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Ants affect citrus pests and their natural enemies in contrasting ways

Diego V. Anjos^{a,*}, Alejandro Tena^b, Helena Maura Torezan-Silingardi^a, Apostolos Pekas^c, Arne Janssen^{d,e}

^a Institute of Biology, Federal University of Uberlândia, Brazil

^b Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada, Spain

^c Instituto Agroforestal Mediterráneo (IAM), Universitat Politècnica de València, Camí de Vera 14, 46022 València, Spain

^d IBED, Department of Evolutionary and Population Biology, University of Amsterdam, The Netherlands

^e Department of Entomology, Federal University of Viçosa, Brazil

HIGHLIGHTS

- We review how ants affect pest abundance, and their natural enemies on citrus crops.
- Ants affect the non-honeydew producing pests, but favour the honeydew producers.
- Ants affect pests with edaphic stages, but favour pests without such stages.
- The identity of the most abundant ant species affect the natural enemies.
- Ants affect the generalist and winged natural enemies of honeydew-producing pests.

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ABSTRACT

Ants are one of the most abundant insect groups in citrus orchards. They help in controlling pests, but they can also facilitate the outbreak of other pests, such as those that produce honeydew. Moreover, ants can also affect natural enemies of pests. Here, we review how ants affect pest abundance, considering whether the pest produces honeydew and whether it spends part of its life-cycle in the soil (edaphic stages). We also investigated the effects of the presence of ants on natural enemies with a meta-analysis and how this effect is modified by honeydew production by the pest, the most abundant ant species, and the specialization and mode of dispersal (winged or wingless) of the natural enemy. The presence of ants decreased the abundance of non-honeydew producing pests, but increased the abundance of honeydew producers. Ants increased the abundance of pests without edaphic stages, but decreased pests with such stages. In addition, the identity of the most abundant ant species affected the abundance of natural enemies; the ants *Iridomyrmex rufoniger* and *Lasius grandis* had a negative impact whereas other ant species did not affect the abundance of natural enemies. Moreover, the presence of ants decreased the abundance of generalist and winged natural enemies of honeydew-producing pests. Our review provides crucial information on the effects of ants on biological control practices in citrus orchards, and increases our understanding of the relationships between ants, pests and their natural enemies.

* Corresponding author.

E-mail address: diegoanjosufop@gmail.com (D.V. Anjos).

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1. Introduction

Ants are one of the most abundant and dominant groups of animals in terrestrial ecosystems, including more than 15,000 described species, and are found in almost all ecosystems (Holldobler and Wilson, 2009). The majority of ant species are omnivorous; they obtain proteins from predation and scavenging and carbohydrates from plant-derived sources such as floral and extrafloral nectar, fruits or from honeydew excreted by hemipterans (Tobin, 1995). The contribution of each of these food sources to the diet of ants determines their role in crop protection. To obtain protein, ants prey upon other arthropods, including important crop pests (Drummond and Choate, 2011; Diamé et al., 2018). For this reason, ants have been included in biological control programs against different pests in diverse crops (van Mele, 2008; Drummond and Choate, 2011; Offenberg, 2015; Diamé et al., 2018). In fact, weaver ants constitute the first recorded case of man-mediated biological control, dating back to 304 CE, and *Oecophylla* sp. workers were employed to suppress citrus pests with a method that is still used nowadays in China (Huang and Yang, 1987; Offenberg, 2015). In contrast, ants may be detrimental to plants because of their mutualistic relationships with honeydew-producing pests in order to obtain carbohydrates (Way, 1963; Stadler and Dixon, 2005; Canedo-Júnior et al., 2019).

Citrus is one of the most important fruit crops in the world, and ants represent the most abundant group of arthropods in this crop. An earlier review reported 295 ant species in citrus in 50 countries (Haney, 1988). In most citrus orchards, ants are structured in communities including dominant and subordinate species (Arnan et al., 2012; Pekas et al., 2011; Retana and Cerdá, 2000). The role of ants in citrus is still controversial. The net outcome of the interaction between ants, citrus pests and natural enemies is determined by several factors (Calabuig et al., 2015). On the one hand, many ant species can protect citrus by preying upon and repelling pests (Stuart et al., 2003; Xiao et al., 2007; Piñol et al., 2009; Bownes et al., 2014). Ants prey on pests in the canopy but also on the soil, and many citrus pests spent part of their life-cycle in the soil, for example, the Mediterranean fruit fly *Ceratitis capitata* Wiedemann, 1824 (Diptera: Tephritidae) (Monzó et al., 2011), the false codling moth *Thaumatotibia leucotreta* Meyrick, 1913 (Lepidoptera: Tortricidae) (Bownes et al., 2014), the mealybug *Delottococcus aberiae* (De Lotto) (Hemiptera: Pseudococcidae) (Pérez-Rodríguez et al., 2018) and several species of thrips (GROUT et al., 1986; Navarro-Campos et al., 2012).

On the other hand, the presence of ants in citrus has been associated with population outbreaks of honeydew-producing pests (since the middle of last century) (Flanders, 1951). Ants collect honeydew excreted by hemipteran pests, and in exchange improve hemipteran colony hygiene, transport hemipterans to new feeding sites, and protect them from their natural enemies (Stadler and Dixon, 2005). In most cases, the outcome of these interactions is the outbreak of honeydew-producing pests, and ants are therefore considered indirect pests in citrus (Flanders, 1951; James et al., 1997; Martínez-Ferrer et al., 2003; Pekas et al., 2010; Tena et al., 2013a; Calabuig et al., 2014). In general, the impact of ants on the population densities of honeydew-producing pests depends mainly on the composition of their honeydew and the ecological context (Tena et al., 2016).

The biology of natural enemies is another important component of the interaction between ants and honeydew-producing pests. Natural enemies of honeydew producers have evolved visual, chemical and behavioural mechanisms that help them cope with ant aggression and they generally attack specific prey species (Völkl, 1994; Völkl and Vohland, 1996; Rasekh et al., 2010). The well-known example in citrus is the predatory coccinellid *Cryptolaemus montrouzieri* Mulsant 1853 (Coleoptera: Coccinellidae), whose larvae mimic the mealybugs on which they feed, thus deceiving the ants tending the mealybug colonies. Thus, these specialist natural enemies might be less affected by ants than generalists (i.e., those attacking honeydew- and non-honeydew producing prey). Similarly, one would expect that winged natural enemies could escape ant aggression more efficiently than wingless ones,

although we are not aware of studies demonstrating this (see Pierre and Idris, 2013).

Finally, ants may also induce population outbreaks of pests that do not produce honeydew in citrus, such as the California red scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) (Moreno et al., 1987; James et al., 1997; Pekas et al., 2010; Calabuig et al., 2014). This is likely the outcome of an indirect interaction, for instance, ants may attack or disrupt natural enemies of these pests while foraging in citrus canopies (Pekas et al., 2010; Calabuig et al., 2014; Dao et al., 2014). Therefore, it is necessary to consider not only the effects of ants on honeydew producers but also on other pests and their natural enemies (Fig. 1A). Identifying these ant effects will allow the design of strategies to boost the positive effects while decreasing the negative effects of ants in citrus (Offenberg, 2015).

Here, our main goal was to review the role of ants in biological control in citrus orchards, specifically to clarify their effects on pests (honeydew-producing or not). In addition, we did a meta-analysis regarding the effects of ants on natural enemies of citrus pests. We also evaluated whether ant effects were modulated by i) the production of honeydew by the pest, ii) the most abundant ant species, iii) specialization of the natural enemies (specialists vs generalists) and iv) the mode of dispersal of the natural enemies (e.g., winged or wingless).

2. Materials and methods

2.1. Data collection and selection criteria

We searched for papers in the ISI Web of Knowledge (www.isiknowledge.com), database using all available years up to September 2020, using the following terms: “ant”, “control” and “citrus” as key words. Our initial search identified 117 studies that were potentially appropriate for review. To complement the dataset, we searched for more studies in recent reviews (see van Mele, 2008; Drummond and Choate, 2011; Offenberg, 2015; Diamé et al., 2018). Eight more studies were identified. From the total of 125 studies, we further selected studies based on the abstract and methods sections using the following criteria: (i) whether the studies investigated effects of ants on the abundance of pests or their natural enemies; (ii) whether they included an appropriate control (access of ants); (iii) whether it concerned field studies.

3. Effect of ants on citrus pests

Owing to the small number of studies found, we decided to review the effects of ants on citrus pests qualitatively and using vote-counting instead of performing a formal meta-analysis (see Friedman, 2001), focusing on studies evaluating the effect of ants on the abundance of pests. A total of 24 studies were considered in this part of the review (Table 1). Citrus pests were categorized to species level, whether they excreted honeydew, and whether they spend part of their life-cycle in the soil, hereafter edaphic pests. When the authors did not provide such information, it was obtained from other literature. In addition, we assessed the effects of ant presence on pest density (decrease, increase or no effect). We used log-linear tests for contingency tables (i.e., GLMs, Crawley, 2013), considering the number of positive and negative results as response variable, and honeydew production and whether pests were edaphic as factors. When ants increased pest abundances this was scored as a positive effect. As only five records of neutral effects were found, these were excluded from the analyses.

4. Effect of ants on natural enemies

Nine studies provided 155 cases of effects of ants on natural enemies of citrus pests (Supplementary materials, References). There were more than 10 cases for each moderator (i.e. factors such as honeydew production, most abundant ant species in crops, specialization of the natural enemies and their mode of dispersal), as recommended by Nakagawa

et al. (2017). We used this dataset for all analyses described below.

We recorded the mean abundance of natural enemies, the variance and the sample size of control (ant access) and treatment (ant exclusion). We also extracted information about the most abundant ant species of orchards for each study. Specialization of natural enemies was considered in relation to their ability to prey on one or a few or more pest species. Hence, parasitic wasps and predators exploiting mainly a single group of pests were considered specialist (e.g., *Phymatosternus lewisii* Crotch, 1874 and *Scymnus posticalis* Sicard, 1913 (Coleoptera: Coccinellidae) on *Toxoptera citricida* Kirkaldy, 1907 (Hemiptera: Aphididae), *Rodolia cardinalis* Mulsant, 1850 (Coleoptera: Coccinellidae) on *Icerya purchasi* Maskell, 1878 (Hemiptera: Monophlebidae)). The rest of the species (e.g., chrysopids, spiders) were considered generalists. The developmental stages of natural enemies that attacked the pests were further classified as winged or wingless. Cases where information on the developmental stage of the natural enemy was not available or where authors did not discriminate among stages were excluded. As above, additional literature was consulted when information was not provided by the authors (Table 2).

We calculated Hedges' g (J-corrected Hedges' d) using the "metafor" package (Viechtbauer, 2010) on R software v. 4.0.1 (R Development Core Team, 2020) as metric of effect size for all analyses, which is

recommended for small sample sizes (Borenstein et al., 2009). Ant exclusion was used as the reference. Negative values of Hedges' g thus refer to cases where ants had a negative effect on natural enemy densities.

4.1. Statistical analysis

We used random-effects models using the "metafor" package (Viechtbauer, 2010) considering Maximum Likelihood on R software v. 4.0.1 (R Development Core Team, 2020). Study identity was used as random factor to control for pseudo-replication. The overall effect size of ants on densities of natural enemies (without moderators) was considered significantly different from zero if the 95% confidence intervals (CI) did not include zero (Borenstein et al., 2009). As there were not enough combinations of most abundant ant species with the other factors, we performed a separate mixed-effects meta-analysis considering only ant species as moderator. Furthermore, we investigated how the effect of ants on natural enemies of citrus pests was modified by three moderators: honeydew production by the pest, specialization and mode of dispersal of natural enemies and their interactions.

To explore the possibility of publication bias, we used funnel plots (graphical approach) and Egger's regression test (statistical approach) (Egger et al., 1997). With Egger's regression test, we maintained the

Fig. 1. (A) Experimental design of studies included in the review and meta-analysis evaluating ant effects on abundance of pests and their natural enemies, respectively. Arrows symbolise direct or indirect ecological interactions mediated by ants and trophic interactions. (B) Global distribution of the number of studies per country included in the review which considers ant effects only on pest abundance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

structure of the original model, but included the variances of *Hedges' g* as a moderator, modified according to [Habeck and Schultz \(2015\)](#). If the intercept of the regression test deviated significantly from zero, the overall relationship between the effect size and its respective variance in each study was considered asymmetrical, therefore, biased ([Sterne and Egger, 2005](#)). Because our dataset consisted of a relatively small number of studies, we used a significance threshold of $\alpha < 0.1$ ([Egger et al., 1997](#)). In addition, we estimated the effect size heterogeneity using I^2 , which describes the percentage of variation across studies due to data heterogeneity ([Higgins et al., 2003](#)).

We found evidence for a publication bias in our dataset, as shown by both the funnel plot ([Supplementary materials Fig. 1A](#)) and the intercept of Egger's regression (intercept = -1.88 ; CI = -2.90 to -0.85 $p < 0.01$). The publication bias may have come from a higher probability of publishing studies with positive results (*file drawer problem*). There was a moderate level of heterogeneity in our model ($I^2 = 66.74\%$) ([Koricheva et al., 2013](#)). We removed three potential outliers until we obtained an unbiased dataset according to Egger's regression model (intercept = -0.85 ; CI = -1.92 to 0.21 ; $p = 0.11$; [Supplementary materials Fig. 1B](#)). After removal of the outliers, there were 152 cases from nine different studies. Model heterogeneity was weakly reduced, but was still within the acceptable range ($I^2 = 63.06\%$) in the new unbiased dataset ([Koricheva et al., 2013](#)). We repeated the meta-analyses with the complete data set (with the outliers) and obtained similar results. Below, we present all results regarding our unbiased dataset (152 cases).

Table 1

Summary of information taken from the 24 studies that considered the effect of ants on the abundance of pests found in the review. (A) Considering honeydew-producing pests and (B) those that do not produce honeydew. See information on the most abundant ant species at [Supplementary materials \(Table S1\)](#).

Edaphic	Pest species	Order	Family	Pest density ^a	Country	Reference
Honeydew-producing pests						
No	Aphids	Hemiptera	Aphididae	- (short)/ + (long term)	Spain	Piñol et al. (2012)
No	Aphids	Hemiptera	Aphididae	-	Spain	Piñol et al. (2009)
No	<i>Toxoptera aurantia</i>	Hemiptera	Aphididae	-	Vietnam	Van Mele et al. 2002
No	<i>Toxoptera citricidus</i>	Hemiptera	Aphididae	-	Vietnam	Van Mele et al. 2002
No	<i>Aleurothrixus floccosus</i>	Hemiptera	Aleyrodidae	n	Brazil	Jesus et al. 2016
No	<i>A. floccosus</i>	Hemiptera	Aleyrodidae	+	Spain	Calabuig et al. (2014)
No	<i>A. floccosus</i>	Hemiptera	Aleyrodidae	+	USA	Moreno et al. (1987)
No	<i>Ceroplastes rubens</i>	Hemiptera	Coccidae	+	Japan	Itioka and Inoue 1996
No	<i>Coccus hesperidum</i>	Hemiptera	Coccidae	+	Australia	James et al. (1997)
No	<i>Coccus viridis</i>	Hemiptera	Coccidae	+	Trinidad	Jutsum et al. (1981)
Unknown	<i>Toumeyella</i> spp	Hemiptera	Liviidae	+	Trinidad	Jutsum et al. (1981)
No	<i>Diaphorina citri</i>	Hemiptera	Pseudococcidae	n	USA	Navarrete et al. 2013
No	<i>Planococcus citri</i>	Hemiptera	Pseudococcidae	+	Spain	Catalán et al. 2016
No	<i>P. citri</i>	Hemiptera	Pseudococcidae	+	Spain	Villalba et al. 2006
No	<i>P. citri</i>	Hemiptera	Aphididae	+	USA	Moreno et al. (1987)
Pests that do not produce honeydew						
Yes	<i>Diaprepes abbreviatus</i>	Coleoptera	Curculionidae	-	USA	Stuart et al. 2003
Yes	<i>Hypomeces squamosus</i>	Coleoptera	Curculionidae	-	Thailand-Vietnam	Offenberg et al. 2013
Yes	<i>H. squamosus</i>	Coleoptera	Curculionidae	-	Thailand	Offenberg et al. (unpublished)
Yes	<i>Ceratitidis capitata</i>	Diptera	Tephritidae	-	South Africa	Bowles et al. (2014)
Yes	<i>Ceratitidis ditissima</i>	Diptera	Tephritidae	-	Ghana	Ativor et al. 2012
Yes	<i>Helicoverpa armigera</i>	Lepidoptera	Noctuidae	-	South Africa	Bowles et al. (2014)
Yes	<i>Thaumatotibia leucotreta</i>	Lepidoptera	Tortricidae	-	South Africa	Bowles et al. (2014)
No	<i>Aonidiella aurantii</i>	Hemiptera	Diaspididae	+	Spain	Calabuig et al. (2014)
No	<i>A. aurantii</i>	Hemiptera	Diaspididae	+/n	Spain	Juan-Blasco et al. (2011)
No	<i>A. aurantii</i>	Hemiptera	Diaspididae	+	Spain	Pekas et al. (2010)
No	<i>A. aurantii</i>	Hemiptera	Diaspididae	+	Australia	James et al. (1997)
No	<i>A. aurantii</i>	Hemiptera	Diaspididae	n	USA	Murdoch et al. 1995
No	<i>A. aurantii</i>	Hemiptera	Diaspididae	+	USA	Moreno et al. (1987)
No	<i>Aonidiella citrina</i>	Hemiptera	Diaspididae	+	USA	Flanders 1945
No	<i>Rhynchocoris humeralis</i>	Hemiptera	Pentatomidae	-	Vietnam	Van Mele et al. 2002
No	<i>Phyllocnistis citrella</i>	Lepidoptera	Gracillariidae	-	Spain	Calabuig et al. (2014)
No	<i>P. citrella</i>	Lepidoptera	Gracillariidae	-	USA	Xiao et al. (2007)
No	<i>P. citrella</i>	Lepidoptera	Gracillariidae	n	USA	Zappala et al. 2007
No	<i>P. citrella</i>	Lepidoptera	Gracillariidae	-	USA	Xiao et al. 2010
Unknown	<i>Papilio</i> spp	Lepidoptera	Papilionidae	-	Vietnam	Van Mele et al. 2002
Unknown	<i>Pyralidae</i> spp	Lepidoptera	Pyralidae	-	Vietnam	Van Mele et al. 2002
	<i>Atta cephalotes</i>	Hymenoptera	Formicidae	-	Trinidad	Jutsum et al. (1981)

^a +: positive effect, -: negative effect, n: no (significant) effect.

Table 2

Number of cases (within normal brackets) per category for each moderator used in the meta-analysis to investigate the effect of ants on natural enemies, with in square brackets the number of studies. N.E. represents the natural enemies. The numbers in bold represent the sample total.

Moderators			
Pest type	Ant species	N.E. specialization	N.E. dispersal
Honeydew producing (89) [6]	<i>Lasius grandis</i> (53) [4]	Generalist (96) [8]	Winged (121) [8]
Non-honeydew producing (39) [2]	<i>Iridomyrmex rufoniger</i> (25) [2]	Specialist (54) [4]	Wingless (29) [5]
	<i>Plagiolepis pygmaea</i> (12) [1]		
	<i>Pheidole pallidula</i> (12) [1]		
	<i>Linepithema humile</i> (9) [1]		
128	111	150	142

5. Results

5.1. Effect of ants on citrus pests

The twenty-four studies comprised 10 countries ([Fig. 1B](#)), considering 15 ant species from 10 different genera ([Supplementary materials](#)

Table S1), and 24 species of citrus pests. California red scale *A. aurantii* (6 of 24 studies, 25%), the citrus leafminer *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae) (4 of 24, 16.6%), woolly whitefly *Aleurothrixus floccosus* Maskell, 1895 (Hemiptera: Aleyrodidae) (3 of 24, 12.5%) and citrus mealybug *Planococcus citri* Risso, 1813 (Hemiptera: Pseudococcidae) (3 of 24, 12.5%) were the most studied pests. Among the 24 pest species, 11 produced honeydew (Table 1). The effect of ant presence on the abundance of honeydew-producing pests was positive in c. 71% of the cases (i.e. ants were associated with increased pest abundance), whereas for pests not producing honeydew, it was positive in c. 35% of the cases, and this difference was significant (Fig. 2A, GLM: $\chi^2 = 4.11$; d.f. = 1; $p = 0.04$). The abundance of edaphic pests was never positively affected by ants, whereas non-edaphic pests were positively affected in 64% of the cases (Fig. 2B, GLM: $\chi^2 = 10.27$; d.f. = 1; $p < 0.01$).

6. Effects of ants on natural enemies

Nine studies (located in Australia, Japan, Spain and USA) with sixty-one species of natural enemies were included in our analysis. The natural enemies belonged to six insect orders (Coleoptera, Diptera, Lepidoptera, Hemiptera, Hymenoptera and Neuroptera) and Arachnida (Araneae and Acari), with parasitic wasps (Hymenoptera) (50 cases), Coleoptera (35) and Araneae (17) being the most frequent groups. The most abundant ant species in citrus crops were *Lasius grandis* Forel, 1909 (53 cases), *Iridomyrmex rufoniger* Lowne, 1865 (25), *Plagiolepis pygmaea* Forel, 1904 (12), *Pheidole pallidula* Smith, 1858 (12), *Linepithema humile* Mayr, 1868 (9) and *Pristomyrmex pungens* Mayr, 1866 (2) (Table 2). Because *P. pungens* was recorded only two times, we removed this species from the analysis with the most abundant ant species.

The presence of ants had a significant overall negative effect on the abundance of natural enemies (Fig. 3, Hedges' $g = -0.23$). In a separate model with only the most abundant ant species, the effect of ants was also significantly negative (Likelihood ratio test (LRT) = 11.83, d.f. = 4,

$p < 0.01$). The species *I. rufoniger* and *L. grandis* decreased the abundance of natural enemies, whereas *L. humile*, *P. pallidula* and *P. pygmaea* had no significant effect on the abundance of natural enemies (Fig. 3).

Including all ant species, we found that the interaction between the production of honeydew by the pest and the specialization of natural enemies had a significant effect on natural enemy abundance (LRT = 10.52, d.f. = 1, $p < 0.01$). The presence of ants was associated with a decrease in the abundance of generalist natural enemies of honeydew-producing pests (Fig. 4A), but not that of other combinations of honeydew production by the pests and the specialization of the natural enemies (Fig. 4A). We also found a significant interaction between the production of honeydew by the pest and the mode of dispersal of natural enemies (LRT = 4.39, d.f. = 1, $p = 0.03$). The presence of ants significantly decreased the abundance of winged enemies of honeydew-producing pests (Fig. 4B), whereas densities of wingless natural enemies of honeydew-producers and both winged and wingless enemies of non-honeydew producing pests were not significantly affected by ants (Fig. 4B).

7. Discussion

Our study shows that the effect of ants on citrus pests depends on the biology of the pest. On the one hand, the presence of ants was often associated with an increase in the abundance of honeydew-producing pests in citrus, but on the other hand, ants were associated with reduced abundance of non-honeydew producing pests. Also, the ants decreased the abundance of pests with edaphic stages in comparison with pests without such at stage. The effect of these two factors was analysed separately because the number of cases was low and, more importantly, because most pests that lack edaphic stages are also honeydew-producing hemipterans, with the exception of the armored scales *A. aurantii* and *Aonidiella citrina* (Coquillett) (Hemiptera: Diaspididae), the pentatomid *Rhynchoris humeralis* (Hemiptera: Pentatomidae) and the moth *P. citrella*. Therefore, our results should be interpreted with caution and, further analyses are needed to study the interaction of these prey characteristics. Such analyses could include other perennial production systems such as orchards and vineyards.

The presence of ants was associated with a twofold increase in the abundance of honeydew-producing pests (Fig. 2A). It is known that the mutualistic interaction between ants and honeydew-producing pests in citrus depends on the interacting species (Tena et al., 2016). For example, mealybugs and soft scales are more heavily tended by ants

Fig. 2. (A) Numbers of records showing positive or negative effects of ants on densities of honeydew-producing or non-honeydew producing pests and (B) on densities of edaphic and non-edaphic pests.

Fig. 3. The overall effect of ant community and separate ant species on natural enemies of citrus pests. Effect sizes and 95% confidence intervals are shown as well the number of cases. Only *Iridomyrmex rufoniger* and *Lasius grandis* decreased the abundance of natural enemies.

Fig. 4. (A) Effect of ants on generalist and specialist natural enemies of citrus pests that produce honeydew or not, and (B) on winged and wingless natural enemies of these citrus pests. Effect sizes and 95% confidence intervals are shown as well as the number of cases. (A) The presence of ants only decreased the abundance of generalist natural enemies of honeydew-producing pests, (B) and the abundance of winged natural enemies of honeydew-producing pests.

than aphids, whiteflies and psyllids (Pekas et al., 2011; Tena et al., 2013a). This variation might depend on the quantity and quality of sugars present in honeydew and on their seasonal availability (Tena et al., 2013b).

Armoured scales are an exception because they are more abundant in the presence of ants although they do not produce any honeydew. This may be because of their coexistence with honeydew producers such as mealybugs, and ants that search for honeydew may accidentally disrupt natural enemies of the scale insects (James et al., 1997; Pekas et al., 2010). As these species spend the entire life-cycle on the canopy, they obtain greater protection by attending ants compared to edaphic pests (Kaneko, 2003; Pekas et al., 2010; Kistner et al., 2017).

The species of edaphic pests considered in this review do not produce any type of reward for the ants (e.g., honeydew), therefore, they are more likely to be preyed upon by ants. Moreover, several species of ants occur in the soil of citrus orchards that do not ascend to the tree canopy, and therefore were difficult to exclude experimentally and consequently were not considered here. These species, however, can prey upon pests in the soil. For example, Pérez-Rodríguez et al. (2021) shows that the terrestrial ant *Aphaenogaster iberica* Emery (Hymenoptera: Formicidae) is the main predator of *C. capitata* pupae in the soil of a citrus orchard. This is likely because the diet of *A. iberica* is more dependent on protein rather than on carbohydrates compared to arboreal ants (Pérez-Rodríguez et al., 2021). Finally, it is worth mentioning that, that pest pupae that spend more time in the soil also have higher rates of predation by

ants (Bowles et al., 2014).

Our meta-analyses show that the presence of ants was associated with a decrease in the abundance of natural enemies. This result, however, depended on the ant species that was most abundant in the orchard, unsurprisingly showing that different ant species have different effects on natural enemies (see Buckley and Gullan, 1991). For instance, *L. grandis* (effect size: -0.47) decreased natural enemy abundance by 145% and 113% compared to *L. humile* (effect size: 0.21) and *P. pallidula* (effect size: 0.06), respectively. *Lasius grandis* is a native and ecologically and behaviourally dominant ant species in Mediterranean citrus orchards (Pekas et al., 2010). Its diet is based on honeydew and it has long periods of activity (Pekas et al., 2010; Piñol et al., 2012). Therefore, it is often associated with a decrease of the abundance of natural enemies (Piñol et al., 2012). The presence of *Linepithema humile*, which is also a dominant species that feeds on honeydew, decreased the abundance of generalist predators of the citrus red mite *Panonychus citri* McGregor, 1916 (Acari: Tetranychidae), but did not affect predatory mites in USA citrus orchards (Haney et al., 1987; Moreno et al., 1987).

Our meta-analysis also shows that in the presence of ants the abundance of generalist natural enemies of honeydew-producing pests is more reduced than that of specialist natural enemies. This might be attributed to the fact that specialist predators and parasitoids have evolved adaptations to deal with ant aggressiveness (Völkl and MacKauer, 1993; Kaneko, 2007). For instance, predatory larvae of the coccinellid *P. lewisii* are chemical camouflaged, therefore, the ants do not attack them (or not as much as they do non-camouflaged predators) (Kaneko, 2007). As another example, the parasitoid of mealybugs *Anagyrus vladimiri* Triapitsyn, formerly known as *A. sp. near pseudococci* (Girault) (Hymenoptera: Encyrtidae), detects and avoids the ant *L. grandis* using the cuticular hydrocarbons left by this ant species (Mouratidis et al., 2021). Lastly, ants had a significant negative effect on the abundance of winged natural enemies of honeydew-producing pests but not on other natural enemies (Fig. 4B). It should be noticed, however, that there were few cases of these other combinations, and effect sizes of ants on wingless natural enemies were comparable to that of winged natural enemies of honeydew-producing pests. It is therefore conceivable that significant negative effects of ants on wingless natural enemies will be detected if more cases would be added.

Knowledge of the relationship between ant species and the main citrus pests is fundamental for optimizing pest management. Our results suggest that ants are associated with increased abundance of honeydew-producing pests but also reduced abundance of pests that spend part of their life cycle in the soil. To manage honeydew-producing pests, it has been suggested to suppress of ants in citrus orchards (Smith et al., 1997; Grafton-Cardwell et al., 2015). Alternative methods to manage ants and honeydew-producing pests include the use of sticky barriers (i.e., partial suppression) or insecticidal paint to exclude ants only from the citrus canopies (Pekas et al., 2010; Juan-Blasco et al., 2011; Calabuig et al., 2014). Excluding the ants, however, also excludes any potential benefit due to ants and other beneficial insects that can use the trunk to climb to the canopy (e.g., *Pilophorus* spp. and dermapterans). Thus, Wäckers et al. (2017) suggested the provisioning of sugars in dispensers placed in the ground, near the trunk, or on the tree branches as a means of disruption of the association between ants and honeydew producers. This resulted in higher abundance of natural enemies in the aphid colonies and a faster reduction of the size of aphid colonies as compared to control trees that received no sugar dispensers. This method has recently been used to successfully increase parasitism rates of the mealybug *P. citri* by the parasitoid *A. vladimiri* and to increase the predation of *C. capitata* pupae in the soil by ants (Pérez-Rodríguez et al., 2021).

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CRediT authorship contribution statement

Diego V. Anjos: Conceptualization, Methodology, Software, Data curation, Writing - review & editing. **Alejandro Tena:** Data curation, Writing - review & editing. **Helena Maura Torezan-Silingardi:** Visualization, Investigation, Writing - review & editing. **Apostolos Pekas:** Visualization, Investigation, Writing - review & editing. **Arne Janssen:** Software, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2021.104611>.

References

- Arnan, X., Cerdá, X., Retana, J., 2012. Distinctive life traits and distribution along environmental gradients of dominant and subordinate Mediterranean ant species. *Oecologia* 170 (2), 489–500.
- Borenstein, M., Hedges, L., Higgins, J., Rothstein, H., 2009. *Introduction to Meta-analysis*. Wiley, Chichester, UK.
- Bowles, A., Moore, S.D., Villett, M.H., 2014. My enemy's enemies: Recruiting hemipteran-tending generalist ants for biological control in citrus orchards by spatial partitioning of foraging webs. *Afr. Entomol.* 22 (3), 519–529.
- Buckley, R., Gullan, P., 1991. More aggressive ant species (Hymenoptera: Formicidae) provide better protection for soft scales and mealybugs (Homoptera: Coccidae, Pseudococcidae). *Biotropica* 3, 282–286.
- Calabuig, A., García-Mari, F., Pekas, A., 2014. Ants affect the infestation levels but not the parasitism of honeydew and non-honeydew producing pests in citrus. *Entomol. Res.* 104 (4), 405–417.
- Calabuig, A., García-Mari, F., Pekas, A., 2015. Ants in citrus: Impact on the abundance, species richness, diversity and community structure of predators and parasitoids. *Agric. Ecosyst. Environ.* 213, 178–185.
- Canedo-Júnior, E.O., Monteiro, A.B., de Queiroz, A.C.M., Silva, G.S., 2019. Aphid-Tending Ants and Their Effects on Natural Enemies Used in the Biological Control. In: Souza, B., Vázquez, L., Marucci, R. (Eds.), *Natural Enemies of Insect Pests in Neotropical Agroecosystems*. Springer, Cham.
- Crawley, M.J., 2013. *The R book*. John Wiley & Sons.
- 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 (2), 137–142.
- Diamé, L., Rey, J.Y., Vayssières, J.F., Grechi, I., Chailleux, A., Diarra, K., 2018. Ants: Major functional elements in fruit agro-ecosystems and natural enemies. *Sustainability* 10 (1), 23.
- Drummond, F., Choate, B., 2011. Ants as natural enemies in agricultural cropping systems. *Terr. Arthropod Rev.* 4 (2), 157–180.
- Egger, M., Smith, G.D., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315, 629–634.
- Flanders, S.E., 1951. The role of the ant in the biological control of homopterous insects. *Can. Entomol.* 83 (4), 93–98.
- Friedman, L., 2001. Why vote-count reviews don't count. *Biol. Psychiatry* 49 (2), 161–162.
- Grafton-Cardwell, E.E., Morse, J.G., O'Connell, N.V., Kallsen, P.A., Haviland, D.R., 2015. UC IPM Pest Management Guidelines: Citrus. Available at: <http://www.ipm.ucdavis.edu/PMG/r107304111.html> (last accessed 19 September 2020).
- Grout, T.G., Morse, J.G., Brawnner, O.L., 1986. Location of citrus thrips (Thysanoptera: Thripidae) pupation: Tree or ground. *J. Econ. Entomol.* 79 (1), 59–61.
- Habeck, C.W., Schultz, A.K., 2015. Community-level impacts of white-tailed deer on understory plants in North American forests: a meta-analysis. *AOB Plants* 7, plv119.
- Haney, P.B., Luck, R.F., Moreno, D.S., 1987. Increases in densities of the citrus red mite, *Panonychus citri* [Acarina: Tetranychidae], in association with the Argentine ant, *Iridomyrmex humilis* [Hymenoptera: Formicidae], in southern California citrus. *Entomophaga* 32 (1), 49–57.
- Haney, P., 1988. Identification, ecology and control of the ants in citrus: a world survey, in: Goren, R., Mendel, K. (Eds.), *Proceedings of the Sixth International Citrus Congress*. Balaban Publishers, Philadelphia/Rehovot, Tel Aviv, Israel.
- Higgins, J.P.T., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. *BMJ* 327, 557–560.
- Holldobler, B., Wilson, E.O., 2009. *The Superorganism*. W. W. Norton & Company Inc, New York, NY, USA.
- Huang, H.T., Yang, P., 1987. The ancient cultivated citrus ant. *BioScience* 37, 665–671.
- James, D.G., Stevens, M.M., O'Malley, K.J., 1997. The impact of foraging ants on populations of *Coccus hesperidum* L. (Hem., Coccidae) and *Aonidiella aurantii* (Maskell) (Hem., Diaspididae) in an Australian citrus grove. *J. App. Entomol.* 121 (1–5), 257–259.
- Juan-Blasco, M., Tena, A., Vanaclocha, P., Cambra, M., Urbaneja, A., Monzó, C., 2011. Efficacy of a micro-encapsulated formulation compared with a sticky barrier for excluding ants from citrus canopies. *J. Appl. Entomol.* 135 (6), 467–472.
- Jutsum, A.R., Cherrett, J.M., Fisher, M., 1981. Interactions between the fauna of citrus trees in Trinidad and the ants *Atta cephalotes* and *Azteca* sp. *J. Appl. Ecol.* 187–195.
- Kaneko, S., 2003. Different impacts of two species of aphid attending ants with different aggressiveness on the number of emerging adults of the aphid's primary parasitoid and hyperparasitoids. *Ecol. Res.* 18 (2), 199–212.
- Kaneko, S., 2007. Larvae of two ladybirds, *Phymatosternus lewisii* and *Scymnus postcalis* (Coleoptera: Coccinellidae), exploiting colonies of the brown citrus aphid *Toxoptera citricidus* (Homoptera: Aphididae) attended by the ant *Pristomyrmex pungens* (Hymenoptera: Formicidae). *Appl. Entomol. Zool.* 42 (2), 181–187.
- Kistner, E.J., Lewis, M., Carpenter, E., Melhem, N., Hoddle, C., Strode, V., Hoddle, M.S., 2017. Digital video surveillance of natural enemy activity on *Diaphorina citri* (Hemiptera: Liviidae) colonies infesting citrus in the southern California urban landscape. *Biol. Control* 115, 141–151.
- Koricheva, J., Gurevitch, J., Mengersen, K., 2013. *Handbook of meta-analysis in ecology and evolution*. Princeton University Press, Princeton, USA.
- Martínez-Ferrer, M.T., Grafton-Cardwell, E.E., Shorey, H.H., 2003. Disruption of parasitism of the California red scale (Homoptera: Diaspididae) by three ant species (Hymenoptera: Formicidae). *Biol. Control* 26 (3), 279–286.
- Moreno, D.S., Haney, P.B., Luck, R.F., 1987. Chlorpyrifos and diazinon as barriers to Argentine ant (Hymenoptera: Formicidae) foraging on citrus trees. *J. Econ. Entomol.* 80 (1), 208–214.
- Mouratidis, A., Vacas, S., Herrero, J., Navarro-Llopis, V., Dicke, M., Tena, A., 2021. Parasitic wasps avoid ant-protected hemipteran hosts via detection of ant cuticular hydrocarbons. *Proc. R. Soc. Lond. [Biol]* 288, 20201684.
- Monzó, C., Sabater-Muñoz, B., Urbaneja, A., Castañera, P., 2011. The ground beetle *Pseudophonus rufipes* revealed as predator of *Ceratitidis capitata* in citrus orchards. *Biol. Control* 56 (1), 17–21.
- Nakagawa, S., Noble, D.W.A., Senior, A.M., Lagisz, M., 2017. Meta-evaluation of meta-analysis: ten appraisal questions for biologists. *BMC Biol.* 15, 1–14.
- Navarro-Campos, C., Pekas, A., Moraza, M.L., Aguilar, A., García-Mari, F., 2012. Soil-dwelling predatory mites in citrus: Their potential as natural enemies of thrips with special reference to *Pezothrips kellyanus* (Thysanoptera: Thripidae). *Biol. Control* 63 (2), 201–209.
- Offenberg, J., 2015. Ants as tools in sustainable agriculture. *J. Appl. Ecol.* 52 (5), 1197–1205.
- Pekas, A., Tena, A., Aguilar, A., García-Mari, F., 2010. Effect of Mediterranean ants (Hymenoptera: Formicidae) on California red scale (Hemiptera: Diaspididae) populations in citrus orchards. *Environ. Entomol.* 39 (3), 827–834.
- Pekas, A., Tena, A., Aguilar, A., García-Mari, F., 2011. Spatio-temporal patterns and interactions with honeydew-producing Hemiptera of ants in a Mediterranean citrus orchard. *Agric. Forest Entomol.* 13 (1), 89–97.
- Pérez-Rodríguez, J., Calvo, J., Urbaneja, A., Tena, A., 2018. The soil mite *Gaeolaelaps* (Hypoaspis) aculeifer (Canestrini) (Acari: Laelapidae) as a predator of the invasive citrus mealybug *Dactylopius citricolus* (De Lotto) (Hemiptera: Pseudococcidae): Implications for biological control. *Biol. Control* 127, 64–69.
- Pérez-Rodríguez, P.A., Tena, A., Wäckers, F.L., 2021. Sugar provisioning for ants enhances biological control of mealybugs in citrus. *Biol. Control*, 104573 (in press).
- Pierre, E.M., Idris, A.H., 2013. Studies on the predatory activities of *Oecophylla smaragdina* (Hymenoptera: Formicidae) on *Pteroma pendula* (Lepidoptera: Psychidae) in oil palm plantations in Teluk Intan, Perak (Malaysia). *Asian Myrmecol.* 5 (1), 163–176.
- Piñol, J., Espadaler, X., Cañellas, N., Pérez, N., 2009. Effects of the concurrent exclusion of ants and earwigs on aphid abundance in an organic citrus grove. *BioControl* 54 (4), 515–527.
- Piñol, J., Espadaler, X., Cañellas, N., 2012. Eight years of ant-exclusion from citrus canopies: Effects on the arthropod assemblage and on fruit yield. *Agric. Forest Entomol.* 14 (1), 49–57.
- R Development Core Team, 2020. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- Rasekh, A., Michaud, J.P., Kharazi-Pakdel, A., Allahyari, H., 2010. Ant mimicry by an aphid parasitoid. *Lysiphlebus fabarum*. *J. Insect Sci.* 10 (1).
- Retana, J., Cerdá, X., 2000. Patterns of diversity and composition of Mediterranean ground ant communities tracking spatial and temporal variability in the thermal environment. *Oecologia* 123 (3), 436–444.
- Smith, D., Beattie, G.A.C., Broadley, R., 1997. *Citrus pests and their natural enemies: Integrated Pest Management in Australia*. Desert Oak Publishing Services, Brisbane, QLD, Australia.
- Stadler, B., Dixon, A.F., 2005. Ecology and evolution of aphid-ant interactions. *Annu. Rev. Ecol. Evol. Syst.* 36, 345–372.
- Sterne, J.A.C., Egger, M., 2005. Regression methods to detect publication and other bias in meta-analysis. In: Rothstein, H., Sutton, A., Borenstein, M. (Eds.), *Publication Bias in Meta-analysis: Prevention, Assessment and Adjustment*. John Wiley & Sons, Chichester, UK, pp. 99–110.
- Stuart, R.J., Jackson, I.W., McCoy, C.W., 2003. Predation on neonate larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in Florida citrus: testing for daily patterns of neonate drop, ant predators and chemical repellency. *Florida Entomol.* 86 (1), 61–72.
- Tena, A., Hoddle, C.D., Hoddle, M.S., 2013a. Competition between honeydew producers in an ant-hemipteran interaction may enhance biological control of an invasive pest. *Entomol. Res.* 103 (6), 714.

- Tena, A., Llcer, E., Urbaneja, A., 2013b. Biological control of a non-honeydew producer mediated by a distinct hierarchy of honeydew quality. *Biol. Control* 67 (2), 117–122.
- Tena, A., Wckers, F.L., Heimpel, G.E., Urbaneja, A., Pekas, A., 2016. Parasitoid nutritional ecology in a community context: the importance of honeydew and implications for biological control. *Curr. Opin. Insect Sci.* 14, 100–104.
- Tobin, J.E., 1995. Ants as Primary Consumers: Diet and abundance in the Formicidae. In: Lowman, M.D., Nadkarni, N. (Eds.), *Ecology and diversity of tropical forest canopy ants*. Academic Press, pp. 129–147.
- van Mele, P., 2008. A historical review of research on the weaver ant *Oecophylla* in biological control. *Agric. Forest Entomol.* 10, 13–22.
- Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. *J. Statist. Soft.* 36, 1–48.
- Vlkl, W., 1994. The effect of ant-attendance on the foraging behaviour of the aphid parasitoid *Lysiphlebus cardui*. *Oikos* 149–155.
- Vlkl, W., MacKauer, M., 1993. Interactions between ants attending *Aphis fabae* spp. *cirsiiacanthoidis* on thistles and foraging parasitoid wasps. *J. Insect Behav.* 6, 301–312.
- Vlkl, W., Vohland, K., 1996. Wax covers in larvae of two *Scymnus* species: do they enhance coccinellid larval survival? *Oecologia* 107 (4), 498–503.
- Wckers, F.L., Alberola, J.S., Garcia-Mar, F., Pekas, A., 2017. Attract and distract: Manipulation of a food-mediated protective mutualism enhances natural pest control. *Agr. Ecosyst. Environ.* 246, 168–174.
- Way, M.J., 1963. Mutualism between ants and honeydew-producing Homoptera. *Annu. Rev. Entomol.* 8 (1), 307–344.
- Xiao, Y., Qureshi, J.A., Stansly, P.A., 2007. Contribution of predation and parasitism to mortality of citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) populations in Florida. *Biol. Control* 40 (3), 396–404.