INTERACTIONS BETWEEN GHOST ANTS AND INVASIVE MEALYBUGS: THE CASE OF *TAPINOMA MELANOCEPHALUM* (HYMENOPTERA: FORMICIDAE) AND *PHENACOCUS SOLENOPSIS* (HEMIPTERA: PSEUDOCOCCIDAE)

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ABSTRACT

Mutualism between ants and honeydew-producing hemipterans is a common phenomenon in ecosystems, and interactions between ants and hemipterans have been extensively studied. The invasive solenopsis mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), and the ghost ant, *Tapinoma melanocephalum* (F.) (Hymenoptera: Formicidae), are widely distributed in China. Previous studies showed that the ghost ant can promote the fitness of the invasive mealybug when natural enemies of the mealybug are excluded. In this study, we investigated the interactions between the ghost ant and the invasive solenopsis mealybug when the mealybug’s natural enemies were included. Our results indicated that the honeydew produced by mealybugs facilitated colony growth of the ghost ant significantly more than by allowing them to feed on the mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Tending by the ghost ant increased mealybug survival on the plants when six-spotted zigzag lady beetle larvae, *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae), were present. Both lady beetle adults and the mealybug parasitoid, *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae), showed strong avoidance responses to ghost ants. The fecundity of lady beetle adults and the hatching rate of their eggs were significantly reduced by ghost ants. Also ghost ant tending appeared to negatively affect parasitoid performance. The number of mummified mealybugs on *Hibiscus rosa-sinensis* L. (Malvales: Malvaceae) in the presence of ghost ants was significantly less than the number without ants.

Key Words: *Aenasius bambawalei*, ant-mealybug mutualism, colony growth, *Cheilomenes sexmaculata*, parasitoid, predator

RESUMEN

El mutualismo entre hormigas y hemípteros que producen mielcilla es un fenómeno común en los ecosistemas, y las interacciones entre las hormigas y hemípteros han sido ampliamente estudiados. La cochinilla invasora solenopsis, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), y la hormiga fantasma, *Tapinoma melanocephalum* (F.) (Hymenoptera: Formicidae), están ampliamente distribuidas en China. Estudios anteriores mostraron que la hormiga fantasma puede promover el desempeño de la cochinilla invasora cuando se excluyen los enemigos naturales de la cochinilla. En este estudio, hemos investigado las interacciones entre la hormiga fantasma y la cochinilla solenopsis invasiva cuando se incluyeron los enemigos naturales de la cochinilla. Nuestros resultados indican que la mielcilla producida por cochinillas facilitó un crecimiento de las colonias de la hormiga fantasma más significativo que permitiendo alimentarse sobre el gusano de la harina, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). El cuidado de las cochinillas por la hormiga fantasma aumentó la sobrevivencia de las cochinillas en las plantas cuando las larvas del cocinelido con seis manchas zigzag, *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae), estuvieron presentes. Tanto los adultos de escarabajos cocinelidos y el parasitóide de la cochinilla, *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae), mostraron fuertes respuestas de evitación a las hormigas fantasmas. La fecundidad de los adultos del cocinelido y la tasa de eclosión de los huevos se redujeron significativamente por las hormigas fantasmas. También el cuidado de las cochinillas por la hormiga fantasma parecía afectar negativamente el desempeño del parasitóide. El número de las cochinillas momificados en *Hibiscus rosa-sinensis* L. (Malvales: Malvaceae) en presencia de hormigas fantasma fue significativamente menor que el número sin hormigas.

Palabras Clave: *Aenasius bambawalei*, mutualismo hormiga-cochinilla, crecimiento de colonias, *Cheilomenes sexmaculata*, parasitoides, depredadores
Interactions between ants and hemipterans are common and ecologically important (Hölldobler 1990; Stadler & Dixon 2005; Styrsky & Eubanks 2007). Ants and hemipterans are often abundant species that often live in the same habitats (Stadler & Dixon 2005); and interactions commonly occur between them (Helms & Vinson 2002; Brightwell & Silverman 2010). Ant-tending can promote hemipteran colonies because ant species protect them from their natural enemies (Daane et al. 2007; Powell & Silverman 2010). In exchange for tending the hemipterans, the ants receive large amounts of honeydew in a consumer-resource mutualism (Holland et al. 2005). Hemipteran honeydew contains sugar and various amino acids (Yao & Akimoto 2002; Helms & Vinson 2008) that can be essential for the ant colony’s growth and survival.

The ghost ant, *Tapinoma melanocephalum*, (F.) (Hymenoptera: Formicidae) is a worldwide invasive species, whose native range is unknown but is believed to be Africa or Asia (Wheeler 1910). Ghost ant populations and infestations are reported in many areas of the United States, as well as in Canada, Puerto Rico, and the Caribbean Islands (Smith & Whitman 1992). *Tapinoma melanocephalum* forms polygyrous colonies that contain huge numbers of workers. Ghost ants show a preference for sweets, sugar, cakes, and syrups. Ghost ant workers frequently tend honeydew-producing insects and scavenged for dead insects in fields (Smith 1965). The ghost ant is highly adaptable in its nesting habits and has been present in China for a long time.

The mealybug, *Phenacoccus solenopsis*, Tinsley (Hemiptera: Pseudococcidae) is native to the US (Fuchs et al. 1991) and has spread throughout the world including Central America, South America, and Africa. It caused serious damage to cotton (*Gossypium* spp.; Malvales: Malvaceae) in India and Pakistan in 2005 (Nagrare et al. 2009). Currently, it is an important invasive species in south China (Lu et al. 2008), where it is frequently found on *Hibiscus rosa-sinensis* L. (Malvales: Malvaceae) and cotton. Zhou et al. (2012a) showed that tending by *Solenopsis invicta* Buren (Hymenoptera: Formicidae), had positive impacts on the fitness of *P. solenopsis* mealybug colonies under laboratory conditions when enemies are absent. However, few studies have attempted to determine whether the *T. melanocephalum* ghost ant also have positive impacts when enemies are present. Previous studies showed that the invasive fire ant, *S. invicta* established a close relationship with *P. solenopsis* in south China (Zhou et al. 2012a). Fire ants frequently protect mealybugs from their natural enemies, and honeydew produced by *P. solenopsis* also facilitates the survival of fire ant colonies (Zhou et al. 2013). Mutualism between these 2 alien species may facilitate the invasion success of both (Zhou et al. 2012a; Zhou et al. 2012b; Zhou et al. 2013). In China, the distribution of *T. melanocephalum* is more extensive than that of fire ants, and interactions between *T. melanocephalum* and mealybugs may produce more profound ecological consequences. However, few studies have investigated the effects of tending by *T. melanocephalum* on *P. solenopsis* and its natural enemies. Also, we wanted to clarify clear how the *T. melanocephalum* ghost ant benefits from the honeydew produced by *P. solenopsis*. In this study, we conducted a series of experiments to examine the effects of *P. solenopsis* honeydew on the growth of *T. melanocephalum* colonies and the effects of *T. melanocephalum* -tending on the survival of the mealybugs and their natural enemies.

**MATERIALS AND METHODS**

**Cotton Plants**

Cotton plants (*Gossypium* spp.; Malvales: Malvaceae) were cultivated in greenhouses in plastic flowerpots (18 cm diam × 17 cm height). Each plant was approximately 25-30 cm in height and had 15-20 true leaves.

**Insects**

Colonies of *P. solenopsis* were collected from a suburb of Guangzhou and fed on cotton. First-instar mealybugs were inoculated on cotton and the colony was raised for several generations. Cotton plants with established mealybug colonies were used for subsequent experiments. Mealybug colonies were reared in the laboratory at 27 ± 2 °C and 60–70% RH and 16:8 h L:D. Twenty colonies of *T. melanocephalum* ghost ants were collected from the campus of South China Agricultural University. Colonies were separated from the soil by dripping water into plastic boxes containing soil and ants until the ants floated to the surface (Jouvenaz et al. 1977). One *T. melanocephalum* sub-colony (approximately 1.0 g fresh weight of ants) from each colony was weighed out by a microbalance (Sartorius, BS, 224S). Each sub-colony included one queen, adult workers, pupae, larvae, and eggs. Each sub-colony was placed in a 9-cm plastic Petri dish, which served as an artificial nest. *T. melanocephalum* sub-colonies were maintained with distilled water plus a 10% honey solution, which was distributed through tubes (1.5 cm diam × 12 cm length) plugged with cotton.

The natural enemies of mealybugs, including the predatory larvae of the six-spotted zigzag lady beetle *Cheilomenes sexmaculata* (F.), (Coleoptera: Coccinellidae), and the parasitoid, *Aenasisus bambawalei* Hayat (Hymenoptera: Encyrtidae), were collected from *H. rosa-sinensis* in the field. The lady beetles were fed mealybug nymphs in the laboratory at 27 °C ± 1 °C and 16:8 h L:D. *Aenasisus*
*bambawalei* were collected as mummified mealybug and isolated in gelatin capsules (10 mm in length) until adult emergence. After emergence, pairs of wasps were established in petri dishes and copulation was observed in all pairings. Female wasps were used in experiments 24 h after mating (Zhou et al. 2013b).

**Experiment 1: Food supply and *T. melanocephalum* Colony Growth**

We prepared 100 Fluon-coated plastic boxes (36 \( \times \) 20 \( \times \) 15 cm) and filled them with soil and transferred potted cotton seedlings into the plastic boxes. A sub-colony of *T. melanocephalum* was then placed into each plastic box, and within 24 h, the ants had left the Petri dish and had constructed a new nest in the soil. The Fluon coating of the inner surfaces of each plastic box prevented the ants from escaping. Our experiments included the following treatments: (1) no food (water supply only); (2) mealworms (*Tenebrio molitor*; Coleoptera: Tenebrionidae), 2 g every 2 days; (3) 15 mL of honey solutions every 2 days; (4) low-density mealybugs (60); and (5) high-density mealybugs (400). Mealybugs were transferred to the potted cotton plants as first instars. A test tube (10 \( \times \) 1.5 cm) filled with distilled water and sealed with a cotton plug was used as a constant source of water. To prevent drying, the soil in each small plastic case was sprayed with water every 3 days. Each treatment was replicated 15 times and after 10 weeks, all surviving ants were extracted, counted, and weighed on a microbalance (Sartorius, BS, 224S).

**Experiment 2: Ant Tending and Predation on *P. solenopsis***

Cotton plants with 15-20 true leaves (30-40 cm tall), were each planted in a plastic boxes filled with soil. Each plastic box was covered with nylon netting surrounded by a cage (70 \( \times \) 70 \( \times \) 100 cm). A sub-colony of *T. melanocephalum* (1 g, 1,000 workers and 1 queen per nest) was placed in each plastic box, and each sub-colony constructed a new nest in the soil as described above (at 27 ± 2 °C and 60–70% RH). Newly laid (approximately 8 h old) *C. sexmaculata* egg clusters of 30-60 eggs were collected and placed in each 10-cm Petri dish in a Fluon coated plastic box (n = 15). This experiment included 2 treatments: (1) 100 ghost ant workers, and (2) no ants. The number of the eggs that hatched in each box was recorded.

**Experiment 3: *Cheilomenes sexmaculata* Egg Viability**

*Cheilomenes sexmaculata* lady beetles were raised on mealybug nymphs in climate-controlled growth chambers under the same physical conditions as described above (at 27 ± 2 °C and 60–70% RH). Newly laid (approximately 8 h old) *C. sexmaculata* egg clusters of 30-60 eggs were collected and placed in each 10-cm Petri dish in a Fluon coated plastic box (n = 15). We transferred 150 third-instar mealybugs to each cotton plant (n = 15) and introduced 2 adult female parasitoids 24 h later. The behavior of the parasitoids was observed continuously and interactions recorded by using a digital video (Sony, HDR-CX240E). The number of visits to mealybugs by the parasitoids within 120 min was recorded. A visit was considered to be the duration of time from when a parasitoid initiated contact with mealybugs until it left, and the duration had to be more than one minute. After 2 weeks, all mealybugs and mummies were counted.

**Experiment 4: Ghost Ants and *Cheilomenes sexmaculata***

The same treatments were used in this experiment as in experiment 2, except that *A. bambawalei* parasitoids were included instead of *C. sexmaculata* larvae. We transferred 150 third-instar mealybugs to each cotton plant (n = 15) and introduced 2 adult female parasitoids 24 h later. The behavior of the parasitoids was observed continuously and interactions recorded by using a digital video (Sony, HDR-CX240E). The number of visits to mealybugs by the parasitoids within 120 min was recorded. A visit was considered to be the duration of time from when a parasitoid initiated contact with mealybugs until it left, and the duration had to be more than one minute. After 2 weeks, all mealybugs and mummies were counted.

**Experiment 5: Performance of *A. bambawalei* in the presence of *T. melanocephalum***

*Cheilomenes sexmaculata* adult females were given a choice to lay eggs in 1 of 2 adjacent areas in each plastic box (36 \( \times \) 20 \( \times \) 25 cm), i.e., an area occupied by *T. melanocephalum* ghost ants, and an area with ghost ants excluded. The plastic boxes were cleaned with alcohol and distilled water to eliminate any residual semiochemicals. Two plastic boxes were placed next to each other, each with an open window in the upper half of the adjoining wall. To prevent ants from crossing from one area into another, Fluon was applied halfway up the inside of each box below the window. However, lady beetles could still access both areas by climbing across or flying across the open window. A 14-cm Petri dish coated with Fluon was placed in the center of each box, and a cotton leaf with 150 second instar mealybugs was placed in the dish. The petiole of a cotton leaf was wrapped with moist cotton to maintain turgor. At the beginning of the experiment, 100 ghost ant workers were placed into each box (n = 15) along with 10 *C. sexmaculata* adults. Fluon was used to prevent mealybugs from escaping and ghost ants from invading. We counted the number of eggs laid in the Petri dishes and the number of lady beetles in each area at 4-h intervals during a 24 h period.
Statistical Analysis

All data were tested for normality using the Shapiro-Wilk test. For the data that were not normally distributed, the Mann-Whitney U-test was used to compare the differences in colony weight between treatments, the differences in mealybug survival with and without ants, differences in hatching rates of lady beetle eggs, and differences in the number of parasitoid visits and numbers of mummified mealybugs. Numbers of lady beetle adults and eggs in areas with and without ants were analyzed using a paired sample t-tests. All statistical analyses were conducted with SPSS version 14.0 (SPSS Inc., Chicago, Illinois).

RESULTS

Food Supply and T. melanocephalum Ghost Ant Colony Growth

Different foods resulted in markedly different ghost ants colony masses among treatments (Fig. 1; $\chi^2 = 49.885, p < 0.001$). There was no significant difference in colony mass between the no food, mealworm and low-density (60 first instars) mealybug treatments ($\chi^2 = 5.105, p = 0.078, df = 2$). Colony mass did not differ significantly between the high-density mealybug and sugar treatments ($U = 103.00, p = 0.694$). However, ghost ant colony mass was significantly greater with high-density mealybugs than with low ($U = 5.00, p < 0.001$).

Ant Tending and P. solenopsis Predation

The results showed that ghost ants exerted a positive effect on mealybug survival when lady beetle larvae were present on the plants. The number of surviving mealybugs on plants with ants was significantly greater than on plants without ants (Fig. 2; $U = 12.00, p = 0.004, 24$ h; $U = 8.50, p = 0.001, 48$ h; $U = 3.00, p < 0.001, 72$ h).

Ant Avoidance Behavior of Cheilomene sexmaculata Lady Beetles

Our results indicated that lady beetle adults strongly avoided plants on which ghost ants were present, and significantly more lady beetles were observed in areas without ants than in areas with ants (Fig. 3A; $t = -8.531, df = 9, p < 0.001$). By 24 h the number of lady beetle eggs in the areas without ants had become significantly greater than in the areas with ants (Fig. 3B; $t = -3.829, df = 9, p = 0.004$).

Ghost Ants and Cheilomene sexmaculata Egg viability

Ghost ants reduced the viability of lady beetle eggs, since significantly fewer hatched in areas with ants than in areas without ants (Fig. 4; $U = 9.00, p = 0.002$).

Performance of Aenasius bambawalei in the Presence of Ghost Ants

Ant tending had a negative impact on the performance of A. bambawalei. There were fewer visits by wasps to mealybugs in the presence of ants (Fig. 5A; $U = 16.50, p = 0.011$) and there were more mealybugs mummified on plants without ants than on plants with ants (Fig. 5B; $U = 4.00, p = 0.001$).
The fire ant *S. invicta* protects *P. solenopsis* from its predators and parasitoids (Zhou et al. 2013). Honeydew produced by *P. solenopsis* improves the colony growth and fitness of *S. invicta* (Zhou et al. 2012). The mutualism between *P. solenopsis* and *S. invicta* facilitates population increases of both species. Our results demonstrate that *T. melanocephalum* and *P. solenopsis* benefit from conditional mutualism. Many studies have shown that honeydew of various hemipterans can support ant colony growth (Porter 1989; Davidson et al. 2004; Abbott & Green 2007). Honeydew produced by *P. solenopsis* was considered an important food resource for *S. invicta* because fire ant worker survival was significantly improved when workers were fed with *P. solenopsis* honeydew (Zhou et al. 2012). However, food derived from animal sources was also essential for colony growth of *S. invicta* (Helms & Vinson 2008). Ghost ant colony mass was significantly greater when the ants had access to *P. solenopsis* as opposed to mealworms. Ant tending facilitates the population growth of hemipterans, not only by reducing predation and parasitism from natural enemies, but also by reducing the risk of fungal infection (Way 1963; Stadler & Dixon 1998; Helms & Vison 2003; Daane et al. 2007). Tending by *S. invicta* can significantly improve the survival of *P. solenopsis* because of ant predation on ladybeetle larvae and interference with parasitoids (Zhou et al. 2013). Our results showed that *T. melanocephalum* defends *P. solenopsis* against its natural enemies. The number of lady beetle adults and mummified mealybugs in the areas without ants were significantly more than in areas with ants (Fig. 3A and Fig. 5B). Both *C. sexmaculata* and *A. bambawalei* strongly avoided ghost ants and the performances of both as natural enemies were decisively impeded. Lady beetle egg hatch was sharply decreased by ghost ants. *Tapinoma melanocephalum* workers effectively utilize their pygidial gland secretions as an alarm-defense system during aggressive encounters with other invaders (Tomalski et al. 1987), and this may have contributed to diminished natural enemy impacts on *P. solenopsis* in the present experiments. Previously we reported that *C. sexmaculata* and *A. bambawalei* attempt to avoid attacks by fire ants (Zhou et al. 2013). In this study, we found that ghost ants rarely attack predators and parasitoids, rather, chemical

**Fig 3.** Effects of ant tending on avoidance behavior of lady beetle adults in a choice test in which lady beetles could move between an area with ants and mealybugs and area with mealybugs but without ants. The numbers of eggs laid by lady beetles are shown in the right panel. ** above the bars indicate statistically significant differences between the treatment and the control (paired sample t-tests, $P = 0.01$).

**Fig 4.** Effects of ghost ants on percent egg hatch of *Cheilomenes sexmaculata* predators. ** above the bars indicate statistically significant differences between the treatment and the control (Mann-Whitney test, $P = 0.01$).
deterrence may be the defense strategy of ghost ants during aggressive encounters. Hübner & Völkl (1996) showed clearly that aphid hyperparasitoids avoid contact with ants. On the one hand, lady beetles avoid ants because the direct attacks by ants. Survival of *C. sexmaculata* on fire ants tended plants was significantly diminished (Zhou et al. 2013). Laying fewer eggs near ants is likely to be adaptation because ants can attack and damage lady beetles, especially larvae (Jiggins et al. 1993; Sloggett & Majerus 2003). On the other hand, ant semiochemicals may also contribute to the avoidance response. Lady beetles may avoid laying eggs in the patches that were attended by ants and the highest density of ant semiochemicals resulted in inhibition of egg laying (Oliver et al. 2008). By assessing ant density relative to aphid abundance, Harmon & Andrew (2007) also showed that lady beetles avoid foraging in patches with ants. Godeau et al. (2003) provided evidence for the ability of the lady beetle *Coccinella magnifica* Redtenbacher (Coleoptera: Coccinellidae) to detect the ant trail pheromone. *Coccinella magnifica* even used wood ant trails to reach aphid colonies (Sloggett et al. 1998).

In conclusion, ghost ants that defend mealybug are rewarded by an abundant honeydew supply, which serves as an essential food resource for colony growth. And ghost ants frequently disturb predators and parasitoids. Ant attacks had negative impacts on the lady beetles and the wasps. Since lady beetles are unable to tolerate ant attacks, they avoid arenas with ants. Performance of *A. bambawalei* parasitoids was significantly reduced by present of ghost ants. Our results demonstrated that a mutually beneficial relationship exists between ghost ants and the invasive mealybug under the pressure of enemies. Therefore, interactions between the 2 species may facilitate the invasion of *P. solenopsis*.

ACKNOWLEDGMENTS

We gratefully acknowledge that this study was supported by the National Natural Science Foundation of China (No. 31401808) and the Fundamental Research Funds for the Central Universities (No. 2014QC024).

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