman, 2000) and degrade rapidly in the environment (Rebenhorst, 1996; Misra and Pavlostathis, 1997).

Apart from their fumigant and acute toxicity against a wide spectrum of insect pests (Huang *et al*., 1997; Shaaya *et al*., 1997; Papachristos *et al*., 2004; Rajendran and Sriranjini, 2008; Michaelakis *et al*. 2009; Wang *et al*., 2009; Michaelakis *et al*., 2011), they can also act as repellents (Jilani *et al*., 1988; Papachristos and Stamopoulos, 2002; Kumar *et al*., 2011; Giatropoulos *et al*., 2012), antifeedants or they can adversely affect the growth rate, reproduction and behaviour of insect pests (Stamopoulos, 1991; Liu and Ho, 1999; Papachristos and Stamopoulos, 2002; Stamopoulos *et al*., 2007; Papachristos and Stamopoulos, 2009; Ebadollahi, 2011).

Insects belonging to the family Tenebrionidae and especially to the genus *Tribolium* are considered major pests of stored products. Both *Tribolium confusum* Jacquelin du Val and *Tribolium castaneum* (Herbst) attack a wide range of starchy materials such as flour, bran or cracked grain kernels, and can occasionally be found in dried fruits, spices and chocolate (Aitken, 1975; Mills and Pedersen, 1990). Moreover, species belong-

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ing to genus *Tribolium* are known to secrete carcinogenic chemicals known as quinones when they occur in large numbers (Hodges et al., 1996).

Although the fumigant action of many essential oil vapours against *T. castaneum* has been studied thoroughly (Kim *et al*., 2010; Liu and Ho, 1999; Mediouni *et al*., 2012; Mohamed and Abdelgaleil, 2008; Mondal and Khalequzzaman, 2010; Rice and Coats, 1994; Safavi and Mobki, 2012), very little relevant information is available on *T. confusum* (Haouas, 2012; Işikber *et al*., 2006; Işikber *et al*., 2009), a very common species in Northern Greece and well adapted at the cold-dry environments of most storehouses (Stamopoulos *et al*., 2007). The two aforementioned species are known to differ in many aspects of their response and susceptibility to xenobiotic compounds (Amos *et al*., 1974; Arthur, 2003). Moreover, essential oils while are generally active against a broad spectrum of stored product pests, interspecific toxicity of individual oils is highly idiosyncratic (Isman, 2000).

The present study was undertaken to investigate the toxicity of *Lavandula hybrida* Rev. (Lamiaceae), *Laurus nobilis* L. (Lauraceae), *Thuja orientalis* L. (Cypressaceae), *Citrus sinensis* Osbeck. (Rutaceae), *Citrus limon* Osbeck. (Rutaceae) and *Origanum vulgare* L. (Lamiaceae) essential oil vapours on *T. confusum* larvae, pupae and adults. The relation between the age, stage and sex of the insect and its vulnerability to these vapours were also studied.

Materials and methods

Biological material

Tribolium confusum larvae, pupae and adults were obtained from laboratory cultures maintained in large glass jars (30 cm height, 8 cm diameter) with wheat flour at 26 ± 1 °C, 65 ± 5% r.h. and a photoperiod of 12 h light, 12 h dark.

Isolation of essential oils

Essential oils were obtained by subjecting plant materials to hydrodistillation using a Clevenger apparatus (Winzer®), from the following plant parts: *L. hybrida* (whole flowering plants), *L. nobilis* (fruits and leaves), *T. orientalis* (fruits), *C. sinensis* (peels from mature fruits), *C. limon* (peels from mature fruits), *O. vulgare* (whole flowering plants). The plants were collected in mid July from the region of Thessaloniki (Northern Greece) except for the fruits of *C. sinensis* and *C. limon*, which were collected in December from the area of Arta (Western Greece). The distilled essential oils were dried over anhydrous sodium sulphate and stored in a freezer at -10°C until use.

Bioassays

In order to test the toxicity of essential oil vapours to the immature stages and adults of *T. confusum*, gastight glass jars of 370 ml volume with metal screw-caps were used as exposure chambers. A small piece (3 x 3 cm) of Whatman No 1 filter paper was attached to the undersurface of the cap to serve as an oil diffuser after the appropriate amount of pure essential oil had been applied. Doses were calculated based on nominal concentrations and assumed 100% volatilisation of the oils in the exposure vessels.

In each jar, 20 insects were placed at the appropriate stage of development i.e. 10-, 25- and 31-days-old larvae, 2-days-old pupae (males and females were exposed separately) and 10- and 60-days-old adults (males and females were exposed separately) were used. Males and females were separated as pupae based on morphological characteristics of genital papillae and kept separately until their use. In females, the genital papillae are pointy, with 2 darker dots on the tip of each, and roughly half the size of the urogomphi whereas those of males are stubby, conjoined and barely noticeable (Park, 1934).

After 48h of exposure to essential oil vapours, the insects were transferred to plastic Petri dishes containing wheat flour. All dead insects were counted by the fourth day, except pupae which were kept for a further 4 days, with those that failed to complete morphogenesis and/or produced developmental intermediates or 'adultoids' (pupal-adult mosaics) being counted as dead. Also the larvae that survived the tests were kept until pupation to examine possible delayed mortality or morphological deformations.

Four to seven doses were tested for each essential oil and developmental stage combination. Each dose was repeated three or four times. Due to differential toxicity, doses of the compounds tested ranged from 0.27 to 165 μl/l of air depending on the essential oil, insect developmental stage, age and sex (based on preliminary tests). All experiments were carried out in an incubator at 26 ± 1°C, 65 ± 5% r.h. and 12 h light/12 h dark photoperiod.

Statistical analysis

Data obtained from each dose-response bioassay were subjected to probit analysis and LC_{50} values and 95% confidence limits were estimated. The comparisons among LC_{50} values were based on the overlap of confidence limits (Finney, 1971). All analyses were conducted using the statistical package SPSS 14.0 (SPSS, 2004)

Results

Fumigant toxicity of essential oils

The fumigant toxicity (LC_{50} values) of the essential oils of *L. hybrida, L. nobilis*, *T. orientalis, C. sinensis*, *C. limon* and *O. vulgare* against immature and adult *T. confusum* is given in Tables 1, 2, 3, 4, 5 and 6, respectively. All but *O. vulgare* essential oils exhibited strong fumigant toxicity to all developmental stages of *T. confusum*. LC_{50} values ranged between 1.8 and 109 μl/l air depending on the essential oil and insect developmental stage, age and sex.

The most susceptible stage of *T. confusum* to the vapours of *L. hybrida* essential oil was the 10-day-old larvae (LC_{50}, 1.8 μl/l air), while the most tolerant was the 31-day-old larvae (LC_{50} 109.9 μl/l air) (Table 1). The estimated LC_{50} values for female and male pupae were very close (37.3 and 38.7 μl/l air, respectively). An increase in the adults' susceptibility to the essential oil vapours with increasing age was recorded, with females being more tolerant than males, especially for 60-day-old adults, where the observed differences were statistically significant.

The most susceptible stage to the vapours of *L. nobilis* oil was that of the 10-day-old larvae, and the most tolerant that of the 25-day-old larvae (Table 2). In the case of pupae, males were more susceptible than females to the essential oil vapours. Also, adult susceptibility to the vapours decreased with increasing age, but no significant differences between the sexes were observed.

The10-day-old larvae were the most vulnerable to the vapours of *T. orientalis* oil, followed by pupae and adults (Table 3). Nevertheless, the calculated LC_{50} values for all stages (except those for the young larvae) could not provide a clear picture of the degree of susceptibility because in most cases the statistical analysis did not reveal significant differences.

A similar case to the *T. orientalis* oil was recorded for *C. sinensis* and *C. limon,* with young larvae being more susceptible than older ones (Tables 4 and 5). A slight (but not statistically significant) decrease in susceptibility of adults to the citrus essential oil vapours with increasing age was observed.

The essential oil of *O. vulgare* showed a completely different effect from all the other essential oils tested. In fact, with the exception of 10-day-old larvae and pupae, its vapours did not provoke mortality even at doses up to 165 μl/l air (Table 6).

Effect on surviving larvae

The larvae that survived exposure to essential oils did not exhibit any noticeable delayed mortality or any kind of morphological abnormalities (data not shown).

Effect on pupae

All the essential oils tested caused a greater or lesser proportion of deformations in *T. confusum* pupae. These morphological deformations were noted in both males and females, with pupae trapped in the puparium, individuals with an appearance intermediate between pupae and adults (adultoids),

Table 1. Fumigant toxicity of *Lavandula hybrida* essential oil to larvae, pupae and adults of *Tribolium confusum.*

Life stage (n[1])	slope ± SE	LC_{50}[2]	95% CL	df	χ^2
Larvae					
10-day-old (420)	0.7 ± 0.1	1.8	1.2 - 2.9	5	4.6
25-day-old (300)	3.6 ± 1.1	59.7	49.9 - 108.3	3	0.9
31-day-old (240)	2.2 ± 0.6	109.9	89.0 - 159.6	2	4.5
Pupae					
Females (300)	3.3 ± 0.5	37.3	32.9 - 42.7	3	3.2
Males (300)	4.2 ± 0.6	38.7	35.0 - 43.2	3	4.8
Adults 10-day-old					
Females (320)	3.9 ± 0.6	81.0	71.1 - 98.6	3	2.1
Males (300)	4.6 ± 0.7	61.0	54.1 - 73.1	3	7.4[3]
Adults 60-day-old					
Females (240)	8.0 ± 1.2	46.3	41.9 - 51.7	2	0.7
Males (280)	6.6 ± 1.0	29.8	25.8 - 34.4	2	2.7

1 Number of insects tested.
2 LC_{50} values are expressed as µl/l air and are considered significantly different when the 95% confidence limits (CL) fail to overlap.
3 Since the goodness-of-fit test is significant (P<0.1), a heterogeneity factor is used in the calculation of confidence limits (CL).

Table 2. Fumigant toxicity of *Laurus nobilis* essential oil to larvae, pupae, and adults of *Tribolium confusum.*

Life stage (n[1])	slope±SE	LC_{50}[2]	95% CL	df	χ^2
Larvae					
10-day-old (240)	0.3 ± 0.5	18.6	15.8 - 21.1	2	3.1
25-day-old (360)	8.8 ± 0.9	86.9	70.3 - 120.3	4	30.4[3]
31- day-old (360)	1.7 ± 0.5	55.6	37.9 - 66.8	4	2.1
Pupae					
Females (240)	4.0 ± 0.7	42.6	38.7 - 48.5	2	0.1
Males (240)	4.9 ± 0.6	33.7	30.5 - 36.7	2	2.3
Adults 10-day-old					
Females (240)	10.9 ± 1.5	62.7	60.3 - 65.0	2	5.8
Males (240)	7.2 ± 0.9	57.8	54.4 - 61.2	2	2.6
Adults 60-day-old					
Females (240)	14.0 ± 1.4	40.5	38.9 - 42.2	2	5.4
Males (280)	14.0 ± 1.4	40.5	38.9 - 42.2	2	5.4

1 Number of insects tested.
2 LC_{50} values are expressed as µl/l air and are considered significantly different when the 95% confidence limits (CL) fail to overlap.
3 Since the goodness-of-fit test is significant (P<0.1), a heterogeneity factor is used in the calculation of confidence limits (CL).

Table 3. Fumigant toxicity of *Thuja orientalis* essential oil to larvae, pupae, and adults of *Tribolium confusum.*

Life stage (n_1)	slope ± SE	LC_{50}[2]	95% CL	df	χ^2
Larvae					
10-day-old (240)	2.0 ± 0.3	20.8	17.0 - 26.2	2	0.2
25-day-old (360)	5.5 ± 0.6	99.2	74.7 - 140.0	4	27.1[3]
31-day-old (300)	7.0 ± 0.7	96.0	90.8 - 102.5	3	2.6
Pupae					
Females (480)	4.3 ± 0.8	66.9	57.5 - 94.6	6	18.9[3]
Males (480)	8.5 ± 0.9	52.0	46.5 - 64.2	6	39.6[3]
Adults 10-day-old					
Females (300)	13.6 ± 1.4	64.6	55.1 - 88.7	3	19.3[3]
Males (300)	12.4 ± 1.4	54.3	52.4 - 57.0	3	3.2
Adults 60-day-old					
Females (240)	9.7 ± 1.1	73.6	70.5 - 76.8	2	4.7
Males (360)	10.0 ± 1.0	53.6	49.7 - 60.7	4	10.6[3]

[1] Number of insects tested.
[2] LC_{50} values are expressed as μl/l air and are considered significantly different when the 95% confidence limits (CL) fail to overlap.
[3] Since the goodness-of-fit test is significant (P<0.1), a heterogeneity factor is used in the calculation of confidence limits (CL).

Table 4. Fumigant toxicity of *Citrus sinensis* essential oil to larvae, pupae and adults of *Tribolium confusum.*

Life stage (n^1)	slope ± SE	LC_{50}[2]	95% CL	df	χ^2
Larvae					
10-day-old (240)	2.0 ± 0.3	19.0	12.3 – 35.6	2	8.8[3]
25-day-old (240)	13.0 ± 1.7	104.5	100.4 - 108.0	2	5.9
31-day-old (300)	4.3 ± 0.5	79.6	45.1 - 105.5	3	11.2[3]
Pupae					
Females (300)	7.8 ± 0.8	42.3	26.8 - 54.9	3	18.1[3]
Males (240)	3.5 ± 0.8	39.4	35.3 - 45.0	2	2.8
Adults 10-day-old					
Females (320)	18.0 ± 2.2	33.1	30.3 - 38.8	3	12.6[3]
Males (240)	20.8 ± 2.4	30.8	22.5 - 36.0	2	6.8
Adults 60-day-old					
Females (240)	10.3 ± 1.2	29.2	28.0 - 30.5	2	2.13
Males (360)	16.6 ± 1.7	27.8	26.2 - 30.3	4	14.7[3]

[1] Number of insects tested.
[2] LC_{50} values are expressed as μl/l air and are considered significantly different when the 95% confidence limits (CL) fail to overlap.
[3] Since the goodness-of-fit test is significant (P<0.1), a heterogeneity factor is used in the calculation of confidence limits (CL).

Table 5. Fumigant toxicity of *Citrus limon* essential oil to larvae, pupae, and adults of *Tribolium confusum.*

Life stage (n[1])	slope ± SE	LC_{50}[2]	95% CL	df	χ^2
Larvae					
10-day-old (240)	4.3 ± 0.5	21.5	12.0 - 51.7	2	5.5[3]
25-day-old (240)	0.03 ± 0.01	77.2	71.7 - 82.5	2	3.2[3]
31-day-old (300)	4.1 ± 0.57	85.0	78.0 - 92.9	3	7.2
Pupae					
Females (240)	0.06 ± 0.01	51.3	48.4 - 54.4	2	2.1
Males (300)	7.0 ± 0.8	45.1	27.2 - 69.9	3	18.8[3]
Adults 10-day-old					
Females (340)	0.2 ± 0.02	35.5	31.9 - 39.5	3	12.5[3]
Males (240)	0.2 ± 0.02	33.7	32.5 - 34.8	2	2.0
Adults 60-day-old					
Females (240)	0.2 ± 0.02	31.9	26.3 - 35.9	2	4.0
Males (240)	0.3 ± 0.03	28.5	27.6 - 29.4	2	3.2

[1] Number of insects tested.
[2] LC_{50} values are expressed as μl/l air and are considered significantly different when the 95% confidence limits (CL) fail to overlap.
[3] Since the goodness-of-fit test is significant (P<0.1), a heterogeneity factor is used in the calculation of confidence limits (CL).

Table 6. Fumigant toxicity of *Origanum vulgare* essential oil to larvae, pupae and adults of *Tribolium confusum.*

Life stage (n[1])	slope ± SE	LC_{50}[2]	95% f.l.	df	χ^2
Larvae					
10-day-old (300)	1.0 ± 0.2	25.6	18.1 - 41.3	3	3.8
25-day-old (120)	-	>165[3]	-	-	-
31-day-old (120)	-	>165[3]	-	-	-
Pupae					
Females (300)	4.0 ± 0.5	74.8	68.1 - 83.7	3	3.5
Males (300)	2.5 ± 0.4	73.0	63.7 - 84.5	3	3.2
Adults 10-day-old					
Females (120)	-	>165[3]	-	-	-
Males (120)	-	>165[3]	-	-	-
Adults 60-day-old					
Females (120)	-	>165[3]	-	-	-
Males (120)	-	>165[3]	-	-	-

[1] Number of insects tested.
[2] LC_{50} values are expressed as μl/l air and are considered significantly different when the 95% confidence limits (CL) fail to overlap.
[3] Indicates the higher dose tested, oregano essential oil vapour failed to provoke mortality even at doses up to 165 μl/l air.

adults with malformed elytra and morphological deformities ranging from aberration of the tarsi to legs reduced to stump-like appendages. For all essential oils, the proportion of the pupae trapped in the puparium was less than that of the adultoids, with the exception of *L. hybrida,* where high doses had the opposite effect (Fig. 1).

Discussion and Conclusions

The essential oil vapours of *L. hybrida, L. nobilis*, *T. orientalis, C. sinensis* and *C. limon* exhibited a strong toxic action against the immature stages and adults of *T. confusum*. The fumigant toxicity of these essential oils depended on the insect developmental stage.

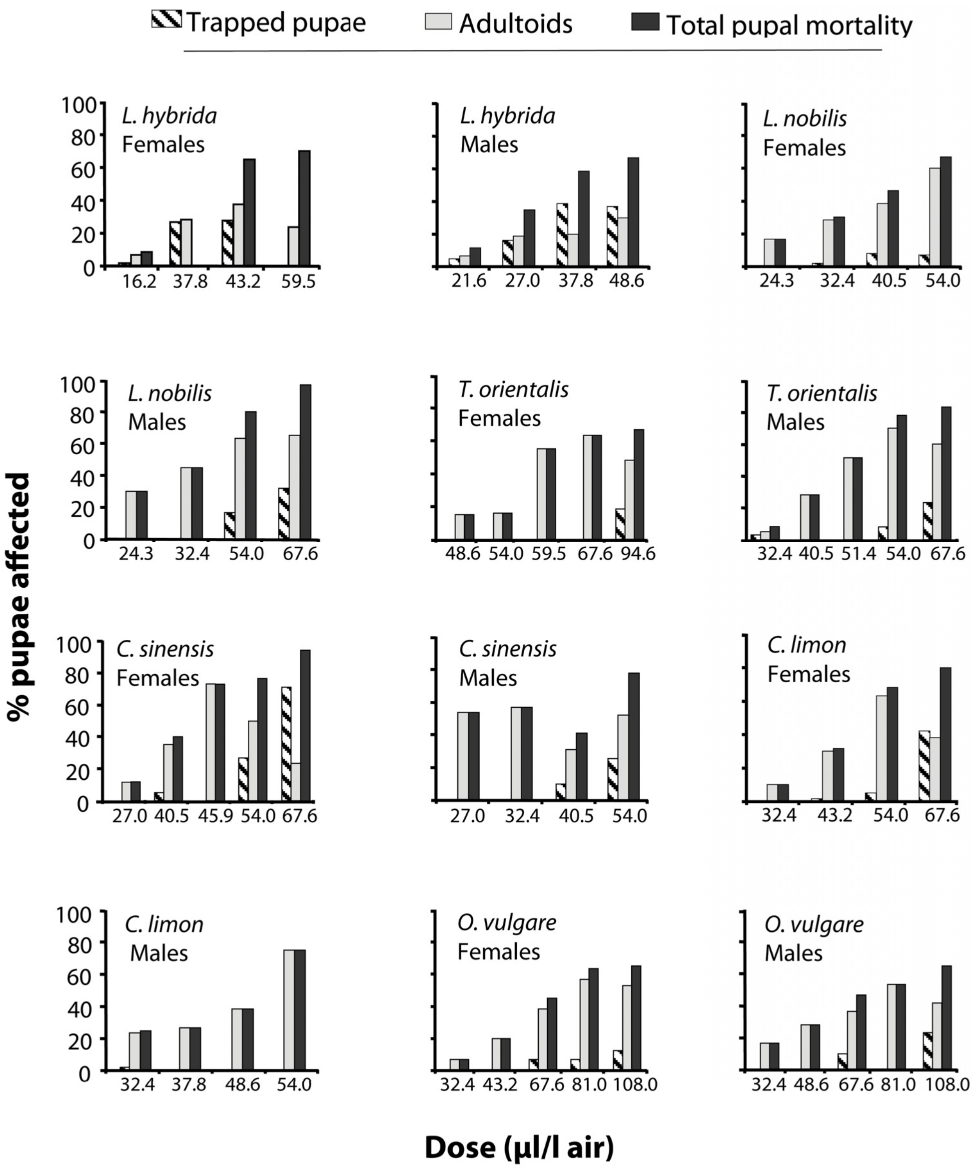

Fig.1. Effects of essential oil vapours on pupae of *Tribolium confusum*.

i.e. *L. hybrida* showed the highest toxic effect against 10 and 25 day-old larvae, *L. nobilis* was the most toxic for 31 day-old larvae and *C. sinensis* and *C. limon* were the most toxic for adults. Lemon and orange essential oils are particularly rich in limonene (Giatropoulos *et al*., 2012) which was found to be one of the most toxic compounds among other monoterpenes against adults of *T. confusum* (Stamopoulos *et al*., 2007).

Origanum vulgare essential oil vapours, with the exception of 10-day-old larvae and pupae, failed to result in mortality even at doses up to 165 μl/l air. Our results concerning *O. vulgare* essential oil, are in correlation with those obtained by Demirel *et al.* (2009) which demonstrated that *O. vulgare*, *O. onites* L. and *O. minutiflorum* L. essential oils possessed the weakest toxicity among eight different essential oils evaluated against *T. confusum*.

According to our findings, tolerance to the essential oils increases as the immature stages grow older, which coincides with exposure data of *T. confusum* larvae to some monoterpenes (Stamopoulos *et al.* 2007) as well as data of *T. castaneum* and *Acanthoscelides obtectus* (Say) larvae to various essential oil vapours (Huang *et al*., 1997; Liu and Ho, 1999; Papachristos and Stamopoulos, 2002). A possible explanation is that the existing difference in body size may be responsible for the variations in susceptibility.

The *L. hybrida, L. nobilis, C. sinensis* and *C. limon* essential oils, in their vapour form, are known to be effective against other stored product insect pests. Essential oils from *L. hybrida, L. nobilis* and *C. sinensis* are effective against the bean weevil *A. obtectus* (Papachristos and Stamopoulos, 2002; Papachristos *et al*., 2004) and essential oils of *C. sinensis* and *C. limon* are effective against adults and larvae of *Callosobruchus maculates F., Sitophilus zeamais Motsch.* and *Dermestes maculatus Deg.* (Don-Pedro, 1996). In all these cases, the LC_{50} values of essential oils were much lower from the one calculated for *T. confusum* in the present work indicating lower susceptibility of *T. confusum* to essential oil vapours compared to other stored product insect pests.

Essential oils of *L. nobilis* from various origins were found to be also effective against adults of *T. castaneum*, but the LC_{50} values reported (ranged from 172 to 217 μl/l air) were much higher than the present one calculated for the adults of *T. confusum* (Jemâa *et al*., 2012). Nevertheless, the *O. vulgare* essential oil vapours have been found to be a highly effective fumigant against the adults of *T. castaneum* with LC_{50} value of 55 mg/l air (Kim *et al*., 2010). These results are indicative of the highly idiosyncratic toxicity of individual oils even between closely related species such as *T. confusum* and *T. castaneum*.

Susceptibility of adults to the essential oils seems to increase with the adult's age, even though this is not statistically proved in most cases. Older insects tend to be more vulnerable to the essential oil vapours, perhaps because they are less able to metabolise and/or detoxify these substances.

In all but one case (*L. hybrida*), male pupae seem to be relatively more susceptible than female pupae although, in most cases, the recorded differences were not statistically significant. Similar slight sex differences in susceptibility were also observed in the adult stage. These differences could be consistent with an innate difference in mode of action rather than with size, especially as the difference in size between males and females of *T. confusum* is almost negligible and cannot justify the divergence in susceptibility observed in other stored product insects (Papachristos and Stamopoulos, 2002; Papachristos *et al*., 2004).

The derailing of morphogenesis at the pupal stage and the appearance of adultoids and mutilated adults could be explained by assuming a direct effect on the insect hormonal system similar to that of the insect growth regulators (IGRs). According to the literature, *L. nobilis* essential oil contains high amounts of 1,8 cineole (Mediouni *et al*., 2012) and that of *L. hybrida* contains 1,8 cineole and linalool (Papachristos *et al*., 2004). Lemon and orange essential oils consist almost of limonene (Giatropoulos *et al*., 2012) and the effects of these essential oils on *T. confusum* pupae morphogenesis could be attributed to the action of some of their monoterpnenes components. Such potent action on pupal morphogenesis has been demonstrated after exposure of *T. confusum* pupae to some monoterpenes (terpinen-4-ol, 1,8-cineole, linalool, R-(+)-limonene and geraniol) (Stamopoulos *et al*., 2007). Moreover, some citrus essential oils (lemon, sweet orange and grape fruit) applied against the late third- and early fourth-instar larvae of *Aedes* (*Stegomyia*) *albopictus* (Skuse 1894), the so-called "Asian tiger mosquito", produced insect growth regulator (IGR)-like properties (Giatropoulos *et al*., 2012). Such effects were also observed by Amos *et al*. (1974) after incorporating various terpenoids into the diet of *T. castaneum* and *T. confusum,* and by other authors working with hydroprene (Bell and Edwards, 1999; Arthur, 2003; Arthur and Dowdy, 2003)

Contrary to what observed for pupae, the larvae that survived the toxicity tests and were kept until pupation to examine possible delayed mortality or morphological deformations did not exhibit such abnormalities. It is known that some terpenoids display activity similar to that exerted by the Juvenile Hormone analogues, when larvae of insects are subjected to their vapours for a long time, performing extra moults and finally developing into either larval-pupal intermediates or normal pupae which produce both morphologically normal adults and adultoids (Amos *et al*., 1974; Semple, 1992). Two likely explanations can be adopted here: either the toxicity tests' limited exposure time (48h) was insufficient for the expression of the aforementioned phenomena or, as Semple (1992) reports, 'larvae of most holometabolous insects such as Lepidoptera and Coleoptera are susceptible only at the end of the last larval instar, while the pupae are susceptible for several hours or at most a few days after the last larval ecdysis'.

Although our findings are preliminary, they could form a basis for further investigation of the questions raised in this work. In particular, additional research is needed to improve our understanding of how essential oil vapours act during morphogenesis, given that the mechanisms underlying the appearance of adultoids and mutilated adults have been, to the best of our knowledge, inadequately studied.

Overall, essential oils tested with the exception of *O. vulgare* essential oil were highly toxic against larvae, pupae and adults of *T. confusum* indicating the potential of their possible utilization as fumigants in protection of stored products in storehouses by reducing the risks associated with the use of synthetic insecticides. However, many aspects of their release kinetics after application and the effect of factors such as temperature, relative humidity and stored product commodities, must be studied to determine whether these substances can be realistically applied as fumigants against stored-product insects in practice.

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Τοξικότητα των ατμών έξι αιθέριων ελαίων σε ανήλικα και ενήλικα άτομα του είδους *Tribolium confusum*

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Περίληψη Προσδιορίστηκε η τοξικότητα των ατμών έξι αιθέριων ελαίων (*Lavandula hybrida*, *Laurus nobilis*, *Thuja orientalis*, *Citrus sinensis*, *Citrus limon*, και *Origanum vulgare*) σε προνύμφες, νύμφες και ενήλικα του είδους *Tribolium confusum*. Τα αποτελέσματα έδειξαν ότι όλα τα αιθέρια έλαια, με εξαίρεση αυτό του είδους *O. vulgare*, ήταν τοξικά για όλα τα στάδια ανάπτυξης του εντόμου. Τη μεγαλύτερη ευπάθεια στα αιθέρια έλαια εμφάνισαν οι προνύμφες ηλικίας 10 ημερών ενώ την μικρότερη οι προνύμφες ηλικίας 25 και 31 ημερών. Οι τιμές των μέσων θανατηφόρων δόσεων (LC_{50}) κυμάνθηκαν από 1,8 έως 109 μl αιθέριου ελαίου ανά l αέρα ανάλογα με το στάδιο ανάπτυξης του εντόμου και το είδος του αιθέριου ελαίου. Η έκθεση των νυμφών στους ατμούς των αιθέριων ελαίων προκάλεσε αναστολή της μορφογένεσης και της εμφάνισης φυσιολογικών ενηλίκων εντόμων.

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Interference between silverleaf nightshade (*Solanum elaeagnifolium* Cav.) and alfalfa (*Medicago sativa* L.) cultivars

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Summary Alfalfa (*Medicago sativa* L.) is the most widely grown forage legume in Greece and other Mediterranean countries. The successful establishment of the crop is crucial for its overall productivity. Weed infestations are a common problem, especially in spring-seeded alfalfa, with silverleaf nightshade (*Solanum elaeagnifolium* Cav.) being one of the most noxious weeds. The main objective of the field experiment conducted in Greece in 2010 and 2011 was to evaluate the differences among three alfalfa cultivars (Gea, Dimitra and Hyliki) regarding their competitiveness against silverleaf nightshade and their forage yield during the first crucial year of crop establishment. Moreover, density and fresh weight data of *S. elaeagnifolium* were also recorded. Our results showed that the presence of this weed caused an annual yield loss ranged from 8 to 26%, depending on the year and the cultivar. In particular, Hyliki was the most productive cultivar, while even with the presence of nightshade it produced significantly higher biomass than the other cultivars (up to 28%). Furthermore, Hyliki was the cultivar with the highest regrowth rate after each cutting. Weed density and biomass were also significantly reduced in the case of Hyliki, while Gea was the less competitive cultivar. The results of the present study confirm that the competitive ability of the alfalfa cultivars might have a substantial range and can be a helpful weed management tool for the growers, especially against noxious species such as *S. elaeagnifolium*.

Additional keywords: alfalfa cultivars, integrated weed management, noxious weeds, regrowth, competition

Introduction

Alfalfa or lucerne (*Medicago sativa* L.), the most widely grown forage legume in Greece, could play a key role in organic crop-livestock systems, owing to its suitability to low input, rainfed conditions, its positive effects on soil fertility and nitrogen balance, and the high protein content and quality of its forage (Entz *et al.*, 2001; Karamanos *et al.*, 2009). The successful establishment of the crop is crucial for its overall productivity (Stout *et al.*, 1992). Established stands of alfalfa are fairly competitive with weeds. However, new alfalfa seedlings are less competitive and more susceptible to weed invasion (Annicchiarico and Pecetti, 2010). In fact, weed competition is one of the most limiting factors during crop establishment and early growth (especially in spring-seeded alfalfa), since the emerging crop plant is not a vigorous competitor and weeds emerging shortly after seeding can reduce alfalfa productivity (Pike and Stritzke, 1984; Fischer *et al.*, 1988; Zimdahl, 2004). The ability of several alfalfa cultivars to suppress weed growth may allow crop producers to reduce total costs (Ominski *et al.*, 1999; Arregui *et al.*, 2001; Dillehay *et al.*, 2011).

During the last years, there have been complaints from several regions of Greece for reduced efficacy of herbicides or increased competitiveness of many alien or recently problematic species (Travlos, 2009; Travlos and Chachalis, 2010). Silverleaf nightshade (*S. elaeagnifolium* Cav.*)* is one of these species, already present for many years in the country (Economidou and Yannitsaros, 1975), with an ongoing dispersal in Greece especially during the last decade, according to extensive weed surveys (Travlos *et al.*, 2011; Travlos, 2013). Silverleaf nightshade has spread in many arid regions of the world

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(Boyd *et al*., 1984) and is a deep-rooted, perennial, broadleaf weed that propagates by seed, root segments, and creeping lateral roots (Cuthbertson *et al*., 1976). Moreover, *S. elaeagnifolium* is now considered as a noxious and invasive alien weed, against which international measures have to be taken in many areas (OEPP/EPPO, 2004).

Quantitative information regarding the potential suppressive effect of alfalfa cultivars on weeds in current cropping systems is rather lacking. Therefore, the objectives of this study were to evaluate the differences among three alfalfa cultivars (Gea, Dimitra and Hyliki) regarding their competitiveness against the noxious weed *S. elaeagnifolium* and their productivity (forage yield) during the first year of crop establishment.

Materials and Methods

A field experiment was conducted during 2010 (and repeated in 2011) in the experimental field of Agricultural University of Athens (37° 59′ 12′′ N, 23° 42′ 96′′ E, 29 m altitude) in order to study the competitive ability of three alfalfa cultivars (Gea, Dimitra and Hyliki) against silverleaf nightshade.

The soil was clay loam (Bouyoucos, 1962), with pH 7.29 (1:1 H_2O), 15 g/kg organic matter (Wakley and Black, 1934) and 160 g/kg $CaCO_3$. Hand-sowing took place at the rate of 20 kg/ha on 28 March, 2010 and 23 March, 2011. The field was fertilized with P_2O_5 and K_2O as recommended by soil analysis for alfalfa (Hall, 2008). Alfalfa crop was mown each time it reached 10% bloom. This resulted in four harvests each year at 65, 100, 145 and 210 days after sowing (DAS). Rhizomes of *S. elaeagnifolium* (5-6 cm in length) were uniformly planted horizontally (12-15 g/m^2 in a depth of 4-5 cm), while other weed species emerged within the experimental area were removed by hand-hoeing.

The experimental design was a split-plot in a randomized complete block with four blocks (replicates). Alfalfa cultivar was the main plot factor and the weed presence (or absence) was the subplot factor. Main plot and subplot sizes were 6 by 4 m and 2.5 by 4 m, respectively.

Irrigation and other common cultural practices were conducted as needed during the growing seasons. Mean monthly temperature and rainfall data are given in Table 1. In each cutting, forage yield was measured, while the total first-year cumulative yield was also recorded. The dry weight of forage was determined after oven drying at 70°C for 48 h. In the same days (65, 100, 145 and 210 DAS) measurements of the density and biomass of silverleaf nightshade were also taken. Visual estimation of regrowth ability of each cultivar was also conducted 15 days after each harvest (cutting) by means of a scale, comparing the most vigorous stands (high and dense, scored as 5) with the lowest and fewer plants (scored as 1).

An analysis of variance (ANOVA) was conducted for all data and differences between means were compared at the 5% level of significance using the Fisher's Protected LSD test. Linear regression was also performed for the three cultivars relating the forage yield and silverleaf nightshade biomass. All statistical analyses were conducted using the Statistica 9 software package (StatSoft, Inc. 2300 East 14th Street, Tulsa, OK 74104, USA).

Results and Discussion

The analysis of variance of our data revealed that alfalfa forage yield and silverleaf nightshade growth (density and biomass production) were significantly affected by the alfalfa cultivar and the presence or absence of the weed. The year was also a significant factor for the forage yield of the crop and for the density of *S. elaeagnifolium* plants, while it had no significant effect on the fresh weight of the weed. Moreover, the interaction between the above-mentioned factors was significant for most parameters except the biomass of silverleaf nightshade on individual plant's level (Table 2).

In particular, the harmful effects of silverleaf nightshade on the forage yield of alfalfa are shown in Table 3. The presence of *S. elaeagnifolium* was responsible for significant reductions, up to 26, 15 and 14% in the annual yields of the cultivars Gea, Dimitra and Hyliki, respectively. The higher yields of all three alfalfa cultivars during the second year of experimentation (2011) compared with the first year (2010) were probably due to the significantly higher precipitation (38%) during 2011 (Table 1). It has also to be noted that the maximum yield reductions were observed in the second and third harvest. This finding may be attributed to the vigorous growth of silverleaf nightshade during summer (mostly July and August) and is in full accordance with previous studies (Travlos, 2012). Our results also proved that even in the presence of silverleaf nightshade, the annual forage yield of Hyliki was 23 to 28% and 12 to 13% higher than the yield of Gea and Dimitra, respectively.

Regarding the density of silverleaf nightshade, the weed was present at densities ranging between 2 and 7 plants/m^2 in the plots of Gea during the first year, while for Hyliki the maximum density was only about 3 plants/m^2 (Table 4). The mean density of the weed plants in the case of Hyliki was 31-34 and 51-57% lower than the corresponding values for Dimitra and Gea, respectively. The more rainfalls during 2011 resulted to a higher weed density compared with the first, drier year of experimentation.

Concerning the weed biomass, the mean growth of individual silverleaf nightshade plants in the plots with Hyliki was 43 and 60% lower than the corresponding values for Dimitra and Gea, respectively. Additionally, in the fourth harvest of alfalfa (210 DAS), the number of *S. elaeagnifolium* plants in the case of Hyliki was 65% lower than that of Gea, while the fresh weight per plant was also significantly lower (82%), as shown in Table 5.

This less vigorous and less dense presence of the weed in the plots of Hyliki is probably due to the recorded faster regrowth rate of this cultivar of alfalfa, compared with the other two cultivars. Indeed, according to our observations the regrowth ability of Hyliki after each harvest was significantly higher (scored as 4) than the other two cultivars (scored as 2). It is considered that differences among alfalfa cultivars in their ability to develop canopy structures early in the season could affect weed emergence (Huarte and Benech Arnold, 2003). Alfalfa has the

Table 1. Mean monthly rainfall and temperature during the field experiment in 2010 and 2011.

Month	Rainfall		Temperature	
	2010	2011	2010	2011
	mm		°C	
January	-	-	-	-
February	-	-	-	-
March	11	25.6	14.4	12.2
April	0	40	17.9	15.5
May	7	40.8	22.2	20.3
June	12	30.4	25.9	25.5
July	0	0	29.3	29.7
August	0	0.6	28.4	28.8
September	22.6	3.4	24.9	26.6
October	81.8	38.4	19	17.5
November	15.6	2.2	18.4	12.3
December	25	100.8	13.7	12
Total	175	282.2	-	-

Table 2. Analysis of variance for alfalfa cultivar (A), presence of *Solanum elaeagnifolium* (S), and year (Y) effects on alfalfa forage yield, density and biomass of the weed plants.

Source	df	Alfalfa forage yield	Silverleaf nightshade density	Silverleaf nightshade biomass
A	2	**	*	*
S	1	**	**	**
A x S	1	*	*	*
Y	2	*	*	ns
Y x A	2	*	*	ns
Y x S	1	*	*	ns
Y x A x S	2	*	*	ns

* Significant at the 0.05 level
** Significant at the 0.01 level

Table 3. Forage yield of three alfalfa cultivars (Gea, Dimitra, Hyliki) (dry weight in tn/ha) with the presence of *Solanum elaeagnifolium* and under a weed-free situation in a two-year field experiment (2010, 2011). Each number represents the mean yield of a cultivar per cutting or the total mean yield of a cultivar in 2010. In parentheses the corresponding values for 2011 are also shown.

With *S. elaeagnifolium*			
	Gea	Dimitra	Hyliki
Cuttings	Dry weight (tn/ha)		
65 DAT	2.84 (3.3) b	3.35 (3.89) a	3.66 (4.04) a
100 DAT	1.88 (2.2) d	2.49 (2.63) cd	2.81 (3.09) c
145 DAT	1.67 (1.88) f	2.21 (2.29) ef	2.58 (2.82) e
210 DAT	2.55 (3.01) h	2.91 (3.04) h	3.36 (3.61) g
Total	8.94(10.39) k	10.96 (11.85) j	12.41 (13.56) i
Without weeds			
	Gea	Dimitra	Hyliki
Cuttings	Dry weight (tn/ha)		
65 DAT	3.72 (3.98) m	4.36 (4.14) l	4.21 (4.29) l
100 DAT	2.66 (2.82) o	2.97 (3.13) no	3.52 (3.38) n
145 DAT	2.35 (2.42) q	2.43 (2.77) q	3.05 (3.25) p
210 DAT	3.38 (3.44) rs	3.15 (3.3) s	3.71 (3.82) r
Total	12.11 (12.66) u	12.91 (13.34) tu	14.49 (14.74) t

Means within a row followed by the same letter are not significantly different ($p < 0.05$) according to Fischer's LSD test.

Table 4. Density of *Solanum elaeagnifolium* plants in plots of three alfalfa cultivars (Gea, Dimitra, Hyliki) in a two-year experimental field (2010, 2011). Each number represents the mean number of weed plants for an alfalfa cultivar per cutting or the total mean number of weed plants for each cultivar in 2010. In parentheses the corresponding values for 2011 are also shown.

Density of *S. elaeagnifolium* (plants/m^2)			
Cuttings	Gea	Dimitra	Hyliki
65 DAT	2.2 (3.1) a	1.2 (2.1) b	1.3 (2.1) b
100 DAT	5.3 (7.2) c	2 (3.6) d	2.2 (3.4) d
145 DAT	7.1 (7.4) e	5.2 (4.5) e	3 (3.1) f
210 DAT	3.4 (4.1) g	3.3 (5.4) g	1.2 (2.1) h
Mean	4.5 (5.45) i	2.9 (3.9) ij	1.9 (2.7) j

Means within a row followed by the same letter are not significantly different ($p < 0.05$) according to Fischer's LSD test.

Table 5. Biomass of *Solanum elaeagnifolium* plants (fresh weight in g/plant) in plots of three alfalfa cultivars (Gea, Dimitra, Hyliki) in a two-year experimental field (2010, 2011). Each number represents the mean fresh weight of weeds for a cultivar per cutting in both experimental years or the total mean fresh weight of weeds for each cultivar.

Fresh weight of *S. elaeagnifolium* (g/plant)			
Cuttings	Gea	Dimitra	Hyliki
65 DAT	2.3 a	2.6 a	2.4 a
100 DAT	9.8 b	6.4 c	5.2 c
145 DAT	12.4 d	8.4 e	4.8 f
210 DAT	11.9 g	8.6 g	2.2 h
Mean	9.1 i	6.5 j	3.7 k

Means within a row followed by the same letter are not significantly different ($p < 0.05$) according to Fischer's LSD test.

ability to suppress weed growth (Peters and Linscott 1988) and appears to exert a long-term effect on weed population dynamics (Ominski *et al*., 1999). Several weeds are expected to be effectively suppressed by *M. sativa*, and thus including alfalfa in crop rotations regime can be part of an integrated weed management strategy for weeds such as *Avena fatua*, *Brassica kaber*, *Cirsium arvense, Rumex crispus*, *Amaranthus quitensis* (Ominski et al. 1999; Huarte and Benech Arnold, 2003).

The inverse linear relationship (R^2 between 0.72 and 0.83) between alfalfa forage yield and *S. elaeagnifolium* biomass found in this study (Fig. 1) suggests that maintaining a high crop biomass is important to avoid the invasion of the silverleaf nightshade and this is in full accordance with previous studies showing a similar relationship between alfalfa and weeds (Grewal, 2010). Moreover, it could be said that the greater suppression of silverleaf nightshade in the case of Hyliki was partly due to the high productivity of this specific cultivar as previously shown in the weed-free situation in Table 3.

In moderate to severe weed infestations, alfalfa yield can be reduced through competition for light, water, and nutrients and the forage quality can be lowered by decrease

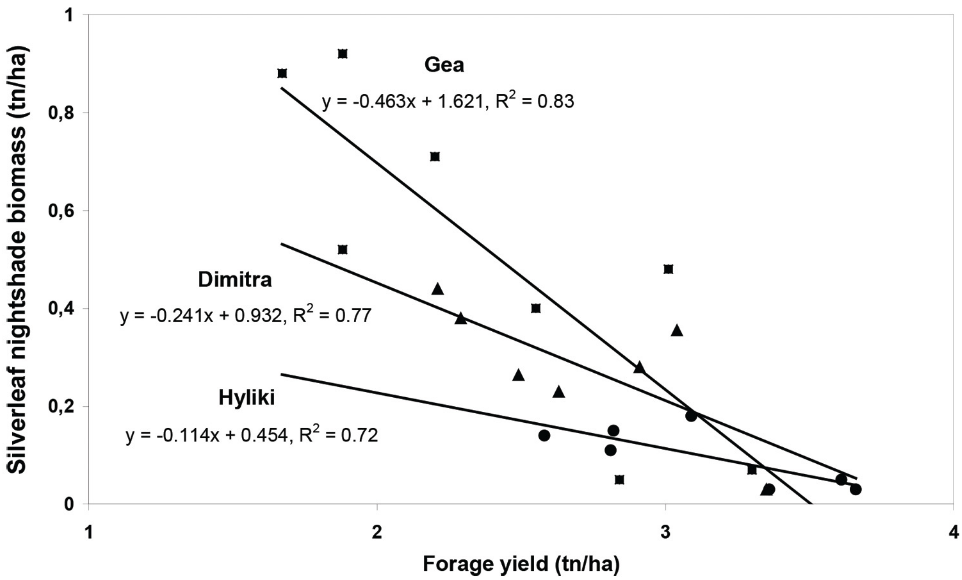

Figure 1. Relationship between forage yield of alfalfa and biomass of Solanum elaeagnifolium for three alfalfa cultivars (Gea, Dimitra, Hyliki) and four harvests during the first year of crop establishment.

in digestibility and protein content of the alfalfa hay (Cords 1973). Usually, alfalfa and weeds are harvested together and thus the quality of the hay is significantly degraded. This degradation is even higher, especially in the case of weeds such as silverleaf nightshade that animals are reluctant to graze because of the spiny leaves and stems (David *et al.*, 1945). For the effective control of silverleaf nightshade, several mechanical (David *et al.*, 1945), biological (Keeling and Abernathy, 1980; Parker, 1986), or chemical methods (Philips and Merkle, 1980; Eleftherohorinos *et al.*, 1993; Westerman and Murray, 1994) have been proposed. However, an integrated weed management approach based on a rotation regime with competitive alfalfa cultivars is rather required for an effective control of noxious species, such as silver nightshade.

Another critical thing to be taken into account is that for a perennial crop like alfalfa, high weed populations in the first year may adversely affect crop yield and quality in subsequent years (Smith *et al.*, 1990). It is well documented that controlling weeds during the establishment year reduces stress on alfalfa and ultimately increases yield in the following years (Stout *et al.*, 1992; Travlos, 2011). Consequently, the careful selection of the most productive and weed competitive alfalfa cultivars could be of great importance during the crucial year of crop establishment.

Conclusions

Our results showed that the weed competitive ability and forage yield of alfalfa cultivars might have a substantial range and should be certainly taken into account. Annual forage yield loss in the case of less weed competitive alfalfa cultivars was high and up to 26%, while after the selection of the most productive and competitive cultivar (i.e. Hyliki) the reduction was significantly lower and forage yield could be up to 28% higher, even with the presence of the noxious silverleaf nightshade. Similar studies should be continued including different alfalfa cultivars, soil and climatic conditions, weed species and weed densities. The ability of alfalfa to suppress weed growth can provide a viable alternative to chemical weed control and allow crop producers to reduce herbicide inputs. Moreover, our results highlight the underestimated importance of alfalfa cultivar selection for a successful establishment of the crop being also effective against noxious weeds. This is important, especially in cases of organic agriculture and in any other agricultural situations where herbicides are missing or they should not be applied.

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Αλληλεπιδράσεις μεταξύ του ζιζανίου σολανό (*Solanum elaeagnifolium* Cav.) και τριών ποικιλιών μηδικής (*Medicago sativa* L.)

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Περίληψη Σε συνθήκες αγρού διερευνήθηκε ο ανταγωνισμός μεταξύ τριών ποικιλιών μηδικής (*Medicago sativa* L.) και του ζιζανίου *Solanum elaeagnifolium* Cav. Συγκεκριμένα, στον αγρό του Εργαστηρίου Γεωργίας του Γ.Π.Α. έγινε πειραματισμός για δύο συνεχή έτη (2010, 2011) με τρεις ποικιλίες μηδικής (Gea, Δήμητρα, Υλίκη). Ίση ποσότητα ριζωμάτων του ζιζανίου ενσωματώθηκε ομοιόμορφα σε κάθε πειραματικό τεμάχιο, ενώ υπήρχαν και πειραματικά τεμάχια χωρίς την παρουσία του ζιζανίου (μάρτυρας). Μελετήθηκε η απόδοση των τριών ποικιλιών κατά τη διάρκεια των τεσσάρων κοπών για το κάθε έτος πειραματισμού, ενώ παράλληλα λαμβάνονταν μετρήσεις της πυκνότητας και του νωπού βάρους και του σολανού. Τα αποτελέσματα έδειξαν ότι το συνολικό ξηρό βάρος της παραγόμενης βιομάζας των ποικιλιών της μηδικής ανά έτος μειώθηκε παρουσία του ζιζανίου *S. elaeagnifolium* σε σχέση με τους μάρτυρες από 8 έως 26% και ανάλογα με τη χρονιά και την καλλιεργούμενη ποικιλία. Από τις τρεις ποικιλίες η Υλίκη ήταν η περισσότερο παραγωγική ακόμη και σε συνθήκες παρουσίας του σολανού με απόδοση σημαντικά υψηλότερη από τις άλλες. Η Υλίκη ήταν επίσης η ποικιλία με τον υψηλότερο ρυθμό αναβλάστησης μετά από κάθε κοπή. Επιπλέον, τα αποτελέσματα έδειξαν ότι η πυκνότητα και το νωπό βάρος του ζιζανίου ήταν σημαντικά μικρότερα στα πειραματικά τεμάχια της Υλίκης σε σχέση με τα αντίστοιχα της Gea. Η μελέτη αυτή επιβεβαιώνει τις διαφορές ως προς την ανταγωνιστική ικανότητα των ποικιλιών της μηδικής έναντι των ζιζανίων και τη σημασία της σωστής επιλογής της κατάλληλης ποικιλίας για την επιτυχή διαχείριση δυσεξόντωτων ζιζανίων όπως το σολανό, ιδιαίτερα μάλιστα σε βιολογικές καλλιέργειες.

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