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Sucrose triggers honeydew preference in the ghost ant, *Tapinoma melanocephalum* (Hymenoptera: Formicidae)

A. M. Zhou[1, 2,]*, *B. Q. Kuang*[2], *Y. R. Gao*[2], *and G. W. Liang*[2]

Abstract

Honeydew produced by hemipterans mediates mutualistic interactions between ants and hemipterans. Previous studies demonstrated that the mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) and the aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) produce abundant honeydew and attract a large number of tending ants. Ghost ants, *Tapinoma melanocephalum* (F.) (Hymenoptera: Formicidae), show a significant preference for mealybug honeydew over aphid honeydew. Although many studies have indicated that the honeydew produced by hemipterans plays an important role in ant–hemipteran interactions, we know little about what triggers ants' foraging preferences. Our results showed that the honeydew produced by both mealybugs and aphids contained fructose, sucrose, trehalose, melezitose, raffinose, and rhamnose. There were no significant difference in the concentrations of the various sugars between mealybugs and aphids, except sucrose. Xylose was present only in mealybug honeydew, and glucose was present only in aphid honeydew. We also found no substantial difference in the excretion frequency and the total weight of honeydew produced per 24 h between mealybugs and aphids. Ghost ants preferred sucrose. In addition, attractiveness of sucrose solutions increased significantly with increasing concentration. These results suggest that sucrose is the trigger for ghost ants' honeydew preference.

Key Words: ant–hemipteran mutualism; sugar composition; sugar concentration

Resumen

La mielcilla producida por hemípteros regula las interacciones mutualistas entre las hormigas y los hemípteros. Los estudios anteriores demostraron que la cochinilla harinosa *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) y el áfido *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) producen mielcilla abundante y atraen a un gran número de hormigas que atienden. Las hormigas fantasma, *Tapinoma melanocephalum* (F.) (Hymenoptera: Formicidae), muestran una preferencia significativa por la mielcilla de la cochinilla sobre la mielcilla del áfido (pulgón). Aunque muchos estudios han indicado que la mielcilla producida por hemípteros juega un papel importante en las interacciones hormiga-hemípteros, sabemos poco acerca de lo que desencadena las preferencias de forrajeo de las hormigas. Nuestros resultados mostraron que la mielcilla producida por las cochinillas y los áfidos contiene fructosa, sacarosa, trehalosa, melezitosa, rafinosa y ramnosa. No hubo diferencia significativa en la concentración de los diversos azúcares entre las cochinillas y los áfidos, excepto la sacarosa. La xilosa estuvo presente sólo en mielcilla de la cochinilla y la glucosa estuvo presente sólo en mielcilla del áfido. También no se encontró una diferencia sustancial en la frecuencia de excreción y el peso total de mielcilla producida por 24 h entre las cochinillas y los pulgones. Las hormigas fantasma prefieren la sacarosa. Además, el atractivo de soluciones de sacarosa aumentó significativamente con el aumento en la concentración. Estos resultados sugieren que la sacarosa es un desencadenante para la preferencia de la hormiga fantasma para la mielcilla.

Palabras Clave: mutualismo de hormiga-hemiptera; composición de azúcar; concentración de azúcar

Mutualism between ants and hemipterans is a common phenomenon in many ecosystems (Helms & Vinson 2002; Simberloff 2006; Brightwell & Silverman 2010). In such a relationship, the ants protect the hemipterans from predators and parasitoids. In return, ants get abundant carbohydrate produced by hemipterans (Davidson et al. 2004; Stadler & Dixon 2005). Honeydew excreted by hemipterans plays a critical role in mutualistic interactions with ants (Way 1963; Baylis & Pierce 1992). The honeydew produced by aphids may be a stimulus for ants to forage on cotton plants (Kaplan & Eubanks 2005). In addition, ants can obtain more honeydew on plants with high densities than on plants with low densities of mealybugs (Zhou et al. 2013a).

Honeydew is essential for ant colony growth and survival because it contains sugars mixed with various amino acids needed by workers and larvae (Porter 1989; Tennant & Porter 1991; Yao & Akimoto 2002; Helms & Vinson 2008; Zhou et al. 2012). Honeydew contains a mixture of monosaccharides, disaccharides, and trisaccharides. Studies have indicated that the intensity of ant foraging can be mediated by the sugar composition of honeydew (Völkl et al. 1999). Trisaccharides in honeydew were commonly attractive to some insect species (Wäckers 2000). Melezitose is preferred by several ant species and is important for ant–aphid relationships (Kiss 1981; Maurizio 1985). For instance, *Lasius niger* (L.) (Hymenoptera: Formicidae) workers preferred trisaccharides over disaccharides and monosaccharides when these sugars were offered in choice tests, and melezitose was the most effective in attracting ants (Völkl et al. 1999). Previous studies also showed that the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), had a specific preference for melezitose in laboratory and field tests (Zhou et al. 2015), whereas it often preferred sucrose over fructose, glucose, maltose, melezitose, raffinose, and xylose in paired tests (Blüthgen & Fiedler 2004).

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Ants distinctly prefer different aphid honeydews; for instance, compared with honeydew produced by *Brachycaudus cardui* (L.), honeydew produced by *Aphis fabae* Scopoli (Hemiptera: Aphididae) is less attractive to *L. niger* (Völkl et al. 1999). Our previous observations found that the ghost ant, *Tapinoma melanocephalum* (F.) (Hymenoptera: Formicidae), preferred the honeydew excreted by the mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) over the honeydew from the aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). Ghost ants visited mealybug-infested plants more frequently than aphid-infested plants (unpublished data). Although several studies demonstrated that ants prefer different hemipteran honeydews (Fischer et al. 1997; Völkl et al. 1999), relatively few studies examined what triggered honeydew preferences in ants. In the present study, we determined the reason why ghost ants preferred mealybug honeydew over aphid honeydew. The specific questions addressed in this work are (1) whether the mealybug *P. solenopsis* produces more honeydew than the aphid *M. persicae*; (2) whether the sugar composition and concentration in honeydew differ between mealybugs and aphids; and (3) whether the ghost ant has sugar preferences.

Materials and Methods

PLANTS AND INSECTS

Tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae), plants were grown in a standard soil from the horticultural farm of South China Agricultural University, Guangzhou, China. Each plant was approximately 20 to 25 cm in height, had 15 to 20 true leaves, and was cultivated in a plastic flowerpot (with upper and lower diameters of 14 and 10 cm, respectively, and a height of 15 cm). The mealybug *P. solenopsis* and aphid *M. persicae* colonies were field collected from tomatoes, then, 20 to 30 individuals were transferred to the potted *S. lycopersicum* plants and reared in a climate chamber. Polygyne colonies of *T. melanocephalum* were collected from a suburb in Guangzhou, China (15 different colonies in total). 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. Each colony was subsequently divided into several colony fragments (3–5 subcolonies) by using an analytical balance (Sartorius BSA 224S, Elk Grove, Illinois, USA). Each small colony included 1 queen and thousands of adult workers (1 g, approximately 3,000 individuals). These colonies were placed in 9 cm plastic Petri dishes as an artificial nest (Zhou et al. 2015). Ant colonies were supplied weekly with fresh live larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and a 10% solution of honey mixed with water (50 mL). All of the colonies of aphids, mealybugs, and ghost ants were reared in the laboratory at 27 ± 2 °C and relative humidity of 60 to 70%.

HONEYDEW PREFERENCES OF *T. MELANOCEPHALUM*

We determined the honeydew preferences of ghost ants in laboratory tests. Ghost ant subcolonies were placed in plastic boxes (with upper and lower diameters of 15 and 11 cm, respectively, and a height of 9 cm) and deprived of any carbohydrate source for 12 h. Ten subcolonies from different colony sources were used in this experiment. To prevent the ants from escaping, Fluon was applied halfway up the inner surface of each box. We transferred 3rd instar mealybugs or aphids to *S. lycopersicum* plants and allowed the mealybugs and aphids to acclimate and feed for 24 h. Each subcolony was then connected to the mealybug-infested and aphid-infested plants simultaneously via 2 plastic tubes (1.5 cm in diameter, 20 cm long) through which the ghost ants could forage for food. After 24 h, we counted the number of the ants that were headed in the direction of the mealybugs or aphids for 10 min. Each treatment was replicated 10 times.

In addition, we tested the honeydew preference of ghost ants in paired choice tests (mealybug honeydew solution versus aphid honeydew solution). Each tested honeydew solution (10 mL, 10% w/v) was adsorbed with a pledget and placed in another plastic box (8 × 8 × 6 cm LWH). The 2 honeydew boxes were connected to the ant colony box by transparent silicone tubes (1 cm diameter and 10 cm length). Ten subcolonies from different colony sources were used in this experiment. After 30 min, we counted the number of the ants that were headed in the direction of the honeydew for 10 min. All tests were replicated 10 times.

WEIGHT OF HONEYDEW PRODUCED BY *P. SOLENOPSIS* AND *M. PERSICAE*

To determine the weight of honeydew produced by mealybugs and aphids, we conducted 2 tests in the laboratory. First, we determined the weight of single honeydew droplets excreted by the mealybugs and aphids. Thirty 3rd instar mealybugs or aphids were transferred to *S. lycopersicum* plants. After 24 h, we chose 1 mealybug or aphid randomly from each colony on the plants, and a honeydew droplet was collected and absorbed with a pre-weighed filter paper strip (1.0 cm long). The weight of each single honeydew droplet was estimated by measuring the weight of the honeydew absorbed onto the filter paper strips (microbalance, Sartorius BT 25S, Elk Grove, Illinois, USA). Each treatment was replicated 10 times. Second, we tested the excretion frequency of mealybugs and aphids (per individual per 24 h). We transferred individual 3rd instars to *S. lycopersicum* leaves, which were placed on agar medium in plastic cups (with upper and lower diameters of 10 and 6 cm, respectively, and a height of 9 cm). All plastic cups were inverted on a filter paper (saffron yellow) that was dyed with bromocresol green solution (0.1% w/w, solvent: alcohol). When honeydew was dropped into the solution, a blue spot appeared on the yellow filter paper. Each filter paper was replaced by a new one every hour (Zhou et al. 2013b). We defined the frequency of excretion as the number of blue spots that appeared in 24 h. This experiment was conducted in the laboratory at 27 ± 1 °C, 70% RH, and a 12:12 h L:D photoperiod. Each treatment was replicated 10 times. The total weight of honeydew produced per mealybug or aphid per 24 h was estimated by multiplying the weight of a single honeydew droplet with the frequency of excretion.

ANALYSIS OF THE HONEYDEW SUGAR COMPOSITION AND CONCENTRATIONS

All *S. lycopersicum* plants on which the mealybugs and aphids fed were at the same developmental stage (contained 15 to 20 true leaves and flowers had not yet opened). The mealybug and aphid densities on the plants were maintained at 20 to 25 individuals per plant. To obtain relatively accurate sugar concentrations, we collected only honeydew that was freshly produced by the mealybugs and aphids. Each sample consisted of honeydew obtained from twenty-five 3rd instar mealybugs or aphids from a single colony. We collected the honeydew directly from the anus of the insects using a microcapillary tube (0.5 µL) (Völkl et al. 1999; Yao & Akimoto 2001). The honeydew was analyzed 48 h after collection by high-performance liquid chromatography. The honeydew sugar concentration and composition were measured in a column (TSK-NH2, 4.6 mm × 250 mm × 5 *µm*) with an apparatus using a differential refraction detector. Sample elution was isocratic, employing 75% acetonitrile and a flow rate of 1 mL/min. The concentrations of 9 sugars (xylose, glucose, fructose, sucrose, maltose, trehalose, melezitose, raffinose, and rhamnose) were analyzed 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 standard sugars. The actual concentrations of the sugars in the samples were estimated by comparing their peak areas with those of standard sugars of known concentrations (Zhou et al. 2015).

SUGAR PREFERENCES OF *T. MELANOCEPHALUM*

The procedures used in this experiment were similar to those used in the experiment to determine honeydew preferences. Here, we set up 2 types of experiments to test the sugar preferences of ghost ants: (i) various single sugars tested concurrently (xylose, glucose, fructose, sucrose, trehalose, melezitose, raffinose, rhamnose, mealybug honeydew, and aphid honeydew; 10% w/v); and (ii) different concentrations of sucrose (10, 20, and 30% w/v). The experimental apparatus included an ant colony box and a sugar box (Fig. 1). Subcolonies from different sources were used in each test. Each tested sugar solution (10 mL) was adsorbed with a pledget and placed in a plastic box (8 × 8 × 6 cm LWH). Each sugar box was connected to the ant colony box by a transparent silicone tube (1 cm diameter and 10 cm length). After 30 min, we counted the number of ants that were headed in the direction of the sugar for 10 min. All tests were replicated 10 times.

STATISTICAL ANALYSES

All data were tested for a normal distribution with the Shapiro–Wilk test. One-way analysis of variance (ANOVA) with a Type III sum of squares or a paired-sample *t*-test was performed to compare the means among 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 for comparing medians was applied. The Mann–Whitney *U* test for multiple comparisons among the different groups was used if the results of the Kruskal–Wallis test showed significant differences at the 0.05 significance level. Paired-sample *t*-tests were used to compare the preferences of ghost ants for mealybug and aphid honeydew. Independent-sample *t*-tests were used to analyze the differences in honeydew concentration, weight of the single honeydew droplets, excretion frequency, and the total volume of honeydew excreted in 24 h between mealybugs and aphids. The differences in sugar preferences of ghost ant were analyzed by the Kruskal–Wallis test. All statistical analyses were conducted with SPSS version 14.0 (SPSS Inc., Chicago, Illinois, USA).

Results

HONEYDEW PREFERENCES OF *T. MELANOCEPHALUM*

Our results showed that mealybug-infested plants were visited about twice as often as aphid-infested plants ($t = -2.496$, df = 9, $P = 0.034$, paired-sample *t*-test; Fig. 2). In addition, honeydew produced by mealybugs was similarly preferred over that produced by aphids ($t = -3.144$, df = 9, $P = 0.012$, paired-sample *t*-test; Fig. 2).

HONEYDEW SUGAR COMPOSITION AND CONCENTRATIONS IN *P. SOLENOPSIS* AND *M. PERSICAE*

The honeydew produced by mealybugs and aphids contained fructose, sucrose, trehalose, melezitose, raffinose, and rhamnose. There was no significant difference in the sugar composition between mealybugs and aphids except sucrose (Table 1). Sucrose concentration in

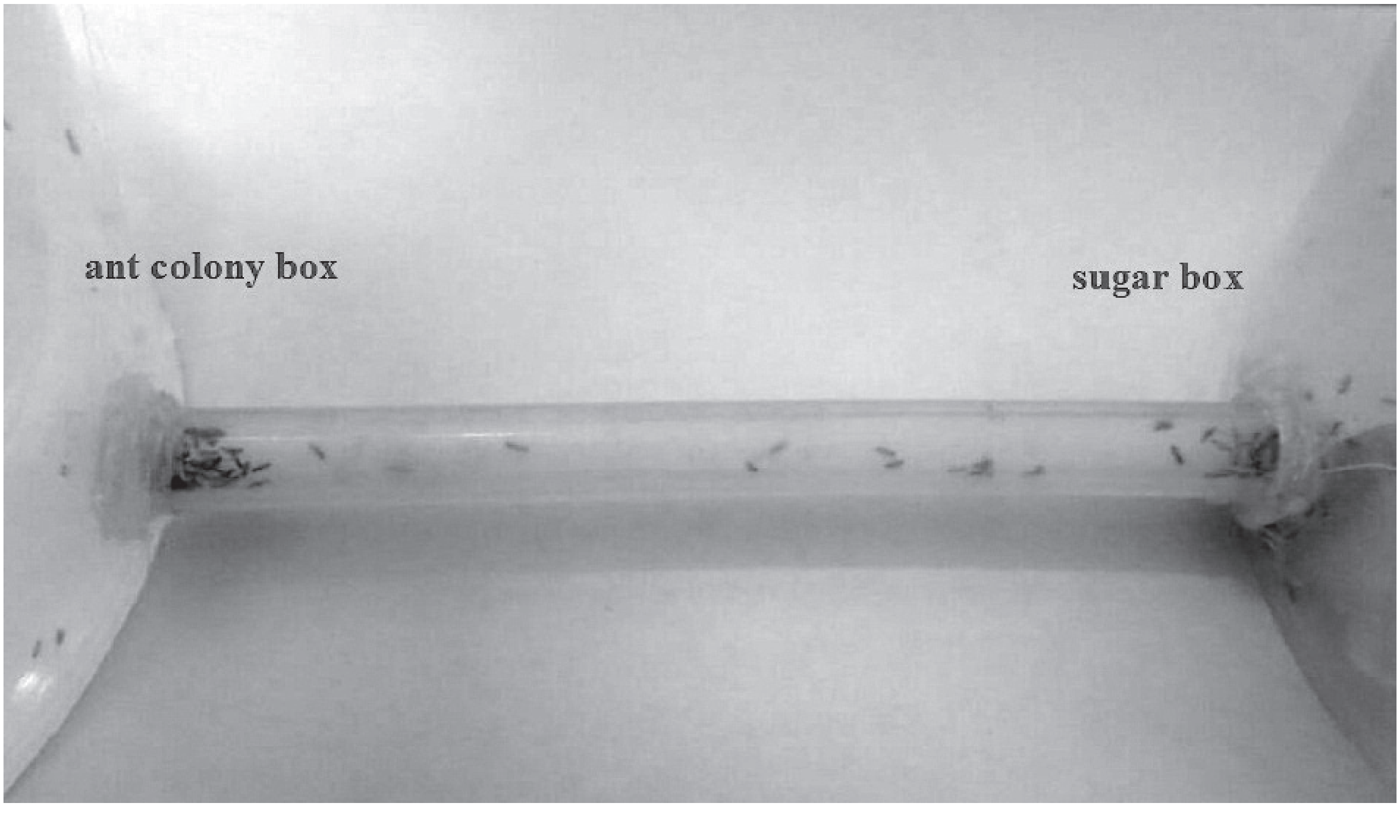

Fig. 1. Experimental apparatus used to evaluate foraging preferences of ghost ants.

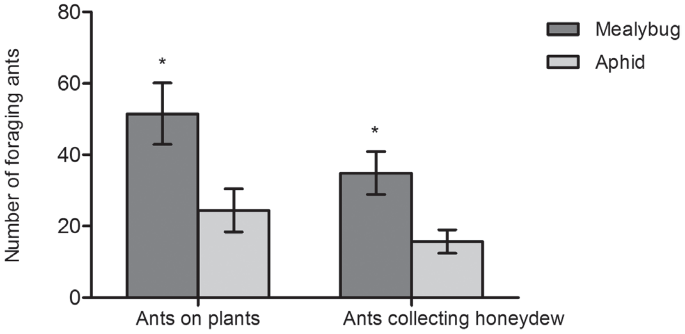

Fig. 2. Foraging preference of ghost ants. The data are presented as the mean ± SE, and an asterisk above the bars indicates statistically significant differences between mealybug and aphid (paired-sample *t*-test, *P* = 0.05).

Table 1. Sugar composition (mean ± SE) of the honeydew produced by *Phenacoccus solenopsis* mealybugs and *Myzus persicae* aphids.

	Sugar concentration (mg/mL)			
Sugar	Mealybug	Aphid	*t*	*P*
Fructose	0.272 ± 0.085	0.455 ± 0.061	−1.750	0.155 NS
Sucrose	2.229 ± 0.494	0.710 ± 0.156	3.065	0.037 *
Trehalose	0.096 ± 0.005	0.147 ± 0.029	−1.726	0.159 NS
Melezitose	0.017 ± 0.003	0.012 ± 0.002	1.200	0.296 NS
Raffinose	0.186 ± 0.047	0.106 ± 0.039	1.299	0.264 NS
Rhamnose	0.114 ± 0.037	0.086 ± 0.011	0.724	0.509 NS
Glucose	—	1.245 ± 0.321	—	—
Xylose	0.026 ± 0.007	—	—	—

P value followed by "NS" indicates no statistically significant differences whereas * indicates significant differences in the sugar composition between mealybug and aphid (independent-sample *t*-test); — indicates the sugar is absent in the honeydew.

mealybug honeydew was significantly higher than that in aphid honeydew (*t* = 3.065, df = 4, *P* = 0.037, independent-sample *t*-test; Table 1: sucrose). In addition, xylose was found only in mealybug honeydew, and glucose was found only in aphid honeydew.

WEIGHT OF HONEYDEW PRODUCED BY *P. SOLENOPSIS* AND *M. PERSICAE*

There was no significant difference in the weight of the single honeydew droplets between mealybugs and aphids (*t* = 0.413, df = 18, *P* = 0.685, independent-sample *t*-test; Table 2). The excretion frequency by mealybugs and aphids was similar (*t* = −1.003, df = 18, *P* = 0.329, independent-sample *t*-test; Table 2). The total volume of honeydew excreted in 24 h was similar in mealybugs and aphids (*t* = −0.312, df = 18, *P* = 0.759, independent-sample *t*-test; Table 2).

SUGAR PREFERENCES OF *T. MELANOCEPHALUM*

Ghost ant trail counts were significantly different among the 10 sugar solutions (χ^2 = 26.624, df = 9, *P* = 0.002; Fig. 3A). Sucrose was visited more frequently than other sugar solutions. No significant differences in trailing behavior was found between mealybug honeydew and aphid honeydew in this experiment (*U* = 24.500, *P* = 0.052, Mann–Whitney *U* test; Fig. 3A). Furthermore, the number of foraging ants significantly increased with increasing concentration of sucrose solutions (χ^2 = 8.615, df = 2, *P* = 0.013; Fig. 3B). High concentrations of sucrose solutions were visited more frequently than low concentrations (*U* = 12.500, *P* = 0.003, Mann–Whitney *U* test; Fig. 3B).

Discussion

Ants are the most common animals consuming extrafloral nectar and honeydew (Buckley 1987; Koptur 1992). Honeydew, a solution of sugars and amino acids that is produced by hemipterans, attracts ants that often protect the hemipterans from potential predators and parasitoids and is considered to be an important food resource for ants because it is rich in energy (Douglas 1993; Tobin 1994; Davidson et al. 2004). Ant–hemipteran interactions are substantially influenced by honeydew produced by hemipterans (Styrsky & Eubanks 2007).

Differences in honeydew composition (qualitative effects) and honeydew production (quantitative effects) seem to be primarily responsible for the hierarchies in ant attendance of different hemipteran species (Völkl et al. 1999). Ants should be expected to focus honeydew collection activities on aphid species that promise a high reward, either in terms of large volumes of honeydew or in terms of the presence of preferred sugars or amino acids (Cushman 1991; Cushman & Addicott 1991). Although several studies have examined sugar preferences of ants (Ricks & Vinson 1970; Sudd & Sudd 1985; Vander Meer et al. 1995; Cornelius et al. 1996; Koptur & Truong 1998; Völkl et al. 1999; Tinti & Nofre 2001), most of the relevant studies focused on the effects of different sugar composition on ant foraging preference, and relatively few studies examined what triggers honeydew preferences in ants. This is therefore the first study to test the qualitative and quantitative effects of honeydew produced by different hemipteran species on sugar preference of ants.

Our results showed that the ghost ant visited mealybug-infested plants more frequently than aphid-infested plants. The difference in honeydew produced by these 2 hemipterans may primarily contribute to the ant-visit preference, because honeydew produced by mealybugs was preferred over that produced by aphids. A similar study indicated that *L. niger* showed marked preferences when collecting honeydew from 3 aphid species, including *Metopeurum fuscoviride* (Hemiptera: Aphididae), *B. cardui*, and *A. fabae* (Fischer & Shingleton 2001). These results suggest that the selective attractiveness of hemipterans to ants may be related to the differences in honeydew quality and quantity. Previous studies determined that the honeydew produced by aphids contained a mixture of monosaccharides, disaccharides, and trisaccharides (Völkl et al. 1999; Fischer & Shingleton 2001; Yao & Akimoto 2001). Our results showed that honeydew of mealybugs and aphids contained fructose, sucrose, trehalose, melezitose, raffinose, and rhamnose, but that the concentration of sucrose in mealybug honeydew was significantly higher than that in aphid honeydew. The pres-

Table 2. Comparison of honeydew excretion behavior between mealybug and aphid.

Honeydew excretion	Mealybug	Aphid	*t*	*P*
Mean weight of honeydew (mg/drop)	1.50 ± 0.23	1.35 ± 0.29	0.413	0.685 NS
Excretion frequency per mealybug (drops/24h)	17.50 ± 1.95	20.60 ± 2.40	−1.003	0.329 NS
Mean weight of honeydew (mg/24h)	27.24 ± 6.06	30.54 ± 8.65	−0.312	0.759 NS

P value followed by "NS" indicates no statistically significant differences. The data are presented as the mean ± SE (independent-sample *t*-test, *P* = 0.05).

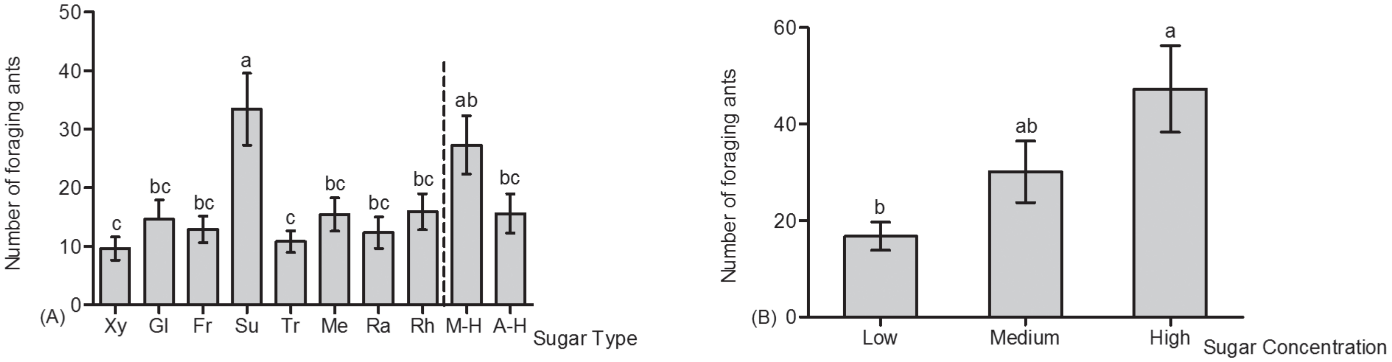

Fig. 3. Foraging preferences of ghost ants (A) when different sugars were offered simultaneously (Xy, Gl, Fr, Su, Tr, Me, Ra, Rh, M-H, and A-H denote xylose, glucose, fructose, sucrose, trehalose, melezitose, raffinose, rhamnose, mealybug honeydew, and aphid honeydew, respectively); and (B) when different concentrations of sucrose were offered simultaneously. The data are presented as the mean ± SE, and different letters above the bars indicate statistically significant differences between the treatments (Mann–Whitney *U* test, *P* = 0.05).

ence of glucose or xylose may be also involved in the ant's preference because glucose was not found in honeydew of mealybugs, and xylose was not found in honeydew of aphids. Sugar composition may be substantially different among different hemipteran species (Fischer & Shingleton 2001). The ability to transform the ingested disaccharide sucrose also differs significantly among hemipterans species (Fisher et al. 1984; Walters & Mullin 1988). For instance, *A. fabae* and *Macrosiphoniella tanacetaria* (Kaltenbach) (Hemiptera: Aphididae) are not able to synthesize oligosaccharides, which may play an important role in aphid osmoregulation (Fisher et al. 1984; Walters & Mullin 1988; Wilkinson et al. 1997). Symbiotic bacteria in hemipterans may also be responsible for the observed differences in the honeydew composition (Baumann et al. 1995; Teo & Woodring 1985).

Both the quantitative and qualitative nutritional characteristics of honeydew probably influence the attractiveness of the hemipterans to ants. Aphid species that produce large volumes of honeydew or excrete ant-preferred sugars are usually preferentially tended by ants (Cushman 1991; Cushman & Addicott 1991). A previous study showed that the volume of honeydew was considerably different among the 4 aphid species *M. fuscoviride* (880 µg per aphid per hour), *B. cardui* (223 µg per aphid per hour), *A. fabae* (133 µg per aphid per hour), and *M. tanacetaria* (46 µg per aphid per hour) (Völkl et al. 1999). However, our results showed that although the sugar composition in honeydew was different between the 2 hemipterans, the weight of a single honeydew droplet, the excretion frequency, and the total volume of honeydew excreted in 24 h were not significantly different between mealybugs and aphids. Thus, the differences in honeydew composition and concentration may be the primary reason for the honeydew preference of ghost ants. Our results showed that ghost ants specifically preferred sucrose. It may be because energetically, sucrose has twice the energy of a monosaccharide. Furthermore, attractiveness of sucrose solutions increased significantly with increasing concentration. The qualitative honeydew production of mealybugs corresponded well with the observed visit frequency by ghost ants. Therefore, we conclude that higher sucrose concentration in mealybug honeydew triggered the honeydew preference in ghost ants. Recruitment of the ant species *Solenopsis geminata* (F.) (Hymenoptera: Formicidae) and *S. invicta* can be influenced substantially by sugar content (Lanza et al. 1993). Ricks & Vinson (1970) found that *Solenopsis saevissima richteri* Forel (Hymenoptera: Formicidae) and *S. invicta* preferred sucrose over xylose, ribose, mannose, arabinose, and galactose. Sucrose was also often preferred by *Anonychomyrma gilberti* (Forel)*, Technomyrmex albipes* (Smith), and *Camponotus vitreus* (Smith) (Hymenoptera: Formicidae) (Blüthgen & Fiedler 2004), whereas melezitose was preferred by several ant species and is important for ant–aphid relationships (Kiss 1981; Maurizio 1985; Völkl et al. 1999).

Our results clearly demonstrated that ghost ants preferred honeydew of mealybugs over that of aphids. This preference may be beneficial to mealybugs in competing for ant tending against aphid species. Previous studies also showed that hemipteran species that occur within the same habitat may compete for the mutualistic services of ants (Bristow 1984, 1991; Cushman 1991). For instance, the presence of conifer aphids (*Cinara* species) on *Picea engelmannii* Parry ex Engelmann (Pinales: Pinaceae) reduced the numbers of ant workers tending *Aphis varians*, which produces less honeydew than conifer aphids (Cushman & Addicott 1991). If colonies of a higher-ranked aphid species were provided, *L. niger* workers changed their behavior and abandoned the lower-ranked species and collected honeydew in colonies of the higher-ranked species (Fischer et al. 2001). As an invasive mealybug in China, *P. solenopsis* is widely associated with ghost ants, and ghost ants strongly defend the mealybugs against their natural enemies (Zhou et al. 2014). Sugar preference of ghost ants may lead to effective protection for mealybugs. Similarlry, Zhou et al. (2015) showed that *S. invicta* exhibited a significant preference for melezitose, and a melezitose-treated arena was visited more intensively by *S. invicta* than arenas treated with other sugars. Therefore, honeydew preference of ghost ants may contribute to the invasion by *P. solenopsis*.

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