



# Ants in citrus: impact on the abundance, species richness, diversity and community structure of predators and parasitoids



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## ABSTRACT

Ants can act as plant biotic defenses, however, in agricultural ecosystems they are often associated with outbreaks of honeydew-producing pests mainly due to the protection they offer to the plant feeders in exchange for honeydew. In this interaction ants may alter the abundance, diversity and community structure of predators and parasitoids. In the present study, we conducted ant-exclusion experiments in three commercial citrus orchards, each one dominated by one ant species (*Pheidole pallidula*, *Lasius grandis* or *Linepithema humile*) during two consecutive years. We then compared the abundance, species richness, diversity and community structure of predators and parasitoids between the ant-allowed and ant-excluded treatments. A total of 176,074 natural enemies belonging to 81 taxa were captured and identified. The abundance of the natural enemies showed a species specific response between treatments. When examining functional groups of natural enemies the abundance of generalist predators decreased while that of parasitoids increased in the ant-allowed treatment. The species richness was significantly lower for predators and higher for parasitoids in the ant-allowed treatment. The Shannon diversity index was not different between treatments for predators, whereas parasitoid diversity was significantly higher in the ant-allowed treatment. Finally, the community structure of predators and parasitoids was not significantly different between treatments. These results suggest that ants in citrus are not associated with a dramatic decrease in natural enemy abundance or biodiversity; on the contrary ants were associated with increased parasitoid species richness and diversity. On the other hand, ants negatively affected the abundance of specific natural enemy species, mainly generalist predators. The impact on these predators might explain the higher pest densities associated with ants in citrus.

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## 1. Introduction

Ants are keystone species affecting directly and indirectly both ecosystem structure and functioning. Ants may act as soil tillers (Folgarait, 1998), seed dispersers (Rico-Gray and Oliveira, 2007), pollinators (Beattie, 1985), predators (Way and Khoo, 1992) and are involved in various mutualisms (Way, 1963). Mutualism has been found to have broad effects on the arthropod community affecting eventually plant health (Kaplan and Eubanks, 2005; Rosumek et al., 2009). One of the best studied mutualisms involving ants is the relationship with honeydew producing Hemiptera, in which ants use the honeydew excreted as an important carbohydrate source and, in turn, protect Hemiptera from their natural enemies (Carroll and Janzen, 1973). As a result, ant-tending may have wider community-level consequences by altering the abundance and distribution of the third (insect predators & primary parasitoids)

(James et al., 1999; Styrsky and Eubanks, 2007) and fourth trophic levels (primary hyperparasitoids) (Kaneko, 2002; Völkl, 1992). These interactions play an important role in agricultural ecosystems since biological control provided by the third trophic level may be negatively affected by ant activity (Daane et al., 2007; Martínez-Ferrer et al., 2003).

The impact of ants on natural enemy abundance, diversity or community structure varies considerably depending (i) on the natural enemy species (ii) on the species of ants involved or (iii) the ecosystem where the study took place (Appendix A.1 of supplementary data and references therein). Whereas several studies have demonstrated a negative impact of ants on the abundance of natural enemies others find no effect or even find positive effects of ants on the community of natural. The same or even greater variability is reported at the species level; natural enemies, even species belonging to closely related taxa, may be affected differently by ants. For example, several studies have shown that ants have a negative impact on certain coccinellid species (Kaplan and Eubanks, 2002), while other coccinellids are not affected (Vanek and Potter, 2010) or even increase their

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densities under ant presence (Daane et al., 2007; Völkl and Vohland, 1996). Likewise, the activity of several parasitoid species is disrupted by ants (Martínez-Ferrer et al., 2003) while others are able to parasitize hemipterans tended by ants (Barzman and Daane, 2001; Völkl, 1994).

Ants are among the most abundant arthropods in citrus and have been demonstrated that they may induce population increases of honeydew and non-honeydew producing pests as a result of their interference with natural enemies (Calabuig et al., 2015, 2013; Dao et al., 2014; Pekas et al., 2011, 2010; Yoo et al., 2013). Several studies have examined the multitrophic interactions involving ants and natural enemies in citrus in different parts of the world. Most of the studies focus on predators from different taxa but little is known about the impact of ants on the diversity and abundance of parasitoids, which are often studied as a single group in the order Hymenoptera. Further, there are no studies examining the impact of ants simultaneously on the whole community of predator and parasitoid species present in citrus orchards. Knowledge about the impact of ants on the abundance, diversity and community structure of all the arthropod natural enemies will provide useful insights and can help us to clarify the role of ants in the citrus agroecosystem.

Thus, we performed an ant-exclusion experiment during two consecutive years in three commercial citrus orchards, each one with a different dominant ant species to test the predictions that the presence of ants in the citrus agroecosystem will be associated with (1) a reduction in the abundance of multiple species of predators and parasitoids, (2) a reduction in the predator and parasitoid species richness and diversity and (3) a change in the community structure of predators and parasitoids.

## 2. Material and methods

### 2.1. Study sites

The study was conducted during two consecutive growing seasons, from April 2011 to November 2012, in three commercial citrus orchards located in an extensive citrus-growing area 30 km south of Valencia, eastern Spain (39° 12' N, 0° 20' W; 39° 11' N, 0° 20' W and 39° 14' N, 0° 15' W). The climate is Mediterranean, with a rainy spring and autumn and a dry winter and summer. The orchards were flood irrigated and weeds were controlled by local application of herbicides (Glyphosate<sup>®</sup>, Bayer CropScience, Spain). Two orchards were of sweet orange *Citrus sinensis* (L.) Osbeck (cv. Navelina) and one of a mixture of two species, sweet orange *C. sinensis* (cv. Navelina) and Clementine mandarin *Citrus reticulata* Blanco (cv. Clementina Fina). In this orchard the trial was performed in the part composed by *C. sinensis* trees. Trees were more than 10 years old in all orchards. No insecticides were sprayed in the previous five years or during the two-year experimental period. In each orchard a single ant species was behaviorally dominant, i.e., ant species that attacks and excludes other ant species from food sources (Pekas et al., 2011). From now on we will refer to the orchards according to the acronym of the predominant ant species present. Thus, in the orchard PP the predominant ant species was *Pheidole pallidula* (Nylander), in the orchard LG it was *Lasius grandis* Forel and in the orchard LH *Linepithema humile* (Mayr) (mixed orchard) was the only ant species present and foraging on the tree canopies (for details see Calabuig et al., 2013).

### 2.2. Experimental design

At each orchard, the experimental design was composed by four replicate blocks, to which a single treatment was applied with two levels (plots): ants allowed and ants excluded. This was

equivalent to 8 plots per orchard each one containing 16 trees (four rows by four trees per row). Ants were excluded in the 16 trees of the ant-excluded plots and left unaffected in the 16 trees of the ant-allowed plots. Only the four central trees of each plot were used for the samplings. With that method we ensure that arthropods captured came from the trees of the same plot and corresponding treatment. Ant-exclusion began in April 2011 and was maintained until November 2012 (19 months). During the first season (2011), ant exclusion was achieved by painting a 25-cm wide band of insecticidal paint in a micro-encapsulated formulation (Inesfly FITO<sup>®</sup> (chlorpyrifos 3%)), Industrias Químicas Inesba S.L., Paiporta, Spain) on the trunk (Calabuig et al., 2013). To ensure that no ants reached the tree canopies, ant-excluded trees were inspected every month and the band was repainted if ants were observed crossing the band. Due to the fact that we observed ants crossing the painted bands in some of the trees during the first growing season we changed the ant exclusion method during the subsequent season. Thus, during 2012, ant exclusion was conducted by applying Tangle-trap<sup>®</sup> (Tanglefoot, Biagro, Valencia, Spain) sticky barrier on the tree trunks. Sticky barriers were inspected every month and, if necessary, the Tanglefoot was renewed; in any case, Tanglefoot was renewed routinely every two months. Trees were pruned periodically and ground vegetation was trimmed to prevent alternative ways for ants to reach the canopies. With both methods ant exclusion was successful in the three experimental orchards (see Calabuig et al., 2013).

### 2.3. Arthropod sampling and classification

Arthropods on the tree canopies were sampled with yellow sticky traps and by using a suction vacuum device. In each plot, one yellow sticky trap (Bug-scan, Biobest<sup>®</sup>), 100 mm × 250 mm, was placed at 1.60 m high in the middle of the plot by hanging it on a twig. Suction samples were taken using a modified vacuum sampler (Komatsu Zenoah Co. HBZ2601) consisting on a reversed leaf-blower with a mesh bag to retain the sample (Tena et al., 2008). The vacuum sampler was applied on the canopies during one minute on each one of the four central trees of the plot. The sticky traps were replaced monthly, from April 2011 to November 2012 whereas suction samples were taken monthly from April to August and bimonthly from September to December in 2011 and 2012. Samples were transferred to the laboratory and maintained in a freezer until their identification.

All natural enemies captured were counted and identified. Most of them were identified to species or morphospecies level, while a few natural enemies were identified to genera or families. The use of morphospecies is a useful tool for studies that require taxonomic identifications of a great number of invertebrates without compromising scientific accuracy (Oliver and Beattie, 1996).

### 2.4. Predator and parasitoid diversity

The natural enemy diversity was measured in each sampling date by calculating the species richness  $S$  (number of species or morphospecies) and the Shannon diversity index  $H'$  (Shannon and Weaver, 1949).

In the calculation of species richness and diversity we included the natural enemies identified to species or morphospecies level pooling data from traps and suction. Species belonging to the fourth trophic level were not included in these analyses.

### 2.5. Statistical analysis

All analyses were performed pooling data from traps and suction samples for each month (i.e., May, June, July, August,

October and December for 2011 and April, May, June, July, August, September and November in 2012). This methodology may pose some problems when studying diversity, as this incorporates a measure of abundance which would be sample method dependent. However, the sampling was consistent on each tree so the bias should be constant, eventually not affecting the results.

To compare the abundance of specific natural enemies in the ant-allowed and the ant-excluded treatments we included only those species represented by more than 100 individuals. Additionally, we compared the abundance of *Pilophorus* sp. (43 individuals captured) in ant-allowed and ant-excluded treatments since we consider this species to be of special interest given the ant

**Table 1**  
Total arthropods captured, arthropods captured in ant-allowed and ant-excluded trees (mean  $\pm$  SE) and effect of ants, globally and for the three orchards separately, during two years, 2011 and 2012, in three citrus orchards.

Arthropods	Total arthropods	Principal prey	Global		Ant effect <sup>a</sup>	Ant effect/Orchard <sup>a</sup>		
			Arthropods/sample			LG	LH	PP
			Ant-allowed	Ant-excluded				
<b>Predators</b>								
Coleoptera								
<i>Cybocephalus</i> sp.	131		0.67 $\pm$ 0.12	0.25 $\pm$ 0.05	+	0	+	0
<i>Ragonycha</i> sp.	235	Generalist	0.59 $\pm$ 0.23	1.03 $\pm$ 0.32	--	0	n.p.	0
Coccinellidae								
<i>Clitosthetus arcuatus</i> Rossi	258	Whiteflies	1.57 $\pm$ 0.13	2.18 $\pm$ 0.18	0	0	0	0
<i>Delphastus catalinae</i> Horn	626	Whiteflies	2.22 $\pm$ 0.38	2.15 $\pm$ 0.49	++	0	0	0
<i>Rodolia cardinalis</i> (Mulsant)	803	<i>Icerya purchasi</i>	2.54 $\pm$ 0.41	3.05 $\pm$ 0.50	0	0	0	-
<i>Scymnus subvillosus</i> (Goeze)	1242	Aphids	4.85 $\pm$ 0.41	3.83 $\pm$ 0.32	0	0	++	0
Diptera								
<i>Platypalpus</i> sp.	1338		3.07 $\pm$ 0.83	6.20 $\pm$ 1.88	0	0	0	0
Heteroptera								
<i>Campyloneura virgula</i> Herrich-Schäffer	745	Generalist	2.04 $\pm$ 0.62	3.13 $\pm$ 0.71	--	0	--	-
<i>Cardiasthetus</i> sp.	368	Generalist	1.03 $\pm$ 0.15	1.53 $\pm$ 0.17	-- <sup>B</sup>	--	0	0
Neuroptera								
Chrysopidae								
<i>Chrysoperla carnea</i> (Stephens)	591	Generalist	1.39 $\pm$ 0.19	2.70 $\pm$ 0.33	-- <sup>B</sup>	--	--	--
Coniopterigidae								
<i>Conwentzia psociformis</i> (Curtis)	4395	Generalist	14.77 $\pm$ 3.65	15.84 $\pm$ 3.39	0	0	-	0
<i>Semidalis aleyrodiformis</i> Stephens	29987	Generalist	107.39 $\pm$ 11.37	101.68 $\pm$ 10.46	0	0	0	0
<b>Parasitoids</b>								
Hymenoptera								
Ceraphronoidea								
Ceraphronidae								
	1083		4.26 $\pm$ 0.54	3.30 $\pm$ 0.34	++	++	0	n.p.
Megaspilidae								
	349		1.18 $\pm$ 0.18	1.25 $\pm$ 0.22	0	0	0	0
Chalcidoidea								
<i>Ablerus</i> sp. <sup>b</sup>	645	Diaspidid parasitoids	3.65 $\pm$ 0.96	0.90 $\pm$ 0.23	++ <sup>B</sup>	n.p.	++ <sup>B</sup>	n.p.
<i>Anagyrus</i> sp.	281	Pseudococcids	1.43 $\pm$ 0.36	0.54 $\pm$ 0.11	++ <sup>B</sup>	++	++	++
<i>Aphelinus</i> sp.	127	Aphids	0.21 $\pm$ 0.04	0.09 $\pm$ 0.02	0	0	0	0
<i>Aphytis chrysomphali</i> (Mercet)	50638	<i>Aonidiella aurantii</i>	167.94 $\pm$ 20.29	184.64 $\pm$ 21.83	0	-	++	0
<i>Aphytis hispanicus</i> (Mercet)	7534	<i>Parlatoria pergandii</i>	35.34 $\pm$ 6.08	17.47 $\pm$ 1.92	++ <sup>B</sup>	++	++ <sup>B</sup>	++
<i>Aphytis melinus</i> DeBach	11694	<i>Aonidiella aurantii</i>	39.96 $\pm$ 5.53	41.50 $\pm$ 7.12	0	0	0	0
<i>Cales noacki</i>	18448	Whiteflies	67.66 $\pm$ 8.16	61.01 $\pm$ 6.71	0	0	+	+
<i>Citrostichus phyllocnistoides</i> (Naranayan)	659	<i>Phyllocnistis citrella</i>	2.74 $\pm$ 0.86	1.86 $\pm$ 0.29	+	0	0	++
<i>Encarsia inquirenda</i> (Silvestri)	3662	<i>Parlatoria pergandii</i>	15.21 $\pm$ 2.16	10.39 $\pm$ 1.22	++	--	++ <sup>B</sup>	++
<i>Encarsia</i> sp. 1	1029		4.50 $\pm$ 0.63	2.71 $\pm$ 0.32	++	++	++ <sup>B</sup>	0
<i>Encarsia</i> sp. 2	178		0.69 $\pm$ 0.34	0.55 $\pm$ 0.19	0	n.p.	0	n.p.
<i>Eretmocerus</i> sp.	102	Whiteflies	0.35 $\pm$ 0.09	0.37 $\pm$ 0.09	0	n.p.	0	0
<i>Marietta</i> sp. <sup>b</sup>	313	Coccid parasitoids	1.36 $\pm$ 0.32	0.83 $\pm$ 0.21	++	0	n.p.	++
<i>Metaphycus flavus</i> (Howard)	8005	Coccids	29.43 $\pm$ 4.32	26.40 $\pm$ 4.03	0	0	++	0
<i>Metaphycus helvolus</i> (Compere)	4355	Coccids	18.83 $\pm$ 3.58	11.64 $\pm$ 1.56	++ <sup>B</sup>	0	++	++ <sup>B</sup>
<i>Microterys nietneri</i> (Motschulsky)	184	Coccids	0.54 $\pm$ 0.11	0.73 $\pm$ 0.13	0	0	+	0
Mymaridae								
	4932	Cicadellidae	19.65 $\pm$ 2.09	14.80 $\pm$ 1.81	++	+	0	+
Cynipoidea								
	286		1.03 $\pm$ 0.17	0.97 $\pm$ 0.18	0	0	0	0
Ichneumonoidea								
Alysinae: Alysini								
<i>Binodoxys</i> sp.	3451	Leaf miners	0.59 $\pm$ 0.08	0.69 $\pm$ 0.08	0	0	0	0
Ichneumonidae								
	1038	Aphids	11.94 $\pm$ 3.33	12.11 $\pm$ 3.09	0	0	0	0
Microgastrinae								
	587	Lepidoptera	3.70 $\pm$ 0.44	3.54 $\pm$ 0.43	0	++	0	0
Other Braconidae								
	270		1.82 $\pm$ 0.23	2.26 $\pm$ 0.30	0	0	0	--
Platygastridae								
Scelionidae								
	10897		39.73 $\pm$ 4.00	36.27 $\pm$ 3.11	0	0	0	0
Proctotrupoidea								
<i>Helorus</i> sp. <sup>b</sup>	309	Crisopids	0.80 $\pm$ 0.14	1.34 $\pm$ 0.18	--	0	--	0

<sup>a</sup> Repeated measures analysis of variance (ANOVA) was used, with treatment as fixed factor, block as random factor and time as repeated measures factor. In the global analysis, orchard was set as random factor. The + indicates a slightly increase in the abundance of the natural enemy in the ant-allowed treatment ( $P < 0.1$ ); ++ indicates a significant increase in the abundance of the natural enemy in the ant-allowed treatment ( $P < 0.05$ ); - indicates a slightly decrease in the abundance of the natural enemy in the ant-allowed treatment ( $P < 0.1$ ); -- indicates a significant decrease in the abundance of the natural enemy in the ant-allowed treatment ( $P < 0.05$ ); n.p. indicates no presence of the natural enemy. LG: *Lasius grandis* orchard; LH: *Linepithema humile* orchard; PP: *Pheidole pallidula* orchard.

<sup>b</sup> Species belonging to the 4<sup>th</sup> trophic level.

<sup>B</sup> Significant effect after sequential Bonferroni correction.

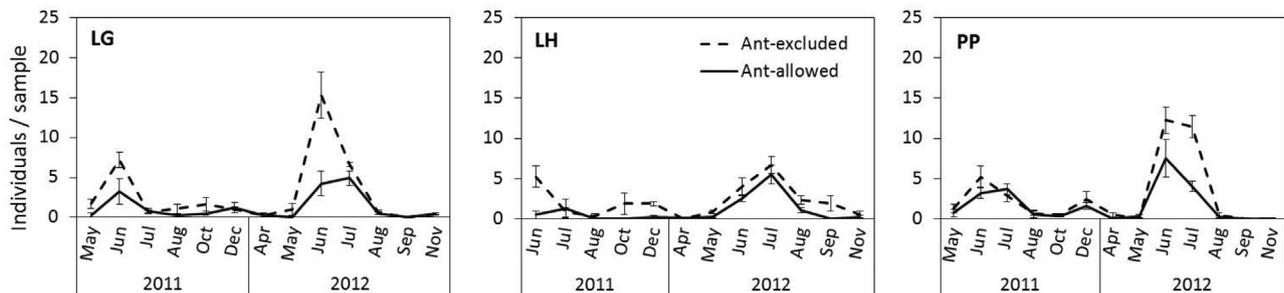
mimetism that it presents. For the species richness (S), the Shannon diversity index (H') and community structure analysis we included all natural enemies identified to species or morphospecies.

We applied repeated measures ANOVA on the abundance of every species, the species richness (S) and the Shannon diversity index (H') either at each orchard or globally, i.e., considering the three orchards together. Treatment (ant-excluded versus ant-allowed) was the fixed factor, orchard (in the global analysis) and block (nested into orchard) were random factors and sampling date was the repeated measures factor. Data were log-transformed in order to meet normality assumptions. To correct for type I error associated with performing multiple significance tests, we applied

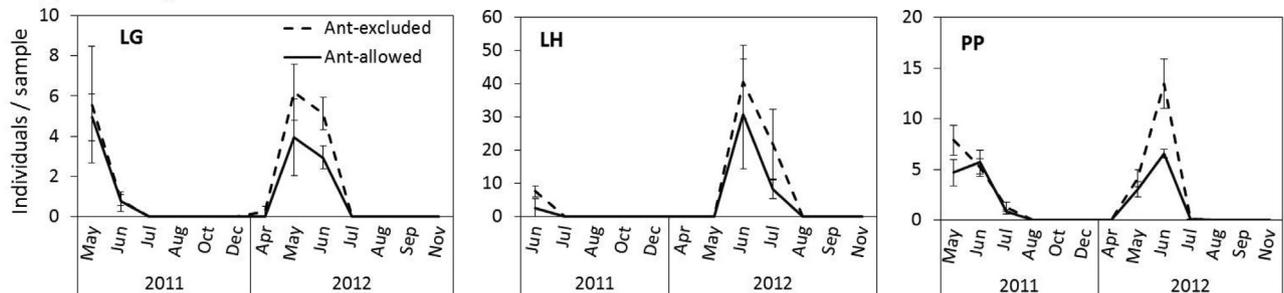
sequential Bonferroni corrections to adjust the significance level (Rice, 1989). However, this method is highly conservative when a high number of tests are performed and its application may greatly inflate the type II error (Moran, 2003). Therefore, we provide both the sequential Bonferroni corrected and uncorrected analyses. All ANOVAS were conducted using Statgraphics 5.1 software (Statgraphics, 1994).

To compare the community structure of natural enemies in ant-allowed and ant-excluded treatments, permutational multivariate analyses of variance (PERMANOVA) were applied to predator and parasitoid abundances (including all the species and morphospecies captured) using the *adonis* function in the *vegan* package (Anderson, 2001) in R Development Core Team (2014). A separate

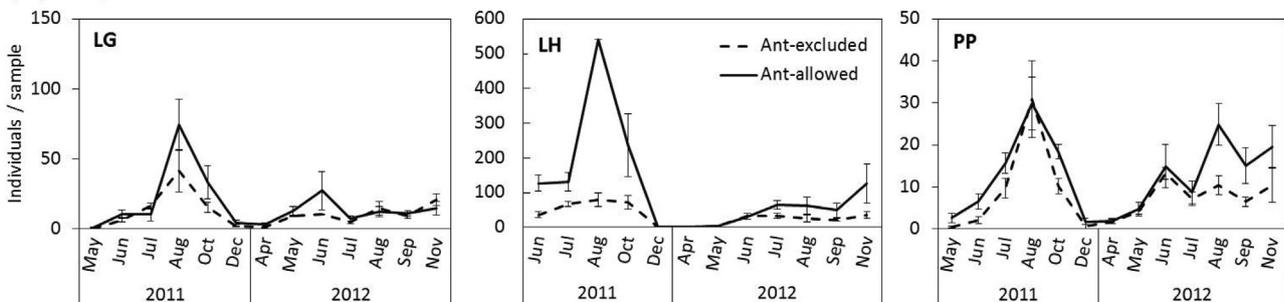
### *Chrysoperla carnea* s.l.



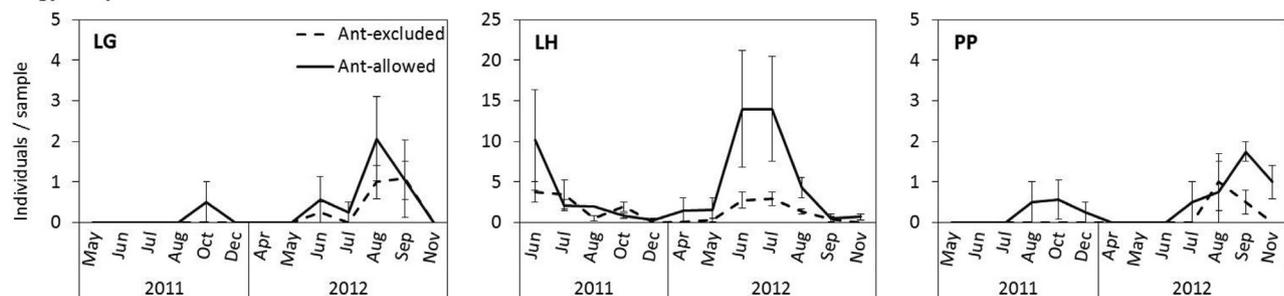
### *Campyloneura virgula*



### *Aphytis hispanicus*



### *Anagyrus* sp.



**Fig. 1.** Abundance of *C. carnea* s.l., *C. virgula*, *A. hispanicus* and *Anagyrus* sp. in ant-allowed and ant-excluded trees during 2011 and 2012. Abundance is measured as the mean number of individuals captured in one sticky trap and four suction (one/tree) in each experimental plot (each plot consisted in 16 trees and only the four central trees were sampled; each orchard contained 8 plots, 4 ant-allowed and 4 ant-excluded).

PERMANOVA was conducted in each orchard for each year. Distance matrices for use in PERMANOVA were constructed using the Bray–Curtis index, and *P*-values were generated using *F*-tests based on sequential sums of squares from 99,999 permutations of the raw data.

### 3. Results

A total of 176,074 natural enemies belonging to 81 taxa were captured and identified in all samplings in the three orchards, including sticky traps and suction of the canopies (Appendix A.2 of Supplementary data). Of them, 39 taxa contained more than 100 individuals: 12 taxa of predators, 24 of parasitoids and 3 belonging to the fourth trophic level (primary hyperparasitoids). These were the taxa included in the comparative analyses of abundance (Table 1) (Appendix A.3 of Supplementary data). Among predators, the most abundant order was Neuroptera and the most abundant species were *Semidialis aleyrodiformis* Stephens and *Conwentzia psociformis* (Curtis) (both Neuroptera: Coniopterygidae). In the case of parasitoids, all of them belonging to the order Hymenoptera, the most abundant group was the superfamily Chalcidoidea, being *Aphytis chrysomphali* (Mercet) and *Cales noacki* Howard (Aphelinidae) the most abundant species (Table 1).

#### 3.1. Abundance of parasitoids and predators

When comparing the abundance of specific species or arthropod taxa between treatments we observed different responses depending on the functional group and species of natural enemy examined. From the 39 comparisons of particular taxa of natural enemies between ant-allowed and ant-excluded trees, 15 (38%) showed a significant difference between treatments (six remained significant after sequential Bonferroni correction, Appendix A.3 of Supplementary data). When separated according to functional groups, the abundance of 42% of the predator and 37% of the parasitoid taxa was affected in the ant-allowed treatment (Table 1). Further, in the comparisons obtained considering orchards individually, the percentage of taxa of natural enemies significantly affected in the ant-allowed treatment was 32% in orchard LH, 25% in orchard PP and 21% in orchard LG (Table 1).

In those cases where we detected significant differences between treatments we observed that in the ant-allowed treatment predator (concretely generalist predator) abundance was usually lower (five species decreased and two increased in at least one orchard), whereas parasitoid abundance was usually

higher (two species decreased and 13 increased in at least one orchard) (Table 1).

Differences in abundance between ant-allowed and ant-excluded treatments occurred in some of the most abundant species of natural enemies all along the sampling period and in the three orchards, as can be seen by examining their seasonal population trend (Fig. 1). For example, the abundance of the generalist predator *Chrysoperla carnea* sensu lato (Stephens) (Neuroptera: Chrysopidae) was consistently lower in the ant-allowed treatment ( $F_{1,12} = 19.26$ ;  $P = 0.0009$ ; sequential Bonferroni corrected  $P < 0.05$ ). On the contrary, the abundance of parasitoids such as *Aphytis hispanicus* (Mercet) ( $F_{1,12} = 24.64$ ;  $P < 0.0001$ ; sequential Bonferroni corrected  $P < 0.05$ ), parasitoid of *Parlatoria pergandii* Comstock (Hemiptera: Diaspididae), and *Anagyrus* sp. (Girault) (Hymenoptera: Encyrtidae) ( $F_{1,10} = 18.96$ ;  $P = 0.0014$ ; sequential Bonferroni corrected  $P < 0.05$ ), parasitoid of pseudo-coccids, remained usually higher in the ant-allowed trees during the sampling period (Fig. 1). Conversely, the response of other species was not so consistent and several taxa showed similar abundance in ant-allowed and ant-excluded treatments (Table 1).

Among true bugs (Heteroptera) the abundance of *Cardiasthetus* sp. (Heteroptera: Anthocoridae) ( $F_{1,12} = 12.29$ ;  $P = 0.0005$ ; sequential Bonferroni corrected  $P < 0.05$ ) and *Campyloneura virgula* (Herrich-Schäffer) (Heteroptera: Miridae) ( $F_{1,5} = 16.36$ ;  $P < 0.0072$ ) (Fig. 1) was significantly lower in the ant-allowed treatment (Table 1). On the other hand, the abundance of the myrmecomorphic *Pilophorus* sp. (Heteroptera: Miridae) was significantly higher in the ant-allowed treatment ( $F_{1,5} = 11.86$ ;  $P < 0.0171$ ).

Species belonging to the 4th trophic level (primary hyperparasitoids, most of them identified as morphospecies) showed mixed responses to ant presence. *Marietta* sp. (Hymenoptera: Aphelinidae), hyperparasitoid of coccid parasitoids, and *Ablerus* sp. (Hymenoptera: Aphelinidae), hyperparasitoid of diaspidid parasitoids, were significantly more abundant in the ant-allowed treatment. *Helorus* sp. (Hymenoptera: Heloridae), a parasitoid of chrysopid eggs, was less abundant in the ant-allowed treatment ( $F_{1,10} = 13.66$ ;  $P = 0.0039$ ).

#### 3.2. Species richness, diversity and community structure of predators and parasitoids

Overall, in the ant-allowed treatment the species richness (*S*) was significantly lower for predators and higher for parasitoids when compared with the ant-excluded trees. The impact of ants was, nevertheless, significant only in one orchard for predators and

**Table 2**  
Impact of ants on Species richness (*S*) (mean ± SE) and Shannon diversity index (*H'*) (mean ± SE) of predators and parasitoids, globally and for the three orchards separately.

Species richness ( <i>S</i> )										
Orchard	Predators					Parasitoids				
	Ant-excluded	Ant-allowed	d.f.	F	P	Ant-excluded	Ant-allowed	d.f.	f	P
LG	7.61 ± 0.37	6.96 ± 0.35	1,71	4.34	0.059*	11.02 ± 0.30	11.76 ± 0.33	1,71	5.5	0.037**
LH	8.42 ± 0.43	8.55 ± 0.42	1,56	0.05	0.828	13.54 ± 0.56	15.50 ± 0.60	1,56	14.08	0.003**
PP	7.35 ± 0.34	7.10 ± 0.38	1,75	0.76	0.401	11.58 ± 0.30	11.27 ± 0.31	1,75	0.78	0.396
Global	7.77 ± 0.22	7.44 ± 0.23	1,250	4.93	0.045**	12.03 ± 0.24	12.59 ± 0.28	1,250	14.02	0.003**

Shannon diversity ( <i>H'</i> )										
Orchard	Predators					Parasitoids				
	Ant-excluded	Ant-allowed	d.f.	F	P	Ant-excluded	Ant-allowed	d.f.	F	P
LG	0.66 ± 0.06	0.62 ± 0.07	1,71	0.16	0.699	1.41 ± 0.04	1.51 ± 0.03	1,71	5.98	0.031**
LH	1.32 ± 0.08	1.45 ± 0.06	1,56	1.99	0.183	1.40 ± 0.05	1.54 ± 0.04	1,56	6.67	0.022**
PP	0.69 ± 0.06	0.60 ± 0.06	1,75	1.50	0.245	1.37 ± 0.05	1.45 ± 0.05	1,75	5.28	0.040**
Global	0.88 ± 0.04	0.84 ± 0.05	1,250	0.09	0.773	1.39 ± 0.03	1.49 ± 0.02	1,250	33.58	<0.001**

Repeated measures analysis of variance (ANOVA) was used, with treatment as fixed factor, block as random factor and time as repeated measures factor. In global analysis, orchard was set as random factor. \*\* indicates a significant effect of ants ( $P < 0.05$ ) and \* indicates a marginally significant effect ( $P < 0.1$ ). LG: *Lasius grandis* orchard; LH: *Linepithema humile* orchard; PP: *Pheidole pallidula* orchard.

two orchards for parasitoids when examining the three orchards separately (Table 2).

The Shannon diversity index ( $H'$ ) was not different between the ant-allowed and ant-excluded treatments in the case of predators, whereas in the case of parasitoids diversity was significantly higher in the ant-allowed treatment both in the global analysis and when the three orchards were analyzed individually (Table 2).

The multivariate test showed that the community of parasitoids changed significantly in the ant-allowed treatment only in 2012 in the orchard LH (Table 3). In the other orchards and/or years the community structure was not different between treatments.

#### 4. Discussion

Our results show that the overall community structure of predators and parasitoids was not significantly different between the ant-allowed and the ant-excluded treatments. When analyzing the effect of ants on the abundance of particular species of natural enemies, there are many cases of significant differences across taxa or species. In general terms, we observed lower numbers of generalist predators and higher numbers of parasitoids in the ant-allowed treatment compared to the ant-excluded treatment. Most crucially, the species richness and diversity of parasitoids were higher in the ant-allowed treatment whereas the diversity of predators was not different between treatments.

##### 4.1. Abundance of predators and parasitoids

Contrary to our prediction, the abundance of most parasitoid species in our study was either not affected by ants or higher in the ant-allowed treatment. This is of particular interest especially if we consider the widely held assumption that ant-attendance offers Hemiptera a protective service against parasitoids (Way, 1963). The higher parasitoid abundance in the ant-allowed treatment seems to be related, at least in some cases, with the impact of ants on the parasitoid host populations, the relaxation of intraguild predation and/or with the ability of the particular species to cope with ant aggression (Barzman and Daane, 2001). Often, honeydew producing pests are more abundant under ant protection and eventually this might explain the higher abundance of their parasitoids in the ant-allowed treatment. In our study, parasitoids of honeydew producing pests, such as the soft scale parasitoids *Metaphycus helvolus* Compere, *Metaphycus flavus* Howard and *Encyrtus* sp. (Hymenoptera: Encyrtidae) or the mealybug parasitoid *Anagyrus* sp. were in general more abundant in the ant-allowed treatment, especially in the orchard LH. Additionally, the lower abundance of predators in ant-allowed trees may result in lower intraguild predation upon parasitized hosts, resulting in increased populations of some parasitoid species.

It was surprising to see that *Encarsia inquirenda* Silvestri and *A. hispanicus* (Hymenoptera: Aphelinidae), parasitoids of *P. pergandii*, an armored scale that does not produce honeydew and therefore is not tended by ants, were more abundant in the ant-allowed

treatment both globally and on each orchard analyzed individually. Apparently, ant presence is associated with increased abundances of *P. pergandii*, as already found for other armored scales (Calabuig et al., 2013; Pekas et al., 2010; Yoo et al., 2013). However, other parasitoids of non-honeydew producers did not follow this trend.

One of the most important findings in our study is the fact that the abundance of the generalist predators was lower in the ant-allowed treatment. It is important to highlight the case of the chrysopid (green lacewing) *C. carnea* sensu lato. This species is a key biological control agent in many agroecosystems and one of the most abundant predators in Mediterranean citrus orchards, preying upon a wide range of pests (García-Marí, 2012). Our results are in agreement with other studies which have also found lower densities of chrysopids in ant presence (James et al., 1999; Kaplan and Eubanks, 2002; Vanek and Potter, 2010). Several authors reported aggressive behavior of different ant species against chrysopids (Vanek and Potter, 2010) or ant predation on chrysopid eggs (Morris et al., 1998) which may result in lower chrysopid populations. Regarding the two most abundant species of predatory Heteroptera (true bugs) *Cardiasthetus* sp. and *Campyloneura virgula* we also registered lower populations in the ant-allowed treatment. In an 8-year study, Piñol et al. (2012b) also found lower abundance of predatory Heteroptera, especially *Cardiasthetus fasciventris*, in the ant-allowed trees. Finally, it is interesting to mention the higher populations of *Pilophorus* sp. in the ant-allowed treatment. This species exhibits ant mimetism that allows it to benefit from ant presence as was already reported in other studies between the ant *L. grandis* and *Pilophorus* sp. (Piñol et al., 2012b; Sánchez and Ortín-Angulo, 2012).

Regarding the impact of ants on coccinellids (ladybirds), which are mostly specialists, our results show great variability depending on the species examined. The response of coccinellids to ant aggression differs between species; some species can cope with ant aggression through morphological, behavioural or chemical adaptations. For example, Völkl and Vohland (1996) found higher populations of *Scymnus* sp. in ant attended resources due to the protective wax cover of the *Scymnus* larvae which allow them to predate upon honeydew producers tended by ants. Wimp and Whitham (2001) found that the aphid-ant mutualism had a negative impact on generalist predators and a positive effect on specialist enemies of aphids. Apparently, specialist predators have evolved the mechanisms necessary in order to deal with ant aggressiveness (Völkl, 1995; Way, 1963) which is not the case for the generalist predators.

The use of ant-exclusion barriers on the trunk might potentially exclude other non-flying predators from climbing on the citrus canopies. In the study area the only predators that could have been excluded by the barriers are earwigs; concretely the species *Forficula auricularia* L. (Dermaptera: Forficulidae). However, this species is of very low abundance in citrus in the study area (Alvis and García-Marí, 2006). In addition we observed no earwigs or other predators on the trunks of the ant-allowed treatment. Thus, we consider that our ant-exclusion method did not affect the abundance of the natural enemies on the canopies.

The abundance of some species from the 4th trophic level was also found to be different between the ant-allowed and ant-excluded treatments. This was apparently related with the abundance of their primary hosts. For example, *Ablerus* sp., hyperparasitoid of *A. chrysomphali*, as well as *Marietta* sp., hyperparasitoid of encyrtids, was more abundant in the ant-allowed treatment. On the other hand, *Helorus* sp., parasitoid of chrysopid eggs, was less abundant in the ant-allowed trees. Several studies have demonstrated that some parasitoids benefit from ant attendance because ants may reduce hyperparasitism by disturbing hyperparasitoids (Völkl, 1992). Additionally, intraguild predation caused by higher abundance of predators in the

**Table 3**

Summary of the PERMANOVA results of the effect of ants on the predators and parasitoids communities in 2011 and 2012 in orchards LG, LH and PP.

Year	Orchard	Predators		Parasitoids	
		R <sup>2</sup>	P	R <sup>2</sup>	P
2011	LG	0.077	0.742	0.105	0.630
	LH	0.301	0.059	0.210	0.288
	PP	0.372	0.057	0.199	0.200
2012	LG	0.050	0.828	0.107	0.544
	LH	0.166	0.371	0.332	0.029**
	PP	0.395	0.085	0.043	0.943

ant-excluded trees, may result in a decrease of the hyperparasitoid populations (Kaneko, 2002). In general, the impact of intraguild predation can create complex patterns of emergent multi-predator effects (Sih et al., 1998). As shown by Woodcock and Heard (2011) the risk of intraguild interactions increases when the domains of the predators overlap which is the case in our study system.

#### 4.2. Species richness, diversity and community structure of predators and parasitoids

To our knowledge, the present study is the first to demonstrate a significant increase on species richness as well as on the Shannon diversity index for parasitoids in the ant-allowed treatment. Previous studies in several ecosystems show usually a decrease of arthropod diversity as a result of ant activity (Human and Gordon, 1997; Wimp and Whitham, 2001). Nevertheless, it is difficult to compare these results with ours given that the previous studies focused on overall arthropod communities including different guilds such as herbivores. On the other hand, Peng and Christian (2013) found that weaver ants either had no impact or increased the diversity of natural enemies in cashew and mango trees.

Our results show that the community structure of predators and parasitoids on the citrus canopies were similar between treatments. Previous studies also found that ants did not affect the overall arthropod communities in vineyards (Chong et al., 2010), peaches (Mathews et al., 2009) or coffee plantations (Philpott et al., 2008). Conversely, Piñol et al. (2012a), in an 8-year exclusion experiment, reported that ants changed the arthropod community in a citrus orchard in some years of their study. Differences in the results obtained in the different studies might be attributed to the species of ants present and/or the characteristics of the experimental orchards.

The present study was not designed to compare the effects of specific ant species on the natural enemy community. Perhaps more replicates, i.e., orchards dominated from each ant species, should take place in order to make any inferences about the effect of the ant species. This however would be logistically very complex to undertake in a field study. Nevertheless, and despite the fact we cannot draw any definitive conclusions when it comes to comparison of species it is interesting to highlight that the impact of the three ant species on the community structure of natural enemies was quite similar. Despite *L. humile* having been described as an aggressive and very disruptive ant species for biological control (Martínez-Ferrer et al., 2003; Way, 1963), in our study we found no significant effects of this invasive species on the community structure of natural enemies. The same can be said of the native species, *L. grandis* and *P. pallidula*. In a previous study it was found that the three ant species induced similar population increases of the herbivore *A. aurantii* and *A. floccosus* (Calabuig et al., 2013).

## 5. Conclusion

In conclusion, our ant exclusion study revealed that ants in citrus were not associated with a dramatic and overall decrease in natural enemy abundance or biodiversity at the community level. The impact of ants on the natural enemies depended mostly on the species of natural enemy; even closely related species showed different or opposite responses to ant activity. In spite of the species specific response of natural enemies, we detected a general tendency related to functional groups: generalist predator abundance decreased whereas parasitoid abundance and diversity increased in the ant-allowed treatment. These results may have practical implications for biological pest control. Despite the fact that ants had no negative impact on the abundance and diversity of

predators and parasitoids at the community level their impact on specific natural enemy species may explain the higher pest densities associated with ant presence in citrus (Calabuig et al., 2013; Dao et al., 2014; Pekas et al., 2010; Yoo et al., 2013).

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agee.2015.08.001>

## References

- Alvis, L., Garcia-Mari, F., 2006. Identification and abundance of ants (Hymenoptera: Formicidae) in citrus trees from Valencia (Spain). IOBC-WPRS Bull. 29, 111–116.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Aust. Ecol. 26, 32–46.
- Barzman, S.M., Daane, M.K., 2001. Host-handling behaviours in parasitoids of the black scale: a case for ant-mediated evolution. J. Anim. Ecol. 70, 237–247. doi: <http://dx.doi.org/10.1111/j.1365-2656.2001.00483.x>.
- Beattie, A.J., 1985. The Evolutionary Ecology of Ant-Plant Mutualisms. Cambridge University Press.
- Calabuig, A., Garcia-Mari, F., Pekas, A., 2013. Ants affect the infestation levels but not the parasitism of honeydew and non-honeydew producing pests in citrus. Bull. Entomol. Res. 104, 405–417. doi: <http://dx.doi.org/10.1017/S0007485313000564>.
- Calabuig, A., Tena, A., Wackers, F.L., Fernandez-Arrojo, L., Plou, F.J., Garcia-Mari, F., Pekas, A., 2015. Ants impact the energy reserves of natural enemies through the shared honeydew exploitation. Ecol. Entomol. doi: <http://dx.doi.org/10.1111/een.12237>.
- Carroll, C., Janzen, D., 1973. Ecology of foraging by ants. Annu. Rev. Ecol. Syst. 4, 231–257.
- Chong, C.S., D'Alberto, C.F., Thomson, L.J., Hoffmann, A.A., 2010. Influence of native ants on arthropod communities in a vineyard. Agric. For. Entomol. 12, 223–232. doi: <http://dx.doi.org/10.1111/j.1461-9563.2010.00472.x>.
- Daane, K.M., Sime, K.R., Fallon, J., Cooper, M.L., 2007. Impacts of Argentine ants on mealybugs and their natural enemies in California's coastal vineyards. Ecol. Entomol. 32, 583–596. doi: <http://dx.doi.org/10.1111/j.1365-2311.2007.00910.x>.
- Dao, H.T., Meats, A., Beattie, G.A.C., Spooner-Hart, R., 2014. Ant-coccid mutualism in citrus canopies and its effect on natural enemies of red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae). Bull. Entomol. Res. 104, 137–142. doi: <http://dx.doi.org/10.1017/S0007485313000187>.
- Development Core Team, R., 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Folgarait, P., 1998. Ant biodiversity and its relationship to ecosystem functioning: a review. Biodivers. Conserv. 7, 1221–1244.
- Garcia-Mari, F., 2012. Plagas de los cítricos. Gestión integrada en países de clima mediterráneo. Phytoma, Valencia.
- Human, K., Gordon, D., 1997. Effects of Argentine ants on invertebrate biodiversity in northern California. Conserv. Biol. 11, 1242–1248.
- James, D.G., Stevens, M., O'Malley, K.J., Faulder, R.J., 1999. Ant foraging reduces the abundance of beneficial and incidental arthropods in citrus canopies. Biol. Control 14, 121–126. doi: <http://dx.doi.org/10.1006/bcon.1998.0678>.
- Kaneko, S., 2002. Aphid-attending ants increase the number of emerging adults of the aphid's primary parasitoid and hyperparasitoids by repelling intraguild predators. Entomol. Sci. 5, 131–146.
- Kaplan, I., Eubanks, M.D., 2002. Disruption of cotton aphid (Homoptera: Aphididae)-natural enemy dynamics by Red Imported Fire ants (Hymenoptera: Formicidae). Environ. Entomol. 31, 1175–1183. doi: <http://dx.doi.org/10.1603/0046-225X-31.6.1175>.
- Kaplan, I., Eubanks, M., 2005. Aphids alter the community-wide impact of fire ants. Ecology 86, 1640–1649. doi: <http://dx.doi.org/10.1890/04-0016>.
- Mathews, C., Bottrell, D., Brown, M., 2009. Extrafloral nectaries alter arthropod community structure and mediate peach (*Prunus persica*) plant defense. Ecol. Appl. 19, 722–730.
- Moran, M.D., 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100, 403–405. doi: <http://dx.doi.org/10.1034/j.1600-0706.2003.12010.x>.
- Morris, T.I., Campos, M., Jervis, M.A., McEwen, P.K., Kidd, N.A.C., 1998. Potential effects of various ant species on green lacewing, *Chrysoperla carnea* (Stephens) (Neuropt., Chrysopidae) egg numbers. J. Appl. Entomol. 401–403. doi: <http://dx.doi.org/10.1111/j.1439-0418.1998.tb01519.x>.
- Oliver, I., Beattie, A., 1996. Invertebrate morphospecies as surrogates for species: a case study. Conserv. Biol. 10, 99–109.

- Pekas, A., Tena, A., Aguilar, A., Garcia-Mari, F., 2010. Effect of Mediterranean ants (Hymenoptera: Formicidae) on California red scale (Hemiptera: Diaspididae) populations in citrus orchards. *Environ. Entomol.* 39, 827–834. doi:http://dx.doi.org/10.1603/EN09207.
- Pekas, A., Tena, A., Aguilar, A., Garcia-Mari, F., 2011. Spatio-temporal patterns and interactions with honeydew-producing Hemiptera of ants in a Mediterranean citrus orchard. *Agric. For. Entomol.* 13, 89–97. doi:http://dx.doi.org/10.1111/j1461-9563.2010.00501.x.
- Peng, R., Christian, K., 2013. Do weaver ants affect arthropod diversity and the natural-enemy-to-pest ratio in horticultural systems? *J. Appl. Entomol.* 137, 711–720. doi:http://dx.doi.org/10.1111/jen.12058.
- Philpott, S., Perfecto, I., Vandermeer, J., 2008. Effects of predatory ants on lower trophic levels across a gradient of coffee management complexity. *J. Anim. Ecol.* 77, 505–511. doi:http://dx.doi.org/10.1111/j.1365-2656.2008.01358.x.
- Piñol, J., Espadaler, X., Cañellas, N., 2012a. Eight years of ant-exclusion from citrus canopies: effects on the arthropod assemblage and on fruit yield. *Agric. For. Entomol.* 14, 49–57. doi:http://dx.doi.org/10.1111/j.1461-9563.2011.00542.x.
- Piñol, J., Ribes, E., Ribes, J., Espadaler, X., 2012b. Long-term changes and ant-exclusion effects on the true bugs (Hemiptera: Heteroptera) of an organic citrus grove. *Agric. Ecosyst. Environ.* 158, 127–131. doi:http://dx.doi.org/10.1016/j.agee.2012.06.004.
- Rice, W., 1989. Analyzing tables of statistical tests. *Evolution* 43, 223–225.
- Rico-Gray, V., Oliveira, P., 2007. *The Ecology and Evolution of Ant-plant Interactions*. University of Chicago Press.
- Rosumek, F., Silveira, F., Neves, F., 2009. Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160, 537–549. doi:http://dx.doi.org/10.1007/s00442-009-1309-x.
- Sánchez, J.A., Ortín-Angulo, M.C., 2012. Abundance and population dynamics of *Cacopsylla pyri* (Hemiptera: Psyllidae) and its potential natural enemies in pear orchards in southern Spain. *Crop Prot.* 32, 24–29. doi:http://dx.doi.org/10.1016/j.cropro.2011.11.003.
- Shannon, C.E., Weaver, W., 1949. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana.
- Sih, A., Englund, G., Wooster, D., 1998. Emergent impacts of multiple predators on prey. *Trends Ecol. Evol.* 13, 350–355.
- Statgraphics, 1994. *Statistical Graphics System, Version 5.1 Plus*. Manugistics, Rockville, MD, USA.
- Styrsky, J.D., Eubanks, M., 2007. Ecological consequences of interactions between ants and honeydew-producing insects. *Proc. R. Soc. B Biol. Sci.* 274, 151–164. doi:http://dx.doi.org/10.1098/rspb.2006.3701.
- Tena, A., Soto, A., Garcia-Mari, F., 2008. Parasitoid complex of black scale *Saissetia oleae* on citrus and olives: parasitoid species composition and seasonal trend. *BioControl* 53, 473–487.
- Völkl, W., Vohland, K., 1996. Wax covers in larvae of two *Scymnus* species: do they enhance coccinellid larval survival? *Oecologia* 107, 498–503. doi:http://dx.doi.org/10.1007/BF00333941.
- Völkl, W., 1992. Aphids or their parasitoids: who actually benefits from ant-attendance? *J. Anim. Ecol.* 61, 273–281.
- Völkl, W., 1994. The effect of ant-attendance on the foraging behaviour of the aphid parasitoid *Lysiphlebus cardui*. *Oikos* 70, 149–155.
- Völkl, W., 1995. Behavioral and morphological adaptations of the coccinellid, *Platynaspis luteorubra* for exploiting ant-attended resources (Coleoptera: Coccinellidae). *J. Insect Behav.* 8, 653–670.
- Vanek, S.J., Potter, D.A., 2010. Ant-exclusion to promote biological control of soft scales (Hemiptera: Coccidae) on woody landscape plants. *Environ. Entomol.* 39, 1829–1837. doi:http://dx.doi.org/10.1603/EN10093.
- Way, M., Khoo, K., 1992. Role of ants in pest management. *Annu. Rev. Entomol.* 37, 479–503.
- Way, M., 1963. Mutualism between ants and honeydew-producing Homoptera. *Annu. Rev. Entomol.* 8, 307–344.
- Wimp, G., Whitham, T., 2001. Biodiversity consequences of predation and host plant hybridization on an aphid-ant mutualism. *Ecology* 82, 440–452.
- Woodcock, B.A., Heard, M.S., 2011. Disentangling the effects of predator hunting mode and habitat domain on the top-down control of insect herbivores. *J. Anim. Ecol.* 80, 495–503. doi:http://dx.doi.org/10.1111/j.1365-2656.2010.01790.x.
- Yoo, H.J.S., Kizner, M.C., Holway, D.A., 2013. Ecological effects of multi-species, ant-hemipteran mutualisms in citrus. *Ecol. Entomol.* 38, 505–514. doi:http://dx.doi.org/10.1111/een.12042.