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

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 secuenciales 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

The fire ant decapitating fly, *Pseudacteon bifidus* Brown and Morrison (Diptera: Phoridae), is a small parasitoid of the tropical fire ant, *Solenopsis geminata* (F.) (Hymenoptera: Formicidae). It occurs in Texas and neighboring regions of Mexico (Plowes et al. 2009). This fly is 1 of more than 20 species of *Pseudacteon* decapitating flies known to parasitize tropical fire ants in their native range (Plowes et al. 2009). Pathogens and parasites of tropical fire ants are of interest as potential biological control agents because *S. geminata* is an invasive pest throughout most of the world's tropics, especially in the islands of the Pacific (Wetterer 2011; Gotzek et al. 2015) where they often cause environmental, agricultural, and health-related problems (Helmly 1970; Bui 1984; Williams & Whelan 1991; Jahn et al. 2003; Krushelnycky et al. 2005; Plentovich et al. 2009; Wauters et al. 2014).

Another 20 or so species of *Pseudacteon* flies are known to parasitize *Solenopsis saevissima* (Hymenoptera: Formicidae) complex ants in South America (Porter & Pesquero 2001; Patrock et al. 2009). Six of these species have been released successfully and established in the United States as self-sustaining biocontrol agents of the red imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Callcott et al. 2011; Plowes et al. 2011, 2012; Porter et al. 2013). Extensive field and laboratory studies with 10 of these South American flies have shown that they were all highly host-specific and only able to parasitize fire ants in the genus *Solenopsis* (Porter & Gilbert 2004). Several of these South American fly species also were able to parasitize native fire ant species from the *S. geminata* complex found in the United States, but rates of parasitism were very poor (Porter 2000; Porter & Gilbert 2004) compared with rates for their natural South American hosts which are all in the *S. saevissima* complex.

As is the case with their South American cousins, *P. bifidus* and other *Pseudacteon* flies, which naturally parasitize *S. geminata* com-

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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
Formicinae		
Camponotus floridanus	0.9 (75)	1.14–1.30
Dolichoderinae		
Nylanderia fulva	(25)	0.51–0.65
Dorymyrmex bureni	0.28 g (about 100 workers) + brood	0.76–0.96
Myrmicinae		
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 floridana	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	0.6–1.4
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 d 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 naïve *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 d.

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

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[a] (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[b]	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 bureni	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[d]	0.6–1.3	0.3 (about 470)	0.4 g	several?	0
Solenopsis invicta[d]	0.6–1.4	1.0 (about 1500) (2 groups)	1.0 g	a few	0[c]
Solenopsis geminata (2 + 6)[d]	0.62–2.35	1.0 (about 1500)	1.0 g	many	2,220
Second Test					
Solenopsis aurea (2 groups)[d]	0.62–1.47	1.0 (about 1500)	1.0 g	a few	13
Solenopsis geminata (8)[d]	0.62–2.35	1.0 (about 1500)	1.0 g	many	about 700

[a]*P. bifidus* can successfully parasitize *S. geminata* workers with head widths from 0.59 to 1.15 mm.
[b]Minor workers only.
[c]Nine larvae were found trapped in unopened host head capsules.
[d]*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.

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 (Callcott 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. catarinae*, 7 – *P. curvatus* females, and 11 – males probably mostly *P. catarinae* 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. catarinae* 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. catarinae* 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. catarinae*, *P. hippeus*) 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; Callcott 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; Callcott 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 & Pesquero 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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