PARASITOID-HOST MATCHING BETWEEN THE LITTLE DECAPITATING FLY *PSEUDACTEON CURVATUS* FROM LAS FLORES, ARGENTINA AND THE BLACK FIRE ANT *SOLENOPSIS RICHTERI*

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Abstract

Matching biotypes of potential biocontrol agents to target host populations can greatly improve the effectiveness of control. This study was designed to determine if the fly *Pseudacteon curvatus* Borgmeier from Las Flores, Buenos Aires Province, Argentina prefers its natural host, the black fire ant, *Solenopsis richteri* Forel. We found that *P. curvatus* strongly preferred *S. richteri* from Argentina, *S. richteri* from the United States, and hybrid (*S. richteri* × *S. invicta*) fire ants from the United States when each was tested against *S. invicta* from the United States. The time to pupation of developing parasitoids was 10% and 21% longer in hybrid and red fire ants than in black fire ants. Parasitism rates, however, were not significantly different among these ant hosts in no-choice parasitism tests.

Key Words: Diptera, Phoridae, Hymenoptera, Formicidae, *Solenopsis invicta*, host preference, hybrid fire ants, biocontrol, biotype

Resumen

Hacer corresponder a biotipos de agentes potenciales de control biológico con poblaciones huéspedes puede, en gran medida, mejorar la eficiencia en el control. Este estudio fue diseñado para determinar si la mosca *Pseudacteon curvatus* Borgmeier de Las Flores, Provincia de Buenos Aires, Argentina, prefiere a su huésped natural, la "hormiga brava" negra, *Solenopsis richteri* Forel. Encontramos que *P. curvatus* prefirió marcadamente a *S. richteri* de Argentina, a *S. richteri* de los EE.UU. y a la forma híbrida (*S. richteri* × *S. invicta*) de los EE.UU. cuando fue comparada con *S. invicta* de los EE.UU. El período hasta pupación de los parasitoides en desarrollo fue 10% y 21% más largo en la forma híbrida y en la "hormiga brava" roja que en la "hormiga brava" negra. Las proporciones de parasitismo, sin embargo, no fueron significativamente diferentes entre los huéspedes en pruebas de parasitismo de no-elección.

Two exotic fire ants have become established in the United States, the black imported fire ant, *Solenopsis richteri* Forel, and the red imported fire ant, *Solenopsis invicta* Buren (Trager 1991). The black imported fire ant is found in northern Mississippi and Alabama where it occupies about 30,000 km² (Shoemaker et al. 1994). The red imported fire ant is found in 11 southeastern states from North Carolina through Texas and occupies about 1,100,000 km² (Callcott & Collins 1996). Between these two species is a broad band of hybridization from the Mississippi River to Atlanta, GA (Shoemaker et al. 1994). Hybrid fire ants occupy about 130,000 km². De-

spite this broad zone of hybridization, red and black imported fire ants are still considered separate species because they apparently do not hybridize in South America (Ross & Trager 1990).

Pseudacteon curvatus Borgmeier is a small decapitating fly from South America that parasitizes *Solenopsis* fire ant workers (Porter 1998). This species was released in several states in the spring of 2000 as a potential biocontrol agent for red and black imported fire ants in the United States (unpublished data). In South America, *P. curvatus* occurs over a very wide range from São Paulo, Brazil westward into Mato Grosso do Sul, Brazil and southward to Buenos Aires Province, Argentina (Borgmeier 1925; SDP-unpublished data). Over this range, *P. curvatus* is known to parasitize at least three species of South American fire ants: *Solenopsis saevissima* (F. Smith), *S. invicta*, and *S. richteri* (SDP-unpublished data).

Host-specificity tests in the United States (Porter 2000) demonstrated that *P. curvatus* flies from Argentina strongly prefer red imported fire ants over the native fire ants *Solenopsis geminata* (Fab.) and *Solenopsis xyloni* (McCook). This preference is not surprising because *P. curvatus* is not a natural parasite of either *S. geminata* or *S. xyloni*. Forced laboratory rearing tests showed that *S. geminata* and *S. xyloni* are both very poor hosts for *P. curvatus* (Porter 2000).

Matching parasitoid biotypes to target host populations can greatly improve the success of biocontrol programs (Van Driesche and Bellows 1996, p. 149). *P. curvatus* flies from Buenos Aires Province, Argentina normally parasitize the black fire ant *S. richteri*. The objective of this study was to determine if *P. curvatus* flies from Buenos Aires Province are better adapted to *S. richteri* (their normal host) than the red fire ant, *S. invicta* (a host in other parts of South America). Results of this study will help us decide where *P. curvatus* should be released in the United States.

<center>MATERIALS AND METHODS</center>

P. curvatus flies used in this study were originally collected from El Toro Ranch southeast of Las Flores, Buenos Aires Province, Argentina in March 1997 (Porter 2000). A few flies from the same location were added to the lab colony several times up to December 1998.

To examine *P. curvatus* preferences for *S. richteri*, *S. invicta,* and hybrid fire ants, 3-hour old and 1-day old flies were introduced into white plastic trays ($42 \times 28 \times 15$ cm) with screened vents and tight-fitting glass lids (described in detail by Porter 2000). We used both 3-hour and 1-day old flies to produce an age mixture similar to what might occur in the field. In the bottom of each tray, were two parallel chambers ($7 \times 30 \times 5$ cm, $l \times w \times h$) for two kinds of ants. Ants were contained in the two bottom chambers by coating the sides with Fluon® CICI, Wilmington, DE).

A small opaque inverted cup (4 cm diameter) was placed on the bottom of each of the two small parallel chambers. These cups were moved back and forth from one end of a chamber to the other with a long aspirator arm (Porter and Alonso 1999) each time most of the ants had crawled under a cup to hide. This procedure kept the ants in both sides trailing continuously from one end of a bottom chamber to the other so the flies always had an opportunity to attack workers of either type of ant.

We used 7 colonies of *S. richteri* from Las Flores, Buenos Aires Province, Argentina, 9 colonies of *S. richteri* from northeastern Mississippi (Tupelo - 4 colonies, Booneville - 3, Corinth - 1, Mayhew - 1), and 7 colonies of hybrid fire ants from around Starkville, MS (USDA Lab - 4, Mayhew - 3). The identities of *S. richteri* and hybrid fire ants from Mississippi were confirmed by gas chromatography (Vander Meer et al. 1985). For each trial *S. richteri* and hybrid fire ants were paired with similar-sized *S. invicta* workers from Gainesville, FL. Different colonies of each kind of ant were used

for each trial to assure that results were not due to differences in the attractiveness of individual colonies. Tests with *S. richteri* fire ants from Argentina were conducted in January 1999. Tests with hybrid fire ants and *S. richteri* from the United States were conducted in June 1999. Tests for all three kinds of ants were run 1-3 weeks after colonies were collected in the field. Voucher specimens have been deposited in the Florida Museum of Arthropods, Gainesville, Florida, USA.

Each test run lasted about 3 h and used 14-18 female flies with an equivalent number of males. Test ants contained 0.25 g of workers (~400) and 0.5 g of brood. The trays were inspected every 10 min and the number of female flies hovering in attack mode over each species of ant was recorded by visual count. Females considered in attack mode hovered 3-10 mm above the ants and oriented to their movements. Males of this species are not attracted to the ants.

To determine if *P. curvatus* flies were equally successful in parasitizing black, hybrid, and red fire ants, we conducted a series of no-choice parasitism tests. The trays used in these tests contained a single solid bottom covered with moistened plaster as described by Porter (2000). Timer motors were used to automatically raise an inverted cup in one end of each tray while lowering a cup at the other end of each tray. This caused the test ants to continuously trail back and forth between the two cups. Timer motors were set to run for 8 h a day (10:00 to 18:00 h).

We conducted 6 trials each with: *S. richteri* from northeastern Mississippi (Corinth - 1 colony, Booneville -2, Tupelo -3), hybrid fire ants from Starkville, MS (3 colonies) and Mayhew, MS (3 colonies), and *S. invicta* from Gainesville, FL (6 colonies). All colonies were collected in June 1999 and used 1-2 weeks after collection. Tests contained 0.5 g of workers (~800) and 1.0 g of brood. Different colonies were used for each test replicate. We used mostly the same colonies for the no-choice parasitism tests as we did for the paired preference tests.

Fifteen to sixteen female flies and an equivalent number of males were added to all no-choice trials on day 1. Tests lasted 2 days. *P. curvatus* adults usually only live a day or two in the attack trays; consequently, most of the flies were dead by the end of the trials. Inactive flies usually live several days longer in the lab. Longevity in the field is unknown, but it is likely to be intermediate between inactive flies and flies in the attack trays. At the end of each trial, worker ants were transferred into small boxes (20 × 2 × 5 cm) with tight-fitting vented lids. Ants were fed fresh sugar water every 3-4 days. We inspected the head capsules of dead workers for fly pupae every 1-2 days for a period of 30 days.

<div align="center">RESULTS</div>

When given a choice in paired tests, about 70% of the *P. curvatus* females preferred to attack black fire ants or hybrid fire ants over red fire ants (Fig. 1). We found highly significant differences in the number of attacking flies for each of the following pairs using paired t-tests: *S. invicta* versus *S. richteri* from Argentina (t = 4.95, d.f. = 6, P = 0.0026), *S. invicta* versus S*. richteri* from Mississippi (t = 3.48, d.f. = 8, P = 0.0083) and *S. invicta* versus hybrid fire ants from Mississippi (t = 7.11, d.f. = 6, P = 0.0004). However, no significant differences were found between the number of flies preferring *S. richteri* from Argentina, *S. richteri* from Mississippi, or hybrid workers (*S. richteri* x *S. invicta*) from Mississippi (all tested against *S. invicta* from Florida; ANOVA, F = 0.81, d.f. = 2,20, P = 0.46).

Once a fly began attacking workers in the choice tests, the average number of oviposition strikes per 15 seconds was respectively 0.67 ± 0.05, 0.87 ± 0.13, 1.15 ± 0.14, and 1.57 ± 0.26 for *S. richteri* workers from Argentina, *S. invicta* from Florida, *S.richteri* from the United States, and hybrid fire ants. The attack rate for hybrid fire ants
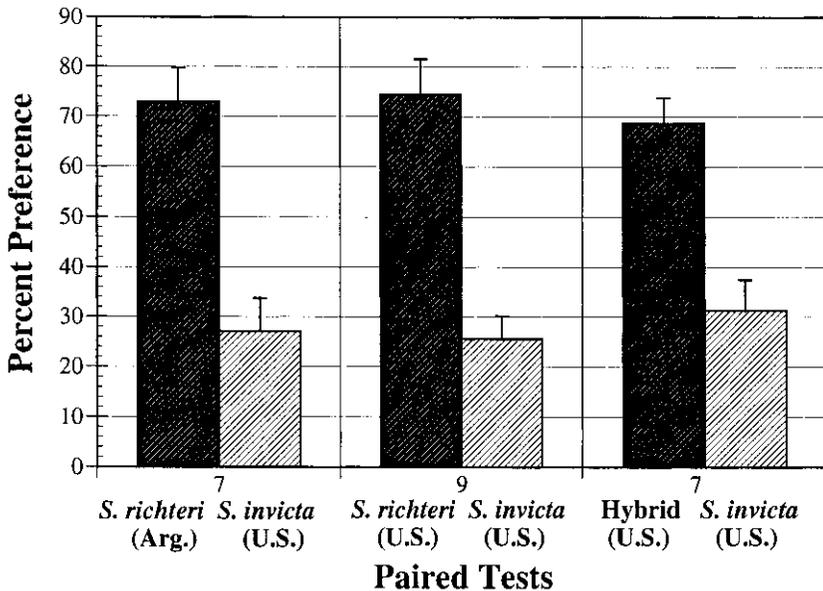
Fig. 1. The percent of female *Pseudacteon curvatus* decapitating flies from Las Flores Argentina preferring to attack *S. richteri* from Argentina, *S. richteri* from Mississippi, or hybrid (*S. richteri* × *S. invicta*) fire ants from Mississippi, each in paired tests with *S. invicta* from Gainesville, Florida. The number of trials is indicated below each pair of bars. Error bars indicate standard errors of the mean.

was significantly higher (Fisher's PLSD, $P < 0.004$; 1-way ANOVA) than rates for either *S. invicta* or *S. richteri* from Argentina. Other pairwise comparisons were not statistically significant. The biological basis and importance of this pattern is not clear.

While *P. curvatus* strongly preferred black and hybrid fire ants when given a choice, significant differences were not found in the number of pupae produced in the no-choice parasitism tests (Table 1, ANOVA, $F = 0.42$, df = 2,15, $P = 0.66$). However, the mean time to pupation varied significantly among hosts (Table 1, ANOVA, $F = 12.6$, df = 2,13, $P = 0.0009$, data were log transformed to equalize variance, two colonies were deleted [*S. invicta* - 1, *S. richteri* - 1] because they each produced less than 40 pupae). The development time to pupation was 21% longer in *S. invicta* than in *S. richteri* and 10% longer in hybrid fire ants than *S. richteri* (Table 1). The mean variability of pupation time (as measured by SD) was also significantly larger for flies developing in *S. invicta* and hybrid fire ants than in *S. richteri* (Table 1; ANOVA, $F = 5.7$, df = 2,13, $P = 0.017$).

DISCUSSION

P. curvatus from Las Flores, Argentina appears to have evolved a specialized relationship with *S. richteri*, its natural host. Specifically, these flies demonstrated a strong preference for *S. richteri* and hybrid fire ants over *S. invicta* (Fig. 1). The fact that hybrid workers were apparently as attractive as *S. richteri* workers suggests that

TABLE 1. PARASITISM RATES OF THE FLY *PSEUDACTEON CURVATUS* ATTACKING DIFFER-
ENT KINDS OF FIRE ANTS AND MEAN DEVELOPMENTAL TIMES OF FLY PUPAE IN
THOSE SPECIES.

	Fire Ant Species (U.S.)[1]		
	S. richteri	Hybrid	*S. invicta*
Pupae Produced/Female Fly (number ± SE)	8.3 ± 1.7 a	9.4 ± 1.0 a	7.6 ± 1.5 a
Mean Development Time (egg to pupae, days ± SE)	12.9 ± 0.2 a	14.2 ± 0.3 b	15.6 ± 0.5 c
Mean Standard Deviation in Devel. Time (days ± SE)	2.2 ± 0.3 a	3.5 ± 0.5 b	4.2 ± 0.4 b

[1]Means within a row with different letters were significantly different (Fisher's PLSD, $P \leq 0.05$).

the source of this attraction is a qualitative trait that is not diminished in the hybrid. However, a one-on-one comparison would be necessary to determine whether the flies prefer black and hybrid fire ants equally. Preferences for specific hosts are likely based on chemical cues (Porter 1998a, b). Which cues these might be are unknown, but black, red, and hybrid fire ants exhibit distinctive differences in their cuticular hydrocarbons, venom alkaloids, and pheromones (Vander Meer et al. 1985, Obin & Vander Meer 1989, Vander Meer & Lofgren 1989). It is notable that a strong preference for *S. richteri* was maintained*,* even after flies had been cultured for 1-2 years (about 8-16 generations) in the lab using exclusively *S. invicta* workers as hosts. Retention of a strong preference for *S. richteri* over this period demonstrates that this preference was not quickly obscured either by behavioral experience or genetic adaptation.

S. richteri populations in the United States are much more likely to have originated from Argentine or Uruguayan port areas rather than landlocked Las Flores (170 km south of Buenos Aires). The fact that the percent preference for *S. richteri* workers from Las Flores, Argentina and the preference for *S. richteri* workers from northeastern Mississippi were quite similar suggests that host preferences are primarily species-level rather than population-level differences. Head-to-head comparisons of fire ant workers from a variety of locations would, of course, be necessary to fully evaluate the extent and nature of parasitoid preferences for different host ant populations.

We found that fly developmental rates increased significantly from *S. richteri* to hybrids to *S. invicta* (Table 1). This relationship is what would be expected by non-dominant genetic hybridization.

In contrast with preferences and developmental rates, rates of parasitism in no-choice laboratory tests were not clearly associated with the type of ant tested (Table 1); perhaps additional replicates would eventually show a modest effect, but this is not certain. Previous tests showed that *P. curvatus* females do not do well at parasitizing the two most common native fire ants in the United States (Porter 2000).

The practical implications of this study are that *P. curvatus* flies from Las Flores, Argentina may do best if they are released onto imported black or hybrid fire ant populations in Alabama, Georgia, Mississippi, and Tennessee. Similarly, *P. curvatus* biotypes collected from regions where they normally parasitize the red fire ant *S. invicta* may be more effective in regions of the United States where this species predominates. Matching specific *P. curvatus* biotypes to their normal host would be especially

important if the host preferences that we observed in the lab are associated with the fly's ability to locate potential hosts at distances of several meters or more when visual abilities are likely to be ineffective. The actual importance of matching *P. curvatus* biotypes to their normal host populations will be evaluated during field releases of this parasitoid that are currently in progress in Florida, Alabama, and Tennessee.

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