Do additional sugar sources affect the degree of attendance of *Dysmicoccus brevipes* by the fire ant *Solenopsis geminata*?

Diana J. Carabalí-Banguero[1], Kris A.G. Wyckhuys[2]*, James Montoya-Lerma[1], Takumasa Kondo[3] & Jonathan G. Lundgren[4]

[1]*Departamento de Biología, Universidad del Valle, Apartado Aéreo, Cali 25360, Colombia,* [2]*CIAT, Centro Internacional de Agricultura Tropical, Apartado Aéreo, Cali 6713, Colombia,* [3]*Corporacion Colombiana de Investigacion Agropecuaria CORPOICA, Palmira, Colombia, and* [4]*USDA-ARS, North Central Agricultural Research Laboratory, Brookings, SD 57006, USA*

Abstract

Mutualistic interactions between ants and Hemiptera are mediated to a large extent by the amount and quality of sugar-rich honeydew produced. Throughout the neotropics, the predaceous fire ant *Solenopsis geminata* (Fabricius) (Hymenoptera: Formicidae) is found in association with colonies of the pineapple mealybug, *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae), which they actively tend and protect from attack by natural enemies. In this study, we evaluate the effects of access to a sucrose solution on the mutualistic association between *S. geminata* and *D. brevipes*. Ten colonies of either species were established, with *D. brevipes* maintained on pumpkin, *Cucurbita maxima* Duchesne (Cucurbitaceae), in screen cages. Five of the *S. geminata* colonies were permitted access to vials with 20% sucrose solution and a pumpkin with 20 adult mealybugs. The remaining ant colonies were allowed access to mealybug-infested pumpkins. Ant colonies with access to the sucrose solution attended mealybugs significantly less than those without additional sugar sources. Mealybug survival rates were similar under both treatments. Total body sugars and fructose were nearly twice as high in ants with access to honeydew and sucrose vs. those with access to honeydew and water. Fructose accumulated on the pumpkins over time in both treatments, suggesting that honeydew was not fully exploited by the ants. In conclusion, *D. brevipes* enjoy lower degrees of ant attendance when *S. geminata* have alternative sources of carbohydrates. We further discuss the significance of these findings for the conservation of predaceous ants and mealybug biological control.

Introduction

Ants have mutualistic associations with a broad range of organisms, including members from the insect families Aphididae, Psyllidae, Cicadellidae, Membracidae, Coccidae, and Pseudococcidae (Perotto et al., 2002; Stadler & Dixon, 2008). As plant sap-feeders, members of these families excrete carbohydrate-rich liquids known as honeydew. These secretions are consumed by various ant species to satisfy their sugar requirements, and in return the ants actively tend those honeydew producers and protect them against a broad set of natural enemies (Perotto et al., 2002; Lundgren, 2009; Mgocheki & Addison, 2009; Nielsen et al., 2010).

By taking part in this facultative mutualism, ants augment their energy reserves and become less dependent upon temporally available plant foods such as floral nectar (Ramírez et al., 2001; Delabie & Fernández, 2003). Also, ample access to carbohydrate sources permits ant colonies to grow in size (Porter, 1989), boost their foraging range, and increase their aggressiveness (Styrsky & Eubanks, 2007). In return, the presence of ants can drastically increase survival rates of honeydew producers (Morales, 2000; McPhee et al., 2012). However, tending ants can also engage in the predation of hemipterans (Cushman, 1991; Franco et al., 2003, 2009), particularly with hemipteran

*Correspondence: Kris A.G. Wyckhuys, CIAT, Apartado Aéreo, Cali 6713, Colombia. E-mail: k.wyckhuys@cgiar.org

species that produce honeydew of inferior quality or quantity (Cushman, 1991).

Many honeydew-producing hemipterans are important agricultural pests, inflicting energetic costs on host plants, reducing overall plant vigor and crop yield, and occasionally transmitting viral diseases (Beattie, 1985; Kondo, 2001; Lambdin, 2008). Also, honeydew droplets form an optimum substrate for sooty mold fungi, which readily interfere with the plant's photosynthesis (Franco et al., 2003; Mera et al., 2010). Aside from a small set of (invasive) pests that are primarily controlled through classical biological control (e.g., Tena et al., 2012), hemipterans are routinely controlled with insecticide sprays. Such tactics frequently cause undesirable side effects on human health, pest resistance development, natural control, and the broader farming environment (Franco et al., 2009). Although biological control can help reduce infestation levels of native and exotic hemipterans alike, its efficacy is highly variable and dependent upon their level of ant attendance (e.g., Cudjoe et al., 1993).

Throughout the (sub-)tropics, the pineapple mealybug, *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae), is a key pest of several agricultural crops, including cacao, pineapple, rice, coffee, soybean, groundnut, sugarcane, oil palm, and citrus (Waterhouse, 1998; Granara, 2009; Chávez, 2010). Mealybug colonies are commonly found in association with different ant species, such as *Iridomyrmex humilis* (Mayr) or *Solenopsis geminata* (Fabricius) (Hymenoptera: Formicidae) (Chávez, 2010). Several biological control programs targeting *D. brevipes* have remained unsuccessful, in part because of protection afforded to the mealybugs by a diversity of ants (Delabie & Fernández, 2003; González-Hernández et al., 2005; Stadler & Dixon, 2008). For example, in Hawaii (USA), the tight association of *D. brevipes* with *S. geminata* and three other ant species has reduced the efficacy of several natural enemies, such as cecidomyiid flies, cocinellid beetles, and multiple parasitoid species (González-Hernández et al., 1999; Blumberg, 2008).

As *D. brevipes*, *S. geminata* is equally well distributed throughout the neotropics, inhabiting agricultural systems and natural ecosystems alike (Perfecto, 1991). A generalist predator and 'keystone' species in certain environments, *S. geminata* shapes the entomofauna through predation upon a multitude of organisms (Perfecto, 1991). In addition to insect prey, *S. geminata* requires carbohydrates as a source of energy for the worker caste, which they obtain by tending hemipterans or harvesting (extra-)floral nectar (Perfecto, 1991; Tennant & Porter, 1991; González-Hernández et al., 2005; Sengupta et al., 2010). Carbohydrate feeding habits of ants such as *S. geminata* are determined by distance, quality, and quantity of the food

resource (Stadler & Dixon, 2008). Hence, the presence of other carbohydrate sources that are more accessible or of superior quality possibly compromise the level of protection provided to hemipterans (Cushman, 1991; Rico-Gray & Morais, 2006; Martinez et al., 2011).

Although it is well understood that honeydew shapes the interaction between *S. geminata* and *D. brevipes*, little is known about the strength and direction of this mutualism under conditions in which other high-quality sugar sources are present. In this study, we assess whether the degree of ant attendance is maintained when *S. geminata* is presented with a high-quality sugar source, in addition to *D. brevipes* honeydew. Under this condition, we also examine whether short-term protein deprivation of ant colonies affects their predation of mealybugs.

Materials and methods

Insect colonies

In March 2011, we collected an aggregation of *D. brevipes* from 121 soursop trees [*Annona muricata* L. (Annonaceae)] and an unidentified tree in the Loranthaceae family, at the research farm of Corporación Colombiana de Investigación Agropecuaria in Palmira, Colombia (CORPOICA; 3.514667°N, 76.316194°W). Mealybugs were transported to laboratories of the Centro Internacional de Agricultura Tropical (CIAT) and established on pumpkins, *Cucurbita maxima* Duchesne (Cucurbitaceae), within a screen cage. Every 2 days, mealybug aggregations were lightly misted using a handheld spray bottle with distilled water. On a biweekly basis, mealybugs were gently transferred to fresh pumpkins to ensure continuity of the aggregation. All pumpkins were obtained from a farm in Caloto, Colombia, and were washed with small amounts of water and soap prior to exposure to mealybugs, to minimize exposure of the mealybugs to any residual pesticides on the pumpkins.

Next, a total of 10 nests of *S. geminata* were excavated from a herbaceous plot at CIAT, in Palmira, Colombia (3.517°N, 76.333°W). Ant colonies were selected that were of similar size, although the exact number of ants within each nest was not assessed. Each ant nest was transferred to a 40 × 25 × 10-cm plastic container, together with surrounding soil and as many worker ants as possible. Petroleum jelly and mineral oil were applied to the upper part of the containers, to prevent ants from escaping. Sides of the plastic containers were covered with black paper to avoid disturbance of the inner structure of the nest, and simulate natural conditions. Each ant colony was provided unlimited access to a 10-cm-diameter Petri dish filled with dry granular cat food, two 25-ml cotton-covered vials with distilled water, and two 25-ml cotton-covered vials

with 20% sucrose (wt/vol) solution (using refined, granulated household sugar). Cat food, water, and sucrose solution were present continuously, and were renewed on a weekly basis. Also, three-fifth instar *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), with crushed headcapsules, were provided weekly as additional food. All ant colonies and mealybug aggregations were maintained at 30 °C, 61.6 ± 3.1% r.h., and L12:D12 photoperiod.

Manipulative assays

Twenty-four hours prior to initiation of the experiments, 20 adult female *D. brevipes* were (gently) brushed on each of 10 fresh pumpkins (12 cm diameter, 10 cm high). Simultaneously, all food resources were removed from *S. geminata* colonies, still allowing access to water. At the onset of the experiment, each *S. geminata* colony was connected by 2.5-cm-diameter tubes to two platforms: one platform containing a *D. brevipes*-infested pumpkin and another platform that either contained five vials filled with distilled water or with 20% sucrose solution. Sucrose has been employed in laboratory studies with a broad range of insect species (e.g., Wäckers, 2001; Luo et al., 2010; Narvaez et al., 2012), and is used as a proxy for naturally occurring sugars such as floral or extra-floral nectar. Also, we counted the mealybugs prior to the onset of the experiment, to ensure that 20 females had established on each pumpkin. Each treatment was established with five replicates, each consisting of a separate ant colony and a *D. brevipes*-infested pumpkin. Hourly for the next 7 h (for a total of nine hourly observation records), we recorded the number of ants foraging on the *D. brevipes* colony and the (water- or sucrose-filled) vials, with each observation lasting 30 s. Observations were started between 08:00 and 09:00 hours. At the end of the experiment, we determined the number of surviving mealybugs on each pumpkin and randomly collected seven worker ants from each colony. As petroleum jelly was applied to the walls of the platform to prevent escape of ants and mealybugs, we assumed that missing mealybugs were effectively removed and preyed upon by ants. Ants were individually placed in 1.5-ml microcentrifuge tubes and frozen at −20 °C, for future processing. Also, to estimate the level of honeydew production by *D. brevipes*, we washed each pumpkin at the end of the experiment with 15 ml of 96% ethanol. The ethanol solution was left to evaporate and was stored at −20 °C for further biochemical analysis. At the end of the experiment, each ant colony was disconnected from its respective platforms and all food resources were re-introduced. The experimental cycle was replicated six times at 48-h intervals (hereafter, these are referred to as bouts). Thus, a total of 48 observations of ant foraging per colony were carried out.

Treatments were randomly assigned to separate ant colonies at any given cycle, and new sets of mealybugs were used for each bout.

Biochemical analyses

Using the frozen ant specimens and ethanol washes of *D. brevipes*-infested pumpkins, we determined sugar content following anthrone assays as defined by Van Handel (1985) and modified by Seagraves et al. (2011). To avoid eventual external contamination of ants with sugar, we washed all specimens with distilled water prior to laboratory processing. Next, each specimen was macerated in 20 μl Ringer solution (0.75 g $NaCl_2$, 0.35 g KCl, 0.28 g $CaCl_2$ in 1 l H_2O), using a sterile plastic pestle. To the sample, we added 450 μl of methanol-chloroform (2:1) and spun the solution for 4 min at 16 100 g in a microcentrifuge. The resulting supernatant was divided between two microcentrifuge tubes for future quantification of fructose (cold anthrone trial) and total sugars (hot anthrone trial). First, samples were heated to 90 °C and evaporated to a volume of 50 μl; 975 μl of anthrone reagent was then added. For the cold anthrone analysis, the subsample was incubated during 1.5 h at 34 °C, whereas for hot anthrone analyses, samples were heated during 15 min at 90 °C. Next, 200 μl from each sample were pipetted into a sterile ELISA plate, for reading at a wavelength of 625 nm in a multi-modal Synergy HT photospectrometer (BioTek, Winooksi, VT, USA). As a negative control for anthrone analysis, we collected a total of 52 ants from our experimental colonies and starved those for 24 h. Five control samples were included per ELISA plate, and their mean absorbance was used as a reference value. Next, we took 10 subsamples of individual starved ants, calculated three times the standard deviation in the absorbances on this single sample, thus obtaining a threshold adjustment. Samples with an absorbance higher than the sum of the reference value and the threshold adjustment were considered positive for fructose or total sugars.

Data analysis

Five colonies were assigned to each of the two treatments. These treatments were observed on six observation bouts. For each bout, ant attendance was recorded hourly for 8 h. The colonies were alternated between treatments on each observation bout to reduce colony-level effects on the experimental outcomes. Note that sugar data were collected from the pumpkins only for the second to fifth observation bout, which reduced the sample sizes for the analyses involving this parameter. The ratio (log-transformed) of ants attending *D. brevipes* mealybugs vs. the alternative food source (water or sucrose, depend-

ing on the treatment) was calculated hourly for 8 h for each colony over six observation bouts (the colony was the experimental unit). Certain sets of data were log-transformed to meet assumptions of normality and homoscedasticity.

A General Linear Model (GLM) was used to simultaneously examine the effects of treatment, hour of observation (Time), and observation bout on the ratio of ants tending the mealybugs vs. the alternative food, as well as interactions among these independent variables. For each experimental bout, t-tests on ant attendance ratios per treatment were run to help explain a significant interaction between experimental bout and treatment. Variable interactions that were non-significant ($\alpha = 0.10$) were omitted from the final model, which ultimately included the individual variables and a bout*treatment interaction term. One-sample t-tests were used to evaluate whether the average ratios (averaged across bouts and hours) observed in the sucrose and water treatments were significantly different from 1 (no preference).

Independent GLMs were used to simultaneously examine the effects of treatment and observation bout on the number of mealybugs killed by the ants, the total sugars detected on the pumpkins (absorbance), the fructose detected on the pumpkins (absorbance), the frequency of detection of total sugars in worker ants (arcsine-root transformed), and the frequency of detection of fructose in worker ants (arcsine-root transformed). Variable interactions that were non-significant ($\alpha = 0.10$) were omitted from the final model, which ultimately excluded all interaction terms in these models. For significant parameters in these models, univariate ANOVAs were used alongside least significant differences among means to distinguish within-parameter variations. All statistics were conducted using SYSTAT 11 (Systat Software, Chicago, IL, USA).

Results

Ant attendance

A total of 6 979 ants were observed foraging on water vials, sucrose solution, or mealybugs. When allowed access to mealybugs and sucrose solution, 20.2% (n = 689) of the ants tended mealybugs, whereas 79.8% (n = 2 668) were associated with the sucrose solution. When only allowed access to mealybugs and water, 70.8% (n = 2 556) of ants were associated with mealybugs and 29.2% (n = 1 056) foraged on water vials.

An ant attendance ratio, calculated on an hourly basis, reflected the number of ants attending *D. brevipes* mealybugs vs. the alternative food source (water or sucrose, depending on the treatment). Mean ($\pm$ SEM) ratios per colony were 1.39 $\pm$ 0.45 and 3.25 $\pm$ 0.89 for the sucrose-

and water–mealybug treatments, respectively. One-sample t-tests revealed no preference of ants for sucrose or mealybugs (t = 0.94, d.f. = 9, P = 0.37), but a strong preference for the mealybugs in the water–mealybug comparison (t = 2.64, d.f. = 9, P = 0.03). Specifically, ant attendance was skewed toward the mealybugs on the first observation bout in the experiment, especially in the water–mealybug comparison (t = −2.73, d.f. = 8, P = 0.03) (Figure 1). None of the remaining experimental bouts had this discrepancy between treatments (P>0.06 for bouts 2–6). Preference was about equal for water and the sucrose solution in the sucrose–mealybug comparison (Figure 1). The ratio quickly diminished in the water–mealybug trials thereafter, leading to a significant interaction between treatment and experimental bout (Table 1, Figure 1). Ant attendance was affected by treatment and the bout of observation (Table 1). Ant attendance ratios were similar across hours of observation (Time) (Table 1).

Mealybug survival and honeydew production

There was no effect of treatment or bout of observation on the number of mealybugs consumed over the 8-h observation period (Table 2). Mean ($\pm$ SEM) numbers of mealybugs surviving (pooled across sample days) were 10.63 $\pm$ 1.23 and 12.93 $\pm$ 1.07 in the sucrose- and water–mealybug treatments, respectively.

There was no effect of bout of observation or treatment on the amount of total sugars produced by the mealybugs (Table 3). The amount of fructose produced by mealybugs
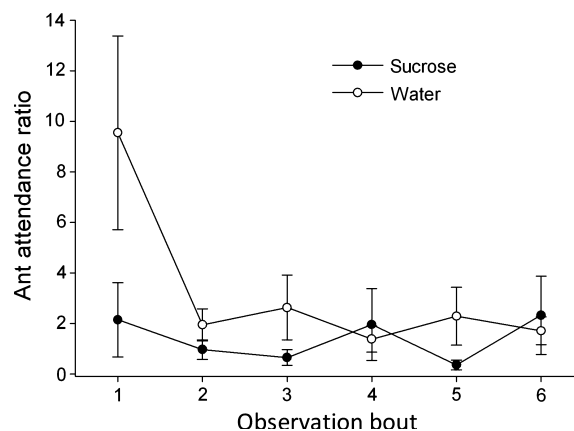

Figure 1 *Solenopsis geminata* mean ($\pm$ SEM; n = 10) attendance ratios per *Dysmicoccus brevipes* colony over the duration of the experiment, when the ants were offered a sucrose or water solution. Observation bout refers to replicate observation day, each conducted independently from one another; data for this figure are pooled across the nine hourly observation records conducted for each observation bout. Ratios of less than one preferred to attend the alternative food relative to the mealybugs.

Table 1 Statistical analysis comparing experimental parameters on the ratio of *Solenopsis geminata* visiting *Dysmicoccus brevipes* vs. alternative food (water or sucrose). Five colonies were monitored in each treatment on each observation day. Specific parameters were observation day (bout; 1–6), hour of observation (time; 1–8), and treatment (water vs. sucrose). Interaction terms in which $P>0.10$ were omitted from the final model

Variable	d.f.	F	P
Bout	5	17.59	<0.001
Time	7	0.19	0.99
Treatment	1	109.19	<0.001
Bout*treatment	5	3.27	0.007
Error	461		

Table 2 Statistical analysis comparing experimental parameters on the number of *Dysmicoccus brevipes* removed by *Solenopsis geminata* when the ants had access to water or sucrose. Five colonies were monitored in each treatment on each observation day. Specific parameters were observation day (bout; 1–6) and treatment (water vs. sucrose). Interaction terms in which $P>0.10$ were omitted from the final model

Variable	d.f.	F	P
Bout	5	0.99	0.43
Treatment	1	2.24	0.14
Error	53		

Table 3 The effects of experimental variables on the quantity of sugars produced by *Dysmicoccus brevipes* tended by *Solenopsis geminata* when the ants had access to water or sucrose. Five colonies were monitored in each treatment on each observation day. Specific parameters included in the model were observation bout (2–5), colony (1–10), and treatment (water vs. sucrose). Hot anthrone detected total sugars, and cold anthrone is specific for fructose. Interaction terms in which $P>0.10$ were omitted from the final model

Variable	d.f.	Hot anthrone		Cold anthrone	
		F	P	F	P
Bout	3	0.33	0.80	3.96	0.02
Treatment	1	0.47	0.50	0.53	0.47
Error	34				

was unaffected by treatment, although there was a significant effect of bout of observation on the quantity produced (as measured by cold anthrone) (Table 3). More fructose was present on the pumpkins as the experiment went on (Figure 2).
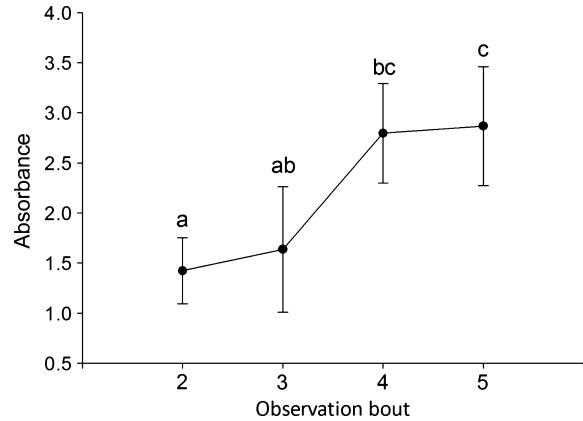

Figure 2 Detectable fructose produced by *Dysmicoccus brevipes* on pumpkins over five exposure bouts of the experiment. Fructose quantities represent honeydew produced and are indirectly presented as mean ($\pm$ SEM; n = 10) absorbance per colony and bout, measured by the cold anthrone test, pooled across treatments. Bouts topped by different letters were significantly different (LSD test: $P<0.05$).

Biochemical analysis of deposited and ingested sugars

Availability of sucrose had an effect on the amount of total sugars and fructose detectable within worker ants (Table 4). Ants with access to sucrose consistently had greater total sugars and fructose proportions than ants offered water alongside *D. brevipes* (Figure 3).

Discussion

The availability of local sugar resources affects the degree to which ants tend mealybugs. In our experiment, this behavioral shift was evident in both the relative degree of ant attendance on the mealybugs in the presence of sucrose or water, and the different nutrient status of the ants with access to sucrose and mealybugs vs. mealybugs and water. In our assays, the degree of ant attendance varied over the duration of the experiment in the water–mealybug comparison. Even though the ants were less attendant on mealybugs when sucrose was available, the ants did not kill the mealybugs when additional sugar sources could be accessed. These results may have positive implications for managing *D. brevipes* populations using biological control. More specifically, lower degrees of ant attendance could create a window of opportunity for other *D. brevipes* natural enemies, such as predators or parasitic wasps.

Ants were more likely to attend mealybugs when honeydew was their sole source of sugar, but their dependence on honeydew diminished over the duration of the experiment and was most evident when other sugar sources were absent (Figure 1). In the mealybug–water treatment, ants

Table 4 The effects of experimental variables on the cumulative quantity of sugars detectable in *Solenopsis geminata* workers when the ants had access to water or sucrose in addition to *Dysmicoccus brevipes*. Five colonies were monitored in each treatment on each observation day. Specific parameters included in the model were observation day (bout; 1–6) and treatment (water vs. sucrose). Hot anthrone detected total sugars, and cold anthrone is specific for fructose. Interaction terms in which P>0.10 were omitted from the final model

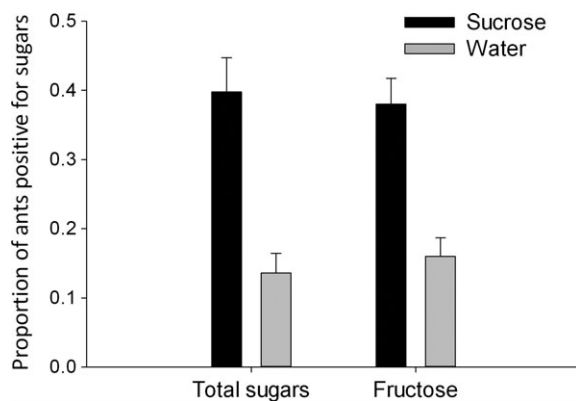| Variable	d.f.	Hot anthrone		Cold anthrone	
		F	P	F	P
Bout	4	2.25	0.08	1.55	0.21
Treatment	1	16.43	<0.001	11.58	<0.001
Error	44				

Figure 3 Sugar contents of *Solenopsis geminata* workers with access to either sucrose or water alongside *Dysmicoccus brevipes*. Bars represent mean (+ SEM) frequencies per colony (n = 10), pooled over experimental days. Total sugars were measured with the hot anthrone assay, and fructose was measured with the cold anthrone assay. Significantly more total sugars and fructose were found in the treatment fed sucrose vs. water (LSD test: P<0.05).

visited the mealybugs 10 times more frequently than the water vials in the first observation bout. This ratio dropped to approximately twice as many visits to the mealybugs vs. the water container for the subsequent five observation bouts (Figure 1), indicating that the mealybugs were less attractive to the ants after their initial exposure. Although there were slightly more ants visiting the mealybugs than the sucrose solution (mean ratio of 1.39:1), this relationship was not significantly different from 1:1, and this ratio was fairly consistent over the six observation bouts (Figure 1). Although ants from both treatments contained sugars and fructose, there was more than twice the amount of sugar-positive ants collected from the sucrose treatment than the water treatment (Figure 3). The observation that

fructose from honeydew was present on the pumpkin throughout the experiment indicates that sugars were not limited in the mealybug–water treatment (Figure 2). In fact, fructose on the pumpkins increased significantly as the experiment progressed (Figure 2), which is in line with the hypothesis that ant consumption of honeydew diminished over the duration of the experiment.

In our assays, we used fructose as an easily quantifiable proxy of honeydew sugar deposited on pumpkins. Although we acknowledge that melezitose is the dominant sugar in honeydew, fructose is equally present in honeydew secreted by several hemipterans (Völkl et al., 1999; Yao & Akimoto, 2001; Detrain et al., 2010). The detection of considerable amounts of fructose on pumpkins colonized by mealybugs showed that this approach effectively indicated honeydew deposition by *D. brevipes*.

We hypothesize that certain aspects of the honeydew of *D. brevipes* on pumpkin fruit were either unattractive or suboptimal for foraging ants. The composition of hemipteran honeydew is greatly affected by host plant and level of ant attendance (Fischer & Shingleton, 2001). Hence, *D. brevipes* honeydew could be more or less nutritionally attractive to *S. geminata*, depending upon its host plant. However, several factors could explain the experimental results involving the initial strong response of *S. geminata* to *D. brevipes* and subsequent reduction in ant tending, the lack of preference for sucrose vs. honeydew, and the higher sugar content in ants fed sucrose rather than honeydew alone. The intensity of mutualism between ants and hemipterans is tightly linked to the level of honeydew production and its nutritional quality (Schumacher & Platner, 2009). Honeydew contains primarily mono-, and oligosaccharides, but also small quantities of lipids, amino acids, and proteins (Lundgren, 2009). Sugars such as sucrose, fructose, glucose, and melezitose all provoke strong recruitment of worker ants (Detrain et al., 2010). But some hemipterans use trisaccharides to make their honeydew less attractive to predatory insects that may harm the honeydew producer (Wäckers, 2000; Hogervorst et al., 2007). Also, honeydew nutrition changes along with the age and status of the hemipterans that produce it (Auclair, 1963; Costa et al., 1999), and the aging mealybug colonies may have altered their honeydew composition over time. Our results echo those of Schumacher & Platner (2009), who found that *Lasius niger* (L.) abandoned aphids when they were provided a choice between a 50% sugarcane juice or *Aphis fabae* Scopoli honeydew.

Although we hypothesize that the honeydew of *D. brevipes* was less abundant, less accessible, and suboptimal to the sucrose solution, we recognize that honeydew was still attractive to the ants (i.e., the ants did not abandon the mealybug colony). Upon discovery of new food sources,

ants may gradually alter their foraging decisions to better accommodate the eventual disappearance and relative quality of a newly discovered food (Rico-Gray & Morais, 2006; Stadler & Dixon, 2008; Morgan, 2009; Kaminski et al., 2010). This so-called 'fidelity' may partially explain the sustained tending of *D. brevipes* in our assays. On the other hand, this 'fidelity' may explain sustained visitation of sucrose vials, as all ant colonies were provided sucrose during times when no experiments were conducted. Also, honeydew may have provided nutrients not present in the sucrose solution. Follow-up research should determine whether mealybug attendance can further be reduced through provision of an external food source that provides other nutrients in addition to sucrose. Long-term studies should also be carried out, to determine whether the shift in foraging patterns (i.e., honeydew and sucrose consumption, mealybug predation) is more pronounced over longer periods of time.

Even though the ants were less likely to attend mealybugs when sucrose was provided, this lack of attendance did not increase predation on the mealybugs. In ant colonies, access to sucrose can rapidly increase overall activity patterns, foraging levels, and aggressiveness (Stadler & Dixon, 2008; Mgocheki & Addison, 2009; Ness et al., 2009). Ants regulate the intake of carbohydrate, fats, and protein sources depending upon the needs of the colony, and we expect that excess consumption of sugar would accompany increased intake of protein (Ness et al., 2009), which was absent when external sugar sources were provided in our assay. Also, sugars are probably infrequently stored by worker ants, which maintains space for other key nutrients, such as lipids and proteins (Trager, 1991). Under conditions of high protein needs of the ant colony and simultaneous availability of low-quality honeydew or excess (alternative) sugar resources, ants sometimes eat hemipterans (Way, 1954; Cushman, 1991). In our study, mealybug survival did not differ between treatments. This could either signal that protein requirements of the *S. geminata* colony were not as high to warrant *D. brevipes* predation (not even under increased sucrose availability), that the (short-term) protein deprivation did not cause major effects on the protein needs of the *S. geminata* colonies, or that mealybugs still provided essential nutrients for the ant colony. However, we note that *D. brevipes* survival over the 8-h observation period was only around 50–60% and that ants were found to carry off mealybug adults. Thus, perhaps the ants were harvesting a proportion of the mealybugs as protein sources regardless of whether a non-honeydew sugar source was provided. The fate of the mealybugs removed in this study remains to be determined, although they were likely already dead or killed by the ants.

Protein needs, and subsequent mealybug predation, directly relate to the size of the ant colony, the presence of a queen, and large quantities of larvae (e.g., Porter, 1989; Portha et al., 2002). Colonies without brood generally show little interest in solid foods, whereas the presence of brood triggers more intense foraging for proteinaceous foods (Portha et al., 2002). In our assays, ant colonies were collected in their entirety, with supposed presence of resident queens and brood. In case the queen died during laboratory establishment of ant colonies, larvae likely matured within 10–15 days and protein needs of the colony could have dropped sharply thereafter (Porter, 1989). A destructive assessment of the size and composition of each colony, at the end of the experiment, undoubtedly would have benefited an interpretation of our findings.

Our work indicates that external sugar sources diminish the level of ant tending of *D. brevipes* by *S. geminata*, and although sugar sources did not increase predation on the mealybugs directly by the ants, diminished attendance could affect mealybug predation rates regardless. As we expect ant-provided protection services to equally diminish under these conditions, we expect that this could create a window of opportunity for the large diversity of natural enemies associated with mealybug species (Blumberg, 2008), and potentially contribute to more effective biological control. In agro-ecosystems, sugar supplementation can be done through provision of floral or extrafloral resources, or sugar applications (e.g., Canas & O'Neil, 1998; Bianchi & Wäckers, 2008). Research that assesses the effect of such external supplementation of sucrose on protection services provided by ants against mealybug natural enemies, and resulting mealybug population dynamics, is warranted. Lastly, this study provides insights in ants' dependence on sugar sources, which could be employed to guide research efforts to conserve predatory ants in a range of (simplified) agro-ecosystems, where they commonly experience suboptimal access to carbohydrate sources.

References

Auclair JL (1963) Aphid feeding and nutrition. Annual Review of Entomology 8: 439–490.

Beattie AJ (1985) The Evolutionary Ecology of Ant-Plant Mutualisms. Cambridge University Press, New York, NY, USA.

Bianchi FJJA & Wäckers FL (2008) Effects of flower attractiveness and nectar availability in field margins on biological control by parasitoids. Biological Control 46: 400–408.

Blumberg D (2008) Review: date palm arthropod pests and their management in Israel. Phytoparasitica 36: 411–448.

Canas LA & O'Neil RJ (1998) Applications of sugar solutions to maize, and the impact of natural enemies on fall armyworm. International Journal of Pest Management 44: 59–64.

Chávez AJ (2010) Biología y Morfología de *Dysmicoccus brevipes* (Cockerell, 1893) (Hemiptera: Pseudococcidae). MSc Thesis, Universidad Nacional Agraria La Molina, Lima, Peru.

Costa HS, Toscano NC, Hendrix DL & Henneberry TJ (1999) Patterns of honeydew droplet production by nymphal stages of *Bemisia argentifolii* (Homoptera: Aleyrodidae) and relative composition of honeydew sugars. Journal of Entomological Science 34: 305–313.

Cudjoe AR, Neuenschwander P & Copland MJW (1993) Interference by ants in biological control of the cassava mealybug *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) in Ghana. Bulletin of Entomological Research 83: 15–22.

Cushman JH (1991) Host-plant mediation of insect mutualisms: variable outcomes in herbivore-ant interactions. Oikos 61: 138–144.

Delabie JHC & Fernández F (2003) Relaciones entre hormigas y «Homópteros» (Hemiptera: Sternorrhyncha y Auchenorrhyncha). Introducción a las Hormigas de la Región Neotropical (ed. by F Fernández), pp. 181–197. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá, Colombia.

Detrain C, Verheggen FJ, Diez L, Wathelet B & Haubruge E (2010) Aphid–ant mutualism: how honeydew sugars influence the behaviour of ant scouts. Physiological Entomology 35: 168–174.

Fischer MK & Shingleton AW (2001) Host plant and ants influence the honeydew sugar composition of aphids. Functional Ecology 15: 544–550.

Franco A, Cárdenas R, Montoya EC & Zenner I (2003) Hormigas asociadas con insectos chupadores en la parte aérea del cafeto. Revista Colombiana de Entomologia 29: 95–105.

Franco JC, Zada A & Mendel Z (2009) Novel approaches for the management of mealybug pests. Biorational Control of Arthropod Pests, Application and Resistance Management (ed. by I Ishaaya & AR Horowitz), pp. 233–278. Springer, New York, NY, USA.

González-Hernández H, Johnson MW & Reimer NJ (1999) Impact of *Pheidole megacephala* (F.) (Hymenoptera: Formicidae) on the biological control of *Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae). Biological Control 15: 145–152.

González-Hernández H, Pandey RR & Johnson MW (2005) Biological characteristics of adult *Anagyrus ananatis* (Gahan) (Hymenoptera: Encyrtidae), a parasitoid of *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae). Biological Control 35: 93–103.

Granara MC (2009) *Dysmicoccus* de la región neotropical (Hemiptera: Pseudococcidae). Revista de la Sociedad de Entomologia de Argentina 68: 11–95.

Hogervorst PAM, Wäckers FL & Romeis J (2007) Effects of honeydew sugar composition on the longevity of *Aphidius ervi*. Entomologia Experimentalis et Applicata 122: 223–232.

Kaminski LA, Freitas AV & Oliveira PS (2010) Interaction between mutualisms: ant-tended butterflies exploit enemy-free space provided by ant-treehopper associations. American Naturalist 176: 322–334.

Kondo T (2001) Las cochillas de Colombia (Hemiptera: Coccoidea). Biota Colombiana 2: 31–48.

Lambdin P (2008) Scale insects and mealybugs (Hemiptera: Coccoidea). Encyclopedia of Entomology (2nd edn.) (ed. by JL Capinera), pp. 3263–3272. Springer, Dordrecht, The Netherlands.

Lundgren JG (2009) Relationships of Natural Enemies and Non-Prey Foods. Springer, Dordrecht, The Netherlands.

Luo SP, Li JC, Liu XX, Lu ZY, Pan WL et al. (2010) Effects of six sugars on the longevity, fecundity and nutrient reserves of *Microplitis mediator*. Biological Control 52: 51–57.

Martinez JJI, Cohen M & Mgocheki N (2011) The response of an aphid tending ant to artificial extra-floral nectaries on different host plants. Arthropod-Plant Interactions 5: 185–192.

McPhee K, Garnas J, Drummond F & Groden E (2012) Homopterans and the invasive European red ant, *Myrmica rubra* (L.). Environmental Entomology 41: 59–71.

Mera YA, Gallego MC & Armbrecht I (2010) Interacciones entre hormigas e insectos en follaje de cafetales de sol y sombra, Cauca-Colombia. Revista Colombiana de Entomologia 36: 116–126.

Mgocheki N & Addison P (2009) Interference of ants (Hymenoptera: Formicidae) with biological control of the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). Biological Control 49: 180–185.

Morales MA (2000) Survivorship of an ant-tended membracid as a function of ant recruitment. Oikos 90: 469–476.

Morgan DE (2009) Trail pheromones of ants. Physiological Entomology 34: 1–17.

Narvaez A, Cancino J, Canal Daza N & Wyckhuys KAG (2012) Effect of different dietary resources on longevity, carbohydrate metabolism, and ovarian dynamics in two fruit fly parasitoids. Arthropod-Plant Interactions 6: 361–374.

Ness JH, Morris WF & Bronstein JL (2009) For ant-protected plants, the best defense is a hungry offense. Ecology 90: 2823–2831.

Nielsen C, Agrawal AA & Hajek AE (2010) Ants defend aphids against lethal disease. Biology Letters 6: 205–208.

Perfecto I (1991) Dynamics of *Solenopsis geminata* in a tropical fallow field after ploughing. Oikos 62: 139–144.

Perotto MC, Buffa LM & Delfino MA (2002) Efectos de la atención de *Camponotus rufipes* (Fabricius) (Hymenoptera,

Formicidae) sobre agregaciones de *Enchenopa sericea* Walk. (Hemiptera, Membracidae). Ecologia Austral 12: 91–97.

Porter SD (1989) Effects of diet on the growth of laboratory fire ant colonies (Hymenoptera: Formicidae). Journal of the Kansas Entomological Society 62: 288–291.

Portha S, Deneubourg JL & Detrain C (2002) Self-organized asymmetries in ant foraging: a functional response to food type and colony needs. Behavioral Ecology 13: 776–781.

Ramírez M, Chacón de Ulloa P, Armbrecht I & Calle Z (2001) Contribución al conocimiento de las interacciones entre plantas, hormigas y homópteros en bosques secos de Colombia. Caldasia 23: 523–536.

Rico-Gray V & Morais HC (2006) Efecto de una fuente de alimento experimental sobre una asociación hormiga hemiptero. Acta Zoologica Mexicana 22: 23–28.

Schumacher E & Platner C (2009) Nutrient dynamics in a tritrophic system of ants, aphids and beans. Journal of Applied Entomology 133: 33–46.

Seagraves MP, Kajita Y, Weber DC, Obrycki JJ & Lundgren JG (2011) Sugar feeding by coccinellids under field conditions: the effects of sugar sprays in soybean. BioControl 56: 305–314.

Sengupta P, Ghorai N & Mukhopadhyay S (2010) Food preference and foraging of fire ant *Solenopsis nitens*. Proceedings of the Zoological Society 63: 73–77.

Stadler B & Dixon AFG (2008) Mutualism: Ants and their Insect Partners. Cambridge University Press, New York, NY, USA.

Styrsky JD & Eubanks MD (2007) Ecological consequences of interactions between ants and honeydew-producing insects. Proceedings of the Royal Society of London B 274: 151–164.

Tena A, Beltra A & Soto A (2012) Novel defenses of *Protopulvinaria pyriformis* (Hemiptera: Coccidea) against its major parasitoid *Metaphycus helvolus* (Hymenoptera: Encyrtidae):

implications for biological control of soft scales. Biological Control 62: 45–52.

Tennant LE & Porter SD (1991) Comparison of diets of two fire ant species (Hymenoptera: Formicidae): solid and liquid components. Journal of Entomological Science 26: 450–465.

Trager JC (1991) A revision of the fire ants, *Solenopsis geminata* group (Hymenoptera: Formicidae: Myrmicinae). Journal of the New York Entomological Society 99: 141–198.

Van Handel E (1985) Rapid determination of glycogen and sugars in mosquitoes. Journal of the American Mosquito Control Association 1: 299–301.

Völkl W, Woodring J, Fischer M, Lorenz MW & Hoffmann KH (1999) Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. Oecologia 118: 483–491.

Wäckers FL (2000) Do oligosaccharides reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect-synthesized honeydew sugars. Oikos 90: 197–201.

Wäckers FL (2001) A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid, *Cotesia glomerata*. Journal of Insect Physiology 47: 1077–1084.

Waterhouse DF (1998) Biological Control of Insect Pests: Southeast Asian Prospects. ACIAR, Canberra, Australia.

Way MJ (1954) Studies on the association of the ant *Oecophylla longinoda* (Latr.) (Formicidae) with the scale insect *Saissetia zanzibarensis* Williams (Coccidae). Bulletin of Entomological Research 45: 113–134.

Yao I & Akimoto SI (2001) Ant attendance changes the sugar composition of the honeydew of the drepanosiphid aphid *Tuberculatus quercicola*. Oecologia 128: 36–43.