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Behavioral and Electroantennogram Responses of Phorid fly *Pseudacteon tricuspis* (Diptera: Phoridae) to Red Imported Fire Ant *Solenopsis invicta* Odor and Trail Pheromone

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The phorid fly, Pseudacteon tricuspis *is an introduced parasitoid of the red imported fire ant,* Solenopsis invicta *in the United States. Previous studies show that phorid flies are attracted to host ant workers at disturbed colonies, to colonies exhibiting aggressive interspecific interactions, and to fire ant mating flights. In a series of behavioral and electroantennogram (EAG) experiments, we confirm the possible use of fire ant odor as cues for host location by* P. tricuspis. *We tested the response of* P. tricuspis *of different sex and mating status to several host-related odor stimuli including live fire ant workers, extracts of worker whole body, head, thorax, and abdomen, and* (E,E)-*α-farnesene, a trail pheromone component of* Solenopsis *fire ants. Results from Y-tube olfactometer bioassays demonstrated the attraction of mated female* P. tricuspis *to live* S. invicta *workers. In addition, extracts of* S. invicta *worker whole body and thorax elicited strong olfactometer response in female flies (mated and unmated) and mated males, but not in unmated males.* Pseudacteon tricuspis *did not show significant attraction to extracts of* S. invicta *worker head and abdomen, or to* (E,E)-*α-farnesene, irrespective of sex and mating status. In EAG experiments, female and male* P. tricuspis *showed significant EAG response to extracts of worker whole body, head, and abdomen, and to a less extent, thorax extract, but not to* (E,E)-*α-farnesene. Females showed slightly greater EAG response than males, but EAG response was not affected by mating status. These results*

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suggest that fire ant thorax is likely the source of kairomones used as host location cues by P. tricuspis*, and support the hypothesis that fire ant worker trail pheromones are not likely used by* P. tricuspis *for host location.*

KEY WORDS: *Pseudacteon tricuspis*; *Solenopsis invicta*; red imported fire ant; olfactometer; electroantennogram; trail pheromone.

INTRODUCTION

Parasitoids use a hierarchy of physical and chemical cues for host location and acceptance (Weseloh, 1981; Vinson, 1984). The majority of research on parasitoid host location has focused on parasitoids of phytophagous insects (e.g., Nordlund *et al.,* 1981; Vet and Dicke, 1992), with little information available on host location by parasitoids of social insects. Ants and other social insects have evolved elaborate systems of intraspecific communication. Although there are tactile, visual and acoustical modalities, pheromonal chemical communication is the primary form (Wilson, 1962). Ants use pheromones for a variety of behavior including nestmate recognition, recruitment of nestmates (trail following), alarm signaling, mating, and attraction of workers to brood and the queen (Wilson, 1962; Williams *et al.,* 1981; Vander Meer *et al.,* 1988; Alonso and Vander Meer, 1997; Vargo, 1997; Ozaki *et al.,* 2005). These pheromonal signals used in intraspecific communication are especially reliable and stable, and may be utilized as kairomones by parasitoids of ants for host location (Brown and Feener, 1991; Feener *et al.,* 1996; Morehead and Feener, 2000).

Pseudacteon phorid flies (Diptera: Phoridae) are parasitoids of ants, and many species are specific to imported fire ants, *Solenopsis* spp. (Porter *et al.,* 1995a; Porter, 1998a; Morrison, 2000). *Pseudacteon* species are commonly referred to as decapitating flies due to their unusual life history (Porter *et al.,* 1995a; 1997; Cônsoli *et al.,* 2001). Females inject a single egg into the thorax of a worker ant. The first instar larva develops in the thorax, whereas the second and third instars develop in the head (Morrison, 2000). Close to pupation, the developing maggot decapitates its living host consuming everything inside the head. Pupation then occurs inside the empty head capsule of the worker host (Porter *et al.,* 1997; Cônsoli *et al.,* 2001). *Pseudacteon tricuspis* Borgmeier is one of a few species of phorid flies that have been introduced in many parts of southern United States for biological control of the invasive red imported fire ant, *Solenopsis invicta* Buren (Gilbert, 1996; Porter *et al.,* 1999).

Phorid flies have been reported to utilize semiochemical cues for ant host location (Brown and Feener, 1991; Feener *et al.,* 1996; Morehead and Feener, 2000). For instance, *Apocephalus paraponerae* (Diptera: Phoridae), a parasitoid of workers of the giant tropical ant, *Paraponera clavata*

(Hymenoptera: Formicidae) was attracted to crushed workers and alarm pheromonal components from worker mandible (Brown and Feener, 1991; Feener *et al.,* 1996). Olfaction has also been suggested as the long range cue used by *Pseudacteon* phorid flies in locating host fire ants (Gilbert and Morrison, 1997; Orr *et al.,* 1997; Porter, 1998b). *Pseudacteon tricuspis* appears to have highly specific host-attraction preferences for *S. invicta* (Gilbert and Morrison, 1997; Porter, 1998a; Morrison and Gilbert, 1999; Porter and Alonso, 1999). Field observations have shown that while *P. tricuspis* is not attracted from a distance to *S. invicta* foraging activity, it is attracted to host workers at disturbed colonies, to colonies exhibiting aggressive interspecific interactions, and to mating flights (Morrison and King, 2004), leading some authors to propose that *P. tricuspis* may be using fire ant worker alarm pheromones/or trail pheromones for host location (Orr *et al.,* 1997; Morrison and King, 2004; Morrison and Porter, 2006). Also, Vander Meer and Porter (2002) reported on the attraction of *P. tricuspis* to volatile compounds released by shaken fire ant workers and suggested the involvement of alarm pheromones in this attraction. However, little is known about the host location cues used by phorid fly parasitoids of fire ants, and we are not aware of any published studies on the olfactory response of *Pseudacteon* species to fire ant odor. One reason for this is the lack of information on the chemical identity of fire ant alarm pheromones. Although sources of alarm pheromones in fire ants have been postulated to include the Dufour's gland and a glandular source in the head (Wilson, 1962), the actual components of fire ant alarm pheromones have not been published to date, making it difficult to test the hypothesis regarding possible use of fire ant alarm pheromones as host location cues by *Pseudacteon* phorid flies.

This paper presents the first direct evidence of semiochemical-mediated attraction of *Pseudacteon* species to fire ant host odor. We evaluated the behavioral (using Y-tube olfactometer) and electroantennogram (EAG) responses of female and male *P. tricuspis* of different mating status to live *S. invicta* workers (behavioral bioassay only), extracts of worker whole body and different body parts, and (*E*,*E*)-α-farnesene, a trail pheromone component of *Solenopsis* fire ants (Vander Meer *et al.,* 1981; 1988; Vander Meer, 1983).

MATERIALS AND METHODS

Insects

Pseudacteon tricuspis were reared on workers of red imported fire ant, *Solenopsis invicta*, at the fire ant rearing facility of the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, U.S.A. as described by Porter *et al*. (1997). Parasitized fire ant worker heads were received in batches and held in a plastic jar (25 × 13 cm) with a lid until emergence. Twice daily, newly-emerged flies were removed with an aspirator, sexed immediately (by using the presence or absence of the distinct female ovipositor), and placed in groups of 2 individuals either of the same sex (unmated individuals) or of opposite sex (mated individuals) in a 6-cm diameter plastic Petri dish. Petri dishes were kept in an incubator at 25 ± 1°C, LD 14:10 h and 70 ± 5% r.h. Sugar (25% sucrose) solution and water were provided in the Petri dishes as previously described (Chen *et al.,* 2005). Adult phorid flies utilized in the experiments were 1–2-day-old.

Red imported fire ants, *Solenopsis invicta* used in this study were collected on the campus of Auburn University (Auburn, Alabama). Mounds were removed and the ants separated from the soil by flooding (Jouvenaz, 1977). Ants were raised in the laboratory using standard rearing procedures (Banks *et al.,* 1981).

Chemical Stimuli

Chemical stimuli consisted of live fire ant workers, extracts of dissected body parts of *S. invicta* workers, and (*E*,*E*)-α-farnesene, a synthetic trail pheromone component of imported fire ants. Tests of extracts of fire ant body parts included whole body, head, thorax, and abdomen (gaster). Fire ant workers from different colonies were chilled to –20°C for 15 min before body parts were separated using micro dissecting scissors and extracted using a method similar to that described by Vander Meer and Alonso (1996). For each body part (e.g. head, thorax or abdomen), 100 dissected individual parts were placed in a 1.5-mL microcentrifuge tube containing 200 μL hexane and crushed with a plastic pestle. After rinsing the pestle with an extra 800 μL hexane (i.e., 100 body parts in 1000 μL hexane), the tube was vortexed, sealed with Parafilm membrane, and kept under laboratory conditions for 24 h. The supernatant of same body part extraction was withdrawn and filtered through glass wool in a pipette. This resulted in 0.1 worker equivalent per μL (WE/μL) solutions. In order to obtain enough materials for testing, extraction of each body part was done in three replicates which were later combined and kept in a freezer at –20°C until used. (*E*,*E*)-α-farnesene (Sigma-Aldrich, St. Louis, MO) was diluted in hexane to give a 0.1 μg/μL solution.

Behavioral Bioassays

A Y-tube olfactometer (Analytical Research Systems, Gainesville, FL) was used to test the attraction of mated and unmated individuals of both sexes of *P. tricuspis* to host-related odor. The system consists of a central tube (13.5 cm long, 24 mm diam.) and two lateral arms (5.75 cm long, 24 mm diam.) which are separately connected to an extending glass tube (14.5 cm long, 19 mm diam.). There is a sieve inlayed in the extending glass tube 5.25 cm away from the connection to prevent escape of insects and to serve as an end point of each lateral arm. Humidified and purified air was passed into the extending glass tube through a Teflon connection at 150 mL/min. To minimize visual distraction for the flies, the Y-tube olfactometer was placed inside a white paper box, which was open on the top (for illumination) and on the front side (for observation). Illumination was provided by vertically hanging an office lamp (20 W, 250 Lux) above (~50 cm high) the olfactometer tube.

The first experiment was conducted to test the attractiveness of live fire ant workers to *P. tricuspis* in a Y-tube olfactometer. This experiment was based on the reports suggesting odor-mediated attraction of *P. tricuspis* to disturbed or shaken fire ant workers (Vander Meer and Porter, 2002; Morrison and King, 2004). Approximately 250–300 mg live *S. invicta* workers were placed into one of the extending glass tube (arms), which was covered with a Teflon connection instantly. The second arm was left blank and served as the control. *Pseudacteon tricuspis* was introduced individually into the central arm of the Y-tube. The initial choice of a fly that responded by walking into one of the arms (choice chambers) and remaining there at least 15 seconds was recorded. If a fly had not made a choice within 2 min of being released, it was removed and discarded. Flies that did not walk into any of the arms were not counted. After five individual flies had been tested, the olfactometer arms were flipped around (180°) to minimize positional effect. After 10 flies had been bioassayed, fire ant workers were discarded and replaced with fresh workers, and the olfactometer set-up was rinsed with soap water, and acetone, and then air-dried. In this and the next experiment, 30 adult *P. tricuspis* of each sex and mating status were bioassayed, and flies were used only once.

A second experiment was conducted to test the attractiveness of body extracts of *S. invicta* workers and (*E*,*E*)-α-farnesene (trail pheromone) to *P. tricuspis* using similar procedures described in the first experiment. A total of five stimuli was tested: extracts of fire ant worker whole body, head, thorax, and abdomen, and (*E*,*E*)-α-farnesene. Each stimulus was delivered as 10-μL sample (resulting in 1 worker equivalent) placed on No. 1 filter paper strips (7 × 40 mm, Whatman® no. 1). After allowing for solvent evaporation (~15 s), the filter paper strip was inserted into one arm of the Y-tube olfactometer. A similar filter paper strip containing a 10-μL aliquot of hexane was inserted into the second arm (solvent control). After three individual flies had been tested, a fresh odor stimulus was used and the olfactometer arms were reversed (180°). After each subset of 6 flies had been bioassayed the olfactometer set-up was rinsed as described above.

A third experiment was conducted in order to confirm the response of *P. tricuspis* to extracts of different body parts of *S. invicta.* This experiment was conducted with freshly extracted body parts from newly-collected fire ant workers. The experimental procedure is as described above for the second experiment. However, only mated individuals of both sexes of *P. tricuspis* were tested ($N = 72$ per sex): unmated individuals of both sexes were not bioassayed. Olfactometer data were analyzed by the use of a chi-square test ($P < 0.05$, SAS Institute, 2004).

Electroantennogram (EAG) Recordings

The EAG techniques used in this study were similar to those previously described (e.g., Dickens, 1984). Glass capillary (1.1 mm I.D.) filled with 0.1 M KCl solution was used as electrodes. The reference electrode was connected to the neck of an isolated head of an adult phorid fly. Due to the observed sexual dimorphism in antennal morphology of *P. tricuspis* in which female antennae bear an arista which is not present on the male antennae (unpublished data), the recording electrode was connected to the cut tip of the antenna (flagellum) in males or the cut tip of the arista in females. Chlorinated silver-silver chloride junctions were used to maintain electrical contact between the electrodes and input of preamplifier. The analog signal was detected through a probe (INR-II, Syntech®, the Netherlands), captured and processed with a data acquisition controller (IDAC-4, Syntech®, the Netherlands), and later analyzed with a software (EAG 2000, Syntech®, the Netherlands) on a personal computer.

Ten-microliter aliquot of each solution was applied to a piece of filter paper strip (7 × 40 mm, Whatman® no. 1). After allowing for solvent evaporation, the impregnated filter paper strip was inserted into a glass Pasteur pipette (~14 cm in length, Fisher Scientific, Pittsburgh, Pennsylvania, U.S.A.) constituting an odor cartridge. The control stimulus was a similar pipette containing a filter paper strip impregnated with a 10 μL aliquot of hexane. The tip of the pipette was placed about 3 mm into a small hole in the wall of a glass tube (13 cm long, 8 mm diameter) oriented towards the antennal preparation (~0.5 cm away from the preparation). In this way, the stimuli were provided as 0.2 s puffs of air into a continuous humidified air stream at 1000 mL/min generated by an air stimulus controller (CS-55, Syntech®, the Netherlands). At least 2 min was allowed between successive stimulations for antennal recovery.

In the first experiment, we tested the EAG response of mated and unmated *P. tricuspis* of both sexes to three doses of fire ant worker whole body extract, (*E*,*E*)-α-farnesene (1 μg dose), and hexane control, for a total of five odor stimuli treatments. A test series of stimuli were applied to an antennal preparation (individual fly) using the following order: hexane control, whole body extract (in a dose order of 0.01, 0.1, 1 worker whole body equivalent (WBE); and (*E*,*E*)-α-farnesene. Recordings were obtained from 12 individuals for each sex and mating combination. In the second experiment, we compared the EAG response of mated females to 1 worker equivalent of whole body, head, thorax, or abdomen extracts, and to (*E*,*E*)-α-farnesene (1 μg dose) and hexane control, for a total of six treatments. A test series of odor stimuli was applied to an antennal preparation using the following order: hexane control, body parts, whole body, and (*E*,*E*)-α-farnesene. Body parts stimuli were presented in the order: 1-2-3, 1-3-2, 2-1-3, 2-3-1, 3-1-2, 3-2-1 (1 = head; 2 = thorax; 3 = abdomen) for every 6 antennal preparations. Whole body extract was presented after body part extracts based on preliminary results showing greater EAG response to whole body extract than to extracts of different body parts. (*E*,*E*)-α-farnesene was presented last since it is the only synthetic chemical compound tested and because the tested dose (1 μg) is considered a high dose. A test series of odor stimuli applied to an antennal preparation was completed within 25 min and the order of odor presentation had no effect on EAG response (personal observation). Furthermore, a fresh isolated-head EAG preparation lasted about 0.5 hour with no noticeable significant decrease in EAG responsiveness over this time period. Twelve individual mated females were used to obtain EAG recordings.

Data from the first EAG experiment were first analyzed by using the standard least squares fit model ($P < 0.05$; SAS Institute, 2004) to determine the effects of odor stimuli, sex, mating, and interactions among these factors on EAG response. Response of female and male flies to each odor stimulus was then compared by using t-test ($P < 0.05$; SAS Institute, 2004). Further analysis of EAG data (from both experiments) was by using analysis of variance (ANOVA) followed by Duncan's multiple comparison tests to compare EAG responses to different odor stimuli ($P < 0.05$; SAS Institute, 2004).

RESULTS

Behavioral Bioassays

Data from the first experiment showed that mated female *P. tricuspis* were significantly attracted to live workers of *S. invicta* ($\chi^2 = 4.8$, $df = 1$, $P = 0.03$, Fig. 1A). However, no significant attraction to live workers was recorded for unmated females ($\chi^2 = 3.3$, $df = 1$, $P = 0.07$, Fig. 1B), mated males ($\chi^2 = 1.2$, $df = 1$, $P = 0.27$, Fig. 1C) or unmated males ($\chi^2 = 1.2$, $df = 1$, $P = 0.27$, Fig. 1D). In the second experiment, we compared the attractiveness of extracts of *S. invicta* worker whole body, head, thorax, and abdomen, and (*E*,*E*)-α-farnesene to *P. tricuspis*. Fire ant worker whole body extract elicited significant attraction in mated females ($\chi^2 = 4.8$, $df = 1$, $P = 0.03$, Fig. 2A), unmated females ($\chi^2 = 4.8$, $df = 1$, $P = 0.03$, Fig. 2B), and mated males ($\chi^2 = 4.8$, $df = 1$, $P = 0.03$, Fig. 2C). Similarly, worker thorax extract induced strong attraction in mated females ($\chi^2 = 8.5$, $df = 1$, $P = 0.004$, Fig. 2A), unmated females ($\chi^2 = 8.5$, $df = 1$, $P = 0.004$, Fig. 2B), and mated males ($\chi^2 = 8.5$, $df = 1$, $P = 0.004$, Fig. 2C). However, no significant attraction of *P. tricuspis* was recorded to head extract, abdomen extract, or (*E*,*E*)-α-farnesene, irrespective of sex and mating status (Figs. 2A–D). Also, none of the odor stimuli was significantly attractive to unmated

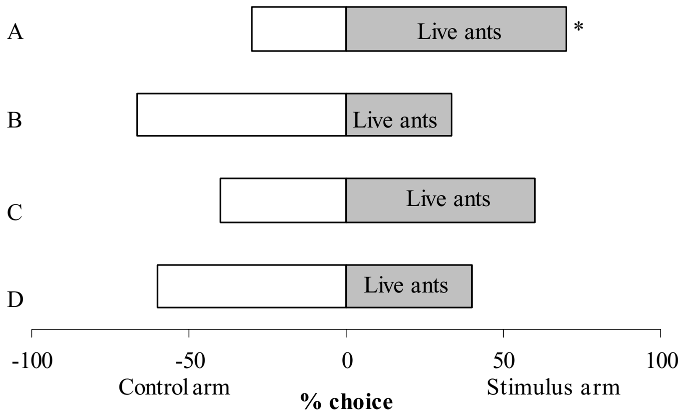
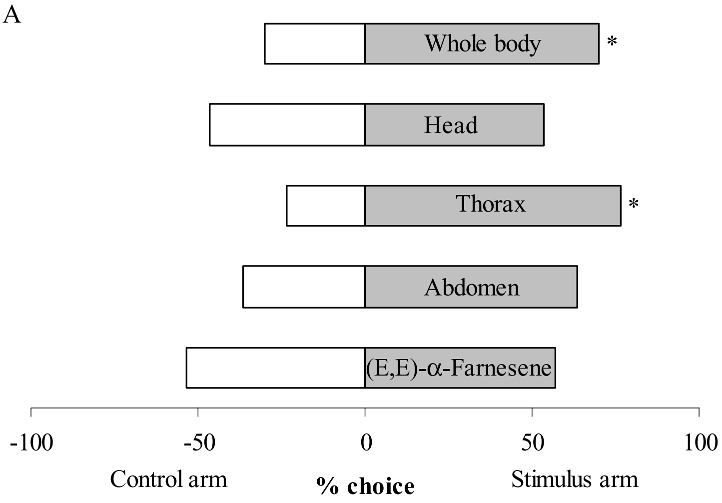


Fig. 1. Response of *Pseudacteon tricuspis* in a Y-tube olfactometer when given a choice between clean air (control) and live *Solenopsis invicta* workers (odor stimulus). (A) Mated female, (B) Unmated female, (C) Mated male, (D) Unmated male. Approximately 250–300 mg of live workers was used as odor stimulus. $N = 30$ individuals per choice test. * Indicates significant difference within a choice test ($P < 0.05$, chi-square).



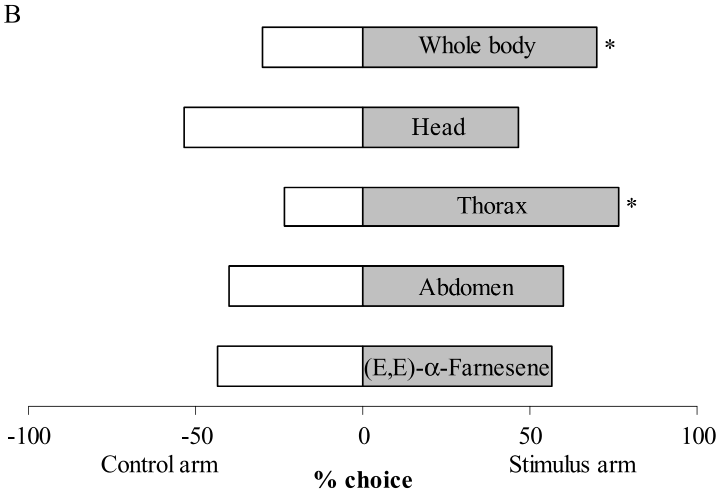
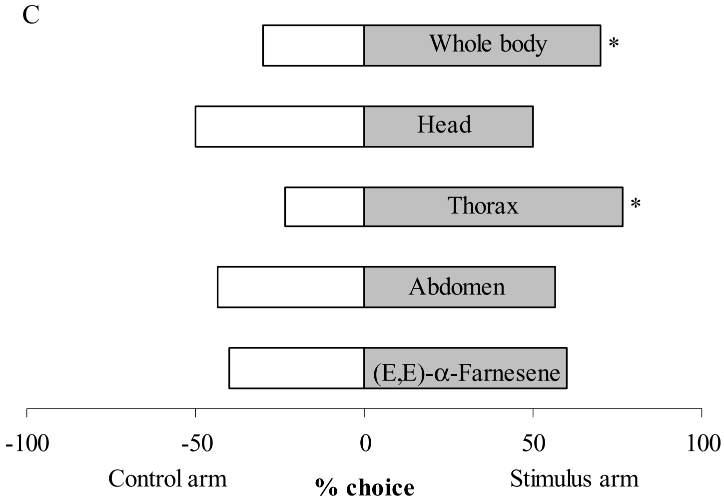


Fig. 2. Response of *Pseudacteon tricuspis* in a Y-tube olfactometer when given a choice between hexane (control) and *Solenopsis invicta* worker extracts (whole body, head, thorax, or abdomen) or (E,E)-α-farnesene (1 μg dose). (A) Mated female, (B) Unmated female, (C) Mated male, (D) Unmated male. Worker extracts were tested at 1 worker equivalent dose. $N = 30$ individuals per choice test. * Indicates significant difference within a choice test ($P < 0.05$, chi-square).



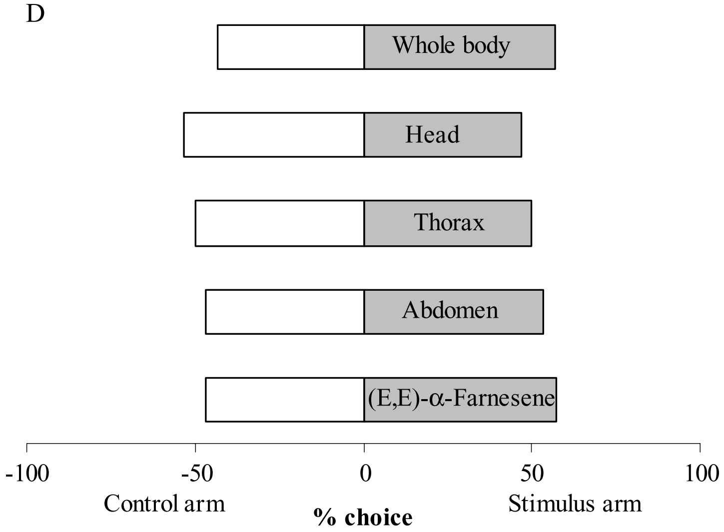


Fig. 2. (*Continued.*)

males (Fig. 2D). Similar results were obtained in the third experiment confirming the attractiveness of extract of worker whole body to mated females ($\chi^2 = 32.0$, $df = 1$, $P < 0.0001$, Fig. 3A) and mated males ($\chi^2 = 24.5$, $df = 1$, $P < 0.0001$, Fig. 3B), as well as the attractiveness of worker thorax extract to mated females ($\chi^2 = 37.6$, $df = 1$, $P < 0.0001$, Fig. 3A) and

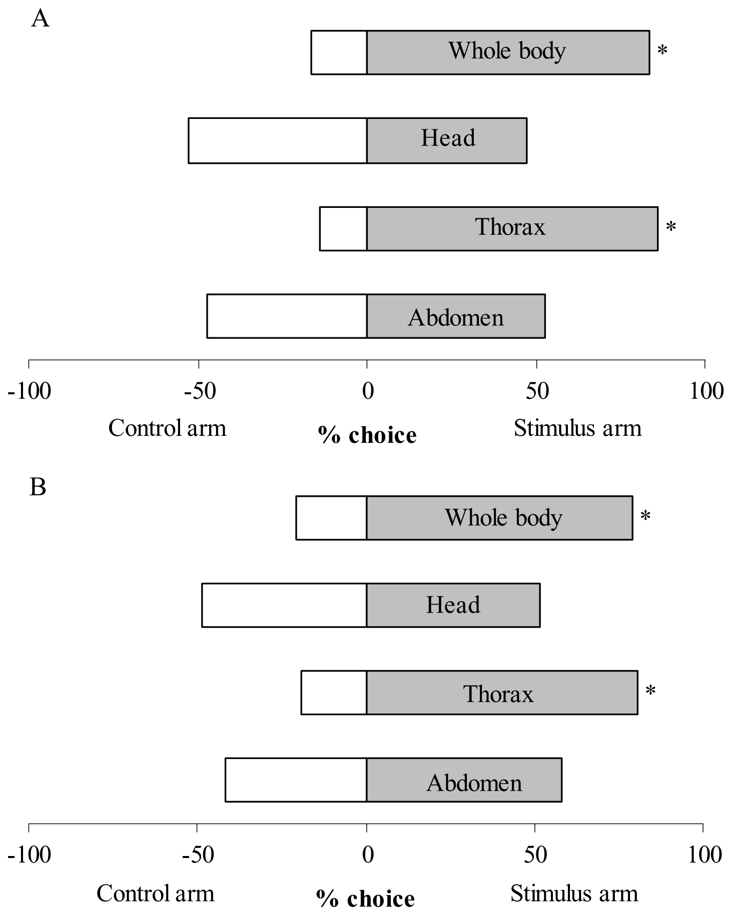


Fig. 3. Response of *Pseudacteon tricuspis* in a Y-tube olfactometer when given a choice between hexane (control) and *Solenopsis invicta* worker extracts (whole body, head, thorax, or abdomen). Experiment repeated with mated female (A) and mated male (B) *P. tricuspis*. Worker body extracts were tested at 1 worker equivalent dose. $N = 72$ individuals per choice test. * Indicates significant difference within a choice test ($P < 0.05$, chi-square).

mated males ($\chi^2 = 26.9$, $df = 1$, $P < 0.0001$, Fig. 3B). In both experiments, extracts of worker head and abdomen, and (E,E)-α-farnesene did not show significant attractiveness to *P. tricuspis,* irrespective of sex or mating status.

Electroantennogram (EAG) Recordings

Pseudacteon tricuspis of both sexes showed typical rapid depolarizing EAG responses (Fig. 4). The EAG amplitude in response to extract of *S. invicta* worker whole body (1 whole body equivalent) was in the range of 2–4 mV. The EAG responses of mated and unmated *P. tricuspis* of both sexes to three doses of *S. invicta* worker whole body extract, and (*E*,*E*)-*α*-farnesene are shown in Fig 5. Standard least squares modeling revealed significant effects of odor stimuli (stimuli treatments consisted of three doses of whole body extracts, (*E*,*E*)-*α*-farnesene, and hexane control) and sex on EAG responses of *P. tricuspis,* resulting in a significant odor stimuli × sex interaction (Table I). However, no significant effect of mating, odor stimuli × mating, sex × mating, or odor stimuli × sex × mating interactions were recorded on EAG response (Table I). Thus, data from unmated and mated individuals of each sex were pooled and analyzed by using ANOVA to test for significant effects of odor stimuli on the EAG response of female and male *P. tricuspis*. Both sexes of *P. tricuspis* showed dose-dependent EAG responses to *S. invicta* whole body extract with the 1 whole body extract (WBE) dose eliciting the greatest EAG responses in females and males (Fig. 5). Response of females to 1 WBE of fire ant whole body extract was significantly greater than response of males to the same

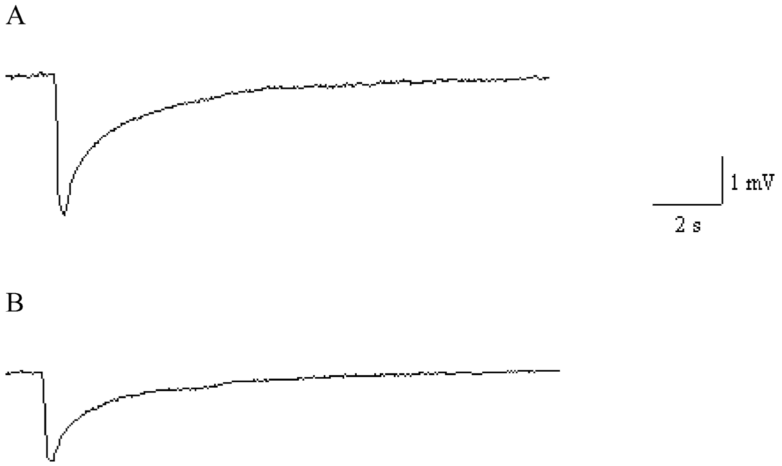


Fig. 4. Typical electroantennogram (EAG) response waveforms of *Pseudacteon tricuspis* to *Solenopsis invicta* worker whole body extract (1 whole body equivalent). (A) Mated female, (B) Mated male. Scales indicate 2 s (horizontal) and 1 mV (vertical).

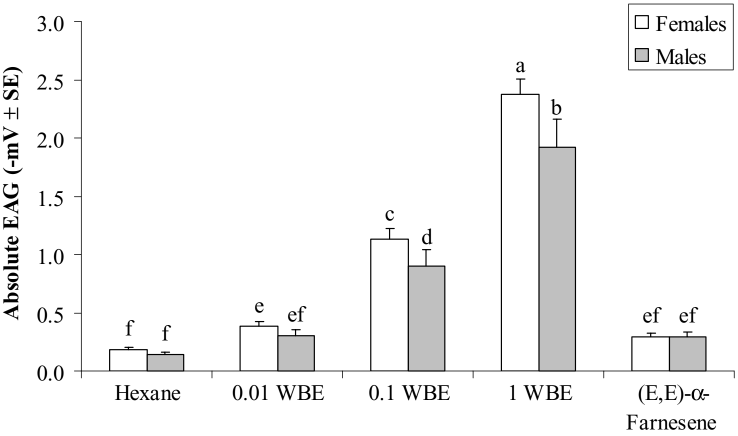


Fig. 5. Electroantennogram (EAG) responses of female and male *Pseudacteon tricuspis* to different doses of *Solenopsis invicta* worker whole body extracts (0.01, 0.1, 1 worker whole body equivalent = WBE) and (E,E)-α-farnesene (1 μg dose). Means followed by different letters are significantly different ($P < 0.05$, Duncan's multiple comparison tests). $N = 24$ individuals per odor stimulus per sex.

odor stimulus ($t = 2.79$, $P = 0.01$). Similarly, females showed significantly greater EAG response than males to the 0.1 WBE ($t = 2.59$, $P = 0.02$) and 0.01 WBE ($t = 2.58$, $P = 0.02$) doses (Fig. 5). However, (E,E)-α-farnesene failed to elicit any significant EAG response in both sexes. This lack of EAG response to (E,E)-α-farnesene is not due to the fact that it was the last odor stimulus presented to an antennal preparation, as results from another experiment also showed no EAG response to (E,E)-α-farnesene

Table I. Standard Least Squares Model Testing for Effects of Odor Stimuli, Sex, Mating, and Interactions of These Variables on EAG Response of *Pseudacteon tricuspis*

Source of variation	DF	MS	F	P
Odor stimuli$^+$	4	32.70	346.82	< .0001*
Sex	1	1.49	15.76	< .0001*
Mating	1	0.01	0.14	0.71
Odor stimuli × Sex	4	0.41	4.37	0.002*
Odor stimuli × Mating	4	0.01	0.09	0.99
Sex × Mating	1	0.03	0.31	0.58
Odor stimuli × Sex × Mating	4	0.01	0.16	0.96

$^+$Odor stimuli consisted of three doses of whole body extracts, (E,E)-α-farnesene (1 μg dose), and hexane control for a total of five treatments.
*Indicates significant variables or interactions at $P < 0.05$.

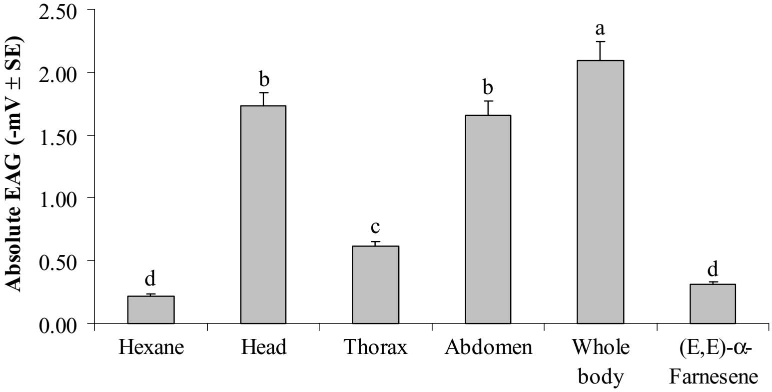


Fig. 6. Electroantennogram (EAG) responses of mated female *Pseudacteon tricuspis* to *Solenopsis invicta* worker extracts (whole body, head, thorax, or abdomen) and (*E*,*E*)-α-farnesene. Worker extracts were tested at 1 worker equivalent dose, while (*E*,*E*)-α-farnesene was tested at a 1 μg dose. Means followed by different letters are significantly different ($P < 0.05$, Duncan's multiple comparison tests). $N = 24$ mated females per odor stimulus.

(at the 0.1 μg and 1 μg doses) when presented alone to a fresh antennal preparation (unpublished data).

In the second EAG experiment, we compared the EAG responses of mated female *P. tricuspis* to 1 worker equivalent of whole body, head, thorax, and abdomen extracts, and to a 1 μg dose of (*E*,*E*)-α-farnesene. Significant differences were recorded in EAG response to the different odor treatments ($F = 56.42$, $df = 4$, $P < 0.0001$). The greatest EAG amplitude was elicited by whole body extract followed by head and abdomen extracts (Fig. 6). The EAG response evoked by thorax extract was significantly greater than the EAG evoked by (*E*,*E*)-α-farnesene or control (hexane) stimulus, but significantly less than the EAG elicited by extracts of whole body, head, and abdomen. The EAG magnitude in response to (*E*,*E*)-α-farnesene was not significantly greater than the EAG elicited by the control stimulus hexane (Fig. 6).

DISCUSSION

Our results confirm the semiochemical-mediated attraction of phorid flies to host fire ant as suggested in previous studies (Orr *et al.,* 1997; Vander Meer and Porter, 2002; Morrison and King, 2004), and support the hypothesis that fire ant worker trail pheromones are not likely used by

P. tricuspis for host location. However, the data do not seem to support the hypothesis regarding the use of fire ant alarm pheromones for host finding by phorid flies, as proposed by some authors (Vander Meer and Porter, 2002; Morrison and King, 2004). The glandular origins of fire ant alarm pheromones have been postulated as mandibular gland located in the head and Dufour's gland located in the abdomen (Obin and Vander Meer, 1994; Vander Meer and Alonso, 1996; Alonso and Vander Meer, 1997). Indeed, two products of the mandibular glands of the giant topical ant, *Paraponera clavata*, 4-methyl-3-heptanone and 4-methyl-3-heptanol, which serve as alarm pheromones in many ant species have been reported as attractants for another species of phorid fly, *Apocephalus paraponerae* (Feener *et al.,* 1996). Given this, one would have expected fire ant head and abdomen extracts, which are likely sources of alarm pheromones, to be highly attractive to *P. tricuspis*. Nevertheless, our olfactometer bioassay results consistently showing that fire ant thorax extract is as attractive to *P. tricuspis* as whole body extract, and significantly more attractive than head or abdomen extracts may imply the use of semiochemicals produced in the fire ant thorax as host location cues by phorid flies.

Of the several known glands associated with fire ant thorax (Phillips and Vinson, 1980; Hölldobler and Engel-Siegel, 1984; Billen, 1990), the paired metapleural glands are a potential candidate glandular source of attractants for phorid flies. These glands are peculiar to the ants (Formicidae) and produce antibiotic secretions that flow out over the cuticle, providing protection (body surface and nest) against microorganisms (Beattie *et al.,* 1986; Cabrera *et al.,* 2004). Another potential glandular source of attractants in the fire ant thorax is the labial gland, which is located in the anterior, lateral region of the prothorax (Phillips and Vinson, 1980), but is considered a cephalic gland because of its opening site in the head (Phillips and Vinson, 1980; Billen, 1990). Female phorid oviposits through a quick jab (a fraction of a second) of its highly specialized and sclerotized ovipositor into the thorax, where the coxal region of the first leg joins the thorax (Feener, 1987; Cônsoli *et al.,* 2001; Wuellner *et al.,* 2002). Thus, the location of phorid eggs appears to fall into the distribution area of host's labial gland (0.5 × 0.5 mm, Phillips and Vinson, 1980). Labial glands contain important digestive enzymes (Paulsen, 1971; Febvay *et al.,* 1984), which may provide a source of nutrients for phorid fly eggs during embryonic development (Cônsoli *et al.,* 2001). Clearly, the hypothesis that the thorax of the fire ant is the likely source of attractants for phorid flies warrants further study. Could the metapleural gland in the ant thorax which is known to secrete an alarm-defense substance in *Crematogaster inflata* (Maschwitz, 1974) also be the source of an alarm-defense substance in *S. invicta,* which may also function as an attractant for phorid flies? Could the attraction of *P. tricuspis* to

S. invicta thorax extracts recorded in the current study, at least in part, explain why female *P. tricuspis* deposits her egg in the thorax of a worker ant rather than in other parts of the body?

The results showing no behavioral or EAG response of *P. tricuspis* to (*E*,*E*)-α-farnesene appears to be in agreement with previous research demonstrating no attraction of phorid flies to foraging fire ant workers' trails (Vander Meer and Porter, 2002; Morrison and King, 2004). The trail pheromone of *S. invicta* is produced in the Dufour's gland (Wilson, 1959), and consists of two key components, two α-farnesenes (*Z,E*- and *E,E*-α-farnesene), and three other components including two homofarnesenes (*Z,Z*- and *Z,E*-3,4,7,11-tetramethyl-1,3,6,10-dodecatetraene) and an allofarnesene (*Z,Z,Z*-3,7,11-trimethyl-2,4,6,10-dodecatertraene) (Vander Meer *et al.,* 1981; Williams *et al.,* 1981; Vander Meer, 1983). Given that only (*E,E*)-α-farnesene was tested in the present study, it is difficult to completely rule out the use of fire ant trail pheromones as kairomones by phorid flies. Nevertheless, since (*E,E*)-α-farnesene alone is capable of inducing trailing behavior in *S. invicta* workers (Vander Meer, 1983), it is doubtful that this compound or other components of the fire ant trail pheromone are used as an attractant by phorid flies. This view is further supported by the results of a separate olfactometer experiment showing no response of phorid flies to different doses of (*E,E*)-α-farnesene (i.e., 0.01, 0.1, 1, 10 μg) (unpublished data).

The different stimuli (live ants or ant extracts) produced slightly different results from phorid flies of different physiological states. Live ants elicited significant behavioral response only in mated females, whereas fire ant body and thorax extracts elicited significant response in all but unmated males. These dissimilar results may possibly be due to different perception-thresholds of phorid flies of different physiological states. For instance, it is possible that the attractant compound(s) are released in low rates by live ants, such that only mated females are capable of responding at that low rate. However, solvent extraction of fire ant body and thorax may yield higher amounts of the attractant compound(s) which are in the range of perception by unmated females and mated males. It is reasonable to predict that mated females are likely to have the lowest perception-threshold given that mated females are likely the primary group involved in host location and hence the most attracted physiological group in nature. The attraction of mated males to fire ant extracts is not entirely surprising. Both sexes of *Pseudacteon* phorid flies are attracted to fire ants and mating occurs while females are searching for ant workers to attack (Feener, 1987; Feener and Brown, 1992; Porter *et al.,* 1995b).

It is not clear why unmated male *P. tricuspis* are not attracted to fire ant workers or body extracts. Porter *et al.* (1997) reported that in a flight box, both male and female *P. tricuspis* hovered a few millimeters above fire ant workers, and the males tracked the movements of ovipositing females rather than fire ant workers. Although the mating status of the males was not determined in the above study, it is possible that newly emerged unmated male phorid flies are preconditioned to respond only to conspecific females and would require further conditioning (in form of mating or experience) in order to respond to fire ant workers. Olfactory learning and effect of experience on response to chemical odors has been demonstrated for various insects including parasitic wasps (e.g., Kaiser and Cardé, 1992; Vet and Papaj, 1992) and flies (e.g., Prokopy *et al.,* 1987; Dubnau and Tully, 1998; Ikeda *et al.,* 2005). In several species of parasitic wasps, only a limited number of odor compounds could elicit behavioral response in inexperienced (naïve) parasitoids; most species are able to expand their array of foraging cues by associative learning (Kaiser and Cardé, 1992; Vet and Papaj, 1992; Wäckers and Lewis, 1994). The hypothesis that newly emerged male phorid flies require odor conditioning or mating experience in order to respond to fire ant odor will be tested in future studies.

The slightly (but significantly) greater EAG response to fire ant odor recorded for females compared to males may be related to the observed sexual dimorphism in antennal morphology of *P. tricuspis*: the female antenna bears an arista which is not present on the male antennae (unpublished data). However, this possibility of sexual dimorphism in response was not obvious in olfactometer tests.

Fire ant worker thorax elicited significant EAG response supporting data from behavioral bioassays. However, head and abdomen extracts which did not induce attraction of *P. tricuspis* in olfactometer bioassays, elicited even stronger EAG response. This data showing disparity between EAG and olfactometer results is not unusual. An electroantennogram does not distinguish between attraction or inhibitory effects, and the magnitude of EAG response does not always correlate with behavior (Löfstedt *et al.,* 1982; Bjostad, 1998). For instance, ethyl vanillin elicited no significant EAG response, but did elicit significant upwind flight in female almond moth, *Ephestia cautella* (Olsson *et al.,* 2005). On the other hand, Lanne *et al.* (1987) reported that some monoterpenes (e.g. *trans*-verbenol) which elicited high EAG response in the pine shoot beetle, *Tomicus piniperda* were unattractive in behavioral experiments. The strong EAG response of *P. tricuspis* to fire ant head and abdomen extracts may be due to the presence of defensive secretions in the head (e.g., mandibular gland secretions) and abdomen (e.g., secretions from Dufour's gland and venom gland), which although may elicit strong EAG response, but may not constitute attractants to phorid flies. Our results showing no attraction of phorid flies to abdomen and head extracts suggest that ant defensive compounds are not likely to be used as attractants by phorid flies. The fact that defensive chemicals are typically produced in high amounts in ants may explain why abdomen and head extracts elicited significantly greater EAG than thorax extract, which is the likely source of attractants used by phorid flies for host location. Further studies will investigate the glandular sources and chemical identity of the attractant compound(s) and possibly elucidate the disparity between our olfactometer and EAG results.

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