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Fire Ant- Hemipteran Mutualisms: Comparison of Ant Preference for Honeydew Excreted by an Invasive Mealybug and a Native Aphid

by

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ABSTRACT

Interaction between ants and honeydew-producing hemipterans is defined as mutualism which is beneficial for both species. Red imported fire ants, *Solenopsis invicta*, who tend the honeydew-producing hemipteran insects, can help reduce their predators and parasites. In return, ants receive honeydew as an important food resource. In this study, we tested the foraging intensity (FI), weight change and honeydew consumption (HC) of *S. invicta* on *Phenacoccus solenopsis*, *Myzus persicae* and infested plants by mixed-colony (both *P. solenopsis* and *M. persicae*) . Our results showed that FI of *S. invicta* was gradually increasing with time on the plants infested by aphids and the mixed-colony, while inverse situation was found on mealybug- infested plants. Within 10 and 15 days, FI on aphid and the mixed-species infested plant was significantly more than that on the mealybug infested plant. We compared the ant weight between the two moving directions, and the result showed that the weight of downward ants was significantly heavier than upward ants except that on the mealybug infested plant after 15 days. The study also indicated that there was no observable difference of HC among the three kinds of honeydew resource in one day and five days, while HC on aphid and the mixed colony infested plant in 10 and 15 days was significantly more than that on mealybug-infested plants.

Key words: *Solenopsis invicta*, *Myzus persicae*, *Phenacoccus solenopsis*, foraging intensity, honeydew consumption.

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INTRODUCTION

Mutualistic interactions between ant species and honeydew-producing hemipteran insects, such as aphids, scales, mealybugs, membracids and lepidopteran larvae, have been described extensively in various ecosystems (Nixon 1951, Buckley 1987b, Buckley 1987a, Holway *et al*. 2002, Ness & Bronstein 2004). Ants tend honey-producing hemipteran insects by reducing not only the predation and parasitism by natural enemies but also the risk of fungal infection. In return, the ants receive abundant honeydew from hemipteran insects as food (Banks & Macaulay 1967, Tilles & Wood 1982, Yao *et al*. 2000, Standler & Dixon 1998). Honeydew excreted by hemipterans is considered to be an important food resource for ants because it contains sugars mixed with various amino acids and energy-rich materials (Hölldobler & Wilson 1990, Douglas 1993, Tobin 1994, Davidson *et al*. 2004). Previous studies showed that *S. invicta* colonies grew substantially larger when supplied with insect prey and honeydew produced by the invasive mealybug *Antonina graminis* (Maskell) (Helms & Vinson 2008). The intensity of mutualism between ants and honeydew-producing hemipterans is involved with various factors, such as host density, host plant quality, species and density of hemipterans and ants (Addicott 1978, Addicott 1979, Auclair 1963, Cushman 1991, Breton & Addicott 1992, Bristow 1984).

Hibiscus rosa-sinensis is commonly infested by ant-tending aphids and mealybugs such as *Myzus persicae* and *Phenacoccus solenopsis* in South China. *M. persicae* is a native species which has abundant population density in the field. The mealybug *P. solenopsis* is native to the US and has spread throughout the world (Fuchs *et al*. 1991). It has a wide geographic distribution and can be found in Central America, South America and Africa (Williams & Willink 1992, Culik & Gullan 2005). Recently, *P. solenopsis* was reported to be an important invasive species in Southern China (Lu *et al*. 2008). The red imported fire ant, *Solenopsis invicta,* is a new invasive pest in South China. Negative effects of *S. invicta* on agriculture and forestry production, human health and poultry production have been reported in South China (Zeng *et al*. 2005). Like the aphid *M. persicae*, we found that *S. invicta* were also attracted by the honeydew-producing *P. solenopsis* in the field. In this study, we compare the FI and HC of *S. invicta* on the plant infested by *M. persicae*, *P.*

solenopsis and the mixed-colony respectively, as well as to test the hypothesis that *S. invicta* had higher FI and HC on a plant infested by mixed-colony than that on a plant which was infested by a single colony of *M. persicae* or *P. solenopsi*s.

MATERIALS AND METHODS

Host plants

H. rosa-sinensis was purchased from a commercial horticultural farm. All plants had 25-30 uninoculated leaves and were approximately 25-30 cm in height. Each plant was cultivated in plastic flowerpots (the diameters of the upper and lower edges were 18 cm and 14 cm, respectively, with a height of 17 cm) in greenhouses.

Mealybugs and Aphids

Colonies of *P. solenopsis* and *Myzus persicae* were fed on *H. rosa-sinensis*. The 1st instar *P. solenopsis* and *M. persicae* nymphs were inoculated on each plant and raised for several generations. All colonies were reared in the laboratory with the temperature maintained at 27 ±2°C and a relative humidity of 60-70%.

Fire ants

Colonies of *S. invicta* were collected from the suburb of Guangzhou and reared in plastic boxes (116 L). All colonies were separated from the soil by dripping water into plastic boxes until the colonies floated (Jouvenaz *et al.* 1977). The ants were removed and reared in plastic boxes with tubes filled with distilled water. Colonies were divided into several small colonies (approximately 1.0 g workers and one queen) measured with a microbalance (Sartorius, BS, 224S). The ants were placed in a 9-cm plastic Petri dish with moist plaster, which served as an artificial nest. The ants were given fresh live *Tenebrio molitor* worms, and a 10% solution of honey mixed with water (50 ml) weekly. The colony was assigned randomly to each experimental treatment.

H. rosa-sinensis seedling leaves were inoculated with 60 3rd instar *P. solenopsis* and *M. persicae*. Artificial nests of *S. invicta* were transferred to plastic cases (40 cm × 28 cm × 22 cm). After 24 h, mealybug and aphid-infected plants were placed into each plastic case. A plastic hose (1.5 cm diameter)

was used to build a bridge between the ants' nest and the basic stem of the plant to allow worker foraging. We randomly collected 30 workers from the bottom stalk as they were moving toward the hemipteran colony since the beginning of the experiment, and 30 more were collected after 1, 5, 10 and 15 days as they were returning from the colony. Weights of the ants before and after foraging were measured with a microbalance (Sartorius, BS, 224S). Workers' weight change of *S. invicta* was viewed as an indirect measure of *P. solenopsis* and *M. persicae* honeydew consumption. Meanwhile, in order to measure the ant foraging intensity, we also counted all the ants present on each plant. In addition, honeydew consumption and foraging intensity of *S. invicta* were studied as was described in the preceding experiments when both mealybugs and aphids were present on the same plant (mixed-colony) (60 3rd instar nymph each hemipteran species). The studies were conducted in an enemy-free laboratory. All treatments were replicated ten times.

STATISTICAL ANALYSIS

Difference of FI and HC of *S. invicta* between mealybug, aphid and mixed-colony inoculated plants and in four different experimental times were analyzed using a one-way ANOVA followed by LSD tests for multiple comparisons. Changes in ant weight between traveling up and down were analyzed with paired-sample t-tests. All statistical analyses were conducted using SPSS, version 14.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Foraging intensity of *S. invicta*

We found that FI on mealybug-infested plants was gradually decreasing, the number of foraging ants in 15 days was significantly smaller than that after one day ($F_{3,36}$=3.406, P=0.028, Fig.1.A). FI on the plants infested by aphids or mixed-colony gradually increased, and the number of foraging ants on aphid-infested plants after 10 and 15 days was larger than that after one day ($F_{3,36}$=3.749, P=0.019, Fig.1.A). There was no marked difference of FI on the plant infested by mixed-colony among the four tests ($F_{3,36}$=1.323, P=0.282, Fig.1.A). In addition, there was no significant difference of FI in one day among the three kinds of honeydew resources ($F_{2,27}$=1.967, P=0.159, Fig.1.B), while the number of foraging workers on the plant infested by aphids

and the mixed-colony in 10 and 15 days was significantly larger than that on mealybug-infested plant after one day ($F_{2,27}$=9.701, P=0.001; $F_{2,27}$=15.042, P=0.000, Fig.1.B).

Honeydew consumption of *S. invicta*

We recorded the difference of the weights of the workers moving in two directions. Our results indicated that the weights of all downward workers were significantly heavier than upward ones on plants inoculated with the three kinds of heipteran species in the four tests, while only the result on mealybug-infested plants in 15 days was significant (Fig.2.A, B and C). We also calculated the HC of *S. invicta* under different honeydew resources for four

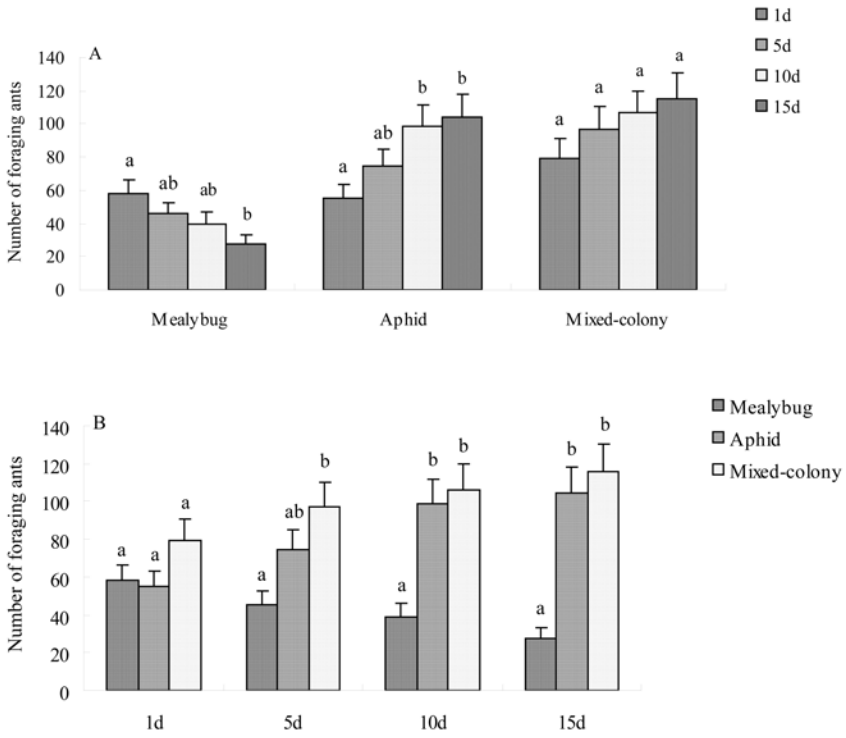

Fig.1.Comparision of the average number (M±SE) of foraging ants per plant (A): the same honeydew resource and different testing time; (B): the same testing time and different honeydew resource. The same letter on bars indicates no significant difference (P≥0.05).

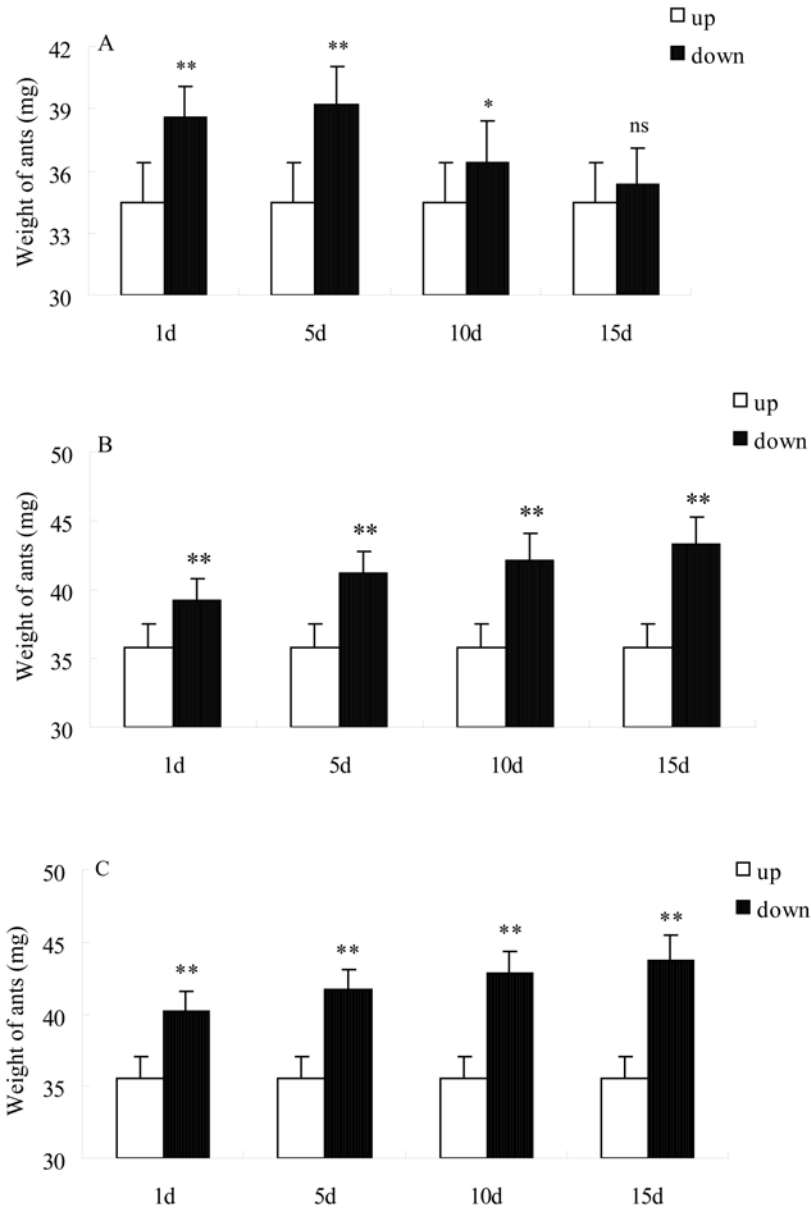

Fig.2. Mean (±SE) weight of workers traveling up (▫) and traveling down (▪) (A): *H. rosa-sinensis* infested with *P. solenopsis* only (B): *H. rosa-sinensis* infested with *M. persicae* only (C): *H. rosa-sinensis* infested with both *P. solenopsis* and *M. persicae*, (* indicate $P<0.05$, ** indicate $P\geq0.01$).

different testing times. The results showed that when plants were inoculated with the mixed-colony, there was no significant difference in HC of *S. invicta* among all the four testing times ($F_{3,36}$=1.157, P=0.339 , Fig.3.A). Compared with HC in one day on aphid-infested plants, HC of *S. invicta* increased after 15 days ($F_{3,36}$=2.398, P=0.084 , Fig.3.A). But the trend on mealybug-infested plants was reversed ($F_{3,36}$=3.688, P=0.021 , Fig.3.A). In addition, there was no observable difference of HC among the three kinds of hemipteran species after one day and five days ($F_{2,27}$=0.361, P=0.700; $F_{2,27}$=0.373, P=0.692, Fig.3.B), while HC on the plants infested by aphids and the mixed-colony after 10 and 15 days was significantly larger than that on mealybug-infested plants ($F_{2,27}$=6.023, P=0.007; $F_{2,27}$=9.292, P=0.001, Fig.3.B).

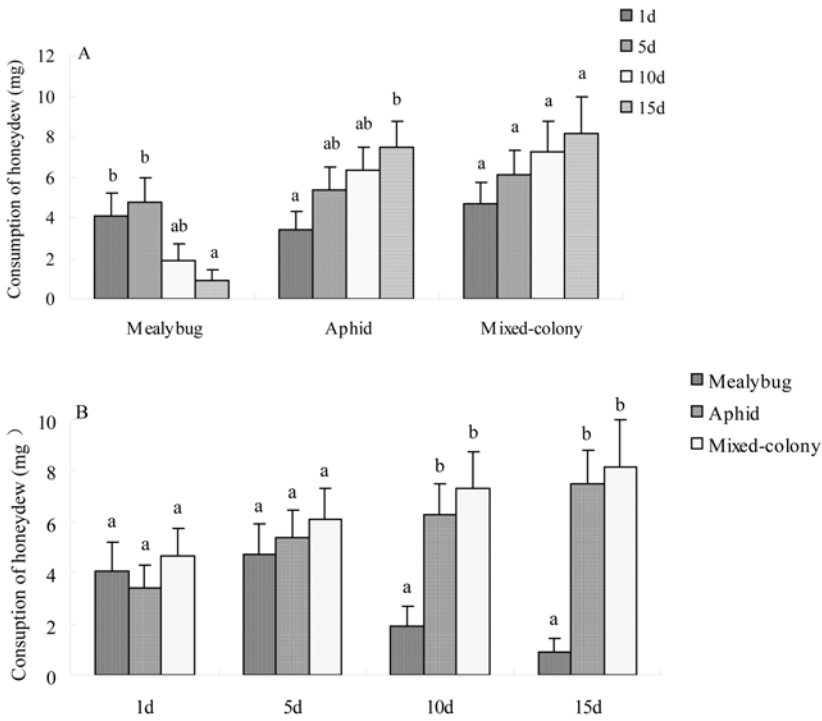

Fig.3.Comparision among the average weight (M±SE) of honeydew consumption
(A): the same honeydew resource and different testing time; (B): the same testing time and different honeydew resource. The same letter on bars indicates no significant difference (P≥0.05).

DISSCUSSION

Our results demonstrated that FI and HC of *S. invicta* decreased on mealybug-infested plants and increased on the plants infested by aphids or the mixed-colony (Fig.1.A, Fig.3.A). Compared with the cases on the plant infested by single-colony, there was no significant increase of FI and HC of *S. invicta* when ants had access to the plants infested by the mixed-colony (Fig.1.B, Fig.3.B). We conclude that FI and HC of *S. invicta* were involved with the quantity of honeydew produced, the more honeydew produced by hemipterans, the more ants attracted to the infested plants. FI and HC of *S. invicta* on the plant infested by aphids and mixed-colony after 10 and 15 days was significantly more than that on mealybug-infested plants after one day (Fig.1.B, Fig.3.B). Those results indicated that the *M. persicae* colony could produce more honeydew than *P. solenopsis* in 10 and 15 days. In addition, the quantities of honeydew produced between *M. persicae* and the mixed-colony showed no significant difference. Different reproductive rates and inter-specific competition between *M. persicae* and *P. solenopsis* could be responsible for the above results. The developmental period of *P. solenopsis* from immature crawler to adult stage (females) was approximately 9-16 days with 23.3-30.2 and 40.5-92.5% RH (Vennila *et al*. 2010). The developmental period of *M. persicae* from nymph to adult was approximately 5-6 days (Liu 1991). A higher reproductive rate of *M. persicae* may lead to decreasing population and fitness of *P. solenopsis* when *M. persicae* and *P. solenopsis* feed on the same plant. This may explain why ants had stronger FI and more HC on the plants infested by mixed colony than on those only infested by *M. persicae*. Our results accorded with the report that ants usually visited a rich hydrocarbon source with higher intensity rather than a less rich one, and would exploit much closer and more worthy sugar resources within their foraging range (Hölldobler & Wilson 1990, Bonser *et al*. 1998, Mailleux *et al*. 2000). Previous studies showed that ants prefer honeydew which contained trisaccharides such as raffinose and melezitose (Vökl *et al*. 1999). *Lasius niger* showed marked preferences when collecting honeydew from three aphid species living on tansy (Fischer *et al*. 2001).

Therefore, difference of honeydew quality between *P. solenopsis* and *M. persicae* may be another reason for the results seen here. However, the composition of honeydew excreted by *P. solenopsis* and *M. persicae* should be further studied.

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