Chemoecology (2015) 25:85–92
DOI 10.1007/s00049-014-0178-x

CHEMOECOLOGY

RESEARCH PAPER

Comparative responses of four *Pseudacteon* phorid fly species to host fire ant alarm pheromone and analogs

Esther Ngumbi · Henry Fadamiro

Received: 2 August 2014 / Accepted: 28 October 2014 / Published online: 11 November 2014

Abstract Several species of *Pseudacteon* phorid flies, including *Pseudacteon cultellatus*, *P. curvatus*, *P. obtusus*, and *P. tricuspis*, have been introduced in the southern United States for biological control of imported fire ants, *Solenopsis* spp. Previous studies showed that *Pseudacteon* flies use the alarm pheromone and venom alkaloids of fire ants to locate host ant workers. A recent study by our group demonstrated attraction of one of the species, *P. tricuspis*, to certain alkylpyrazine analogs of fire ant alarm pheromone. If effective, alkylpyrazine analogs of fire ant alarm pheromone may provide cost-efficient alternatives as phorid fly attractants. To test the hypothesis that other phorid fly species are attracted to alkylpyrazine analogs of fire ant alarm pheromone, we compared electroantennogram (EAG) and behavioral responses of four *Pseudacteon* species, *P. cultellatus*, *P. curvatus*, *P. obtusus*, and *P. tricuspis*, to the commercially available alarm pheromone isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine) and six structurally related alkylpyrazine analogs at physiologically relevant doses (ranging from picogram to 10 μg). The alarm pheromone isomer and two analogs, 2,3-diethyl-5-methyl pyrazine and 2 ethyl-3,5 + 6 methyl pyrazine, elicited the strongest EAG activity in all four species at all tested doses. Results from four-choice olfactometer bioassays testing the above three EAG-active compounds showed that all four species were strongly attracted to the alarm pheromone isomer at doses ranging from 0.001 to 1 μg. The flies also showed significant attraction to the two analogs (2,3-diethyl-5-methyl pyrazine and 2 ethyl-3,5 + 6 methyl pyrazine) compared to the solvent control but not as strong as the attraction elicited by the alarm pheromone isomer. These results confirm that multiple species of phorid flies use fire ant alarm pheromone as a host location cue. However, the flies are not strongly attracted to the tested alkylpyrazine analogs of the alarm pheromone.

Keywords *Pseudacteon cultellatus* · *Pseudacteon curvatus* · *Pseudacteon obtusus* · *Pseudacteon tricuspis* · Fire ants alarm pheromone · Alkylpyrazine analogs

Handling Editor: Paulo H G Zarbin.

E. Ngumbi (✉) · H. Fadamiro
Department of Entomology and Plant Pathology, Auburn University, 36849 Auburn, AL, USA
e-mail: enn0002@tigermail.auburn.edu

Introduction

Pseudacteon phorid flies (Diptera: Phoridae) are key parasitoids of imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae), in their native South America (Patrock et al. 2009; Porter and Pesquero 2001). Several species have been introduced in the southern United States for the classical biological control of imported fire ants (Porter et al. 2004; Graham et al. 2003; Plowes et al. 2011; Porter and Calcaterra 2013). To date, six phorid fly species are reported to have successfully established in the southern United States (Porter and Calcaterra 2013). These include *Pseudacteon tricuspis* Borgmeier, a medium-large species (Porter et al. 2004); *P. curvatus* Borgmeier, a small species (Graham et al. 2003; Vazquez et al. 2006); *P. obtusus* Borgmeier, a medium-large species (Plowes et al. 2011); and *P. cultellatus* Borgemeier, a small species (Porter and Calcaterra 2013). *Pseudacteon* phorid flies are highly specific to imported fire ants (Gilbert and Morrison 1997; Porter et al. 1995; Porter and Alonso 1999; Porter and Gilbert 2004).

Previous research by our group and others have provided direct evidence of odor-mediated attraction of *P.*

tricuspis to fire ant workers (Vander Meer and Porter 2002; Chen and Fadamiro 2007; Chen et al. 2009; Sharma et al. 2011; Chen et al. 2012). Specifically, recent studies by our group showed that fire ant venom alkaloids (both *cis* and *trans*) are key attractants for *P. tricuspis* (Chen et al. 2009). Following successful identification of fire ant alarm pheromone component, 2-ethyl-3,6(or 5)-dimethyl pyrazine (Vander Meer et al. 2010), the role of fire ant alarm pheromone in mediating fire ant-phorid fly interactions was investigated. Sharma et al. (2011) showed that *P. tricuspis* was attracted to the commercially available mixture of 2-ethyl-3,6(or 5)-dimethyl pyrazine (herein referred to as alarm pheromone isomer) and six other structurally related alkyl pyrazine analogs of the alarm pheromone. Recently, Sharma and Fadamiro (2013) reported that fire ant alarm pheromone and venom alkaloids act in concert to attract *P. tricuspis*. However, it remains unclear if other species of *Pseudacteon* phorid flies use fire ant alarm pheromone and/or venom alkaloids for host location, like *P. tricupis*.

To test the hypothesis that other phorid fly species are attracted to fire ant alarm pheromone and its alkylpyrazine analogs, we compared electroantennogram (EAG) and behavioral responses of *P. cultellatus*, *P. curvatus*, *P. obtusus*, and *P. tricuspis* to the commercially available alarm pheromone isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine) and six structurally related alkylpyrazine analogs at varying doses. At least two of the tested alkyl pyrazine analogs have been shown to elicit significant EAG and behavioral response in *P. tricuspis*. If effective, these alkylpyrazine analogs may provide cost-efficient alternatives as phorid fly attractants. First, we tested the EAG response of the four *Pseudacteon* species to the alarm pheromone and six alkyl pyrazine analogs. The most active compounds were then further tested in EAG and olfactometer bioassays at physiologically relevant doses.

Materials and methods

Insects

Pseudacteon cultellatus, *P. curvatus*, *P. obtusus*, and *P. tricuspis* were reared at the phorid fly rearing facility of the USDA-APHIS-PPQ-CPHST Laboratory/Florida DPI, Gainesville, FL, USA as described by Porter et al. (1995). 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, 70 ± 5 % RH and 14:10 h (L:D) photoperiod. Newly emerged flies were removed daily with an aspirator and placed in groups of 2–4 individuals in a 6-cm diameter plastic Petri dish. Sugar solution (10 %) and water were provided in the Petri dishes (Chen et al. 2009). Adult-mated female phorid flies 1–2 days old were used for the experiments.

Test chemicals

The responses of *P. cultellatus*, *P. curvatus*, *P. obtusus*, and *P. tricuspis* to the commercially available 2-ethyl-3,6(or 5)-dimethyl pyrazine (i.e., alarm pheromone isomer) (<95 %) and six related alkylpyrazine analogs with methyl and/or ethyl chain modifications, were tested in EAG and olfactometer bioassays. The alkylpyrazine analogs tested were: 2,3-diethyl-5-methyl pyrazine (<95 %), 2,3-dimethyl pyrazine (99 %), 2-ethyl-3-methyl pyrazine (<98 %), 2-ethyl-3,5 + 6-methyl pyrazine (98 %), 2,5-dimethyl pyrazine (<98 %), and 2,3-diethyl pyrazine (<98 %). The compounds 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.

EAG experiments

EAG tests were conducted following the techniques and procedures previously described by Chen et al. (2009) and Sharma et al. (2011). 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 the parasitoid (*P. cultellatus*, *P. curvatus*, *P. obtusus*, and *P. tricuspis*), 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 the preamplifier. The analog signal was detected through a probe (INR-II; Syntech®, The Netherlands), captured, 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). The solvent was allowed to evaporate for ~10 s. The odor impregnated filter paper strip was 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 were completed within 25–30 min (depending upon experiment).

Two EAG experiments were conducted. The first experiment was conducted to screen seven commercially available pyrazine compounds: 2-ethyl-3,6(or 5)-dimethyl pyrazine (i.e., alarm pheromone isomer), 2,3-diethyl-5-methyl pyrazine, 2,3-dimethyl pyrazine, 2-ethyl-3-methyl pyrazine, 2-ethyl-3,5 + 6-methyl pyrazine, 2,5-dimethyl pyrazine, and 2,3-diethyl pyrazine for their ability to elicit EAG activity in female *P. cultellatus*, *P. curvatus, P. obtusus*, and *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. In the second EAG experiment, the selected compounds were tested at five physiologically relevant doses (1 pg, 0.0001, 0.001, 0.01, and 0.1 μg). For the first experiment, recordings were obtained from 10 individual flies per dose. For the second experiment, recordings were obtained from 15 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, data were checked for normal distribution and analyzed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer HSD comparison test ($P < 0.05$; JMP1 7.0.1, SAS Institute 2007) to establish significant differences among the treatments.

Olfactometer bioassays

The behavioral responses of female *P. cultellatus*, *P. curvatus*, *P. obtusus*, and *P. tricuspis* to the three most EAG-active pyrazines were tested in four-choice olfactometer bioassays. The olfactometer apparatus and procedures were as previously described by Sharma et al. (2011). 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, USA). 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. The three compounds were compared with hexane control in five separate (dose) experiments at 0.0001, 0.001, 0.01, 0.1, or 1 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 15 times. All experiments were conducted at 25 ± 1 °C, 40–60 % r.h., and between 12:00 and 16:00 h, the time of day for high phorid flies activity (Pesquero et al. 1996). Olfactometer data were analyzed using analysis of variance (ANOVA) followed by Tukey–Kramer HSD comparison test ($P < 0.05$; JMP1 7.0.1, SAS Institute 2007) to establish significant differences among the treatments.

Results

EAG experiments

Data from the first EAG experiment revealed significant differences among the tested compounds (Table 1). For all four species, the pheromone isomer (2-ethyl-3,6(or 5)-dimethyl pyrazine) and two other analogs (2,3-diethyl-5-methyl pyrazine, 2 ethyl-3,5 + 6 methyl pyrazine) elicited significantly greater EAG response than the hexane control at all three doses (0.1, 1, and 10 μg doses; Table 1).

In the second experiment, the three most EAG-active compounds in Experiment 1 (i.e., alarm pheromone isomer, 2,3-diethyl-5-methyl pyrazine, and 2 ethyl-3,5 + 6 methyl pyrazine) were tested further at five doses (1 pg, 0.0001, 0.001, 0.01, and 0.1 μg). For *P. cultellatus* (Fig. 1a) and *P. curvatus* (Fig. 1b), the alarm pheromone isomer elicited significantly greater EAG response than the hexane control or 2,3 diethyl-5-methyl pyrazine at three lower doses (0.0001, 0.001, and 0.1): *P. cultellatus* (0.0001 μg, $F = 11.99$, $df = 3$, $P < 0.001$; 0.001 μg ($F = 17.07$, $df = 3$, $P < 0.001$; 0.01 μg ($F = 26.67$, $df = 3$, $P < 0.001$); *P. curvatus* (0.0001 μg, $F = 29.25$, $df = 3$, $P < 0.001$; 0.001 μg ($F = 48.27, df = 3, P < 0.001$; 0.01 μg ($F = 44.42, df = 3$, $P < 0.001$). For *P. obtusus* (Fig. 1c) and *P. tricupis* (Fig. 1d), the alarm pheromone isomer elicited significantly greater EAG response than the hexane control or 2,3 diethyl-5-methyl pyrazine only at the two lowest doses (0.0001 and 0.001 μg): *P. obtusus* (0.0001 μg, $F = 12.33$, $df = 3$, $P < 0.001$; 0.001 μg ($F = 16.08$, $df = 3$, $P < 0.001$); *P. tricupis* (0.0001 μg, $F = 19.86$, $df = 3$, $P < 0.001$; 0.001 μg ($F = 47.20, df = 3, P < 0.001$).

Olfactometer bioassays

Similar to the EAG results, significant differences were recorded in the behavioral response of each of the four

Table 1 Mean (mv ± SE) EAG response of *P. cultellatus*, *P.curvatus*, *P. obtusus*, and *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-Ethyl-3,5 + 6-methyl pyrazine	2,5-Dimethyl pyrazine	2,3-Diethyl pyrazine	*F*	*P*
P. cultellatus										
0.1 μg	0.25 ± 0.03b	0.41 ± 0.06a	0.39 ± 0.04a	0.18 ± 0.03b	0.21 ± 0.03b	0.35 ± 0.04a	0.18 ± 0.02b	0.25 ± 0.07b	11.86	<0.001
1 μg	0.22 ± 0.05c	0.46 ± 0.13ab	0.54 ± 0.16a	0.20 ± 0.07c	0.31 ± 0.08bc	0.50 ± 0.16ab	0.18 ± 0.04c	0.24 ± 0.06c	6.69	<0.001
10 μg	0.20 ± 0.04b	0.47 ± 0.12a	0.31 ± 0.10b	0.18 ± 0.04b	0.35 ± 0.09ab	0.48 ± 0.16a	0.19 ± 0.05b	0.25 ± 0.07b	7.49	<0.001
P. curvatus										
0.1 μg	0.27 ± 0.03b	0.77 ± 0.11a	0.66 ± 0.10a	0.33 ± 0.06b	0.42 ± 0.05b	0.78 ± 0.12a	0.31 ± 0.05b	0.31 ± 0.04b	18.99	<0.001
1 μg	0.31 ± 0.03c	0.91 ± 0.12a	0.85 ± 0.09a	0.39 ± 0.06c	0.55 ± 0.09b	0.92 ± 0.14a	0.33 ± 0.04c	0.41 ± 0.06bc	26.31	<0.001
10 μg	0.28 ± 0.02e	0.91 ± 0.11ab	0.66 ± 0.12bcd	0.45 ± 0.06ed	0.75 ± 0.14abc	0.98 ± 0.14a	0.33 ± 0.06e	0.54 ± 0.11cde	11.69	<0.001
P. obtusus										
0.1 μg	0.17 ± 0.01c	0.36 ± 0.04a	0.36 ± 0.03a	0.19 ± 0.02c	0.27 ± 0.03b	0.35 ± 0.03a	0.19 ± 0.02c	0.21 ± 0.02c	21.21	<0.001
1 μg	0.21 ± 0.02d	0.55 ± 0.09ab	0.64 ± 0.11a	0.33 ± 0.05c	0.45 ± 0.08b	0.52 ± 0.07b	0.25 ± 0.03cd	0.29 ± 0.05cd	14.90	<0.001
10 μg	0.22 ± 0.02c	0.55 ± 0.08a	0.35 ± 0.05b	0.37 ± 0.06b	0.55 ± 0.07a	0.56 ± 0.08a	0.36 ± 0.04b	0.38 ± 0.04b	13.05	<0.001
P. tricuspis										
0.1 μg	0.85 ± 0.15b	1.81 ± 0.35a	2.07 ± 0.37a	0.78 ± 0.19b	1.31 ± 0.16b	2.03 ± 0.37a	0.71 ± 0.14b	0.93 ± 0.21b	11.83	<0.001
1 μg	0.87 ± 0.13b	2.02 ± 0.35a	1.96 ± 0.30a	0.90 ± 0.12b	1.35 ± 0.19b	1.87 ± 0.27a	0.80 ± 0.17b	0.90 ± 0.12b	14.34	<0.001
10 μg	0.74 ± 0.10c	2.01 ± 0.37a	1.81 ± 0.44a	1.38 ± 0.34ab	1.71 ± 0.34a	1.91 ± 0.29a	0.90 ± 0.17bc	1.44 ± 0.29ab	7.02	<0.001

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 ($N = 10$; $P < 0.05$, Tukey–Kramer HSD test)

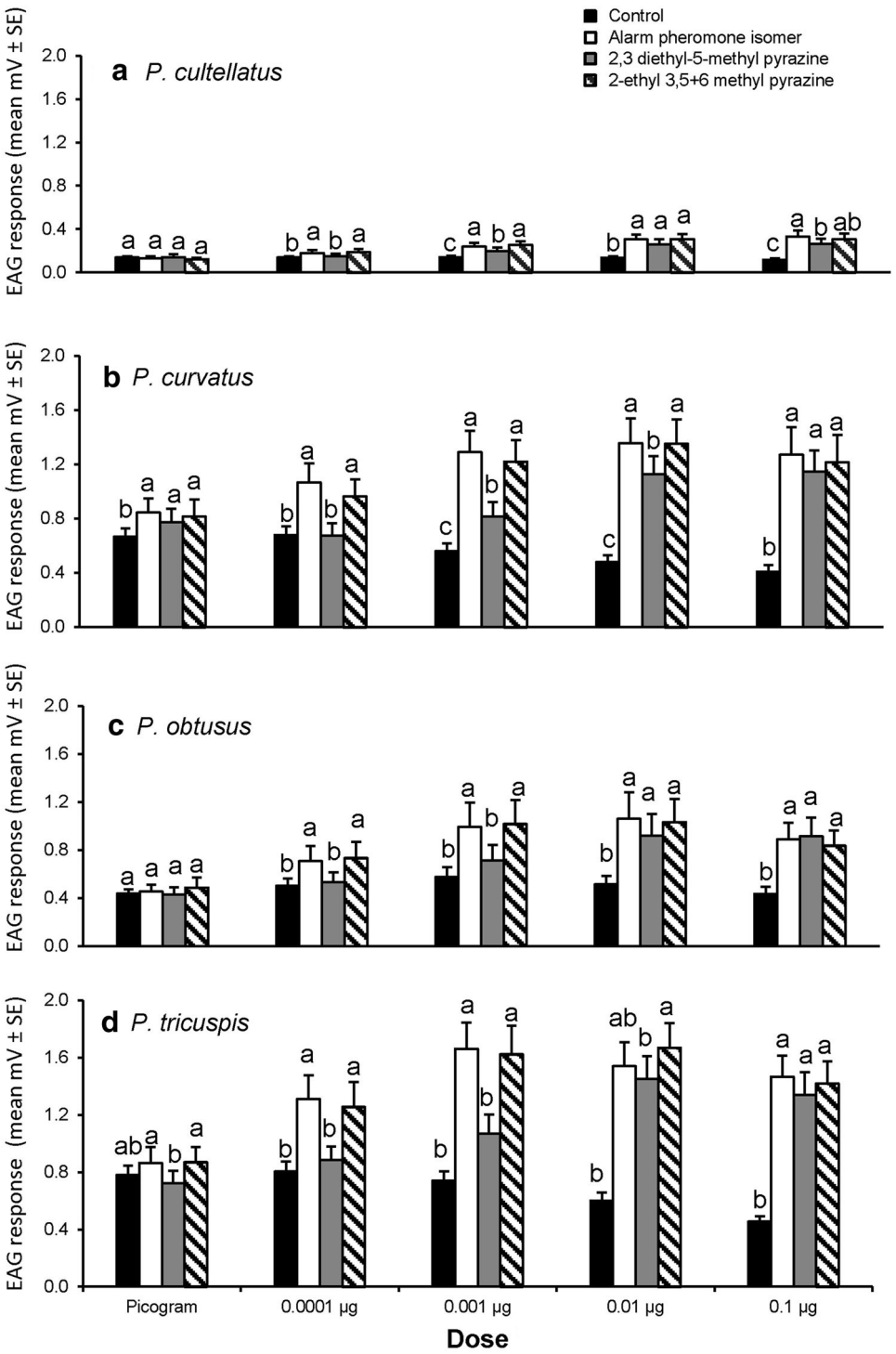

Fig. 1 EAG responses (mV ± SE) of *P. cultellatus* (**a**), *P. curvatus* (**b**), *P. obtusus* (**c**), and *P. tricupsis* (**d**) females to the alarm pheromone isomer (2-ethyl-3,6 (or 5)-dimethyl pyrazine) and two analogs (2,3-diethyl-5-methyl pyrazine and 2-ethyl-3,5 + 6 methyl pyrazine) at five doses. (Control = hexane). $N = 15$

species to the treatments at most of the doses (Fig. 2). *Pseudacteon cultellatus* flies were significantly more attracted to the alarm pheromone isomer compared to 2,3-diethyl-5-methyl pyrazine, 2-ethyl 3,5 + 6-dimethyl pyrazine, or the hexane control at four of the tested doses (0.001, 0.01, 0.1, and 1 µg): *P. cultellatus* (0.001 µg, $F = 21.33$, $df = 3$, $P < 0.001$; 0.01 µg ($F = 8.33$, $df = 3$, $P = 0.0002$; 0.1 µg ($F = 15.61$, $df = 3$, $P < 0.001$; 1 µg ($F = 21.33$, $df = 3$, $P < 0.001$) (Fig. 2a). *P. curvatus* were significantly more attracted to the pheromone isomer compared to 2,3-diethyl-5-methyl pyrazine, 2-ethyl 3,5 + 6-dimethyl pyrazine, and hexane control at 1 µg: *P. curvatus* (1 µg, $F = 20.84$, $df = 3$, $P < 0.001$) (Fig. 2b). *Pseudacteon obtusus* flies were significantly more attracted

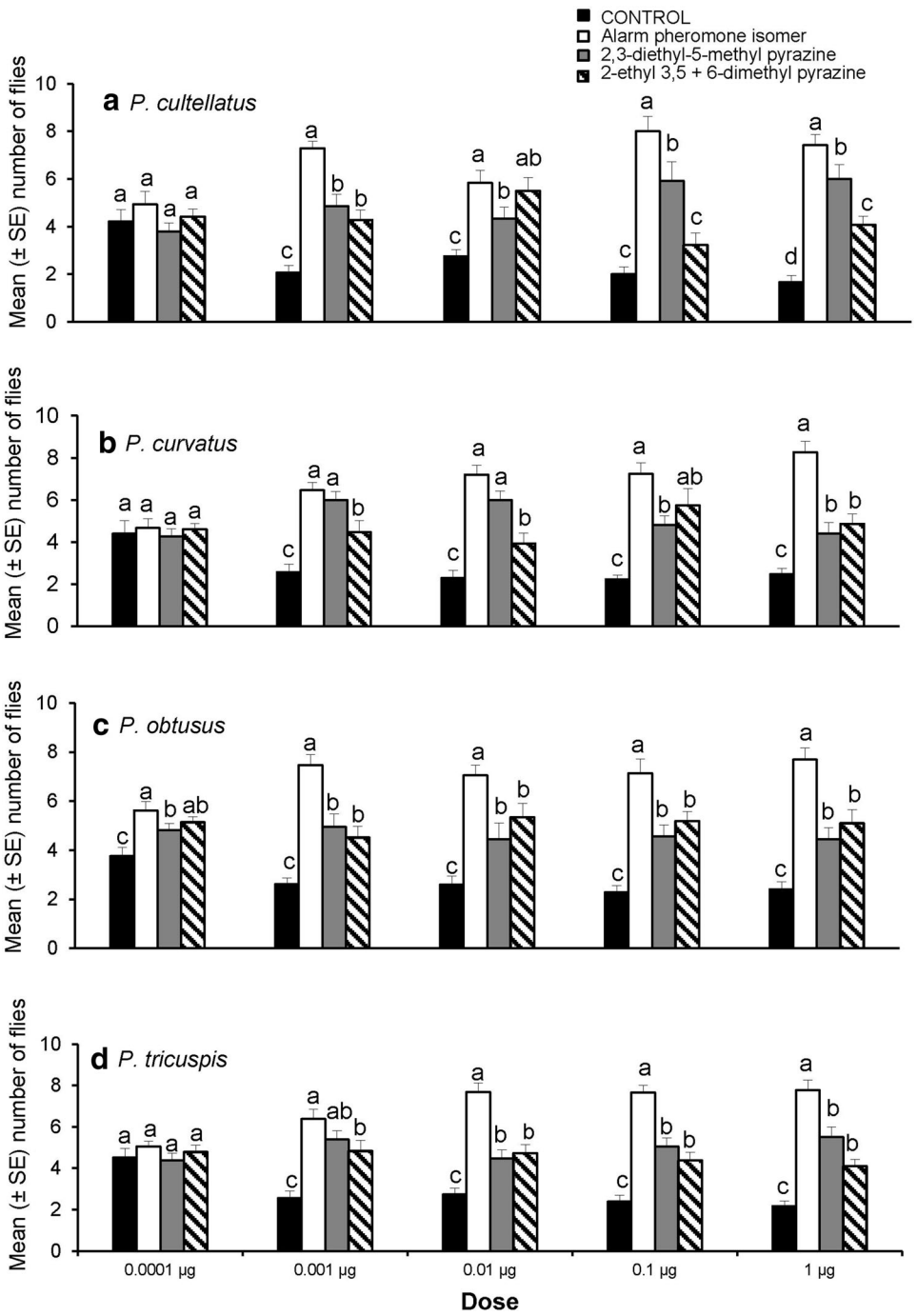

Fig. 2 Response of *P. cultellatus* (**a**), *P. curvatus* (**b**), *P. obtusus* (**c**), *and P. tricupsis* (**d**) females in a four-choice olfactometer bioassay to the alarm pheromone isomer (2-ethyl-3,6 (or 5)-dimethyl pyrazine) and two analogs (2,3-diethyl-5-methyl pyrazine and 2-ethyl 3,5 + 6 methyl pyrazine) at five doses. (Control = hexane). $N = 15$

to the alarm pheromone isomer compared to 2,3-diethyl-5-methyl pyrazine, 2-ethyl 3,5 + 6-dimethyl pyrazine, or the hexane control at four of the tested doses (0.001, 0.01, 0.1, and 1 µg): *P. obtusus* (0.001 µg, $F = 33.73$, $df = 3$, $P < 0.001$; 0.01 µg ($F = 10.60$, $df = 3$, $P < 0.001$; 0.1 µg ($F = 29.00$, $df = 3$, $P < 0.001$; 1 µg ($F = 26.77$, $df = 3$, $P < 0.001$) (Fig. 2c). For *P. tricuspis*, the flies were significantly more attracted to the alarm pheromone isomer than to the two analogs (2,3-diethyl-5-methyl pyrazine and 2-ethyl 3,5 + 6-dimethyl pyrazine) or the hexane control at three of the tested doses (0.01, 0.1, and 1 µg): *P. tricuspis* (0.01 µg ($F = 25.52$, $df = 3$, $P < 0.001$; 0.1 µg ($F = 34.14$, $df = 3$, $P < 0.001$; 1 µg ($F = 36.11$, $df = 3$, $P < 0.001$) (Fig. 2d).

Discussion

All four tested phorid species (*P. cultellatus*, *P. curvatus*, *P. obtusus*, and *P. tricuspis*) showed significant EAG

response and behavioral attraction to fire ant alarm pheromone (2-ethyl-3,6-dimethyl pyrazine) and two analogs (2-ethyl-3,6(or 5)-dimethyl pyrazine and 2 ethyl-3,5 + 6 methyl pyrazine). These results confirm the involvement of fire ant alarm pheromone in the attraction of *Pseudacteon* phorid flies to their fire ant hosts, as previously demonstrated for *P. tricuspis* (Vander Meer and Porter 2002; Sharma et al. 2011). The EAG results were confirmed by the olfactometer bioassays, which demonstrated strong attraction of the four phorid fly species to the alarm pheromone isomer. Taken together, our EAG and olfactometer bioassay results confirm that fire ant alarm pheromones are important host location cues used by phorid flies (Vander Meer et al. 2010; Sharma et al. 2011). Other important host location cues used by phorid flies to locate their hosts are venom alkaloids (Chen et al. 2009) or a combination of fire ant alarm pheromone and venom alkaloids (Sharma and Fadamiro 2013).

In this study, we tested different doses, ranging from very low (1 pg) to moderate doses (0.1–1 μg). Vander Meer et al. (2010) reported the alarm pheromone produced by a single fire ant worker to be ca. 300 pg. Our results revealed that the picogram dose did not elicit significant differences between the treatment and control, suggesting that low doses may be hard to detect by the parasitoids. Previous reports showing that low doses (ca. 30 pg) of the alarm pheromone elicit behavioral activity in fire ant workers (Regnier and Wilson 1968; Wilson 1958; Vander Meer et al. 2010) may not imply that these low doses will be attractive to phorid flies. Phorid flies most likely are responding to the fire ant alarm pheromone from long range; hence, it is possible that phorid flies have evolved to respond to moderate and/or higher doses of the alarm pheromone relative to fire ant workers. Alarm pheromones have a low molecular weight and are highly volatile (Billen and Morgan 1998; Vander Meer et al. 2010). Long range cues need to be highly volatile. Therefore, alarm pheromones, which are reported to be produced by fire ant workers in response to disturbance, are likely the long range cues that are used by phorid flies to locate their fire ant hosts. Once closer to the source, phorid flies likely use venom alkaloids and other cues, including cuticular hydrocarbons, for host location and preference (Vander Meer et al. 1985; Chen et al. 2009; Sharma et al. 2011; Chen et al. 2012).

Our results showed that all the tested phorid flies showed greater EAG attraction to the fire ant alarm pheromone. This was also true in behavior experiments, suggesting that *Pseudacteon* phorid flies, which attack fire ants, have likely evolved similar host locating mechanisms involving the use of fire ant alarm pheromone. The use of alarm pheromones as cues for host location may be adaptive for phorid flies if their hosts, *S. invicta* workers, are more abundant or more available for parasitization in situations where alarm pheromones are released. Our results showing significant attraction of *P. cultellatus*, *P. curvatus*, *P. obtusus*, and *P. tricuspis* females to the fire ant alarm pheromone and closely related analogs suggest the potential for the development of control strategies for *S. invicta*.

In this study, we tested both the fire ant alarm pheromone and seven other closely related alkyl pyrazine analogs. Out of the seven alkyl pyrazine analogs, two analogs (2 ethyl-3,5 + 6 methyl pyrazine and 2,3-diethyl-5-methyl pyrazine) consistently elicited both EAG and behavior attraction to the parasitoids. The positive and consistent activity of the two analogs is consistent with results reported by Sharma et al. (2011). However, it is interesting to note that none of the two compounds has been reported as a component of the fire ant alarm pheromones (Sharma et al. 2011). It is possible that these analogs are constituents of other important pheromones produced by the fire ants, including recruitment and queen-produced recognition pheromones. Alternatively, these analogs could be minor components of the fire ant alarm pheromone, which are yet to be described. It is important to note that the chemical identification of fire ant alarm pheromone has been an elusive subject (Vander Meer et al. 2010), and therefore, the first description of the fire ant alarm pheromone could be the beginning of the discovery and identification of other additional fire ant alarm pheromone components. Several di- and tri-alkyl substituted pyrazines have been reported as known components of alarm pheromones of other ant species including *Wasmannia auropunctata* (Roger) (Showalter et al. 2010), *Eutetramorium mocquerysi* Emery (Tentschert et al. 2000), *Iridomyrmex humilis* (Mayr) (Cavill and Houghton 1974), and *Odontomachus brunneus* (Longhurst et al. 1978). These highly active alkyl pyrazine analogs are commercially available and could be used together with the fire ant alarm pheromone isomer in formulating effective attractants of phorid flies to improve biological control of fire ants.

Taken together, our results confirm that phorid flies use fire ant alarm pheromone to locate fire ant workers (Vander Meer and Porter 2002; Morrison and King 2004; Morrison and Porter 2006). It is also plausible that other fire ant semiochemicals, including venom alkaloids (Chen et al. 2009), may be part of the suite of semiochemicals that are used by the phorid flies to find their fire ant hosts. Future studies are needed to clearly understand how the fire ant alarm pheromone, the two active alkyl pyrazine analogs, and other semiochemicals, including venom alkaloids and cuticular hydrocarbons interact to influence the process of host location in different phorid fly species. Our results form foundational studies and provide a strong basis for future studies that will be designed to help unravel the

complexity of semiochemical-mediated interactions between imported fire ants and their natural enemies.

Acknowledgements We thank Amy Bass (USDA-APHIS-PPQ-CPHST Laboratory/Florida DPI, Gainesville, FL, USA) for the supply of *Pseudacteon* phorid flies for this study, and Olufemi Ajayi for help with rearing and separating parasitoids.

References

Billen J, Morgan ED (1998) Pheromone communication in social insects: sources and secretions. In: Vander Meer RK, Breed MD, Espelie KE, Winston ML (eds) Pheromone communication in social insects ants, wasps, bees, and termites. Westview, Boulde, pp 3–33

Cavill GWK, Houghton E (1974) Some pyrazine derivatives from the argentine ant, *Iridomyrmex humilis*. Aust J Chem 27:879–889

Chen L, Fadamiro HY (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, Sharma KR, Fadamiro HY (2009) Fire ant venom alkaloids act as key attractants for the parasitic phorid fly, *Pseudacteon tricuspis* (Diptera: Phoridae). Naturwissenschaften 96:1421–1429

Chen L, Ochieng S, He X, Fadamiro HY (2012) Comparing electroantennogram and behavioral responses of two *Pseudacteon* phorid fly species to body extracts of black, red, and hybrid imported fire ants, *Solenopsis* spp. J Insect Physiol 58:1360–1367

Gilbert LE, Morrison LW (1997) Patterns of host specificity in *Pseudacteon* parasitoid flies (Diptera, Phoridae) that attack *Solenopsis* fire ants (Hymenoptera: Formicidae). Environ Entomol 26:1149–1154

Graham LCF, Porter SD, Pereira RM, Dorough HD, Kelley AT (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

Longhurst C, Baker R, Howse PE, Speed W (1978) Alkylpyrazines in ponerine ants: their presence in three genera, and caste specific behavioral responses to them in *Odontomachus troglodytes*. J Insect Physiol 24:833–837

Morrison LW, King JR (2004) Host location behavior in a parasitoid of imported fire ants. J Insect Behav 17:367–383

Morrison LW, Porter SD (2006) Post-release host-specificity testing of *Pseudacteon tricuspis*, a phorid parasitoid of *Solenopsis invicta* fire ants. Biocontrol 51:195–205

Patrock RJW, Porter SD, Gilbert LE, Folgarait PJ (2009) Distributional patterns of *Pseudacteon* associated with the *Solenopsis saevissima* complex in South America. J Insect Sci 9:17

Pesquero MA, Campiolo S, Fowler HG, Porter SD (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 RM, Lebrun EG, Gilbert LE (2011) Introduction of the fire ant decapitating fly *Pseudacteon obtusus* in the United States: factors influencing establishment in Texas. Biocontrol 56:295–304

Porter SD, Alonso LE (1999) Host specificity of fire ant decapitating flies (Diptera: Phoridae) in laboratory oviposition tests. J Econ Entomol 92:110–114

Porter SD, Calcaterra LA (2013) Dispersal and competitive impacts of a third fire ant decapitating fly (*Pseudacteon obtusus*) established in North Central Florida. Biol Control 64:66–74

Porter SD, Gilbert LE (2004) Assessing host specificity and field release potential of fire ant decapitating flies (Phoridae: Pseudacteon). In: Van Driesche RG, 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, pp 152–176

Porter SD, Pesquero MA (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 SD, Pesquero MA, Campiolo S, Fowler HG (1995) Growth and development of *Pseudacteon* phorid fly maggots (Diptera: Phoridae) in the heads of *Solenopsis* fire ant workers (Hymenoptera: Formicidae). Environ Entomol 24:475–479

Porter SD, Nogueira de Sa LA, Morrison LW (2004) Establishment and dispersal of the fire ant decapitating fly *Pseudacteon tricuspis* in North Florida. Biol Control 29:179–188

Regnier FE, Wilson EO (1968) The alarm-defense system of the ant *Acanthomyops claviger*. J Insect Physiol 14:955–970

SAS Institute (2007) JMP 7.0.1. Cary, NC, USA

Sharma KR, Fadamiro HY (2013) Fire ant alarm pheromone and venom alkaloids act in concert to attract parasitic phorid flies, *Pseudacteon* spp. J Insect Physiol 59:1119–1124

Sharma KR, Vander Meer RK, Fadamiro HY (2011) Phorid fly, *Pseudacteon tricuspis*, response to alkylpyrazine analogs of a fire ant, *Solenopsis invicta*, alarm pheromone. J Insect Physiol 57:939–944

Showalter DN, Troyer EJ, Aklu M, Jang EB, Sideburst MS (2010) Alkylpyrazines: alarm pheromone components of the little fire ant, *Wasmannia auropunctata* (Roger) (Hymenoptera, Formicidae). Insect Soc 57:223–232

Tentschert J, Bestmann HJ, Holldobler B, Heinze J (2000) 2,3-Dimethyl-5-(2(-propyl) pyrazine), a trail pheromone component of *Eutetramorium mocquerysi* Emery (1899) (Hymenoptera: Formicidae). Naturwissenschaften 87:377–380

Vander Meer RK, Porter SD (2002) Fire ant, *Solenopsis invicta*, worker alarm pheromones attract *Pseudacteon* phorid flies. In: Proceedings of the 2002 imported fire ant conference, Athens, pp 77–80

Vander Meer RK, Lofgren CS, Alvarez FM (1985) Biochemical evidence for hybridization in fire ants. Florida Entomol 68:501–506

Vander Meer RK, Preston CA, Choi MY (2010) Isolation of a pyrazine alarm pheromone component from the fire ant, *Solenopsis invicta*. J Chem Ecol 36:163–170

Vazquez RJ, Porter SD, Briano JA (2006) Field release and establishment of the decapitating fly *Pseudacteon curvatus* on red imported fire ants in Florida. Biocontrol 51:207–216

Wilson EO (1958) A chemical releaser of alarm and digging behavior in the ant *Pogonomyrmex badius* (Latreille). Psyche 65:41–51