Effects of a Native Ant, *Pristomyrmex pungens* (Hymenoptera: Formicidae) on the Population Dynamics and Spatial Distribution of the Invasive Mealybug *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae)

by

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ABSTRACT

The mealybug *Phenacoccus solenopsis* has been recently reported as a serious invasive insect pest in China. Earlier investigations suggest that some species of native ants may tend *P. solenopsis* in the field, and affect the population number and spatial distribution of *P. solenopsis*. To confirm this, we studied the relationship between *P. solenopsis* and the native ant *Pristomyrmex pungens* on *Dendranthema morifolium* plants in a garden spot. We found there was a significant positive correlation between population numbers of *P. solenopsis* and *P. pungens*, and the less the distance from nest, the greater the quantity of *P. solenopsis*. Additionally, the mean number of *P. solenopsis* increased significantly in the presence of *P. pungens*. *P. solenopsis* and *P. pungens* had strong crowding tendencies and these individuals were attracted to each other on all investigated dates except on August 6. Our results suggest the presence of *P. pungens* has effects on the population numbers and spatial distribution of *P. solenopsis* on *D. morifolium* plants, and this relationship between them may have an important effect on the population explosion and dispersal of the invasive mealybug.

Key words: mealybug, biological invasion, spatial distribution, *Phenacoccus solenopsis*, *Pristomyrmex pungens*

INTRODUCTION

Invasive species are now recognized as one of the most important threats to ecosystems (Vermeij 1996). Approximately 520 invasive species have entered

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China in the last century, and 50 species are listed as being among "100 of the world's worst invasive alien species," according to the World Conservation Union. Natural environments in China have suffered substantial damage from biological invasions. Recently, the mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has been recognized to be a problem of agricultural and ornamental plants in China. *P. solenopsis* was described originally from the U.S.A in 1898 (Tinsley 1898), and so far, it has been reported in other countries, such as Brazil, Pakistan and India (Hodgson *et al.* 2008). *P. solenopsis* was first reported in China in Guangzhou on *Hibiscus rosa-sinensis* (Wu & Zhang 2009). *P. solenopsis* feeds on numerous crops, weeds, ornamentals and medical plants, and adults and nymphs cause severe damage on leaves, fruit, stems and branches by feeding on phloem sap and exuding honeydew (Hodgson *et al.* 2008). It had been reported that 154 species of crops, flowers, and weeds were damaged by *P. solenopsis* (Arif *et al.* 2009). Such a wide host range may expedite invasion process of *P. solenopsis*, but the mechanisms associated with invasions remain unknown.

Some honeydew-producting species, such as aphids, mealybugs and some Lepidoptera larvae have a mutualistic relationship with ants (Way 1963). Mutualistic relationships could affect their spatial dispersion, and even improve the invasion of alien species (Simberloff & Von Holle 1999; Richardson *et al.* 2000; Bruno *et al.* 2003). Previous studies have focused mainly on the interactions between honeydew-producing hemipterans and invasive ants (Ness & Bronstein, 2004; Kaplan & Eubanks, 2005; Huang *et al.* 2010). However, interactions between invasive mealybugs and native ants have been less considered. Helms and Vinson (2002, 2003, 2008) reported that the invasive mealybug *Antonina graminis* may be an important nutritional resource for the invasive ant *Solenopsis invicta* in the southeast United States; in addition, the mealybug occurrence increased significantly with increasing proximity to *S. invicta* mounds. We have observed some species of native ants (e.g. *Pristomyrmex* sp., *Plagiolepis* sp., and *Tetramorium* sp.) tending the invasive mealybug *P. solenopsis* on *Dendranthema morifolium*, *Portulaca grandiflora*, *Torenia fournieri*. But this phenomenon has not been investigated. Hence, it is necessary to determine whether there is a positive correlation between the population numbers of native ant and *P. solenopsis*, and whether the distance between them affects the population number of *P. solenopsis*. In addition, it may be important to assess the spatial distribution patterns.

In this study, we investigate the tending native ant *Pristomyrmex pungens* (Hymenoptera: Formicidae) on *P. solenopsis* associated with *Dendranthema morifolium* (Asterales: Asteraceae) plants, and assess the relationship between population numbers of *P. pungens* and *P. solenopsis*. We also focus on the spatial distribution patterns of *P. pungens* and *P. solenopsis* during the growing period of *D. morifolium*. Our results will present an apparently important association occurs between the invasive mealybug *P. solenopsis* and the native ant, and that such associations may be an important effect on the population explosion and dispersal of the invasive mealybug.

MATERIALS AND METHODS

Study site

Our study was conducted at a garden spot in the Flower Research and Development Centre, Zhejiang Academy of Agricultural Sciences, Hangzhou, China. An area (180 m^2, 30 m in length and 6 m in width) with nearly two thousand pots of *D. morifolium* plants (20 pots per row and 100 pots per line) was chosen for our test. The upper diameter, bottom diameter and height of pots are 13.0 cm, 9.5 cm and 12.0 cm, respectively. The tested area is surrounded by some weed species, such as *Eleusine indica*, *Plantago asiatica*, *Conyza canadensis*.

Field investigation

From September to October, 2011, 300 pots of *D. morifolium* were chosen randomly, and the presence of *P. pungens* and *P. solenopsis* on each plant was investigated. Then, the amount of *P. pungens* and *P. solenopsis* which occurred simultaneously on the same plant were recorded. In order to determine whether the distance affects the quantity of *P. solenopsis*, distance from pots to ant nests with 10 levels (0.5m, 1.0m, 1.5m, 2.0m, 2.5m, 3.0m, 3.5m, 4m, 4.5m, and 5.0m) was designed, and 100 pots of *D. morifolium* in total was chosen, then the quantity of *P. solenopsis* was recorded.

System investigation

In order to make clear the effect of *P. pungens* on the population numbers and spatial-distribution patterns of *P. solenopsis*, a site nearly 30 m^2 was chosen. The site was divided into five quadrats (parallel and continual); each quadrant consisted of 30 pots of *D. morifolium* plants (total, 150 pots). The population numbers of *P. pungens* and *P. solenopsis* on *D. morifolium* plant in each quadrat were recorded. The test was performed on August 6, August 11, August 19, August 26 and September 2, 2011, respectively. Another site (ca. 30 m^2) with no *P. pungens* was used as the control.

Statistical analyses

The correlation between the population numbers of *P. pungens* and *P. solenopsis* was assessed using simple linear-regression analysis, then via an F-test. The different population numbers of *P. solenopsis* in the presence or absence of *P. pungens* on five investigating dates were analyzed using one-way analysis of variance (ANOVA), then via Duncan's test. The different population numbers of *P. solenopsis* between the presence and absence of *P. pungens* on each investigating date were compared using Student's t test. Iwao's patchiness regression (Iwao 1968) was used to determine the spatial distribution patterns of them, then via F-test. All statistical analyses were performed using the Statistical Package for Social Sciences, version 14.0.

RESULTS

Relationship between the population numbers of *Pristomyrmex pungens* and *Phenacoccus solenopsis*

At the end of September, 2011, the occurrence rate of *P. pungens* and *P. solenopsis* was investigated on 300 pots of *D. morifolium*. The rate of co-occurrence of *P. pungens* and *P. solenopsis* on *D. morifolium* plants was 18.0% (i.e. 54 pots), and the rate of neither *P. pungens* nor *P. solenopsis* presented on *D. morifolium* plants was 70.7% (i.e. 212 pots); 27 and 7 pots *D. morifolium* (9.0% vs. 2.30%) only carrying *P. solenopsis* and *P. pungens*, respectively (Table 1). In addition, there was an extremely significant positive correlation between quantities of *P. solenopsis* and *P. pungens* ($P < 0.001$) (Fig. 1). We also found the

Table 1. Survey results of coexisting of *Pristomyrmex pungens* and *Phenacoccus solenopsis* on *Dendranthema morifolium* plants.

	Presence of *P. pungens*		Absence of *P. pungens*	
	Pot (plant) number	Percentage (%)	Pot (plant) number	Percentage (%)
Presence of *P. solenopsis*	54	18.0	27	9.0
Absence of *P. solenopsis*	7	2.3	212	70.7

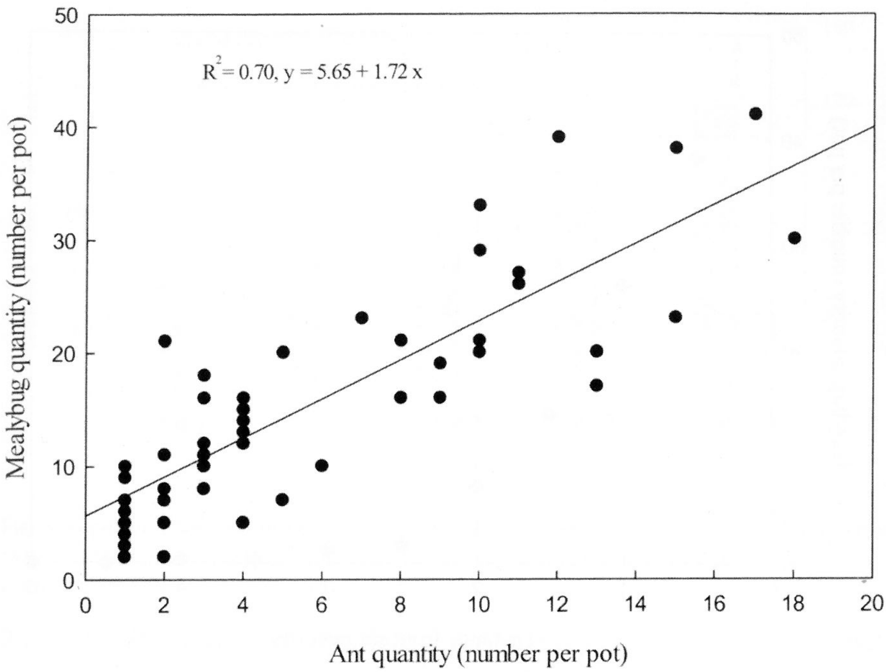

Fig. 1. Correlation between the population numbers of *Pristomyrmex pungens* and *Phenacoccus solenopsis* on *Dendranthema morifolium* plants ($F = 120.48$, $df = 53$, $P < 0.001$).

Table 2. Spatial distribution patterns of *Pristomyrmex pungens* and *Phenacoccus solenopsis*. In Iwao's patchiness regression analysis, α parameter is the intercept that provides a measure of the crowding tendency, and β is the slope that describes the pattern in which the organism inhabits the environment (Iwao 1968).

Species	Date	Iwao's patchiness regression ($m^* = \alpha + \beta m$)	Regression coefficient R	P
	August 6	$m^* = -0.3666 + 3.7300\ m$	0.9133	< 0.05
	August 11	$m^* = 0.7925 + 4.0283\ m$	0.9629	< 0.01
P. pungens	August 19	$m^* = 1.7465 + 2.4944\ m$	0.9557	< 0.01
	August 26	$m^* = 1.3651 + 3.0253\ m$	0.8648	< 0.05
	September 2	$m^* = 2.848 + 2.5356\ m$	0.9260	< 0.05
	August 6	$m^* = -0.2889 + 4.7988\ m$	0.8946	< 0.05
	August 11	$m^* = 0.9525 + 3.8639\ m$	0.9702	< 0.01
P. solenopsis	August 19	$m^* = 20.2903 + 1.9908\ m$	0.9881	< 0.01
	August 26	$m^* = 29.7870 + 1.3013\ m$	0.9258	< 0.05
	September 2	$m^* = 19.6119 + 1.9263\ m$	0.9263	< 0.01

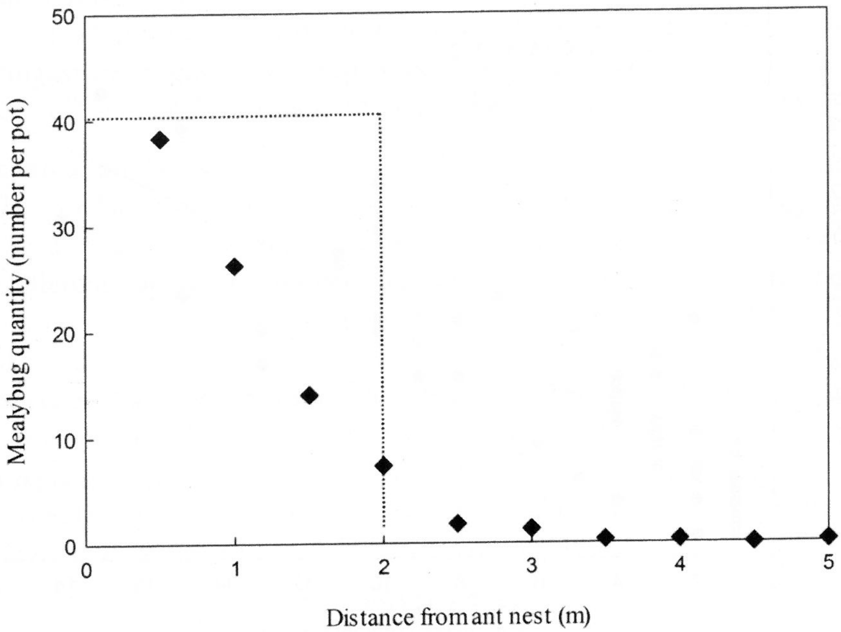

Fig 2. Mean number of *Phenacoccus solenopsis* at different distances from *Pristomyrmex pungens* nests.

distance from *P. pungens* nest affected the quantity of *P. solenopsis*. *P. solenopsis* was almost found no more than 2 m from *P. pungens* nest (Fig. 2).

Effects of *Pristomyrmex pungens* on the population numbers and spatial distribution patterns of *Phenacoccus solenopsis*

The mean number of *P. solenopsis* increased significantly in the presence of *P. pungens* from August 6 to September 2, 2011. The number of *P. solenopsis* in the presence of *P. pungens* on September 2 (374.2±30.5 per quadrat) was ca. 7.3 times more than that on August 6 (51.0±4.6 per quadrat) (Fig. 3, $F = 55.7$, $P < 0.001$). But the number of *P. solenopsis* in the absence of *P. pungens* (=control) on September 2 (85.4±6.9 per quadrat) was only ca. 1.7 times more than that on August 6 (49.6±5.0 per quadrat) (Fig. 3, $F = 10.8$, $P < 0.01$). Comparing the data between presence and absence of *P. pungens* on each investigating date, we found that there was a remarkable significance on August 19 ($t = 10.7$, $P < 0.01$), August 26 ($t = 9.2$, $P < 0.01$), and September

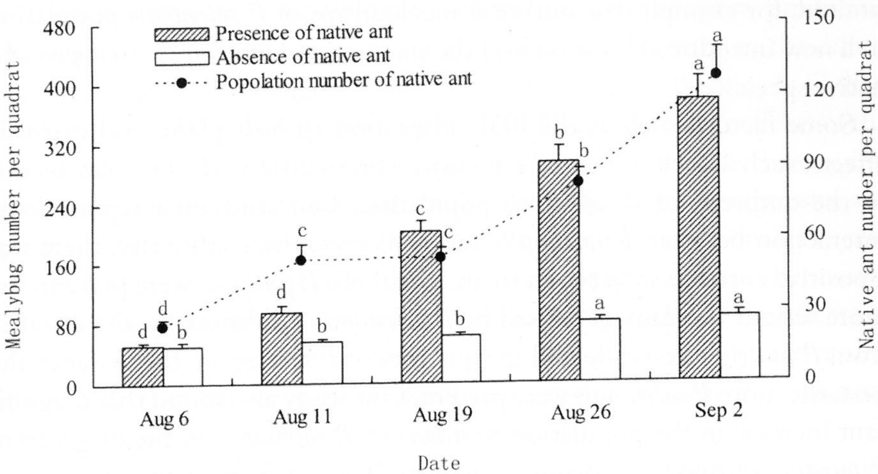

Fig. 3. Population numbers of *Phenacoccus solenopsis* in the presence and absence of *Pristomyrmex pungens* sites from August 6 to September 2, 2011. The different letters in same series indicate that the means are different at a $P = 0.05$ level (Duncan's test).

2 ($t = 9.3$, $P < 0.01$), and significance on August 11 ($t = 3.9$, $P < 0.05$), with no significance on August 6 ($t = 0.2$, $P > 0.05$).

The relationship between mean crowding (m^*) and mean densities (m) of *P. pungens* and *P. solenopsis* was described using Iwao's patchiness regression from August 6 to September 2, 2011, which revealed that *P. pungens* had strong crowding tendencies (Iwao's $\beta > 1$) and that individuals were attracted to each other (Iwao's $\alpha > 0$) on each investigate date except on August 6; the baseline component of population distribution was individuals. However, on August 6, the spatial distribution pattern of *P. solenopsis* was aggregation (Iwao's $\beta > 1$), and the individuals were repelled each other (Iwao's $\alpha < 0$); the results on other investigate dates were the same with those of *P. pungens* (Table 2).

DISCUSSION

Since *P. solenopsis* was first reported in Guangzhou, China in 2008, it has rapidly spread throughout nearly 38 districts and cities from 9 provinces in China (Wu & Zhang, 2009). So far, host plant species and biological character of *P. solenopsis* have been widely studied (Aheer *et al.* 2009; Vennila *et al.* 2010). By contrast, other relevant studies on the mealybug are quite limited, for example, the outbreak mechanisms of *P. solenopsis* population in a new introduced location and the interaction between *P. solenopsis* and native species.

Some factors, such as the high adaptation to host plant, and extrinsic effects, such as lack of natural enemies or mutualism with ants, may be due to the outbreak of *P. solenopsis* population. Our study first reported the interaction between *P. solenopsis* and *P. pungens*, indicating that there was a positive correlation between them. The more *P. pungens* were present, the more serious the damage caused by *P. solenopsis*. Additionally, the distance from *P. pungens* nests affected the quantity of *P. solenopsis*. The closer to the nest, the more *P. solenopsis* were present. Our study also found that a significant increase in the population numbers of *P. solenopsis* in the presence of *P. pungens* during the growing periods of *D. morifolium* plants. We suggest that there is a positive effect of *P. pungens* on the population numbers of *P. solenopsis*. In addition, the spatial distribution pattern of *P. pungens* and *P. solenopsis* were basically aggregations. Thus, we suggest that there may be a strong attraction between them.

We made an interesting observation that *P. pungens* frequently used dead leaves to cover the older nymphs of *P. solenopsis* on the host plant. A similar phenomenon can be found in the Helms and Vinson (2002) report. They found that the invasive ant *S. invicta* tended Homopterans extensively and actively constructed shelters around them; these shelters were common in the southeast United States, and may offer protection from weather and/or Homoptera predators and parasites, or even offer protection for tending *S. invicta* workers. Additionally, Huang *et al* (2010) reported that *S. invicta* frequently moved their mounds to the proximity of the mung bean-carrying aphids in the field. Thus, we hypthesize that this may be attributed to the native ant attempting to protect their nonmoving food sources or tend *P. solenopsis* as well. Compared with other studies about the invasive ant *S. invicta*, we suspect the protective capability of the native ant for food sources is not stronger than *S. invicta*. Otherwise, one more points can not be explained in our study: why did *P. pungens* move 1st or 2st instar nymphs of *P. solenopsis* into their mounds? It has already been established that ants can move Homoptera between plants (Vinson & Scarborough 1991; Michaud & Browning 1999), but aspects about this behavior of ants remain unknown.

The degree of protection provided by ants attending to honeydew-producing Hemipterans varies among native ant species, primarily owing to the differences among species in aggressiveness and territoriality (Buckley & Gullan 1991, Kaneko 2003) For example Sipura (2002), compared the effects of the interactions between aphids and two native ant species on the herbivory and growth of two willow species, and found that only the more aggressive ant species had measurable effects on plant growth. In our observation, *P. pungens* is more active and strongly defended territories compared to another native ant, e.g. *Plagiolepis* sp. Therefore it may be useful to compare the effects of ant-invasive mealybug interactions involving different species of native ants on each others' colony development and the success of the mealybug invasion, even on the arthropod community structure.

Ants have a profound impact on the arthropod community in their ecosystem, and the mutualistic relationship between ants and honeydew-producting Hemipteran insects is common and important (Wilson 1971; Way & Khoo 1992). However, effects of the mutualistic relationship on pest management in the field are argued extensively. In our study, we suggest that the population numbers of *P. solenopsis* increase with attending *P. pungens* on *D. morifolium* plants, and this relationship may be important in *P. solenopsis* success at newly introduced locations. Thus, future studies should focus on the outbreak mechanisms of *P. solenopsis* population with the ant-mealybug interactions, and the ecological factors that influence the consequences of ant-mealybug interactions to provide greater insight into the role of positive species interactions in food web dynamics and greater predictability of the direct and indirect effects of herbivores and natural enemies on host plants.

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