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Phorid fly, *Pseudacteon tricuspis*, response to alkylpyrazine analogs of a fire ant, *Solenopsis invicta*, alarm pheromone

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ABSTRACT

The phorid fly, *Pseudacteon tricuspis* Borgmeier, is a parasitoid of the red imported fire ant, *Solenopsis invicta* Buren. This fly has been reported to use fire ant chemicals, specifically venom alkaloids and possibly alarm pheromone to locate its host. A recent study identified 2-ethyl-3,6-dimethyl pyrazine as a component of the alarm pheromone of S. invicta. To determine the possible involvement of this fire ant alarm pheromone component in mediating fire ant-phorid fly interactions, we tested electroantennogram (EAG) and behavioral responses of *P. tricuspis* females to the commercially available mixture of 2-ethyl-3,6-dimethyl pyrazine and its 3,5-dimethyl isomer, as well as six structurally related alkylpyrazine analogs at varying doses. *Pseudacteon tricuspis* females showed significant EAG response to 2-ethyl-3,6(or 5)-dimethyl pyrazine (herein referred to as pheromone-isomer) at all doses, 0.001–10 μg. Among the tested alkylpyrazine analogs, 2,3-diethyl-5-methyl pyrazine showed significant EAG activity at 0.1 and 1 μg. 2,3-dimethyl pyrazine also showed significant EAG activity at 0.1 μg. Results of four-choice olfactometer bioassays demonstrated significant attraction of *P. tricuspis* females to the pheromone-isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine) at all tested doses (0.01, 0.1, 1 and 10 μg). The analogs, 2,3-diethyl-5-methyl pyrazine and 2,3-dimethyl pyrazine were significantly better than the control at the higher doses (0.1, 1 and 10 μg). The pheromone-isomer was significantly better than both analogs at two doses, 0.1 and 1 μg. These results confirm that the reported fire ant alarm pheromone component plays a role in mediating attraction of phorid flies to host workers. Venom alkaloids were previously shown to attract *P. tricuspis*; therefore, we propose that fire ant alarm pheromones may act in tandem or synergistically with venom alkaloids to attract phorid fly parasitoids to fire ant workers.

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1. Introduction

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is a major introduced species in the southern United States, inhabiting over 320 million acres (Williams et al., 2003). Fire ants 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. The decapitating phorid fly, *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae) is one of a suite of parasitoids of fire ants, which was the first to be introduced from its native South America to the southern United States for biological control of *S. invicta* (Orr et al., 1997; Porter and Alonso, 1999; Gilbert et al., 2008). Like other related phorid fly species, *P. tricuspis* is highly specific to imported fire ants (Gilbert and Morrison, 1997; Porter and Alonso, 1999), and its attraction to *S. invicta* workers has been demonstrated in the field (Gilbert and Morrison, 1997; Orr et al., 1997; Morrison and King, 2004) and laboratory (Vander Meer and Porter, 2002; He and Fadamiro, 2009).

Recent studies have provided direct evidence of odor-mediated attraction of *P. tricuspis* to *S. invicta* (Vander Meer and Porter, 2002; Chen and Fadamiro, 2007; Chen et al., 2009). Vander Meer and Porter (2002) demonstrated that short-lived chemicals released by shaken fire ant workers acted to both alarm fire ant workers and attract *P. tricuspis* parasitoids. Chen and Fadamiro (2007) reported the electroantennogram (EAG) and behavioral responses of both sexes of *P. tricuspis* to live *S. invicta* workers and body extracts, confirming the use of fire ant odor as host location cues by *P. tricuspis*. Follow-up studies demonstrated the attraction of *P. tricuspis* to two venom alkaloid fractions (*cis* and *trans* alkaloid fractions) from *S. invicta* (Chen et al., 2009). The authors proposed a possible role for fire ant alarm pheromone (Vander Meer and Porter, 2002) together with venom alkaloids in mediating attraction of phorid flies to fire ants (Chen et al., 2009). The recent identification of 2-ethyl-3,6-dimethyl pyrazine as a component of

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the alarm pheromone of *S. invicta* by Vander Meer et al. (2010) has presented an opportunity to investigate the possible role of fire ant alarm pheromone in mediating interactions between fire ants and phorid flies. The identified alarm pheromone component of fire ant, 2-ethyl-3,6-dimethyl pyrazine is available commercially as a mixture with the 2-ethyl-3,5-dimethylpyrazine isomer (Aldrich Chemical Co, Milwaukee, WI, USA). The isomer also has alarm activity and does not appear to interfere with the activity of the natural isomer (Vander Meer et al., 2010). Thus, there is potential for field use of fire ant alarm pheromones as a phorid fly attractant.

Previous research has showed that semiochemical homologs and analogs could elicit biological activity in insects comparable to the natural compounds (Kapitsky and Zhukvoskaya, 1999; Francke et al., 2002; Solari et al., 2007; Plettner and Gries, 2010), and may provide effective, cost-efficient alternatives to the use of natural semiochemicals in pest management (Renou and Guerrero, 2000). Thus, the main objective of this study was to test the response of *P. tricuspis* to the commercially available 2-ethyl-3,6(or 5)-dimethyl pyrazine (mixture of the 3,5- and 3,6-dimethyl isomers, herein referred to as pheromone-isomer) and related di- and tri-alkylpyrazine analogs, since these compounds may serve as kairomones for parasitic phorid flies. First, we tested the EAG response of *P. tricuspis* to the pheromone-isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine) and six related alkylpyrazine analogs. The most promising compounds were then selected for further testing in EAG and olfactometer bioassays. Identification of attractants for phorid flies would enhance field monitoring of this important group of biological control agents.

2. Materials and methods

2.1. Insects

Pseudacteon tricuspis were reared on workers of *S. invicta* at the phorid fly-rearing facility of the USDA-APHIS-PPQCPHST Laboratory/Florida DPI, Gainesville, FL, USA as described by Porter et al. (1997). Parasitized fire ant worker heads were received in batches and kept in a plastic jar (25 cm × 13 cm) covered using a lid with mesh, until emergence in the incubator at 25 ± 1 °C, LD 14:10 h and 70 ± 5% r.h. Newly emerged *P. tricuspis* flies were removed daily with an aspirator and placed in groups of 2–4 individuals in a 6-cm diameter plastic Petri dish. Sucrose solution (25%) and water were provided ad libitum in the Petri dishes (Chen et al., 2009). Adult, mated phorid flies 1–2 day old were utilized for the experiments.

2.2. Test chemicals

We tested the response of *P. tricuspis* to the commercially available 2-ethyl-3,6(or 5)-dimethyl pyrazine (i.e. pheromone-isomer) and six related alkylpyrazine analogs with methyl and/or ethyl chain modifications. The pheromone-isomer is a mixture of the fire ant alarm pheromone component, 2-ethyl-3,6-dimethyl pyrazine (40%) and its isomer, 2-ethyl-3,5-dimethyl pyrazine (60%). The compounds and their chemical structures are shown in Table 1. 2-Ethyl-3,6(or 5)-dimethyl pyrazine (<95%), 2,3-dimethyl pyrazine (99%), 2,6-dimethyl pyrazine (98%) 2,3-diethyl-5-methyl pyrazine (<95%), 2,5-dimethyl pyrazine (<98%), 2,3-diethyl pyrazine (<98%), and 2-ethyl-3-methyl pyrazine (<98%) were purchased from Sigma® Chemical Co. (St. Louis, MO, USA). Solutions of each compound were prepared in HPLC grade hexanes and stored at −20 °C until use.

2.3. EAG experiments

The EAG tests were conducted following the techniques and procedures previously described by Chen and Fadamiro (2007) and Chen et al. (2009). Glass capillaries (1.1 mm ID) filled with Ringer solution were used as electrodes. The reference electrode was connected to the neck of an isolated head of a *P. tricuspis* female, and the recording electrode was connected to the cut tip of the arista. Ag–AgCl 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®), and later analyzed with computer software (EAG 2000; Syntech®).

A 10 μl aliquot of each test compound (in hexane) was applied onto a filter paper strip (15 mm × 10 mm, Whatman® no. 1) and the solvent was allowed to evaporate for ~10 s. The odor impregnated filter paper strip was then inserted into a glass Pasteur pipette (~14 cm in length, Fisher Scientific, Pittsburgh, PA, USA) constituting an odor cartridge. The solvent control was hexane. The stimuli were provided as 0.2 s puffs of air into a continuous humidified air stream at 800 ml/min generated by an air stimulus controller (CS-55; Syntech®, The Netherlands). At least 2 min were allowed between each puff for the recovery of antennal receptors.

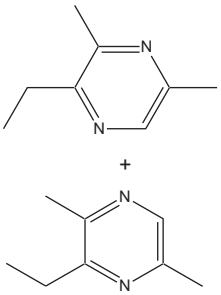
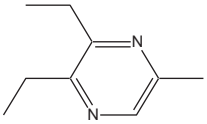
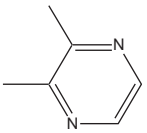
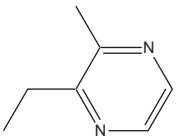
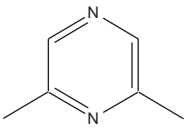
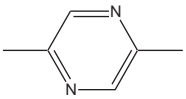
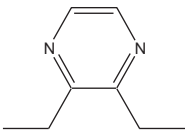
A test series of the pyrazines of the same dose were applied to a single antennal preparation in the following order: hexane control, test compounds (presented in a random fashion), and hexane control. A test series of odor stimuli applied to an antennal preparation was completed within 25–30 min (depending upon experiment) and the order of odor presentation had no effect on EAG response. Furthermore, a fresh isolated-head EAG preparation lasted about 45 min with no noticeable decrease in EAG responsiveness over this time period.

Two EAG experiments were conducted. The first experiment was conducted to screen seven commercially available pyrazine compounds (Table 1) for their ability to elicit EAG activity in female *P. tricuspis*. The compounds were tested at three doses (0.1, 1 and 10 μg). The three most promising compounds, which elicited significant EAG responses in the first experiment, were then selected for further EAG testing in the second experiment. Compounds were tested at five doses (0.001, 0.01, 0.1, 1, and 10 μg). For each experiment, recordings were obtained from 13 individual flies per dose. For analysis, EAG response to the hexane control (average of two recordings per antennal preparation) was compared to the EAG amplitudes elicited by the test compounds. For each experiment, EAG data was determined to be normally distributed and then analyzed using one-way 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 treatments.

2.4. Olfactometer bioassays

A four-choice olfactometer bioassay was used to test the behavioral response of female *P. tricuspis* to the three most biologically active pyrazines (2-ethyl-3,6 (or 5)-dimethyl pyrazine, 2,3-diethyl-5-methyl pyrazine, and 2,3-dimethyl pyrazine), as determined in EAG tests. The olfactometer apparatus and procedures were as previously described by Chen et al. (2009) with minor modifications. Briefly, the apparatus consisted of a central chamber (20 cm long × 20 cm wide × 20 cm high) connected to four cylindrical glass jars or "arms" (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.

Table 1
Structures of the tested alkylpyrazine analogs of *S. invicta*'s alarm pheromone.

No	Name	Structure	Molecular weight	Molecular formula
1.	2-Ethyl-3,6(or 5)-dimethyl pyrazine (pheromone-isomer)		136.19	$C_8H_{12}N_2$
2.	2,3-Diethyl-5-methyl pyrazine		150.22	$C_9H_{14}N_2$
3.	2,3-Dimethyl pyrazine		108.14	$C_6H_8N_2$
4	2-Ethyl-3-methyl pyrazine		122.17	$C_7H_{10}N_2$
5.	2,6-Dimethyl pyrazine		108.14	$C_6H_8N_2$
6.	2,5-Dimethyl pyrazine		108.14	$C_6H_8N_2$
7.	2,3-Diethyl pyrazine		136.19	$C_8H_{12}N_2$

The three compounds were compared with hexane control in four separate (dose) experiments at 0.01, 0.1, 1 or 10 μg dose. Each treatment was delivered as 10-μl sample impregnated on filter paper squares (1 × 1 cm, Whatman® no. 1). After allowing for solvent evaporation (~10 s), the filter paper square was inserted into its designated olfactometer arm. For each test (replicate), 20 female 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 walk into any of the arms within 15 min were scored as "non-responders" and were 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. Each experiment (dose) was replicated 16 times. All tests were conducted at 25 ± 1 °C, 40–60% r.h., and between 12:00 and 16:00 h, the time of day for high *P. tricuspis* activity (Pesquero et al., 1996). Olfactometer data was normally distributed and 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 treatments.

3. Results

3.1. EAG experiments

In the first experiment, significant differences were recorded in the EAG response of *P. tricuspis* females to the various treatments at all tested doses (0.1 μg: $F = 4.77$, df = 7, 103, $P < 0.0001$; 1 μg: $F = 9.43$, df = 7, 103, $P < 0.0001$; 10 μg: $F = 7.63$, df = 7, 103, $P < 0.0001$) (Table 2). At the lowest tested dose (0.1 μg), the pheromone-isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine) and two analogs (2,3-diethyl-5-methyl pyrazine and 2,3-dimethyl pyrazine), elicited significant EAG response compared to the hexane control for at least one dose. However, only the pheromone-isomer and 2,3-diethyl-5-methyl pyrazine elicited significantly greater EAG response than the hexane control at all three doses (0.1, 1.0, and 10 μg doses; Table 2). Thus, the pheromone-isomer and the two most-active analogs (2,3-diethyl-5-methyl pyrazine and 2,3-dimethyl pyrazine) were selected for further EAG testing at five doses.

Table 2
Mean (mV ± SE) EAG response of *P. tricuspis* females to alkylpyrazine analogs of *S. invicta*'s alarm pheromone at three doses.

Doses	Control (hexane)	2-Ethyl-3,6(or 5)-dimethyl pyrazine	2,3-Diethyl-5-methyl pyrazine	2,3-Dimethyl pyrazine	2-Ethyl-3-methyl pyrazine	2,6-Dimethyl pyrazine	2,5-Dimethyl pyrazine	2,3-Diethyl pyrazine	*F*	*P*
0.1 μg	0.48 ± 0.04c	0.91 ± 0.08a	0.90 ± 0.08a	0.88 ± 0.10ab	0.80 ± 0.11abc	0.62 ±0.09abc	0.60 ± 0.04abc	0.55 ± 0.05bc	4.77	<0.001
1 μg	0.57 ± 0.05c	1.02 ± 0.09a	0.87 ± 0.07ab	0.73 ± 0.07bc	0.59 ± 0.06c	0.47 ± 0.05c	0.56 ± 0.06c	0.48 ±0.05c	9.43	<0.001
10 μg	0.40 ± 0.05c	0.99 ± 0.10a	0.90 ± 0.08ab	0.67 ± 0.08bc	0.61 ± 0.06bc	0.58 ± 0.07c	0.57 ± 0.05c	0.53 ± 0.06	7.63	<0.001

Note: 2-Ethyl-3,6(or 5)-dimethyl pyrazine is the pheromone-isomer while the other compounds are analogs. Means in the same row having no letter in common are significantly different ($n = 13$; $P < 0.05$, Tukey–Kramer HSD test).

In the second experiment, the pheromone-isomer elicited significantly greater EAG response in *P. tricuspis* females compared to the hexane control or 2,3-dimethyl pyrazine at 0.001 μg ($F = 4.06$, df = 3, 51, $P < 0.01$) and 0.01 μg ($F = 5.08$, df = 3, 51, $P < 0.003$) doses (Fig. 1). At the 0.1 and 1 μg dose, the pheromone-isomer and 2,3-diethyl-5-methyl pyrazine evoked significantly greater EAG response than the hexane control or 2,3-dimethyl pyrazine (0.1 μg: $F = 18.78$, df = 3, 51, $P < 0.0001$; 1 μg: $F = 6.14$, df = 3, 51, $P < 0.001$). At the highest tested dose (10 μg), EAG response to the pheromone-isomer was only significantly greater than EAG response to hexane control ($F = 4.40$, df = 3, 51, $P < 0.008$), but not significantly greater than EAG response to the other two compounds (Fig. 1).

3.2. Olfactometer bioassays

Significant differences were recorded in the behavioral response of *P. tricuspis* females to the treatments at all four tested doses (0.01 μg: $F = 6.10$, df = 7, 63, $P < 0.001$; 0.1 μg: $F = 86.23$, df = 3, 63, $P < 0.0001$; 1 μg: $F = 126.14$, df = 3, 63, $P < 0.0001$; 10 μg: $F = 39.46$, df = 3, 63, $P < 0.0001$). The flies were significantly more attracted to the pheromone-isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine) compared to the hexane control at all tested doses. The flies also showed significantly greater attraction to the pheromone-isomer than to the two analogs (2,3-diethyl-5-methyl pyrazine and 2,3-dimethyl pyrazine) at 0.1 and 1 μg (Fig. 2). Both pyrazine analogs elicited significantly greater behavioral response in *P. tricuspis* females compared to the control at the higher doses of 0.1, 1 and 10 μg (Fig. 2).

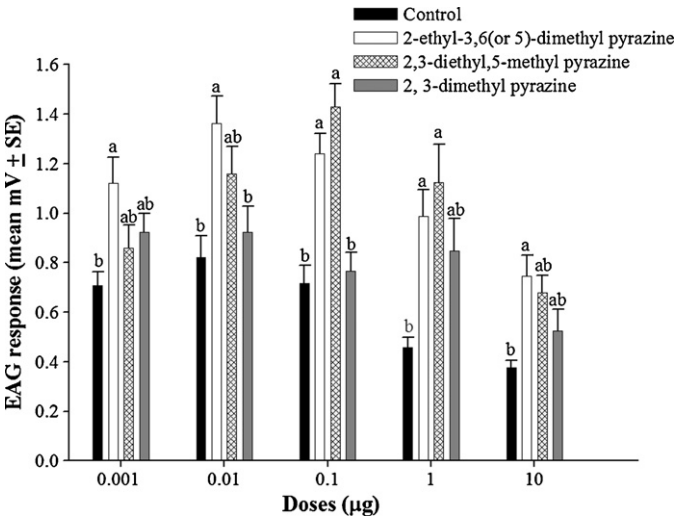


Fig. 1. EAG response (mV ± SE) of *P. tricuspis* females to the pheromone-isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine) and two analogs (2,3-diethyl-5-methyl pyrazine and 2,3-dimethyl pyrazine) at five doses. (control = hexane). Means ($n = 13$) for the same dose having no letter in common are significantly different ($P < 0.05$, Tukey–Kramer HSD test).

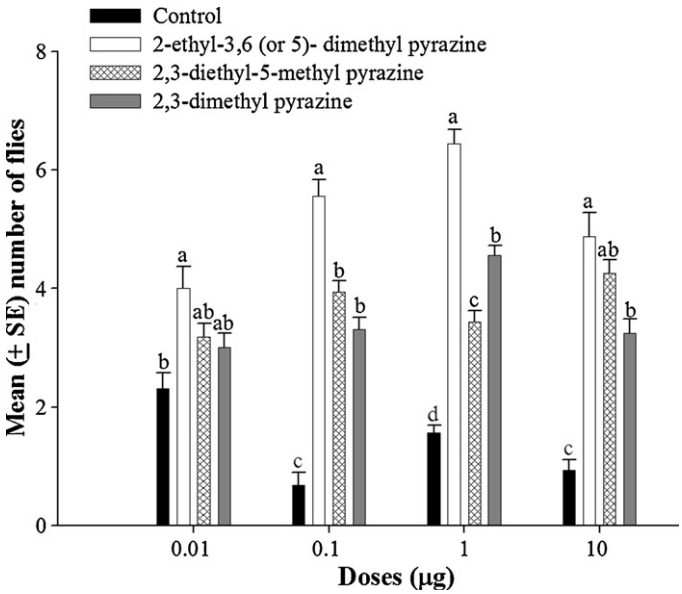


Fig. 2. Response of *P. tricuspis* females in a four-choice olfactometer bioassay to the pheromone-isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine), two analogs (2,3-diethyl-5-methyl pyrazine and 2,3-dimethyl pyrazine) and control (hexane) at four doses. Figure shows mean (±SE) number of flies attracted per 15 min. 20 flies were released per test and replicated 16 times. Means for the same dose having no letter in common are significantly different ($P < 0.05$, Tukey–Kramer HSD test).

4. Discussion

The results of the EAG and olfactometer experiments demonstrated the response of *P. tricuspis* females to 2-ethyl-3,6(or 5)-dimethyl pyrazine (pheromone-isomer), the commercially available mixture of 2-ethyl-3,6-dimethyl pyrazine (*S. invicta* alarm pheromone component) and its 3,5-dimethyl isomer, and supports a previous report that host alarm pheromones are possibly involved in the attraction of *P. tricuspis* to their fire ant hosts (Vander Meer and Porter, 2002). In the EAG experiments, *P. tricuspis* females exhibited the highest EAG response to the pheromone-isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine) followed by the analog, 2,3-diethyl-5-methyl pyrazine. Schönrogge et al. (2008) also reported EAG response of the hover fly, *Microdon mutabilis* L., to the alarm pheromone components of its host ant, *Myrmica scabrinodis* Nylander. The EAG results were confirmed by the results of the olfactometer bioassays, which demonstrated strong attraction of *P. tricuspis* to the pheromone-isomer and also to two analogs, 2,3-diethyl-5-methyl pyrazine and 2,3-dimethyl pyrazine. The recorded attraction to the analogs, 2,3-diethyl-5-methyl pyrazine and 2,3-dimethyl pyrazine, demonstrates that other pyrazine isomers also elicit behavioral activity in *P. tricuspis*; however, the identified alarm pheromone maintains greater activity at lower concentrations. These results support previous

reports that phorid flies use the fire ant alarm pheromone for host location (Vander Meer and Porter, 2002; Morrison and King, 2004; Morrison and Porter, 2006), and are in agreement with previous reports on the role of alarm pheromones of ants and certain other insect species as attractants (kairomones) for their natural enemies (Feener et al., 1996; Schönrogge et al., 2008; Verheggen, 2008; Witte et al., 2010). For instance, *Apocephalus paraponerae* Borgmeier (Diptera: Phoridae), was shown to exhibit attraction to the alarm pheromone of its host, *Paraponera clavata* (F.) (Hymenoptera: Formicidae) (Feener et al., 1996). Similarly, a recent study by Witte et al. (2010) reported on the attraction of the European ant-decapitating fly, *Pseudacteon brevicauda* Schmitz (Diptera: Phoridae) to the alarm pheromone of its host, *Myrmica rubra* Linnaeus (Hymenoptera: Formicidae).

Pseudacteon tricuspis showed significant response to the alkylpyrazines at low to moderate doses (0.1–1 μg) but showed reduced response at the highest tested dose (10 μg). Vander Meer et al. (2010) reported that the concentration of the alarm pheromone, 2-ethyl-3,6-dimethyl pyrazine is lower in the mandibular gland extracts of workers of *S. invicta* compared to the other castes. Previous studies showed that *P. tricuspis* females were more attracted to worker ants compared to alates, and that alates were rarely attacked by the flies (Williams and Banks, 1987; Porter, 1998; Smith and Gilbert, 2003). Pesquero et al. (1993) reported 33.3% of observed *S. saevissima* mating flights attracted *P. tricuspis* parasitoids. This is not surprising, since mating flight initiation is characterized by frenzied worker and alate activity attributed to release alarm pheromone (Alonso and Vander Meer, 1997). *P. tricuspis* females have been observed attacking gynes (Pesquero et al., 1993), but there is no evidence of the parasitoids successfully developing in gynes.

Of the seven tested alkylpyrazines, the pheromone-isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine) elicited the greatest biological activity in *P. tricuspis*, which is not surprising since this mixture contains the natural fire ant alarm pheromone, 2-ethyl-3,6-dimethyl pyrazine (Vander Meer et al., 2010). The positive activity of *P. tricuspis* to the two analogs (2,3-diethyl-5-methyl pyrazine and 2,3-dimethyl pyrazine) is interesting, because neither compound has been identified as a components of fire ant alarm pheromone. Numerous di- and tri-alkyl substituted, pyrazines are known components of alarm pheromones of other ant (Hymenoptera: Formicidae) species including, *Iridomyrmex humilis* (Mayr) (Cavill and Houghton, 1974), *Eutetramorium mocquerysi* Emery (Tentschert et al., 2000), and *Wasmannia auropunctata* (Roger) (Showalter et al., 2010). It is possible that both analogs are unidentified components of the alarm pheromone of *S. invicta*, or the observed response of *P. tricuspis* to both compounds may simply be due to their structural similarity to 2-ethyl-3,6-dimethyl pyrazine, the alarm pheromone component of *S. invicta*. Semiochemical analogs and structurally related compounds have been reported to elicit biological activity in insects similar to that elicited by natural compounds (Kapitsky and Zhukvoskaya, 1999; Francke et al., 2002; Solari et al., 2007; Plettner and Gries, 2010). Although, structure–activity is not a focus of the present study, 2,3-diethyl-5-methyl pyrazine is more structurally related to 2-ethyl-3,6-dimethyl pyrazine than the remaining four compounds. It is therefore possible that *P. tricuspis* receptor neurons, tuned to the alarm pheromone (2-ethyl-3,6-dimethyl pyrazine), also respond to these two related alkylpyrazine analogs. Receptor neurons tuned to pheromone components have also been shown to respond to pheromone analogs in many other insects (Berg et al., 1995; Kapitsky and Zhukvoskaya, 1999; Nikonov et al., 2001). Our results show that there are commercially available and biologically active alkylpyrazine analogs of the natural alarm pheromone of *S. invicta*, 2-ethyl-3,6-dimethyl pyrazine that could lead to cost effective application of phorid fly attractants to improve rearing and monitoring.

Our results have clearly demonstrated the attraction of *P. tricuspis* to the alkylpyrazine alarm pheromone component of *S. invicta* and two analogs. In a previous study we demonstrated attraction of *P. tricuspis* to fire ant venom alkaloids (Chen et al., 2009). Based on these findings, we propose a role for both the alarm pheromones and venom alkaloids of fire ants in phorid fly host location. Our specific proposal is that both chemicals act in tandem or synergistically to attract phorid flies to ant workers (Chen et al., 2009). It is plausible that fire ant alarm pheromones, which are highly volatile short-chain carbon compounds in the 100–200 molecular weight range (Vander Meer and Alonso, 1998), are utilized as long-range host location cues by *P. tricuspis*, whereas the larger and less volatile venom alkaloids are used as medium-/short-range host location chemical cues. Alternatively, fire ant venom alkaloids and alarm pheromones may show the additive effect to attract *P. tricuspis* and other *Pseudacteon* species to fire ant workers. Ongoing studies will test the above hypotheses regarding the individual and combined role of fire ant alarm pheromones and venom alkaloids in mediating phorid fly host location.

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