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HOST SPECIFICITY OF FOUR *PSEUDACTEON* SPP. (DIPTERA: PHORIDAE), PARASITOIDS OF FIRE ANTS IN ARGENTINA (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Several South American species of *Pseudacteon* have been released for biocontrol of red imported fire ants *Solenopsis invicta* in the U.S. Here we provide additional data from host specificity tests on 4 additional candidate species, *P. nocens*, *P. nudicornis*, *P. cultellatus*, and *P. obtusus*, all of which are components of multi-species complexes that occur within Argentinean *Solenopsis* populations. All 4 species were tested with sequential, no choice exposures to the red imported fire ant *S. invicta*, and the tropical fire ant, *S. geminata*. Levels of intrageneric specificity ranged from moderate to high and all 4 species showed greater specificity than some *Pseudacteon* species already approved for release.

Key Words: biological control, parasitism, *Solenopsis geminata*, *Solenopsis invicta*, *Pseudacteon*

RESUMEN

Varias especies de fóridos *Pseudacteon* provenientes de Sur América han sido introducidas en los Estados Unidos para utilizar como control biológico de la hormiga roja de fuego *Solenopsis invicta*. En esta publicación mostramos los resultados de pruebas de especificidad de hospedero realizadas en otros cuatro fóridos, *P. nocens*, *P. nudicornis*, *P. cultellatus* y *P. obtusus* que son parte del grupo de especies normalmente asociadas con poblaciones de *Solenopsis* en Argentina. Las pruebas de especificidad consistieron en la exposición secuencial de las moscas a las hormigas de fuego, *S. invicta* y *S. geminata*. El grado de especificidad de hospedero en las cuatro especies de fóridos varió entre moderado y alto pero fue siempre mayor que el presentado por algunas de las especies aprobadas para liberar como control biológico en los Estados Unidos.

Translation provided by the authors.

After their introduction in the early 20th century, the South American fire ants, *Solenopsis invicta* Buren and *S. richteri* Forel, quickly expanded their range in the United States (US). Today, *S. invicta* (red imported fire ant) occupies the southern states of US and Puerto Rico (Callcott & Collins 1996), is spreading south into Mexico (Sanchez et al. 2005), and is predicted to expand more than 100 km northward in the US due to anticipated climate changes (Morrison et al. 2005). The success of the imported fire ants as invasive species is probably due, in part, to the absence of the natural enemies that have coevolved with this species in South America (Porter et al. 1997). Some enemies left behind include phorid flies of the genus *Pseudacteon* (Diptera: Phoridae) that parasitize and eventually kill ant workers and may likely contribute to decrease population densities to the levels found in their homeland (Porter et al. 1997).

In the US, 3 *Pseudacteon* species, *P. tricuspis*, *P. curvatus*, and *P. litoralis*, have been released as part of the biological control program that seeks to reduce fire ant densities to levels with less ecological and economical impact (Porter & Gilbert 2004). In order to achieve this goal, more species and specific biotypes (geographic distinctive population) are necessary so phorid communities could resemble more closely those that exist in South America. Including more species could, for example, expose ants throughout the day and year, increase the range of workers size exposed, and affect ants in foraging trails as well as disturbed mounds (Pesquero et al. 1996; Orr et al. 1997; Morrison et al. 1997; Folgarait & Gilbert 1999; Folgarait et al. 2003, 2005b). It could offer the possibility of choosing species or biotypes locally adapted to ecological or climatic conditions similar to those where flies are going to be released (Folgarait et al. 2003; Calcaterra et al. 2005: Folgarait et al. 2005a).

Species and biotypes of *Pseudacteon* from Brazil and Argentina have been tested for their host specificity in laboratory conditions (Gilbert & Morrison 1997; Porter & Alonso 1999; Morrison &

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Gilbert 1999; Vazquez et al. 2004). Their rates of attack have been low when tested against the North America-native fire ant *S. geminata* with the exceptions of *P. curvatus* and *P. borgmeieri* that exhibit moderate and higher rates, respectively (Gilbert & Morrison 1997; Morrison & Gilbert 1999). However, in spite of those attacks, only *P. curvatus* and *P. obtusus* developed in *S. geminata* but with apparent low success (Porter & Gilbert 2004).

Here we evaluated 4 additional species or biotypes of *Pseudacteon* for their inclination to attack North America-native fire ants, *P. nudicornis* Borgmeier, *P. nocens* Borgmeier, *P. cultellatus* Borgmeier, and *P. obtusus* Borgmeier. We used individuals for a large biotype of *P. obtusus* from the Corrientes province in Argentina instead of the small biotype from Campinas (Brazil) tested before by Morrison & Gilbert (1999). Large females *P. obtusus* in Argentina have a mean body length of 1.395 mm (SD = 0.142 mm, n = 10, P.J.F. unpublished data), about 35% larger than the small Argentinean biotype and the Brazilian *P. obtusus* tested in Morrison & Gilbert (1999) (0.90 ± 0.082 mm, n = 10; 0.91 ± 0.087 mm, n = 4, respective body length of both small biotypes, P.J.F. and L.E.G. unpublished data). Male morphological differences between large and small biotypes have been reported before (Porter & Pesquero 2001). Moreover, recent phylogenetic analysis with 2 mitochondrial and 1 nuclear gene show that both biotypes are genetically distinct and probably constitute different species (Kronforst et al. 2006). Types used for the description of *P. obtusus* were collected in La Plata (Argentina) and have a body length of approximately 1.3 mm (Borgmeier 1925). According to this, flies from the Argentinean large biotype tested here should maintain the name *P. obtusus*.

Materials and Methods

Individuals of 4 species of *Pseudacteon* (Diptera: Phoridae) flies were brought in from Argentina to the quarantine facility at the University of Texas, Brackenridge Field Laboratories (BFL), between June 2003 and April 2004. Flies were either collected directly or were reared as progeny from females attacking *S. invicta* in Argentina. Fly rearing was done in the Centro de Estudios e Investigaciones at the Universidad Nacional de Quilmes (Buenos Aires, Argentina). *P. nudicornis* were collected from the Reserva Ecológica Costanera Sur in Buenos Aires Province (34.37'S and 58.22'W), *P. obtusus* around Mercedes in the Corrientes Province (27. 78'S and 58.05'W), and *P. nocens* and *P. cultellatus* near Brea Pozo in Santiago del Estero province (28.27'S and 63.95'W). Some *P. cultellatus* came in February 2006 from collections by S.D. Porter in Corrientes, Argentina.

Host specificity in these phorid species was tested with the red imported fire ant *S. invicta* and the tropical fire ant *S. geminata* Fabricius, denoted hereafter as exotic and native fire ants, respectively. Exotic and native are adjectives given with regard to the US ant biota. Polygyne colonies (multiple queens) for *S. invicta* were collected from Travis, Williamson, Wharton, La Salle, and Bexar counties in central Texas. Polygyne colonies of *S. geminata* were obtained in Travis, Lampasas, and Mill counties, also in Texas. Colonies were transported to BFL, set in a rearing room at 30°C and 12:12 (L:D) cycle, and fed with frozen crickets, sugar water, and water *ad libitum*.

Female flies were tested upon arrival at the quarantine facility within 3 or 4 days after field collection. When not used in tests, they were kept humid and chilled at ca. 10°C in the dark to keep them alive for longer time. Fly specificity tests were done in plastic flight boxes ($15.5 \times 9.5 \times 5$ cm) (henceforth denoted as arenas), containing either *S. geminata* or *S. invicta*. Arenas were lined inside with Fluon (Polytetrafluoroethylene) to prevent ants from escaping and the top was covered with clear glass. The bottoms of the plastic boxes were covered with a layer of plaster 1 to 2 cm deep. The plaster was moistened every day before the beginning of tests. A small hole covered by a rubber sheet in one of the sides of the arena (about 4 cm high) allowed the introduction of flies. Plastic boxes were used only for 1 ant host species to avoid the occurrence of confounding odor from the other host.

Oviposition rates were recorded in arenas that held 1 to 5 g of unsieved ants of either species (≈500-5000 ants). Approximately half of the workers used had head widths of 0.8 mm or less (0.49 ± 0.06 mm), which is one of the characteristic traits of polygyne fire ant colonies (Morrison & Gilbert 1998). Ants were fed daily with a mealworm and had continuous supply of sugar water and water. One 10 cm diameter × 1.5-cm deep plastic jar lid with a lateral hole was put inside each arena to give the ants a place to hide. This lid was moved manually before introducing the flies and after 10 min within each test to keep ants moving constantly around the arena, facilitating fly attack behavior. Arenas were placed under fluorescent lamps and their internal temperature fluctuated between 26 and 29°C.

Pseudacteon attack behavior and development has been studied elsewhere (Morrison et al. 1997; Porter 1998a). Here, two behaviors were observed as indicators of female attack motivation. 'Approaches' were recorded when flies hovered over the dorsum of an ant and followed it for at least a few seconds. An 'Attack' was designated when females attempted to oviposit in the ant's thorax, which usually immobilizes the ant for a short time.

Two types of sequential no choice tests were designed in order to test for *Pseudacteon* host specificity and to measure rates of attack on *S. invicta*

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and *S. geminata*. *Pseudacteon obtusus*, *P. nocens*, and *P. cultellatus* were tested with the first type of tests. Here a single female was introduced into a *S. invicta* arena for a maximum of 15 min to test her motivation to oviposit. Behavior in these arenas varied among individual flies. Only individuals that actively approached and attacked at least 1 ant during those 15 min were considered to be motivated to oviposit and therefore transferred to subsequent arenas. The rate of attacks in this and other arenas was measured as the number of attacks from each female per min during a 5-min period, counted from the first attack. After that, the fly was immediately transferred to an arena containing *S. geminata* for additional 15 min. There, the number of approaches and the attack rate were recorded. In most cases, the fly survived and it was transferred immediately to a second *S. invicta* arena to determine whether it was still motivated to attack. In this third arena flies were observed for maximum15 min or, if they attacked, for 5 min counted from the first attack. The main purpose of this was to test for both handling effects and energy limitations when the flies were transferred between arenas and after some time of active attacks. Individual females were tested only once.

Pseudacteon nocens and *P. nudicornis* were tested in a second type of experimental test. In the former type of tests, some attacks were observed on *S. geminata* ants. The aim of the second type of experiments was to test whether the attacks on the native fire ant were either due to mistakes (after the flies were motivated to attack *S. invicta*) or due to low host specificity. Here 1 female fly was first introduced for 15 min into a *S. geminata* arena and the rate of approaches and attacks recorded. The fly was then transferred to a *S. invicta* arena to test for her motivation to oviposit. If the fly did not attack after 15 min the test was aborted and the female was kept in a cool and dark environment for later testing. If the fly attacked, the rate of attack was measured for 5 min and then it was transferred to the next arena. The final arena for this type of experiments had either *S. geminata* or *S. invicta*. Half of the flies that attacked *S. invicta* were transferred to a different *S. invicta* arena to test for the handling effect and limitations of energy. The other half were transferred to *S. geminata* arenas in order to compare the rate of approach and attacks on this species of ants before and after motivation with *S. invicta*.

The time elapsed between the introduction of the fly in a *S. invicta* arena and the time of the first attack (orientation time) was measured and then compared between the first and second exposure to the exotic fire ant with the non-parametric Mann-Whitney test (STATISTICA for Windows 1999). The mean attack rates, measured as the mean number of attacks per female per min, were compared by the non-parametric Wilcoxon matched pair test (STATISTICA for Windows 1999).

To reduce any effect of minor differences in light or temperature conditions, individual flies started the sequential tests in different *S. invicta* or *S. geminata* arenas. Phorid flies where transferred between arenas with an aspirator similar to the one described in Gilbert & Morrison (1997). They were gently aspirated from one arena to a tube whose bottom was replaced by mesh, and then released immediately to the next arena. Weak and disoriented flies usually walked among the ants after release and were easily killed. To decrease this handling effect, we let the fly go out of the tube at their own pace, which often happens in less than 1 min.

To determine whether flies developed inside the ants attacked, both species of ants were monitored for more than 90 d after their exposure to phorids or until all ants died. Forty days is about the median development period for all the species of phorids tested (Folgarait et al. 2002a). Dead ants were examined for pupae 3 times a week and daily for fly eclosion. Ants were kept in a quarantine room at 27°C and 12L:12D cycle.

RESULTS

Females of the *Pseudacteon* species *P. obtusus*, *P. cultellatus*, and *P. nudicornis* showed high degrees of host specificity for the red imported fire ant *S. invicta*. The former two species were tested with the sequential no choice tests: *invicta-geminata-invicta*, whereas *P. nudicornis* females were exposed to *S. geminata* before being transferred to the motivation arenas with the exotic fire ant. When exposed to *S. invicta* in the motivation arenas, *Pseudacteon* females of these 3 species attacked ants with a rate of about 3 attacks per min (Tables 1 and 2). The same flies were then transferred to *S. geminata* arenas. There, 13% of *P. obtusus*, 46% *P. cultellatus*, and all *P. nudicornis* females were still highly motivated and followed ants displaying the pursuing behavior called 'Approach'. In most cases, flies approached ants after being moved from the *S. invicta* arenas for the first few min, but displayed no activity, afterwards for the rest of the observation period. In spite of those approaches only 1 female of *P. obtusus*, 4 *P. cultellatus* (Table 1) and none of *P. nudicornis* (Table 2) attacked *S. geminata* ants. Fig. 1 shows the percentage of the females exposed to *S. geminata* that attacked those ants, and compares the percentages with those obtained for *P. curvatus*, *P. tricuspis*, and *P. litoralis*, species of phorid flies currently used for biocontrol of exotic fire ants in the US.

Females of the 2 *Pseudacteon* species that attacked *S. geminata*, *P. obtusus* and *P. cultellatus*, however did not attempt to oviposit more than twice, about 10% of the number of attacks per female on *S. invicta*. There were not enough females attacking both *S. geminata* and *S. invicta*

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TABLE 1. APPROACH AND ATTACK RATES OF *PSEUDACTEON NOCENS*, *P. OBTUSUS*, AND *P. CULTELLATUS* IN THE FIRST TYPE OF SEQUENTIAL HOST SPECIFICITY TESTS.

	P. nocens	*P. obtusus*	*P. cultellatus*
Initial exposure to *S. invicta* (motivation)			
No. of individuals attacking	61	8	24
Attack rate	3.27 ± 3.20 (44)	3.47 ± 2.3	4 ± 2.51 (20)
Exposure to *S. geminata*			
No. individuals approaching/No. exposed	48/61	1/8	11/24
Approach rate	8.85 ± 12.88	0.2	1.36 ± 1.58
No. of individuals attacking/No. exposed	22/61	1/8	4/24
Attack rate	0.62 ± 0.44	0.4	0.2 ± 0
Final exposure to *S. invicta*			
No. individuals attacking	36/54	4/6	14/21
Attack rate	3.55 ± 3.07	1.76 ± 2.14	3.85 ± 2.18

Attack and approach rates are given as the mean ± SD of rates calculated from independent females. Rates are the number of attacks or approaches per min after 5 or 15-min exposure respectively. In 1 experimental period flies were transferred to *S. geminata* arenas immediately after they attacked *S. invicta* twice. In those cases rates of attack in the motivation arena were not calculated. Therefore when the number of flies used for mean calculations were different than total number of individuals attacking, this number is written in brackets.

to do statistical tests on this subset of flies. Nevertheless, when all females were considered, numbers of attacks per female per min to the native fire ants were always lower than those to *S. invicta* (Table 1).

After being exposed to the native fire ants, females were subsequently transferred to *S. invicta* arenas to test for handling effects and energy limitations. Flies attacking in this last step of the test demonstrated that lack of attraction to *S. geminata* was not due to either the disturbance of being relocated or due to lack of energy. More than 60% of the *P. obtusus*, *P. cultellatus*, and *P. nudicornis* females attacked in the second *S. invicta* arenas (Tables 1 and 2), but they did it in a lower rate than when first exposed to these ants ($Z = 1.98$, $P = 0.05$, Wilcoxon matched pair test, $n = 15$, data from the 3 species combined). *Pseudacteon nudicornis* first and second exposure to *S. geminata* showed that more flies hovered over ants after being motivated to attack in *S. invicta* than they did otherwise. In addition, rates of

TABLE 2. APPROACH AND ATTACK RATES OF *PSEUDACTEON NOCENS* AND *P. NUDICORNIS* IN THE SECOND TYPE OF SEQUENTIAL HOST SPECIFICITY TESTS.

	P. nocens	*P. nudicornis*
Initial exposure to *S. geminata*		
No. individuals approaching/No. exposed	2/10	1/6
Approach rate	2.17 ± 1.46	0.6
No. of individuals attacking/	0/10	0/6
Attack rate	0	0
Initial exposure to *S. invicta*		
No. of individuals attacking	10/10	6/6
Attack rate	4.92 ± 2.74	3.16 ± 1.48
Subsequent exposure to *S. geminata*		
No. individuals approaching/No. exposed	4/4	3/3
Approach rate	5.05 ± 6.17	3.91 ± 2.3
No. of individuals attacking/No. exposed	1/4	0
Attack rate	0.2	0
Subsequent exposure to *S. invicta*		
No. individuals attacking	4/4	1/1
Attack rate	2.7 ± 1.83	2.8

Attack and approach rates are given as the mean ± SD of the rates calculated from independent females. Rates are the number of attacks or approaches per min after 5 or 15-min exposure, respectively.

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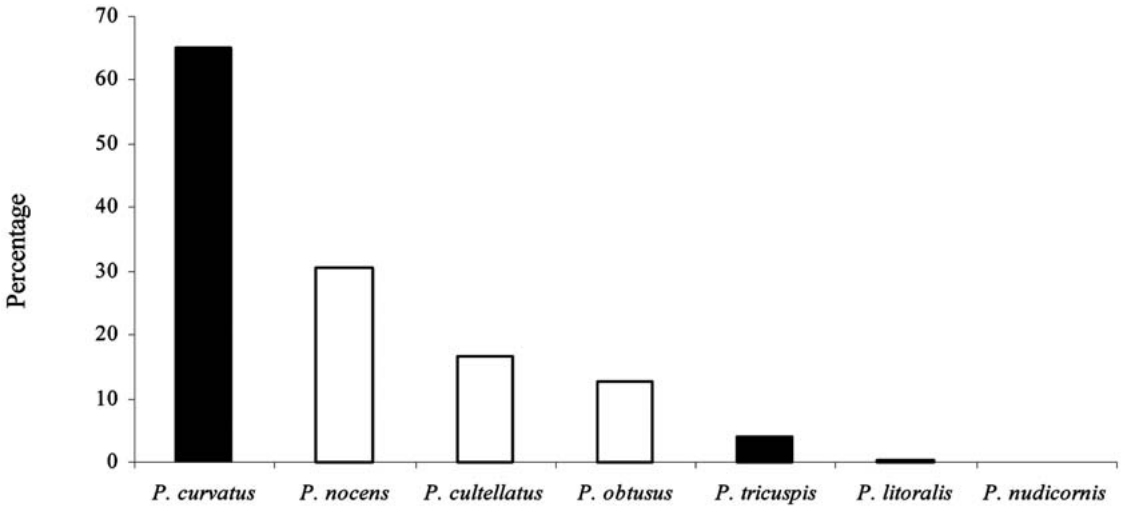


Fig. 1. Percentage of *Pseudacteon* females that attacked *S. geminata* ants in host specificity tests. *Pseudacteon nocens* percentage was calculated using both types of sequential tests. Value for *P. nudicornis* is zero. Percentages for *P. curvatus*, *P. tricuspis* and *P. litoralis* (bars in black), species currently released in the US for biocontrol of exotic fire ants, are data from Gilbert and Morrison (1997).

approach in both arenas were different, with 1 female approaching the native fire ants 10 times in the first exposure and 3 females approaching 94, 25, and 57 times in the second trial (Table 2).

Pseudacteon nocens was tested using both the sequential test: *invicta-geminata-invicta* and the tests where flies were exposed to *S. geminata* first (Tables 1 and 2). *Pseudacteon nocens* host specificity for *S. invicta* was lower than that seen in the other *Pseudacteon* species described above. From the 61 females exposed to *S. geminata* in the first type of tests, 78% approached and 36% attacked these ants at least once (Table 1).

The behavior of *P. nocens* females was very similar in the second type of tests. Those showed that, once motivated in *S. invicta* arenas, *P. nocens* females readily approached and some proportion of them attacked the native fire ants (Table 2). In contrast, females exposed first to *S. geminata* exhibited little motivation to approach and none of the 10 females exposed attacked (Table 2). The majority of females first introduced into *S. geminata* arenas flew to the top of the arena or stood most of the time on the box wall ignoring the presence of ants. The same females, when moved to motivational *S. invicta* arenas, were attracted to the ants as soon as 26 s after introduction (2.19 minutes ± 2.54, mean ± SD orientation time). Flies that were then moved to another *S. invicta* arena started to attack as soon as 7 s after being transferred (1.74 ± 2.09 mean ± SD orientation time). In contrast with the first exposure to the native fire ants, those flies transferred from motivation *S. invicta* to *S. geminata* arenas started approaching ants very quickly, and 1 was observed attacking (Table 2). Rates of approach in both *S. geminata* arenas were different, with a lower rate in the first exposure (although statistic tests were not done because of the small sample size). Females hovered on top of ants 17 and 48 times in the first and 34, 107, 7, and 50 times in the second exposure to *S. geminata*.

Considering both types of tests, out of the 71 *P. nocens* females exposed to *S. geminata*, 31% attempted to oviposit in those ants (Fig. 1). Nevertheless, the mean number of attacks per female per min to the native fire ants was very low and comparable to numbers found for the other *Pseudacteon* species tested here (Table 1). Females of *P. nocens* that attacked both species of ants did so in a significantly reduced rate in *S. geminata* than in the exotic fire ant ($Z = 3.15$, $P = 0.002$, $n = 17$, Wilcoxon matched pair test). In contrast, for this phorid species, neither, the attack rates or the orientation times differed in their first and second exposure to *S. invicta* ($Z = 1.62$, $P = 0.11$, $n = 30$ and $Z = 0.02$, $P = 0.98$, $n = 19$, Wilcoxon matched pair test for rates of attack and orientation time respectively).

In the choice tests only about 40% (17-66%) of females initially tested attacked at least 1 *S. invicta* ant in the motivation arena and were transferred to subsequent arenas. We did not use these percentages to compare to those in subsequent exposures to *S. invicta* because several causes besides lack of motivation contributed to the low percentage of females useful for the specificity tests. In most cases, for example, after released, flies were killed by ants in the arena or died prematurely due perhaps to causes related to their transport from the field to BFL.

From the 69 oviposition attempts observed in *S. geminata* by *P. nocens*, only 3 pupae developed.

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One pupa was found in the native fire ant attacked by *P. cultellatus*. No adult flies emerged from these pupae. No pupae were observed in *S. geminata* colonies attacked by *P. obtusus*. Nevertheless, because of the limited sample size, the possibility of larval development in *S. geminata* by the phorid species studied here cannot be eliminated. In addition, attempts to rear those phorid species in *S. invicta* were largely unsuccessful at the time when the tests were done. For example, only 1.5 pupae per female were produced from 349 *P. nocens* that attacked *S. invicta* repeatedly for few hours (during a 5-month period), and only 58% of those pupae developed successful to adults.

Discussion

The species of *Pseudacteon* tested here showed high and moderate degrees of host specificity. Low percentages of *P. obtusus*, *P. cultellatus*, and none of the *P. nudicornis* females attacked *S. geminata* even though they were already motivated to attack *S. invicta*. Those females that attacked *S. geminata*, however, did so very infrequently (no more than 2 attacks in 15 min). In contrast, 31% of *P. nocens* females attempt to oviposit in *S. geminata* after being exposed to exotic fire ants. These attacks, though, could be attributed to mistakes because flies only did so after exposure to their usual host, *S. invicta*, while none of the females attempted to oviposit on *S. geminata* if exposed to this ant first. Even though one third of the tested *P. nocens* females attacked the native fire ants, they did so at about 1/6 the frequency they did in *S. invicta* arenas. This lower rate of attack can not be attributed only to handling effects and energy limitations because the frequencies of attacks in *S. invicta* arenas before and after exposure to *S. geminata* were not different.

Despite of the small sample size of females of *P. obtusus*, *P. cultellatus*, and *P. nudicornis* used, our results of host specificity are similar to those for other species of *Pseudacteon* reported elsewhere (Gilbert & Morrison 1997; Porter & Alonso 1999; Morrison & Gilbert 1999; Folgarait et al. 2002b; Vazquez et al. 2004). They add to the body of evidence that indicates that *Pseudacteon* species from South America are highly specific in the *S. saevissima* group of fire ants. It is also becoming apparent that some of these flies may mistakenly approach and even try to oviposit on other hosts. However, it seems that *Pseudacteon* offer a low risk to native species in the *S. geminata* group if introduced as biocontrol agents of red and black imported fire ants (Porter & Pesquero 2001; Porter & Gilbert 2004).

The species studied here have many characteristics that made them worth considering for further evaluation for classical biocontrol. They showed high to moderated degrees of host specificity to *S. invicta*, in all cases greater than those exhibited by species already released in the US. They attacked and developed successfully in both exotic fire ant species *S. invicta* and *S richteri* (Folgarait et al. 2002a, 2002b, 2005b, 2006) but with the exception of *P. obtusus* (Porter & Gilbert 2004) none of them have been reported to develop in native fire ants. In addition, development times and temperature conditions have been thoroughly studied for laboratory rearing and they show high rates of success (Folgarait et al. 2002a, 2002b, 2005b, 2006).

Finally, these 4 species of *Pseudacteon* could enhance in many ways the effect of the species already released as biocontrol agents of exotic fire ants. *Pseudacteon obtusus* and *P. nudicornis*, for example, are known to be attracted to workers in foraging trails in addition to disturbed mounds (Orr et al. 1997; Folgarait et al. 2005b). In contrast, *P. tricuspis*, *P. litoralis*, *P. curvatus* (species already introduced to U.S.), *P. cultellatus*, and *P. nocens* are found more frequently in disturbed mounds, mating flights or when ants engage in fights, likely cued by alarm pheromones (Orr et al. 1997; Folgarait et al. 2002a; Morrison & King 2004; Folgarait et al. 2006).

Fly size varies considerably among *Pseudacteon* species and is positively related with the range of worker sizes they parasitize (Morrison et al. 1997). *Pseudacteon cultellatus*, *P. nudicornis*, and some *P. nocens* are small flies that attack smaller than average sized workers (Folgarait et al. 2002a, 2006). This could be especially favorable in regions were polygyne fire ant colonies with low worker mean sizes predominate. These *Pseudacteon* species could complement parasitism by the already introduced small species *P. curvatus*, or replace it in regions not favorable for this species. *Pseudacteon cultellatus*, *P. nudicornis*, and *P. curvatus* frequently coexist in phorid communities in Argentina (Folgarait et al. 2005a). *Pseudacteon obtusus* (large biotype) and some *P. nocens*, on the other hand, are big flies that coexist with *P. tricuspis* in Argentina and probably will complement their effect over imported fire ants in the U.S.

Therefore, the species of *Pseudacteon* studied here are good candidates for further evaluation for introduction to the U.S. for fire ant biocontrol. They (1) show high and moderate specificity for *S. saevissima* group species of fire ants, (2) are widely distributed in their homeland, and probably locally adapted to several climatic conditions that can be matched in the southern US, (3) can be reared in the laboratory, and (4) exhibit different sizes and host searching strategies, therefore complementing the species of phorids already released as biocontrol.

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