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Biologia 70/4: 504—515, 2015
Section Zoology
DOI: 10.1515/biolog-2015-0050

A qualitative and quantitative study of thrips (Thysanoptera) on alfalfa and records of thrips species on cultivated and wild *Medicago* species of Greece

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Abstract: Foliage and litter samples of twelve *Medicago* species (medics) (Fabaceae) were collected for the study of Thysanoptera from mainland and insular Greece between 2007–2013. The species composition, population dynamics and spatial distribution of thrips were also evaluated based on two similarly managed experimental plots, except the number of cuttings, within an alfalfa hay field in Kopais Valley (Central Greece) between 2007–2008. Nine thrips species were recorded from nine medics, two of which (*Pseudodendrothrips mori* and *Sericothrips bicornis*) are new to the Greek fauna. Eight species of Thysanoptera were recorded on alfalfa and five species in the rest of the medics, among which *Medicago strasseri* is an endemic shrub of Crete. Data regarding the quantitative part of the study demonstrated that *Frankliniella occidentalis*, which was the most abundant thrips species in both plots, presented a seasonal pattern of population fluctuation and also tended to aggregate. The mean population density of this species significantly differed between the above-mentioned plots, but no such a difference was estimated for the larvae of Thripidae.

Key words: Thysanoptera; *Medicago*; Greece; population dynamics; spatial distribution

Introduction

Thrips (Insecta: Thysanoptera) comprise about 6,000 species throughout the world and many of them are considered very serious crop pests, whereas other species are mycophagous and predatory (Reynaud 2010). Thrips can be found everywhere on plants apart from roots. Phytophagous species prefer to infest the non-woody aerial parts of plants, where predatory thrips can be also found seeking for prey (Kirk 1997a, b). Some species of Thysanoptera exhibit a quite complicated role on plants, such as western flower thrips, *Frankliniella occidentalis* Pergande, 1895 (Thripidae), which can damage crops directly, transmit plant viruses, control mite populations or even pollinate (Kirk 1997a).

Although many scientific studies have dealt with recording injurious thrips species associated with crops (e.g., Lewis 1997b), there is a parallel need to enrich our knowledge with faunistic surveys over non-cultivated plant species and Thysanoptera or even reexamine the thysanopteran fauna of crops in a region and how this changes over time. The recording of thrips species usually reveals new potential crop pests and shows the diversity of habitats in which thrips can be found. For instance, it is known that thrips species, which are crop pests, are generally vagile and migrate from the surrounding vegetation of crops to crop plants building usually there high population densities (Mound 1997). In some cases thrips can cause significant loss of yield and several agricultural practices are used to control them. For instance, Trdan et al. (2006) studied how intercropping could reduce the damage caused by onion thrips, *Thrips tabaci* Lindeman, 1889 (Thripidae), in the production of onions in Slovenia and they concluded that white clover (*Trifolium repens* L.) was the most effective plant species to result in high yields of onions in comparison to buckwheat (*Fagopyrum esculentum* Moench.), lacy phacelia (*Phacelia tanacetifolia* Benth.) and orchard grass (*Dactylis glomerata* L.). There are also increasing data that non-cultivated plants, including those of field margins or forests, contribute to the conservation of beneficial insects' populations near crops, such as thrips (Morandin et al. 2014).

Apart from studying the role of thrips on plants and other habitats (e.g., litter, fungi) the knowledge of the population dynamics and spatial distribution of these insects on host plants is indispensable for planning strategies to control them, particularly in crop monocultures, where these pests are recorded in high population densities (Kirk 1997b). For instance, Chyzik & Ucko (2002) studied the seasonal abundance of *F. occidentalis* found in the flowers of 22 plant species in the Arava Valley, Israel, stressing that western flower thrips infested many plant species developing high population densities for many months.

Alfalfa (*Medicago sativa* L. ssp. *sativa*) (Fabaceae) is considered the most important hay crop for livestock. The genus *Medicago* comprises 87 mostly perennial and annual species, also known as medics (Small 2011). Thirty-four species have been recorded so far from this genus in Greece located in natural ecosystems as well as in anthropogenic environments (Thanopoulos 2007). The latter include urban areas and alfalfa crops particularly in Central Greece. According to Konsiotou (2005) about 129,400 ha in Greece are covered with alfalfa producing 12–30 t/ha of hay in irrigated fields. Many species of Thysanoptera have been recorded on alfalfa worldwide including the genera *Apterothrips, Frankliniella*, *Haplothrips*, *Odontothrips* and *Thrips* (Lewis 1997b; Ripa et al. 2009; Ábrahám 2012). For instance, Trdan (2003) reported the presence of the thripid species *Frankliniella intonsa* (Trybom, 1895), *Odontothrips confusus* Priesner, 1926 and *Thrips physapus* L., 1761 on alfalfa in Slovenia. Although there are some reports of thrips species on alfalfa in Greece (Jenser & Tzanakakis 1985; Emmanouel et al. 1994; Deligeorgidis 2002), little is known about their presence on other medics worldwide. For instance, Thanopoulos et al. (2008) recorded unidentified thrips larvae on *Medicago strasseri* Greuter, Matthäs and Risse, an endemic shrub of Crete. The knowledge of thrips species on alfalfa from several parts of Greece, which have not been surveyed in the past, as well as the study of the not so well known thysanopteran fauna of other medics, which can trigger future studies to emphasize risks for medics as potential host plants of thrips, was a motivation to conduct the current study.

Many studies have also dealt with the spatial distribution of thrips on several plants species in Greece, such as cotton (*Gossypium hirsutum* L.) (Deligeorgidis et al. 2002), and other parts of the world. For instance, Trdan et al. (2005) investigated the distribution of *Aeolothrips intermedius* Bagnall, 1934 (Aeolothripidae) in various parts (e.g., flowers or ears of cereals) on several plant species. Kumar et al. (2014) studied the vertical and horizontal distribution of *Scirtothrips dorsalis* Hood, 1919 (Thripidae) on cotton, peanut (*Arachis hypogaea* L.) and pepper (*Capsicum annum* L.). Finally, the within-tree distribution of *Pezothrips kellyanus* Bagnall, 1916 (Thripidae) nymphs in citrus canopies was studied by Planes et al. (2014). However, few studies have investigated their spatial distribution and population dynamics on alfalfa (e.g., Ripa et al. 2009).

The objectives of the present study were to record the Thysanoptera in the foliage and litter of *Medicago* species from various ecosystems in Greece and estimate the abundance, population fluctuation and spatial distribution of thrips found in the foliage of cultivated alfalfa in Greece.

Material and methods

Sampling procedure

Foliage samples were collected from alfalfa hay fields from many parts of Greece between 2007–2013. To study the population dynamics and spatial distribution of thrips in the foliage of cultivated alfalfa, two hay plots, differing only in the number of cuttings and not being treated with pesticides, were selected in Kopais Valley within the Experimental Station of the Agricultural University of Athens. These plots, approximately 1,000 m^2 each, were 100 m apart and sown with the Greek alfalfa cultivar "Iliki" on 31 March 2006. The first one (plot 1) was usually cut once a month, between April and October every year, whereas the second one (plot 2) was harvested almost bimonthly during the same time period. In total, 36 foliage samples, each consisting of the terminal 15 cm of ten shoots (Lewis 1997a), were randomly collected once a month from each plot between January 2007 and December 2008.

The sampling procedure in alfalfa hay fields in other regions of Greece was the same with that in Kopais Valley, but only ten foliage samples were collected from each of these fields. Twelve litter samples were also randomly collected from the soil surface of each field with a metallic quadrat (14 cm × 16 cm). Spontaneous alfalfa and herbaceous medics were cut off at ground level and one litter sample was usually collected underneath each sampled plant. Finally, the terminal 15 cm of shoots were cut off from shrub medics, since the sampled plants were often big, and one litter sample was usually collected underneath each plant.

All foliage and litter samples were placed in plastic bags labelled with the name of plant species and sampling date, locality, geographic coordinates and the type of ecosystem, and transferred to the laboratory. Figure 1 presents a map with the sampling sites throughout Greece used for this study.

Extraction and identification of Thysanoptera

The extraction of thrips from samples was carried out using the Berlese method (Lewis 1997a). The extracted individuals were then stored in 70% ethanol and counted under a stereoscopic microscope. Since identification keys for preadult stages may be not useful (Moritz et al. 2002) and the identification of larval stages of Thysanoptera is either impossible (Brunner et al. 2002), problematic (Mehle & Trdan 2012) or limited to the second instar larvae of some species (Vierbergen et al. 2010), only adult Thysanoptera were identified to the species level based on published literature (Bournier 1960; Palmer et al. 1992; Mound et al. 1976; Mound & Kibby 1998; CSIRO 2015). The larvae of Thysanoptera were only identified up to the family level using the identification key of Moritz (1994).

Population dynamics and spatial distribution of Thysanoptera on alfalfa

The monthly population fluctuation of species and larvae of Thysanoptera collected from the alfalfa plots in Kopais Valley between 2007–2008 was presented as the mean number of individuals/10 shoots (± SEM) (Fig. 2). The number of individuals collected were also analyzed and compared at α

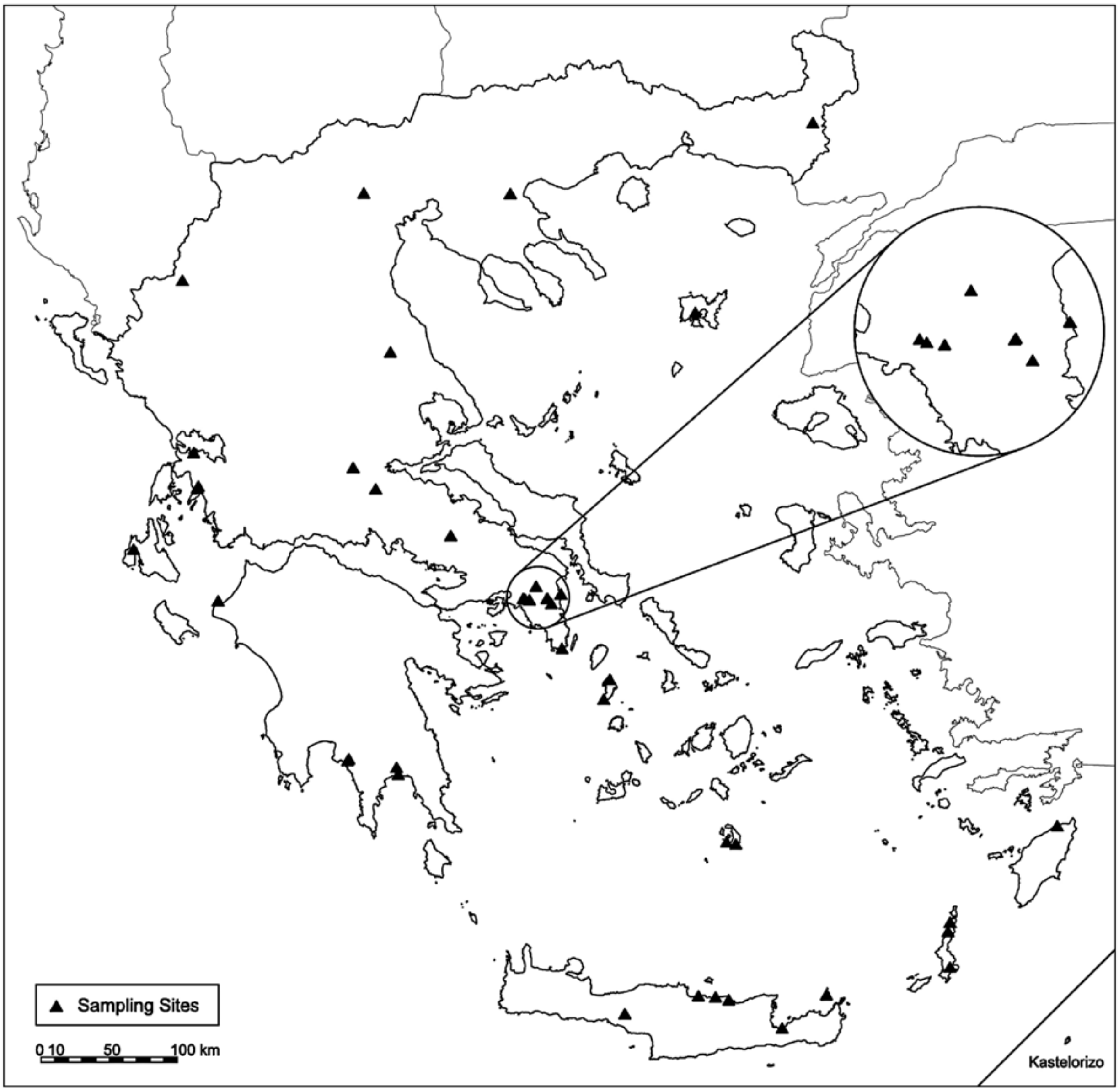

Fig. 1. Map of Greece with all sampling sites of *Medicago* species between 2007–2013 for the study of Thysanoptera

= 0.05 using the GLM of SAS JMP 7.0.1. statistical package. For the data analysis the larvae of Thysanoptera were combined into a single group (Reitz et al. 2003; Baez et al. 2011; Wang et al. 2014).

The spatial distribution of the thrips species recorded in Kopais Valley was estimated using Taylor's power law and Iwao's regression of patchiness. Taylor's power law (1961) presents the linear regression between variance and sample mean after the logarithmic transformation of the equation: $s^2 = a\bar{x}^b$. In this equation s^2 is the variance, a is a coefficient depending on the sample size, the sample mean ($\bar{x}$) and b the slope of the straight line which is used to define spatial distribution as uniform ($b < 1$), random ($b = 1$) and aggregated ($b > 1$) (Davis 1994). The equation of Iwao's regression of patchiness (1968), $\dot{x} = a + b\bar{x}$, represents the regression of the mean crowding, $\dot{x}$, and the mean, $\bar{x}$. Parameter a is called the index of basic contagion and parameter b is the density contagiousness coefficient. Parameter a is the average number of other individuals present in the same quadrat or sampling unit with a given individual and parameter b is the index of aggregation of the population (Davis 1994; Subramanyam & Hagstrum 1996). If $a > 0$, the basic component is a colony, if $a = 0$ the basic component is a single individual and if $a < 0$ there is a tendency of repulsion among individuals. Parameter b measures the spatial distribution of the clumps. If $b > 1$ the clumps are aggregated, if $b = 1$ the clumps are randomly distributed and if $b < 1$ the clumps are distributed regularly (Davis 1994). The values of a and r (correlation coefficient of the line) were tested for departure from 0 and b for departure from 1 using Student's t-test at $n - 2$ degrees of freedom (Davis 1994) ($\alpha = 0.05$).

Results

A total of 14,614 individuals (larval and adult stages of thrips) were collected from 1,931 foliage samples and 198 litter samples of medics from many parts of Greece

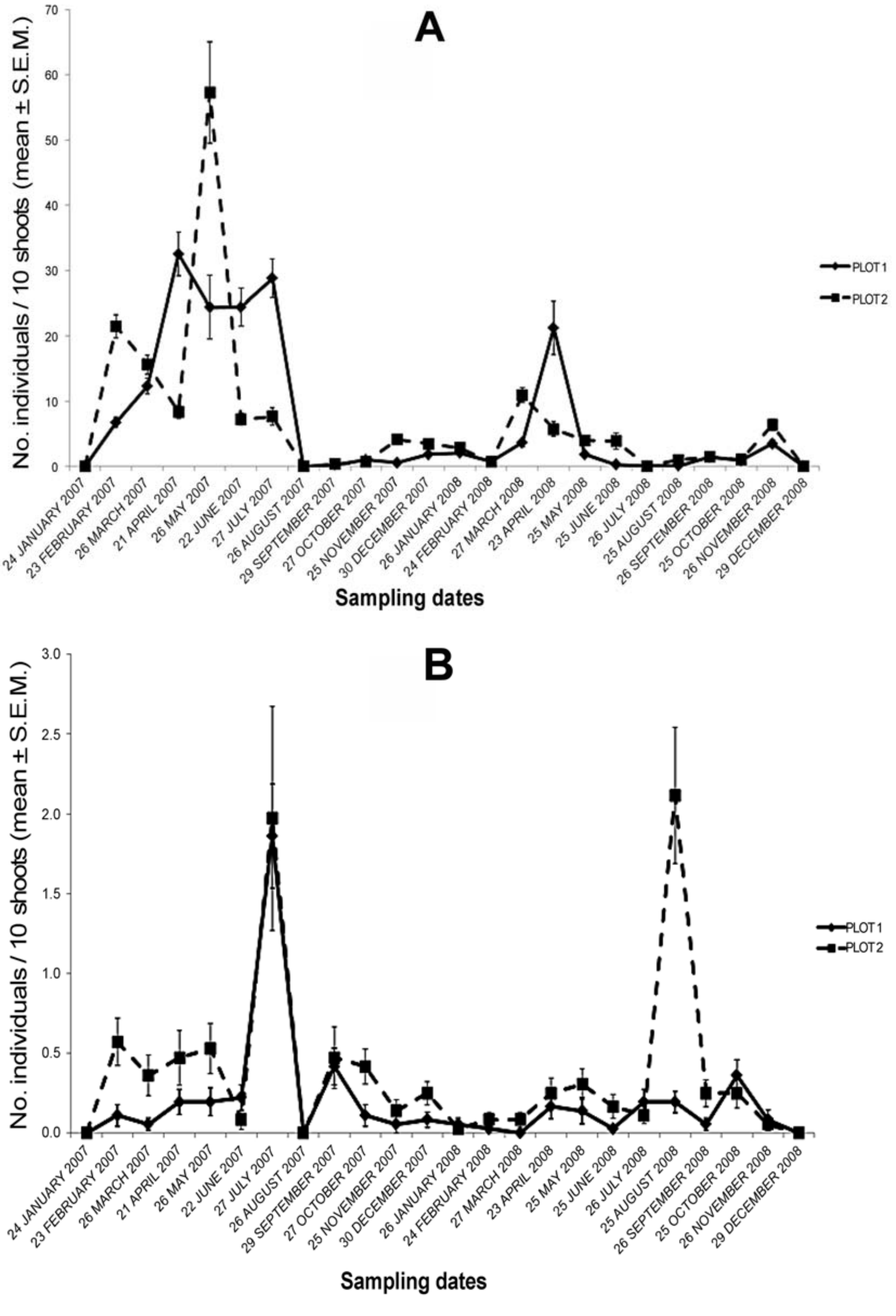

Fig. 2. Monthly fluctuation of the mean population density of thripid larvae (A) and *Frankliniella occidentalis* (B) in alfalfa hay plots (1 and 2) in Kopais Valley (Central Greece) between 2007–2008

between 2007–2013 (Appendix 1, 2). Thrips were collected from eleven out of twelve species of *Medicago,* except *Medicago rigidula* (L.) All. All Thysanoptera belonged to the families Aeolothripidae, Phlaeothripidae and Thripidae. Eight species of Thysanoptera were recorded in the foliage and four species in litter samples. Eight thrips species were recorded in alfalfa, whereas five species in the rest of the medics. *Thrips tabaci* was commonly found among medics followed by *F. occidentalis* (Appendix 1, 2).

As regards Kopais Valley, 6,256 individuals were collected from plot 1 and 6,255 were collected from plot 2 between 2007–2008. Almost the same thrips species were recorded in the foliage of both plots (Ap-

Table 1. Parameters of Taylor's power law and Iwao's regression of patchiness for the study of the spatial distribution of *Frankliniella occidentalis* in the foliage of alfalfa hay plots 1 and 2 in Kopais Valley (Central Greece) between 2007–2008

		Taylor's power law			Iwao's regression of patchiness		
Plot	n^1	log $(a)^2$	b^3	r^4	a^5	b^6	r^7
1	20	0.16 ± 0.06*	1.09 ± 0.06*	0.95*	0.12 ± 0.08	1.42 ± 0.18*	0.77*
2	21	0.38 ± 0.06*	1.37 ± 0.08*	0.93*	–0.32 ± 0.30	3.43 ± 0.43*	0.77*

Explanations: [1] Number of mean – variance and mean – mean crowding pairs used in the regressions; [2,3,4] Parameters of Taylor's power law. Parameters log (a) and b (± S.E.) and correlation coefficient *r;* [5,6,7] Parameters of Iwao's regression of patchiness. Parameters a and b (± S.E.) and correlation coefficient *r;* * significant difference of parameters log (a), a and r from 0 and parameter b from 1 in both models ($\alpha = 0.05$, *t*-test) at $n - 2$ degrees of freedom.

pendix 1). *Frankliniella occidentalis* made up approximately 88.29% of adults in plot 1 and 93.02% of adults in plot 2. The larvae of Thripidae were more abundant than the adults with a similar population fluctuation in both plots and with higher densities in spring and lower ones during summer, autumn and winter (Fig. 2A). The population fluctuation of *F. occidentalis* was similar between the two plots, although higher population densities were recorded in summer than in spring during the two-year study (Fig. 2B). The mean population density of thripid larvae was not significantly different between the plots (Plot 1: 7.0232 ± 0.4961, Plot 2: 6.8495 ± 0.5364, df = 1, $\chi^2 = 0.0565$, $P = 0.8121$). In contrast, the mean population density of *F. occidentalis* significantly differed between the two plots, being higher in plot 2 (Plot 1: 0.1921 ± 0.0220, Plot 2: 0.3712 ± 0.0424, df = 1, $\chi^2 = 13.9852$, $P = 0.0002$). Taylor's power law fitted better to the data compared to Iwao's regression of patchiness, but both methods estimated as aggregated the spatial distribution of *F. occidentalis* (Table 1). The basic component of the population of *F. occidentalis* was a single individual within each foliage sample (parameter a of Iwao's regression of patchiness not significantly different from zero in both plots) (Table 1).

Discussion

Taking into account that thrips can be found almost on any part of plants many authors erroneously consider "host plant" every plant on which adult thrips can be recorded (Mound 1997). According to Monteiro (2002) a "host plant" is a plant species which thrips use to feed on, lay their eggs and develop. Thrips species recorded on plants can be vagrants, but published studies rarely distinguish plant species serving for foraging from those that constitute reproductive hosts for thrips (Ripa et al. 2009). Therefore, the term "host plant" is poorly defined and not always used correctly and this is the reason why we use it with concern in the present study.

About 106 species of Thysanoptera have been reported so far from the Greek mainland (Fauna Europaea 2015), although there is no complete checklist of the thrips species of Greece. In the present study thrips species recorded from foliage and litter samples of several medics of Greece are presented. Quantitative data concerning thrips collected from the foliage of two alfalfa hay experimental plots in Kopais Valley (Central Greece) are also given. In the current study nine species of Thysanoptera were recorded, among which two new species to the Greek fauna, *Pseudodendrothrips mori* Niwa, 1908 and *Sericothrips bicornis* (Karny, 1910) of the Thripidae. The first species is reported to infest the genera *Morus* and *Ficus* (Moraceae) (CSIRO 2015) and is spread worldwide including Italy, France, Slovenia and Spain (DAISIE 2015), but the occurrence of one individual of this species on alfalfa must be considered accidental. *Sericothrips bicornis* is quite spread in Europe and is associated with plant species of the Fabaceae, except medics (Andjus et al. 2008). In the present study it was found in a litter sample of *Medicago lupulina* L. as well as in foliage samples of alfalfa. *Bebelothrips latus* Buffa, 1909 (= *Trachythrips flavicinctus* Bournier, 1960) (Phlaeothripidae) has been recorded in litter samples of *Quercus ilex* L. in France by Bournier (1960) and under trees and bushes of *Quercus trojana* Webb in Italy (de Marzo 2005). This species is also known from Spain, found in forests of *Juniperus* (Berzosa & Maroto 1986) and is considered a soil–dwelling species (de Marzo 2005) as well as a hyphae feeder (Marullo & de Grazia 2013). It has been also recorded on Adelfi islets in North Sporades (Aegean Sea) with no other information (GBIF 2015). *Haplothrips aculeatus* (F., 1803) (Phlaeothripidae) has been also recorded on the island of Skiathos in North Sporades (Aegean Sea) without any other information (GBIF 2015). This species has been found on alfalfa in Croatia (Raspudić et al. 2009), Slovenia (Trdan 2002) and Tuscany (Italy) (Conti 2009) and is known for feeding on grassy weeds, cereals and sugar cane in Asia and Europe (Lewis 1997b). *Ceratothrips frici* (Uzel, 1895) (Thripidae) is associated with the family Asteraceae and *Chirothrips manicatus* (Haliday, 1836) (Thripidae) is usually recorded on species of the Poaceae (Mound et al. 1976; Trdan 2003) and *Asparagus officinalis* L. (Trdan 2003). *Ceratothrips frici* is a synonym of *Tenothrips frici* (Uzel, 1895) (Bhatti 1990), which has been recorded in Greece by Deligeorgidis (2002) on tomato (*Lycopersicon esculentum* Mill.). Both *C. frici* and *C. manicatus* have been also recorded by Jenser & Tzanakakis (1985) in Central Macedonia; the first one on *Hypericum perforatum* L. (Clusiaceae) as well as on an unidentified species of *Tragopogon* (Poaceae), whereas no report of plant species is given over *C. manicatus* by the same authors. The latter species has been recorded in Central Macedonia by Argyriou & Kourmadas (1985) captured with sticky traps. This species has been also found by Deligeorgidis (2002) in many parts of Greece on plant species of the genera *Amaranthus*, *Hordeum*, *Lycopersicon*, *Quercus* and *Trifolium*. The predatory species *A. intermedius*, which is the most common of the genus *Aeolothrips* in Europe, is considered a significant species of biological control (Trdan 2005). It has been recorded by Argyriou & Kourmadas (1985), Jenser & Tzanakakis (1985) and Deligeorgidis (2002) on many cultivated and spontaneous plants of mainland Greece. The polyphagous species *F. occidentalis*, which is also considered a pest of ornamental plants (Trdan 2003), has been recorded on many plant species in Greece, except medics (Deligeorgidis 2002; Anagnou-Veroniki et al. 2008). However, alfalfa is one of many host plants of *F. occidentalis* (Lewis 1997b; Ripa et al. 2009) along with *T. tabaci* (Lewis 1997b). The latter has been also recorded on alfalfa in Greece by Emmanouel et al. (1994) and Deligeorgidis (2002). Other thrips species that have been recorded on alfalfa in Greece include *Sericothrips staphylinus* Haliday, 1836,, an unidentified species of the genus *Haplothrips* from Kopais Valley (Emmanouel et al. 1994) as well as *Thrips angusticeps* Uzel, 1895 from other parts of mainland Greece (Deligeorgidis 2002). Studies of the arthropod fauna of cultivated alfalfa in Greece have mainly emphasized insect pests (Lykouressis et al. 1991) and mites (Emmanouel et al. 1994; Badieritakis et al. 2012; Badieritakis et al. 2014).

Most of the thrips collected from the foliage of cultivated alfalfa in Kopais Valley (Central Greece) were thripid larvae. *Frankliniella occidentalis* was the most abundant species among adults and we speculate that a large number of the larvae collected belonged to this species. It is known that the Thripidae are an important family of phytophagous species (Kirk 1997a). However, the current study does not intent to treat the population of thripid larvae collected as if they constituted one biological species, since the species composition of these larvae is unknown and all thrips species do not share the same bioecological traits and habits. Thripid larvae and *F. occidentalis* tented to present a seasonal population fluctuation pattern in cultivated alfalfa in Kopais Valley. Thripid larvae presented high population densities in spring and lower ones in the rest of the year. Spring in Kopais Valley provides thrips with favorable temperature and humidity conditions, since it is known that these insects are sensitive to dry conditions and heat (Kirk 1997b). In late autumn and winter only dry leaves and hard shoots usually remain on alfalfa, which cannot support thrips populations sufficiently. In contrast, the population of *F. occidentalis* peaked in summer, although in quite low numbers. Deligeorgidis et al. (2002) also reported that the population of *F. occidentalis* peaked in July and August in a cotton field in the area of Terpsithea (Larissa, Central Greece). The larvae of *F. occidentalis* are more sensitive to high temperatures followed by adult females (Kirk 1997b).

Although the present study did not aim to investigate the impact of the different number of cuttings of cultivated alfalfa on thrips populations, some conclusions can be drawn over the presented results. The literature survey provides many reports about the reestablishment of the population densities of thrips in the long term between cut and uncut cultivated areas. Stoltz & McNeal (1982) reported that the population of *F. occidentalis* increased slightly in adjacent bean fields just after cutting alfalfa hay fields, during a three-year study, although their population rapidly recovered in alfalfa. Hossain et al. (2002) mentioned a significant movement of arthropod pests and natural enemies from cut alfalfa hay plots to adjacent uncut ones just after harvesting, but this directional movement was not significant three days after harvesting. Therefore, we assume that the population of thripid larvae reestablished in plot 1 in the long term, but this was not the case with the adults of *F. occidentalis* which were possibly intensely disturbed by the frequent harvesting of alfalfa in plot 1. However, more replications are needed to fully investigate the role of the number of cuttings of alfalfa on thrips populations.

The spatial distribution of *F. occidentalis* in the aforementioned plots was estimated as aggregated based on parameter b of both methods. Parameter a of Iwao's regression of patchiness did not significantly differ from zero, possibly due to the low population density of *F. occidentalis* in both plots. So far, many relevant scientific studies have reported an aggregated pattern of spatial distribution of thrips species on plants. Thrips tend to aggregate, no matter their biological stage (juveniles or adults) and the sampling system used (Deligeorgidis et al. 2002; Mateus et al. 2004; Seal et al. 2006; Sedaratian et al. 2010). However, Kirk (1997b) stressed that the usual occurrence of aggregated patterns in thrips populations may arise from the heterogeneous sampling units, which does not necessarily reflect their real spatial distribution, which might be random. Ripa et al. (2009) stated that *F. occidentalis* was intensely aggregated in the terminal buds during the non-flowering phase as well as in the flowering inflorescences in alfalfa crops in Central Chile.

To conclude, only alfalfa is a host plant for *F. occidentalis* and *T. tabaci*, whereas some thrips species recorded in the present study are associated with other plant families. It is not clear if the rest of the phytophagous thrips species found on the medics sampled are simply vagrants or use the latter as host plants. Despite this fact, the current study contributes to the knowledge of the Greek fauna of Thysanoptera and presents data relating to the population ecology of thrips in cultivated alfalfa, which are generally scarce.

Acknowledgements

The authors would like to thank the members of staff of the Farm Unit of the Agricultural University of Athens (AUA) for their support in Kopais Valley and Prof. Gábor Jenser for identifying *H. aculeatus* and *S. bicornis*. The authors are also grateful to the Laboratory of Plant Breeding and Biometry of AUA for their help with SAS JMP 7.0.1., Dr. Eleftheria V. Kapaxidi, Dr. Spiros A. Antonatos and doctoral students Ioanna Ch. Lytra and Georgios I. Chintzoglou for helping with the samplings and Dr. Anthony Prokos for designing the map of sampling sites.

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Received September 5, 2014
Accepted February 12, 2015