The Fire Ant Decapitating Fly, Pseudacteon bifidus (Diptera: Phoridae): Host Specificity and Attraction to Potential Food Items

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The fire ant decapitating fly, *Pseudacteon bifidus* (Diptera: Phoridae): host specificity and attraction to potential food items

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**Abstract**

The tropical fire ant, *Solenopsis geminata* (F.) (Hymenoptera: Formicidae), is an invasive pest throughout most of the tropics, especially on islands in the Pacific. Natural enemies such as the fire ant decapitating fly, *Pseudacteon bifidus* Brown and Morrison (Diptera: Phoridae), offer the potential for use as self-sustaining biological control agents provided they are host specific and do not cause other unintended problems. This paper provides details of sequential field and laboratory choice tests with host and non-host ants, as well as tests with a variety of potential food items to which adult flies may be attracted. Results of these tests showed that *P. bifidus* flies are highly host specific to tropical fire ants and that they are not attracted to ants in other genera. Even other species of fire ants are unlikely to be parasitized by this fly in the field. Furthermore, tests with a variety of food items demonstrated that *P. bifidus* is not likely to be a nuisance to humans because it is not attracted to carrion, feces, fruits, or various kinds of human food items. In short, *P. bifidus* would not likely cause unintended problems if it were used as a self-sustaining biological control agent of invasive tropical fire ants.

**Key Words:** *Solenopsis geminata*; host range; diet preferences; biological control

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**Resumen**

La hormiga de fuego tropical, *Solenopsis geminata* (F.) (Hymenoptera: Formicidae), es una plaga invasora en la mayor parte de los trópicos, especialmente en las islas del Pacífico. Enemigos naturales como la mosca decapitadora de la hormiga brava, *Pseudacteon bifidus* Brown y Morrison (Diptera: Phoridae), ofrecen el potencial de usarlos como agentes autosostenibles de control biológico siempre que sean específicos al hospedero y no cause otros problemas no deseados. Este artículo provee detalles de pruebas sucesivas de elección en el campo y laboratorio con hormigas hospederas y no hospederas, así como, pruebas con una variedad de posibles alimentos que podrían usarse para alimentar las mosquitas adultas. Resultados de estas pruebas mostraron que las mosquitas *P. bifidus* son altamente específicas para las hormigas de fuego tropicales y que no son atraídas por hormigas de otros géneros. Incluso, es poco probable que hormigas de fuego de otras especies sean parasitadas por esta mosca en el campo. Además, las pruebas con una variedad de alimentos demostraron que no es probable que *P. bifidus* sea una molestia para los seres humanos ya que no es atraída a la carroña, heces, frutas u otros tipos de alimentos humanos. En conclusión, es poco probable que *P. bifidus* causaría problemas no deseados si fuese utilizada como un agente de control biológico autosostenible de las hormigas de fuego tropicales invasoras.

**Palabras Claves:** *Solenopsis geminata*; rango de hospederos; preferencias de dieta; control biológico

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plex fire ants in the United States, appear to have narrow host ranges because none of them have been collected attacking either red or black imported fire ants (S. invicta and S. richteri Forel [Hymenoptera: Formicidae]) (Porter & Gilbert 2004). Nevertheless, host ranges of Pseudacteon flies that attack S. geminata complex ants have not yet been experimentally tested. The primary objective of this study was to use a centrifugal testing procedure (Briese 2005) to document the host range of P. bifidus using ants closely related and more distantly related to S. geminata, their normal host.

Additionally we tested the diet preferences of adult flies to determine whether they had the potential to be a nuisance pest or a mechanical vector of diseases. Host-specificity and diet information are important because they will help assess whether this fly can be deployed safely as a self-sustaining natural enemy of tropical fire ants in regions where these ants are invasive pests.

Materials and Methods

COLLECTION AND REARING

The P. bifidus flies used in this study were collected in Dimmit County about 16 km north of Catarina, Texas, on 1 May 2014. Field host-specificity tests were conducted at the same site and day as the original collection. Laboratory host-specificity and food attraction tests were conducted in Gainesville, Florida, using laboratory-reared flies.

Rearing was conducted as described by Porter & Plowes (submitted). Briefly, emergence, mating, and host parasitization occurred in 2 large attack boxes (2 × 1 × 0.6 m) with clear plastic tops (Vogt et al. 2003; Porter & Plowes submitted). Temperature in these boxes was regulated at 27.0 ± 0.5 °C during the day by heat from 300 W halogen lamps controlled by a thermostat. Relative humidity in the attack boxes was continuously maintained at 87 ± 4% RH by circulating moist air into the attack box from a lower bay containing a vaporizer controlled by a humidistat (Porter et al. 2013; Porter & Plowes submitted).

Newly emerged adult decapitating flies entered the attack box from a side chamber which contained trays of puparia. Wicks with sugar water (10% by weight) were attached to the tops of the attack boxes (Porter & Plowes submitted) to provide food for adult flies. We used S. geminata workers collected from the area around Gainesville, Florida, as hosts for the flies. After 4 to 5 d, potentially parasitized workers were removed from the large attack boxes and placed in small holding boxes (25 × 23 × 8 cm) at about 27 °C with vented lids and moisture blocks that maintained relative humidity above 95% (Porter et al. 2013). Dead workers with pupariating larvae were collected daily and placed on moist plaster trays stored in high humidity holding boxes at room temperature (about 23.5 °C).

SEQUENTIAL FIELD TEST OF HOST-SPECIFICITY

This field test was conducted by sequentially setting out host and non-host ant species to determine which species would attract P. bifidus and several other native Pseudacteon species at the Dimmit County test site (Plowes et al. 2009). We began the test with S. geminata, the normal host, to confirm that flies were present, followed simultaneously by a variety of ant species, mostly in other genera (see Table 1). We then brought back S. geminata to confirm flies were still present and finally presented the desert fire ant, Solenopsis aurea Wheeler (Hymenoptera: Formicidae) (Table 1), a close congener of S. geminata. Solenopsis aurea workers were not tested simultaneously with other non-host species because we did not want the presence of S. aurea to mask potential attraction to other species that were likely to be considerably less attractive.

In order to concentrate phorid decapitating flies at a single location, 10 trays with S. geminata workers (from Florida) were initially set out around the Dimmit County collection site. These trays with their attacking flies were then carried to a single location at 1430 h where the flies were shooed out over a tray (43 × 56 × 8 cm) with a single large laboratory colony of S. geminata ants from Texas (Table 1). Pseudacteon flies observed hovering over ants in this tray were counted at 5, 10, and 15 min of exposure. This tray was then removed and flies were shooed out.

Next, 12 trays (25 × 23 × 8 cm) with non-host ants were set out in about 7 min (Table 1; 2 of the trays contained S. invicta fire ants). Two of us (SDP, RMP) periodically agitated these ants to keep them active and carefully inspected each tray for flies at 5, 10, 15, and 20 min after they had been set out. All flies observed hovering over the ants were collected with an Allen aspirator for later identification. All ants used

Table 1. Ant species used in the sequential field test of Pseudacteon decapitating fly host-specificity. A large colony of the tropical fire ant (Solenopsis geminata), the normal host, was initially set out followed by 11 other species presented simultaneously, then the S. geminata colony again, and finally the desert fire ant, Solenopsis aurea. Test ant species were selected to have similar head widths as S. geminata workers parasitized by the local community of decapitating fly species (Plowes et al. 2009).

| Subfamily | Species                        | Worker amount, g (number) | Head width range, mm |
|-----------|--------------------------------|---------------------------|----------------------|
| Formicidae| Camponotus floridanus          | 0.9 (75)                  | 1.14–1.30            |
|          | Nylania fulva                  | (25)                      | 0.51–0.65            |
|          | Dorymyrmex bureni              | 0.28 g (about 100 workers) + brood | 0.76–0.96 |
| Myrmicina| Pogonomyrmex badius            | 1.11 (200)                | 1.45–2.30            |
|          | Tetramorium bicarinatum        | 0.12 g (about 100)        | 0.71–0.88            |
|          | Crematogaster laeviuscula      | 1.73 g (about 1,000)      | 0.88–1.08            |
|          | Cyphomyrmex rimosus            | (35)                      | 0.69–0.76            |
|          | Trachymyrmex septentrionalis   | 0.44 g (about 250)        | 0.78–1.02            |
|          | Pheidole floridan              | 0.58 g (>50 majors, about 200 minors) + brood | 0.47 & 0.88 |
|          | Pheidole dentata               | 0.92 g (about 1,000) + brood | 0.6 & 1.3 |
|          | Solenopsis invicta (2 groups)  | about 7 g workers and brood, each | 0.6–1.4 |
|          | Solenopsis aurea               | 0.84 g workers            | 0.65–1.4             |
|          | Solenopsis geminata (Texas)    | 15–20 g workers and brood | 0.62–2.35            |
in this test possessed head widths in the range known to be parasitized by the *Pseudacteon* species occurring at the site (Plowes et al. 2009). Head widths of a small sample of workers from each species in this test and the subsequent test were measured with either a wedge micrometer (Porter 1983) or an ocular micrometer.

At the end of 20 min, the non-host test ants were removed and the large *S. geminata* colony was returned. All flies returning to this colony were collected after 5, 10, 15, and 20 min. At the end of these collections, we set out a tray with *S. aurea* workers (Table 1) and released the flies just captured.

Ants which had attracted *Pseudacteon* flies were returned to the laboratory in Florida and placed in vented holding boxes (25 × 23 × 8 cm) as described above. Dead ants were collected daily from 7 to 22 h after exposure to fly attacks and inspected for fly puparia, or dissected if maggots appeared to be present.

**SEQUENTIAL LABORATORY HOST-SPECIFICITY TESTS**

The sequential laboratory host-specificity tests were different from the field tests above in that hundreds of flies were used and we started by providing naive *P. bifidus* flies with a selection of non-host ant species to determine whether they would attempt to oviposit in them. Non-host species were then followed by *S. geminata* host ants to confirm their motivation to oviposit, and to compare rates of parasitism. These tests were conducted in the large attack boxes described above.

**First Test**

Nine species of non-host ants were used in this test (Table 2). Test ants were put into the large attack box at 1600 h on the first day and observed every 30 min until the automatic refuge cups were shut down for the evening at 1800 h (Porter & Plowes submitted). Observations every 30 min resumed the second day at 1000 h when the lifter cups began operating and continued until 1830 h. The 2 groups of *S. invicta* ants were removed at 1300 h on the second day leaving only the 8 other test species (Table 2). Two groups of *S. geminata* ants each from separate colonies were added to the test box at the end of the second day (1800 h), after which the other test ants were immediately removed. Six additional groups of *S. geminata* (separate colonies) were added on the third day and observations of fly activity were made every 30 min from 1000 to 1200 h when observations were terminated. We used a new set of *S. geminata* colonies the third day to increase our sample size of colonies and because the first 2 colonies were badly stressed by the large numbers of fly attacks on the evening of the previous day.

Flies used with the non-host ants were 0 to 34 h old to assure a variety of ages. Based on the number of puparia used and emergence rates, we estimate that about 2,300 flies emerged into the attack box during this test. All puparia were removed from the attack box in the afternoon of the second day so that no new flies emerged on the third day.

During this experiment, we recorded the number of strikes per 15 s for female flies attempting to oviposit in ant workers. Upon removal from the attack box, all ants exposed to fly attacks were placed in the vented holding boxes, described above. Dead or dying workers were collected daily.

**Second Test**

A second specificity test was conducted with *P. bifidus* and 2 groups of *Solenopsis aurea* Wheeler from 2 colonies collected from the Coachella Valley, California (Table 2). As noted above, this desert fire ant is a close relative of *S. geminata*, the normal host of *P. bifidus*. The setup and procedures were similar to those described earlier except that the attack box contained several hundred flies that were 48 to 72 h old when *S. aurea* workers were introduced for 3 h at 1000 h. These workers were monitored for 3 h, as above, after which they were removed and set up so they could be checked for *P. bifidus* puparia. We then introduced 8 trays of *S. geminata* workers at 1330 h and monitored them for an additional 2.25 h. These ants were left in the attack box for the remainder of the day.

**FOOD ATTRACTION TESTS**

Tests were conducted in the same large attack box used in the sequential laboratory choice tests above. No ants were present during food attraction tests. In the first test, we set out samples of the following potential food items: beef liver, pork, chicken, shrimp, hot dog, mozzarella cheese, refried beans, shortbread cookie, tomato, green

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Table 2. Laboratory host-range tests of the decapitating fly *Pseudacteon bifidus* with its normal host, the tropical fire ant (*Solenopsis geminata*), and 10 other species of ants.

| Species                  | Mean head widths* (mm ± SD) | Worker weight, g (number) | No. ant larvae & pupae | No. observed attacks | No. resulting puparia |
|--------------------------|-----------------------------|----------------------------|------------------------|----------------------|-----------------------|
| **First Test**           |                             |                            |                        |                      |                       |
| Odontomachus brunneus    | 1.77 ± 0.06                 | 0.45 (49)                  | 3 pupae                | none                 | 0                     |
| Camponotus floridanus*   | 1.18 ± 0.03                 | 0.81 (100)                 | about 30               | none                 | 0                     |
| Nylanderia fulva         | 0.59 ± 0.04                 | 1.2 (about 1,400)         | a few                  | none                 | 0                     |
| Dorymyrmex burenii       | 0.88 ± 0.06                 | 0.28 (about 250)          | none                   | none                 | 0                     |
| Trachymyrmex septentrionalis | 0.92 ± 0.07               | 0.15 (120)                 | none                   | none                 | 0                     |
| Cyphomyrmex rimosus      | 0.72 ± 0.02                 | 0.12 (169)                 | none                   | several?             | 0                     |
| Aphaenogaster ashmeadi   | 0.93 ± 0.02                 | 1.0 (about 500)           | about 20               | none                 | 0                     |
| Pheidole dentata*        | 0.6–1.3                     | 0.3 (about 470)           | 0.4 g                  | several?             | 0                     |
| Solenopsis invicta*      | 0.6–1.4                     | 1.0 (about 1500) (2 groups) | 1.0 g                  | a few                | 0                     |
| Solenopsis geminata (2 + 6)* | 0.62–2.35                  | 1.0 (about 1500)          | 1.0 g                  | many                 | 2,220                 |
| **Second Test**          |                             |                            |                        |                      |                       |
| Solenopsis aurea (2 groups)* | 0.62–1.47               | 1.0 (about 1500)          | 1.0 g                  | a few                | 13                    |
| Solenopsis geminata (8)*  | 0.62–2.35                 | 1.0 (about 1500)          | 1.0 g                  | many                 | about 700             |

*P. bifidus can successfully parasitize *S. geminata* workers with head widths from 0.59 to 1.15 mm.

Minor workers only.

Nine larvae were found trapped in unopened host head capsules.

*Pheidole* is a dimorphic genus and *Solenopsis* is a polymorphic genus, so giving a range of sizes is more meaningful for assessing host potential than giving a mean ± SD.

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bean, potato, sweet corn, banana, apple, orange, mango, sugarcane, dog feces, human feces, 10% honey water, and 5 wet lab tissues. These food items (2–4 g each item) were arranged in a 5 × 5 array, each on a 5 cm diam cup lid with about 10 cm between lid centers. The 5 wet lab tissues were included to determine if attraction to food items differed from attraction to moisture alone. Meats, vegetables, and fruits were presented raw. Test items were distributed randomly on the array. The P. bifidus flies used in the test varied from recently emerged to 2 d old. Sugar water tubes were removed 18 h before the start of this test. Observations were conducted every 5 min from 1100 to 1200 h.

A second test was conducted using the methods above with a 4 × 5 array which included over-ripe fruit: plum, mango, avocado, and pear (2 each); ripe armadillo carrion (2); and wet lab tissues (10).

STATISTICAL TESTS

Two-tailed t-tests were used to compare oviposition strike rates for P. bifidus flies attacking several species of fire ants in the laboratory. Each replicate was a 15-s observation of an individual fly selected haphazardly from among all active flies during the observations. A 2-tailed exact binomial test (http://vassarstats.net/binomialX.html) was used to evaluate the significance of a male biased sex ratio in flies emerging from S. aurea hosts.

Results

SEQUENTIAL FIELD TEST OF HOST-SPECIFICITY

Within 5 min after releasing Pseudacteon flies over the large test tray with S. geminata workers, 18 flies were observed hovering and attacking. The numbers of flies in this tray remained steady until the end of a 15 min observation period. After swapping in all 12 trays with non-host test ants (Table 1) and removing the tray with S. geminata workers, no Pseudacteon flies were observed hovering in any of the trays with non-Solenopsis ants during the 2nd observation period, despite 20 min of close observations. Not surprisingly, however, we did collect 18 Pseudacteon flies while they attacked imported fire ant workers (S. invicta) in 2 trays, but all of these flies were South American species (14 – P. curvatus Borgmeier, 3 – P. nocens Borgmeier, 1 – P. obtusus Borgmeier [all Diptera: Phoridae]) which had been released in Texas as self-sustaining biological control agents for this invasive pest ant (Caldcott et al. 2011; Plowes et al. 2011, 2012). After replacing the non-host test ants with the large S. geminata colony, we observed attacking flies return within 15 to 20 s. Over a period of 20 min, 33 Pseudacteon flies were collected (13 – female P. bifidus, 2 – female P. caritanae, 7 – P. curvatus females, and 11 – males probably mostly P. catariniae because males of the other 2 species are not attracted to fire ant hosts). After removing the large S. geminata colony, all of the flies collected above were released over an S. aurea fire ant colony; however, none of the flies were seen hovering over the S. aurea workers during 5 min of observations before the S. geminata colony was returned for a second time. About 10 min later, 1 P. catariniae fly was observed attacking S. aurea workers for 10 to 15 min; however, no puparia were produced from these S. aurea workers.

About 2,900 Pseudacteon puparia resulted from several h of attacks on the 10 S. geminata colonies mentioned above (95% P. bifidus, 4% P. caritanae Plowes et al., and 1% P. hippeus Plowes et al. [all Diptera: Phoridae]). Interestingly, no P. curvatus flies emerged from any of the puparia resulting from field attacks on S. geminata workers even though some P. curvatus females had been attracted to trays with the S. geminata workers as noted above.

SEQuENTIAL LABORATORY HOST-SPECIFICITY TESTS

First Test

In the first laboratory test (Table 2), none of the P. bifidus flies that emerged on the first day (8–9 h old) were observed attacking any of the 9 species of test ants. However, the next morning 1-d-old flies were observed attacking S. invicta workers (mean 1.6 ± 1.4 flies per tray, SD) from 10:00 AM until 1:00 PM when the S. invicta workers were removed (Table 2). Over the course of the second day (10:00 AM to 6:00 PM), several flies were observed hovering over and tracking the Dorymyrmex ants, the Pheidole ants, and 1 fly was observed hovering over the Cyphomyrmex ants (all Hymenoptera: Formicidae). Several possible oviposition attempts were observed with the Pheidole and Cyphomyrmex ants (Table 2). No fly activity was observed with the other 5 species of ants either before or after the S. invicta ants were removed.

At 6:00 PM on the second day, 2 groups of S. geminata ants were added to the attack box. Within seconds, both the boxes were filled with too many attacking female flies to count accurately, but the total in both boxes was probably over 80 and perhaps as many as 150. On the third day at 10:00 AM, 49 flies were observed attacking ants in 8 S. geminata trays. By 10:30 AM, the number of attacking flies dropped to 15, and after that numbers varied between 4 and 6. The oviposition strike rate of hovering flies was about 4 times higher for flies hovering over their normal host, S. geminata, compared to the imported fire ant, S. invicta (2.32 ± 0.18 versus 0.56 ± 0.12 strikes per 15 s; ± SE, n = 37 and 27 observation periods, respectively (2-tailed t-test: t = 5.48; df = 62; P < 0.0001).

Second Test

In the specificity test with S. aurea (10:00 AM to 1:00 PM; Table 2), we observed 10 to 20 P. bifidus flies attacking S. aurea workers for the first 30 to 40 min, after which the number of attacking flies averaged only 1 in the 2 test trays. In contrast, when we removed the 2 trays of S. aurea workers and replaced them with 8 trays of S. geminata workers (1:30 PM to 3:45 PM), we initially observed about 110 flies attacking. The number of attacking flies dropped to 44 after 30 min and then gradually fell to 14 after 2.25 h. The mean strike rate for females attacking S. aurea was 0.29 ± 0.11 per 15 s (± SE, n = 28 observations) and the mean strike rate for females attacking S. geminata (n = 24) was 2.25 ± 0.28 per 15 s (2-tailed t-test: t = 6.78; df = 50; P = 0.0001).

PARASITIZATION RATES

None of the non-Solenopsis ants in the first laboratory test produced any fly puparia (Table 2) even though they had been exposed to hundreds of female flies for more than 24 h. The S. invicta ants produced 9 larvae from somewhat over 3 h of attacks (Table 2). More significantly, P. bifidus larvae appeared to be incapable of successfully pupariating in S. invicta hosts because they all failed to push away the mouthparts and were found dead, trapped inside their host head capsules. In contrast, the 8 trays with S. geminata workers in the first laboratory test produced 2,220 healthy puparia after about 24 h of exposure (Table 2).

The S. aurea ants in the second test produced 13 P. bifidus puparia (9–12 d after oviposition; Table 2) plus 4 larvae that failed to pupariate. By way of comparison, approximately 700 P. bifidus puparia resulted from S. geminata workers during this test. A few more puparia may have been produced if we had left the S. aurea workers in the attack boxes as long as the S. geminata workers (3 versus 6 h), but the number of puparia would not have increased much because after the 1st h, an average of only 1 fly remained active in the 2 S. aurea trays.
Adult flies emerged from 85% of the puparia developing in the S. aurea workers (11 of 13) and 91% of these flies were male (10 of 11), a percentage that was significantly higher than the 58% males normally found (Porter & Plowes submitted) when S. geminata workers are the host (2-tailed Exact Binomial test, $P = 0.045$).

**ADULT FOOD ATTRACTION TESTS**

None of the flies landed on any of the test items in the 1st food attraction test even though we observed an average of 81 ± 10 (SD) P. bifidus flies actively flying around the top of the box or resting on refuge cup string rigging in the box. Many additional flies likely were present, but not counted. Occasionally, flies were observed flying 5 to 10 cm above the test arrays and several flies landed on the small lids containing the test food items, but did not proceed to contact food items.

In the second food attraction test, 1 fly landed on 1 of the 2 mango samples. During this test, we observed an average of 108 ± 11 P. bifidus flies active in the box as above.

**Discussion**

**HOST-SPECIFICITY**

We used a centrifugal testing procedure (Briese 2005) to assess the host specificity of P. bifidus flies using ants both closely related to their normal host and ants more and more distantly related. We found that P. bifidus was not attracted to non-Solenopsis ants presented in the field. Furthermore, no Pseudacteon species known to parasitize Solenopsis fire ants has been observed to parasitize ants in another genera despite extensive field observations and laboratory tests (Porter & Gilbert 2004; Weissflog et al. 2008).

In the field, neither P. bifidus nor either of 2 additional native Pseudacteon species (P. catariniae, P. hippaeus) were attracted to the workers of the imported fire ant S. invicta. Furthermore, many years of extensive monitoring of introduced Pseudacteon phorid populations that parasitize S. invicta fire ants in the United States (Gilbert et al. 2008; LeBrun et al. 2009; Calcott et al. 2011) have failed to result in a single case where P. bifidus either has been captured attacking S. invicta in the field or been observed to parasitize S. invicta in the field. The same can be said for those Pseudacteon species that parasitize S. geminata in the United States, even after more than 60 yr of opportunities to switch to S. invicta (Plowes et al. 2009; Calcott and Collins 1996). In our field test, P. bifidus also was not attracted to workers of S. aurea, a closely related fire ant species in the same complex as S. geminata, their normal host. Several P. curvatus females were attracted to S. geminata workers during the field host-specificity test, but no P. curvatus flies emerged from puparia produced by exposure to attacks in the field.

The laboratory no-choice specificity tests generally supported the results of the field specificity tests (Table 2). Large numbers of P. bifidus females showed almost no interest in the 8 species of non-Solenopsis ants tested. The few flies that were occasionally observed hovering over non-Solenopsis ants may have done so because of their visual similarity to fire ants. Under field conditions it is unlikely that the flies would be able to locate ants in other genera because Pseudacteon flies use host-specific fire ant alarm pheromones and aerosolized venom to locate their hosts (Chen et al. 2012). The rare oviposition attempts we observed in the laboratory with P. bifidus failed to produce any parasitized workers in non-Solenopsis genera (Table 2). These results are similar to observations with other Pseudacteon species that attack fire ants (Porter & Gilbert 2004).

**Pseudacteon bifidus** was not successful in completing its lifecycle on the imported fire ant S. invicta. A few percent of the hundreds of P. bifidus females present did hover over S. invicta workers and were able to successfully oviposit in them. Nevertheless, all developing larvae were unable to pupate (Table 2), apparently due to an incompatibility with host physiology or morphology. Flies attacking S. invicta workers produced only 9 dead larvae compared with more than 2,220 puparia for those attacking S. geminata workers. Furthermore, rates of oviposition attempts with S. invicta were only 1/4 the rate observed for S. geminata.

In contrast, P. bifidus was able to successfully parasitize S. aurea workers (11 puparia total) but the parasitism rate was only a small fraction of the rate observed for S. geminata workers (Table 2). Also, the sex ratio was highly skewed to males (91%) compared with the normal sex ratio, which is only slightly male-biased (58%). Curiously, the sex of some Pseudacteon species appears to be determined by the nature of the host, not fly genetics (Morrison et al. 1999).

As with S. invicta workers discussed above, oviposition attempts by P. bifidus females attacking S. aurea workers were considerably less than S. geminata workers. In short, there is strong natural selection against attempting to oviposit in non-host congeners like S. invicta and S. aurea because of the extremely low rates of success. Similarly, some South American Pseudacteon species will attack and occasionally parasitize fire ants in the North and Central American S. geminata species group (Porter & Gilbert 2004; Estrada et al. 2006), but the outcomes are always very poor compared to their normal South American fire ant hosts (Porter 2000; Vazquez et al. 2004; Vazquez & Porter 2005).

Despite extensive tests under laboratory conditions with 6 Pseudacteon species that parasitize fire ants, none including P. bifidus has ever been able to parasitize an ant in another genera (Porter & Gilbert 2004; Tables 1, 2). These failures are likely the result of incompatibilities associated with: (1) the use of species-specific ant defensive pheromones for host location (Sharma & Fadamiro 2013), (2) highly specialized ovipositors (Porter & Pesqueiro 2001), (3) the need to neutralize immune responses to developing larvae, (4) pupal morphology which is closely adapted to host head morphology (Porter 1998), (5) physiological control of host decapitation (Consoli et al. 2001), and (6) the ability to assume “zombie-like” control of host behavior in preparation for pupation (Henne & Johnson 2007).

In summary, our laboratory and field host-specificity tests, together with similar tests of other Pseudacteon species, provide strong evidence that P. bifidus will not be able to successfully parasitize ants in other genera. Furthermore, the highly specialized life-history of Pseudacteon fire ant decapitating flies explains why these flies are likely to be highly specific to certain species.

**ADULT FEEDING AND ATTRACTION TO FOOD**

Access to sugar water and water have been shown to increase the longevity of adult Pseudacteon flies (Chen & Fadamiro 2006), and flies will ingest sugars if they run across them (Porter 1998). However, P. bifidus flies showed no attraction to any of the food items presented even though they were held without access to food for 18 h. Pseudacteon adults will ingest water or honey water if they land on a moist surface, but in the laboratory they were not attracted to various kinds of fruits, vegetables, meats, prepared foods, feces, or carrion. Several species of Pseudacteon phorids that attack fire ants in South America have shown a similar lack of interest in food arrays presented in laboratory tests (Porter & Gilbert 2004). Furthermore, neither P. bifidus nor any of more than 40 congeners that parasitize fire ants in North and South America have ever been reported to be a nuisance in the field.

In summary, the host-specificity tests discussed earlier and the adult food attraction tests indicate that P. bifidus would be neither a
threat to other ants nor a nuisance to people if it were introduced as a self-sustaining biological control agent against invasive populations of tropical fire ants.

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