Bulletin of Insectology ***72** (1): 143-149, 2019*
ISSN 1721-8861 eISSN 2283-0332

Aphid species composition in populations from citrus orchards in a region of the island of Crete

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Abstract

Citrus tristeza virus (CTV) is one of the most serious diseases affecting citrus trees worldwide. CTV is dispersed in the field by various aphid species at different transmission efficiencies. As a result, any information about aphid species composition in citrus orchards is essential for epidemic prognosis and disease management. CTV was firstly detected in Greece in 2000 but, extended information on the prevalence of the aphid species in CTV-infected citrus orchards is currently missing. Here, we report data from an 8-year survey carried out over the last decade on the aphid species frequencies infesting citrus orchards in the only CTV transmission hot-spot (Chania region) of the island of Crete. In 9,500 wingless adult females collected mainly in spring-early summer, five aphid species were recorded, including the second most efficient CTV vector *Aphis gossypii* Glover, and the less efficient vectors *Aphis spiraecola* Patch, *Aphis aurantii* (Boyer de Fonsicolombe), *Myzus persicae* (Sulzer) and *Aphis craccivora* Koch. *Aphis citricidus* (Kirkaldy), the most efficient vector of CTV, was not detected. In all years, the most prevalent species was *A. spiraecola* (mean frequency 82.5%) followed by *A. gossypii* (mean frequency 13.5%), which was recorded in six of the eight years of the survey. Both species may play a significant role to the CTV spread in Chania region. As the determination of aphid transmission efficiencies for the prominent CTV isolate (GR-1825) is pending, the current study brings forward a panel of five CTV-aphid vector species in this area. The presence of the relatively efficient vector *A. gossypii* at low-moderate frequencies, in a CTV hotspot is a matter of concern and while monitoring and eradication of CTV infected trees continues, a first detection of *A. citricidus* or any increase of the *A. gossypii* prevalence would have a negative impact on containment measures.

Key words: *tristeza*, *Aphis spiraecola*, *Aphis gossypii*, orange, mandarin, vectors.

Introduction

Aphids can be serious pests in citrus-growing areas under favourable environmental conditions, due to high reproductive rates that allow several generations per year, and the production of winged adults that can spread rapidly and migrate to great distances. Aphids are responsible for direct and indirect damages to host-plants (Blackman and Eastop, 1994; 2000; Barbagallo *et al*., 2017). In citrus trees, direct damage resulting from sap-sucking includes leaf deformation. Indirect damages include the secretion of honeydew, which promotes the development of sooty moulds, and most importantly the transmission of viral diseases (Barbagallo *et al*., 2017). *Citrus tristeza virus* (CTV; Family: Closteroviridae, Genus: *Closterovirus*) causes one of the most destructive diseases of citrus (Bar-Joseph *et al*., 1989; Moreno *et al*., 2008). CTV is disseminated by grafting of virus-infected plant material and from infected trees by some aphid species (Hemiptera Aphididae) in a semi-persistent manner (Bar-Joseph *et al*., 1989; Marroquín *et al*., 2004; Moreno *et al*., 2008). According to previous reports, by early 2000 CTV had killed >55 million trees in the Mediterranean Basin (Marroquín *et al*., 2004). In Greece, CTV was firstly detected in 2000, most notably in imported sweet orange [*Citrus sinensis* (L.)] cv. Lane Late trees grafted on Carrizo citrange [*C. sinensis* × *Poncirus trifoliata* (L.)], in Argolida, southern Greece and in Chania region of the island of Crete (Dimou *et al*., 2002; Dimou and Coutretsis, 2009). Timely detection of CTV infected trees and the prompt application of eradication measures has managed to restrict CTV infections in Greece, although there is a serious concern for future introductions due to the lack of attention in the circulation of nursery plant material and customs controls. In the case of Crete, a 18-year survey showed that CTV is regularly found in its western region. Genetic analyses suggest two distinct CTV introductions corresponding to a mild (identical to the Spanish isolate T385) and a severe (GR1825) isolate. The former currently appears scattered in the form of isolated infections, whereas the latter is prominent in one particular geographical location (Shegani *et al*., 2012; Owen *et al*., 2014; Livieratos *et al*., unpublished data).

The most efficient vector of CTV world-wide is the aphid *Aphis citricidus* (Kirkaldy) (Roistacher and Bar-Joseph, 1987; Yokomi *et al*., 1989; 1994). *Aphis gossypii* Glover, while less effective, is also an efficient vector of many CTV strains (Cambra *et al*., 2000). Yokomi *et al*. (1989; 1994) demonstrated that *A. citricidus* is up to 25 times more efficient vector at transmitting some isolates of CTV than *A. gossypii*. *A. gossypii* is the main vector of CTV in Spain, Israel, Italy, Morocco, in some citrus growing areas in California (USA), and in other locations where *A. citricidus* is absent (Raccah and Loebenstein, 1976; Hermoso De Mendoza *et al*., 1984;

Yokomi *et al*., 1989; Cambra *et al*., 2000; Marroquín *et al*., 2004; Afechtal *et al*., 2015; Davino *et al*., 2015). Other vectors of CTV such as *Aphis aurantii* (Boyer de Fonsicolombe) (Norman and Grant, 1953; Hermoso De Mendoza *et al*., 1984), *Aphis spiraecola* Patch (Norman and Grant, 1953) (Hermoso de Mendoza *et al*., 1988), *Myzus persicae* (Sulzer), *Aphis craccivora* Koch and *Uroleucon jaceae* (L.) (Varma *et al*., 1960; 1965), although less efficient than *A. citricidus* and *A. gossypii*, can predominate in some areas. For instance, *A. spiraecola* is the major aphid species contributing to CTV spread in Morocco (Elhaddad *et al*., 2016).

In Greece nine aphid species have been found to infest citrus trees, i.e., *A. craccivora*, *A. gossypii*, *A. spiraecola*, *Aulacorthum solani* (Kaltenbach), *Brachycaudus helichrysi* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), *M. persicae*, *Rhopalosiphum maidis* (Fitch) and *T. aurantii* (Argyriou, 1969; Kavallieratos and Lykouressis, 1999). However, to our knowledge, in Greece no studies have been conducted of the aphid species composition in citrus orchards in the areas where CTV has been detected.

The present study was designed to address which aphid species colonize citrus orchards in the region of Chania, Crete, an important citrus production region of Greece, where CTV infection has been detected since 2000 (Dimou *et al*., 2002; Dimou and Coutretsis, 2009).

Materials and methods

Aphid sampling

The study was conducted in citrus orchards, located 15 km south of Chania, in a representative citrus production area in western Crete, Greece. In this area, CTV infected trees have been found since 2000. Aphids populations were monitored to determine the species composition in four citrus orchards in October 2016, May 2017 and June 2017. Preliminary surveys had shown that aphid infestation of citrus trees is heaviest during these months.

Two categories of orchards were selected in terms of management system, one complying with organic standards according to EU legislation (Council Regulation -EC- 834/2007), and the second complying with EU Common Agricultural Policy (CAP) framework describing conventional farming. In each category one orchard was planted with the variety ‘Washington Navel’ sweet orange *C. sinensis* and the other with the ‘Nova’ mandarin hybrid variety [*Citrus clementina* hort. ex Tan. × (*Citrus reticulata* Blanco × *Citrus paradisi* Macfad)]. The orchards had an average size of 0.55 ha, ranging from 0.2 to 1 ha (100-500 trees per orchard), considered typical for the area. During the sampling period, only basic cultivation techniques were performed (fertilization, weed destroying, pruning, irrigation) and no insecticide was applied.

In October 2016 and May-June 2017, samples were taken every seven or 15 days for a total of 7 samplings starting from October 7, 2016. The four plots were assessed for aphid occurrence by direct sampling of established colonies on the leaves of young shoots, since the aphids develop only on new citrus growth (flush). From each orchard 40 young shoots, 10 cm long approximately, were randomly collected on each sampling date (20 randomly selected trees and two young shoots per tree) with a total of 1,050 shoots collected. The samples from each tree were placed separately in self-sealing plastic bags, slightly inflated, containing a piece of paper towel to absorb excessive moisture. Bags were put in insulated plastic containers, containing ice packs, and transferred to the laboratory. On each sampling date, the total number of aphid individuals (nymphs, wingless adults, winged adults) per shoot was counted. Most of the adult aphids collected were identified to species.

The surveys were continued in autumn 2017 and spring 2018, although they were not regular and extensive. The aim was to examine the frequency of the prevailing aphid species. Ten wingless adult aphids from each of the 113 randomly collected young shoots (10-15 adults per shoot) from the aforementioned orchards in October-November 2017 (81 shoots) and April-May 2018 (32 shoots) were identified to species.

We also present data for aphid species frequencies from surveys conducted in various citrus orchards in the Chania region before 2016 (2008, 2009, 2011, 2013 and 2014). Most of the orchards were planted with *C. sinensis* and a few with *C. paradisi*, *C. reticulata* and *Citrus limon* (L.). A number (257-717) of wingless adult aphids were collected and identified from 100-180 infested young shoots (two shoots from 10 trees per orchard from 5-9 conventional orchards) in the spring of each year (in May of the years 2008-2013 and in April 2014). In all these five years, 700 infested shoots were collected, and 2,416 wingless adult aphids were identified to species.

Species identification

In all samplings, a number of adults were preserved in tubes filled with one part lactic acid (75% w/w): two parts ethyl alcohol (95%) until examined. Species identification was based on the keys provided by (Blackman and Eastop, 2000) and the specimens were examined directly under a dissecting scope (KONUS CRYSTAL-45). A few hundreds of specimens were also examined after permanent slide preparation under a phase contrast microscope (Leica DRMB) for validation purposes.

Statistical analysis

Data from counts of aphid specimens (pooled data from adults and nymphs from all species) were compared between orchards and varieties using the non-parametric Scheirer-Ray-Hare test (equivalent to 2-way ANOVA) because the data deviated from normality (Shapiro-Wilk normality test). The data from the two sampling periods, i.e. October and May-June were analysed separately. The frequencies of the aphid species identified were compared using the χ^2 test (with Yates' correction). When χ^2 test returned a significant value, pairwise comparisons were performed using the Bonferroni correction. Fisher's exact test was used in one case because the analysis returned that χ^2 approximation may be incorrect. All the analyses were conducted using R (R Core Team, 2017).

Table 1. Mean number of aphids per shoot (adults and nymphs: pooled data from all species; adults: *A. spiraecola* and *A. gossypii*) collected from sweet orange (Washington Navel variety) and mandarin (Nova variety) trees in four orchards in Chania, Crete, Greece during October 2016 and May-June 2017.

Orchard	Variety	October				May-June			
		N	Mean		S.E.	N	Mean		S.E.
All species adults and nymphs									
Conventional	Nova	120	58.1		6.9	160	64.2		7.0
Conventional	Washington Navel	120	19.8		2.5	140	56.4		8.6
Organic	Nova	130	26.6		4.0	160	52.8		4.7
Organic	Washington Navel	80	51.5		14.5	140	34.5		6.6
Conventional	Both	240	39.0	a	3.9	300	60.6	a	5.5
Organic	Both	210	36.1	b	6.1	300	44.2	b	4.2
Both	Nova	250	41.7	a	4.0	320	58.5	a	4.8
Both	Washington Navel	200	32.5	b	6.1	280	45.5	a	4.9
A. spiraecola									
Both	Nova	250	2.8	a	0.4	320	6.0	a	0.7
Both	Washington Navel	200	2.1	b	0.4	280	3.5	a	0.4
A. gossypii									
Both	Nova	250	1.3	a	0.2	320	3.1	a	0.4
Both	Washington Navel	200	0.7	b	0.2	280	1.6	b	0.3

N = number of shoots examined; means followed by a different letter (comparisons between orchards and between varieties, pooled data) differed significantly by Scheirer-Ray-Hare test.

Results

A total of 48,369 aphid individuals (both adults and nymphs of all species) were counted during autumn 2016 and spring 2017, 27,516 and 20,853 individuals in the conventional and organic orchards respectively. Table 1 illustrates the number of aphid individuals (adults and nymphs of all species and adults of *A. spiraecola* and *A. gossypii*) per shoot that were collected in each orchard during the sampling period. In the sampling period of October, the differences between orchards (conventional: 39.0 *vs*. organic: 36.1 aphids per shoot; H = 7.353, df = 1, P = 0.007 - pooled data from both varieties) and between varieties (Nova: 41.7 *vs*. W. Navel: 32.5; H = 5.224, df = 1, P = 0.022 - pooled data from both orchard categories) were significant. Their interaction was found significant (H = 4.236, df = 1, P = 0.040) and we also analysed each factor separately using Kruskal-Wallis *H* test. Significant differences between varieties was found only in the conventional orchard (Nova: 58.1 *vs*. W. Navel: 26.6; χ^2 = 11.338, df = 1, P = 0.0008). The differences between orchards were significant only for the Nova variety (conventional: 58.1 *vs*. organic: 26.6; χ^2 = 12.571, df = 1, P = 0.0004). The application of the Scheirer-Ray-Hare test on the dataset from the period of ‘May-June’ showed that the cultivation practices in the orchards had a significant effect on the mean number of aphids per shoot collected (conventional: 51.0 *vs*. organic 40.9; H = 8.808, df = 1, P = 0.003 - pooled data from both varieties). The differences between varieties (H = 0.134, df = 1, P = 0.705) and the interaction between orchard and variety (H = 0.010, df = 1, P = 0.922) were not significant.

To investigate further the effect of varieties on aphid infestation we analysed separately the data of the two most frequent aphid species recorded (see below), i.e. *A. spiraecola* and *A. gossypii*. In the sampling period of October, a significantly higher mean number of adults per shoot of both species were collected from Nova than from W. Navel variety (*A. spiraecola*: 2.8 *vs*. 2.1, H = 4.452, df = 1, P = 0.035; *A. gossypii*: 1.3 *vs*. 0.7, H = 3.983, df = 1, P = 0.046 - pooled data from both orchard categories). The interaction between orchard category and variety was significant for both species (*A. spiraecola*: H = 14.833, df = 1, P < 0.001; *A. gossypii*: H = 7.763, df = 1, P = 0.005). The analysis with the Kruskal-Wallis *H* test showed that in the conventional orchards then mean number of adults per shoot was significant higher in Nova than in W. Navel variety for both aphid species (*A. spiraecola*: 4.1 *vs*. 1.1, χ^2 = 17.673, df = 1, P < 0.001; *A. gossypii*: 1.8 *vs*. 0.4, χ^2 = 10.674, df = 1, P = 0.001). On the contrary, the differences between varieties were not significant in the organic orchards for either of the two aphid species (*A. spiraecola*: χ^2 = 2.700, df = 1, P = 0.132; *A. gossypii*: χ^2 = 0.546, df = 1, P = 0.460). In the sampling period of May-June, the mean number of adults per shoot of both species was higher in Nova than in W. Navel variety, although the difference was significant only for *A. gossypii* (*A. spiraecola*: 6.0 *vs*. 3.5, H = 1.7832, df = 1, P = 0.182; *A. gossypii*: 3.1 *vs*. 1.6, H = 9.283, df = 1, P = 0.002 - pooled data from both orchard categories). The interaction between orchard category and variety was not significant for either of the two species (*A. spiraecola*: H = 0.014, df = 1, P = 0.906; *A. gossypii*: H = 0.033, df = 1, P = 0.856).

Figure 1 illustrates the percentages of aphid individuals (total number of adults and nymphs) collected in October 2016, May and June 2017 (= no. of individuals collected in each month / total no. of individuals collected during the whole sampling period). In both type of orchards more aphids were found in May-June than in October (34.5-39.6% *vs*. 24.3-28.9% of the total aphids found).

The frequencies of the aphid species collected during the whole sampling period are shown in table 2. A total

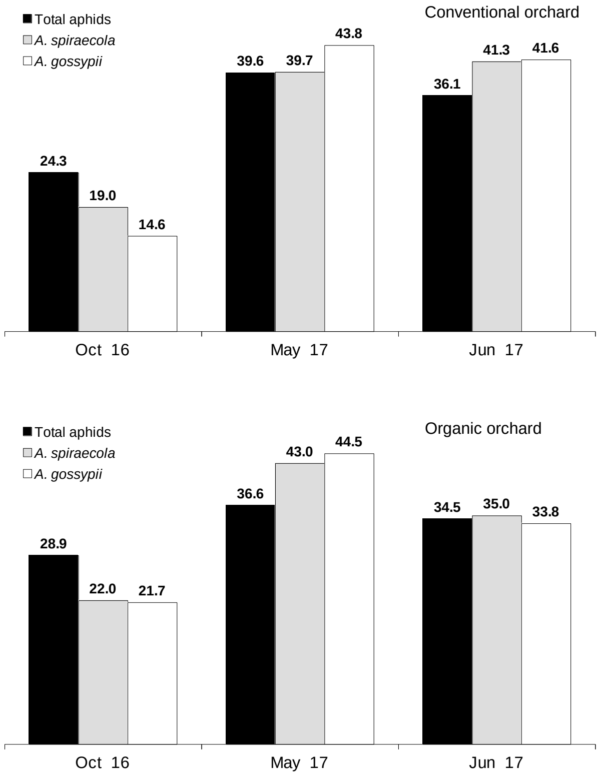

Figure 1. Frequency (%) of total aphids (adults and nymphs of all species), *A. spiraecola* (adults) and *A. gossypii* (adults) (= no. of individuals in each month / total no. of individuals in the whole sampling period) in trees from various organic and conventional orchards in autumn 2017 and spring 2018.

of 6,290 wingless adult aphids (3,760 and 2,530 adults in conventional and organic orchards respectively) were identified and five aphid species were found (*A. spiraecola*, *A. gossypii*, *A. aurantii*, *A. craccivora* and *M. persicae*) at significant different frequencies ($P < 0.001$, χ^2 test) in both organic and conventional orchards (pooled data from both varieties; a similar trend was observed when data were analysed according to variety and sampling season - data not shown). The most frequent species was *A. spiraecola* (64.2% in the total sample) (χ^2 test, $P < 0.001$ in all pairwise comparisons) followed by *A. gossypii* with a 2-fold lower frequency (30.5% in the total sample), while the three remaining species showed much lower frequencies (< 3.6%). This trend was found in both cultivation systems, although the frequency of *A. spiraecola* was significant higher in organic than in conventional orchards (68.8 *vs.* 61.1%; $\chi^2 = 38.919$, df = 1, $P < 0.0001$).

The seasonal trend of the two most frequent aphid species (*A. spiraecola* and *A. gossypii*) followed the trend of total aphids collected, i.e. most of *A. spiraecola* and *A. gossypii* adults were collected in May-June (figure 1). The Pearson's correlation coefficients were highly significant between the frequencies of total aphids and *A. spiraecola* (conventional orchard: R = 0.875, N = 7, P = 0.005; organic orchard: R = 0.977, N = 7, $P < 0.001$) as well as between total aphids and *A. gossypii* (conventional orchard: R = 0.900, N = 7, P = 0.002; organic orchard: R = 0.15, N = 7, P = 0.001).

All the adult aphids (1,300) collected in the additional samplings of infested shoots in autumn 2017 (81 shoots) and spring 2018 (32 shoots) were *A. spiraecola*.

The surveys that were conducted before 2016 showed similar results, although the samples were collected only in the spring of each year. A total of three aphid species were identified (*A. spiraecola*, *A. gossypii*, *A. aurantii*) and the predominant aphid species was by far *A. spiraecola* (χ^2 test or Fisher's test, $P < 0.001$ in all pairwise comparisons) for all years. The frequency of *A. spiraecola* ranged from 67.6% in 2009 to 99.6% in 2008. *A. gossypii* was present in all pre-2016 years except 2008 (4.7-19.9%) and *A. aurantii* in three years (0.4-13.6%) (table 3).

Discussion

The data from the long-term surveys presented here, shed light on the frequencies of the prevailing aphid species in citrus orchards in the Chania region of Crete, where CTV is present since 2000. In surveys performed annually since 2001, a number of trees were detected as being CTV positive each year, suggesting disease spread by aphids in the field (Livieratos *et al.*, unpublished data). A total of five (*A. spiraecola*, *A. gossypii*, *A. aurantii*, *M. pescicae* and *A. craccivora*) of the nine aphid species that have been recorded on citrus in Greece were identified, with relative frequencies that varied between years and all of these have been reported to be CTV vectors. However, the most efficient CTV vector worldwide, *A. citricidus*, was not detected. *A. gossypii* which is ranked as the second most efficient vector of CTV was

Table 2. Frequencies of aphid species collected from sweet orange and mandarin trees in conventional and organic orchards in Chania, Crete, Greece during October 2016 and May-June 2017.

	Conventional			Organic			Total		
	%	CI	CI	%	CI	CI	%	CI	CI
A. spiraecola	61.09 a	59.51	62.65	68.81 a	66.97	70.62	64.20 a	63.00	65.38
A. gossypii	32.77 b	31.27	34.29	27.00 b	25.27	28.77	30.45 b	29.31	31.60
A. aurantii	5.61 c	4.90	6.40	1.78 c	1.30	2.37	4.07 c	3.60	4.59
A. craccivora	0.53 d	0.33	0.82	2.02 d	1.50	2.64	1.13 d	0.88	1.42
M. persicae	0.00 e	0.00	0.10	0.40 e	0.19	0.73	0.16 e	0.08	0.29

CI = 95% confidence intervals; 3,760 and 2,530 adult aphids were identified from conventional and organic orchards respectively; frequencies within columns followed by a different letter differ significantly ($P < 0.001$, χ^2 test).

Table 3. Frequencies of aphid species collected from citrus orchards trees in Chania, Crete, Greece during the last ten years.

Year Season		*A. spiraecola*		*A. gossypii*		*A. aurantii*		*M. persicae*		*A. craccivora*	
2008 Spring	%	99.608	a			0.392	b				
(N = 510)	CI	98.591				0.048					
	CI	99.952				1.409					
2009 Spring	%	67.643	a	19.944	b	12.413	c				
(N = 717)	CI	64.081		17.078		10.088					
	CI	71.059		23.060		15.052					
2011 Spring	%	71.930	a	14.474	b	13.596	c				
(N = 456)	CI	67.563		11.374		10.586					
	CI	76.010		18.043		17.089					
2013 Spring	%	95.213	a	4.787	b						
(N = 376)	CI	92.539		2.862							
	CI	97.138		7.461							
2014 Spring	%	95.331	a	4.669	b						
(N = 257)	CI	91.986		2.436							
	CI	97.564		8.014							
2016 Autumn	%	70.968	a	28.906	b	0.127	c				
(N = 1581)	CI	68.661		26.680		0.015					
	CI	73.196		31.210		0.456					
2017 Spring	%	58.489	a	30.178	b	8.800	c			2.532	d
(N = 2409)	CI	56.491		28.350		7.699				1.942	
	CI	60.466		32.056		10.003				3.241	
2017 Summer	%	65.522	a	31.783	b	1.826	c	0.435	d	0.435	d
(N = 2300)	CI	63.539		29.882		1.319		0.209		0.209	
	CI	67.465		33.730		2.460		0.798		0.798	
2017 Autumn	%	100									
(N = 810)	CI	99.546									
	CI	100									
2018 Spring	%	100									
(N = 320)	CI	98.854									
	CI	100									

N = number of adult wingless females identified to species; CI = 95% confidence intervals; frequencies within rows followed by a different letter differ significantly (P < 0.001, χ^2 test; Fisher's exact test for 2016).

recorded at low to moderate frequencies (4.7-31.8%) and was detected in six of the eight years of the survey. The most frequent species was clearly *A. spiraecola* with high to very high frequencies in all years (58.5-100%). This finding is supported by the extended and detailed samplings that were performed in the last two years (2016-2018), where a much higher number of adults were identified than the pre-2016 years and samples were examined from orchards with different cultivation systems (conventional and organic). The absence of *A. gossypii* in some of the years during this study was unexpected, without any obvious justification. However, changes in plant protection strategies and/or differential selection pressure by biological control agents (e.g., different parasitisation rates) among aphid species on citrus that have been well documented in Greece (Kavallieratos and Lykouressis, 1999; Kavallieratos *et al*., 2004) may be involved. In any case, the observed sharp fluctuations of the frequencies of *A. gossypii* among years requires further investigation. The other three species (*A. aurantii*, *M. persicae* and *A. craccivora*) were present at low frequencies or were absent throughout the survey.

The results from the regular samplings in 2016 and 2017 showed that the populations of the two main aphid species (*A. spiraecola* and *A. gossypii*) were higher in May-June compared to October. This could be due to the increased number of young shoots available for colonization by aphids in this period, because these are the months when the greatest vegetative growth of citrus occurs in the study area.

The main pathway of CTV introductions (entry, establishment, and dispersion) in new regions is the movement of infected material, followed by local spread by its natural vector in a semi-persistent manner (Vidal *et al*., 2012). In the Chania region, CTV was firstly detected in 2000, having been introduced through several accidental utilization of CTV-infected propagation material from Spain (Dimou *et al*., 2002; Dimou and Coutretsis, 2009). Although eradication measures have been applied, a few disease *foci* still remain and constitute a source of viral inoculum for uninfected citrus orchards. The results of the present study suggest that *A. spiraecola* (the predominant vector species) and *A. gossypii* (less frequent but the second most efficient vector after *A. citricidus*) are the major aphid vectors contributing to CTV spread in Chania citrus orchards, as is the case in other citrus growing regions of the world where *A. citricidus* is absent (Raccah and Loebenstein, 1976; Hermoso De Mendoza *et al*., 1984; Yokomi *et al*., 1989; Cambra *et al*., 2000; Marroquín *et al*., 2004; Elhaddad *et al*., 2016). Nevertheless, further studies to estimate the incidence of viruliferous aphids of these species in Chania region are necessary to confirm their importance in CTV spread.

Another point of discussion is that our results demonstrate geographical variation in the prevailing aphid species in citrus orchards in Greece. Kavallieratos *et al*. (2002) reported *A. gossypii* to be the predominant aphid species found in sweet orange and tangerine orchards in southern Greece in 1996-1997 (4.4-24.5 -fold higher mean numbers than the second species, *A. solani* or *A. spiraecola*). In latter surveys (2000-2001) in a citrus orchard in central Greece, Kavallieratos *et al*. (2004) found *A. aurantii* to be the predominant species (6.9-7.7 -fold higher mean numbers than the second species, *A. gossypii*). In comparison to these data, the mean frequencies of *A. spiraecola* and *A. gossypii* over the 8-year survey were 82.5 and 13.5% (6.1-fold difference, table 3) respectively. It is worth mentioning that a change in predominant aphids in citrus orchards has been reported in Spain, where *A. aurantii* and *A. spiraecola* predominated until 1987, but since then *A. gossypii* has become dominant (Cambra *et al*., 2000). These findings suggest that large scale and long-term surveys in various regions of Greece are needed to monitor and clarify the frequencies of the predominant aphid vectors of CTV, especially in CTV hot-spots. It would be valuable for CTV management to know where *A. gossypii*, a relatively efficient vector is common, or whether inefficient vectors such as *A. spiraecola* and *A. aurantii* predominate, as well as their seasonal variation.

Our study has revealed differences in the aphid populations (adults and nymphs from all aphid species) among orchards with different cultivation systems. Higher populations were recorded in conventionally managed orchards rather than in organically cultivated orchards. Various factors could be responsible for these differences, among these insufficient chemical control programs or the adverse effects of chemical insecticides on the aphid natural enemies would seem the most probable. The analysis showed a significant effect of variety on the aphid infestation levels (adults and nymphs from all aphid species) during the 2016-2017 surveys, but only in conventional orchards in the sampling period of October, where more aphids were collected in mandarin than in sweet orange. Looking separately at data from the two most frequent species, a higher number of aphids per shoot was recorded in mandarin than in sweet orange, although the differences were not always significant. These findings agree with those of Marroquín *et al*. (2004) who found that clementine mandarin was the most visited by aphids compared to sweet orange and other citrus species. Similarly, Cambra *et al*. (2000) found that *A. gossypii* preferential alighted on clementine mandarin than sweet orange. The authors explained this by a preferential alighting of aphids on clementine, most probably because the shoots remained tender and succulent longer in this variety than in sweet orange. By contrast, Kavallieratos *et al*. (2004) based on data from aphid samples from citrus orchards suggested that *A. gossypii* did not show a preference for orange or tangerine trees. The use of different citrus varieties as an additional tool for the management of aphid vectors and subsequently CTV in the Chania region is an interesting preposition, although further research is needed which should also take into account required orchard characteristics and market demands.

In conclusion, the present study shows that the predominant aphid species on mandarin and sweet orange trees in the Chania region of western Crete, a hot-spot for CTV transmission, was *A. spiraecola* followed by *A. gossypii*. Both species are vectors of CTV and may play a significant role to the spread of the virus in this area. However, as *A. citricidus* species is now present in continental Portugal and Spain, it may pose a serious threat for Greek citriculture. There have been well documented examples of aphid species invasions in Greece the last decade (Tsitsipis *et al*., 2005) which have been attributed to the globalization of the commerce. Therefore, monitoring of the aphid species composition in citrus orchards should be continued, in the framework of CTV management, with a particular aim the early detection of *A. citricidus* and the increase of the *A. gossypii* frequencies which is considered the second most efficient vector of CTV.

Acknowledgements

The authors thank Despina Tzobanoglou for the help in field samplings. We also thank Carolyn Owen for the language revision of the manuscript.

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Received January 2, 2019. Accepted April 30, 2019.