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1 **Running head: indirect effects in food webs**

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

4 **Authors:** A. Calabuig¹; A. Tena²; F. Wäckers³; L. Fernández-Arrojo⁴; F.J. Plou⁴; F. Garcia-
5 Marí¹; A. Pekas^{1,3*}

6 ¹ Instituto Agroforestal Mediterráneo (IAM), Universitat Politècnica de València

7 ² Unidad Asociada de Entomología UJI-IVIA-CIB CSIC, Centro de Protección Vegetal y
8 Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Valencia, Spain

9 ³ R&D Department Biobest Belgium

10 ⁴ Instituto de Catálisis y Petroleoquímica, Consejo Superior de Investigaciones Científicas
11 (CSIC), 28049 Madrid, Spain

12

13 * Corresponding author: Dr. Apostolos Pekas

14 R&D Department Biobest Belgium N.V.

15 Ilse Velden 18,2260, Westerlo, Belgium

16 Tel: +32 14 257 980

17 Fax: +32 14 257 982,

18 E-mail address: tolis@biobest.be

19 **Abstract**

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

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

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

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

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

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

42 Introduction

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

61 Honeydew is a sugar-rich fluid excreted by plant feeders (mostly hemipteran species) after
62 feeding on phloem sap. Honeydew is especially relevant in agricultural ecosystems where it is the
63 principal carbohydrate source since the presence of other sugar sources, such as nectar, is limited and
64 variable in space and time, being available almost exclusively during the flowering season (Wäckers
65 *et al.*, 2008). Honeydew contains a mixture of phloem sugars, such as sucrose, fructose and glucose,
66 and oligosaccharides synthesized by the plant feeders, such as erlose and melezitose (Völkl *et al.*,

67 1999; Wäckers, 2000). Its composition makes honeydew an important carbohydrate source for a
68 wide range of insects in the field, among which ants hold a predominant position (Hölldobler &
69 Wilson, 1990; Wäckers, 2005). Most ant species are omnivorous and obtain protein from animal
70 matter and carbohydrates from plant products such as floral and extrafloral nectar, food bodies, plant
71 sap and above all honeydew (Way, 1963; Carroll & Janzen, 1973; Tobin, 1994). Honeydew is crucial
72 for the ant colony growth and, usually, honeydew producers thrive when ant-tended (Hölldobler &
73 Wilson, 1990). In fact, honeydew exploitation is associated with behavioural dominance in ants:
74 dominant ant species exclude subordinate species and monopolize the honeydew sources (Blüthgen
75 *et al.*, 2004; Pekas *et al.*, 2011).

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

88 The citrus agro-ecosystem provides a suitable environment for several honeydew producers
89 (Garcia-Marí, 2012) that are usually ant-attended (Pekas *et al.*, 2011; Tena *et al.*, 2013a) and also
90 harbours a complex of naturally occurring parasitoids and predators (Garcia-Marí, 2012). In the
91 present study, we test the hypothesis that ants might impact the energy reserves of predators and

92 parasitoids through the exploitation of honeydew: positively, by increasing the opportunities for
93 honeydew feeding due to the positive feedback between the ants and the abundance of the honeydew
94 producers or negatively, due to the direct competition ants exert by monopolizing the honeydew
95 sources. To test our hypothesis we carried out an ant-exclusion experiment in a citrus agroecosystem
96 where we related the level of ant activity with the energy reserves and history feeding of individual
97 natural enemies. We included in the study two of the most important entomophagous arthropods in
98 terms of abundance and biocontrol potential on the citrus canopy belonging to two different guilds,
99 one parasitoid and one predator species.

100 *Study system:*

101 We conducted our study in the main Mediterranean citrus-growing area (Valencia, Spain), where the
102 two most abundant and widely distributed ant species are the native *Lasius grandis* (Forel) and
103 *Pheidole pallidula* (Nylander) (Cerdá *et al.*, 2009; Pekas *et al.*, 2011). Both species are behaviourally
104 dominant and are in close association with honeydew producers; they are active from March until
105 November, with *L. grandis* peaking its activity in June and *P. pallidula* in July-August (Pekas *et al.*,
106 2011). The most abundant honeydew producers present in Mediterranean citrus orchards are the
107 citrus aphid *Aphis spiraecola* Patch (Hemiptera: Aphididae) in early spring, soft scales such as
108 *Coccus hesperidum* L. and *Saissetia oleae* Olivier (Hemiptera: Coccidae), and the citrus mealybug
109 *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) during summer, whereas the most abundant
110 honeydew producer in autumn is the woolly whitefly *Aleurothrixus floccosus* (Maskell) (Hemiptera:
111 Aleyrodidae) (Pekas *et al.*, 2011; Tena *et al.*, 2013c).

112 Parasitoids of genus *Aphytis* (Hymenoptera: Aphelinidae) are the most important natural enemies of
113 the California red scale (CRS) *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), a major pest in
114 citrus worldwide (Rosen & DeBach, 1979). *Aphytis* are synovigenic ectoparasitoids that engage in
115 host-feeding for egg maturation (Heimpel & Collier, 1996; Heimpel *et al.*, 1997b); however, host

116 feeding cannot substitute sugar feeding because CRS contains very low amounts of sugar (Tena *et*
117 *al.*, 2013c). In the lab, in the absence of sugar feeding, *Aphytis* fecundity and longevity are seriously
118 compromised and survival does not exceed three days (Avidov *et al.*, 1970; Heimpel *et al.*, 1997b;
119 Tena *et al.*, 2013b). Furthermore, Tena *et al.* (2013c) demonstrated that honeydew is the main sugar
120 source for *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) in the field, despite the fact that its
121 host does not produce honeydew. Honeydew feeding enhances the longevity and realized fecundity
122 of *A. melinus* (Tena *et al.*, 2013b). The species of our study, *Aphytis chrysomphali* Mercet
123 (Hymenoptera: Aphelinidae), is native to the Mediterranean and is one of the most important
124 parasitoids of CRS (Pekas *et al.*, 2010a). Most probably it also feeds on honeydew in the field,
125 though this has never been demonstrated.

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

136 **Materials and Methods**

137 *Study site and experimental design*

138 The study was conducted in a commercial citrus orchard of sweet orange *Citrus sinensis* L. Osbeck
139 (cv. Navelina) of approximately 0.7 ha, located in an extensive citrus-growing area 30 km south of

140 Valencia, eastern Spain (39° 12'2" N, 0° 20'52" W). The climate is Mediterranean, with a rainy
141 spring and autumn and a dry winter and summer. The orchard was flood irrigated and weeds were
142 controlled by local application of herbicides (Glyphosate®, Bayer CropScience, Spain). No
143 insecticides were sprayed in the previous nine years, or during the experiment. According to previous
144 studies, the ant species foraging on the tree canopies were *P. pallidula*, *Plagiolepis schmitzii* and *L.*
145 *grandis* (Pekas *et al.*, 2010b, 2011).

146 The experimental design was a randomized block with eight replicates (blocks) of two adjacent
147 treatments (plots): ant-allowed and ant-excluded trees. Each treatment contained 16 trees (four rows
148 by four trees) . . . Ants were excluded in the 16 trees but only the four central trees were used for the
149 samplings. Ant-exclusion began in January 2013 and was maintained until November 2013 by
150 placing sticky barriers based on Tangle-trap® (Tanglefoot, Biagro, Valencia, Spain) on the tree
151 trunks at 30 cm above ground. Tanglefoot was applied using a spatula on a 15 cm wide adhesive
152 plastic tape fixed around the trunk. Sticky barriers were inspected every month and if necessary the
153 Tanglefoot was renewed; in any case, Tanglefoot was renewed routinely every two months. Trees
154 were pruned periodically and ground vegetation was trimmed to prevent alternative ways for ants to
155 reach the canopies.

156 *Ant activity*

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

163 *Honeydew producers*

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

169 *Sampling parasitoids and predators*

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

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

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

181 *HPLC sugar analysis*

182 Prior to the analysis, the samples were homogenized in the ethanol solution using a pestle. Then, the
183 ethanol was evaporated in a vacuum centrifuge for 60-120 min. After that, the samples were
184 rehydrated by adding 20µl of ethanol 70% and 80µl of Milli-Q water for *A. chrysomphali* and 200µl
185 of ethanol 70% and 800µl of Milli-Q water for *C. carnea* s.l. The samples were mixed by vibration
186 and filtered (0.2 µm PVDF membrane). Analysis was carried out by high performance anion-
187 exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). 25µl of

188 each sample were injected into a ICS3000 Dionex system (Dionex Corp., Sunnyvale, CA) consisting
189 of a SP gradient pump, an AS-HV autosampler and an electrochemical detector with a gold working
190 electrode and Ag/AgCl as reference electrode. All eluents were degassed by flushing with helium. A
191 pellicular anion-exchange 4 x 250 mm Carbo-Pack PA-1 column (Dionex) connected to a CarboPac
192 PA-1 guard column was used at 30 °C. For eluent preparation, MilliQ water and 50% (w/v) NaOH
193 (Sigma-Aldrich) were used. Daily reference curves were obtained for sorbitol, mannitol, trehalose,
194 galactose, glucose, sucrose, mannose, fructose, melezitose, raffinose, erlose and maltose by injecting
195 calibration standards with concentrations of 2.5, 5, 7.5 and 10 ppm of each of these sugars. The
196 peaks were analysed using Chromeleon software. Identification of the different carbohydrates was
197 done based on standards commercially available.

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

200 *Interpretation of HPLC data*

201 The “total sugar content” for each insect was obtained from the sum of the concentrations of all the
202 sugars detected and adjusted by the hind tibia length, expressed as $\mu\text{g}/\text{mm}$ hind tibia length (Tena *et*
203 *al.*, 2013c).

204 In order to determine whether an insect had fed on sugar, we used the total sugar content and the
205 glucose-fructose ratio as classification parameters (Steppuhn & Wäckers, 2004) . The glucose-
206 fructose ratio was calculated as the glucose fraction of the sum of both monosaccharides. An insect
207 was classified as “sugar-fed” when the total sugar content was above an established threshold and the
208 glucose-fructose was below an established threshold. The thresholds used to classify an insect as
209 “sugar-fed” were obtained from previous studies with laboratory insects. For *A. chrysomphali*, we
210 used the thresholds obtained for the sibling species *A. melinus* established at 1.5 $\mu\text{g}/\text{mm}$ hind tibia
211 length for total sugar content and 0.63 for glucose-fructose ratio (Tena *et al.*, 2013c). For *C. carnea*
212 s.l., we used the thresholds obtained by Hogervorst *et al.* (2007) for the total sugar content and

213 glucose-fructose ratio, established at 59.2 and 0.85 $\mu\text{g/lacewing}$ respectively. Individuals with total
214 sugar content below or glucose-fructose ratio above the established thresholds were considered as
215 “unfed or starved”. The erlose-melezitose ratio can be used to determine recent honeydew
216 consumption for some species (Hogervorst *et al.*, 2007; Tena *et al.*, 2013c). We used the erlose-
217 melezitose ratio to determine “honeydew consumption” by *A. chrysomphali* using the threshold
218 obtained in laboratory by Tena *et al.* (2013c) for *A. melinus*, which was established at 0.32.

219 *Statistical analysis*

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

224 In the ant-excluded treatment the tanglefoot barriers were very effective in completely excluding the
225 ants from climbing to the canopy, however, ant activity was null or very low in some of the ant-
226 allowed trees. Therefore, for the analysis average ant activity per plot was used as explanatory
227 variable in place of the categorical treatment variables ant-exclusion and ant-allowed (see Yoo *et al.*
228 2013).

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

233 We also applied generalized linear mixed modeling techniques assuming Gamma error variance for
234 the total sugar content and binomial error structure for sugar-feeding or honeydew-feeding
235 occurrence to construct models with ant activity as the explanatory variable and block as random

236 factor using the *glmer* function (Bates, 2010). In all models, ant activity from the four sampled trees
237 in the ant-allowed treatment was averaged to obtain a mean for each plot. Different models were
238 constructed for each sampling date given that ant activity as well as the composition of species of the
239 honeydew producers was significantly different among seasons. All statistical analyses were
240 conducted with R (R Development Core Team, 2014).

241 **Results**

242 *Ant activity*

243 In the ant-excluded treatment, the sticky barriers excluded the ants from climbing to the canopy; ant
244 activity was null in all the trees and samplings dates. In the ant-allowed trees, three ant species were
245 identified foraging on the canopies: *P. pallidula* (accounting for the 56% of the total ants counted),
246 *P. schmitzii* (31%) and *L. grandis* (13%). Ant activity was different among seasons ($F_{2, 21} = 44.42$; P
247 < 0.0001), being significantly higher in summer (11.7 ± 1.8 ants/min) than in spring (6.4 ± 1.1
248 ants/min) or autumn (1.5 ± 0.3 ants/min) (Fig 1).

249 *Abundance of honeydew producers*

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

255 The species composition of the honeydew producers differed among seasons. In spring, the principal
256 honeydew producers were *Ceroplastes sinensis* Del Guercio (Hemiptera: Coccidae) (accounting for
257 the 40% of the total honeydew producers) and *Icerya purchasi* Maskell (Hemiptera: Monophlebidae)

258 (36%), whereas the whitefly *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae) was the
259 most abundant species in summer (90%) and autumn (70%) (Fig. 2).

260 *Sugar spectrum of natural enemies*

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

264 *Effect of ants on total sugar content*

265 Pooling all the captured individuals, the total sugar content of *A. chrysomphali* in the field ranged
266 from 0.15 to 17.12 $\mu\text{g}/\text{hind tibia length}$, with a mean value of $3.94 \pm 0.29 \mu\text{g}/\text{hind tibia length}$. A
267 significant negative relationship between ant activity and the total sugar content of *A. chrysomphali*
268 was found in summer ($\chi^2 = 5.88$, $P = 0.015$) (Fig. 3). Ant activity had a marginally significant effect
269 on the total sugar content of *A. chrysomphali* in spring ($\chi^2 = 3.55$, $P = 0.059$) whereas no significant
270 relationship between ant activity and the total sugar content was found in autumn ($\chi^2 = 0.168$, $P =$
271 0.682).

272 The total sugar content of *C. carnea* s.l. ranged from 1.6 to 337.8 $\mu\text{g}/\text{hind tibia length}$, with a mean
273 value of $66.7 \pm 9.4 \mu\text{g}/\text{hind tibia length}$ (pooling all the captured individuals). The relation between
274 ant activity and total sugar content of *C. carnea* s.l. was non-significant in spring ($\chi^2 = 2.58$, $P =$
275 0.108) or summer ($\chi^2 = 0.05$, $P = 0.82$). It is also important to highlight the very big (4 times) higher
276 total sugar content in *C. carnea* s.l. individuals captured in summer in comparison with the ones
277 captured in spring.

278 *Effect of ants on sugar-feeding occurrence*

279 Overall, out of 146 *A. chrysomphali* captured in the field in the three seasons, 65% was classified as
280 sugar-fed. Ant activity had a marginally negative significant effect on the occurrence of “sugar-fed”

281 individuals of *A. chrysocephala* in summer ($\chi^2 = 3.64$, $P = 0.056$) whereas no significant relationship
282 between ant activity and sugar feeding occurrence was found in spring ($\chi^2 = 0.23$, $P = 0.630$) or
283 autumn ($\chi^2 = 0.26$, $P = 0.607$).

284 Overall, out of 47 *C. carnea* s.l. captured in the field in the three seasons, 55.3% was classified as
285 sugar-fed. The effect of ant activity on the sugar feeding occurrence for *C. carnea* s.l. was negative
286 in spring ($\chi^2 = 4.82$, $P = 0.028$) (Fig. 4) whereas it was non-significant in summer ($\chi^2 = 1.12$, $P =$
287 0.290).

288 *Effect of ants on honeydew-feeding occurrence*

289 Overall, out of 146 *A. chrysocephala* captured in the field in the three seasons, 24.7% was classified
290 as honeydew-fed. Honeydew-feeding in *A. chrysocephala* was negatively correlated with ant activity
291 in summer ($\chi^2 = 4.99$, $P = 0.026$) (Fig. 5), whereas no relationship was found in spring ($\chi^2 = 0.06$, P
292 = 0.808) or autumn ($\chi^2 = 2.13$, $P = 0.144$).

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

296 **Discussion**

297 Our study delves into the understanding of indirect, multi-trophic interactions mediated by honeydew
298 excreted by plant feeders testing the hypothesis that the mutualistic relationship between ants and
299 honeydew producers may impact positively or negatively the energy reserves and access to
300 honeydew of natural enemies. Herein, for the first time we provide evidence that ants can have a
301 negative impact on the energy reserves and access to honeydew of the parasitoid *A. chrysocephala*,
302 and the predator *C. carnea* s.l. Nevertheless, this ant interference depends on the level of ant activity.
303 The impact of ant activity on the energy reserves of natural enemies is likely to be widespread in

304 natural and managed ecosystems with potential effects for the arthropod community composition and
305 biological control.

306 Regarding the interference of ants with the total sugar content of *A. chrysomphali*, we found a
307 negative relationship in summer, the period when both ant activity and abundance of honeydew
308 producers peaked. In the same way, the sugar and honeydew-feeding incidence by *A. chrysomphali*
309 was negatively affected by ant activity in summer. Several studies have demonstrated aggressive
310 behavior of ants against parasitoids whose effects depend on the parasitoid species; there are
311 parasitoids that are highly susceptible to ant aggression (Way, 1963; Kaneko, 2003) whereas others
312 can cope with or even benefit in the presence of ants (Völkl, 1994; Barzman & Daane, 2001).
313 *Aphytis* spp. apparently fall into the former category given that ants have been documented to prey
314 upon them in the field (Heimpel *et al.*, 1997a) or disturb them during host-feeding and oviposition
315 (Martínez-Ferrer *et al.*, 2003). Honeydew sources in citrus orchards are commonly ant tended.
316 Therefore, *Aphytis* should elude any encounter with ants in order to avoid a possible attack when
317 reaching a honeydew source to feed upon. *Aphytis melinus* females spend more than 300 seconds
318 feeding on a honeydew bout (Tena *et al.*, 2013b). Assuming that *A. chrysomphali* spends a similar
319 time feeding on honeydew, it is likely that ants may interfere with the sugar acquisition by *A.*
320 *chrysomphali* during that feeding time. The impact of ant activity on the feeding history and energy
321 reserves may be more significant for small parasitoids such as *Aphytis* species because of their
322 limited capacity to engage in long flights (Campbell, 1976; Zappalà *et al.*, 2012) to exploit other non-
323 tended honeydew sources. Finally, our results show that the effect of ants on the energy reserves of
324 parasitoids depended on the level of ant activity. Therefore, we expect that this interference may be
325 more pronounced when honeydew producers are tended by aggressive ant species and with high
326 levels of activity, as is the case of many invasive ant species (Holway *et al.*, 2002). All this being
327 said, we propose that ant interference might be more detrimental for those parasitoid species that are

328 not tolerant to ant aggression, have limited dispersion capacity and/or whose host does not excrete
329 honeydew.

330 Overall, the energy reserves of the predator *C. carnea* s.l. were not affected by ant activity either in
331 spring or in summer. On the other hand, ant activity reduced the incidence of sugar-feeding
332 occurrence in spring. We suggest that this result may be interpreted in terms of honeydew
333 availability in combination with the behavior of *C. carnea* s.l. As the populations of the honeydew
334 producers were very low in spring, sugar availability was scarce. Under this scenario, adult *C. carnea*
335 s.l. may find occasional honeydew droplets in absence of ants which would increase little their sugars
336 levels but would be enough to distinguish them as sugar-fed. Nevertheless, under conditions of
337 increased ant activity, it is likely that most honeydew sources will be occupied by ants, leaving very
338 little chance to *C. carnea* s.l. adults to feed on sugars. In summer, the higher availability of
339 honeydew allows lacewings to feed reaching high energy reserves, even though the presence of ants.
340 The flight behavior, in particular the migration flights of *C. carnea* s.l. might give an additional
341 explanation for the results obtained in our study. In the first three nights after emergence, the
342 lacewings fly downwind in adaptive dispersal flights, irrespective of the availability of food (Duelli,
343 1980a, 1980b). This behavior implies that young adults captured after these flights will show very
344 low levels of energy reserves regardless the availability of honeydew or the disturbance of ants.
345 Later, the sexually mature adults respond to kairomones signaling honeydew and perform a stepwise
346 flight against the wind to approach the source of attractant (Duelli, 1980b). Although larvae and
347 adults of green lacewings are heavily attacked by ants and the adults show a strong tendency to avoid
348 ants (Bartlett, 1961) the capacity of adults for dispersion apparently plays an important role in
349 overcoming the disturbance by ants in summer since they can search for non-ant tended honeydew
350 sources. For example, adult *Chrysopa carnea* (= *Chrysoperla carnea* s.l.) may move up to 1 km away
351 from the release point after one day (Duelli, 1980b). Consequently, the energy reserves of *C. carnea*

352 s.l. are less susceptible to be influenced by the level of ant activity compared to that of *Aphytis*
353 parasitoids.

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

365 All in all, we report a novel interaction between ants and natural enemies mediated by the shared
366 honeydew exploitation. As already known, honeydew collecting ants may modify natural enemies
367 abundances (Bartlett, 1961; James *et al.*, 1999), enhance hemipteran populations (Bartlett, 1961;
368 Samways, 1990) and, eventually, impact plant health (Rosumek *et al.*, 2009). Herein, we report for
369 the first time, that ants can affect negatively the energy reserves, sugar and honeydew feeding of
370 entomophagous arthropods. This type of interaction may be widespread in various ecosystems with
371 important consequences at the community-level and with practical implications for the biocontrol
372 services the natural enemies provide (Eubanks & Finke, 2014). Absence of sugar feeding has been
373 found to be detrimental for the fitness of many species of predators and parasitoids and is likely to
374 affect also their abundance, the abundance of the herbivores and ultimately plant quality resulting in
375 top-down trophic cascades (Pace *et al.*, 1999). These trophic cascades may be particularly important
376 in agroecosystems, where the availability of sugar sources is usually limited in space and time

377 (Wäckers *et al.*, 2008) resulting in a deficient pest control. Ideally, this lack of sugar sources may be
378 compensated by means of artificial sugar sprays (Wade *et al.*, 2008) and/or habitat management to
379 enhance the functional biodiversity that will support the beneficial organisms to maximize the
380 ecosystem services provided (Olson & Wäckers, 2006).

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- 547

548 **Captions:**

549 **Fig. 1.** Mean (\pm 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 (\pm SE) number of hemipteran honeydew producers per sampling unit in representative
552 days of spring, summer and autumn. Means with different letter differ significantly at $P < 0.05$.

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

556 **Fig. 4.** Relationship between ant activity (ants/minute) and sugar-feeding occurrence by *Chrysoperla*
557 *carnea* s.l. in representative days of spring. (Open circles, ant-allowed trees; solid circles, ant-
558 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).

562

563 **Table 1.** Total amount of sugars ($\mu\text{g}/\text{mm}$ tibia length) of *Aphytis chrysomphali* and *Chrysoperla*
564 *carnea* s.l. captured in the field (number of individuals is shown in parentheses).

565

Sugar	Total sugar ($\mu\text{g}/\text{mm}$ tibia length)	
	<i>Aphytis chrysomphali</i> (145)	<i>Chrysoperla carnea</i> (47)
Erlose	0.170 ± 0.041	1.351 ± 0.471
Maltose	0.183 ± 0.017	3.477 ± 0.891
Raffinose	0.002 ± 0.001	0.576 ± 0.133
Melezitose	0.511 ± 0.099	7.381 ± 1.824
Melibiose	0.021 ± 0.003	2.856 ± 0.503
Sucrose	0.716 ± 0.107	4.885 ± 1.190
Mannose	0	0.157 ± 0.034
Fructose	1.100 ± 0.099	19.728 ± 3.797
Glucose	1.105 ± 0.073	22.146 ± 1.847
Galactose	0.005 ± 0.002	0.050 ± 0.040
Trehalosae	0.045 ± 0.018	0.381 ± 0.130
Mannitol	0.068 ± 0.008	1.250 ± 0.204
Sorbitol	0.011 ± 0.004	1.307 ± 0.223
Total	3.936 ± 0.290	66.687 ± 9.389

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