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Original Article

Ants (Hymenoptera: Formicidae) reduce the density of *Cacopsylla pyri* (Linnaeus, 1761) in Mediterranean pear orchards

Juan Antonio Sanchez, Aline Carrasco-Ortiz, Elena López-Gallego & Michelangelo La Spina

Abstract

Ants have a strong impact on the population dynamics of sap-sucking hemipterans, but their role in pest control in pear orchards is still to be clarified. The pear psyllid, *Cacopsylla pyri* (Linnaeus, 1761), is the main pest of pear trees in the Mediterranean area and temperate Europe. Integrated pest management and biological control are good alternatives for the control of psyllids. The aim of this work was to determine the effect of ants on the control of the psyllid. This was investigated in one pear orchard through an experiment involving a randomised block design with two factors: (1) ant exclusion – with and without – and (2) psyllid density – three levels. The pear trees accessible to ants (namely *Lasius grandis* Forel, 1909) had significantly lower psyllid abundances than those from which ants were excluded; the effect of ants was significant for the three density levels of the psyllid assayed. Ant abundance showed a non-linear relationship with honeydew; an increase in ant abundance until approximately 5% of the leaf surface was impregnated with honeydew, with a subsequent decrease at higher values, was predicted. The abundance of coccinellids and anthocorids was significantly lower in the presence of ants. Ants had a strong effect, lowering the numbers of psyllids. Overall, ants seem to contribute positively to biological pest control in pear orchards. The non-linear numerical response of ants may have important implications in the control of psyllids; the washing of excessive honeydew from trees is advised to improve the predatory response of ants.

Key words: *Lasius grandis*, psyllids, sap-sucking hemipterans, population dynamics, biological control, ant exclusion, honeydew.

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Introduction

Many ant species use colonies of honeydew-producing hemipterans as their principal source of carbohydrates (Hölldobler & Wilson 1990, Delabie 2001). In return, ants benefit their hemipteran partners by providing protection against their natural enemies, increasing their fecundity, and reducing competition with other herbivores (Del-Claro & Oliveira 2000, Flatt & Weisser 2000, Billick & al. 2007, Smith & al. 2008, Stadler & Dixon 2008, Cheng & al. 2015). This relationship is frequently cited as one of the most classical examples of mutualism in the world of insects (Bronstein 1994a, Delabie 2001, Stadler & Dixon 2008). However, the response of ants in the context of honeydew-producing hemipterans is not straightforward, their behaviour being influenced by the ecological context (Bronstein 1994b, Sakata 1999, Del-Claro & Oliveira 2000, Mooney & Agrawal 2008). For instance, ants are known to predate on their hemipteran partners when their sugar needs are fulfilled or to increase predation of low-rewarding species in the presence of more-rewarding ones (Sakata 1999, Fischer & al. 2001, Billick & al. 2007, Styrsky & Eubanks 2007). In this sense, ant-hemipteran interactions are not always beneficial for the two partners, especially for the hemipterans, covering a wide spectrum from antagonism to mutualism (Bronstein 1994b, Offenberg 2001, Billick & al. 2007, Petry & al. 2012).

Ants have always been a group of insects difficult to label in relation to their role in agricultural crops. Because of their protective behaviour, ants interfere negatively with many groups of natural enemies and, generally, they have been regarded as disrupters of the biological control of myrmecophilous hemipterans and other phytophagous pests in fruit-tree orchards (Erler 2004, Yoo & al. 2013, Calabuig & al. 2015). However, many cases in the literature report a positive effect of the ant-hemipteran mutualism on biological control, with few studies documenting a

reduction in plant fitness (Eubanks & Styrsky 2006, Morris & al. 2018). Although ants are among the insects with the greatest impact on the structure of the populations of terrestrial invertebrates (Hölldobler & Wilson 1990, Styrsky & Eubanks 2007, Stadler & Dixon 2008, Feng & al. 2015), they have rarely been taken into account in relation to pest control in pear orchards and their role in the control of psyllids is still to be clarified (Paulson & Akre 1992, Sanchez & Ortin-Angulo 2012, Sanchez & al. 2019).

Cacopsylla pyri (Linnaeus, 1761) (Hemiptera: Psyllidae) is the main pest of pear orchards in the Mediterranean area and temperate Europe (Stamenkovic & al. 2001, Silva & al. 2005). This pear psyllid produces direct damage, by feeding on pear shoots, and indirect damage, by the transmission of phytoplasma; besides, it produces a great amount of honeydew that is colonised by sooty mould, reducing the photosynthetic activity and defoliating trees (Blomquist & Kirkpatrick 2002, Silva & al. 2005, Sule & al. 2007). Integrated pest management (IPM) has become the most sustainable alternative for the control of the pear psyllid due to the restriction of the application of chemicals and the development of resistances (Vilajeliu & al. 1998, Buès & al. 2003, Civolani & Pasqualini 2003, Erler 2004). Currently, the control of *C. pyri* in European IPM pear orchards is mainly based on the release and/or conservation of *Anthocoris nemoralis* (Fabricius, 1794) (Hemiptera: Anthocoridae) (Scutareanu & al. 1999, Sigsgaard & al. 2006). However, because of its delayed numerical response, *A. nemoralis* often fails to prevent psyllid outbreaks (Sarasua & al. 1994, Vilajeliu & al. 1998, Scutareanu & al. 1999, Erler 2004). Many other groups of natural enemies are known to spontaneously colonise and multiply in pear orchards when the pressure of insecticides is reduced (Vilajeliu & al. 1998, Solomon & al. 2000, Erler 2004, Sanchez & Ortin-Angulo 2012). However, because of their generalist character, their specific role in the regulation of the populations of psylllids is practically unknown and difficult to assess (Symondson & al. 2002, Sanchez & Ortin-Angulo 2012). Among these natural enemies, ants have been reported recently as key species for the control of psyllids in Mediterranean pear orchards (Sanchez & al. 2019).

The aim of this work was to determine the impact of ants on the regulation of the populations of the pear psyllid, *Cacopsylla pyri*, and the numerical response of ants as a function of the abundance of honeydew in pear orchards. The null hypothesis was that ants will have no effect on the population dynamics of the psyllid and, therefore, no significant differences will be registered in the abundance of the psyllid between pear trees with and without ant-exclusion barriers. In relation to the numerical response of ants, a linear increase in the abundance of ants on pear trees as a function of the availability of honeydew was predicted.

Materials and methods

Location and setting-up of experimental plots: The assay was carried out in one pear orchard of 6400 m², with eight rows of 80 pear trees each, located in the area of Las Encebras (38° 23' 20" N, 1° 14' 28" W) in the municipality of Jumilla (Murcia, Spain), from 12 April to 30 July 2012. This area has a continental Mediterranean climate with an average precipitation around 300 mm per year and an average annual temperature of 15 °C; generally, most of the rainfall takes place in autumn (September - December). The trees were 2 - 2.5 m tall and did not touch each other, being 4 m apart between lines and 2 m apart within lines. The orchard was split longitudinally in three blocks of two lines each; the blocks were separated by another line of trees. Three plots of 20 pear trees each (i.e., 2 lines of 10 trees) were established at random within each of the three blocks. The orchard was conducted under conventional agricultural practices in the previous years, but it was dedicated exclusively to the present experiment during 2012; no chemical treatments other than those specified in the experimental settings below were applied along the assay.

Experimental settings and sampling: In each of the three plots of each block, the psyllid density was regulated at three levels (T1, T2 and control) using different intensities of spraying. T1 plots were sprayed once (12 April 2012) with paraffinic oil (83%), T2 plots were sprayed twice with paraffinic oil (83%) (12 April and 4 May 2012), and control plots were not sprayed at all. Paraffinic oil (83%) was used for spraying because it is harmless to predators (Contreras & al. 2006, Sanchez & Ortin-Angulo 2012). In the buffer zones on the outside of the experimental plots, the psyllid was kept under control by the regular spraying (i.e., six treatments along the duration of the experiment) of abamectin (1.8%) and paraffinic oil (83%). A plastic film was placed along the sides of the experimental plots to avoid the drift of insecticide when the buffer trees were treated. Ant-exclusion barriers were placed on half (i.e., 10) of the trees, selected at random, in each of the nine experimental plots. A 20-cm-wide band of yellow paper tape, with the sticky surface on the outside, was placed on the middle of each trunk. The bands were impregnated with a solution of glue (40%) (Roe-Glue, Massó Animal Health, Barcelona) and an organic solvent (60%) that was applied with a brush once a week; the paper bands were replaced once a month, or more frequently if needed.

The sampling for the estimation of the abundance of all arthropods was performed following the indications of Sanchez & Ortín-Angulo (2011). The samplings were carried out weekly from 12 April to 30 July 2012. The numbers of ants and other predators were counted visually in situ on five shoots (approx. 20 cm long) of each of the 20 trees of each of the nine plots (i.e., 10 trees with and 10 without ant exclusion). One fully developed upper leaf of each of these shoots was selected for the counting of the psyllids (nymphs and adults, separately), which was performed using a hands-free magnifying glass (1.8 - 4.8 ×). On each of those leaves, the percentage of leaf covered by honeydew (×) was estimated using the following scale (honeydew index): 1 ($0 < \times \leq 1\%$), 2 ($1 < \times \leq 5\%$), 3 ($5 < \times \leq 10\%$), 4 ($10 < \times \leq 25\%$), 5 ($25 < \times \leq 50\%$) and

6 (50 < × ≤ 100%). In addition, after the visual sampling, the 10 trees with and without ant exclusion of each plot were sampled separately by beating for the estimate of the abundances of *Cacopsylla pyri* adults, ants and other predators; for this purpose, entomological funnels – diameter 28 cm – equipped with a 100-ml plastic beaker at the tip for the collection of the sample were used (Sanchez & Ortin-Angulo 2011). For this sampling, one branch (2 - 3 cm in diameter) from each of the 10 trees of each plot was hit at the base three times with a wooden stick, placing the funnel below for the collection of the sample. The beakers with the samples were taken to the laboratory in refrigerated containers for processing and the identification of the arthropods. *Cacopsylla pyri* was identified following the Hodkinson & White (1979) keys to Psylloidea and *Pilophorus gallicus* Remane, 1954 (Hemiptera: Miridae) according to Josifov (1989) and Rieger (2006). Anthocoridae, ants, and Chrysopidae were identified using the keys provided by Péricart (1972), Martínez & al. (1985) and Collingwood (1978), and Monserrat (2016), respectively.

Analysis of data: The abundance of psyllids and ants – as dependent variables – both in the visual and beating samplings was tested in function of the level regulation of the psyllid density (T1, T2 and control) and ant exclusion (excluded / non-excluded) – as fixed factors – using Generalized Linear Mixed Models (GLMM), with block and date of sampling as random factors. Additionally, tree was included as a random factor in the visual sampling. In the case of the visual sampling, the dependent variable was the average of the number of psyllids (nymphs + adults) on the five leaves of the same tree or the average number of ants on the five shoots. The function "glmmPQL" (library "MASS") was used to perform the analyses in R, with the log normal family for the psyllid and the negative binomial distribution for the ants (R Development Core Team 2017). In the beating sampling, the dependent variable was the number of psyllid adults or ants captured on the beating of the 10 trees of each treatment (i.e., with and without ant exclusion) on each plot and date; psyllid nymphs were not included because beating is not a proper method for the sampling of nymphs (Sanchez & Ortin-Angulo 2011). The analyses were performed using the function "lmer" (library "lme4") (Bates & al. 2015). In this case, psyllid data were found to be normally distributed but ant data were transformed by the natural logarithm of (× + 1) to correct their deviation from normality. For all GLMM the contrast among the three levels of spraying was performed with the function "glth" (library "multcomp") (R Development Core Team 2017).

GLMM with the function "glmmPQL", with the quasipoisson distribution as family, were used to explain the variation in the honeydew index per leaf -dependent variable- as a function of the level of regulation of the psyllid density and ant exclusion, as fixed factors. The honeydew index per leaf was the average of the five leaves sampled in a tree. Block, date of sampling and tree were introduced in the models as random factors.

The numerical response of the ants was explained as a function of the honeydew index using Generalized Additive Models (GAM) (Hastie & Tibshirani 1990), with the "gam" function (library "mgcv") (R Development Core Team 2017). The average number of ants per shoot in the plots where ants were allowed access to pear trees was used as a dependent variable; entries with values of zero were excluded from the matrix and the data were transformed by the natural logarithm.

The effect of the variables ant-exclusion barriers and level of regulation of the psyllid density on the abundance of predators, that is, *Pilophorus gallicus* (Remane, 1954) (Hemiptera: Miridae), *Forficula auricularia* Linnaeus, 1758 (Dermaptera: Forficulidae), spiders, chrysopids, coccinellids and anthocorids, was tested by analysis of variance using the function "aov" (library "stats") (R Development Core Team 2017). Only the data from the beating sampling were used because these predators were rarely observed during the visual sampling. Because of the low abundances, in each block, all the captures from the 16 sampling dates in each of the six treatments (i.e., the combination of the two levels for ant exclusion and the three levels for the regulation of the psyllid density) were summed for each group of predator. The post-hoc analyses for the separation of the means were performed with the Tukey test using the function "TukeyHSD" available in the "stats" package- (R Development Core Team 2017). For this and all the previous statistical analyses, the significance level was established at $\alpha = 0.05$.

Results

Abundance of ants and other groups of predators: The ant-exclusion method worked properly, with very few ants registered on the trees with exclusion barriers along the experiment (Figs. 1 and 2). The diversity of ants was low; *Lasius grandis* Forel, 1909 (Hymenoptera: Formicidae) was the predominant species, representing 98.3 and 88.1% of the ants registered visually on shoots and in the beating samples, respectively. In contrast, *Plagiolepis* sp. represented 1.7 and 11.9% of the ants on shoot and beating samples, respectively. Just one or two individuals of *Tapinoma nigerrimum* (Nylander, 1856) (Hymenoptera: Formicidae) and *Camponotus* sp. were collected during the beating of the branches. All these ant species nest in the soil and commute between the soil and the pear trees.

In the visual sampling, the abundance of ants on the trees without exclusion barriers increased progressively between the end of May and the beginning of July, reaching density peaks of 4.8 ± 1.2, 1.8 ± 1.2 and 1.6 ± 0.5 ants per shoot (mean ± standard error) in T1, T2 and control plots, respectively (Fig. 1). In the beating sampling, the lowest ant abundances were also registered in T2 and control plots; the ant density peaks in July at T1 (379.3 ± 95.2) and T2 (73.6 ± 39.2), while in control plots it peaked by mid May (112.3 ± 36.4 ants per 10 beaten trees) (Fig. 2). Differences between the ant-excluded and ant-allowed treatments were clearly significant, for the abundance of ants estimated using both the visual sampling of shoots

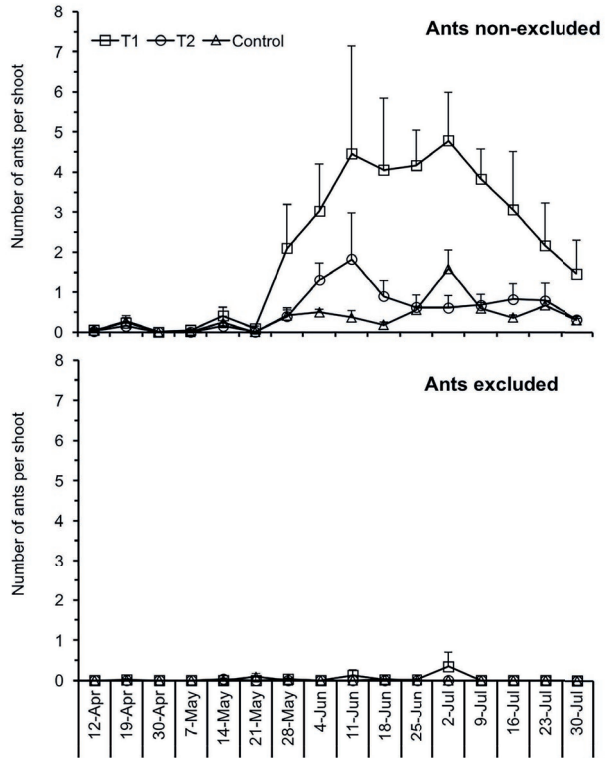

Fig. 1: Number of ants per shoot (mean ± standard error) in plots with different levels of regulation of the psyllid density (control, T1 and T2), in trees with (lower graph) and without (upper graph) ant-exclusion barriers.

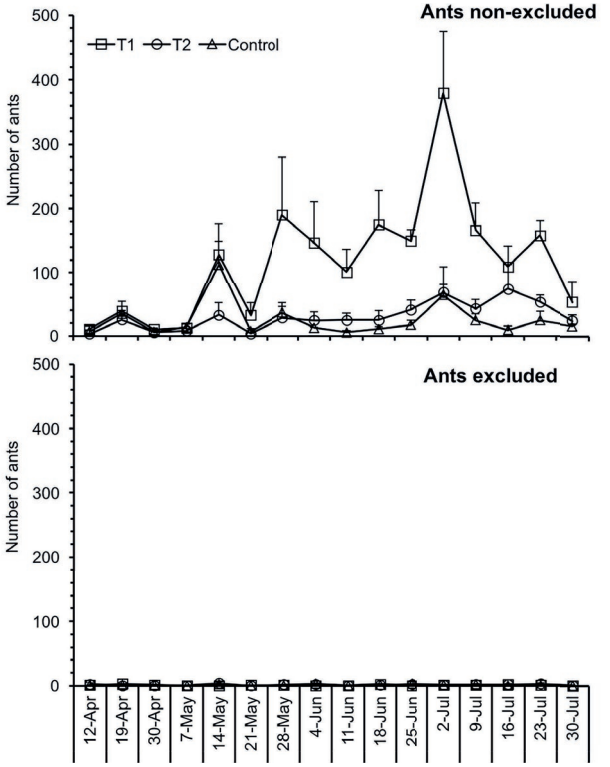

Fig. 2: Number of ants counted after the beating of 10 trees (mean ± standard error) in plots with different levels of regulation of the psyllid density (control, T1 and T2), in trees with (lower graph) and without (upper graph) ant-exclusion barriers.

[$\chi^2(1) = 154.3$, $P < 0.001$] and the beating method [$\chi^2(1) = 785.3$, $P < 0.001$]. The level of regulation of the psyllid population had also a significant effect on the abundance of ants on pear trees (Figs. 1 and 2). The ants showed the lowest abundances in the trees with the highest pear psyllid densities, being their highest abundances registered in trees with intermediate densities of psyllids. On the visual sampling, overall significant differences were found in the abundance of the ants among levels [$\chi^2(1) = 94.4$, $P < 0.001$], with a significantly higher number of ants in T1 than in T2 and control trees (Tukey contrast, $P < 0.001$). Similar results were obtained with the beating sampling, with overall significant differences in ant abundance among levels [$\chi^2(2) = 41.9$, $P < 0.001$]. The number of ants collected in the beating samples in T1 differed significantly from those in T2 and the control (Tukey contrast, $P < 0.001$), but no significant difference was found between T2 and the control (Tukey contrast, $P = 0.479$).

The abundance of ants on pear shoots showed a non-linear response to the abundance of honeydew on pear leaves (Fig. 3). It increased until the honeydew index reached a value of about two (i.e., 1 - 5% of the leaf surface impregnated with honeydew), decreasing progressively thereafter. The relationship between the abundance of ants on pear shoots and the honeydew index was satisfactorily explained by a GAM model ($R^2 = 0.372$; $F = 14.0$; $df = 5$; $P = 0.001$). Figure 3 shows the prediction of the model with the standard error for the prediction of the means.

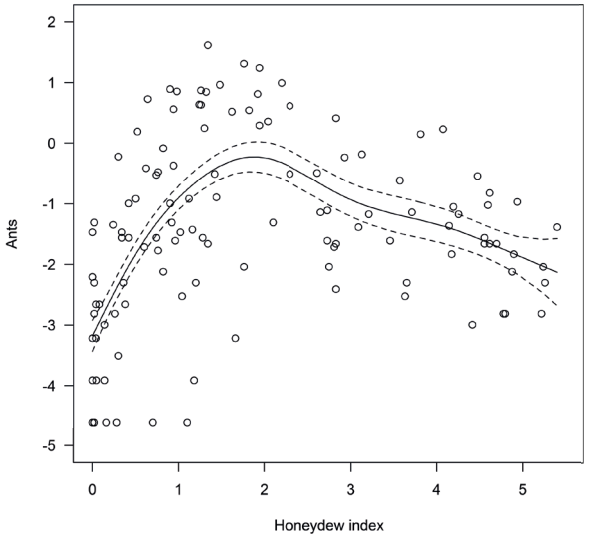

Fig. 3: Number of ants (data transformed by the natural logarithm) as a function of the honeydew index. The curves represent the prediction of the GAM model (solid line) and the standard error for the prediction (dotted lines).

Other groups of arthropods were much less abundant than ants and were mostly registered in the beating sampling (Fig. 4). In these samples, the representation of the most common predators – such as *Pilophorus gallicus* (1.46%), *Forficula auricularia* (0.90%), spiders (1.17%),

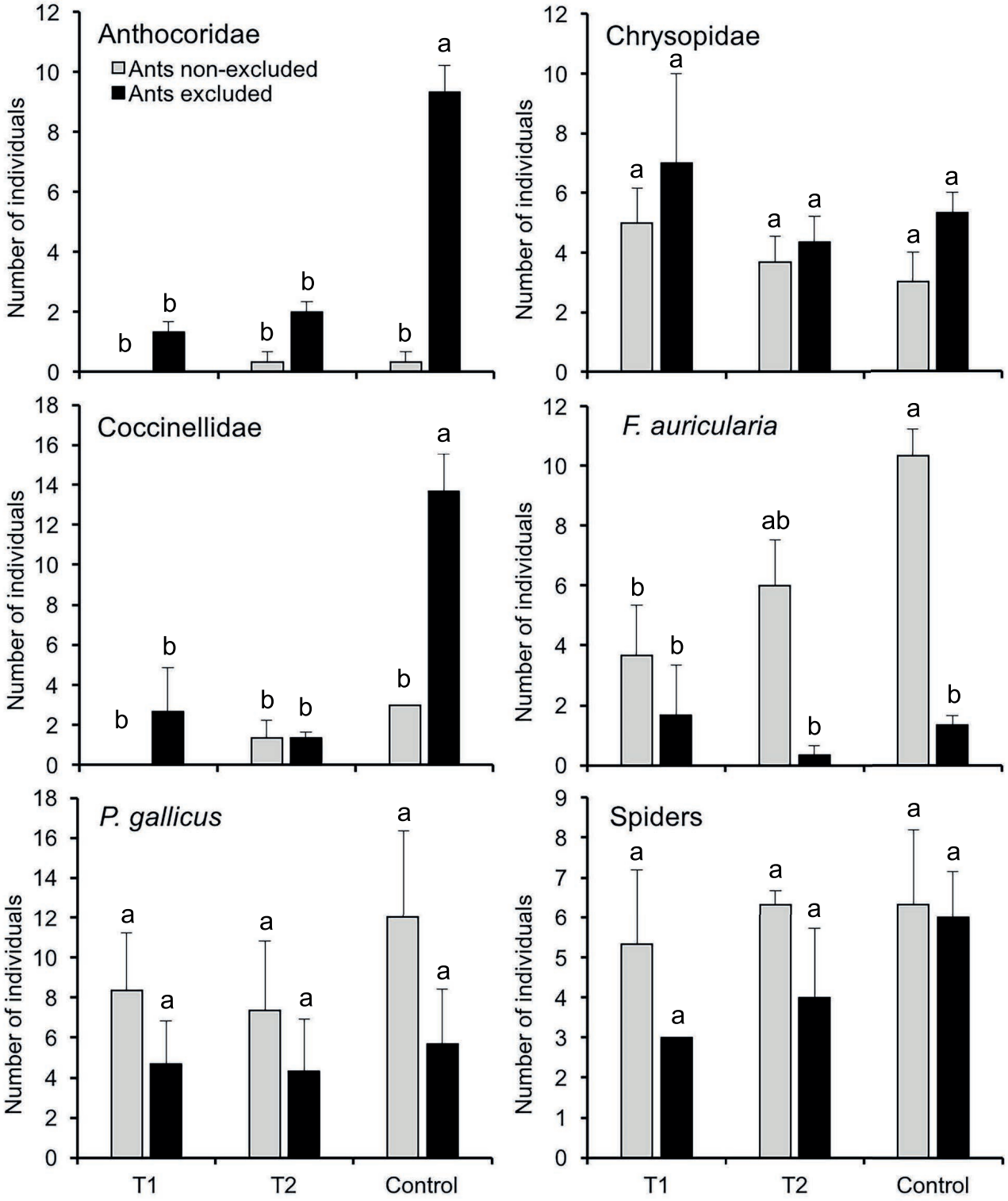

Fig. 4: Total number of individuals of the main groups of predators (mean ± standard error) captured using the beating sampling in trees with and without ant-exclusion barriers for the different levels of regulation of the psyllid density (T1, T2 and control). The same letters at the top of bars indicate no significant differences on the mean values (Tukey test, P > 0.05).

chrysopids (1.37%), coccinellids (0.66%) and the anthocorids *A. nemoralis* and *Orius* spp. (0.44%) – was very low in relation to the abundance of ants (94%). Ant exclusion and the level of the regulation of the psyllid density, as well as the interaction between these two variables, had a significant effect on the abundance of Anthocoridae and Coccinellidae (Fig. 4, Tab. 1). The abundance of these predators in trees with ant exclusion at the highest psyllid density (i.e., the control) was significantly higher than in the rest of the treatments (Fig. 4). In contrast, the abundance

Tab. 1: Analysis of variance of the individuals of various groups of predators as a function of ant exclusion, levels of regulation of the psyllid density, and their interaction. Df, degree of freedom (numerator, denominator); MS, mean of squares.

Taxa	Variables	Df	MS	F-value	P-value
Anthocoridae	Ant exclusion	1, 12	72.00	68.21	< 0.001
	Regulation level	2, 12	31.06	29.42	< 0.001
	Interaction	2, 12	28.17	26.68	< 0.001
Chrysopidae	Ant exclusion	1, 12	12.50	1.88	0.196
	Regulation level	2, 12	7.39	1.11	0.362
	Interaction	2, 12	1.17	0.18	0.842
Coccinellidae	Ant exclusion	1, 12	88.89	19.51	< 0.001
	Regulation level	2, 12	98.00	21.51	< 0.001
	Interaction	2, 12	46.22	10.15	0.003
Forficula auricularia	Ant exclusion	1, 12	138.90	31.25	< 0.001
	Regulation level	2, 12	17.39	3.91	0.049
	Interaction	2, 12	18.39	4.14	0.043
Pilophorus gallicus	Ant exclusion	1, 12	84.50	2.89	0.115
	Regulation level	2, 12	14.89	0.51	0.614
	Interaction	2, 12	4.67	0.16	0.854
Spiders	Ant exclusion	1, 12	12.50	2.21	0.163
	Regulation level	2, 12	6.00	1.06	0.377
	Interaction	2, 12	2.00	0.35	0.710

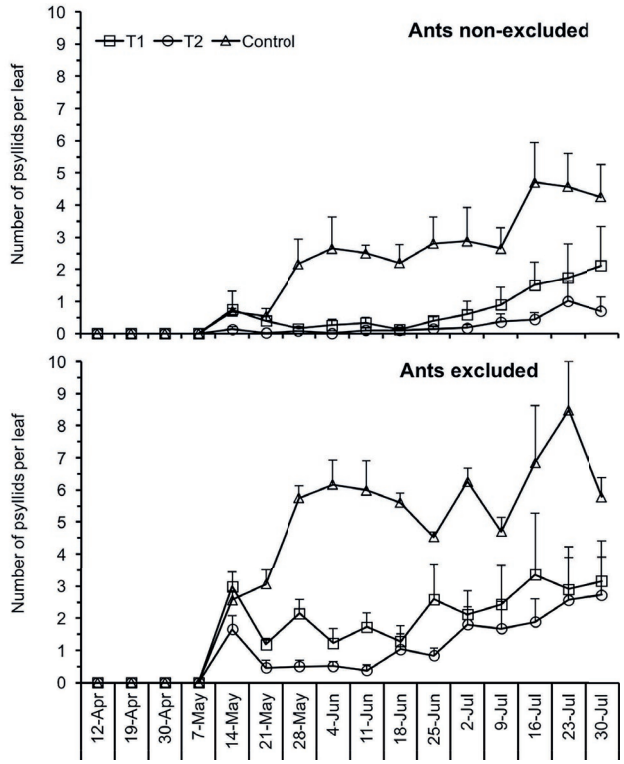

Fig. 5: Number of psyllids (nymphs + adults) per shoot (mean ± standard error) in plots with different levels of regulation of the psyllid density (control, T1 and T2), in trees with (lower graph) and without (upper graph) ant-exclusion barriers.

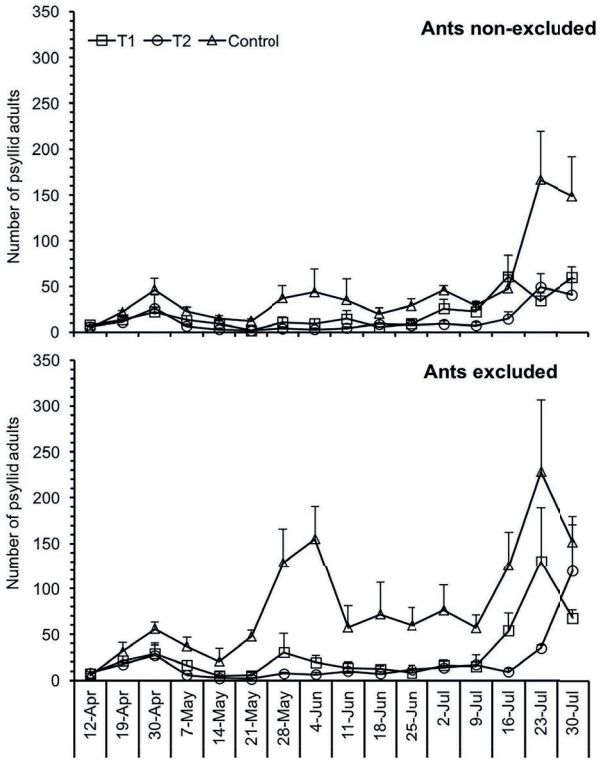

Fig. 6: Number of psyllid adults counted after the beating of 10 trees (mean ± standard error) in plots with different levels of regulation of the psyllid density (control, T1 and T2), in trees with (lower graph) and without (upper graph) ant-exclusion barriers.

of *F. auricularia* in control trees without exclusion barriers was significantly higher than in the rest of the treatments, with the exception of T2 (Fig. 4, Tab. 1). No significant differences were found in the abundance of Chrysopidae, *P. gallicus* and spiders among treatments (Fig. 4, Tab. 1).

Effects of ant exclusion and the intensity of spraying on the abundance of the pear psyllid: Ant exclusion had a strong effect on the abundance of *Cacopsylla pyri* (Figs. 5 & 6). The nymphs represented 93.5% of the total number of psyllids (nymphs + adults) on leaves. In trees without exclusion barriers, the number of psyllid on leaves increased from mid May until the end of the experiment, with increasing density peaks from T2 (1.0 ± 0.6), T1 (2.1 ± 1.2), and the control (4.7 ± 1.2 psyllids per leaf). In the trees where ants were excluded, the trend of population dynamics of the psyllid was similar to the trees with free ant access, but the psyllid densities were higher (Fig. 5). In T1 and T2 plots, the abundance of psyllid reached a primary peak in mid May (T1 = 3.0 ± 0.5, T2 = 1.7 ± 0.4 psyllids per leaf), and a secondary one in the second half of July (T1 = 3.4 ± 1.9, T2 = 2.7 ± 1.2 psyllids per leaf). In contrast, the density of psyllids in control plots increased progressively to reach only one peak (8.5 ± 1.5 psyllids per leaf) at the end of July (Fig. 5). The presence of other honeydew producers was anecdotic; only 40 Aphis sp. were registered on all the leaves sampled over the experiment. The abundance of psyllids registered on the treatments with ant-exclusion barriers was significantly higher than in those where ants had free access [$\chi^2(1) = 94.4$, $P < 0.001$]. The level of regulation of the psyllid had also a significant effect on its abundance on leaves [$\chi^2(2) = 1438.2$, $P < 0.001$], with significant differences found among the three levels (Tukey contrast, $P < 0.001$). The psyllid density was highest in the plots that were not sprayed (control), followed by those that were sprayed once (T1) and then those that were sprayed twice (T2) with paraffinic oil (Fig. 5).

In the beating method, the psyllid adults were already detected in the first sampling (Fig. 6). In the trees without ant exclusion barriers, the number of psyllid adults increased progressively, with some ups and downs, since April until the end of the experiments in the lasts weeks of July. The density peak was higher in the control (166.7 ± 52.9), than in T1 (61.0 ± 23.5) and T2 (49.0 ± 15.4 psyllid adults per 10 beaten trees) (Fig. 6). In the trees with ant exclusion, the density of psyllid adults was higher than in those where ants had free access to the trees (Fig. 6). The density of psyllid adults in control plots increased progressively since the beginning of April to reach one primary peak (128.7 ± 37.2) in June and a secondary one (151.0 ± 28.5 psyllid adults per 10 beaten trees) at the end of July. In T1 and T2 plots the density of the psyllid adults was lower than in control plots, reaching peaks at the end of July of 130.0 ± 59.5 and 120.3 ± 49.8 psyllid adults for T1 and T2, respectively. The abundance of psyllid adults in the beating samples was significantly higher when ants were excluded than when ants were given free access to the trees [$\chi^2(1) = 18.4$, $P < 0.001$] (Fig. 6). The

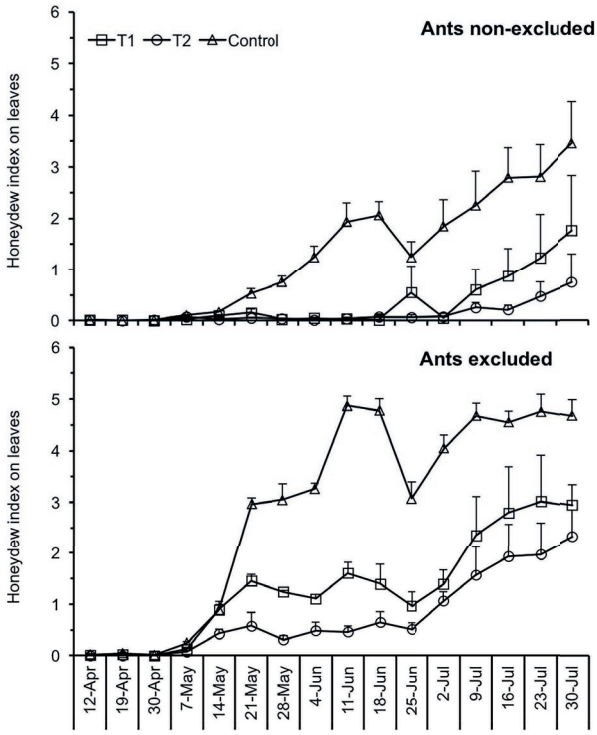

Fig. 7: Honeydew index (mean ± standard error) in plots with different levels of regulation of the psyllid density (control, T1 and T2), in trees with (lower graph) and without (upper graph) ant-exclusion barriers.

regulation of the psyllid density had also a significant effect on the number of psyllid adults on the beating samples [$\chi^2(2) = 211.7$, $P < 0.001$] (Fig. 6). Significant differences were found among the three levels of the regulation of the psyllid density (Tukey contrast, $P < 0.001$), with the abundance of adults progressively decreasing from the control to levels T1 and T2.

The incidence of honeydew on leaves increased in a parallel way to the density of psyllids. When ants were excluded, the overall average honeydew index was 1.33 ± 0.24, 0.77 ± 0.21 and 2.87 ± 0.04 in T1, T2 and control plots, respectively. In the presence of ants, the average honeydew index was reduced to 0.34 ± 0.22, 0.14 ± 0.07 and 1.32 ± 0.23 in T1, T2 and control plots respectively. The regulation of the psyllid density had a significant effect on the incidence of honeydew [$\chi^2(1) = 1488.4$, $P < 0.001$], with significant differences among the three levels (Tukey contrast, $P < 0.001$), both with and without ant exclusion. Besides, the incidence of honeydew was significantly higher when ants were excluded than when they had free access to the pear trees [$\chi^2(1) = 666.0$, $P < 0.001$] (Fig. 7).

Discussion

Ants have a strong impact on the structure of the communities of terrestrial arthropods (Hölldobler & Wilson 1990, Styrsky & Eubanks 2007, Stadler & Dixon 2008). In the present work, ants had a significant effect on the population dynamics of the psyllid *Cacopsylla pyri* in a pear orchard. Contrary to our null hypothesis, the pear trees accessible to ants had significantly lower psyllid

abundances than those from which ants were excluded. This effect was registered for all three population density levels of the psyllid assayed. Ants have been reported, on several occasions, as the main biological agent behind the reduction of psyllid populations (Paulson & Akre 1992, Sanchez & al. 2019). In the present work, because of the outstandingly high number of ants in trees without exclusion barriers, in comparison with other groups of potential psyllid predators, and the almost total absence of ants from trees with exclusion barriers, it was concluded that the ants were the main biological agent behind the reduction in the number of psyllids in the former case. Previous work showed that ants preyed on psyllids and had a negative effect on the population growth rate of the psyllid in pear orchards (Sanchez & al. 2019).

The behaviour of ants depends largely on the ecological context (Billick & al. 2007, Smith & al. 2008, Stadler & Dixon 2008). The scientific literature shows a wide disparity in relation to the impact of ants on the population growth of myrmecophilous hemipterans. On the one hand, in many cases both aphids and ants benefited from their partnership, with high correlations between the population increases of the two mutualists (Flatt & Weisser 2000, Kaplan & Eubanks 2005, Miñarro & al. 2010, Powell & Silverman 2010, Mestre & al. 2016). On the other hand, other studies reported negative effects of ants on sap-sucking hemipterans such as aphids and psyllids (Fischer & al. 2001, Offenberg 2001, Billick & al. 2007, Sanchez & al. 2019). Moreover, on some occasions, ants have been reported to show opposite effects simultaneously, both by preying or protecting and reducing the predation of natural enemies on myrmecophilous hemipterans (Sakata 1999, Yao & al. 2000, Billick & al. 2007, Sanchez & al. 2019).

The occurrence of sugary liquid food (i.e., honeydew) is one of the main factors accounting for the presence of ants on plant surfaces (Rico-Gray & Oliveira 2007), and many ant species patrol colonies of sap-sucking hemipterans that contribute highly to the fulfilment of their sugar demands (Hölldobler & Wilson 1990, Stadler & Dixon 2008). However, the activity of ants does not seem to show a linear response to the availability of honeydew (Grover & al. 2007, Petry & al. 2012). In the present work, the highest number of ants were registered in plots with intermediate levels of honeydew, but not in those were honeydew was highly abundant. Contrary to our second working hypothesis, the number of ants in pear trees showed a non-linear relationship with the amount of honeydew on pear trees. The same behaviour was observed in *Linepithema humile* (Mayr, 1868) (Hymenoptera: Formicidae), which increased its activity at intermediate levels of honeydew and strongly reduced it at high levels (Grover & al. 2007). Other authors have also found that carbohydrate-fed ants strongly reduce their activity and foraging on plants (Offenberg 2001, Petry & al. 2012). This kind of behaviour could be explained by the lower number of workers that would be needed to collect the same amount of food when excess honeydew is present (Stadler & Dixon 2005). In addition, the accumulation of honeydew could hamper the movement of ants in the canopy of pear trees (Sanchez & al. 2019).

Ants are known to have a strong impact on many groups of natural enemies in Mediterranean fruit-tree orchards (Miñarro & al. 2010, Piñol & al. 2012, Calabuig & al. 2015, Mestre & al. 2016). In this work, the abundance of the most common groups of predators, such as mirids (i.e., *Pilophorus gallicus*), anthocorids, spiders, chrysopids, *Forficula auricularia* and coccinellids, was very low in comparison with the abundance of ants. In addition, a significant reduction in the abundance of coccinellids and anthocorids was registered in the pear trees to which ants were allowed access, in comparison to the trees from which ants were excluded. These results are in agreement with those of other authors that reported a reduction in the populations of predatory hemipterans and coccinellids in the presence of ants (Piñol & al. 2012, Calabuig & al. 2015). The earwig *F. auricularia* was the only predator whose population was significantly greater on the pear trees without exclusion barriers, while no significant effect was registered for chrysopids, *P. gallicus* or spiders. The reduction in the abundance of earwigs can be attributed to the sticky barriers, which are known to exclude ground-dwelling insects (Piñol & al. 2010). Other authors have reported higher populations of *Pilophorus* species and a decrease in the numbers of chrysopids and spiders in the presence of ants (Kaplan & Eubanks 2002, Vanek & Potter 2010, Piñol & al. 2012, Calabuig & al. 2015). In the present work, *P. gallicus* and spiders were slightly more abundant in trees without exclusion barriers, and chrysopids less abundant in trees with free access of ants. However, these differences were not significant and these observations cannot be regarded as conclusive because of the low abundances at which these predators were found in the field.

Ants play a principal role in the regulation of the psyllid populations in pear orchards in southern Spain. The results of the present work corroborate the findings of previous experiments (Sanchez & al. 2019), which reported a decrease in the densities of psyllids associated with an increase in the number of ants in pear orchards along a four-year study. In this previous work, the control of psyllids by ants was inferred from the analysis of population dynamics data and laboratory experiments; in the present work, we have corroborated these findings through ant-exclusion experiments. In spite of the negative impact of ants on several groups of natural enemies, overall, ants were found to contribute positively to biological pest control. Other authors have also reported an improvement in biological control in crops where ants interact with honeydew-producing hemipterans (Eubanks & Styrsky 2006, Morris & al. 2018). However, as it has been reported for other agroecosystems (Rivera-Salinas & al. 2018), the outcome of the ant-psyllid interaction may be largely conditioned by the availability of honeydew. The negative influence of excess honeydew on the activity of ants may have important implications in relation to the

control of the psyllid. Other authors have also reported that the excess of honeydew produced by psyllids disrupts the predatory behaviour of anthocorid bugs (Ge & al. 2020). Low-impact intervention, such as the use of soap for washing honeydew off pear trees, could improve the predatory response of ants. Future work will determine how the predation by ants varies in response to changes in the availability of honeydew.

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