

## **Effects of *Solenopsis invicta* (Hymenoptera: Formicidae) Tending on the Probing Behavior of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae)**

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# EFFECTS OF *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE) TENDING ON THE PROBING BEHAVIOR OF *PHENACOCCLUS SOLENOPSIS* (HEMIPTERA: PSEUDOCOCCIDAE)

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## ABSTRACT

The involvement of red imported fire ant *Solenopsis invicta* Buren in the probing behavior of the mealybug *Phenacoccus solenopsis* Tinsley was investigated. The electrical penetration graph (EPG) technique was applied to compare the different probing behavior of ant-tended and untended adult female mealybugs on hibiscus seedlings. The results showed that the duration of the E1, E2, and G waveforms for the mealybugs from both treatments were not significantly different. However, the E1 waveform (which indicates that the stylets have reached phloem and salivation has begun) appeared earlier in ant-tended mealybugs, with a shortened duration of the path waveform (C). The percentage of mealybugs with E2 (phloem) and G (xylem) waveforms were not significantly changed after fire ant tending, whereas apparent increases of E1e and F waveforms were observed. Therefore, the results suggested that *S. invicta* has significant effects on the probing behavior of *P. solenopsis*.

**Key Words:** Electrical penetration graph, solenopsis mealybug, red imported fire ant, mutualism

## RESUMEN

Se investigó el papel que juega la hormiga de fuego roja importada *Solenopsis invicta* Buren en el comportamiento alimentario de la cochinilla *Phenacoccus solenopsis* Tinsley. Se aplicó la técnica de gráfico de la penetración eléctrica (GPE) para comparar los diferentes comportamientos de alimentación de las hembras adultas de la cochinilla sobre plántulas de hibiscus atendidas o no atendidas por las hormigas. Los resultados mostraron que la duración de las E1, E2 y G formas de onda para las cochinillas de ambos tratamientos no fueron significativamente diferentes. Sin embargo, la forma de la onda E1 (lo que indica que los estiletes han alcanzado salivación del floema) apareció anteriormente en las cochinillas atendidas por hormigas, con una menor duración de la ruta de la forma de onda (C). El porcentaje de las cochinillas con formas de onda E2 (floema) y G (xilema) no se cambiaron significativamente después de ser atendidas por la hormiga de fuego, mientras que se observó un aumento aparente en las formas de onda E1e y F. Por lo tanto, los resultados sugieren que *S. invicta* tiene efectos significativos en el comportamiento de alimentación de la cochinilla *P. solenopsis*.

**Palabras Clave:** gráfico eléctrica penetración, cochinilla *Solenopsis*, la hormiga roja de fuego importada, mutualismo

Symbiotic and mutualistic relationships between insects are common ecological phenomena. A typical widely studied example is the mutualism between ants and honeydew-producing insects (Del-Claro & Oliveira 1996; Huang et al. 2010; Buckley 1987; Stadler & Dixon 1998; Stadler & Dixon 1999; Stadler & Dixon 2005; Helms & Vinson 2008). Ant tending protects honeydew-producing hemipterans from their natural enemies and removes excreted honeydew rapidly, reducing fungal and

microbial infections. The ants, in turn, obtain honeydew from the plant, which serves as their most important food resource (Stadler & Dixon 1998; Yao et al. 2000). This mutually beneficial relationship between the ants and honeydew-producing hemipterans promotes the development of each other's population. In addition, previous studies suggest that ant tending may affect the feeding behavior of honeydew-producing hemipterans (Rauch et al. 2002; Zhou et al. 2012a).

*Solenopsis invicta* exists in a variety of ecosystems (Vinson 1997; Holway et al. 2002). The species is said to have a mutualistic relationship with honeydew-producing hemipterans (aphids and scale insects) by defending them against their natural enemies (Zhou et al. 2013). This relationship directly affects the predation range of *S. invicta* as well as its interference and predation intensity on other arthropods (Vinson 1997; Holway et al. 2002). Several reports have shown that fire ants facilitate aphid population growth on different crops, such as, soybean, corn, cotton, and cabbage (Eubanks 2001; Kaplan & Eubanks 2002; Diaz et al. 2004). *Phenacoccus solenopsis* Tinsley originated from the United States and has been considered an important invasive species in South China (Wu & Zhang 2009), where its habitats in the field overlap with those of *S. invicta* with which it has a mutualistic relationship (Zhou et al. 2012a; Zhou et al. 2012b). The excretion frequency of mealybugs was significantly increased and an apparent decrease in the weight of honey dew droplets occurred in the presence of *S. invicta*. However, ant tending has no significant impact on the total weight of the honeydew excreted by mealybugs (Zhou 2012). However, no evidence has been obtained to prove that the change in excretion frequency was caused by a change in the probing behavior of *P. solenopsis*. Therefore, we hypothesized that mutualism between these 2 invasive species may change the probing behavior of *P. solenopsis*.

The electrical penetration graph (EPG) technique was designed to transform stylet activities of insects in leaves into a visual signal; the technique allows real-time tracking of the probing behavior of insects and records an accurate location of each stylet in the plant tissue (Tjallingii & Esch 1993; Yan 2003). EPG technology has been widely used for investigating the probing behavior of a variety of sucking insects (Huang et al. 2012; Tjallingii & Esch 1993; Zhang et al. 2009; Yan 2003). Zhang et al. (2009) suggested that elevated  $O_3$  concentration may change the growth, nutritional contents, and secondary chemicals of the host plants and result in the change of probing behavior of cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). Zhou et al. (2004) revealed that aqueous extracts of plants have very significant effects on the probing behavior of *A. gossypii*. EPG technology was first used by Calatayud et al. (1994) to record the probing behavior of mealybugs (*Phenacoccus manihoti* Matile-Fertero). Cid & Fereres (2010) similarly recorded the waveforms that were associated with stylet penetration behavior and the tissue locations of the citrus mealybug *Planococcus citri* Risso. Huang et al. (2012) was the first to monitor the stylet penetration behavior of *P. solenopsis* on cotton using the EPG technique (direct current, DC, system). Typical waveforms of A, B, C, and pd (combined

pathway),  $E_1$  and  $E_2$  (phloem), F (obstructed stylet mechanics), and G (xylem) were identified and further characterized.

Previous studies found no significant difference in the probing behavior of aphids between ant-tended and untended individuals (Rauch et al. 2002). Our preliminary observation revealed that the presence of *S. invicta* changed the probing sites of *P. solenopsis* from the back of the leaves of the host plants to the stem, but whether this change reflects a change in the probing behavior is unknown. To test the hypothesis that the presence of *S. invicta* causes changes in the probing behavior of *P. solenopsis*, EPG was applied to compare the difference in the probing behavior of ant-tended and untended adult female mealybugs on hibiscus seedlings.

## MATERIALS AND METHODS

### Organisms

The *Hibiscus rosa-sinensis* L. (Malvales: Malvaceae) seedlings were purchased from a local nursery and grown in flower pots filled with potting soil. The diam of the upper and lower bottom of the pots were 17 cm and 12 cm, respectively, whereas the height of the pots was 14 cm. All plants had 15 true leaves and were approximately 40 cm to 60 cm tall. Each flower pot was placed in a plastic container (50 × 40 × 16 cm), covered with nylon netting, and enclosed in a cage (70 × 70 × 100 cm).

Colonies of *P. solenopsis* were provided by the Laboratory of Insect Ecology in South China Agricultural University. These insects were fed on *H. rosa-sinensis*. Plants with established mealybug colonies were used for subsequent experiments. *Solenopsis invicta* colonies were collected within the campus of South China Agricultural University and established in the laboratory for bioassays. The ants were fed weekly with fresh *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and honey water. All colonies were maintained at 25 ± 2 °C, 60% to 70% RH, and 16:8 h L:D.

### Experimental Setup for Fire Ant Tending

A total of 300 mealybugs, including nymphs of each instar and adults, were placed on the hibiscus seedling leaves, and a certain amount of petroleum jelly (Vaseline®) was applied on the base of the plants to prevent the mealybugs and fire ants from escaping. Approximately 5,000 individual fire ants (weighing 5 g) were placed in a plastic box (22 × 16 × 6 cm) with a daily supply of distilled water; these individuals were composed of workers and larvae (workers: larvae = 5:1) with one queen. A petri dish (7 cm diam) with moist plaster was used as an artificial nest. After the mealybugs had colonized the plants, the potted

plants and the artificial ant nest were placed in a large plastic box (48 × 40 × 16 cm). Five *T. molor* larvae and water were fed to the fire ants every day. For the bioassay, a plastic hose was used to provide a bridge between the ant nest and the plant seedlings to allow worker foraging. Ten days after setting-up the interaction, the adult female mealybugs were collected for EPG. Mealybugs reared without *S. invicta* were designated as control.

#### Electrical Monitoring of Probing Behavior of *P. solenopsis* on *H. rosa-sinensis*

Experiments were conducted inside a Faraday cage in the laboratory at  $25 \pm 2$  °C and 50% to 70% RH. The stylet penetration activities of mealybugs were recorded using a 4-channel DC-EPG system (Giga-4; EPG Systems, Wageningen, The Netherlands). The recording method of Cid & Fereres (2010) and Huang et al. (2012) was used, with minor modifications. Mealybug adults (that were not in a probing position) of uniform size were carefully collected from the rearing plants. The dorsal wax was partially brushed off with a fine brush to attach the insect electrode using a droplet of water-based silver glue (Provided by Prof. Fengming Yang, Henan Agricultural University, China); the electrode was made of a thin gold wire (18 µm in diam, 2 cm long). The insects were then transferred to an empty petri dish for 3 min for the complete drying of the glue. Recording lasted for approximately 6 h under fluorescent light. Output signals were stored on a personal computer hard disk mediated by an A/D USB device (Di158&710; Dataq Instruments, Akron, Ohio, USA) at 100 Hz sampling using the EPG Stylet+d (version 1.10) software (Entomology Laboratory, Wageningen University, The Netherlands). On each plant, 22 and 30 recordings were made for the treatment and the control, respectively, for the characterization and analysis of EPG signals using the EPG Stylet + version 1.20 software. The waveforms of the *P. solenopsis* EPG on *H. rosa-sinensis* were characterized according to the relative amplitude, frequency voltage level (extra- or intracellular), and main electrical origin [resistance (R) or electromotive force (emf)] (Tjallingii 1990; Cid & Fereres 2010; Huang et al. 2012).

#### Statistical Analysis

Given that the obtained data did not follow a normal distribution, the Mann-Whitney and  $\chi^2$  tests were used to compare the differences between the ant-tended and the untended treatment at a 0.05 significance level. All statistical analyses were conducted using SPSS (version 14.0; SPSS Inc., Chicago, Illinois, USA).

## RESULTS

#### Variables Analyzed

All the variables analyzed in this study are listed in Table 1, which corresponds to the commonly recognized waveforms (Fig. 1), as defined in Huang et al. (2012). Aside from the total time of penetration in the sieve elements with saliva secretion (E1) and sap ingestion (E2), the xylem elements (G) were the central interest in this study. The total time of waveform occurrence (E1e), np (no penetration), and stylet's penetration difficulty mechanics (F) were likewise investigated. Results showed that the E1 and E2 waveforms often alternated, and only a few mealybugs produced the E2 waveform. Potential drops (pd), including the pd1 and pd2 waveforms, were observed frequently during the C waveform (grouped A, B, and C). pd2 was usually followed by pd1, which was similar with the E1e waveform. The duration of E1e varied significantly.

#### Effects of Fire Ant-Tending on Aphid Behavior

Based on the analysis of the EPG characteristics of mealybugs with or without the presence of fire ants, the total time of the E1 and E2 waveforms exhibited no significant change ( $E_1$ :  $U = 239$ ,  $P = 0.087$ ;  $E_2$ :  $U = 288.5$ ,  $P = 0.166$ ). the numbers of the E1 and E2 waveforms were not significantly different between the treatments and the controls ( $E_1$ :  $U = 275$ ,  $P = 0.296$ ,  $E_2$ :  $U = 292.5$ ,  $P = 0.211$ ). There were no significant differences in the number ( $U = 285$ ,  $P = 0.184$ ) and total duration ( $U = 293.5$ ,  $P = 0.282$ ) of G waveform between the ant-tended and untended mealybugs. The total time of penetration ( $U = 144$ ,  $P = 0.001$ ) and the path wave ( $U = 180$ ,  $P = 0.005$ ) were shortened after fire ant-tending, whereas the number of C waveforms increased significantly ( $U = 173$ ,  $P = 0.003$ ). The total duration of the np and E1e waveforms were significantly prolonged (np:  $U = 131$ ,  $P < 0.0001$ ; E1e:  $U = 186$ ,  $P = 0.001$ ). The number of np ( $U = 141.5$ ,  $P < 0.0001$ ) and E1e ( $U = 185$ ,  $P = 0.001$ ) waveforms were increased, whereas the number ( $U = 241$ ,  $P = 0.03$ ) and total time ( $U = 236$ ,  $P = 0.022$ ) of the F waveform was significantly extended after fire ant-tending. Compared with the untended treatments, the time of first penetration ( $U = 229.5$ ,  $P = 0.053$ ) and the number of pd waveforms ( $U = 270.5$ ,  $P = 0.27$ ) showed no significant change. The time to the first sieve element penetration with saliva secretion (E1) brief ( $U = 178$ ,  $P = 0.004$ ), but the time to first sieve elements with sap ingestion (E2) was not significantly different ( $U = 299$ ,  $P = 0.262$ ). The durations of first F ( $U = 248$ ,  $P = 0.046$ ) and E1e ( $U = 181$ ,  $P = 0.001$ ) waveforms were significantly prolonged, while there were no significant difference in the times to the first G ( $U = 299$ ,  $P = 0.333$ )

TABLE 1. BEHAVIORAL PARAMETERS QUANTIFIED FROM EPG-RECORDINGS OF *PHENACOCCLUS SOLENOPSIS* FEEDING ON *HIBISCUS ROSA-SINENSIS*.

Variable	Unit	Definition
NWCEI	—	Number of waveform C events per insect
NWE1CEI	—	Number of waveform E1 events per insect
NWE2CEI	—	Number of waveform E2 events per insect
NWGCEI	—	Number of waveform G events per insect
NWFCEI	—	Number of waveform F events per insect
NWE1eCEI	—	Number of waveform E1e events per insect
E1 <sub>total</sub>	Min	Total time of penetration in the sieve elements with saliva secretion (E1) per insect
E2 <sub>total</sub>	Min	Total time of penetration in the sieve elements with sap ingestion (E2) per insect
G <sub>total</sub>	Min	Total time of penetration in the xylem elements (waveform G) per insect
P <sub>total</sub>	Min	Total time except np (no penetration) per insect
Path <sub>total</sub>	Min	Total time of pathway penetration (A+B+C+pd) per insect
E1e <sub>total</sub>	Min	Total time of the occurrence of waveform E1e per insect
np <sub>total</sub>	Min	Total time of the occurrence of waveform np (no penetration) per insect
F <sub>total</sub>	Min	Total time of the occurrence of waveform F per insect
n <sub>pd</sub>	—	Number of waveform pd per insect
n <sub>np</sub>	—	Number of waveform np (no penetration) per insect
1 <sup>st</sup> P	Min	Time to first penetration (waveform C) per insect
1 <sup>st</sup> E1	Min	Time to first sieve element penetration with saliva secretion per insect
1 <sup>st</sup> E2	Min	Time to first sieve elements with sap ingestion per insect
1 <sup>st</sup> Pd	Min	Time to waveform Pd event per insect
1 <sup>st</sup> G	Min	Time to waveform G event per insect
1 <sup>st</sup> F	Min	Time to waveform F event per insect
1 <sup>st</sup> E1e	Min	Time to waveform E1e event per insect
%E2	—	Percent of mealybugs produce waveform E2
%G	—	Percent of mealybugs produce waveform G
%E1e	—	Percent of mealybugs produce waveform E1e
%F	—	Percent of mealybugs produce waveform F

and pd ( $U = 241, P = 0.107$ ) waveforms between treatment and control (Table 2).

Furthermore, the percentage of mealybugs that produced the E2 and G waveforms did not change significantly (E2:  $\chi^2 = 0.343, P = 0.558$ ; G:  $\chi^2 = 0.735, P = 0.386$ ), whereas the percentages of mealybugs that exhibited E1e and F waveforms were increased (E1e:  $\chi^2 = 6.831, P = 0.009$ ; F:  $\chi^2 = 5.147, P = 0.023$ ) (Table 2).

DISCUSSION

The mutualistic relationship between *S. invicta* and *P. solenopsis* has been shown to promote the development of their respective populations (Zhou et al. 2012a), reduce the effect of natural enemies on mealybug populations (Zhou et al. 2013), and hinder honeydew collection activities of native ants (Zhou et al. 2012c). Although this mutually beneficial relationship affects the honeydew excretion of mealybug, whether this relationship changes the mealybug feeding behavior on the host plant was unknown. To answer this question, we compared the EPG behavior before and after the mealybugs were tended by the fire ants.

The results verified that *S. invicta* did not significantly reduce mealybug feeding, because there were no significant differences observed between the ant-tended and untended treatments in terms of the number and total durations of the waveforms (E1, E2, and G). These results are consistent with a previous study by Zhou et al. (2012a), which reported that the interaction between *S. invicta* and *P. solenopsis* did not change the amount of mealybug honey excreted. Therefore, food intake was not increased, but a change in the mealybug probing behavior was observed. In the presence of *S. invicta* the total duration of the path wave was significantly shortened as well as the time of first sieve element penetration with saliva secretion (E1), whereas the duration of the F waveform was not significantly different between the ant-tended and untended treatments. These results implied that fire ant tending changed the probing behavior of the mealybugs and increased the acceptability of the host plants. Therefore, our results confirmed the scientific hypothesis that the presence of *S. invicta* causes changes in the probing behavior of *P. solenopsis*.

The np waveform generally represents “no penetration” by insects. Only a few researchers



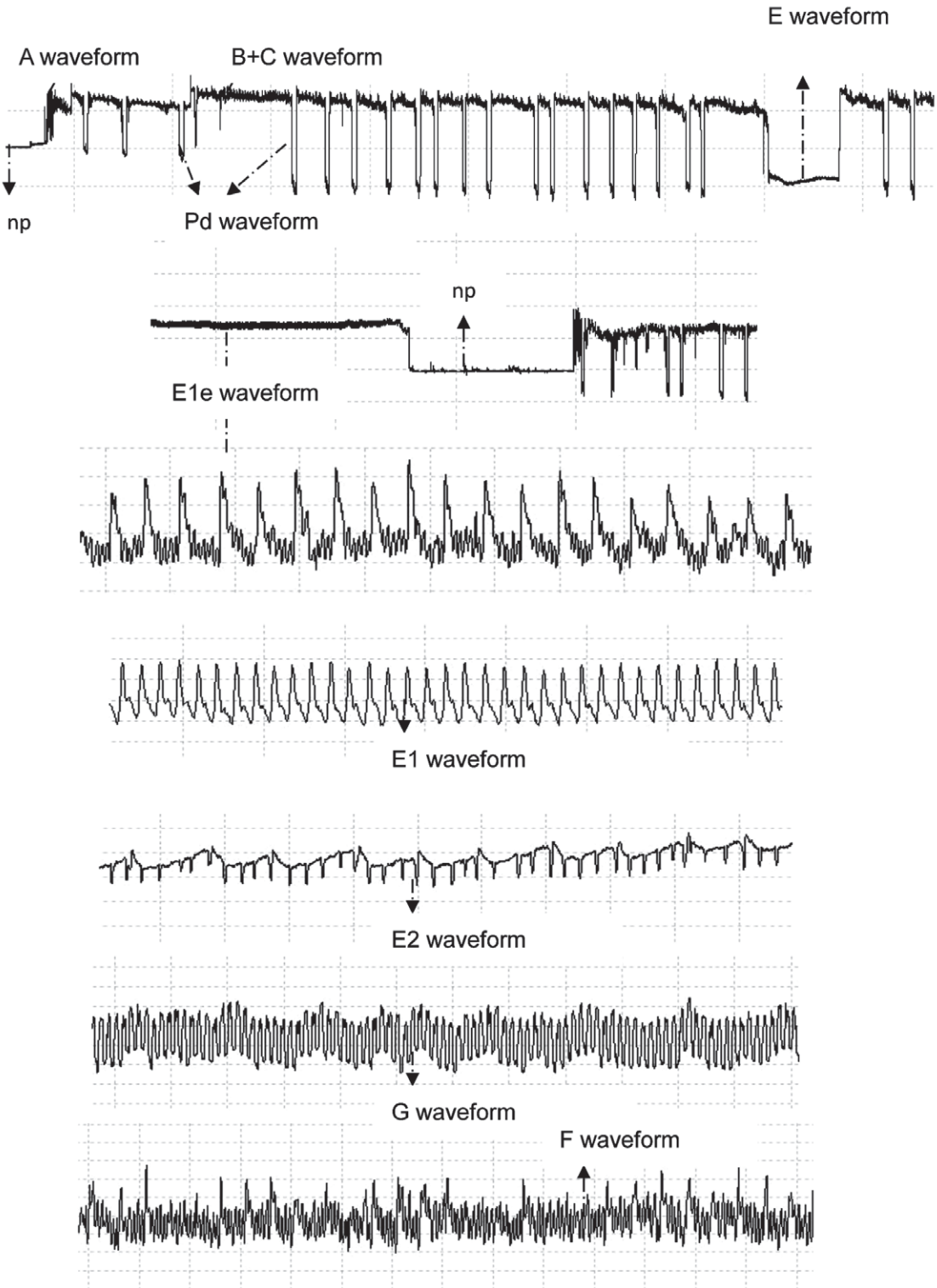


Fig. 1. DC-EPG waveforms recorded for *Phenacoccus solenopsis* probing on *Hibiscus rosa-sinensis*.

TABLE 2. VARIOUS BEHAVIORAL PARAMETERS (MEAN ± SE) OF *PHENACOCCLUS SOLENOPSIS* PROBING ON *HIBISCUS ROSA-SINENSIS* IN RED IMPORTED FIRE ANT-TENDED AND UNTENDED TREATMENTS.

Variable <sup>1</sup>	Tended treatment	Untended treatment	P	U	χ <sup>2</sup>
NWCEI	4.68 ± 0.55 b	2.90 ± 0.36 a	0.003	173	
NWE1CEI	1.59 ± 0.42 a	1.83 ± 0.28 a	0.296	275	
NWE2CEI	0.27 ± 0.27 a	0.30 ± 0.14 a	0.211	292,5	
NWGCEI	0.32 ± 0.14 a	0.10 ± 0.06 a	0.184	285	
NWFCEI	0.73 ± 0.27 b	0.23 ± 0.12 a	0.03	241	
NWE1eCEI	0.82 ± 0.24 b	0.17 ± 0.11 a	0.001	185	
E1 <sub>total</sub>	55.85 ± 17.12	86.81 ± 14.94	0.087 <sup>NS</sup>	239	
E2 <sub>total</sub>	1.28 ± 1.28	8.39 ± 4.07	0.166 <sup>NS</sup>	288.5	
G <sub>total</sub>	19.21 ± 10.50	18.92 ± 10.79	0.282 <sup>NS</sup>	293.5	
P <sub>total</sub>	214.57 ± 19.11	307.98 ± 15.74	0.001 <sup>*</sup>	144	
Path <sub>total</sub>	130.67 ± 13.94	201.59 ± 9.1	0.005 <sup>*</sup>	180	
E1e <sub>total</sub>	57.41 ± 17.05	11.92 ± 7.88	0.001 <sup>*</sup>	186	
np <sub>total</sub>	43.84 ± 6.95	12.05 ± 2.76	< 0.001 <sup>*</sup>	131	
1 <sup>st</sup> P	9.97 ± 2.67	5.7 ± 1.52	0.053 <sup>NS</sup>	229.5	
1 <sup>st</sup> E2	6.10 ± 6.10	29.25 ± 14.60	0.262 <sup>NS</sup>	299	
1 <sup>st</sup> E1	72.14 ± 16.18	145.32 ± 18.20	0.004 <sup>*</sup>	178	
1 <sup>st</sup> Pd	67.95 ± 16.52 a	28.31 ± 3.28 a	0.107	243	
1 <sup>st</sup> G	44.43 ± 21.74 a	17.04 ± 9.77 a	0.333	299	
1 <sup>st</sup> F	45.21 ± 17.63 b	21.95 ± 11.61 a	0.046	248	
1 <sup>st</sup> E1e	55.66 ± 15.60 b	9.79 ± 6.48 a	0.001	181	
F <sub>total</sub>	65.89 ± 22.20	19.95 ± 11.60	0.022 <sup>*</sup>	236	
n <sub>pd</sub>	30.82 ± 5.08	38.80 ± 4.57	0.270 <sup>NS</sup>	270.5	
n <sub>np</sub>	3.05 ± 0.42	1.17 ± 0.32	< 0.001 <sup>*</sup>	141.5	
%E2	4.5%	13.3%	0.558 <sup>NS</sup>		0.343
%G	22.7%	10%	0.386 <sup>NS</sup>		0.735
%E1e	40.9%	10%	0.009 <sup>*</sup>		6.831
%F	40.9%	13.33%	0.023 <sup>*</sup>		5.147

Variables are defined in Table 1.  
<sup>\*</sup> and <sup>NS</sup> indicate data in a given row are significantly ( $P < 0.05$ ) and not significantly ( $P > 0.05$ ) different from each other, respectively, according to Mann-Whitney test or  $\chi^2$  test.

have reported on the repellent phenomenon that occurs when the np waveform was significantly prolonged in a specific case (Zhou et al. 2004). In this study, the total duration and number of np waveforms in the ant-tended treatments were significantly increased. Host-plant rejection was not observed, but a physiological or behavioral change may have occurred in ant-tended mealybugs. Another possible reason is that fire ants may forage and tend the mealybugs at night under artificial conditions, suggesting a shift in the feeding time of mealybugs from daytime to nighttime. Daytime EPG signals were recorded in this study, thereby causing the np waveforms to frequently occur for longer durations, which suggests that the mealybugs may develop a different honeydew-producing biorhythm as a result of fire ant tending (Zhou 2012).

To date, the biological significance of the E1e waveform remains controversial. Tjallingii (1990) suggested that the E1e waveform might represent the watery salivation activity that normally occurs during E1 in sieve elements. Huang et al.

(2012) argued that the specific *P. solenopsis* E1e patterns associated with potential drops may represent different activities. However, the voltages of E1e waveforms in this study were generally similar to those of C waveforms, and occasionally they were accompanied by F waveforms, but they were not always associated with pd waveforms. Our results showed that the duration of the E1e waveform varies from 8 to 9 min and may last up to a few hours. Only 10% of untended mealybugs exhibited the E1e waveform, whereas an increase to 40.9% was observed in the ant-tended treatments. The total duration of the E1e waveform was significantly prolonged by fire ant tending, although further study is needed to elucidate the effect of this change on specific behaviors.

The F waveform, its duration, and the percentage of mealybugs that produced the E1e waveforms showed an apparent increase, thereby indicating a change in the probing behavior of the ant-tended mealybugs. This is the result of a change in the feeding positions of the mealybugs after the ants began tending them. The mealybugs produced honey-

dew of different compositions when tended by ants. Fire ant tending increased the frequency of honeydew excretion by the mealybugs, but the sizes of the honeydew drops were smaller than in the untended treatments. The percentage of mealybugs that produced F waveforms increased with fire ant tending, which suggested that mealybugs searched for new feeding positions to increase the frequency of honey excretion, even when they encountered mechanical resistance by the host plants.

Our results provide behavioral proof of the indirect impact of mutualism between *S. invicta* and *P. solenopsis*. Also, this study gives a scientific basis for the evaluation of some of the ecological consequences of red imported fire ant invasion.

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