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Management system affects the occurrence, diversity and seasonal fluctuation of Auchenorrhyncha, potential vectors of *Xylella fastidiosa*, in the olive agroecosystem

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Abstract

Occurrence, seasonal abundance and diversity of *Xylella fastidiosa* Wells *et al*. potential vectors species and other Auchenorrhyncha were studied in olive orchards under different management systems. Auchenorrhyncha were monitored monthly in 6 olive groves at Istiea (Central Greece) and Chania (South Greece) for 13 months using malaise traps and sweeping-net, as a sampling strategy to collect even the most, or the least-mobile Auchenorrhyncha within the orchards. Olive orchards in Chania had a lower abundance and diversity in *X. fastidiosa* potential vectors, as well as in total Auchenorrhyncha. The abandoned olive orchard hosted the highest population of potential vectors in Chania, but the lowest in Istiea, compared with the respective cultivated one. The organic farms were more diverse in Auchenorrhyncha compared to the other management systems in both regions. Weed control provided the most probable explanation for the significant Auchenorrhyncha population fluctuation among olive orchards. However, insecticide applications against olive fruit fly did not affect Auchenorrhyncha abundance. The conventional orchard in Istiea displayed the highest abundance and species richness of the potential vectors of *X. fastidiosa*, mainly during autumn, when dried weeds remained in the orchard after herbicide application.

Key words: olive quick decline syndrome (OQDS), potential vector, spittlebugs, Aphrophoridae, conventional, organic, abandoned.

Introduction

Olive is one of the most emblematic cultivations in the Mediterranean basin and a significant source of income for agricultural areas. In Greece, olive is one of the most important crops, covering over 900 thousand ha with more than 170 million trees. This area represents 17% of total cultivated land and 75% of the fruit tree plantations (Hellenic Statistical Authority, 2020). These data highlight the magnitude of the potential devastating effects on the agricultural sector in case of a serious pathogen such as the quarantine bacterium *Xylella fastidiosa* Wells *et al*. establishes and spreads in Greece.

Among the three relevant olive management in Greece {conventional [complying with EU Common Agricultural Policy (CAP)], organic [according to European Union (EU) legislation (Council Regulation (EC) 834/2007)] and integrated (according to the agri-environmental and sustainable development requirements of the EC 2078/ 92 and 1257/99)}, the conventional and organic systems are the most common regimes. In conventional management systems, synthetic insecticides are widely used against the key -*Bactrocera oleae* Rossi- as well as other secondary pests of olives [i.e. *Prays oleae* (Bernard), *Closterotomus trivialis* (Costa), *Rhynchites cribripennis* Desbrochers, *Saissetia oleae* (Olivier), *Parlatoria oleae* (Colvee)], while weed control is based on synthetic herbicides. On the other hand, in the organic management system, weed control involves conservation of ground cover vegetation (i.e. *Oxalis pes-caprae*) to suppress noxious weeds, mechanical means for weed destruction, and limited use of insecticides compatible with the organic farming (Berg *et al*., 2018). *X. fastidiosa* is a gram-negative bacterial plant pathogen that causes the devastating disease of "olive quick decline syndrome" (OQDS). It has considerable genotypic and phenotypic diversity and has been classified, to date, into four accepted subspecies: *X. fastidiosa fastidiosa*, *X. fastidiosa pauca*, *X. fastidiosa multiplex* and *X. fastidiosa sandyi.* In 2013, *X. fastidiosa* ssp. *pauca* was detected in Europe in the Lecce region of Apulia, Italy causing OQDS that affected 10,000 ha of olive trees leading to quarantine measures being imposed around the infected area (EFSA Panel on Plant Health, 2015). Up to date, infections of different *X. fastidiosa* subspecies have been reported in France, Spain, Portugal and Germany (EPPO, 2016; 2018; Marchi *et al*., 2018; Saponari *et al*., 2019; EFSA Panel on Plant Health *et al*., 2019).

X. fastidiosa is transmitted exclusively by xylem-feeding Auchenorrhyncha (Hemiptera) belonging to the families Cicadellidae (sharpshooters), Aphrophoridae (spittlebugs) and Cercopidae (froghoppers) (Frazier and Freitag, 1946; Hewitt and Houston, 1946; Redak *et al*., 2004; Severin, 1947). According to Purcell (1989) and more recently to EFSA (Di Serio *et al*., 2019), every xylem fluid-feeding hemipteran should be regarded as a potential vector of the bacterium. In Europe, among a total of 15 species tested, the pathogen has been recently detected in individuals belonging to the xylem feeders *Philaenus spumarius* (L.), *Philaenus italosignus* Drosopoulos et Remane, *Latilica tunetana* (Matsumura) and *Neophilaenus campestris* (Fallen) (Hemiptera Aphrophoridae) collected from olive orchards in Italy (Ben Moussa *et al*., 2016; Cavalieri *et al*., 2019; Panzavolta *et al*., 2019). Although it was previously thought that only xylem-feeders could be infected by the bacterium, Elbeaino *et al*. (2014) reported that the phloem feeder *Euscelis lineolatus* (Brulle) (Hemiptera Cicadellidae), collected from the infected area of Apulia carried the bacterium - even though it has not been demonstrated that this species (as well as other phloem feeders) is capable of transmitting it. Similar results were also reported from the study of Ben Moussa *et al*. (2016). The fact that *E. lineolatus* was found to host the bacterium indicated that specialized phloem feeders, such as Deltocephalinae leafhoppers, could come into contact with xylem vessels during feeding and become infected. This hypothesis was proved by Chuche *et al*. (2017), who found that many individuals of the phloem feeder, *Scaphoideus titanus* Ball (Hemiptera Cicadellidae), reached the grapevine xylem tissue quickly and probed it for extended periods. The potential of phloem-feeders to act as potential *X. fastidiosa* vectors highlights the importance of investigating not only the xylem feeders, but also the entire Auchenorrhyncha fauna. This being the case, research priorities are focused on species composition of the Auchenorrhyncha fauna, identification of potential vectors, observation of their abundance in olive crops across the country, and their seasonal fluctuation and voltinism. In Italy, while an abundance of data concerning Auchenorrhyncha fauna exists in general (Guglielmino *et al*., 2000; 2005; 2015), knowledge of the Auchenorrhyncha populations in olive orchards before the introduction of *X. fastidiosa* was scarce. However, following the introduction of *X. fastidiosa* in Europe, studies on the Auchenorrhyncha fauna and the host preference in olive orchards were prioritized and very soon accomplished not only in Italy (Elbeaino *et al*., 2014; Ben Moussa *et al*., 2016; Cornara *et al*., 2017; Dongiovanni *et al*., 2019; Bodino *et al*., 2020), but also in other European countries (Lopes *et al*., 2014; Morente *et al*., 2018; Tsagkarakis *et al*., 2018; Albre and Gibernau, 2019; Antonatos *et al*., 2020).

Previous studies on Auchenorrhyncha in olive orchards in Greece, have confirmed the presence of potential *X. fastidiosa* vectors with the most important representatives being *P. spumarius* and *N. campestris.* Molecular analysis of field-collected adults has confirmed the absence of the bacterium in them (Tsagkarakis *et al*., 2018; Antonatos *et al*., 2020). However, studies of Auchenorrhyncha fauna should continue, in order to gain a better knowledge of the seasonal appearance and host preference of potential vectors. Moreover, it is essential to understand the ecology and biology of these insects in order to establish effective control strategies as a means of mitigating the risk or/and countering a possible OQDS outbreak in Greece.

A wide range of plants can host *X. fastidiosa* potential oligophagous or polyphagous vectors (Halkka *et al*., 1977; Thompson, 1994). The most known vector in Europe, *P. spumarius*, is a very polyphagous insect with more than 500 host-plants (Ossiannilsson, 1981; Cornara *et al*., 2018). Its nymphs are often found on herbaceous plants that are more abundantly available, such those of the genera *Galium*, *Silene*, *Sonchus* (Latini *et al*., 2019; Bodino *et al*., 2020). Similarly, other spittlebugs of *Philaenus* and *Neophilaenus* genera in their nymphal stage are found on herbaceous plants (Drosopoulos *et al*., 2010; Maryańska-Nadachowska *et al*., 2010). After eclosion, spittlebugs disperse around the olive agroecosystem and infest olive trees during the phenological stages of inflorescence emerging and development of flowers (Bodino *et al*., 2020). Apart from the two descriptions of the most preferred host plants by spittlebug juvenile stages in Italy (Dongiovanni *et al*., 2019; Bodino *et al*., 2020), an extended comparison on the occurrence and seasonal abundance of the total Auchenorrhyncha population at the adult stage among olive orchards with different management systems is still lacking. Furthermore, it is essential to compare a conventional with an organic or even an abandoned olive orchard, to determine if the farming practices affect the population size and the richness of the Auchenorrhyncha fauna. In this study, the Auchenorrhyncha of olive orchards, located in Central (Euboea) and Southern (Crete) Greece, was investigated over a 13-month period. The investigation included the monitoring of Auchenorrhyncha species in different management systems, as well as in abandoned orchards. The hypothesis of the study was that less intensified agricultural management, or the absolute lack of management practices, generally supports greater taxa abundance and diversity, while farming practices applied under conventional olive production are expected to reduce the Auchenorrhyncha community. Therefore, the main objectives of this study were to identify and record the occurrence, abundance and diversity of *X. fastidiosa* potential vector species and other Auchenorrhyncha, in conventional, organic and abandoned olive orchards in Greece.

Materials and methods

In our study, Auchenorrhyncha population parameters in two representative olive producing regions in Greece under different weed and insect management systems with a focus on potential *X. fastidiosa* vectors, were studied.

Sampling areas

The study took place in six pilot olive orchards located in 2 different representative olive production regions in Greece: Istiea, Euboea region (Central Greece) and Chania, Crete (South Greece). Each study region included three neighbouring orchards with different management systems: one complying with organic standards according to European Union (EU) legislation (Council Regulation (EC) 834/2007), the second complying with EU Common Agricultural Policy (CAP) framework describing conventional farming, and the third being abandoned. The characteristics of the pilot olive orchards, in terms of location, olive variety, management systems, farming practices (soil management and pesticide) and weed species, are shown in table 1. All the sampling orchards were part of a wider and continuous olive tree cultivation area

Table 1. Olive orchard characteristics and different cultivation practices applied to each system.

	Istiea			Chania		
	Conventional	Organic	Abandoned	Conventional	Organic	Abandoned
Coordinates	38°57'47.7"N 23°09'03.0"E	38°58'06.8"N 23°07'36.3"E	38°57'46.7"N 23°08'37.7"E	35°28'56.5"N 23°39'49.6"E	35°26'3058"N 23°45'4173"E	35°29'1962"N 23°49'1508"E
Trees / cultivar[1]	700 / AM	280 / KA	120 / AM	100 / KO	100 / KO	80 / KO
Weed control[2]	SHS	MTT	Not applied	RM	MTT	Not applied
Application time[3]	5, 8	3, 6, 10	-	5, 10	3, 10	-
Main weed species[4]	MA, AV, CA, SO, SH	AV, SH, CI, PA	AV, OX, CN, SA, AS	GS, AV, FV, SO, SH	AV, SO, SH, GS, PL	PI, RO, CC, LA, AV, SO
Pesticide a.i.[5]	λC	SP	Not applied	λC + TH	KA	Not applied
Application time[3]	7, 9	7, 9	-	6, 7, 9	6, 9	-

[1]Cultivar: AM = Amfissis, KA = Kalamon, KO = Koroneiki; [2]Weed control: SHS = Synthetic herbicide spraying, MTT = Mechanical tiller attached on a tractor, RM = Rotary mower; [3]Application time in months of 2018: 1 = January, 2 = February, etc.; [4]Weed species: MA = *Malva* spp., AV = *Avena* spp., CA = *Calendula arvensis*, SO = *Sonchus oleraceus*, SH = *Sorghum halepense*, CI = *Cichorium intybus*, PA = *Phragmites australis*, OX = *Oxalis pes-caprae*, CN = *Calamintha nepeta*, SA = *Sinapis arvensis*, AS = *Asparagus* spp., GS = *Glebionis segetum*, FV = *Foeniculum vulgare*, PL = *Plantago* spp., PI = *Pistacia lentiscus*, RO = *Rosmarinus officinalis*, CC = *Cistus creticus*, LA = *Lavandula angustifolia*; [5]Pesticide a.i.: λC = λ-cyhalothrin 10%, SP = spinosad 0.024%, TH = thiacloprid 24%, KA = kaolin.

with all the surrounding vegetation being olive trees, with only sporadic presence of other trees, shrubs, or other cultivations.

Weed management

In one of the conventional orchards, weeds were controlled by herbicide sprayings throughout the orchard, while in the other one by throughout mowing. In the organic orchards, weeds were controlled by tillage, while in the abandoned orchards no weed control had been applied for about five years prior to the study. Additionally, tillage treatment in organic orchards was applied between and within the tree rows with a distance of at least two meters from the tree bark.

Collection and identification of insects

A white-colour malaise trap was installed in the centre of each olive orchard in order to study the occurrence, seasonal appearance and abundance of Auchenorrhyncha. The dimensions of the malaise trap were 176 cm height at the top end, 110 cm height at the lower end, 165 cm length and 180 cm width, with a 165 × 110 cm^2 interception area. In each trap, a 600-ml plastic container was attached with 98% ethanol as the preservation fluid. The containers were collected at monthly intervals from all the orchards (10th April 2018 to 7th April 2019, 13 insect sample collections). In addition, in order to determine the occurrence of Auchenorrhyncha in specific host plants, samples were taken from olive trees and weeds of the three different orchards, within a radius of 50 m from the malaise trap. During these samplings, which were realized at the same time of the container collection, the more dominant weeds were sampled with entomological sweeping net (39 cm diameter). Each weed species was sampled with 12 sweeps performed on 2 different spots in each orchard. For the olive trees, five groups of 6 trees (30 trees in total, all neighbouring to the malaise trap) were chosen, and each tree's canopy was swept twice. The collected Auchenorrhyncha were aspirated, killed and preserved in ethanol 98%. The collected insects were transferred for identification to the Laboratory of Agricultural Zoology and Entomology of the Agricultural University of Athens (AUA) and the Laboratory of Entomology of the Institute of Olive Tree, Subtropical Plants and Viticulture, Chania. The identification of the captured Auchenorrhyncha was based on taxonomic keys (Ribaut, 1936; 1952; Ossiannilsson, 1978; 1981; 1983; Holzinger *et al*., 2003; Biedermann and Niedringhaus, 2009; Gnezdilov *et al*., 2014; Dmitriev, 2020). Moreover, the entomological collection of the Greek Auchenorrhyncha fauna of the late Sakis Drosopoulos, which is established in the Laboratory of Agricultural Zoology and Entomology of the AUA, was used for comparison and was a very useful tool. To identify the species, male genitalia were dissected and kept in KOH (10%) for 24 h, except for Typhlocybinae, which were kept for 2 h. Subsequently, genitalia were mounted on glass slides with a cavity containing in glycerol and observed under a stereoscopic microscope. Females without a distinct taxonomical character were initially identified at the genus or subfamily level. If all the specimens of a genus in a specific sampling belonged to one species, the female specimens of this genus were added to that species, after being examined for their morphological characters. In the case that the females belonged to more than one species, they were reported as the genus, or subfamily followed by 'sp.', according to its morphotype (e.g., *Mocydiopsis* sp.).

Auchenorrhyncha diversity

The potential vector individuals that were collected were categorized using the criteria of dominance and frequency (Curry, 1973; Cusack *et al*., 1975; Emmanuel, 1977). 'Dominance' indicates the percentage of individuals of a given taxon, compared with the total number of individuals of all taxa found. Hence, a given taxon is classified as 'dominant', 'influent' or 'recedent', if it constitutes >10, 5-10 or <5% of the total number of individuals, respectively. Similarly, three categories are recognized about the 'frequency' of occurrence of an insect taxon in the samples. A taxon is classified as 'constant', 'accessory' or 'accidental', if it occurs in >50, 25-50 or

<25% of the total number of samples, respectively.

Simpson's Diversity Index was used to show differences in diversity among the different regions and management systems (Simpson, 1949):

$$D = 1 - \sum_{i}^{s} \frac{ni(ni-1)}{N(N-1)}$$

ni = number of individuals of a single Auchenorrhyncha species;

N = total number of individuals of all Auchenorrhyncha species.

The value of this index ranges between 0 and 1, and the greater the value, the greater the sample diversity. Thus, the index represents the probability of two individuals selected randomly from a sample belonging to different species.

Results

Auchenorrhyncha occurrence

A total of 7,188 of Auchenorrhyncha adults were collected from the three olive orchards in Istiea and 1,321 from Chania, during the whole sampling period. Numbers of Auchenorrhyncha catches fluctuated among olive orchards (table 2).

X. fastidiosa potential vectors occurrence

During the 13-month sampling period, 393 potential vector adults were collected in total from all the olive orchards of the study: 337 from Istiea and 56 from Chania. From the spittlebugs, the most abundant species was *P. spumarius* (n = 225), followed by *N. campestris* (n = 95), *Lepyronia coleoptrata* (L.) (n = 19) and *Neophilaenus lineatus* (L.) (n = 2). Aphrophoridae was the most abundant family in individuals (n = 341) and species richness (4 species: *P. spumarius*, *N. campestris*, *N. lineatus*, *L. coleoptrata*), while Cercopidae was represented exclusively by *Cercopis sanguinolenta* (Scopoli) (n = 52). It has to be mentioned that from the 127 potential vector individuals collected with the sweep-net, only 3 were collected from the olive tree canopy (October 2018, conventional orchard in Istiea), while all the other were collected from the surrounded weeds.

Auchenorrhyncha seasonal fluctuation

Istiea

Auchenorrhyncha collection showed asynchronous peaks in the different olive management systems, except for the peak in May of 2018 (figure 1). In the conventional orchard, the Auchenorrhyncha captures were highest in September (n = 1,036), while two lower peaks were observed in May (n = 376) and November (n = 314) of 2018. In the organic orchard, the highest peak was observed in May (n = 381), however two lower peaks were observed in November (n = 156) and September (n = 149) of 2018. In the abandoned orchard, the total collected individuals showed three peaks, in May (n = 864), August (n = 396) and October (n = 311) of 2018. Only a few adults were collected between December and March in the conventional and the organic orchard (figure 1). At the same period, Auchenorrhyncha individuals were collected from the abandoned orchard, though in low numbers.

Table 2. Auchenorrhyncha population in olive orchards with different cultivation practices in two different sampling areas.

		Istiea			Chania		
		Conventional	Organic	Abandoned	Conventional	Organic	Abandoned
Cicadomorpha	Cicadellidae	2,760	1,223	2,722	321	356	351
	Agallinae	(28)	(36)	(150)	(2)	(4)	(6)
	Aphrodinae	(0)	(17)	(19)	(1)	(0)	(6)
	Deltocephalinae	(1,163)	(830)	(458)	(155)	(230)	(181)
	Dorycephalinae	(0)	(0)	(2)	(0)	(0)	(0)
	Hecalinae	(1)	(2)	(0)	(0)	(0)	(0)
	Iassinae	(4)	(0)	(2)	(0)	(0)	(0)
	Idiocerinae	(0)	(0)	(0)	(1)	(4)	(7)
	Macropsinae	(1)	(0)	(52)	(0)	(0)	(0)
	Megopthalminae	(1)	(1)	(1)	(1)	(3)	(1)
	Typhlocybinae	(1,562)	(337)	(2,038)	(161)	(115)	(150)
	Aphrophoridae	157	88	40	4	25	27
	Cercopidae	25	21	6	0	0	0
Fulgoromorpha	Delphacidae	35	11	38	20	32	9
	Flattidae	0	0	8	0	0	0
	Issidae	8	5	7	33	62	78
	Cixiidae	0	8	26	0	0	0
	Caliscelidae	0	2	0	0	0	0
	Tettigometridae	0	0	0	0	0	2
	Dictyopharidae	0	3	1	0	0	1
Total Auchenorrhyncha		2,985	1,361	2,848	378	475	468

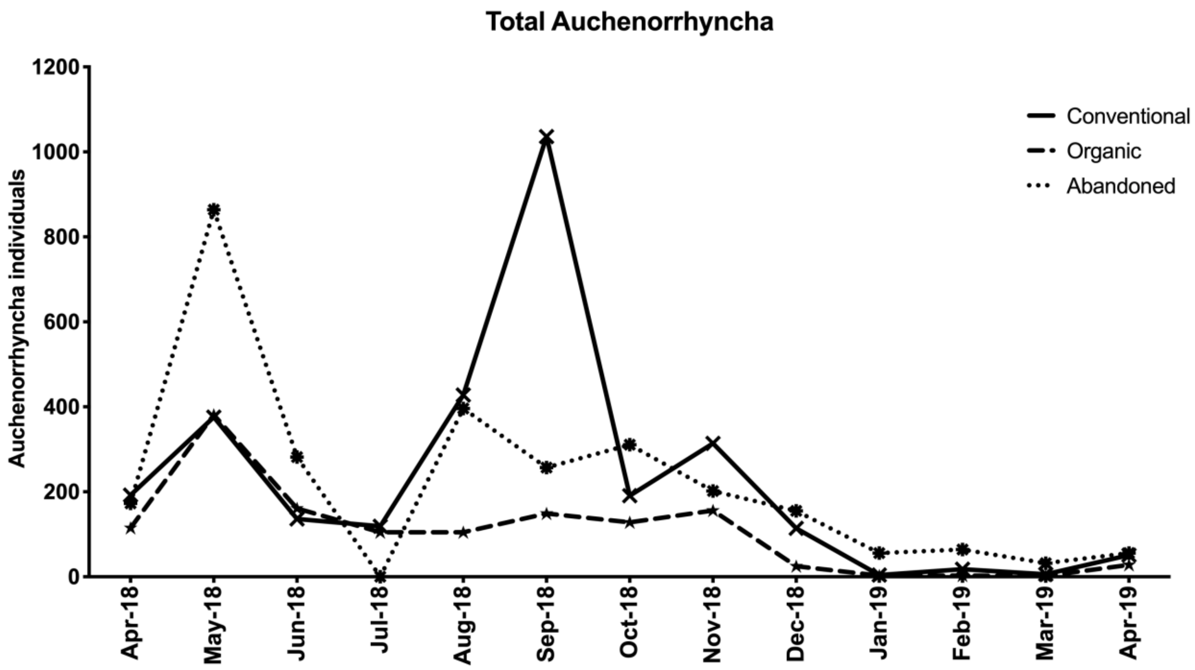

Figure 1. Total number of adults of Auchenorrhyncha captured with malaise traps and sweeping-net from a conventional, an organic and an abandoned olive orchard in Istiea (Central Greece) during the 13-month survey.

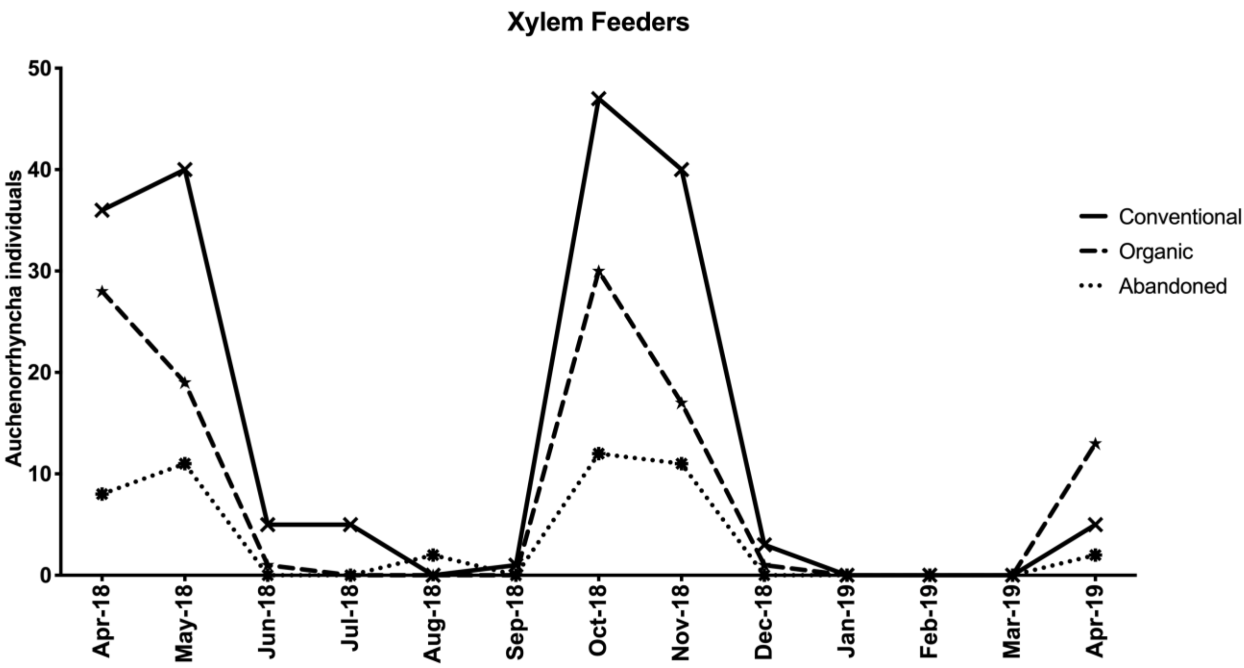

Figure 2. Total number of adults of xylem-feeders captured with malaise traps and sweeping-net from a conventional, an organic and an abandoned olive orchard in Istiea (Central Greece) during the 13-month survey.

In the conventional orchard 182 xylem-feeders were collected, 157 Aphrophoridae and 25 Cercopidae. Their collection showed the highest peak in October 2018 (n = 47), followed by April (n = 36), May (n = 40) and November (n = 40) 2018 peaks (figure 2), with most abundant species being *P. spumarius* (table 3). *C. sanguinolenta* was only present in the samples of April and May. In the organic orchard 109 xylem-feeder individuals were captured (88 Aphrophoridae and 21 Cercopidae), showing two discrete peaks in October and April of 2018 (30 and 28 individuals, respectively) (figure 2). Similarly, in the conventional orchard, *C. sanguinolenta* was present in samples only in April and May. The most abundant xylem-feeder was *N. campestris* (table 3). Interestingly, in the abandoned orchard xylem-feeder's collection was the lowest among the olive orchards, peaking in October 2018 (n = 12) and remaining at the same level in November, with another peak in May 2018 (n = 11) (figure 2). From the 46 individuals

Table 3. Total number, dominance and frequency of potential vectors collected with malaise trap and sweep net from olive orchards with different cultivation systems in Istiea (Central Greece).

Species	Conventional			Organic			Abandoned		
	N	DO	FR	N	DO	FR	N	DO	FR
Lepyronia coleoptrata	14	0.47	17.86	0	0	0	5	0.18	10.71
Neophilaenus campestris	37	1.24	14.29	47	3.45	21.43	9	0.31	7.14
Neophilaenus lineatus	2	0.07	7.14	0	0	0	0	0	0
Philaenus spumarius	104	3.48	25.00	41	3.01	21.43	26	0.91	10.71
Cercopis sanguinolenta	25	0.84	10.71	21	1.54	3.57	6	0.21	7.14

N = number of individuals captured; DO = Dominance (%): percentage of individuals of each taxon among the total number of individuals found; FR = Frequency (%): percentage of each species occurrence in the total number of samplings.

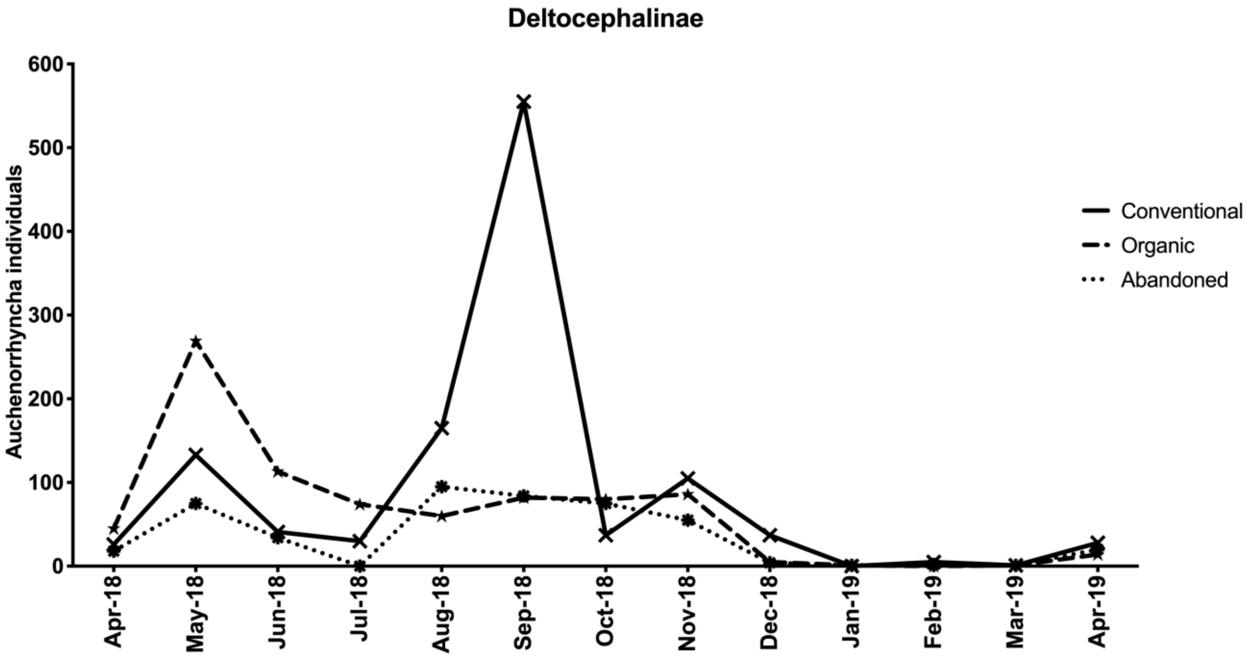

Figure 3. Total number of adults of Deltocephalinae captured with malaise traps and sweeping-net from a conventional, an organic and an abandoned olive orchard in Istiea (Central Greece) during the 13-month survey.

captured, 40 were spittlebugs (26 *P. spumarius*, 9 *N. campestris* and 5 *L. coleoptrata*) and 6 froghoppers (*C. sanguinolenta*) (table 3).

Deltocephalinae population dynamics generally followed the population trend of total Auchenorrhyncha in all orchards sampled (figure 3). In the conventional orchard, their collection showed the highest peak in September 2018 with 555 individuals out of 1,097 collected in total. In the organic orchard, 777 individuals were collected, with their captures peaking in May 2018 (n = 269). In the abandoned orchard, the Deltocephalinae collection reached 399 individuals in total and showed one slight peak in August 2018 (n = 95).

C h a n i a

Total adult Auchenorrhyncha number peaked at very close intervals in the orchards with different management system. Generally, it peaked in late spring-early summer, declined during summer and increased again during fall in all the orchards (figure 4). In the conventional orchard, the Auchenorrhyncha collection was the lowest among the three orchards. It showed the highest peak in May 2018 (n = 60) and two lower peaks in September (n = 46) and November (n = 48) of 2018. In the organic orchard, the highest peak was observed in May (n = 87), while two lower peaks occurred in October (n = 77) and December (n = 78) of 2018. In the abandoned orchard, even though a high peak existed in May (n = 70), the highest peak occurred in September (n = 89) of 2018. Moreover, two lower peaks were observed in November 2018 (n = 44) and in February 2019 (n = 24). In all the orchards, there was an almost equal distribution of the collected Auchenorrhyncha adults collected between spring and fall. It has to be mentioned that the Auchenorrhyncha collection was relatively high in early and mid-winter months, especially in the organic and the abandoned orchard (figure 4).

In Chania, the only xylem-feeders collected belong to Aphrophoridae family. In the conventional orchard, only 4 xylem-feeder individuals were collected, all *P. spumarius*: 2 in April 2018 and 2 in April 2019 (figure 5, table 4). In the organic orchard 25 xylem-feeder individuals were

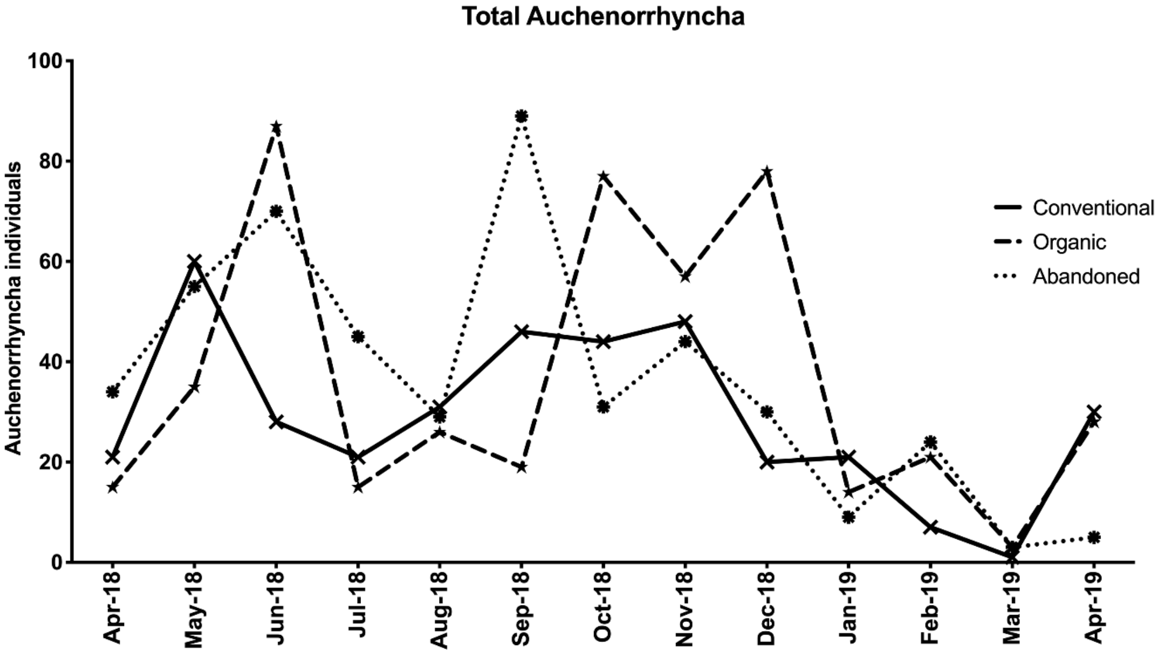

Figure 4. Total number of adults of Auchenorrhyncha captured with malaise traps and sweeping-net from a conventional, an organic and an abandoned olive orchard in Chania (South Greece) during the 13-month survey.

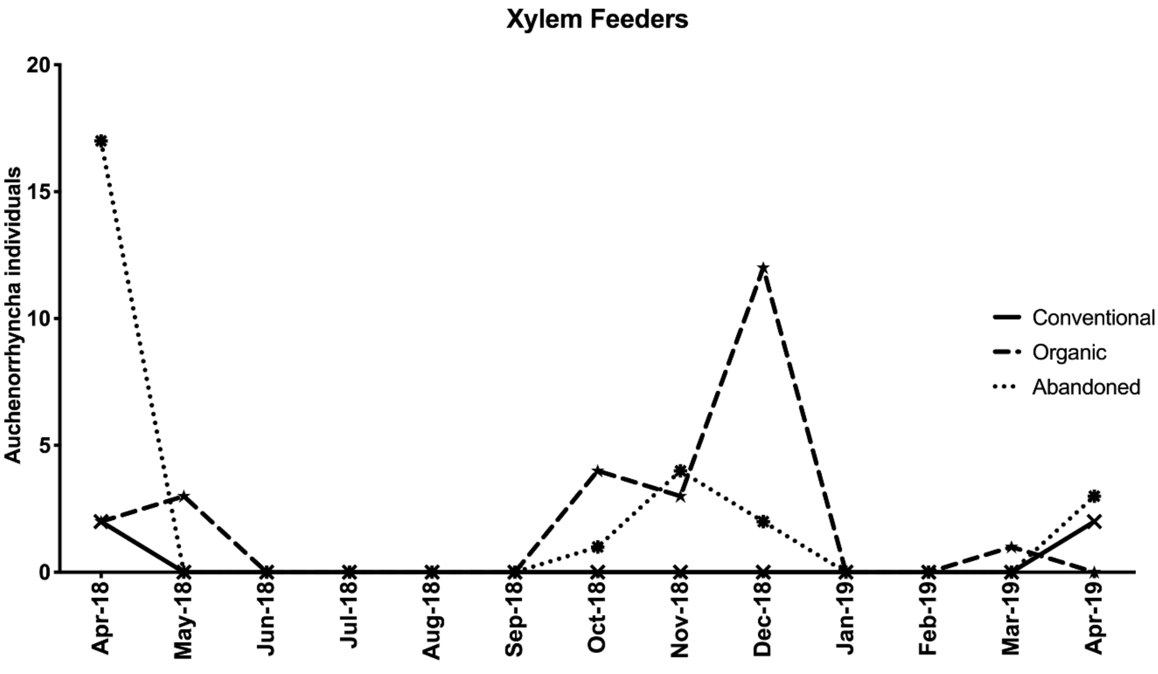

Figure 5. Total number of adults of xylem-feeders captured with malaise traps and sweeping-net from a conventional, an organic and an abandoned olive orchard in Chania (South Greece) during the 13-month survey.

captured (24 *P. spumarius*, 1 *N. campestris*), showing the highest peak in December (n = 12) and two lower peaks in May (n = 3) and October (n = 4) of 2018 (figure 5, table 4). The highest xylem-feeder collection was observed in the abandoned orchard in April 2018 (n = 17) with a smaller peak occurring in November 2018 (n = 4) (figure 5). The total number of xylem-feeders collected was 27 (26 *P. spumarius*, 1 *N. campestris*) (table 4).

Similarly to Istiea's orchards, Deltocephalinae population dynamics followed, in general, the population trend of total Auchenorrhyncha in all the sampled orchards of Chania (figure 6). In the conventional orchard, their collection showed a slight peak in May 2018 (n = 28), with the highest peak observed in October 2018 with 35 individuals, out of 148 collected in total. In the organic orchard, 224 individuals were collected, with their collection peaking in December 2018 (n = 53), with a lower peak in June 2018 (n = 34). In the abandoned orchard, the Deltocephalinae collection was 174 individuals in total and showed two clear peaks, in September (n = 49) and June (n = 29) of 2018.

Table 4. Total number, dominance and frequency of potential vectors collected with malaise trap and sweep net from olive orchards with different cultivation systems in Chania (South Greece).

Species	Conventional			Organic			Abandoned		
	N	DO	FR	N	DO	FR	N	DO	FR
Lepyronia coleoptrata	0	0	0	0	0	0	0	0	0
Neophilaenus campestris	0	0	0	1	0.21	7.69	1	0.21	7.69
Neophilaenus lineatus	0	0	0	0	0	0	0	0	0
Philaenus spumarius	4	1.05	15.38	26	5.05	46.15	26	5.56	38.46
Cercopis sanguinolenta	0	0	0	0	0	0	0	0	0

N = number of individuals captured; DO = Dominance (%): percentage of individuals of each taxon among the total number of individuals found; FR = Frequency (%): percentage of each species occurrence in the total number of samplings.

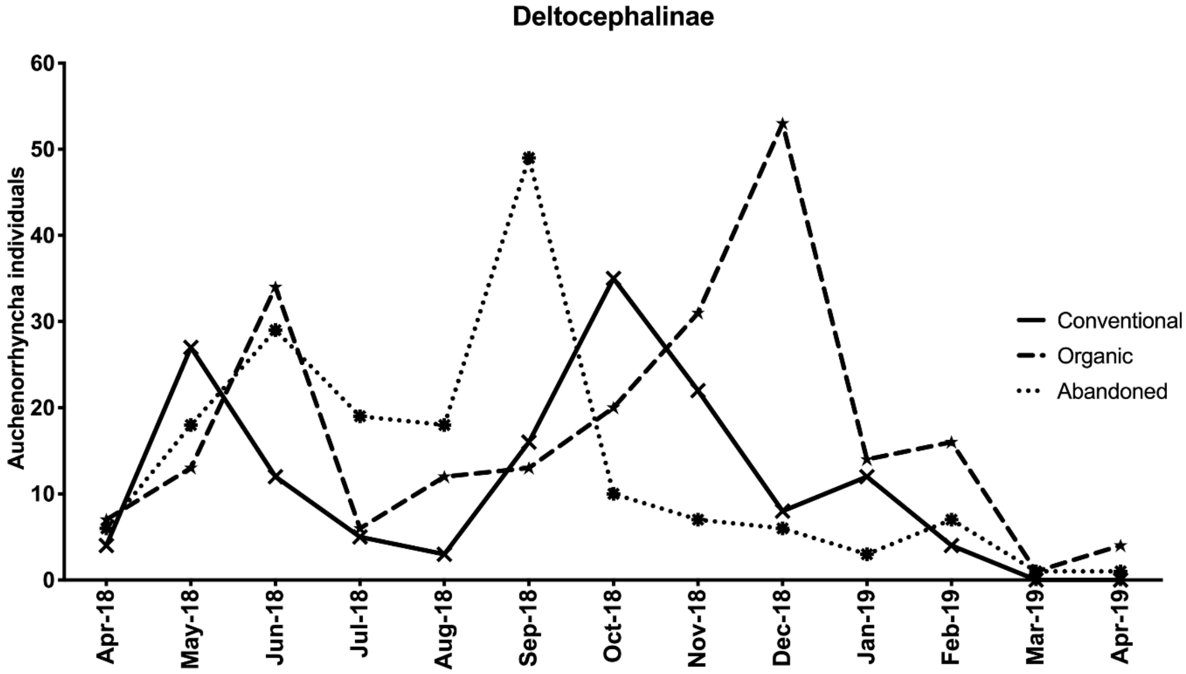

Figure 6. Total number of adults of Deltocephalinae captured with malaise traps and sweeping-net from a conventional, an organic and an abandoned olive orchard in Chania (South Greece) during the 13-month survey.

X. fastidiosa potential vectors diversity

Diversity of potential vector species analysis in terms of dominance and frequency (tables 2, 3) revealed none of the species as dominant, while only *P. spumarius* was influent in the organic and the abandoned orchard in Chania. All the other species were recedent in the examined orchards. In the Istiea conventional orchard 5 potential vector species were collected (table 3). *P. spumarius* was accessory in frequency, while *L. coleoptrata*, *N. campestris*, *N. lineatus* and *C. sanguinolenta* were accidental. In the organic orchard of the same area, 3 vector species were found in the samples (*P. spumarius*, *N. campestris* and *C. sanguinolenta)* all accidental in frequency (table 3). In the abandoned orchard, four potential vector species, all accidental in frequency, were collected: *P. spumarius*, *N. campestris*, *L. coleoptrata* and *C. sanguinolenta* (table 3). The sampled Chania olive orchards were less diverse in potential vector species, since only *P. spumarius* (accessory) and *N. campestris* (accidental) were collected from the organic and the abandoned orchard (table 4). The conventional orchard in the same area was the least diverse of all, since only *P. spumarius* was collected accidentally in frequency (table 4).

Simpson's diversity index in Istiea was the greatest in the organic orchard (D_{ORGIST} = 0.899), followed by the conventional ($D_{CONVIST}$ = 0.824) and the abandoned orchard (D_{ABIST} = 0.815). In Chania, the organic orchard was again the most diverse (D_{ORGCHA} = 0.905), followed by the abandoned (D_{ABCHA} = 0.883) and the conventional orchard ($D_{CONVIST}$ = 0.779).

Discussion

Differences in occurrence, abundance, species diversity and population fluctuation were recorded between the 2 different regions.

Overall population levels and diversity

The total Auchenorrhyncha collection recorded in Istiea (Central Greece) was almost five times higher than in Chania (Crete, South Greece). This is in accordance with the findings of Tsagkarakis *et al*. (2018) who reported that the Auchenorrhyncha collection in Central Greece (Fthiotida region) was higher than that in South Greece (Chania, Crete). Apart from the fact that Crete is a quite distant from the mainland island -a fact that could be responsible for the lack of certain species- other critical factors responsible for this difference may be higher relative humidity (RH) and cumulative rainfall recorded in Istiea compared to Chania, especially during the summer (66.86% RH - 20.7 mm in Istiea, 56.26% RH - 9.8 mm in Chania). Hashem *et al*. (2009), Sharma and Singh (2012) and Pinedo-Escatel and Moya-Raygoza (2018) confirmed the positive impact of moisture to the Auchenorrhyncha collection levels which favours weed food-plant availability and saves insects from high water stress.

Incidence of potential *X. fastidiosa* vectors

Six European potential vectors of *X. fastidiosa* (EFSA Panel on Plant Health, 2015) were present in the samples: *P. spumarius*, *P. italosignus*, *N. campestris*, *N. lineatus*, *L. coleoptrata* and *C. sanguinolenta.* Three of them, *P. italosignus*, *Philaenus spumarius* and *N. campestris* have been identified in Italy (Elbeaino *et al*., 2014). Individuals from all species -except *P. italosignus*- were collected from Istiea orchards, while only *P. spumarius* and *N. campestris* were present in samples from Chania. Generally, the most abundant aphrophorid in both sampling regions of the study was *P. spumarius*, with the exception of *N. campestris* which was the most abundant in the organic orchard in Istiea. In Chania, spittlebug collection was very low (only 56 individuals totally) whereas in Istiea, their collection was much higher (285 adult individuals). Their contribution to the total Auchenorrhyncha population was 3.96% in Istiea and 4.23% in Chania, corresponding to a 3.99% and 4.92% of the total Cicadomorpha collected, respectively. These results are closer with previous studies conducted in Greek olive orchards by Tsagkarakis *et al*. (2018), Kalaitzaki *et al*. (2019) and Koufakis *et al*. (2019) reporting population levels of spittlebugs less than 1.5% of the total Cicadomorpha, while they differ significantly compared to results by Antonatos *et al*. (2020) reporting population levels of Aphrophoridae as high as 24% of the total Cicadomorpha.

In general, the collection of Aphrophoridae was low during the whole sampling period, with one remarkable peak in the conventional orchard in Istiea (late October). No spittlebugs were collected with the sweep-net from the tree canopy in the organic and the abandoned orchards in both areas. In the conventional orchard of Istiea, all Aphrophoridae individuals were collected from weeds except for only three that were collected from the tree canopy during the collection peak. The low number of collected spittlebugs from the olive tree canopy is in agreement with Morente *et al*. (2018) and Bodino *et al*. (2020), who reported that the peak of spittlebug abundance on olive trees occurs during the phenological stages of inflorescence emerging and development of flowers, due to the high content of amino acids in xylem at these stages in contrast to stages during water stress periods (Drossopoulos et Niavis, 1988; Bodino *et al*., 2019). The high water stress could also explain the extremely low collection of spittlebug adults during summer in all the orchards in Istiea, and their total absence in Chania. As previously described, all the sampling orchards were part of a wider and continuous olive tree cultivation area, thus all the surrounding vegetation were olive trees, with only sporadic presence of other trees and shrubs (e.g. *Quercus* sp., *Acer* sp. etc.).

Effect of pest management strategies on population fluctuation

Weed management

It is well accepted that pest management strategies, especially those concerning the application of pesticides, have a significant effect on the fauna of the ecosystem. For example, Trivellone *et al*. (2012) reported a significant correlation between high insecticide and herbicide pressure and negative consequences on population composition and behaviour of leafhopper communities found in vineyards. Specifically, only highly mobile species managed to escape the consequences of pesticide applications and frequent tillage and rapidly recolonized the managed area by taking advantage of the temporary lack of competition with low mobile species. This seems to be the case in the present study with collection peaks occurring after herbicide applications in the conventional orchard in Istiea (especially in the fall), with the most abundant species being some highly mobile Typhlocybinae and Deltocephalinae [*Zyginidia pullula* (Boheman), *Cicadulina bipunctata* (Melichar) and *Balclutha frontalis* (Ferrari)]. No such collection peak was observed in the conventional orchard in Chania, where herbicide applications were substituted by weed- cuttings using a rotary mower twice during the study period, probably due to the drier climate.

In the case of the organic orchard in Istiea, population curves were much smoother and only low population fluctuations occurred after tillage, with the most abundant species being *E. lineolatus*. This difference could be due to a marginal effect of the organic orchard where edges remained undisturbed and acted as an insect species reservoir, since tillage only affected areas within tree rows, in contrast to herbicide applications in the conventional orchard which included spraying of orchard margins. This probably contributed to the fact that the Auchenorrhyncha diversity remained high: edge grasses are habitats of highly diverse populations of leafhoppers both during wet and the dry season (Pinedo-Escatel and Moya-Raygoza, 2018). A lower difference was recorded between the organic and the conventional orchard in Chania, with the organic Auchenorrhyncha population being slightly higher than the conventional. This can be probably explained by the fact that, although no herbicides were used, weed-cutting was more thorough in the conventional orchard leaving no undisturbed weed reservoirs in the margins.

Spittlebug population trends in the different olive management systems can provide valuable information about their ecology. An outbreak of Aphrophoridae species (*P. spumarius* and *N. campestris*) occurred in the conventional orchard in Istiea during October-November of 2018. It must be noted that weeds in the conventional orchard were controlled exclusively by herbicide applications, with the last herbicide application taking place in September of 2018 while no tillage took place. Cornara *et al*. (2018) reported that removal of ground cover either by mowing, soil tillage or herbicides within and in the surroundings of olive orchards drastically reduced nymphs residing on weed remains and thus the insect collection. However, specifically in the herbicide application, where dried weeds remained in the orchard giving spittlebugs an ideal substrate for oviposition (Weaver and King, 1954), the population remained at high levels. This could be the reason for our results concerning the high abundance of spittlebug adults (almost 90% females) collected from dry weeds -probably due to oviposition purposes- in contrast to only three collected on olive tree canopy. In light of this, the authors suggest that tilling of orchards during the fall season could deter adult spittlebug movement towards the orchards by depriving insects from their preferable weed oviposition substrate, complementing EFSA Panel on Plant Health *et al*. (2019) and Dongiovanni *et al*. (2018) recommendation for winter and spring for spittlebug nymphal population reduction. During spring, spittlebug population levels in the cultivated orchards were much lower than in fall, which is in disagreement with Bodino *et al*. (2020), who reported that spittlebug adults were more abundant in olive agroecosystem soon after their emergence in May/June. Spittlebug preference in specific host-plants, as given by Dongiovanni *et al*. (2019) could explain this difference. Specifically, *P. spumarius* showed preference for some genera of the families Asteraceae, Fabaceae, and Apiaceae, while *N. campestris* preferred genera of the Poaceae family (Dongiovanni *et al*., 2019). In our study the abundance of these weeds in these orchards was low because of the weed management applications in the cultured orchards (conventional and organic) during April and May, probably indicating that adults readily leave the studied areas due to unfavourable conditions resulting in a reduced spittlebug population.

Fruit fly management

No evidence was found supporting that olive fruit-fly bait sprayings influenced Auchenorrhyncha population, xylem-feeders included. A possible reason was that the overall sprayed surface of the tree canopy was just $1/8^{th}$ of the total tree canopy surface in the orchard (the protocol for olive fruit-fly bait sprayings is to spray the north part of the canopy of every second tree in the orchard). Another possible explanation could be the timing of the bait-spraying applications (summer-early fall), that did not coincide with significant levels of the spittlebug population (almost zero during the dry Greek conditions). These results provide a potential preliminary answer to the (EFSA Panel on Plant Health *et al*., 2019) question whether olive fruit-fly bait sprayings can have a significant impact against *X. fastidiosa* potential vectors.

Non-management impact on spittlebug population

Contrary to Mizell *et al*. (2003), who report that an agroecosystem with less-intensified control measures and greater diversity of weeds has higher numbers of leafhoppers, neither the total population nor the diversity of the leafhoppers in the abandoned orchards were the highest in the present study - although this comparison could be limited since abandoned orchards do not necessarily have greater diversity of weeds. Actually, the abandoned orchards were second in abundance in both sampling areas while they exhibited the lowest diversity indexes in Istiea and the second lowest in Chania. Additionally, in the abandoned orchards of both areas no remarkable population fluctuation was recorded, with the exception of Typhlocybinae (*Eupteryx* spp.), where a population upshift was occasionally observed. The results on Auchenorrhyncha diversity are in agreement with Sanderson *et al*. (2019), who reported that the minimum Auchenorrhyncha biodiversity was found in shrub habitats within the first four years after cutting, compared with these been cut between 8-12 years.

Specifically, differences were observed between the two studied regions as far as the abandoned olive orchards are concerned: In Istiea, spittlebug population was the lowest compared with the cultivated ones, without any remarkable population peak. On the contrary, the highest spittlebug population was observed in the abandoned olive orchard compared with the cultivated olive orchards in Chania. The variation in the weed flora composition (Nickel, 2003), especially in the woody plant species in the sampled orchards, may be the explanation for this difference. The characteristic of the abandoned orchards is the high numbers of woody weeds between and within the olive tree rows limiting the herbaceous soil cover which, especially during spring, reduce the available niche for the spittlebug nymphs. Studies indicate that wild woody weeds within the olive agroecosystem may host a considerable number of spittlebugs (Morente *et al*., 2018; Santoiemma *et al*., 2019; Bodino *et al*., 2020). In our study this was the case for Chania, especially during spring and fall wet seasons, but not for Istiea. *Pistacia lentiscus* was of the most dominant woody weed species in Chania orchard, belonging to one of the most preferable host genus according to Bodino *et al*. (2020). None of the most preferable shrubs was present in the abandoned orchard in Istiea, where the most dominant woody weeds recorded belong to the *Rubus* and *Satureja* genera. In the same orchard the highest spittlebug population was observed mostly in fall rather than in spring. This difference can be additionally justified by the high presence of *O. pes-caprae* (the second most dominant weed in the abandoned orchard of Istiea in spring) which according to Dongiovanni *et al*. (2019) does not favour hosting of *P. spumarius.*

It has to be noted that, although froghoppers were the most abundant xylem-feeders in the cultivated orchards in spring, Cercopidae were absent in the abandoned orchards. This absence and the fact that Cercopidae population numbers decreased after weed control on conventional orchards, can be explained by the preference of these species for grassland and herbs that were absent or destroyed (Dietrich, 2009). Furthermore, Cercopis species hibernate in the nymph stage and thus they are present very early (in spring) with adults found only during the summer which could explain the absence of adults collected during spring.

Monitoring methods

Following the emergence of *X. fastidiosa* as a devastating olive tree pathogen, an increasing interest for reliable and cost-effective monitoring methods of *X. fastidiosa* vectors belonging to Auchenorrhyncha has been developed by the scientific community. In the present study as well as in a previous one from Greece (Tsagkarakis *et al.*, 2018), a malaise trap was used in combination with sweeping-net. Malaise traps have certain advantages, including consistency and reliability in catching a flying population, which they fully realized in the present study proving once more that they constitute a very effective tool for capturing large numbers of a diverse array of species. However, they have the limitations of any one single method and need to be complemented with other monitoring methods as indicated by the differences of the spittlebugs collected by the sweep-net at the same dates and same orchards. Malaise traps were efficient for collecting highly mobile insects during migration e.g. to the dried woods in the Istiea conventional orchard during October-November. On the other hand, contrary to malaise traps, sweeping-net is a very effective method for directional sampling in order to determine the population density of spittlebugs from their host plants. Taking these facts into account, the authors suggest that future investigations on the Auchenorrhyncha occurrence would benefit from a sampling strategy which combines different sampling methods, malaise trapping and sweeping-net included (Tsagkarakis *et al*. 2018).

Conclusions

The present study provides information about the occurrence, population fluctuation and species richness of Auchenorrhyncha, with emphasis on *X. fastidiosa* potential vectors in olive orchards in two regions of Greece under conventional, organic and in the absence of a management system. Management systems were diversified in terms of pest management, specifically olive fruit-fly and weed control. The orchards with the highest Auchenorrhyncha species diversity were the organic ones, with that in Istiea having the lowest abundance in Auchenorrhyncha individuals. On the contrary, the conventional orchard of Istiea displayed the highest abundance both in total Auchenorrhyncha and in Aphrophoridae in particular, probably due to the presence of dry weeds following herbicide applications which provided the spittlebugs an ideal substrate for oviposition. This suggests that a targeted soil tillage during fall in the dry agroecosystems of the Mediterranean olive orchards could reduce spittlebug migration to the olive orchards by removing available oviposition sites for the adults. This practice supplements the proposed in previous studies tilling during spring and winter and should contribute to the reduction of spittlebug nymphal population of the following year (Cornara *et al.*, 2018; Dongiovanni *et al.*, 2018). Despite this, the presence of wild vegetation around and within the olive orchards is not discouraged as in the present study it deterred xylem-feeders from migrating to the tree canopy. Effective weed management should benefit from those two observations in order to determine the correct timing and implementation of weed control. Furthermore, the strong water stress could explain the extremely low population of spittlebug adults during summer in all the orchards in Istiea and their total absence in Chania. No significant impact of the insecticide control applied against olive fruit-fly using bait sprayings to the potential vectors population was found most likely because sprays were applied during summer-early fall when the spittlebug population was almost zero. Finally, the significance of abandoned olive orchards, as spittlebug reservoirs, was found to be dependent on the woody weed species present.

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David Theodorou and Ioannis Koufakis performed the experiments, made the draft insect identifications and wrote the paper draft; Zoi Thanou, Ekaterini Chaldeou and Dimitrios Afentoulis made the confirmed the precise insect identifications and wrote the paper draft, Argyro Kalaitzaki designed the experiments, analysed the data and wrote the paper; Antonios Tsagkarakis conceived and designed the experiments, analysed the data and wrote the paper. All authors have read and agreed to the published version of the manuscript.

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