ANT TENDING IMPAIRS PERFORMANCE OF AENASIASIS BAMBAWALEI BY MANIPULATING THE HONEYDEW COMPOSITION PRODUCED BY PHENACOCCUS SOLENOPTIS

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Abstract

Honeydew produced by hemipterans is known as a possible kairomonal resource for parasitoids. The application of artificial honeydew effectively improves the performance of natural enemies. Aenasius bambawalei is a particularly dominant and aggressive endoparasitoid of the invasive mealybug Phenacoccus solenopsis. Our previous study showed that tending by the ghost ant Tapinoma melanocephalum significantly reduced the parasitism of A. bambawalei. We hypothesize that ghost ant tending influences host location of parasitoids by manipulating the composition of the honeydew produced by mealybugs. In this study, we tested whether the honeydew composition differs between treatments with and without ant attendance and whether changes in the honeydew influence the performance of A. bambawalei. Our results show that the sucrose concentration increased significantly in the ant-attendance treatment but decreased when ant attendance was switched to an ant-exclusion treatment; the inverse was true for the glucose concentration. Compared with the plastic honeydew treatment (mealybug with ant attendance), parasitoids spent much more time searching, had longer lifespans and showed higher parasitism on filter papers treated with natural honeydew (mealybug without any pre-treatment) and those treated with convalescent honeydew (mealybug having experienced ant attendance and then switched to ant exclusion). These results support the hypothesis that ant tending influences the performance of parasitoids by manipulating honeydew composition.

Introduction

Mutualism, a common and important ecological phenomenon characterized by beneficial interactions between two species (Bronstein 1994; Begon et al. 1996; Stachowicz 2001), between ants and hemipterans, has been widely recognized in multiple ecosystems (Helms and Vinson 2002; Simberloff 2006; Brightwell and Silverman 2010). In such relationships, ants feed on large amounts of honeydew excreted by hemipterans (Stachowicz 2001; Davidson et al. 2004; Stadler and Dixon 2005); in exchange, the ants protect the hemipterans from predators and parasitoids and reduce the risk of fungal infection (Way 1963; Stadler and Dixon 1998; Helms and Vinson 2003; Daane et al. 2007).

Hemipterans clearly benefit from ant tending through protection from enemies. For example, Argentine ants (Linepithema humile) are frequently associated with an obscure mealybug, Pseudococcus viburni, and they lower the densities of its encyrtid parasitoids Pseudaphycus flavidulus and Leptomastix epona (Daane et al. 2007). Tapinoma sessile was reported to be effective at protecting Aphis gossypii from its natural enemy parasitoids.
enemies *Aphidius colemani* (Powell and Silverman 2010). Kaplan and Eubanks (2002) also demonstrated that *S. invicta* suppressed both *Chrysoperla carnea* and *Hippodamia convergens* larval predation of *Aphis gossypii* in greenhouse experiments. Our previous study also showed that the ghost ant *Tapinoma melanocephalum* negatively affected the performance of the mealybug parasitoid *Aenasius bambawalei*: the number of mummified mealybugs per plant in the presence of ghost ants was significantly less than the number without ants (Zhou et al. 2014). Although it is widely accepted that ant tending reduces the risk of predation and parasitism for hemipterans, the mechanism underlying this effect remains relatively poorly studied. Many studies have demonstrated that direct killing or interference by ants is an important mechanism by which ants negatively affect predators (Jiggins et al. 1993; Sloggett and Majerus 2003; Oliver et al. 2008; Zhou et al. 2013, 2014). However, for parasitoids, studies demonstrating the mechanism by which ants influence parasitoid performance remain insufficient.

*Aenasius bambawalei* Hayat is a solitary endoparasitoid of the invasive mealybug *Phenacoccus solenopsis* (Hayat 2009; Fand et al. 2011; Suroshe et al. 2013) and is the most dominant and aggressive parasitoid reported to date (Kumar et al. 2009; Suroshe et al. 2014). *A. bambawalei* is also reported as an important enemy of the *P. solenopsis* in China (Chen et al. 2011). Our previous work indicated that the invasive ant *Solenopsis invicta* frequently protects *P. solenopsis* from *A. bambawalei* and that parasitism of *A. bambawalei* was significantly decreased on ant-tended plants (Zhou et al. 2012, 2013). Although *A. bambawalei* showed strong avoidance responses to ants that tended mealybugs (Zhou et al. 2014), no clear negative effect was observed on the survival of *A. bambawalei* (Zhou et al. 2013). Hübner and Völk (1996) reported that aphid hyperparasitoids jump from their position when ants make physical contact; indeed, the excellent jumping ability of hyperparasitoids prevents their exposure to certain dangers. These results may suggest that the significant decrease in parasitism by parasitoids is unlikely to be caused by direct kill by ants.

When searching for hosts, parasitoids can increase their search efficiency if they concentrate their efforts at sites that are most likely to harbour their hosts (Zaborski et al. 1987). Shaltiel and Ayal (1998) reported that kairomones act as crucial factors in host location, and many studies have demonstrated that honeydew can act as a kairomonal resource for parasitoids (Shimron et al. 1992; Romeis and Zebitz 1997). Furthermore, the application of artificial honeydew effectively contributes to retaining natural enemies in the target areas and increases the percentage of parasitism or predation (Jacob and Evans 1998; Lewis et al. 1998; Mandour et al. 2006). In ant-hemipteran mutualism, honeydew is essential for ant colony growth and survival because it contains sugar mixed with various amino acids (Yao and Akimoto 2002; Helms and Vinson 2008). Moreover, honeydew and its single sugars produced by hemipterans as crucial signals or kairomones play an important role in host location for parasitoids (Budenberg 1990; Mandour et al. 2003; Leroy et al. 2011, 2012, 2014). For example, *Encarsia bimaculata* uses honeydew produced by *Bemisia tabaci*, as well as nymphs, exuviae or dead adults, as kairomonal sources in host location (Mandour et al. 2003).

A growing body of research has demonstrated that ant tending has significant effects on the honeydew composition produced by aphids, for pairs including *Lasius niger* and *Brachycnidaus cardui* (Vökl et al. 1999), *Formica yessensis* and *Tuberculatus quercicola* (Yao and Akimoto 2001), *Lasius niger* and *Chaitophorus populnei* (Fischer and Shingleton 2001), *Lasius niger* and *Aphis fabae* (Woodring et al. 2004), and *Solenopsis invicta* and *Phenacoccus solenopsis* (Zhou et al. 2015). However, whether tending by the ghost ant *Tapinoma melanocephalum* can alter the honeydew composition produced by the invasive mealybug *P. solenopsis* remains unknown, as is whether such altered honeydew can influence host location by *A. bambawalei*. In this study, we conducted laboratory experiments to test the following questions: (i) Is there plasticity in the composition of honeydew produced by mealybugs with and without tending by ghost ants? (ii) Does the changed honeydew influence the searching behaviour, longevity and parasitism of *A. bambawalei*?

**Materials and Methods**

**Plants and insects**

All of the *Solanum tuberosum* plants were grown in defined soil from the horticultural farm. Each plant was approximately 30–35 cm in height, contained 10–15 true leaves and was cultivated in a plastic flowerpot (with upper and lower diameters of 14 cm and 10 cm, respectively, and a height of 15 cm). The *P. solenopsis* colony was collected in the field from cotton plants. A total of 60 to 80 individuals from the colony were transferred to the potted *S. tuberosum* plants and reared in a climate-controlled chamber. Newly established sizable polygyne colonies of *T. melanocephalum* were collected from a suburb of Guangzhou.
The parasitoid *Aenasius bambawalei* was collected from cotton plants in the field. *A. bambawalei* arrived as mummified mealybugs (parasitized by *A. bambawalei*), which were separated into gel capsules (10 mm in length) until adults emerged. After emergence, the wasps were sexed and randomly paired. Copulation was observed in all pairings, and fertilized female wasps were used in subsequent experiments 24 h after the initial pairing. All ghost ant colonies were separated from the soil by dripping water into the plastic boxes until the colonies floated (Jouvenaz et al. 1977). The ants were then removed and reared in plastic boxes with tubes filled with distilled water. The colonies were subsequently divided into several small colonies (approximately 1.0 g each) using a microbalance (Sartorius BSA 224S). Each small colony included one queen and several adult workers (1 g, approximately 3000 individuals). These colonies were placed in 9-cm plastic Petri dishes as an artificial nest (Zhou et al. 2015). Ant colonies were supplied with fresh live worms (*Tenebrio molitor*) and a 10% solution of honey mixed with water (50 ml) weekly. All of the colonies of mealybugs and ghost ants were reared in the laboratory at 27°C, with 60%-70% relative humidity.

**Plasticity in honeydew sugar composition and concentration**

In this experiment, we tested the plasticity of honeydew sugar composition and concentration in response to changes in the ant tending condition. All potted *S. tuberosum* plants were reared in a greenhouse (27°C, relative humidity 75%, LD 16:8). To eliminate interference by other hemipterans, such as aphids and whitefly, all potted plants were covered with nylon netting and surrounded by a cage (80 × 80 × 110 cm). The potted plants were randomly arranged in our study. A total of 50 third-instar mealybugs were transferred to each caged plant. The honeydew produced by mealybugs was collected three times. At 24 h after the mealybugs were transferred, we collected honeydew for the first time. We collected honeydew directly from the anus of the mealybug using a microcapillary tube (0.5 μl). After this collection, an artificial nest (one queen and 1 g of workers) of *T. melanocephalum* was then connected to each potted plant via a plastic tube (1.5 cm in diameter) through which the ant workers can travel to forage for honeydew. This treatment is called an ant-attendance treatment and enabled worker ants to approach the mealybug colonies directly through the tubes. In the ant-attendance experiment, the ants were deprived of all carbohydrate sources for 24 h to ensure that they would show a strong demand for sugars. After rearing for 15 days, honeydew produced by the mealybugs was collected for the second time in the same manner as in the first collection. After this collection, each mealybug colony was subjected to a switch in treatment. After the ant-attendance treatment, ant visitation was prohibited. We removed the connecting plastic tube to ensure that there was no access to the mealybug colony for foraging ants. This treatment is called an ant-exclusion treatment. After rearing for a further 15 days, we collected the honeydew for the third time, in the same manner as in the first collection. New mealybugs were used for honeydew analysis in the three collections, because new generation of *P. solenopsis* have emerged when we proceeded the second and the third collection in our experimental condition. For honeydew collection, we used mealybugs fed on a defined developmental stage of *S. tuberosum* (stems and leaves were completely developed, and flowers had not yet opened). The mealybug density on the leaves was maintained at a constant level (25–30 individuals per plant). This experiment was repeated 8 times.

The obtained honeydew was analysed via high-performance liquid chromatography (HPLC). The sugar concentration and composition of the honeydew were measured in a column (TSK-NH2, 4.6 mm × 250 mm × 5 μm, Wako Pure Chemical, Osaka, Japan) with an apparatus using a differential refraction detector (Shimadzu Corp., Kyoto, Japan). Sample elution was isocratic, employing 75% acetonitrile (ACN) and a flow rate of 1 ml/min. The concentrations of seven sugars (xylose, glucose, fructose, sucrose, trehalose, melezitose and raffinose) were analysed through this method, and the retention time of each sugar was measured. The sugars in the honeydew were identified by comparing the retention times of the sample sugars with those of sugar standards. The actual concentrations of the sugars in the samples were estimated by comparing their peak areas with those of standard sugars of known concentrations.

**Searching response of parasitoids to mealybug honeydew**

To collect the fresh honeydew, third-instar mealybugs were transferred to potted *S. tuberosum* plants (30 individuals per plant) as in the first experiment, and honeydew produced by a total 2400 mealybugs for 24 h was collected. The mealybugs experienced the same treatment as in the first experiment. Fresh honeydew secreted by mealybugs was collected and placed in Petri dishes (7 cm diameter). Then, 5 ml of a 50%
ethanol solution was poured into each Petri dish and allowed to mix for 10 min. The resulting solution was then concentrated to 2 ml under 60°C. Filter paper (10 cm diameter) was placed in a clean Petri dish, and 400 µL of the concentrated honeydew solution was pipetted onto the centre of the paper. This was equivalent to the amounts excreted by 480 mealybugs over 24 h. After the ethanol had evaporated, a parasitoid was introduced at the centre of the paper. The time between the parasitoid’s initial contact with the centre to flying off was recorded. In this experiment, we used four types of solution to test the searching response of parasitoids: (i) natural honeydew only (honeydew produced by mealybugs which experienced no ant tending), (ii) plastic honeydew (the honeydew produced by mealybugs which tended by ghost ants), (iii) convalescent honeydew (the honeydew produced by mealybugs which were subjected to a switch in treatment, and ant visitation was prohibited) and (iv) double-distilled water only (blank control). Each treatment was repeated 15 times. The bioassays were conducted at 09:00 h at 28°C, 75% relative humidity and 16:8 LD.

Effect of mealybug honeydew on performance of parasitoids

To evaluate whether ant tending reduces parasitoids performance by manipulating mealybug honeydew composition, we determined the effects of honeydew composition on parasitoids survival and parasitism. First, we tested the effects of honeydew composition on A. bambawalei survival. Newly emerged females of A. bambawalei were collected and kept individually in glass tubes (10 cm X 2 cm), each of which contained 1 ml honeydew sugar solution and was sealed with a cotton plug. Each honeydew sugar solution included 30% (w/v) honeydew sugar, whether natural honeydew, plastic honeydew or convalescent honeydew; and double-distilled water (blank control). Honeydew solutions were tested as kairomones for A. bambawalei. Sugar solutions were renewed every 2 days. We checked each live parasitoid daily and recorded its survival. Each treatment was repeated 20 times. Second, the effects of honeydew composition on parasitism by parasitoids were also determined. A total of 80 third-instar mealybugs were transferred to each potted plant; plants were covered by nylon cages as in experiment 1. Each cage contained four plants, and four fertilized parasitoids were introduced and released in the middle of the cage. After 24 h, 30% (w/v) honeydew solutions were sprayed onto the caged plants using a mini sprayer. The honeydew solution treatments were as follows: (i) natural honeydew only, (ii) plastic honeydew, (iii) convalescent honeydew and (iv) double-distilled water only (blank control). Every 2 days, the plants were sprayed with 10 ml of the indicated solution. This experiment was repeated 15 times. After 2 weeks, all surviving mealybugs and mummified mealybugs on each plant were counted and recorded. The tests were conducted in a greenhouse at 28°C, 75% relative humidity and 16:8 LD.

Statistical analysis

All data were tested for normal distribution using the Shapiro–Wilk test. One-way analysis of variance (ANOVA) with a type III sum of squares or an independent sample t-test was performed to compare the means among all of the measured variables when the data were normally distributed and possessed similar variances. For data that were not normally distributed, the nonparametric Kruskal–Wallis test was used for comparing medians. The Mann–Whitney U-test was used for multiple comparisons among the different groups if the results of the Kruskal–Wallis test showed significant differences at the 0.05 significance level. All statistical analyses were conducted with SPSS, version 14.0 (SPSS Inc., Chicago, IL, USA).

Results

Plasticity in the sugar composition and sugar concentration of honeydew

Our results revealed that honeydew from all treatments contained melezitose, raffinose, sucrose, trehalose, glucose, xylose and fructose (Table 1). The honeydew sugar concentration changed with ant attendance. The concentration of sucrose increased significantly in ant-attendance treatment and decreased when ant attendance was switched to the ant-exclusion treatment (F_{2,21}=6.222, P=0.008, Table 1). In contrast, honeydew droplets showed a considerable decrease in the glucose concentration in ant-attendance treatment, which increased significantly when ant attendance was switched to ant-exclusion treatment (F_{2,21}=6.391, P=0.007, Table 1). The switch in treatment had no effects on the concentrations of melezitose, raffinose, trehalose, xylose and fructose.

Searching response of parasitoids to mealybug honeydew

The searching response of parasitoids differed significantly among the honeydew treatments (χ^2=43.493, d.f. = 5, P < 0.001, Kruskal–Wallis test, Fig. 1).
Table 1 Sugar composition (mean ±SE) of the honeydew produced by mealybugs

<table>
<thead>
<tr>
<th>Sugar composition</th>
<th>Mealybug only exclusion</th>
<th>Ant attendance</th>
<th>Ant</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melezitose</td>
<td>0.034 ± 0.009a</td>
<td>0.029 ± 0.011a</td>
<td>0.031 ± 0.007a</td>
<td>0.105</td>
<td>0.901 ns</td>
</tr>
<tr>
<td>Raffinose</td>
<td>0.043 ± 0.010a</td>
<td>0.026 ± 0.007a</td>
<td>0.040 ± 0.010a</td>
<td>0.975</td>
<td>0.394 ns</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.190 ± 0.166b</td>
<td>1.956 ± 0.264a</td>
<td>0.983 ± 0.172b</td>
<td>6.222</td>
<td>0.008**</td>
</tr>
<tr>
<td>Trehalose</td>
<td>0.177 ± 0.052a</td>
<td>0.210 ± 0.085a</td>
<td>0.159 ± 0.058a</td>
<td>0.147</td>
<td>0.864 ns</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.969 ± 0.250a</td>
<td>0.940 ± 0.220b</td>
<td>2.054 ± 0.264a</td>
<td>6.391</td>
<td>0.007**</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.587 ± 0.173a</td>
<td>0.626 ± 0.161a</td>
<td>0.669 ± 0.126a</td>
<td>0.071</td>
<td>0.931 ns</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.652 ± 0.129a</td>
<td>0.354 ± 0.111a</td>
<td>0.607 ± 0.134a</td>
<td>1.657</td>
<td>0.215 ns</td>
</tr>
</tbody>
</table>

*ns, no statistically significant differences of the sugar composition between the treatments (P > 0.05).
**Statistically significant differences in the sugar composition between the treatments (P < 0.05). Sugar concentration values followed by different letters in the same line indicate significant differences among the treatments (P < 0.05).

Fig. 1 Differences in searching times (seconds) of parasitoids on honeydew- and sugar-treated filter papers and on blank control filter papers. Natural, plastic and convalescent indicate natural honeydew, plastic honeydew and convalescent honeydew, respectively. Results are expressed as the means ± SE. Bars sharing the same letters indicate no significant differences between the treatments (Mann–Whitney U-test, P > 0.05).

Parasitoids spent significantly longer searching on filter papers with natural honeydew or convalescent honeydew than on filter papers treated with plastic honeydew (U = 36,000, P = 0.001; U = 52,500, P = 0.011, respectively; Mann–Whitney U-test, Fig. 1). There were no significant differences in searching times of parasitoids between natural honeydew treatment and convalescent honeydew treatment (U = 96,500, P = 0.512, Mann–Whitney U-test, Fig. 1). A significant difference in searching times of parasitoids was observed between the plastic honeydew treatment and the blank control (U = 20,500, P < 0.001, Mann–Whitney U-test, Fig. 1).

Effects of mealybug honeydew on parasitoid performance

The tested honeydew and honeydew sugars in this study differed significantly in their effects on the longevity of parasitoids ($\chi^2=87.953$, d.f.=10, P < 0.001, Kruskal–Wallis test, Fig. 2). In comparison with control treatment, natural honeydew, convalescent honeydew, sucrose, glucose and fructose significantly extended the longevity of the parasitoids (U = 10,000, P < 0.001; U = 13,500, P < 0.001; U = 86,000, P = 0.002; U = 52,500, P < 0.001, U = 47,000, P < 0.001, respectively; Mann–Whitney U-test, Fig. 2). Longevity of the parasitoids in natural honeydew and convalescent honeydew treatments was much longer than that in plastic honeydew treatment (U = 33,500, P < 0.001, U = 36,500, P < 0.001, respectively; Mann–Whitney U-test, Fig. 2). However, there were no significant differences in the effect on parasitoid longevity among plastic honeydew, melezitose, raffinose, trehalose, xylose and control treatments.

In addition, our results indicate that the percentage of parasitism by parasitoids was significantly different among the honeydew treatments ($\chi^2=34.555$, d.f.=3, P < 0.001, Kruskal–Wallis test, Fig. 3). The percentage of parasitism in natural honeydew and convalescent honeydew treatments was significantly greater than that in plastic honeydew treatment (U = 41,000, P = 0.002, U = 58,500, P = 0.023, respectively; Mann–Whitney U-test, Fig. 3). The percentage of parasitism in plastic honeydew treatment was also significantly higher than that in control treatment (U = 25,000, P < 0.001; Mann–Whitney U-test, Fig. 3).

Discussion

Many studies have considered honeydew produced by hemipterans to be a contact kairomone and an arrestant for predators and parasitoids (Bouchard and Cloutier 1984; Romeis and Zebitz 1997; Han and...
Chen 2002; Mandour et al. 2007; Leroy et al. 2012; Tranfaglia and Dfga 2013). Honeydew produced by hemipterans contains sugars including glucose, fructose, sucrose, trehalose, glucose, xylene and fructose, which is consistent with the results of previous studies (Völkl et al. 1999; Fischer and Shingleton 2001; Yao and Akimoto 2001). Honeydew composition exhibits high plasticity under ghost ant tending. The concentration of sucrose increased significantly in ant-attendance treatment, but it dropped when ant attendance was removed (Table 1). In contrast, glucose concentration exhibited a significant decrease in ant-attendance treatment and then increased significantly when ant attendance was switched to the ant-exclusion treatment (Table 1). Although similar studies have also demonstrated that tending of aphids by Lasius niger, for example, alters the composition of the aphid honeydew, the most marked changes were in the concentrations of glucose and melezitose (Fischer and Shingleton 2001). Yao and Akimoto (2002) also showed that the glucose concentration in aphid honeydew was significantly lower with F. yessensis tending. Our study is the first to demonstrate that honeydew composition exhibits significant plasticity under the switched ant-attendance treatment.

Our results show that honeydew produced by mealybugs contains melezitose, raffinose, sucrose, trehalose, glucose, xylene and fructose, which is consistent with the results of previous studies (Völkl et al. 1999; Fischer and Shingleton 2001; Yao and Akimoto 2001). Honeydew composition exhibits high plasticity under ghost ant tending. The concentration of sucrose increased significantly in ant-attendance treatment, but it dropped when ant attendance was removed (Table 1). In contrast, glucose concentration exhibited a significant decrease in ant-attendance treatment and then increased significantly when ant attendance was switched to the ant-exclusion treatment (Table 1). Although similar studies have also demonstrated that tending of aphids by Lasius niger, for example, alters the composition of the aphid honeydew, the most marked changes were in the concentrations of glucose and melezitose (Fischer and Shingleton 2001). Yao and Akimoto (2002) also showed that the glucose concentration in aphid honeydew was significantly lower with F. yessensis tending. Our study is the first to demonstrate that honeydew composition exhibits significant plasticity under the switched ant-attendance treatment.

Honeydew strongly entices natural enemies and appears to provide an important contact semiochemical cue for natural enemies. Host location by insect parasites is regulated by a series of physical and chemical cues (Vinson 1985, 1991). As a contact kairomone, aphid honeydew stimulates host location in several aphid parasites (Gardner and Dixon 1985; Budenberg and Powell 1992; Shaltiel and Ayal 1998). The kairomonal effect of cornicle secretion can stimulate both host location and oviposition (Grasswitz and Paine 1992). For example, both female and male parasites of Aphidius rhopalosiphi responded to cereal aphid honeydew on filter paper discs by greatly increasing visit times. Moreover, their response increased with increasing concentrations of honeydew (Budenberg 1990). On the honeydew-treated filter paper, the aphid parasitoid Aphidius sp. searched actively, with much turning and reduced walking speeds. The parasitoid exhibited arrestment, antennal examination and ovipositor probing (Han and Chen 2002). Honeydew elicited oviposition by Episyrphus balteatus, and the number of eggs laid by E. balteatus increased with increasing honeydew concentration. In addition, Episyrphus balteatus females landed more frequently on wheatears contaminated with honeydew than on clean wheatears (Budenberg and Powell 1992).

Our results also showed that the natural honeydew produced by P. solenopsis stimulated the searching...
response of *A. bambawalei*, which was consistent with previous studies. Furthermore, compared with the plastic honeydew, parasitoids spent longer searching on filter papers treated with natural honeydew and convalescent honeydew (Fig. 1). The longevity of the parasitoids in natural honeydew and convalescent honeydew treatment was much longer than that in plastic honeydew treatment (Fig. 2). In addition, sucrose, glucose and fructose significantly extended the longevity of the parasitoids. A similar study also showed that glucose, sucrose, fructose, trehalose and trehalulose treatments extended the lifespan of the parasitoids by factors of 8.4, 8.1, 6.3, 6.1 and 4.2, respectively, but that melezitose and natural honeydew did not show comparable effects (Mandour et al. 2007). However, in our experiments, natural honeydew contributed most to the parasitoids’ performance. This contradiction may be explained by the different honeydew preferences among parasitoid species. Our results clearly show that glucose is attractive for *A. bambawalei*. However, glucose concentration decreased significantly under the ant-attendance treatment. These results show that ghost ant tending influenced the physiological status of *P. solenopsis* by changing the honeydew composition produced by *P. solenopsis*, which may suggest that ant tending reduces the effect of honeydew as an important kairomone for host location of parasitoids. The resulting percentages of parasitism by parasitoids in natural honeydew and convalescent honeydew treatments were significantly higher than that in plastic honeydew (Fig. 3), supporting the hypothesis that ant tending reduces the performance of parasitoids through plasticity in honeydew composition. Both quality and quantity of the honeydew produced by hemipterans play an important role in the stability and outcomes of hemipteran–ant–enemy interactions (Addicott 1978; Sakata 1995). Our previous study showed that when tended by *S. invicta*, honeydew composition produced by *P. solenopsis* changed significantly. However, the total weights of honeydew excreted by mealybugs was indistinctive between with ant tending and without ant tending (Zhou et al. 2015). Yao and Akimoto (2001) also found that the total volume of honeydew produced under ant tending did not differ significantly from that in untended aphids. These results may suggest that ant tending impairs performance of parasitoids by manipulating the honeydew quality rather than honeydew quantity produced by hemipterans.

The present study demonstrates that honeydew from a potato-infesting mealybug plays a role in host-finding by the parasitoid *A. bambawalei*. A similar study also showed that the parasitoid *Aphidius nigripes* spent more time searching on potato plants with aphid honeydew than on fresh plants, and water extracts of aphid honeydew applied to the surface of foliage or to filter paper discs also increased the searching time and affected the locomotor behaviour of parasitoids (Bouchard and Cloutier 1984). Saad and Bishop (1976) showed that potato plants with artificial honeydew attracted many aphid predators in the field. Therefore, it would certainly be worthwhile to identify the behaviourally active materials in natural honeydew. Our results contribute to an understanding of the interactions involved in the ant–hemipterans–parasitoid system, and these findings may provide a new approach for the use of specific artificial honeydews in the control of mealybugs by enhancing the effectiveness of parasitoids.

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