



Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Fire ant alarm pheromone and venom alkaloids act in concert to attract parasitic phorid flies, *Pseudacteon* spp.

Kavita R. Sharma^{a,b}, Henry Y. Fadamiro^{a,*}^a Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849, USA^b Department of Entomology, 900 University Avenue, Riverside, CA 92521, USA

ARTICLE INFO

Article history:

Received 13 May 2013

Received in revised form 16 August 2013

Accepted 24 August 2013

Available online 11 September 2013

Keywords:

Phorids

Solenopsis invicta

Alarm pheromone

Venom alkaloids

Olfactometer

ABSTRACT

Pseudacteon tricuspidis, *Pseudacteon obtusus* and *Pseudacteon curvatus* are three species of parasitic phorid flies (Diptera: Phoridae), which have been introduced as classical biological control agents of imported, *Solenopsis* fire ants (Hymenoptera: Formicidae) in the southern USA. Previous studies demonstrated the behavioral response of *P. tricuspidis* to the venom alkaloids and alarm pheromone of the fire ant, *S. invicta*. In the present study, we compared the responses of *P. tricuspidis*, *P. obtusus* and *P. curvatus* to *Solenopsis invicta* alarm pheromone, venom alkaloids, or a mixture of both chemicals in four-choice olfactometer bioassays. The main hypothesis tested was that the fire ant alarm pheromone and venom alkaloids act in concert to attract *Pseudacteon* phorid flies. Both sexes of all three *Pseudacteon* species were attracted to low doses of the fire ant alarm pheromone or venom alkaloids (i.e. 1 ant worker equivalent) alone. However, the flies were significantly more attracted to a mixture of both chemicals (i.e., 1:1 mixture of alarm pheromone + alkaloids) than to either chemical. The results suggest an additive rather than a synergistic effect of combining both chemicals. Comparing the fly species, *P. tricuspidis* showed relatively greater attraction to *cis* alkaloids, whereas the alkaloid mixture (*cis* + *trans*) was preferred by *P. obtusus* and *P. curvatus*. In general, no key sexual differences were recorded, although females of *P. tricuspidis* and *P. obtusus* showed slightly higher response than conspecific males to lower doses of the alarm pheromone. The ecological significance of these findings is discussed, and a host location model is proposed for parasitic phorid flies involving the use of fire ant alarm pheromone and venom alkaloids as long range and short range attractants, respectively.

Published by Elsevier Ltd.

1. Introduction

Two fire ant species (Hymenoptera: Formicidae) were accidentally introduced from South America into the southern USA in the past century. The black imported fire ant, *Solenopsis richteri* Forel was introduced in the 1910s followed by the introduction of the red imported fire ant, *Solenopsis invicta* Buren in the early 1930s. Hybridization between both fire ant species has been documented in Alabama, Mississippi, Georgia, and Tennessee (Ross et al., 1987; Vander Meer et al., 1985). Together, both fire ant species and their hybrid form are estimated to be responsible for almost \$6 billion annually in damage repair and control costs (Lard et al., 2006), as well as unquantified medical and environmental impacts on native organisms. Fire ants have also recently become a global problem detected in several countries and regions (Wetterer, 2013).

The search for a sustainable solution to the fire ant problem in the southern USA in the 1990s led to the identification of parasitic *Pseudacteon* phorid flies (Diptera: Phoridae) as key natural enemies of fire ants in South America (Gilbert et al., 2008; Porter and Alonso,

1999). As a result, several species of *Pseudacteon* phorid flies, including *Pseudacteon tricuspidis* Borgmeier, *P. curvatus* Borgmeier and *P. obtusus* Borgmeier, were introduced from South America in the past two decades for biological control of fire ants in the USA (Gilbert 1996; Graham et al., 2003; Plowes et al., 2009; Porter and Gilbert, 2004; Vazquez and Porter, 2005).

Pseudacteon spp. are highly specific to fire ants (Porter and Alonso, 1999; Porter and Gilbert, 2004), and their attraction to fire ant workers has been demonstrated in the field (Gilbert and Morrison, 1997; Morrison and King, 2004; Orr et al., 1997) and laboratory (He and Fadamiro, 2009; Vander Meer and Porter, 2002). *Pseudacteon* flies that attack *Solenopsis* fire ants typically do not attack ants in other genera, and showed an attack preference for introduced fire ants over native fire ants (Gilbert and Morrison, 1997; Porter, 1998; Porter and Alonso, 1999; Folgarait et al., 2002). Furthermore, different *Pseudacteon* species show different levels of host specificity and preferences. For example, Morrison and Porter (2006) reported that *P. tricuspidis* readily attacked *S. invicta* but rarely parasitized native ants such as *S. geminata*, or other ant species. Among the introduced fire ant species, *P. tricuspidis* showed greater preference for *S. invicta* and the *S. invicta* × *S. richteri* hybrid compared to *S. richteri* (He and Fadamiro, 2009).

* Corresponding author. Tel.: +1 334 844 5098; fax: +1 334 844 5005.

E-mail address: fadamhy@auburn.edu (H.Y. Fadamiro).

Attraction of phorid flies to fire ant workers was later confirmed to be mediated by fire ant odor (Vander Meer and Porter, 2002; Chen and Fadamiro, 2007). Two recent studies have attempted to identify the specific chemical cues mediating the interaction between *Pseudacteon* spp. and fire ants. First, Chen et al. (2009) demonstrated the attraction of *P. tricuspis* to the venom alkaloids of *S. invicta*. The recent identification of 2-ethyl-3,6-dimethyl pyrazine as alarm pheromone component of *Solenopsis* fire ants (Vander Meer et al., 2010) paved the way for testing the role of fire ant alarm pheromone in phorid fly host location. Subsequently, it was shown that *P. tricuspis* was attracted to 2-ethyl-3,6(or 5)-dimethyl pyrazine, a commercially available mixture of fire ant alarm pheromone component (2-ethyl-3,6-dimethyl pyrazine) and its isomer (Sharma et al., 2011). These findings led us to propose that both the fire ant alarm pheromone and venom alkaloids likely act in concert to attract *Pseudacteon* spp. to fire ant workers (Sharma et al., 2011).

In the present study, we tested the above hypothesis by evaluating the responses of three species of phorid flies, *P. tricuspis*, *P. obtusus* and *P. curvatus*, to the alarm pheromone and venom alkaloids of *S. invicta*. Specifically, four choice olfactometer bioassays were used to compare the response of both sexes of each species to 2-ethyl-3,6(or 5)-dimethyl pyrazine (alarm pheromone), venom alkaloids, or a mixture of both chemicals to determine the possibility of an additive or a synergistic interaction between fire ant alarm pheromone and venom alkaloids in attracting phorid flies.

2. Materials and methods

2.1. Insects

The three species of *Pseudacteon* phorid flies, *P. tricuspis* (Jaguarina biotype from Brazil), *P. obtusus* (large biotype) and *P. curvatus* (Formosa biotype from Argentina), evaluated in the study were reared on the workers of *S. invicta* at the USDA-APHIS, Center for Medical, Agricultural and Veterinary Entomology laboratory in Gainesville, Florida, USA, as described by Porter et al. (1997). Parasitized fire ant worker heads were received in batches and maintained in a plastic jar with a mesh lid (25-cm diameter × 13-cm high) at 26 ± 1 °C, 75 + 5% RH and LD 14:10 h photoperiod. Emerging flies were removed twice daily with an aspirator and species identity and sex were confirmed under a stereomicroscope by using appropriate morphological characters such as the presence of an ovipositor in females (Porter, 1998; Porter and Pesquero, 2001). Newly-emerged flies of each species were placed in groups of two individuals of the same sex (mated individuals) in a 6-cm diameter plastic Petri dish, and provided with 10% sucrose solution. One-day old flies were used for the tests.

2.2. Test chemicals

Two types of chemical stimuli were evaluated in this study, *S. invicta* alarm pheromone and venom alkaloids. The alarm pheromone component of *S. invicta*, 2-ethyl-3,6-dimethyl pyrazine, is commercially available as 2-ethyl-3,6(or 5)-dimethyl pyrazine, which is a mixture of 2-ethyl-3,6-dimethyl pyrazine (40%) and its isomer, 2-ethyl-3,5-dimethyl pyrazine (60%). This alarm pheromone-isomer, 2-ethyl-3,6(or 5)-dimethyl pyrazine (<95%), which was previously shown as attractive to *P. tricuspis* (Sharma et al., 2011), was purchased from the Sigma® Chemical Co. (St. Louis, MO) and tested as the fire ant alarm pheromone. Solutions of this compound were prepared in HPLC grade hexanes to obtain the three doses (0.1, 1 and 10 µg) and stored at -20 °C until use. *S. invicta* worker venom alkaloid fractions (*cis* and *trans*) were extracted, isolated and identified as described by Chen and Fadamiro

(2009a,b) and Chen et al. (2009). One worker ant equivalent (WE) of the venom alkaloids (*cis*, *trans*, or a 1:1 mixture of *cis* + *trans*) was evaluated, based on a previous study (Chen et al., 2009).

2.3. Olfactometer bioassay protocols

A four-choice olfactometer was used to test the behavioral response of both sexes of *P. tricuspis*, *P. obtusus* and *P. curvatus* to different doses and combinations of the fire ant alarm pheromone and venom alkaloids in three major experiments. The olfactometer system and protocols were as described by Chen et al. (2009) and Sharma et al. (2011). Briefly, the glass olfactometer system consisted of one central chamber (20 cm long × 20 cm wide × 20 cm high) connected to four cylindrical arms on each side (19 cm long × 11 cm wide). The orifices of the olfactometer were connected through Teflon-glass tube connectors to four pumps on an air delivery system equipped with a vacuum pump (ARS, Inc., Gainesville, FL). Purified air was drawn at a constant rate of 300 ml/min through each of the four arms and removed by suction via the vacuum pump through the central orifice at the rate of 1300 ml/min. The apparatus was positioned under a fluorescent light source (~100 lux) for uniform lighting.

Each stimulus (or hexane control) was delivered as 10-µl sample impregnated on filter paper strips (1 × 1 cm, Whatman® no. 1). After allowing for solvent evaporation (~15 s), the filter paper strip was inserted into its designated olfactometer arm. Phorid flies (1-day old) were released at the top of the central chamber. The flies were observed continuously for 15 min, and those found in each arm were counted and removed. Flies that did not choose one of the four arms within 15 min were considered as “non-responders” and not included in the analysis. After each test, the olfactometer was cleaned with hexane and acetone and the arms were rotated (90°) to minimize positional effect. All experiments were conducted at 25 ± 1 °C, 50–60% RH, and between 12:00 h and 16:00 h, the time of day for high phorid fly activity (Pesquero et al., 1996). For each experiment, 20 phorid flies were released per replication and replicated 10 (Experiment 1 and 2) or 15 (Experiment 3) times using new flies (i.e. an individual fly was tested only once).

2.3.1. Experiment 1. Response of *Pseudacteon* spp. to the alarm pheromone of *S. invicta*

The responses of both sexes of *P. tricuspis*, *P. obtusus* and *P. curvatus* to 2-ethyl-3,6(or 5)-dimethyl pyrazine, the commercially available synthetic alarm pheromone isomer of *S. invicta* (hereafter referred to as alarm pheromone) were evaluated in a series of four-choice olfactometer bioassays. In each bioassay three doses of the pheromone (0.1, 1 and 10 µg) were compared with a solvent control (hexane). Each species and sex was evaluated in separate tests for a total of six experiments.

2.3.2. Experiment 2. Response of *Pseudacteon* spp. to the venom alkaloids of *S. invicta*

The responses of both sexes of *P. tricuspis*, *P. obtusus* and *P. curvatus* to the *S. invicta* venom alkaloid fractions (*cis* or *trans*) and a 1:1 mixture of both were evaluated in a series of four-choice olfactometer bioassays. The aim was to determine the most preferred venom alkaloid fraction (or mixture) for each phorid fly species and sex. The venom alkaloid fractions (*cis* or *trans*) and a 1:1 mixture of *cis* + *trans* were tested at a dose equivalent to one fire ant worker equivalent (1 WE) and compared with a solvent control (hexane), as described by Chen et al. (2009). Each species and sex was evaluated in separate tests for a total of six experiments.

2.3.3. Experiment 3. Response of *Pseudacteon* spp. to mixture of the alarm pheromone and venom alkaloids of *S. invicta*

To determine if the fire ant alarm pheromone and venom alkaloids act in tandem or synergistically to attract phorid flies, the responses of both sexes of *P. tricuspidis*, *P. obtusus* and *P. curvatus* to the *S. invicta* alarm pheromone (2-ethyl-3,6(or 5)-dimethyl pyrazine), venom alkaloids, or a 1:1 mixture of both chemicals were compared with a solvent control (hexane). A synergistic effect is defined as an effect in which the mixture of the alarm pheromone and venom alkaloids elicited an attraction greater than the sum of the attraction elicited by each individual component, while an additive effect is defined as an effect in which the mixture elicited an attraction similar to or less than the sum of the attraction elicited by each individual component. Each species and sex was evaluated in separate tests for a total of six experiments. The most preferred alarm pheromone dose (optimum dose) and venom alkaloid fraction determined in the preceding experiments for each sex and species of phorid flies were selected for testing in each experiment. For both sexes of *P. tricuspidis*, a mixture of 0.1 μg of the alarm pheromone and *cis* alkaloid fraction (1 WE) were tested. For both sexes of *P. obtusus* and *P. curvatus*, a mixture of 0.1 μg of the alarm pheromone and the alkaloid mixture (1 WE of *cis* + *trans* alkaloids) was evaluated.

2.4. Statistical analysis

Olfactometer data were not normally distributed and thus were first normalized by using the square-root transformation ($\sqrt{x} = 0.5$). Transformed data were then analyzed by using analysis of variance (ANOVA) followed by Tukey–Kramer HSD comparison test ($P < 0.05$; JMP[®] 7.0.1, SAS Institute, 2007) to establish significant differences among the different treatments evaluated in each experiment.

3. Results

3.1. Experiment 1. Response of *Pseudacteon* spp. to the alarm pheromone of *S. invicta*

All three phorid fly species showed a dose-related response to the alarm pheromone (2-ethyl-3,6(or 5)-dimethyl pyrazine) of *S. invicta* (Fig. 1). *Pseudacteon tricuspidis* females were significantly more attracted to the lowest dose (0.1 μg) of the alarm pheromone than to highest dose (10 μg) or solvent control ($F = 32.83$, $df = 3.36$, $P < 0.0001$) (Fig. 1A). Males also showed significantly greater response to all three doses of the alarm pheromone compared with solvent control ($F = 12.53$, $df = 3.36$, $P < 0.0001$) (Fig. 1B). *Pseudacteon obtusus* females were significantly more responsive to the lowest dose than to higher doses or solvent control ($F = 39.00$, $df = 3.36$, $P < 0.0001$) (Fig. 1A). Similarly, the 0.1 μg and 1 μg doses elicited the greatest attraction in males ($F = 29.76$, $df = 3.36$, $P < 0.0001$) (Fig. 1B). *Pseudacteon curvatus* also showed significantly greater attraction to the alarm pheromone compared with solvent control (females: $F = 9.85$, $df = 3.36$; $P < 0.0001$; males: $F = 14.65$, $df = 3.36$, $P < 0.0001$) (Fig. 1A and B). In general, *P. curvatus* was relatively less responsive to the alarm pheromone than the other two species.

3.2. Experiment 2. Response of *Pseudacteon* spp. to the venom alkaloids of *S. invicta*

For *P. tricuspidis*, both sexes exhibited significant attraction to the *cis* and *trans* fractions of the *S. invicta* venom alkaloids and their mixture compared with solvent control (females: $F = 31.49$, $df = 3.36$, $P < 0.0001$; males: $F = 25.45$, $df = 3.36$, $P < 0.0001$)

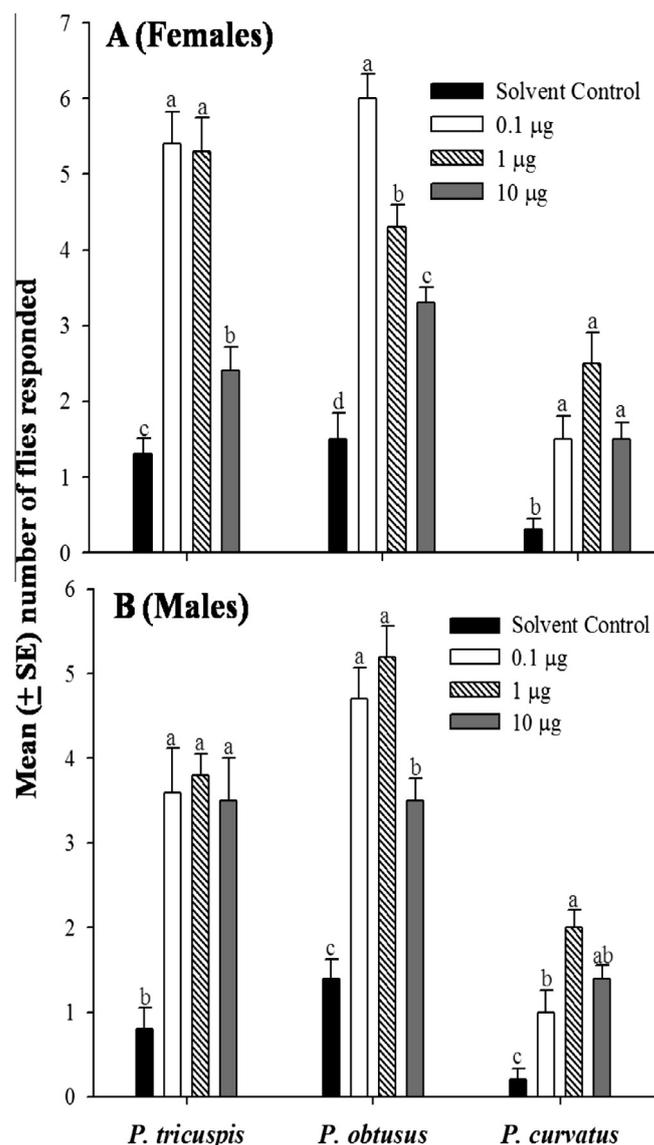


Fig. 1. Response of *Pseudacteon tricuspidis*, *P. obtusus* and *P. curvatus* in a four-choice olfactometer to varying doses (0.1, 1 and 10 μg) of the alarm pheromone-isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine, simply referred to as pheromone) of *S. invicta*. (A) Females; (B) Males. Figure shows mean (\pm SE) number of flies attracted per 15 min. 20 flies were released per test and replicated 10 times. Means having no letter in common are significantly different ($P \leq 0.05$, Tukey–Kramer HSD test).

(Fig. 2A). Among the three venom alkaloid treatments, females were significantly more attracted to the *cis* fraction than to the *trans* fraction or the mixture. Males were also significantly more attracted to the *cis* fraction and to the mixture than to the *trans* fraction (Fig. 2B).

Pseudacteon obtusus females were significantly more attracted to the mixture than to either fraction or solvent control ($F = 30.38$, $df = 3.36$, $P < 0.0001$) (Fig. 2A). *Pseudacteon obtusus* males were also strongly attracted to the mixture and the *cis* fraction ($F = 32.35$, $df = 3.36$, $P < 0.0001$) (Fig. 2B). *Pseudacteon curvatus* females ($F = 25.81$, $df = 3.36$, $P < 0.0001$) (Fig. 2A) and males ($F = 15.55$, $df = 3.36$, $P < 0.0001$) (Fig. 2B) were also significantly attracted to all three venom alkaloid treatments compared with solvent control. However, the mixture elicited the highest attraction in both sexes.

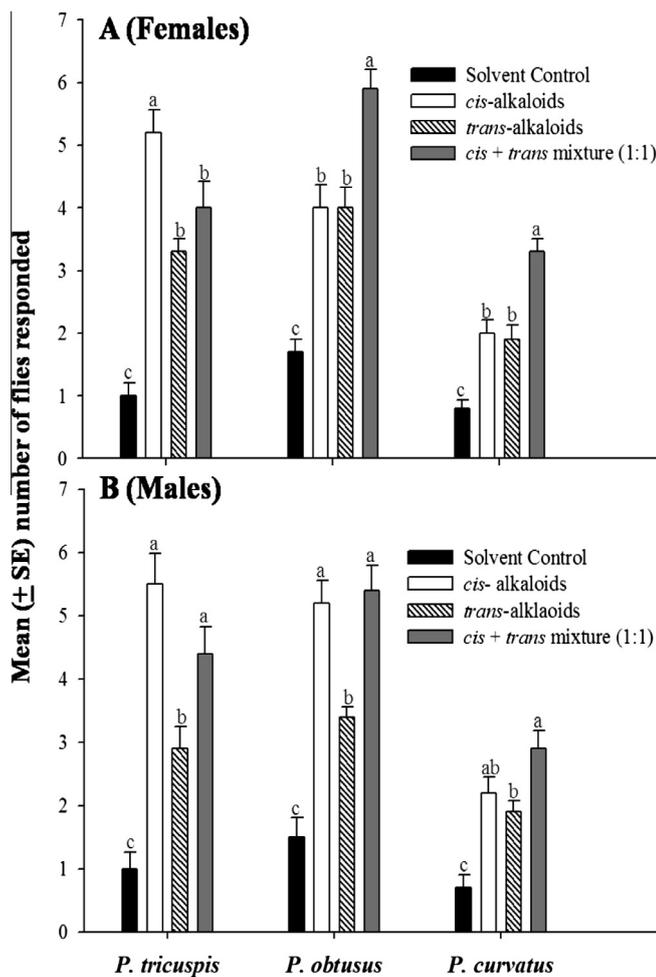


Fig. 2. Response of *Pseudacteon tricuspis*, *P. obtusus* and *P. curvatus* in a four-choice olfactometer to the *S. invicta* venom alkaloid fractions (*cis* or *trans*) or a 1:1 mixture of both (*cis* + *trans*) fractions. (A) Females; (B) Males. Figure shows mean (\pm SE) number of flies attracted per 15 min to 1 worker equivalent of each alkaloid treatment. 20 flies were released per test and replicated 10 times. Means having no letter in common are significantly different ($P \leq 0.05$, Tukey–Kramer HSD test).

3.3. Experiment 3. Response of *Pseudacteon* spp. to mixture of the alarm pheromone and venom alkaloids of *S. invicta*

Both sexes of *P. tricuspis* were significantly more attracted to the mixture of the alarm pheromone (0.1 μ g) and *cis* alkaloid (preferred alkaloid fraction) than to either chemical alone (females: $F = 63.04$, $df = 3.56$, $P < 0.0001$; males: $F = 62.66$, $df = 3.56$, $P < 0.0001$) (Fig. 3A and B). *Pseudacteon obtusus* also responded significantly more to the mixture of the alarm pheromone (0.1 μ g) and *cis* + *trans* alkaloids (preferred alkaloid) than to either chemical (females: $F = 45.73$, $df = 3.56$, $P < 0.0001$; males: $F = 45.40$, $df = 3.56$, $P < 0.0001$) (Fig. 3A and B). Similar results were also recorded for *P. curvatus*. Females ($F = 32.97$, $df = 3.56$, $P < 0.0001$) and males ($F = 33.74$, $df = 3.56$, $P < 0.0001$) were significantly more attracted to the mixture of the alarm pheromone (0.1 μ g) and *cis* + *trans* alkaloids (preferred alkaloid) than to either chemical (Fig. 3A and B). In summary, all three species were significantly more attracted to the mixture of the venom alkaloid and alarm pheromone than to either chemical but the data suggest only an additive effect of combining the two chemicals.

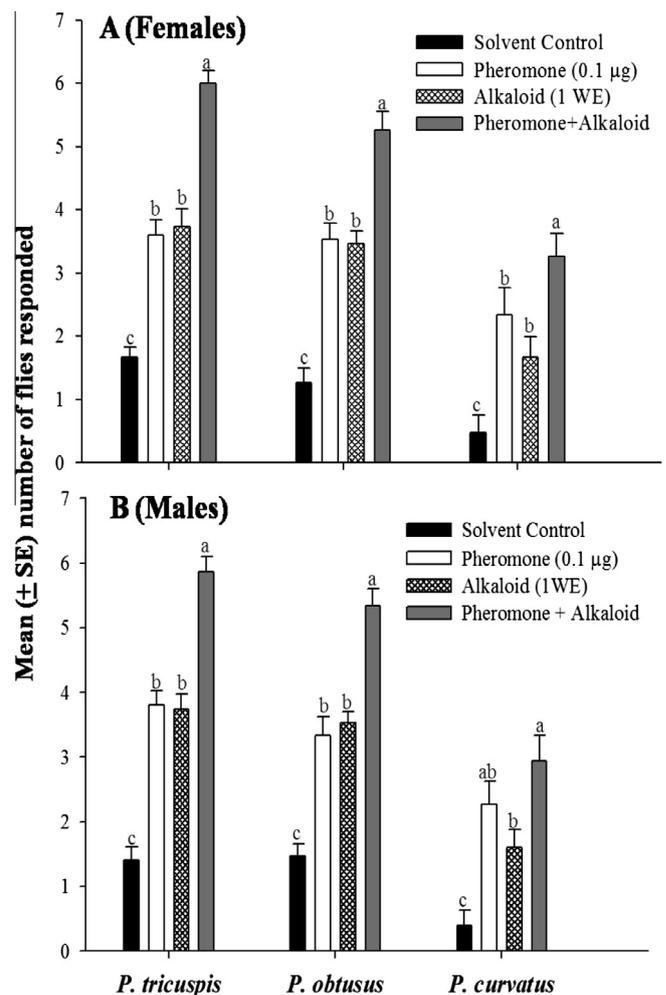


Fig. 3. Response of *Pseudacteon tricuspis*, *P. obtusus* and *P. curvatus* in a four-choice olfactometer to the alarm pheromone-isomer (pheromone), venom alkaloids (alkaloid), and to the mixture of pheromone and alkaloid (pheromone + alkaloid) of *S. invicta*. (A) Females; (B) Males. Figure shows mean (\pm SE) number of flies attracted per 15 min. 20 flies were released per test and replicated 15 times. Means having no letter in common are significantly different ($P \leq 0.05$, Tukey–Kramer HSD test). Note: the alarm pheromone (pheromone) was tested at a dose of 0.1 μ g for all species; the preferred venom alkaloid fraction for each *Pseudacteon* species as determined in experiment 2 was tested: *cis* alkaloid for *P. tricuspis*, and a 1:1 mixture of *cis* + *trans* alkaloids for *P. obtusus* and *P. curvatus*.

4. Discussion

All three phorid fly species, *P. tricuspis*, *P. obtusus* and *P. curvatus*, were attracted to the fire ant alarm pheromone or venom alkaloids alone (at the tested doses), but were significantly more attracted to a mixture of both chemicals. The data suggest an additive effect of combining the fire ant alarm pheromone and venom alkaloids as attractants for phorid flies, since the mixture elicited an attraction similar to or less than the sum of the attraction elicited by each individual component. These results are consistent with previous reports suggesting that the fire ant alarm pheromone (Morrison and King, 2004; Morrison and Porter, 2006; Sharma et al., 2011; Vander Meer and Porter, 2002; Vander Meer et al., 2010) or venom alkaloids (Chen et al., 2009) may serve as host location cues for phorid flies, and support our hypothesis that both the alarm pheromone and venom alkaloids act in concert to attract phorid flies to fire ant workers (Chen et al., 2009; Sharma et al., 2011). Another phorid fly species, *Pseudacteon formicarum* Verrall,

uses formic acid, the defense secretion of host formicine ants in the genus *Lasius*, as a host finding cue (Maschwitz et al., 2008; Weissflog et al., 2008). In contrast, *Pseudacteon brevicauda* Schmitz, was shown to respond to the mandibular gland components (alarm pheromone source) of its ant host, *Myrmica rubra* Linnaeus, but not to its non-volatile defensive secretion (Witte et al., 2010). However, this is the first demonstration of the combined role of the alarm and defensive secretions of ants as attractants for their natural enemies.

All three phorid fly species were attracted to both the fire ant alarm pheromone and venom alkaloids, suggesting that parasitic *Pseudacteon* phorid flies which attack fire ants have evolved similar host finding mechanisms involving the use of alarm and defensive secretions of their hosts. However, minor differences were recorded in the preference of the flies for the *cis* vs. *trans* alkaloids. While *P. tricuspsis* showed relatively greater attraction to the *cis* alkaloids, as previously reported (Chen et al., 2009), the alkaloid mixture (*cis* + *trans*) was preferred by both *P. obtusus* and *P. curvatus*. The relative alkaloidal preference of the phorid fly species may be explained by their host preferences in relation to fire ant size. Both *P. obtusus* and *P. curvatus* prefer the intermediate-sized fire ant workers as host, whereas *P. tricuspsis* prefers the larger-sized fire ant workers (Fowler, 1997). Deslippe and Guo (2000) reported that abundance and ratio of venom alkaloids vary with ant size, with larger ants producing more alkaloids (and consequently more *cis* alkaloids) than smaller ants. Although there is no direct evidence to date that host preference in phorid flies is mediated by the amount of alkaloids produced by different fire ant species, it is possible that *P. tricuspsis*, which parasitizes larger-sized ants that produce higher amounts of alkaloids (and consequently more *cis* alkaloids), may have evolved preference for the *cis* alkaloids. The results may also suggest possible attraction of *P. tricuspsis* to native fire ants such as *Solenopsis xyloni* and *S. geminata*, since the *cis* alkaloids predominate in both species (Brand et al., 1972). In their study of host specificity of *Pseudacteon* species in Argentina, Folgarait et al. (2002) reported that *P. tricuspsis* attacked *S. geminata*, but did not successfully complete development. In contrast, Morrison and Porter (2006) simultaneously presented *S. geminata* and *S. invicta* to *P. tricuspsis* and observed no parasitization on the former. Thus, it is likely that the ratios of the alkaloids and presence of some minor novel alkaloids (Chen and Fadamiro, 2009a,b), rather than abundance of *cis* alkaloids, play a major role in mediating phorid fly host preference.

Like the females, males of the phorid fly species were also attracted to both fire ant alarm pheromone and venom alkaloids. Odor-mediated attraction of male phorid flies to fire ant workers has also been demonstrated in the laboratory (He and Fadamiro, 2009) and field (Porter, 1998; Smith and Gilbert, 2003). Although males do not parasitize fire ant workers, they are attracted to fire ant odor probably for mate location (Chen et al., 2009; Morrison and King, 2004). In *P. tricuspsis* and possibly some other species, mating occurs as females search for ant workers to attack (Porter, 1998). Furthermore, there is no evidence to date for a sex pheromone in phorid flies. Thus, it is possible that male phorid flies have evolved to use the fire ant alarm and defense secretions as mate finding cues. Feener et al. (1996) also reported that both sexes of another phorid fly, *Apocephalus paraponerae* Borgmeier, were attracted to freshly killed *Paraponera clavata* (Fabricius, 1775) workers, and suggested that male flies used hosts as feeding or mating sites.

Vander Meer et al. (2010) estimated that *S. invicta* produces ca. 300 pg of the alarm pheromone per worker and reported that this amount was enough to elicit alarm response in conspecific workers. In the present study, we tested relatively higher doses than the dose reported by Vander Meer et al. (2010). The three doses tested were selected based on preliminary data which showed that

the phorid fly species were significantly more attracted to the alarm pheromone at the dose range of 0.1–10 µg than at lower (≤ 0.01 µg) or higher (100 µg) doses (Sharma et al., 2010; unpublished data). Thus, the results suggest that the physiologically relevant dose of the alarm pheromone perceived by fire ant workers is lower than that perceived by phorid flies. This is plausible given that the alarm pheromone is perceived by fire ants at the colony level (Vander Meer and Alonso, 1998; Wilson, 1976), while it is perceived by phorid flies from long range. Thus, it is conceivable that phorid flies might have evolved to respond to higher doses of the alarm pheromone compared to fire ant workers. Furthermore, whereas one worker equivalent of the alarm pheromone may be enough to trigger an alarm reaction in conspecific workers, phorid flies might require higher doses since they typically respond from long range to fire ants at the population level. Future studies are necessary to further evaluate the attraction of phorid flies to the attractants in field situations and to confirm the hypothesis that phorid flies have evolved to respond to higher doses of the alarm pheromone at the population level.

Our results demonstrated that the alarm pheromone and venom alkaloids of fire ants act in concert to attract parasitic phorid flies that attack fire ants. This finding is not unexpected since venom release is part of the alarm response and probably a component of the complex alarm odor (Vander Meer et al., 2002). The mandible is the source of volatile alarm pheromones in fire ants (Vander Meer et al., 2010), whereas the venom alkaloids are produced in the poison (venom) gland (Chen et al., 2009) and may be released into the air or directly injected into the attacker with the stinger. Given that both chemicals are produced in different parts of the body, it is likely that they are released independently rather than simultaneously by fire ant workers when disturbed or engaged in territorial fighting or related aggressive behaviors. Thus, it is conceivable that parasitic phorid flies would have evolved to use both chemicals as kairomones for host location, since their release would have consistently signaled presence of fire ant workers. The lack of an observable synergistic effect of combining the alarm pheromone and venom alkaloids may be because the alarm pheromone-isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine), rather than the pure compound (2-ethyl-3,6-dimethyl pyrazine), was tested in this study. Similarly, venom alkaloid extracts were tested due to the difficulty in obtaining synthetic fire ant alkaloids. Thus, it is possible that an evaluation of the pure alarm pheromone and synthetic venom alkaloids might provide better results.

Taken together, we propose a model in which both the chemical cues, fire ant alarm pheromone and venom alkaloids act in tandem to attract parasitic phorid flies. The cues which phorid flies use to locate the fire ant hosts from long distance need to be highly volatile to find host habitat. Thus, the highly volatile pyrazine alarm pheromone (Vander Meer et al., 2010), which is probably released first by workers in response to disturbance, is likely the long-range attractant for parasitic phorid flies. Closer to the source, the longer chain and less volatile venom alkaloids are probably used as medium-/short-range cues for host location and preference (Chen et al., 2009; Sharma et al., 2011). In other words, the pyrazine alarm pheromone serves as a general host location cue from long range, while the venom alkaloids may play a major role in host preference and discrimination at close range. The above proposed model may also hold for parasitoids of some other genera of ants that release volatile defensive secretions and for termite species parasitized by phorid flies (Disney, 1986). Although visual and other chemical cues such as cuticular chemicals may also play a role in host discrimination and acceptance, the use of venom alkaloids and alarm pheromone for host location is likely the functional reason why *Pseudacteon* flies are rarely attracted to ants in other genera (Porter and Gilbert, 2004; Morrison and Porter, 2006). Future studies will

test the above hypotheses regarding the specific role of the fire ant alarm pheromones and venom alkaloids as phorid fly attractants, and the contributions of visual and other cues to the process.

Acknowledgements

We thank Debbie Roberts and Amy Croft (USDA–APHIS–PPQ–CPHST Laboratory/Florida DPI, Gainesville, FL, USA) for the supply of adult *Pseudacteon* flies for this study, and Li Chen for manuscript review.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jinsphys.2013.08.010>.

References

- Brand, J.M., Blum, M.S., Fales, H.M., McConnell, J.G., 1972. Fire ant venoms: comparative analyses of alkaloidal components. *Toxicon* 10, 259–271.
- Chen, L., Fadamiro, H.Y., 2007. Behavioral and electroantennogram responses of phorid fly *Pseudacteon tricuspis* (Diptera: Phoridae) to red imported fire ant, *Solenopsis invicta* odor and trail pheromone. *J. Insect Behav.* 20, 267–287.
- Chen, L., Fadamiro, H.Y., 2009a. Re-investigation of venom chemistry of *Solenopsis* fire ants. I. Identification of novel alkaloids in *S. richteri*. *Toxicon* 53, 469–478.
- Chen, L., Fadamiro, H.Y., 2009b. Re-investigation of venom chemistry of *Solenopsis* fire ants. II. Identification of novel alkaloids in *S. invicta*. *Toxicon* 53, 479–486.
- Chen, L., Sharma, K.R., Fadamiro, H.Y., 2009. Fire ant venom alkaloids act as key attractants for the parasitic phorid fly, *Pseudacteon tricuspis* (Diptera: Phoridae). *Naturwissenschaften* 96, 1421–1429.
- Deslippe, R.J., Guo, Y.-J., 2000. Venom alkaloids of fire ants in relation to worker size and age. *Toxicon* 38, 223–232.
- Disney, R.H.L., 1986. Two remarkable new species of scuttle-fly (Diptera: Phoridae) that parasitize termites (Isoptera) in Sulawesi. *Syst. Entomol.* 11, 413–422.
- Feener, D.H., Jacobs, L.F., Schmidt, J.O., 1996. Specialized parasitoid attracted to a pheromone of ants. *Animal Behav.* 51, 61–66.
- Folgarait, P.J., Bruzzone, O.A., Patrock, R.J.W., Gilbert, L.E., 2002. Developmental rates and host specificity for *Pseudacteon* parasitoids (Diptera: Phoridae) of fire ants (Hymenoptera: Formicidae) in Argentina. *J. Econ. Entomol.* 95, 1151–1158.
- Fowler, H.G., 1997. Morphological prediction of worker size discrimination and relative abundance of sympatric species of *Pseudacteon* (Diptera: Phoridae) parasitoids of the fire ant, *Solenopsis saevissima* (Hymenoptera: Formicidae) in Brazil. *J. Appl. Entomol.* 121, 37–40.
- Gilbert, L.E., 1996. Prospects of controlling fire ants with parasitoid flies: the perspective from research based at Brackenridge field laboratory. In: Cohen, W.E. (Ed.), *Texas Quail Short Course II*. Texas Agr Ext Ser. Texas A&M University, Kingsville, pp. 77–92.
- Gilbert, L.E., Morrison, L.W., 1997. Patterns of host specificity in *Pseudacteon* parasitoid flies (Diptera, Phoridae) that attack *Solenopsis* fire ants (Hymenoptera: Formicidae). *Environ. Entomol.* 26, 1149–1154.
- Gilbert, L.E., Barr, C.L., Calixto, A.A., Cook, J.L., Drees, B.M., Lebrun, E.G., Patrock, R.J.W., Plowes, R.M., Porter, S.D., Puckett, R.T., 2008. Introducing phorid fly parasitoids of red imported fire ant workers from South America to Texas: outcomes vary by region and by *Pseudacteon* species released. *Southwestern Entomol.* 33, 15–29.
- Graham, L.C.F., Porter, S.D., Pereira, R.M., Dorough, H.D., Kelley, A.T., 2003. Field releases of the decapitating fly *Pseudacteon curvatus* (diptera: phoridae) for control of imported fire ants (Hymenoptera: Formicidae) in Alabama, Florida, and Tennessee. *Florida Entomol.* 86, 334–339.
- He, X., Fadamiro, H.Y., 2009. Host preference in *Pseudacteon* phorid flies: response of *P. tricuspis* and *P. curvatus* to black, red and hybrid imported *Solenopsis* fire ants in multiple choice bioassays. *Biocontrol* 51, 116–121.
- Lard, C.F., Schmidt, J., Morris, B., Estes, L., Ryan, C., Bergquist, D., 2006. An Economic Impact of Imported Fire Ants in the United States of America. Texas A&M University, College Station, Texas. <http://fireantecon.tamu.edu>.
- Maschwitz, U., Weissflog, A., Seebauer, S., Disney, R.H.L., Witte, V., 2008. Studies on European ant decapitating flies (Diptera: Phoridae): I. Releasers and phenology of parasitism of *Pseudacteon formicarum*. *Sociobiology* 51, 127–140.
- Morrison, L.W., King, J.R., 2004. Host location behavior in a parasitoid of imported fire ants. *J. Insect Behav.* 17, 367–383.
- Morrison, L.W., Porter, S.D., 2006. Post-release host-specificity testing of *Pseudacteon tricuspis*, a phorid parasitoid of *Solenopsis invicta* fire ants. *Biocontrol* 51, 195–205.
- Orr, M.R., Seike, S.H., Gilbert, L., 1997. Foraging ecology and patterns of diversification in dipteran parasitoids of fire ants in South Brazil. *Ecol. Entomol.* 22, 305–314.
- Pesquero, M.A., Campiolo, S., Fowler, H.G., Porter, S.D., 1996. Diurnal patterns of ovipositional activity in two *Pseudacteon* fly parasitoids (Diptera: Phoridae) of *Solenopsis* fire ants (Hymenoptera: Formicidae). *Florida Entomol.* 79, 455–457.
- Plowes, R.M., Lebrun, E.G., Brown, B.V., Gilbert, L.E., 2009. A Review of *Pseudacteon* (Diptera: Phoridae) that parasitize ants of the *Solenopsis geminata* complex (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* 102, 937–958.
- Porter, S.D., 1998. Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). *Florida Entomol.* 81, 292–309.
- Porter, S.D., Alonso, L.E., 1999. Host specificity of fire ant decapitating flies (Diptera: Phoridae) in laboratory oviposition tests. *J. Econ. Entomol.* 92, 110–114.
- Porter, S.D., Gilbert, L.E., 2004. Assessing host specificity and field release potential of fire ant decapitating flies (Phoridae: *Pseudacteon*), in: Van Driesche, R.G., Murray, T., Reardon, R. (Eds.), *Assessing Host Ranges for Parasitoids and Predators Used for Classical Biological Control: A Guide To Best Practice*. FHTET-2004-03, USDA Forest Service, Morgantown, West Virginia, pp. 152–176.
- Porter, S.D., Pesquero, M.A., 2001. Illustrated key to *Pseudacteon* decapitating flies (Diptera: Phoridae) that attack *Solenopsis saevissima* complex fire ants in South America. *Florida Entomol.* 84, 691–699.
- Porter, S.D., Williams, D.F., Patterson, R.S., 1997. Rearing the decapitating fly *Pseudacteon tricuspis* (Diptera: Phoridae) in imported fire ants (Hymenoptera: Formicidae: *Solenopsis*) from the United States. *J. Econ. Entomol.* 90, 135–138.
- Ross, K.G., Vander Meer, R.K., Fletcher, D.J.C., Vargo, E.L., 1987. Biochemical phenotypic and genetic studies of two introduced fire ant species and their hybrid (Hymenoptera: Formicidae). *Evolution* 41, 280–293.
- SAS Institute, 2007. JMP® 7.0.1. Cary, NC, USA.
- Sharma, K.R., Vander Meer, R.K., Fadamiro, H.Y., 2011. Phorid fly, *Pseudacteon tricuspis*, response to alkylpyrazine analogs of a fire ant, *Solenopsis invicta*, alarm pheromone. *J. Insect Physiol.* 57, 939–944.
- Smith, C.R., Gilbert, L.E., 2003. Differential attraction of a parasitoid to dead host ants. *Florida Entomol.* 86, 479–480.
- Vander Meer, R.K., Alonso, L.E., 1998. Pheromone directed behavior in ants, pp. 159–192. In: Vander Meer, R.K., Breed, M., Winston, M., Espelie, K.E. (Eds.), *Pheromone Communication in Social Insects*. Westview Press, Boulder, CO, p. 368.
- Vander Meer, R.K., Porter, S.D., 2002. Fire ant, *Solenopsis invicta*, worker alarm pheromones attract *Pseudacteon* phorid flies, in: *Proceedings of the 2002 Imported Fire Ant Conference*, Athens, GA, pp. 77–80.
- Vander Meer, R.K., Lofgren, C.S., Alvarez, F.M., 1985. Biochemical evidence for hybridization in fire ants. *Florida Entomol.* 68, 501–506.
- Vander Meer, R.K., Slowik, T.J., Thorvilson, H.G., 2002. Semiochemicals released by electrically stimulated red imported fire ants, *Solenopsis invicta*. *J. Chem. Ecol.* 28, 2585–2600.
- Vander Meer, R.K., Preston, C.A., Choi, M.-Y., 2010. Isolation of a pyrazine alarm pheromone component from the fire ant, *Solenopsis invicta*. *J. Chem. Ecol.* 36, 163–170.
- Vazquez, R.J., Porter, S.D., 2005. Re-confirming host specificity of the fire ant decapitating fly *Pseudacteon curvatus* (Diptera: Phoridae) after field release in Florida. *Florida Entomol.* 88, 107–110.
- Weissflog, A., Maschwitz, U., Seebauer, S., Disney, R.H.L., Seifert, B., Witte, V., 2008. Studies on European ant decapitating flies (Diptera: Phoridae): II. Observations that contradict the reported catholicity of host choice by *Pseudacteon formicarum*. *Sociobiology* 51, 87–94.
- Wetterer, J.K., 2013. Exotic spread of *Solenopsis invicta* Buren (Hymenoptera: Formicidae) beyond North America. *Sociobiology* 60, 50–55.
- Wilson, E.O., 1976. The organization of the colony defense in the ant *Pheidole dentate* Mayr (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* 1, 63–81.
- Witte, V., Disney, R.H.L., Weissflog, A., Maschwitz, U., 2010. Studies in European ant-decapitating flies (Diptera: Phoridae): ant alarm pheromone as host finding cue in *Pseudacteon brevicauda*, a parasite of *Myrmica rubra* (Formicidae: Myrmicinae). *J. Nat. History* 44, 905–912.