Title: Ants impact the energy reserves of natural enemies through the shared honeydew exploitation

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1. Ants as well as many species of parasitoids and predators rely on sugar-rich foods such as honeydew to fulfil their energetic needs. Thus, ants and natural enemies may interact through the shared honeydew exploitation.

2. Here we performed ant-exclusion experiments in a citrus orchard to test the hypothesis that ants may impact the energy reserves of predators and parasitoids through the competition for honeydew sources. Using high performance liquid chromatography (HPLC) we related the level of ant activity with the energy reserves and history feeding of individual specimens collected in the field during representative days of spring, summer and autumn.

3. Out of 145 Aphytis chrysomphali parasitoids captured in the field, 65% were classified as sugar-fed and 24.7% as honeydew-fed. In summer, when ant activity peaked, there is a significant negative correlation between the level of ant activity and the total sugar content and honeydew feeding incidence by A. chrysomphali.

4. Out of 47 individuals of the predator Chrysoperla carnea s.l., captured in the field, 55.3% were classified as sugar-fed. We found a significant negative effect of the level of ant activity on the sugar feeding incidence by C. carnea in spring.

5. This study provides evidence that ants can interfere with the energy reserves of natural enemies. This interaction may be widespread in various ecosystems with important consequences for the arthropod community composition and with practical implications for biological control given that absence of sugar feeding is detrimental for the fitness of many species of predators and parasitoids.

**Key words.** Ant hemiptera mutualism, Aphytis chrysomphali, biological control, Crysoperla carnea, multitrophic interactions
Introduction

Ecological communities are complex systems that consist of species interacting directly and indirectly (Miller, 1994; Bascompte et al., 2006; Ohgushi, 2008; Eubanks & Finke, 2014). It has been long known that sugar rich food sources, such as floral and extrafloral nectar or honeydew excreted by plant feeders, mediate species interactions over several trophic levels. For example, pollinators interact with birds (Laverty & Plowright, 1985) or ants (LeVan et al., 2014) when they share a common nectar source. Extrafloral nectar sources may affect the plant, the herbivore and the herbivore’s natural enemies composition and abundances at the community level (Rudgers & Gardener, 2004). Lately, the importance of honeydew in shaping multitrophic interactions has gained increased attention (Kaplan & Eubanks, 2005; Styrsky & Eubanks, 2007; Yoo et al., 2013). Honeydew can be involved in a protective mutualism; ants protect the plant feeders from their natural enemies in exchange for honeydew (Way, 1963; Carroll & Janzen, 1973; Hölldobler & Wilson, 1990). Honeydew is a valuable energy source for numerous organisms including the third trophic level, i.e. natural enemies such as predators and parasitoids, in natural (Zoebelein, 1956) and agricultural ecosystems. In this context, ants and natural enemies may interact through the shared energy sources in the form of honeydew. Understanding this interaction will provide useful insights from an ecological but also applied perspective given that it may impact the fitness of the natural enemies and eventually the efficacy of biological control. However, no studies have examined the potential impact of ants on the energy reserves of predators and parasitoids.

Honeydew is a sugar-rich fluid excreted by plant feeders (mostly hemipteran species) after feeding on phloem sap. Honeydew is especially relevant in agricultural ecosystems where it is the principal carbohydrate source since the presence of other sugar sources, such as nectar, is limited and variable in space and time, being available almost exclusively during the flowering season (Wäckers et al., 2008). Honeydew contains a mixture of phloem sugars, such as sucrose, fructose and glucose, and oligosaccharides synthesized by the plant feeders, such as erlose and melezitose (Völkl et al.,
Its composition makes honeydew an important carbohydrate source for a wide range of insects in the field, among which ants hold a predominant position (Hölldobler & Wilson, 1990; Wäckers, 2005). Most ant species are omnivorous and obtain protein from animal matter and carbohydrates from plant products such as floral and extrafloral nectar, food bodies, plant sap and above all honeydew (Way, 1963; Carroll & Janzen, 1973; Tobin, 1994). Honeydew is crucial for the ant colony growth and, usually, honeydew producers thrive when ant-tended (Hölldobler & Wilson, 1990). In fact, honeydew exploitation is associated with behavioural dominance in ants: dominant ant species exclude subordinate species and monopolize the honeydew sources (Blüthgen et al., 2004; Pekas et al., 2011).

Honeydew is not only crucial for ants. A broad range of entomophagous arthropods, including parasitoids and predators, uses honeydew as an energy source (Jervis & Kidd, 1986; Jervis et al., 1993; Wäckers, 2001; Steppuhn & Wäckers, 2004; Tena et al., 2013c). Honeydew consumption enhances the longevity (Wäckers, 2001; Wäckers et al., 2008) and fecundity of parasitoids (Faria et al., 2008; Tena et al., 2013b), eventually resulting in increased efficacy of biological control (Faria et al., 2008; Wäckers et al., 2008). Therefore, it is likely that honeydew, due to its availability, nutritional quality and impact on fitness, may mediate direct and indirect competitive interactions between ants and parasitoids or predators. For example, ants may affect negatively the natural enemies by excluding them from the honeydew sources in the same way ants have been found to exclude floral visitors from nectar sources (Lach, 2007). On the other hand, predators and parasitoids may benefit if the probabilities for sugar feeding increase due to the higher abundance of the honeydew producers under ant-attendance.

The citrus agro-ecosystem provides a suitable environment for several honeydew producers (Garcia-Marí, 2012) that are usually ant-attended (Pekas et al., 2011; Tena et al., 2013a) and also harbours a complex of naturally occurring parasitoids and predators (Garcia-Marí, 2012). In the present study, we test the hypothesis that ants might impact the energy reserves of predators and
parasitoids through the exploitation of honeydew: positively, by increasing the opportunities for honeydew feeding due to the positive feedback between the ants and the abundance of the honeydew producers or negatively, due to the direct competition ants exert by monopolizing the honeydew sources. To test our hypothesis we carried out an ant-exclusion experiment in a citrus agroecosystem where we related the level of ant activity with the energy reserves and history feeding of individual natural enemies. We included in the study two of the most important entomophagous arthropods in terms of abundance and biocontrol potential on the citrus canopy belonging to two different guilds, one parasitoid and one predator species.

Study system:

We conducted our study in the main Mediterranean citrus-growing area (Valencia, Spain), where the two most abundant and widely distributed ant species are the native *Lasius grandis* (Forel) and *Pheidole pallidula* (Nylander) (Cerdá et al., 2009; Pekas et al., 2011). Both species are behaviourally dominant and are in close association with honeydew producers; they are active from March until November, with *L. grandis* peaking its activity in June and *P. pallidula* in July-August (Pekas et al., 2011). The most abundant honeydew producers present in Mediterranean citrus orchards are the citrus aphid *Aphis spiraecola* Patch (Hemiptera: Aphididae) in early spring, soft scales such as *Coccus hesperidium* L. and *Saissetia oleae* Olivier (Hemiptera: Coccidae), and the citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) during summer, whereas the most abundant honeydew producer in autumn is the woolly whitefly *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae) (Pekas et al., 2011; Tena et al., 2013c).

Parasitoids of genus *Aphytis* (Hymenoptera: Aphelinidae) are the most important natural enemies of the California red scale (CRS) *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), a major pest in citrus worldwide (Rosen & DeBach, 1979). *Aphytis* are synovigenic ectoparasitoids that engage in host-feeding for egg maturation (Heimpel & Collier, 1996; Heimpel et al., 1997b); however, host
feeding cannot substitute sugar feeding because CRS contains very low amounts of sugar (Tena et al., 2013c). In the lab, in the absence of sugar feeding, *Aphytis* fecundity and longevity are seriously compromised and survival does not exceed three days (Avidov et al., 1970; Heimpel et al., 1997b; Tena et al., 2013b). Furthermore, Tena et al. (2013c) demonstrated that honeydew is the main sugar source for *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) in the field, despite the fact that its host does not produce honeydew. Honeydew feeding enhances the longevity and realized fecundity of *A. melinus* (Tena et al., 2013b). The species of our study, *Aphytis chrysomphali* Mercet (Hymenoptera: Aphelinidae), is native to the Mediterranean and is one of the most important parasitoids of CRS (Pekas et al., 2010a). Most probably it also feeds on honeydew in the field, though this has never been demonstrated.

Neuroptera belonging to the family Chrysopidae are among the most abundant generalist predators present in Mediterranean citrus (Garcia-Marí, 2012). *Chrysoperla carnea* sensu lato (Stephens) (Neuroptera: Chrysopidae) is the most important Neuropteron species in agricultural ecosystems (Stelzl & Devetak, 1999). *Chrysoperla carnea* s.l. larvae prey upon aphids (Hemiptera: Aphididae), tetranychid mites (Acari: Tetranychidae), whiteflies (Hemiptera: Aleyrodidae) and the citrus leaf miner (Lepidoptera: Gracillariidae) in citrus (Garcia-Marí, 2012), whereas adults are non-predaceous and consume nectar, pollen and honeydew (Principi & Canard, 1984; Hogervorst et al., 2007). Sheldon and MacLeod (1971) reported that honeydew is a major food source for adult *C. carnea* s.l. in the field. Honeydew enhances fecundity (Finney, 1948; Neumark, 1952) and acts as an arrestment stimulus for *C. carnea* s.l. (McEwen et al., 1993).

**Materials and Methods**

**Study site and experimental design**

The study was conducted in a commercial citrus orchard of sweet orange *Citrus sinensis* L. Osbeck (cv. Navelina) of approximately 0.7 ha, located in an extensive citrus-growing area 30 km south of
Valencia, eastern Spain (39º 12’2” N, 0º 20’52” W). The climate is Mediterranean, with a rainy spring and autumn and a dry winter and summer. The orchard was flood irrigated and weeds were controlled by local application of herbicides (Glyphosate®, Bayer CropScience, Spain). No insecticides were sprayed in the previous nine years, or during the experiment. According to previous studies, the ant species foraging on the tree canopies were *P. pallidula*, *Plagiolepis schmitzii* and *L. grandis* (Pekas et al., 2010b, 2011).

The experimental design was a randomized block with eight replicates (blocks) of two adjacent treatments (plots): ant-allowed and ant-excluded trees. Each treatment contained 16 trees (four rows by four trees). Ants were excluded in the 16 trees but only the four central trees were used for the samplings. Ant-exclusion began in January 2013 and was maintained until November 2013 by placing sticky barriers based on Tangle-trap® (Tanglefoot, Biagro, Valencia, Spain) on the tree trunks at 30 cm above ground. Tanglefoot was applied using a spatula on a 15 cm wide adhesive plastic tape fixed around the trunk. 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.

**Ant activity**

Ant activity was defined as the number of ants (all species) moving up and down crossing an imaginary horizontal line on the tree trunk during one minute. We monitored ant activity by observing the trunk of the four central trees on each plot between 10:00 and 14:00 a.m., a period of the day when ants are actively foraging on the canopies (Pekas et al., 2011). Ant activity was monitored on the same day that the natural enemies were collected (see below) in spring (3 June), summer (10 August), and autumn (5 November).

**Honeydew producers**
To determine the honeydew sources present in the orchard, a 0.52 m-diameter ring was randomly thrown on the four compass directions (N, S, E and W) of the canopy of the sampled trees and the number of honeydew producers in the ring was counted (Tena et al., 2013c). The sampling unit consisted of the four rings thrown per each tree. Honeydew producers were sampled within the same week that ant activity was monitored.

*Sampling parasitoids and predators*

Adult parasitoids and predators were collected between 11.00 and 14.00 hours. To collect adult *Aphytis* spp., the branches were hand-beaten in order to make arthropods fall onto the white surface. *Aphytis* were then captured with a brush soaked in ethanol and transferred to an Eppendorf with ethanol 70% to preserve them individually.

To collect the predators, we actively searched on the canopies. When adult *C. carnea* s.l. were detected, we captured them within a plastic recipient and transferred them individually into an Eppendorf with ethanol 70%. No *C. carnea* s.l. individuals were captured in the autumn sampling.

In the laboratory, the hind tibia length of each individual used in the HPLC analyses, was measured under a stereomicroscope in order to adjust the results to arthropod size (Tena et al., 2013c). Each arthropod was maintained individually in Eppendorf tubes with ethanol 70% and stored at 5°C until HPLC analysis.

*HPLC sugar analysis*

Prior to the analysis, the samples were homogenized in the ethanol solution using a pestle. Then, the ethanol was evaporated in a vacuum centrifuge for 60-120 min. After that, the samples were rehydrated by adding 20μl of ethanol 70% and 80μl of Milli-Q water for *A. chrysomphali* and 200μl of ethanol 70% and 800μl of Milli-Q water for *C. carnea* s.l. The samples were mixed by vibration and filtered (0.2 μm PVDF membrane). Analysis was carried out by high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). 25μl of
each sample were injected into a ICS3000 Dionex system (Dionex Corp., Sunnyvale, CA) consisting of a SP gradient pump, an AS-HV autosampler and an electrochemical detector with a gold working electrode and Ag/AgCl as reference electrode. All eluents were degassed by flushing with helium. A pellicular anion-exchange 4 x 250 mm Carbo-Pack PA-1 column (Dionex) connected to a CarboPac PA-1 guard column was used at 30 °C. For eluent preparation, MilliQ water and 50% (w/v) NaOH (Sigma-Aldrich) were used. Daily reference curves were obtained for sorbitol, mannitol, trehalose, galactose, glucose, sucrose, mannose, fructose, melezitose, raffinose, erlose and maltose by injecting calibration standards with concentrations of 2.5, 5, 7.5 and 10 ppm of each of these sugars. The peaks were analysed using Chromeleon software. Identification of the different carbohydrates was done based on standards commercially available.

A total of 46 (spring), 44 (summer) and 55 (autumn) A. chrysomphali were analysed. The total number of C. carnea s.l. analysed was 25 (spring) and 22 (autumn).

**Interpretation of HPLC data**

The “total sugar content” for each insect was obtained from the sum of the concentrations of all the sugars detected and adjusted by the hind tibia length, expressed as µg/mm hind tibia length (Tena et al., 2013c).

In order to determine whether an insect had fed on sugar, we used the total sugar content and the glucose-fructose ratio as classification parameters (Steppuhn & Wäckers, 2004). The glucose-fructose ratio was calculated as the glucose fraction of the sum of both monosaccharides. An insect was classified as “sugar-fed” when the total sugar content was above an established threshold and the glucose-fructose was below an established threshold. The thresholds used to classify an insect as “sugar-fed” were obtained from previous studies with laboratory insects. For A. chrysomphali, we used the thresholds obtained for the sibling species A. melinus established at 1.5 µg/mm hind tibia length for total sugar content and 0.63 for glucose-fructose ratio (Tena et al., 2013c). For C. carnea s.l., we used the thresholds obtained by Hogervorst et al. (2007) for the total sugar content and
glucose-fructose ratio, established at 59.2 and 0.85 µg/lacewing respectively. Individuals with total sugar content below or glucose-fructose ratio above the established thresholds were considered as “unfed or starved”. The erlose-melezitose ratio can be used to determine recent honeydew consumption for some species (Hogervorst et al., 2007; Tena et al., 2013c). We used the erlose-melezitose ratio to determine “honeydew consumption” by A. chrysomphali using the threshold obtained in laboratory by Tena et al. (2013c) for A. melinus, which was established at 0.32.

Statistical analysis

We used a one-way ANOVA to check for differences in ant activity and abundance of honeydew producers among seasons. Normality assumption was assessed using Shapiro test, and homoscedasticity assumption was assessed with Levene test. When necessary, data were log transformed in order to fulfil normality and homoscedasticity assumptions.

In the ant-excluded treatment the tanglefoot barriers were very effective in completely excluding the ants from climbing to the canopy, however, ant activity was null or very low in some of the ant-allowed trees. Therefore, for the analysis average ant activity per plot was used as explanatory variable in place of the categorical treatment variables ant-exclusion and ant-allowed (see Yoo et al. 2013).

We applied generalized linear mixed modeling techniques assuming Gamma error variance to construct a model with the abundance of honeydew producers as depended variable, ant activity as the explanatory variable and block as random factor. Abundance of honeydew producers from the four sampled trees was averaged to obtain a mean for each plot.

We also applied generalized linear mixed modeling techniques assuming Gamma error variance for the total sugar content and binomial error structure for sugar-feeding or honeydew-feeding occurrence to construct models with ant activity as the explanatory variable and block as random
factor using the *glmer* function (Bates, 2010). In all models, ant activity from the four sampled trees in the ant–allowed treatment was averaged to obtain a mean for each plot. Different models were constructed for each sampling date given that ant activity as well as the composition of species of the honeydew producers was significantly different among seasons. All statistical analyses were conducted with R (R Development Core Team, 2014).

**Results**

**Ant activity**

In the ant-excluded treatment, the sticky barriers excluded the ants from climbing to the canopy; ant activity was null in all the trees and samplings dates. In the ant-allowed trees, three ant species were identified foraging on the canopies: *P. pallidula* (accounting for the 56% of the total ants counted), *P. schmitzii* (31%) and *L. grandis* (13%). Ant activity was different among seasons (*F* \(2, 21 = 44.42; P < 0.0001\)), being significantly higher in summer (11.7 ± 1.8 ants/min) than in spring (6.4 ± 1.1 ants/min) or autumn (1.5 ± 0.3 ants/min) (Fig 1).

**Abundance of honeydew producers**

Honeydew producers were present in the three seasons in the ant-allowed and ant-excluded treatments. The abundance of the honeydew producers was different among seasons, being significantly higher in summer than spring and autumn (*F* \(2, 45 = 19.1; P < 0.0001\)) (Fig. 2). Ant activity increased the abundance of honeydew producers in summer (*\(\chi^2 = 7.93, P = 0.005\)*), whereas no relationship was found in spring (*\(\chi^2 = 0.26, P = 0.607\)*) or autumn (*\(\chi^2 = 0.001, P = 0.965\)*).

The species composition of the honeydew producers differed among seasons. In spring, the principal honeydew producers were *Ceroplastes sinensis* Del Guercio (Hemiptera: Coccidae) (accounting for the 40% of the total honeydew producers) and *Icerya purchasi* Maskell (Hemiptera: Monophlebidae)
(36%), whereas the whitefly Aleurothrixus floccosus (Maskell) (Hemiptera: Aleyrodidae) was the most abundant species in summer (90%) and autumn (70%) (Fig. 2).

Sugar spectrum of natural enemies

The sugar spectrum revealed that glucose and fructose were the predominant sugars for A. chrysomphali and C. carnea s.l. (Table 1). In addition, the two species contained the honeydew specific sugars erlose and melezitose.

Effect of ants on total sugar content

Pooling all the captured individuals, the total sugar content of A. chrysomphali in the field ranged from 0.15 to 17.12 µg/hind tibia length, with a mean value of 3.94 ± 0.29 µg/hind tibia length. A significant negative relationship between ant activity and the total sugar content of A. chrysomphali was found in summer ($\chi^2 = 5.88, P = 0.015$) (Fig. 3). Ant activity had a marginally significant effect on the total sugar content of A. chrysomphali in spring ($\chi^2 = 3.55, P = 0.059$) whereas no significant relationship between ant activity and the total sugar content was found in autumn ($\chi^2 = 0.168, P = 0.682$).

The total sugar content of C. carnea s.l. ranged from 1.6 to 337.8 µg/hind tibia length, with a mean value of 66.7 ± 9.4 µg/hind tibia length (pooling all the captured individuals). The relation between ant activity and total sugar content of C. carnea s.l. was non-significant in spring ($\chi^2 = 2.58, P = 0.108$) or summer ($\chi^2 = 0.05, P = 0.82$). It is also important to highlight the very big (4 times) higher total sugar content in C. carnea s.l. individuals captured in summer in comparison with the ones captured in spring.

Effect of ants on sugar-feeding occurrence

Overall, out of 146 A. chrysomphali captured in the field in the three seasons, 65% was classified as sugar-fed. Ant activity had a marginally negative significant effect on the occurrence of “sugar-fed”
individuals of *A. chrysomphali* in summer ($\chi^2 = 3.64, P = 0.056$) whereas no significant relationship between ant activity and sugar feeding occurrence was found in spring ($\chi^2 = 0.23, P = 0.630$) or autumn ($\chi^2 = 0.26, P = 0.607$).

Overall, out of 47 *C. carnea* s.l. captured in the field in the three seasons, 55.3% was classified as sugar-fed. The effect of ant activity on the sugar feeding occurrence for *C. carnea* s.l. was negative in spring ($\chi^2 = 4.82, P = 0.028$) (Fig. 4) whereas it was non-significant in summer ($\chi^2 = 1.12, P = 0.290$).

**Effect of ants on honeydew-feeding occurrence**

Overall, out of 146 *A. chrysomphali* captured in the field in the three seasons, 24.7% was classified as honeydew-fed. Honeydew-feeding in *A. chrysomphali* was negatively correlated with ant activity in summer ($\chi^2 = 4.99, P = 0.026$) (Fig. 5), whereas no relationship was found in spring ($\chi^2 = 0.06, P = 0.808$) or autumn ($\chi^2 = 2.13, P = 0.144$).

Despite the fact that the mere presence of erlose and melezitose cannot be used for determination of honeydew feeding, we detected a considerable percentage of *C. carnea* s.l. (63.8% of individuals captured in spring and summer) with presence of honeydew signature sugars.

**Discussion**

Our study delves into the understanding of indirect, multi-trophic interactions mediated by honeydew excreted by plant feeders testing the hypothesis that the mutualistic relationship between ants and honeydew producers may impact positively or negatively the energy reserves and access to honeydew of natural enemies. Herein, for the first time we provide evidence that ants can have a negative impact on the energy reserves and access to honeydew of the parasitoid *A. chrysomphali*, and the predator *C. carnea* s.l. Nevertheless, this ant interference depends on the level of ant activity. The impact of ant activity on the energy reserves of natural enemies is likely to be widespread in
natural and managed ecosystems with potential effects for the arthropod community composition and biological control.

Regarding the interference of ants with the total sugar content of *A. chrysomphali*, we found a negative relationship in summer, the period when both ant activity and abundance of honeydew producers peaked. In the same way, the sugar and honeydew-feeding incidence by *A. chrysomphali* was negatively affected by ant activity in summer. Several studies have demonstrated aggressive behavior of ants against parasitoids whose effects depend on the parasitoid species; there are parasitoids that are highly susceptible to ant aggression (Way, 1963; Kaneko, 2003) whereas others can cope with or even benefit in the presence of ants (Völkl, 1994; Barzman & Daane, 2001).

*Aphytis* spp. apparently fall into the former category given that ants have been documented to prey upon them in the field (Heimpel et al., 1997a) or disturb them during host-feeding and oviposition (Martínez-Ferrer et al., 2003). Honeydew sources in citrus orchards are commonly ant tended. Therefore, *Aphytis* should elude any encounter with ants in order to avoid a possible attack when reaching a honeydew source to feed upon. *Aphytis melinus* females spend more than 300 seconds feeding on a honeydew bout (Tena et al., 2013b). Assuming that *A. chrysomphali* spends a similar time feeding on honeydew, it is likely that ants may interfere with the sugar acquisition by *A. chrysomphali* during that feeding time. The impact of ant activity on the feeding history and energy reserves may be more significant for small parasitoids such as *Aphytis* species because of their limited capacity to engage in long flights (Campbell, 1976; Zappalà et al., 2012) to exploit other nontended honeydew sources. Finally, our results show that the effect of ants on the energy reserves of parasitoids depended on the level of ant activity. Therefore, we expect that this interference may be more pronounced when honeydew producers are tended by aggressive ant species and with high levels of activity, as is the case of many invasive ant species (Holway et al., 2002). All this being said, we propose that ant interference might be more detrimental for those parasitoid species that are
not tolerant to ant aggression, have limited dispersion capacity and/or whose host does not excrete honeydew.

Overall, the energy reserves of the predator *C. carnea* s.l. were not affected by ant activity either in spring or in summer. On the other hand, ant activity reduced the incidence of sugar-feeding occurrence in spring. We suggest that this result may be interpreted in terms of honeydew availability in combination with the behavior of *C. carnea* s.l. As the populations of the honeydew producers were very low in spring, sugar availability was scarce. Under this scenario, adult *C. carnea* s.l. may find occasional honeydew droplets in absence of ants which would increase little their sugars levels but would be enough to distinguish them as sugar-fed. Nevertheless, under conditions of increased ant activity, it is likely that most honeydew sources will be occupied by ants, leaving very little chance to *C. carnea* s.l. adults to feed on sugars. In summer, the higher availability of honeydew allows lacewings to feed reaching high energy reserves, even though the presence of ants.

The flight behavior, in particular the migration flights of *C. carnea* s.l. might give an additional explanation for the results obtained in our study. In the first three nights after emergence, the lacewings fly downwind in adaptive dispersal flights, irrespective of the availability of food (Duelli, 1980a, 1980b). This behavior implies that young adults captured after these flights will show very low levels of energy reserves regardless the availability of honeydew or the disturbance of ants. Later, the sexually mature adults respond to kairomones signaling honeydew and perform a stepwise flight against the wind to approach the source of attractant (Duelli, 1980b). Although larvae and adults of green lacewings are heavily attacked by ants and the adults show a strong tendency to avoid ants (Bartlett, 1961) the capacity of adults for dispersion apparently plays an important role in overcoming the disturbance by ants in summer since they can search for non-ant tended honeydew sources. For example, adult *Chrysopa carnea* (=*Chrysoperla carnea* s.l.) may move up to 1 km away from the release point after one day (Duelli, 1980b). Consequently, the energy reserves of *C. carnea*
s.l. are less susceptible to be influenced by the level of ant activity compared to that of *Aphytis*
parasitoids.

It is also important to highlight that the energy reserves and incidence of sugar or honeydew feeding were never enhanced in the presence of ants neither for *A. chrysomphali* nor for *C. carnea* s.l. This is even more surprising if we consider the fact that the present as well as other studies conducted in citrus (Moreno *et al.*, 1987; Calabuig *et al.*, 2013; Yoo *et al.*, 2013) have demonstrated that under increased ant activity levels there are greater numbers of honeydew producers on the citrus canopies. This means that predators and parasitoids, in spite of ant interference, could potentially have higher probabilities of accessing honeydew sources in the presence of ants. However, this was not the case in our study. Possibly the direct ant competition might explain this result given that in citrus orchards colonies of honeydew producers are usually ant tended (Pekas *et al.*, 2011). Moreover, competition with other honeydew feeders such as pollinators and other unintended consumers might also have an influence (Wäckers & Fadamiro, 2005).

All in all, we report a novel interaction between ants and natural enemies mediated by the shared honeydew exploitation. As already known, honeydew collecting ants may modify natural enemies abundances (Bartlett, 1961; James *et al.*, 1999), enhance hemipteran populations (Bartlett, 1961; Samways, 1990) and, eventually, impact plant health (Rosumek *et al.*, 2009). Herein, we report for the first time, that ants can affect negatively the energy reserves, sugar and honeydew feeding of entomophagous arthropods. This type of interaction may be widespread in various ecosystems with important consequences at the community-level and with practical implications for the biocontrol services the natural enemies provide (Eubanks & Finke, 2014). Absence of sugar feeding has been found to be detrimental for the fitness of many species of predators and parasitoids and is likely to affect also their abundance, the abundance of the herbivores and ultimately plant quality resulting in top-down trophic cascades (Pace *et al.*, 1999). These trophic cascades may be particularly important in agroecosystems, where the availability of sugar sources is usually limited in space and time.
(Wäckers et al., 2008) resulting in a deficient pest control. Ideally, this lack of sugar sources may be compensated by means of artificial sugar sprays (Wade et al., 2008) and/or habitat management to enhance the functional biodiversity that will support the beneficial organisms to maximize the ecosystem services provided (Olson & Wäckers, 2006).

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References


548 Captions:

549 Fig. 1. Mean (±SE) ant activity (number of ants per minute) in representative days of spring, summer
550 and autumn. Means with different letter differ significantly at $P < 0.05$.

551 Fig. 2. Mean (± SE) number of hemipteran honeydew producers per sampling unit in representative
days of spring, summer and autumn. Means with different letter differ significantly at $P < 0.05$.

552 Fig. 3. Relationship between the level of ant activity (ants/minute) and the total sugar content
553 (µg/mm tibia length) of *Aphytis chrysomphali* in representative days of summer. (Open circles, ant-
554 allowed trees; solid circles, ant-excluded trees).

555 Fig. 4. Relationship between ant activity (ants/minute) and sugar-feeding occurrence by *Chrysoperla*
556 carnea s.l. in representative days of spring. (Open circles, ant-allowed trees; solid circles, ant-
557 excluded trees).

559 Fig. 5. Relationship between ant activity (ants/minute) and honeydew-feeding occurrence by *Aphytis*
560 chrysomphali in representative days of summer. (Open circles, ant-allowed trees; solid circles, ant-
561 excluded trees).
Table 1. Total amount of sugars (µg/mm tibia length) of *Aphytis chrysomphali* and *Chrysoperla carnea* s.l. captured in the field (number of individuals is shown in parentheses).

<table>
<thead>
<tr>
<th>Sugar</th>
<th><em>Aphytis chrysomphali</em> (145)</th>
<th><em>Chrysoperla carnea</em> (47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erlose</td>
<td>0.170 ± 0.041</td>
<td>1.351 ± 0.471</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.183 ± 0.017</td>
<td>3.477 ± 0.891</td>
</tr>
<tr>
<td>Raffinose</td>
<td>0.002 ± 0.001</td>
<td>0.576 ± 0.133</td>
</tr>
<tr>
<td>Melezitose</td>
<td>0.511 ± 0.099</td>
<td>7.381 ± 1.824</td>
</tr>
<tr>
<td>Melibiose</td>
<td>0.021 ± 0.003</td>
<td>2.856 ± 0.503</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.716 ± 0.107</td>
<td>4.885 ± 1.190</td>
</tr>
<tr>
<td>Mannose</td>
<td>0</td>
<td>0.157 ± 0.034</td>
</tr>
<tr>
<td>Fructose</td>
<td>1.100 ± 0.099</td>
<td>19.728 ± 3.797</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.105 ± 0.073</td>
<td>22.146 ± 1.847</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.005 ± 0.002</td>
<td>0.050 ± 0.040</td>
</tr>
<tr>
<td>Trehalose</td>
<td>0.045 ± 0.018</td>
<td>0.381 ± 0.130</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.068 ± 0.008</td>
<td>1.250 ± 0.204</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>0.011 ± 0.004</td>
<td>1.307 ± 0.223</td>
</tr>
<tr>
<td>Total</td>
<td>3.936 ± 0.290</td>
<td>66.687 ± 9.389</td>
</tr>
</tbody>
</table>
Aphytis chrysomphali

Total sugar content (µg/mm tibia)

Ants / min

Summer