Effects of Honeydew of *Phenacoccus solenopsis* on Foliar Foraging by *Solenopsis invcta* (Hymenoptera: Formicidae)

by

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ABSTRACT

The olfactory response of fire ants to plant leaves, mealybugs and the honeydew excreted by mealybugs was tested with a Y-tube olfactometer. The foraging activities of fire ants on three plants were also measured. Our results showed that plant leaves and mealybugs alone had no significant attraction to the fire ant workers, while fire ants could be obviously attracted by honeydew. The selection rate of fire ants on honeydew of *Hibiscus rosa-sinensis*, cotton (*Gossypium spp.*) and tomato (*Solanum lycopersicum*) was 60.22%, 57.45% and 64.29% respectively. When mealybugs were present on plants, fire ant workers foraged more frequently on the plants than controls ($P<0.05$). As to different plants, fire ants preferred foraging on tomato (66.3 per plant) to *Hibiscus rosa-sinensis* (50.4 per plant) and cotton (45.1 per plant). However, there was no significant difference in foraging frequency of fire ants on the three kinds of plant, with 24.9, 22.9 and 32.3 ants foraging per five minutes respectively.

Key words: *Solenopsis invicta*, *Phenacoccus solenopsis*, honeydew, preference, foraging activity.

INTRODUCTION

The excretions of Homopterans, called honeydew, are rich in hydrocarbons, amino acids, protein and water (Way 1963, Fischer *et al.* 2002), and held a special attraction to ant species (Way 1963, Buckley 1987). Ants attend honey-producing Homopteran insects by reducing not only the predation and parasitism by natural enemies but also the risk of fungal infection. In return, the ants obtain excreted honeydew from Homopteran insects as food (Way 1954, Banks & Macaulay 1967, Tilles & Wood 1982, Yao *et al.* 2000, Standler & Dixon 1998).

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Fire ants have invaded a variety of ecosystems and habitats (Vinson 1997, Holway *et al.* 2002), and established a variety of mutual-beneficial relations with honey-producing Homopteran insects, such as aphids and scale insects which excrete honeydew to attract fire ants to forage, while the Homopterans benefit by having the fire ants ward off potential predators (Eubanks 2001, Eubanks *et al.* 2002, Harvey & Eubanks 2004, Kaplan & Eubanks 2005). Our preliminary results of field investigations show that the honeydew produced by *Phenacoccus solenopsis*, a new invasive pest in South China (Wu & Zhang, 2009) has a strong attraction for the red imported fire ant, *Solenopsis invcita*. But the mutual relationship between the fire ant and mealybug remains unknown. This study tested the effects of honeydew of *P. solenopsis* produced in three plants on foliar foraging by *S. invcta* indoors, which would be helpful for understanding the relationship between these two invasive species.

MATERIALS AND METHODS

Insects

S. invcta colonies and *P. solenopsis* were collected from the suburb of Guangzhou and maintained in the laboratory for bioassays. The collected ants were fed with a mixture of 10% honey and live insects (*Tenebrio molitor* L.). A test tube (25 mm×200 mm), which was filled partially with water and plugged with cotton, was used as a water source. *P. solenopsis* was fed on *H. rosa-sinensis*. Ants and mealybugs were maintained in the laboratory at 25 ± 2°C.

Plants

We grew cotton and tomato seedlings whose seeds were purchased from the local market and *P. solenopsis* seedlings were purchased directly from a nursery. All seedlings of the three plants were used for bioassay at the height of 55-60cm.

Olfactometer

The Olfactometer mainly consisted of the following accessories which were connected with the odorless silicone tube: circulating water pumps, Y-tube tube boom, distilled water bottles, and odor source bottles. The length and diameter of the main and side arms were 19cm and 2.5cm respectively, with

75° as the angle of two sides. Gauze was fixed in the side arms to block the path to the odor bottles (Fig. 1).

Bioassay

Olfactory response test

The tested component was put into one of the odor source bottles, and distilled water was put into the other one as a control. 100 fire ant workers were induced to the main arm, and the number of workers which went through one or the other of the side arms and stayed for 30 seconds (regarded as reaction to the odor) were recorded for ten minutes after the beginning of the test. 10 replicates were used for each treatment. 95% ethanol was used to clean the olfactometer after each replicate, then it was washed with distilled water, and dried. The two side arms were exchanged in turn for treatments and control.

The odor sources tested in the experiments were as follows: (1) Five mealybugs, one of each instar; (2) One leaf (approximately 38 cm^2) of each plant species with or without (3) honeydew of *P. solenopsis*. To collect the honeydew, we induced five 3rd instar mealybugs on the leaves in a Petri dish, and we wrapped the petiole with moist cotton. Mealybugs were removed after 24h and the leaves with honeydew were used for the olfactory response test.

Selection Rate (%) was considered to be = (Number of ants selecting the treatment odor / Total number of ants in in control and treatment recordings) × 100%

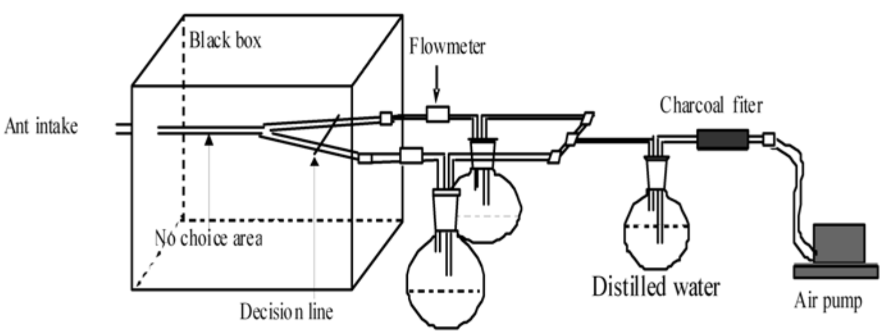

Fig. 1 Sketch map of Y-tube olfactometer

Foraging activity of *S. invcta*

Plant seedlings were cultivated in plastic pots of uniform size (diameter of upper bottom and lower bottom were 17cm and 12cm respectively, with the height as 14cm). The ektexine was painted with fluon to prevent ants from climbing up to plants from the outer surface of pots. 30 3rd instar mealybugs were induced to the seedling leaves, and a certain amount of Vaseline was deposited on the base of the plants to prevent escape by the mealybugs. We placed the pot and ant's artificial nest in a big plastic box (50cm×40cm×16cm) after the mealybugs colonized on the plants for 24h. One gram (about 950-1000 individuals) workers and one queen were placed in a plastic box (19cm×12cm×8cm) as an artificial nest, with water supplied everyday. When the bioassay began, the artificial nest was placed in the big box, and a plastic hose was used to build a bridge between the ants' nest and plant seedling for the foraging of workers. 24 hours later, we counted the number of the ants foraging on the seedlings. Meanwhile, we recorded the number of ants passing the "foraging bridge" per five minutes. Ten replicates were conducted.

Statistical analysis

T-test for paired data was used to compare the selection rate of workers between the treatment and control. Variations of foraging activity of workers among three plants were analyzed using analysis of variance. All the statistical analyses were conducted using the SPSS13.0 software package.

RESULTS

Olfactory response of *S. invicta*

Compared with control, we found that all of the leaves of three plants tested had no obvious attraction on the fire ant workers ($P>0.05$). The results in Fig. 2 indicated that the selection rate in both of the treatment and control for the same plant were nearly 50%.

Compared with controls, all nymphs and adults of *P. solenopsis* had no significant attraction for the fire ant workers ($P>0.05$). The results in Fig. 3 indicated that the selection rate in both of the treatment and control in the same plant were nearly 50%.

However, the honeydew excreted by the mealybugs attracted the fire ants significantly ($P<0.05$), while no difference showed in treatments among the three plant species. The selection rate of workers on the treatment and control

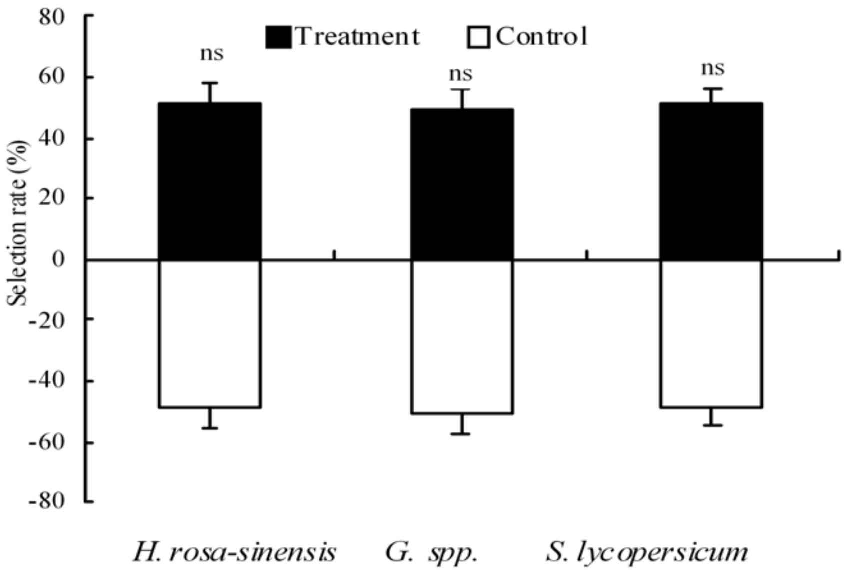

Fig. 2 Selectivity of fire ants to three plant species leaves (ns on bars indicates no significant difference between the treatment and control in the same plant)

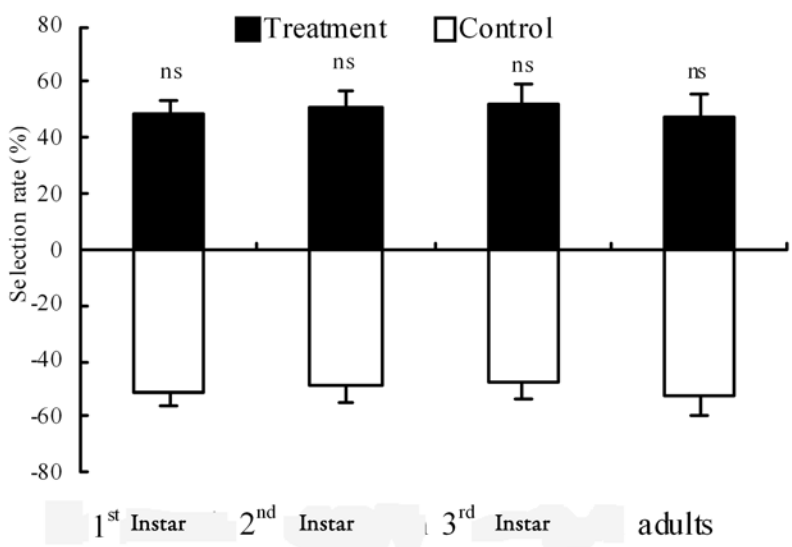

Fig. 3 Selectivity of fire ants to mealybugs (ns on bars indicates no significant difference between the treatment and control in the same stage of *P. solenopsis*)

in *H. rosa-sinensis*, *G. spp.*, and *S. lycopersicum* were 60.22% and 39.78%, 57.45% and 42.55%, and 64.29%and 35.71% respectively (Fig. 4).

Foraging activity of *S. invcta*

Foliar foraging numbers of fire ants increased more than 2-fold when mealbugs were present compared with control (Fig. 5; P>0.05). Numbers of foraging workers on the tomato plants was significantly more than on cotton and *Hibiscus* when *P. solenopsis* was present. The number of foraging workers in the treatment and control on *H. rosa-sinensis*, *G. spp.*, *S. lycopersicum* were 50.4/seedling and 16.6/seedling, 45.1/seedling and 12.5/seedling, and 66.3/seedling and 14.4/seedling respectively (Fig. 5).

There was a significant difference in foraging frequency of fire ants when mealybugs were present compared to when they were absent (Fig. 6; P>0.05), while there was no difference among the three plant species treatments. The number of foraging workers in the treatment and control on *H. rosa-sinensis*, *G. spp.*, *S. lycopersicum* were 24.9 and 8.6 per five minutes, and 22.9 and 10.1/seedling, per five minutes, 32.3/seedling and 9.7 per five minutes respectively (Fig. 6).

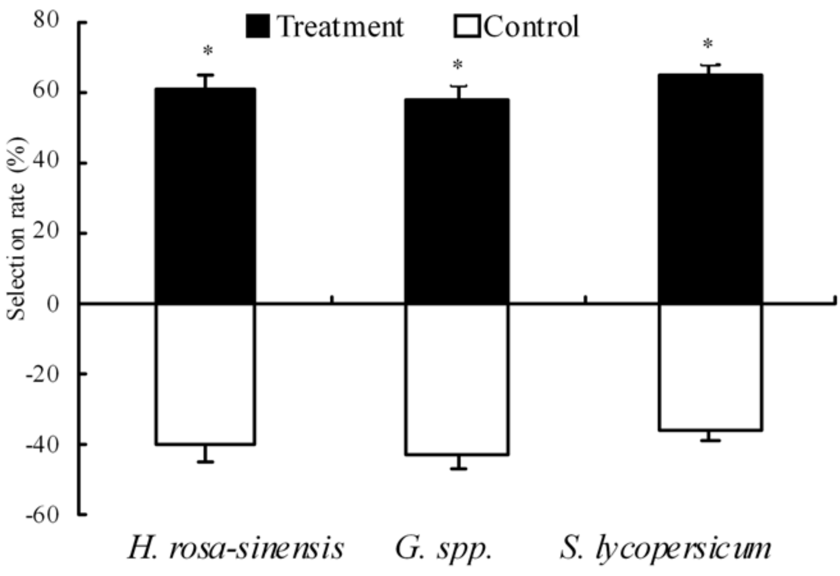

Fig. 4 Selectivity of fire ants to honeydew in three plants (* on bars indicates significant difference between the treatment and control in the same plant)

DISCUSSION

We conclude that what attracted the fire ants was the honeydew excreted by *P. solenopsis* but not the mealybugs themselves. Honeydew also facilitated foliar foraging by *S.invcta*, while the foraging preference on tomatoes indicated

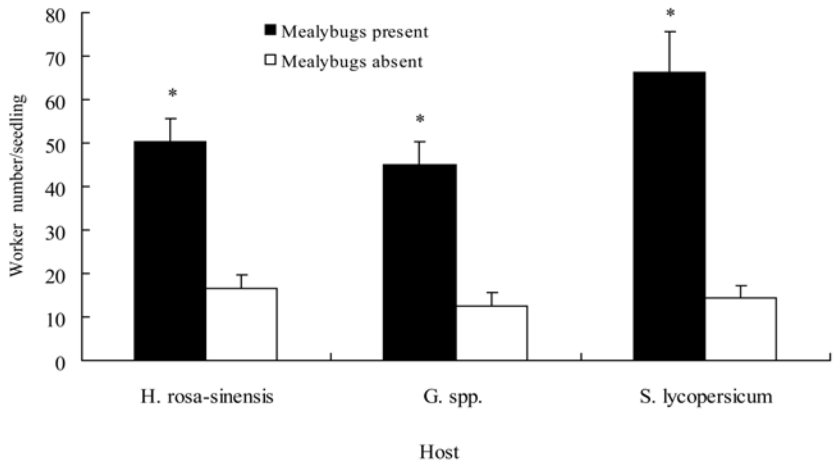

Fig. 5 Effects of mealybugs on quantity of foraging ants on plants (* on bars indicates significant difference between the treatment and control in the same plant)

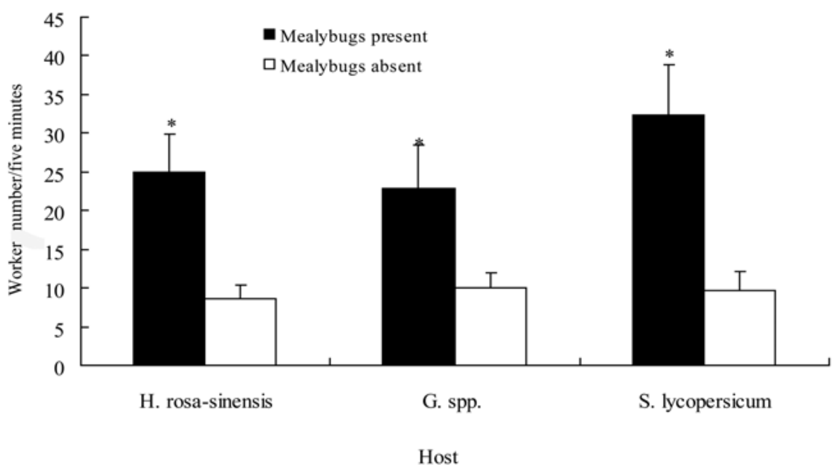

Fig. 6 Effects of mealybugs on foraging frequency of foraging ants (* on bars indicates significant difference between the treatment and control in the same plant)

that the preference of *P. solenopsis* for this species might affect the relationship between the fire ant and mealybug (*P. solenopsis* preferred to feed on tomato and excreted more honeydew, which supplied more food for the fire ants). Our results corroborate precious reports that honeydew could stimulate the foraging activity of fire ants and more workers were attracted to cottons with higher density of aphids (Kaplan & Eubanks 2005).

Homopteran honeydew contains a variety of carbohydrates such as monosaccharides, disaccharides and polysaccharides. The polysaccharides can include pine trisaccharide, raffinose, and melezitose which may have some special activity on invasive ants (Völkl *et al.* 1999). The chemical difference among the honeydew produced on different plants may also explain the fact that fire ants foraged more on tomato than the other two plants. Therefore, further studies on honeydew and nectar composition will contribute to a clearer understanding of the interactions between fire ants and *P. solenopsis*.

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