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# 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

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ABSTRACT

Host preferences in both sexes of *Pseudacteon tricuspis* Borgmeier (Jaguariuna biotype) and *Pseudacteon curvatus* Borgmeier (Formosa biotype) and their relative attraction to the imported fire ants (IFA), *Solenopsis invicta* Buren (red IFA), *Solenopsis richteri* Forel (black IFA) and *S. invicta* × *S. richteri* hybrids (hybrid IFA) were investigated in two separate experiments utilizing multiple choice flight bioassays. The results of both experiments clearly showed that both sexes of the Jaguariuna biotype of *P. tricuspis* could distinguish among the three IFA species and demonstrated greater preference for hybrid IFA and red IFA. This conclusion is supported by a variety of data collected on the number of fly visits, attack rate, and hovering duration (Experiment 1), and on the number of trapped flies (Experiment 2), which showed that black IFA is the least preferred of the three species. Similar results were recorded for the Formosan biotype of *P. curvatus*, although the data were not as strongly conclusive. Females of this biotype spent a significantly greater amount of time in hovering mode over red IFA and hybrid IFA compared to black IFA, but the other data were not significant. The red IFA is the natural host of both phorid fly biotypes and our results suggest that both biotypes may have evolved a specialized relationship with red IFA including an ability to discriminate it from related fire ants. These results are discussed in relation to the possible role of fire ant chemicals in mediating host preferences in phorid flies, contributions of male phorid flies to fire ant biocontrol, and the practical implications of the key findings.

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## 1. Introduction

Two species of imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae) were accidentally introduced from South America into southern United States (U.S.) in the past century. The black imported fire ant (black IFA), *Solenopsis richteri* Forel was introduced around 1918 followed by the introduction of the red imported fire ant (red IFA), *Solenopsis invicta* Buren in the early 1930s. Both species are believed to have entered the U.S. through the port of Mobile, Alabama, spreading northward in Alabama and into neighboring states (Wilson, 1958). Hybridization between *S. invicta* and *S. richteri* (producing hybrid IFA) has been documented in Alabama, Mississippi, Georgia, and Tennessee (Vander Meer et al., 1985; Ross et al., 1987a,b; Diffie et al., 1988; Vander Meer and Lofgren, 1988; Shoemaker et al., 1996). These invasive fire ants are now widely distributed throughout the southern U.S. inhabiting more than 320 million acres (Williams et al., 2003) and causing a multitude of problems for humans, domestic animals, and agriculture in the region.

Four species of *Pseudacteon* decapitating phorid flies (Diptera: Phoridae) have been introduced from South America in the past decade as classical biological control agents of imported fire ants in southern U.S. (Feener and Brown, 1992; Orr et al., 1995; Gilbert, 1996; Porter et al., 1995a; Porter and Alonso, 1999; Porter and Gilbert, 2004; Gilbert et al., 2008). The two key species were *Pseudacteon tricuspis* Borgmeier and *Pseudacteon curvatus* Borgmeier (Porter and Gilbert, 2004; Vazquez and Porter, 2005). One of the early successful introductions of phorid flies in southern U.S. involved a biotype of *P. tricuspis* collected from Jaguariuna, Brazil in 1996 on red IFA (Porter and Alonso, 1999; Porter et al., 2004; Pereira and Porter, 2006). This biotype is now well established in several southern states (Pereira and Porter, 2006). In addition, two biotypes of the second species, *P. curvatus* have been released in the region. A biotype from Las Flores, Argentina was released to control populations of black IFA and hybrid IFA in Alabama, Mississippi and Tennessee (Graham et al., 2003; Vogt and Streett, 2003; Parkman et al., 2005). A second biotype from Formosa, Argentina, which was also collected on red IFA, was released on red IFA in Florida, South Carolina and Texas (Vazquez et al., 2004; Davis and Horton, 2005; Gilbert et al., 2008).

Both species of phorid flies (*P. tricuspis* and *P. curvatus*) were shown in pre-release laboratory host range tests to be highly specific to imported fire ants and minimal nontarget effects on native fire ants were predicted (Gilbert and Morrison, 1997; Porter and Alonso, 1999; Vazquez et al., 2004). Field studies also showed that

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the flies were not attracted to native fire ants or ants from other genera (Porter, 1998b; Morrison and Porter, 2005; Vazquez and Porter, 2005), and their highly specialized behavior and life history make them unlikely threats to other arthropods (Porter, 1998a; Porter and Gilbert, 2005).

Matching biotypes of potential biological control agents to target host populations can significantly improve the effectiveness of biological control agents (Van Driesche and Bellows, 1996; Porter and Briano, 2000; Porter and Gilbert, 2005). However, very little is known about the host preferences of the different species and biotypes of phorid flies. In perhaps the only published study on parasitoid-host matching in phorid flies, Porter and Briano (2000) showed that the Las Flores, Argentina biotype of *P. curvatus* preferred black IFA and hybrid IFA over red IFA. However, this study was carried out using no-choice and pair-wise choice bioassays making it impossible to compare attraction of the flies to the three imported fire ant species/forms in a single test.

Parasitism has been used as the ultimate parameter for determining host specificity and preference in phorid flies (Porter and Briano, 2000; Vazquez et al., 2004). However, studies have showed that parasitism rates of phorid flies on ants are consistently less that 5% in the field (Feener, 1981, 1988; Morrison et al., 1997; Morrison and Porter, 2005), suggesting that the direct effect of ant mortality in the field due to phorid flies is very low. On the contrary, pre-parasitization behaviors of phorid flies (e.g., hovering and attack attempts) resulted in a 50% decrease in food retrieval by fire ants (Morrison, 1999), suggesting that these behaviors may play very important roles in the effectiveness of phorid flies as ant biological control agents.

Aside from their primary role in mating with females, little is known about the contributions of male phorid flies to ant biological control. In *P. tricuspis*, both sexes are attracted to fire ants and mating occurs while females are searching for ant workers to attack (Porter, 1998a). Male and female *P. tricuspis* also display similar hovering behavior over fire ants (Porter, 1998a). Furthermore, response of both sexes of *P. tricuspis* to odor of live *S. invicta* workers and their body extracts have been reported (Chen and Fadamiro, 2007). These findings suggest that *P. tricuspis* males may be providing more contributions than previously thought to the overall biological control effects of phorid flies in the field. However, in *P. curvatus*, mating occurs elsewhere and males are unlikely to contribute much to biological control of fire ants since they are not attracted to fire ant workers (Wuellner et al., 2002).

The main objective of this study was to determine host preferences in both sexes of *P. tricuspis* (Jaguariuna biotype) and *P. curvatus* (Formosa biotype) by testing their relative attraction to the three imported fire ant species/forms in multiple choice flight bioassays. Males of *P. tricuspis* were tested because they are attracted to fire ants (Porter, 1998a). Thus, we hypothesized that *P. tricuspis* males will demonstrate similar host preferences as conspecific females. However, *P. curvatus* males were tested simply for comparison and to confirm a previous report which suggested that they are not attracted to fire ants (Wuellner et al., 2002). Host preference was evaluated using pre-parasitization behavioral parameters such as number of phorid flies attracted to ants, number of fly visits to ants, number of attacks on ants per minute (attack rate), and the amount of time spent by flies in hovering mode over ants (hovering duration).

## 2. Materials and methods

### 2.1. Imported fire ants

Colonies of black IFA and hybrid IFA were collected in northern Alabama and western Tennessee (USA) in spring 2008. Red IFA colonies were collected on the campus of Auburn University, Auburn, Alabama. Ant workers were collected by transferring about 1 l of soil (containing workers, broods and the queen) from each mound into 1-gallon (3.785-l) Rubbermaid plastic jars coated with Fluon® (ICI, Wilmington, DE) to prevent escape. Workers collected from each colony were maintained in their nest soil in the laboratory at 25 ± 1 °C, 50 ± 10% relative humidity, on a 14:10 h light:dark cycle and fed 10% sucrose solution and crickets. The colonies were maintained for 1–2 months after collection before the tests. Colonies were separated and identified as red IFA, black IFA and hybrid IFA by analysis of worker venom alkaloids and cuticular hydrocarbon profiles using gas chromatography (Vander Meer et al., 1985), as recently described by Fadamiro et al. (2009).

### 2.2. Phorid flies

*Pseudacteon tricuspis* (Jaguariuna biotype from Brazil) and *P. curvatus* (Formosa biotype from Argentina) used in this study were reared on the workers of red IFA at the fire ant rearing facility of the USDA-ARS Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, U.S.A. as previously described (Porter et al., 1997) and shipped to our laboratory as parasitized fire ant worker heads. Parasitized fire ant worker heads were received in batches and held in a plastic jar (25 × 13 cm) with a lid until emergence. Jars were kept in a growth chamber at 25 ± 1 °C, L:D 14:10 h and 75 ± 5% r. h. Twice daily, newly-emerged flies were removed 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 of females (Porter, 1998a; Porter and Pesquero, 2001). One-day old flies which had been provided 10% sucrose solution for 3 h were used for the tests.

### 2.3. Experiment 1

A methodology similar to the one described by Porter and Briano (2000) was used to evaluate preferences of *P. tricuspis* and *P. curvatus* for the three imported fire ant species/forms (red IFA, black IFA and hybrid IFA). The main difference was that our experiments were multiple choice bioassays compared to the no-choice and dual choice bioassays used by Porter and Briano (2000). The tests were conducted in white plastic trays (42 × 33 × 16 cm) with screened vents and tight-fitting transparent glass lids (Porter, 2000; Porter and Briano, 2000). Four opaque plastic cups (8 cm diameter × 5 cm high; 150 ml) with the inner sides coated with Fluon® (to contain the ants) were placed in the bottom of each tray, one in each of the four corners of the tray. For each trial, workers of red IFA, black IFA and hybrid IFA of similar size were kept separately in each cup (1 species per cup). The fourth cup was left blank and served as negative control. The location of each cup was randomly determined and re-randomized during each replication. Test ants containing 0.25 g of workers (∼400) and several broods were placed in each cup. Test ants were first placed in the cup and allowed to acclimate for 30 min before phorid fly release. Twenty one-day old female or male *P. tricuspis* or *P. curvatus* were released through screened vents into the plastic tray without removing the glass lid cover. Separate tests were conducted for each phorid fly species and sex combination. Each test lasted 1 h and the tray was inspected continuously over this duration by a single observer. Tests were conducted between 12:00 and 16:00 h, the time of day for high phorid fly activity (Pesquero et al., 1996). Each phorid fly species and sex combination was replicated 16 times using four colonies of each fire ant species (4 replicates per colony). The number of visits made by flies to each cup (ant treatment) was recorded by visual count (fly visits). "Visiting" flies typically hovered over each cup containing "attractive" ants.

The total amount of time (min) spent by test flies in hovering mode over each ant species (hovering duration) was also calculated. Also, the number of "attacks" made by each hovering female fly on test ants was recorded per minute (attack rate). "Attack" was designated when hovering female flies attempted to oviposit in the ant's thorax, which usually immobilizes the ant for a short time (Estrada et al., 2006).

### 2.4. Experiment 2

A second experiment was conducted to further determine host preferences in both sexes of the two phorid fly species by comparing the numbers attracted to the three imported fire ant species at the end of a 7-h exposure duration in a multiple choice "trapping" bioassay, which was a variant of the tanglefoot-coated perch PTS tri-stand trap (Puckett et al., 2007). The bioassay was similar to the one described above (Experiment 1) but a key difference was that phorid flies that were attracted to each ant species (cup) were trapped and counted at the end of the test rather than the real-time observations made in Experiment 1. The same white plastic trays and opaque plastic cups (both coated on the inner side with Fluon®) described in Experiment 1 were used. However, each cup contained a layer of plaster to maintain high humidity. Plaster was made by using 2:1 mixture of pottery plaster (U.S. Gypsum Co., Chicago, IL) and water. One inverted glass tube (1.7 cm diameter × 6.4 cm high) was stuck in the centre of the plaster before the plaster became dry. Fluon® was coated on the base part of the glass tube as a 2 cm strip to prevent ants from climbing. Tangle-trap insect brush-on coating (The Tanglefoot Company, Grand Rapids, MI, USA) was coated on the remaining part of the glass tube to glue (trap) attracted flies. Plaster was moistened with water before each test. Four cups (each with a trap) were thus placed in the bottom of each tray as described in Experiment 1. Cups were randomly assigned to each ant species while the fourth cup served as negative (blank) control. Test ants of each species containing 0.25 g of workers (~400) and several broods were placed in each cup. Thirty 1-day old female or male *P. tricuspis* or *P. curvatus* were released through screened vents into the plastic tray. The number of flies glued on each glass tube during the first 2 min after release of flies were removed and discounted since the flies had not acclimated in the box and those glued to the glass tube could have been accidentally trapped during fly release. Afterwards, the set up (test) was left for 7 h at the end of which the number of flies glued on each glass tube (trapped flies) were counted and recorded. Separate tests were conducted for each phorid fly species and sex combination. Each phorid fly species and sex combination was replicated 12 times (one replicate per day) using three colonies of each fire ant species (4 replicates per colony). The location of each cup (trap) was re-randomized during each daily replication.

### 2.5. Statistical analysis

Data on number of fly visits and hovering duration (Experiment 1), and number of trapped flies (Experiment 2) were not normally distributed and transformations did not adequately correct this anomaly. Thus, these data were analyzed using the Kruskal-Wallis non-parametric test ($P < 0.05$; JMP® 7.0.1, SAS Institute 2007). Designations of significantly different pairs of treatment means were made using the non-parametric Wilcoxon signed-rank test for each paired comparison, similar to the procedure used by Obin and Vander Meer (1989). Only the treatment pairs shown to be significant in this follow-up test were designated significant in Section 3. Data on attack rates (Experiment 1) were log-transformed and analyzed by using the analysis of variance (ANOVA) followed by the Tukey-Kramer HSD comparison test ($P < 0.05$; JMP® 7.0.1, SAS Institute, 2007).

## 3. Results

### 3.1. Experiment 1

When presented with the three imported fire ants in a multiple choice flight bioassay, females of *P. tricuspis* made more visits to hybrid IFA and red IFA than to black IFA or the blank control ($\chi^2_2 = 20.51$, $df = 3$, $P = 0.0001$, Fig. 1). A similar preference trend was also recorded for male *P. tricuspis*: significantly more visits were made to hybrid IFA than to black IFA or control ($\chi^2_2 = 15.63$, $df = 3$, $P = 0.0014$, Fig. 1). However, the numbers of visits made by *P. tricuspis* males to red IFA were only numerically (but not significantly) greater than the number of visits made to black IFA (Fig. 1). Females of *P. curvatus* also made significantly greater numbers of visits to hybrid IFA and red IFA than to control ($\chi^2_2 = 12.57$, $df = 3$, $P = 0.05$, Fig. 1), but these numbers were not statistically different from the number of visits made to black IFA. In contrast, no significant differences were recorded in the numbers of visits made by *P. curvatus* males to the four treatments ($\chi^2_2 = 5.93$, $df = 3$, $P = 0.115$, Fig. 1). In general, the numbers of visits made to black IFA were not significantly greater than to the control, and fewer visits were made by *P. curvatus* compared to *P. tricuspis*.

Significant differences were recorded in the number of attacks per minute (attack rate) made by hovering female *P. tricuspis* on the different ant treatments. The attack rate on hybrid IFA was significantly greater than on black IFA ($F = 3.35$, $df = 2$, $P = 0.05$, Fig. 2). Females of *P. curvatus* also made more attacks on hybrid IFA and red IFA but these were not statistically different, possibly due to a generally lower attack rate by this species ($F = 1.91$, $df = 2$, $P = 0.17$, Fig. 2). Consistent with the results for the first two behavioral parameters, the amount of time spent by *P. tricuspis* females in hovering mode over ants (hovering duration) was significantly higher for hybrid IFA than for black IFA ($\chi^2_2 = 11.14$, $df = 2$, $P = 0.004$, Fig. 3). A similar result was also recorded for *P. tricuspis* males ($\chi^2_2 = 7.72$, $df = 2$, $P = 0.021$, Fig. 3). Significant differences were also recorded in the hovering duration of *P. curvatus* females ($\chi^2_2 = 8.45$, $df = 2$, $P = 0.015$, Fig. 3) but not for males ($\chi^2_2 = 2.53$, $df = 2$, $P = 0.282$, Fig. 3). Hovering durations of female *P. curvatus* were significantly higher on hybrid IFA and red IFA than on black IFA (Fig. 3). In general, hovering durations of *P. tricuspis* females were 2–3 times greater than durations recorded for female *P. curv-*

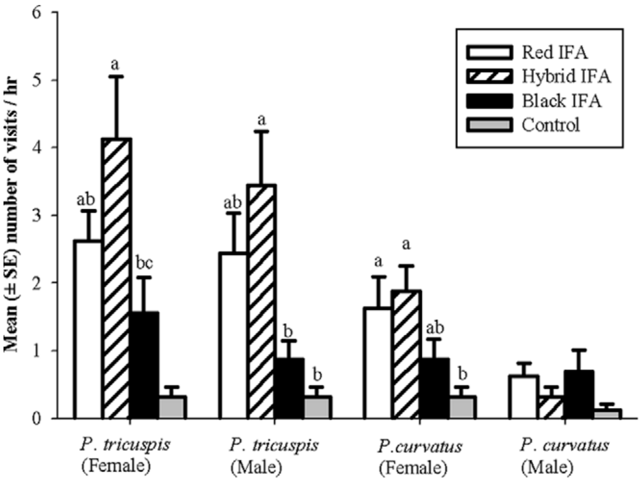

**Fig. 1.** Number of visits made by phorid flies (fly visits) to cups containing different species of fire ants. Figure shows mean (±SE) number of fly visits per hour per replicate. Each replicate consisted of 20 phorid flies and replicated 16 times. Means for the same species and sex combination having no letter in common are significantly different ($P < 0.05$).

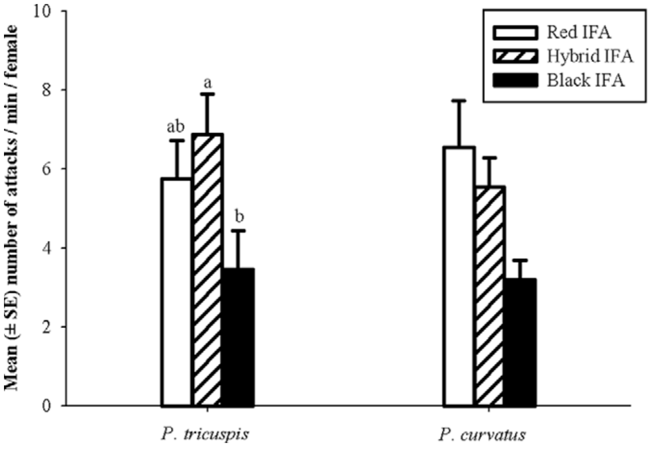

**Fig. 2.** Attack rates of female phorid flies on different species of fire ants. Figure shows mean (±SE) number of attacks per minute per female. Means for the same species having no letter in common are significantly different ($P \leqslant 0.05$).

*atus*, and males of the latter species rarely hovered over the ants (Fig. 3).

### 3.2. Experiment 2

The mean numbers of *P. tricuspis* females glued to red IFA (5.13 ± 0.62) and hybrid IFA (4.87 ± 0.68) traps were significantly higher than the numbers glued to black IFA (3.13 ± 0.40) or control (2.5 ± 0.34) traps ($\chi^2_2 = 12.87$, *df* = 3, *P* = 0.005, Fig. 4). The numbers of *P. tricuspis* males glued to the traps were numerically higher for red IFA but not significantly different among the three ant species ($\chi^2_2 = 7.72$, *df* = 3, *P* = 0.05, Fig. 4). However, no significant differences were recorded in the numbers of *P. curvatus* females ($\chi^2_2 = 5.67$, *df* = 3, *P* = 0.128, Fig. 4) and males ($\chi^2_2 = 5.95$, *df* = 3, *P* = 0.114, Fig. 4) glued to the different treatments. In general, greater numbers of *P. tricuspis* (~52% and 47% of released females and males, respectively) were glued to the treatments than *P. curvatus* (~25% and 23% of released females and males, respectively), probably suggesting higher activity of *P. tricuspis*. Alternatively, the results could suggest that *P. tricuspis* spend relatively more time "perching" than attacking ant workers.

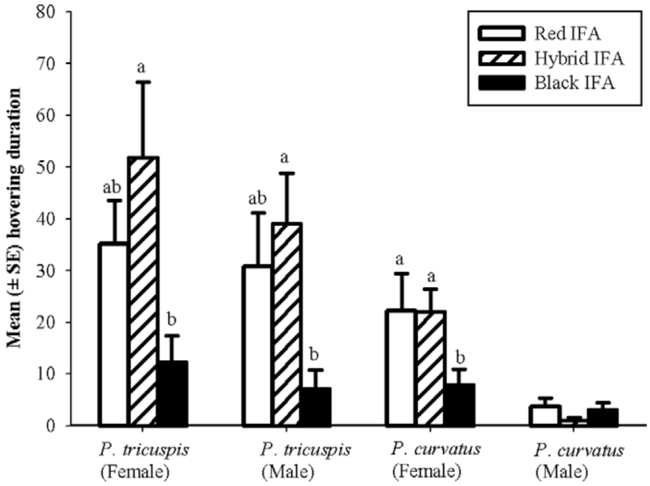

**Fig. 3.** Amount of time spent by phorid flies in hovering mode (hovering duration) over different species of fire ants. Figure shows mean (±SE) hovering duration (min) per replicate. Each replicated consisted of 20 phorid flies and replicated 16 times). Means for the same species and sex combination having no letter in common are significantly different ($P < 0.05$).

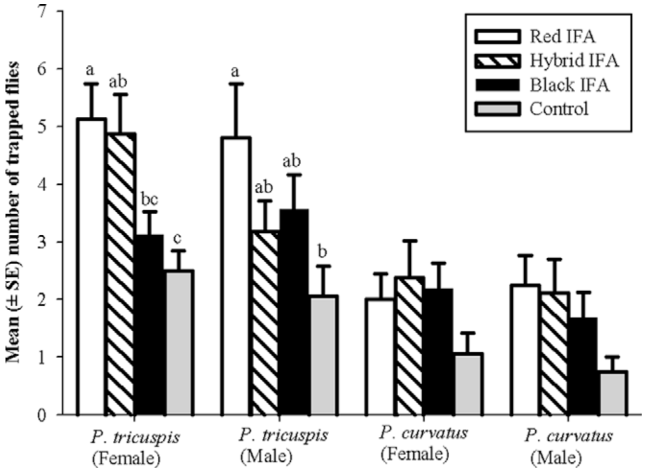

**Fig. 4.** Numbers of female phorid flies glued to glass sticky traps (trapped flies) positioned within cups containing different species of fire ants. Figure shows mean (±SE) number of trapped flies per replicate. Each replicate consisted of 30 phorid flies and replicated 12 times. Means for the same species and sex combination having no letter in common are significantly different ($P \leqslant 0.05$).

## 4. Discussion

The results of the two experiments showed that both sexes of the Jaguariuna biotype of *P. tricuspis* could distinguish among the three imported fire ant species/forms and demonstrated greater preference for hybrid IFA and red IFA. This conclusion is supported by the variety of data collected on the number of fly visits, attack rate, and hovering duration from the first experiment, and on the number of trapped flies from the second experiment, which showed that black IFA is the least preferred of the three species. The results showing preference of this *P. tricuspis* biotype for red IFA over black IFA is not surprising considering that red IFA is the natural host of the Jaguariuna biotype (Porter and Alonso, 1999) and both species might have coevolved with each other. However, the data which showed that this biotype equally preferred red IFA and hybrid IFA but preferred hybrid IFA over black IFA is somewhat surprising given that red and black IFA do not hybridize in South America (Ross and Trager, 1990), making it unlikely for phorid flies to have evolved a specialized relationship with hybrid IFA.

Our results on the females of the Formosan biotype of *P. curvatus* are however, not as conclusive. Only the data on hovering duration showed a clear preference of females of this biotype for red IFA and hybrid IFA over black IFA. Data obtained for the other parameters (e.g., number of fly visits and number of attacks per minute) only showed a trend for greater preference of females of this biotype for red IFA and hybrid IFA. Also, no significant results were recorded for the males. Like the Jaguariuna biotype of *P. tricuspis*, the Formosa biotype of *P. curvatus* was collected in South America also on red IFA (Vazquez et al., 2004; Vazquez and Porter, 2005). Therefore, a significant preference of this biotype for its natural host would not have been surprising. The non-significant results obtained for this *P. curvatus* biotype may be related to its relatively lower activity compared to the *P. tricuspis* biotype tested in this study. For instance, only about a quarter of *P. curvatus* females released in the second experiment responded to the treatments compared to about half of *P. tricupis* females which responded. In general, the results for both phorid fly biotypes tested in this study are consistent with those recorded for the Las Flores biotype of *P. curvatus*, which was reported to prefer black IFA (its natural host) and hybrid IFA over red IFA (Porter and Briano, 2000). It is unclear why the two biotypes tested in the present study and the Las Flores biotype of *P. curvatus* tested by Porter and Briano (2000) all showed preference for hybrid IFA even though hybrids are not known to occur in the native range of these flies in South America. Host preference has been defined as the genetically based tendency to respond to particular host cues (Clements, 1999). Host location and preference in phorid flies is likely mediated by ant chemical cues (Porter, 1998a, b; Chen and Fadamiro, 2007) but the source and identity of such cues remain unknown. Studies have revealed distinctive qualitative and quantitative differences among the three imported fire ant species in the chemical profiles of their cuticular hybrocarbons, venom alkaloids, and pheromones (Vander Meer et al., 1985; Obin and Vander Meer, 1989; Vander Meer and Lofgren, 1989; Chen and Fadamiro, 2009a,b). The possible role of these chemicals in mediating phorid fly–fire ant interactions, in particular host location and preferences in phorid flies, is the subject of ongoing research in our laboratory. The equal attraction of both phorid fly biotypes to red IFA and hybrid IFA suggests that the cues mediating the attraction are qualitative traits which are well conserved in the hybrid. Assuming that host preference in phorid flies is more genetic than facultative (Porter, 2000) and is in fact mediated by host chemical cues (Porter, 1998a,b; Chen and Fadamiro, 2007), the gene(s) regulating such chemical cues would be expected to be conserved in the hybrids. Nonetheless, the mechanisms mediating the preference of both phorid fly biotypes for hybrid IFA merit further investigation.

It is possible that the preference of both biotypes tested in this study for red IFA is due to the fact that they were reared on this host. Some parasitoids have been reported to show increased response to the odor of the host on which they were reared (Hérard et al., 1988), and this phenomenon can be explained by the "chemical legacy hypothesis" (Thorpe and Jones, 1937; Corbet, 1984). However, the high preference of both biotypes for hybrid IFA suggests that this phenomenon is unlikely the main factor behind these results.

Some of the pre-parasitization behaviors (i.e., hovering duration and attack rate) of phorid flies documented in this study may contribute significantly to their performance as biocontrol agents of fire ants (Morrison, 1999) given that direct mortality of ants via parasitism by phorid flies is very low in the field (Feener, 1981, 1988; Morrison et al., 1997; Morrison and Porter, 2005). Our results on the attack rates of both species/biotypes are generally similar to those previously reported by other authors (Porter and Briano, 2000; Vazquez et al., 2004; Vazquez and Porter, 2005).

Another interesting part of this study which had not been previously tested in detail is the host preference of phorid fly males and their potential contributions to fire ant biological control. Males of the biotype of *P. tricuspis* tested in this study exhibited similar hovering and attack behavior as conspecific females, as reported by Porter (1998a) and Smith and Gilbert (2003). Our data also indicated that male *P. tricuspis* have the same innate host preferences as their females. These results are also consistent with those from a previous study which demonstrated response of both sexes of *P. tricuspis* to fire ant odor (Chen and Fadamiro, 2007). Furthermore, the stereotypical C-shaped defensive posture typically displayed by fire ant workers in response female phorid flies (Obin and Vander Meer, 1985; Porter et al., 1995b; Porter, 1998a) was also observed in the present study in response to hovering male *P. tricuspis*, as well as the females. This ant defensive behavior elicited by hovering female phorid flies has been reported to decrease ant foraging (Orr, 1992; Morrison, 2000), and thus, it is likely that hovering males will have a similar effect on ant foraging. In addition, hovering behavior of male *P. tricuspis* may elicit production of volatile chemical cues by fire ants, which are used by female *P. tricuspis* for ant host location (Morrison and King, 2004). Altogether, these findings suggest that *P. tricuspis* males may be contributing more than previously thought to biological control of fire ants. In contrast, however, *P. curvatus* males were minimally attracted to fire ants, as previously reported (Porter and Briano, 2000; Wuellner et al., 2002), and rarely did display hovering behavior over the ants. Further studies are necessary to confirm and quantify the biocontrol impact of males of *P. tricuspis* and other similar species (e.g., *P. obtusus*) which mate while females are searching for ant workers to attack (Porter, 1998a).

In conclusion, our results indicated that both biotypes of phorid flies tested in this study showed strong preferences for red IFA, their natural host, as well as for hybrid IFA. This suggests that they may have evolved a specialized relationship with their natural host including an ability to discriminate it from similar fire ants. These findings imply that the biogeographic distribution of fire ants and parasitoid-host matching must be considered to enhance the effectiveness of phorid flies as fire ant biocontrol agents. Phorid fly biotypes (such as the ones tested in this study) with preference for red IFA and hybrid IFA are likely to perform better when released within the distribution ranges of both ant species, but may not perform as well on black IFA.

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## References

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. Journal of Insect Behavior 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.

Clements, A.N., 1999. The Biology of Mosquitoes. CABI Publishing.

Corbet, S.A., 1984. Insect chemosensory responses: a chemical legacy hypothesis. Ecological Entomology 10, 143–153.

Davis, T.S., Horton, P.M., 2005. Release and spread of the fire ant decapitating fly *Pseudacteon curvatus* in South Carolina. Annual Imported Fire Ant Conference, April 2005, Gulfport, Mississippi, USA.

Diffie, S., Vander Meer, R.K., Bass, M.H., 1988. Discovery of hybrid fire ant populations in Georgia and Alabama. Journal of Entomological Science 23, 187–191.

Estrada, C., Patrock, R.W., Folgarait, P.J., Gilbert, L.E., 2006. Host specificity of four *Pseudacteon* spp. (Diptera: Phoridae), parasitoids of fire ants in Argentina (Hymenoptera: Formicidae). Florida Entomologist 89, 462–468.

Fadamiro, H.Y., He, X., Chen, L., 2009. Aggression in imported fire ants: an explanation for shifts in their spatial distributions in southern United States? Ecological Entomology, in press, doi:10.1111/j.1365.2311.2009.01102.x.

Feener, D.H., 1981. Competition between ant species: outcome controlled by parasitic flies. Science 214, 815–817.

Feener, D.H., 1988. Effects of parasites on foraging and defense behavior of a termitophagous ant, *Pheidole titanis* Wheeler (Hymenoptera: Formicicdae). Behavioral Ecology and Sociobiology 22, 421–427.

Feener, D.H., Brown, B.V., 1992. Reduced foraging of *Solenopsis geminata* (Hymenoptera: Formicidae) in the presence of parasitic *Pseudacteon* spp. (Diptera: Phoridae). Annals of the Entomological Society of America 85, 80–84.

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.), Proceedings of a Second Conference on Quail Management. Texas Agricultural Extension Service, 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). Environmental Entomology 26, 1149–1154.

Graham, L.C., 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 Entomologist 86, 334–339.

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 Entomologist 33, 15–29.

Hérard, F., Keller, M.A., Lewis, W.J., Tumlinson, J.H., 1988. Beneficial arthoropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson. Journal of Chemical Ecology 14, 1579–1606.

Morrison, L.W., 1999. Indirect effects of phorid fly parasitoids on the mechanisms of interspecific competition among ants. Oecologia 121, 113–122.

Morrison, L.W., 2000. Mechanisms of *Pseudacteon* parasitoid (Diptera: Phoridae) effects on exploitative and interference competition in host *Solenopsis* ants (Hymenoptera: Formicidae). Annals of the Entomological Society of America 93, 841–849.

Morrison, L.W., Dall'Aglio-Holvorcem, C.G., Gilbert, L.E., 1997. Oviposition behavior and development of *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). Environmental Entomology 26, 716–724.

Morrison, L.W., King, J.R., 2004. Host location behavior in a parasitoid of imported fire ants. Journal of Insect Behavior 17, 367–383.

Morrison, L.W., Porter, S.D., 2005. Testing for population-level impacts of introduced *Pseudacteon tricuspis* flies, phorid parasitoids of *Solenopsis invicta* fire ants. Biological Control 33, 9–19.

Obin, M.S., Vander Meer, R.K., 1985. Gaster flagging by fire ants (*Solenopsis* spp.): functional significance of venom dispersal behavior. Journal of Chemical Ecology 11, 1757–1768.

Obin, M.S., Vander Meer, R.K., 1989. Between and within-species recognition among imported fire ants and their hybrids (Hymenoptera: Formicidae): application to hybrid zone dynamics. Annals of the Entomological Society of America 82, 649–652.

Orr, M.R., 1992. Parasitic flies (Diptera: Phoridae), influence foraging rhythms and caste division of labor in the leaf-cutter ant. *Atta cephalotes* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology 30, 395–402.

Orr, M.R., Seike, S.H., Benson, W.W., Gilbert, L.E., 1995. Flies suppress fire ants. Nature 373, 292–293.

Parkman, P., Vail, K., Rashid, J., Oliver, J., Pereira, R.M., Porter, S.D., Shires, M., Haun, G., Powell, S., Thead, L., Vogt, J.T., 2005. Establishment and spread of *Pseudacteon curvatus* in Tennessee. Annual Imported Fire Ant Conference, April 2005, Gulfport, Mississippi, USA, pp. 111–112.

Pereira, R.M., Porter, S.D., 2006. Range expansion of the fire ant decapitating fly, *Pseudacteon tricuspis*, 8–9 years after releases in north florida. Florida Entomologist 89, 536–538.

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 Entomologist 79, 455–457.

Porter, S.D., 1998a. Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). Florida Entomologist 81, 292–309.

Porter, S.D., 1998b. Host-specific attraction of *Pseudacteon* flies (Diptera: Phoridae) to fire ant colonies in Brazil. Florida Entomologist 81, 423–429.

Porter, S.D., 2000. Host specificity and risk assessment of releasing the decapitating fly, *Pseudacteon curvatus*, as a classical biocontrol agent for imported fire ants. Biological Control 19, 5–47.

Porter, S.D., Alonso, L.E., 1999. Host specificity of fire ant decapitating flies (Diptera: Phoridae) in laboratory oviposition tests. Journal of Economic Entomology 92, 110–114.

Porter, S.D., Briano, J., 2000. Parasitoid-host matching between the little decapitating fly *Pseudacteon curvatus* from Las Flores, Argentina and the black fire ant *Solenopsis richteri*. Florida Entomologist 83, 422–427.

Porter, S.D., Fowler, H.G., Campiolo, S., Pesquero, M.A., 1995a. Host specificity of several *Pseudacteon* (Diptera: Phoridae) parasites of fire ants (Hymenoptera: Formicidae) in South America. Florida Entomologist 78, 70–75.

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., Reardon, R. (Eds.), Assessing Host Ranges for Parasitoids and Predators Used for Classical Biological Control: A Guide to Best Practice. Forest Health Technology Enterprise Team-2004-03. USDA Forest Service, Morgantown, West Virginia, pp. 152–176.

Porter, S.D., Gilbert, L.E., 2005. Parasitoid case history: an evaluation of methods used to assess host ranges of fire ant decapitating flies. Second international symposium on biological control of arthropods. Davos, Switzerland, pp. 634–650.

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 Entomologist 84, 691–699.

Porter, S.D., Vander Meer, R.K., Pesquero, M.A., Campiolo, S., Fowler, H.G., 1995b. *Solenopsis* (Hymenoptera: Formicidae) fire ant reactions to attacks of *Pseudacteon* flies (Diptera: Phoridae) in southeastern Brazil. Annals of the Entomological Society of America 88, 570–575.

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. Journal of Economic Entomology 90, 135–138.

Porter, S.D., Nogueira de Sa, L.A., Morrison, L.W., 2004. Establishment and dispersal of the fire ant decapitating fly *Pseudacteon tricuspis* in North Florida. Biological Control 29, 179–188.

Puckett, R.T., Calixto, A., Barr, C.L., Harris, M., 2007. Sticky traps for monitoring *Pseudacteon* parasitoids of *Solenopsis* fire ants. Environmental Entomology 36, 584–588.

Ross, K.G., Trager, J.C., 1990. Systematics and population genetics of fire ants (*Solenopsis saevissima* complex) from Argentina. Evolution 44, 2113–2134.

Ross, K.G., Vander Meer, R.K., Fletcher, D.J.C., Vargo, E.L., 1987a. Biochemical phenotypic and genetic studies of two introduced fire ant species and their hybrid (Hymenoptera: Formicidae). Evolution 41, 280–293.

Ross, K.G., Vargo, E.L., Fletcher, D.J.C., 1987b. Comparative biochemical genetics of three fire ant species in North America, with special reference to the two social forms of *Solenopsis invicta* (Hymenoptera: Formicidae). Evolution 41, 979–990.

Shoemaker, D.D., Ross, K.G., Arnold, M.L., 1996. Genetic structure and evolution of a fire ant hybrid zone. Evolution 50, 1958–1976.

Smith, C.R., Gilbert, L.E., 2003. Differential attraction of a parasitoid to dead host ants. Florida Entomologist 86, 479–480.

Thorpe, W.H., Jones, F.G.W., 1937. Olfactory conditioning and its relation to the problem of host selection. Proceedings of the Royal Society of London B 124, 56–81.

Van Driesche, R.G., Bellows Jr., T.S., 1996. Biological Control. Chapman and Hall, New York.

Vander Meer, R.K., Lofgren, C.S., 1988. Use of chemical characters in defining populations of fire ants, *Solenopsis saevissima* complex, (Hymenoptera: Formicidae). Florida Entomologist 71, 323–332.

Vander Meer, R.K., Lofgren, C.S., 1989. Biochemical and behavioral evidence for hybridization between fire ants, *Solenopsis invicta* and *Solenopsis richteri* (Hymenoptera: Formicidae). Journal of Chemical Ecology 15, 1757–1765.

Vander Meer, R.K., Lofgren, C.S., Alvarez, F.M., 1985. Biochemical evidence for hybridization in fire ants. Florida Entomologist 68, 501–506.

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 Entomologist 88, 107–110.

Vazquez, R.J., Porter, S.D., Briano, J.A., 2004. Host specificity of a biotype of the fire ant decapitating fly *Pseudacteon curvatus* (Diptera: Phoridae) from Northern Argentina. Environmental Entomology 33, 1436–1441.

Vogt, J.T., Streett, D.A., 2003. Establishment of *Pseudacteon curvatus* Borgmeier in Mississippi. Imported Fire Ants Conference Proceedings, pp. 58.

Williams, D.F., Oi, D.H., Porter, S.D., Pereira, R.M., Briano, J.A., 2003. Biological control of imported fire ants. Annals of the Entomological Society of America 49, 144.

Wilson, E.O., 1958. Origin of the variation in the imported fire ant. Evolution 7, 262–263.

Wuellner, C.T., Porter, S.D., Gilbert, L.E., 2002. Eclosion, mating, and grooming behavior of the parasitoid fly *Pseudacteon curvatus* (Diptera: Phoridae). Florida Entomologist 85, 563–566.