

## Host Specificity of a Biotype of the Fire Ant Decapitating Fly *Pseudacteon curvatus* (Diptera: Phoridae) from Northern Argentina

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**ABSTRACT** We tested the host specificity of *Pseudacteon curvatus* Borgmeier from Formosa, Argentina, on North American colonies of the red imported fire ant, *Solenopsis invicta* Buren, and the native fire ants *Solenopsis geminata* (F.) and *Solenopsis xyloni* McCook. No-choice tests showed that *P. curvatus* hovered over and attacked all three species of fire ants. The number of pupae successfully completing development to adult flies in the trials was  $5.03 \pm 1.55$  (mean  $\pm$  SE) per female fly in *S. invicta*,  $0.66 \pm 0.24$  per female fly in *S. xyloni*, and 0 per female fly in *S. geminata*. Paired preference tests showed that *P. curvatus* preferred to hover over *S. invicta* instead of *S. xyloni*  $77 \pm 3\%$  (mean  $\pm$  SE) of the time and preferred *S. invicta* over *S. geminata*  $87 \pm 4\%$  of the time. The oviposition attempts of active female *P. curvatus* were 2.8 times higher on *S. invicta* than on *S. xyloni* and 16 times higher on *S. invicta* than on *S. geminata*. These results demonstrate that this new biotype of *P. curvatus* is more host specific to North American red imported fire ants than a previous biotype collected from black imported fire ants.

**KEY WORDS** biocontrol, host range, *Solenopsis invicta*, *Solenopsis xyloni*, *Solenopsis geminata*

BEFORE AN EXOTIC BIOLOGICAL control agent is released in the field, the degree of host specificity must be assessed to determine whether that agent will likely parasitize nontarget species (Marohasy 1998, van Klinken 2000, van Klinken and Heard 2000, Browne and Withers 2002). Phorid flies of the genus *Pseudacteon* Coquillett are being studied as potential biological control agents because many are common parasitoids of imported fire ants. *Pseudacteon* species that parasitize fire ants seem to be specific to fire ants (Borgmeier and Prado 1975, Disney 1994). Field tests in South America have demonstrated that *Pseudacteon* flies are not attracted to ants in other genera (Porter et al. 1995). Furthermore, most *Pseudacteon* flies in Brazil and Argentina seem to be specific to fire ants in the *saevissima* complex of the genus *Solenopsis* (Gilbert and Morrison 1997, Morrison and Gilbert 1999, Porter and Alonso 1999). However, a few flies in some of these species will parasitize fire ants in the *geminata* complex (Porter et al. 1995, Gilbert and Morrison 1997, Porter 2000).

*Pseudacteon curvatus* Borgmeier is a small decapitating fly from South America that normally parasitizes fire ant workers in the *saevissima* complex (Borgmeier 1925, Williams and Whitcomb 1974, Porter et al. 1995). In its native habitat, *P. curvatus* is distributed over a large geographical area from São Paulo, Brazil, westward into Mato Grosso do Sul, Brazil, and southward to Buenos Aires Province, Argentina (Porter and Pesquero 2001, Folgarait et al. 2004). *P. curvatus* flies reach peak abundances during the summer in South America (January–March; Fowler et al. 1995, Folgarait et al. 2003) and North America (July–September; F. Graham, personal communication). Mating occurs on the ground in the morning (Wuellner et al. 2002). Studies on oviposition behavior have shown that female *P. curvatus* flies attack workers that are significantly smaller than the colony mean (Morrison et al. 1997). Oviposition behavior consists of flies hovering in attack mode 3–5 mm above their host; orienting themselves to workers; and diving in to strike the thorax of workers, injecting eggs via an ovipositor.

In a previous series of host specificity tests, a *P. curvatus* biotype that was collected from the black imported fire ant, *Solenopsis richteri* Forel, in Las Flores, Argentina, was able to attack and develop successfully in two native fire ants: *Solenopsis geminata* (F.) and *Solenopsis xyloni* McCook (Gilbert and Morrison 1997, Porter 2000). Parasitism rates were very low in *S. geminata*, indicating that this ant would not be a good host (Porter 2000). However, parasitism rates on *S. xyloni* in the laboratory reached minimum levels at which *P. curvatus* might be able to sustain a population in the field (Porter 2000). Because im-

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ported fire ants are the number one enemy of native fire ants and because *P. curvatus* is a much greater threat to imported fire ants than native fire ants, Porter (2000) argued that releasing these flies would most likely benefit native fire ants rather than hurt them. Subsequently, the Las Flores biotype was approved for field release in 2001.

Field releases of this biotype successfully established populations in Alabama and Mississippi on hybrid fire ants (*S. richteri* × *S. invicta*) and black imported fire ants, but failed in Florida on red imported fire ants (Graham et al. 2003, Vogt and Street 2003). Field releases of the Las Flores biotype seem to have failed on red imported fire ants because this fly was too host specific. Although no-choice laboratory tests showed that the Las Flores biotype parasitized red and black imported fire ants equally, host preference tests revealed that the Las Flores biotype strongly preferred *S. richteri* and hybrid fire ants when tested against *Solenopsis invicta* Buren (Porter and Briano 2000, Folgarait et al. 2002). The preference for black imported fire ants was not unexpected because the Las Flores biotype was originally collected from black fire ants in South America.

Because the Las Flores biotype failed to establish on *S. invicta* populations in the United States, a new biotype of *P. curvatus* was collected from *S. invicta* fire ants in Formosa, Argentina. The objective of this study was to determine whether this new biotype of *P. curvatus* was sufficiently host specific to be released in the field with existing permits obtained from previous tests by using the Las Flores biotype. We also compared our results with the previous Las Flores biotype study.

### Materials and Methods

The new biotype of *P. curvatus* flies was collected attacking *S. invicta* fire ants 35 km northwest of Formosa, Argentina, by S.D.P. and J.A.B. (October 2001). Flies were collected by setting up several trays (42 by 28 by 15 cm, Panel Control Corp., Detroit, MI) containing several thousand fire ants. *P. curvatus* flies were allowed to attack the fire ant workers for 4–5 h while the workers ran from one side of the tray to the other as previously done by Porter (2000). These workers were airfreighted to our quarantine facility in Gainesville, FL. Flies were reared in a large self-contained, climate-controlled attack box exposing fire ants to attacks similar to the one described by Vogt et al. (2003).

**No-Choice Tests with Native Fire Ants.** To determine whether the Formosa biotype of *P. curvatus* will actively attack and develop in native *Solenopsis* fire ants, no-choice trials were conducted with *S. xyloni* and *S. geminata*. Ten plastic trays (42 by 28 by 15 cm; Panel Control Corp.) were used in the no-choice tests, each with screened vents and tight-fitting glass lids similar to those described by Porter and Alonso (1999). The trays contained a single solid bottom covered with a 2–3-cm layer of moistened plaster to maintain high humidity. Plaster was made by using a 1:1

mixture of pottery plaster (U.S. Gypsum Co., Chicago, IL) and plaster wall patch (DAP Inc., Baltimore, MD). Plaster was moistened before each test run. Before moistening, the plaster bottoms were scraped to remove residues left behind from previous use. When test trays were reused, native fire ants were not used in trays that had been used by imported fire ants (and vice versa) unless the plaster bottoms were replaced. This procedure avoided confounding results with odors previously deposited on the plaster bottoms.

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 per d (1000–1800 hours). A small piece of laboratory tissue (Kimwipes, Kimberly Clark, Roswell, GA) moistened in 1 M sugar water solution served as a food source for the flies, and a bunch of artificial flowers in the center of each tray provided a perching location. The laboratory was maintained at 27–28°C.

Trials were conducted in a complete randomized design with ant species as treatments and trials serving as experimental replication. Seven trials were conducted with *S. geminata*, six trials with *S. xyloni*, and eight control trials with *S. invicta*. The laboratory colonies of *S. xyloni* were collected from California (July 2001) and *S. invicta* and *S. geminata* colonies were collected near Gainesville, FL (August–September 2002). Workers from test colonies were sieved through a U.S. standard 20-mesh sieve to separate out the smaller workers that are preferred by *P. curvatus* females. Each test group contained 0.5 g of small workers and 1.0 g of brood. Different colonies were used for each trial to ensure that results were not due solely to differences in the attractiveness of individual colonies.

Flies were aspirated with an Allen-type double chamber aspirator from a holding box, retained in vials, knocked down with CO<sub>2</sub>, and separated by sex within 20 s on a cold table at which point they were placed into vials according to sex. Fifteen to 20 newly emerged female flies and an equivalent number of males (for mating) were added to all no-choice trials over a period of 2 d (treatments and controls always received equal numbers). Flies were added on two consecutive days to increase fly numbers and reduce effects of temporal variation. Trials lasted 4 d to cover the complete life span of ovipositing flies (1–2 d). Flies were introduced into the trays via an injection port as described by Porter and Alonso (1999). On each trial date, the number of female flies hovering in attack-mode over test ant species were recorded every 10 min over a continuous period of 2–3 h between 1100 and 1700 hours (EST), the time period that flies are most active. An average from the observations of female flies in attack-mode was taken for each tray and used in a one-way analysis of variance (ANOVA). *P. curvatus* males do not hover over ants (Wuellner et al. 2002). All flies were dead by the end of the 4-d trials. After tests, ants were removed from the trays, retained in small boxes (20 by 12 by 5 cm) with tight-fitting vented (2 by 3-cm) lids, and inspected for pupating

flies every other day for a period of 35 d. Inside each retainment box, we placed a small 3-cm block of moist plaster and a nest tube with water held in the end by a cotton ball (16 by 125 mm). Ants were fed fresh sugar water every 2 d. We removed dead workers from the small boxes and placed them inside condiment cups (4 oz) with moist plaster bottoms where the larvae could pupate. Determination of pupating flies in ant head capsules was made by looking for a sclerotized cap flanked by two respiratory horns as described by Porter (1998). The total number of pupae produced in each ant species was divided by the 15 to 20 female flies used in the trials to produce an average pupae production rate per female fly. Pupae were held for a total of 25 d to determine rates of adult emergence.

**Paired Preference Tests.** Host preferences of Formosa *P. curvatus* flies were examined as paired difference tests consisting of seven paired trials with *S. invicta* and *S. xyloni* (August–September 2002) and six paired trials with *S. invicta* and *S. geminata* (September 2002). Trials were conducted in three white plastic trays (42 by 28 by 15 cm, Panel Control Corp.) with screened vents and tight-fitting glass lids. In the bottom of each tray, two long side-by-side holes were cut and two smaller trays were glued (30 by 7 by 5 cm) as described by Porter (2000). This configuration produced two parallel chambers in the bottom of the big tray that allowed the testing of two species of ants at the same time. Ants were contained in the two bottom trays by coating their sides with Fluon (AGC Chemicals Americas Inc., Bayonne, NJ). To maintain high humidity, four moistened 3 by 3 by 4-cm sponges were placed in the corners of the test trays and a 1-cm-thick layer of hard plaster (Castone, Dentsply, York, PA) was poured into the bottom of both bottom trays. The plaster and sponges were moistened before each test run. Small 20-cm desk fans were directed toward the vents of the test boxes so that high humidity did not cause condensation on the glass lid or the sides of the trays.

Flies were introduced into the trays as described previously. A small opaque inverted cup (4 cm in diameter) with a large wire loop glued to the top was placed on the plaster in each of the two bottom trays. These cups were moved back and forth from one end of a tray to the other with a plastic rod each time most of the ants had crawled under a cup to hide. This procedure kept the ants trailing continuously from one end of a bottom tray to the other so that the flies always had an opportunity to attack the ants. Smaller workers were obtained by sieving as described above. We used 0.3 g of workers and 0.6 g of brood for each test group.

Each test used ants from a different colony and received 10–15 female flies and an equivalent number of males. Trials lasted 3 h during which time we recorded the number of females hovering in attack-mode over each ant species every 10 min during 1100–1500 hours (EST) to produce an average number of females in attack-mode. When possible, we recorded the number of oviposition attempts in 20-s intervals for individual flies hovering in attack-mode over each

group of ants to produce an average rate of attack per minute per attacking female. When the test boxes were reused, workers from one species of fire ant were not placed in a side that had been used by another species. At the end of each trial, worker ants were transferred to small boxes and checked for pupating flies as described in no-choice tests. The head capsules of dead workers were inspected for fly larvae or pupae every 1–2 d for a period of 25 d so that most larvae had time to complete development in their host. The total number of pupae produced per ant trial was divided by number of females used to produce an average production rate. Voucher specimens of flies have been deposited in the Florida Collection of Arthropods, Gainesville, FL.

**Statistical Analysis.** A one-way ANOVA was used to evaluate differences between treatment means in attacking activity during the no-choice tests. Pupal production, from all three species of ants in the no-choice tests, was compared across experiments using a Kruskal–Wallis test. A  $\chi^2$  test was used to evaluate the percentage of pupae that completed development in the no-choice tests. Data did not receive transformation. In the preference tests, a two-tailed, paired *t*-test was used to compare fly activity (hovering in attack-mode and attack rates) and pupal production; however, a Wilcoxon signed-rank test was used to compare pupal production in one paired trial of the preference tests. All analyses were conducted using Minitab 13 (Minitab Inc. 2003).

## Results

**No-Choice Tests with Native Fire Ants.** The number of flies hovering in attack-mode over *S. invicta* workers was not significantly different than the number over either *S. xyloni* or *S. geminata* workers (Fig. 1A;  $1.40 \pm 0.28$  [mean  $\pm$  SE] versus  $1.08 \pm 0.25$  and  $0.90 \pm 0.42$ ;  $F = 0.60$ ;  $df = 2, 12$ ;  $P = 0.56$ ). In the no-choice trials, *P. curvatus* flies successfully developed in *S. xyloni* workers but failed to develop successfully in *S. geminata* (Fig. 1B). The mean number of pupae produced per female fly in *S. invicta* workers was 7 times higher than the mean number for pupae produced from *S. xyloni* workers (Fig. 1B;  $5.03 \pm 1.55$  [mean  $\pm$  SE] versus  $0.66 \pm 0.24$ ,  $H = 13.31$ ,  $df = 2$ ,  $P < 0.001$ , Kruskal–Wallis test). The percentage of pupae that successfully developed to adult flies was 65% in *S. invicta* (349/535) and 18% in *S. xyloni* (10/56) (likelihood ratio  $\chi^2 = 47.72$ ,  $df = 1$ ,  $P < 0.001$ ).

**Paired Preference Tests.** *P. curvatus* strongly preferred *S. invicta* over either species of native fire ant in the preference tests (Fig. 2). Female flies that were hovering in attack-mode preferred to hover over *S. invicta* 77% of the time rather than *S. xyloni* (Fig. 2A;  $1.66 \pm 2.6$  [mean  $\pm$  SE] versus  $0.54 \pm 2.0$  flies per observation,  $t = 7.58$ ,  $df = 6$ ,  $P < 0.001$ , paired *t*-test). Similarly, hovering female flies in attack-mode preferred to hover over *S. invicta* 87% of the time rather than *S. geminata* (Fig. 2A;  $3.00 \pm 4$  [mean  $\pm$  SE] versus  $0.52 \pm 4$  flies per observation,  $t = 3.74$ ,  $df = 4$ ,  $P = 0.02$ , paired *t*-test). The attack rate was 2.8 times higher for

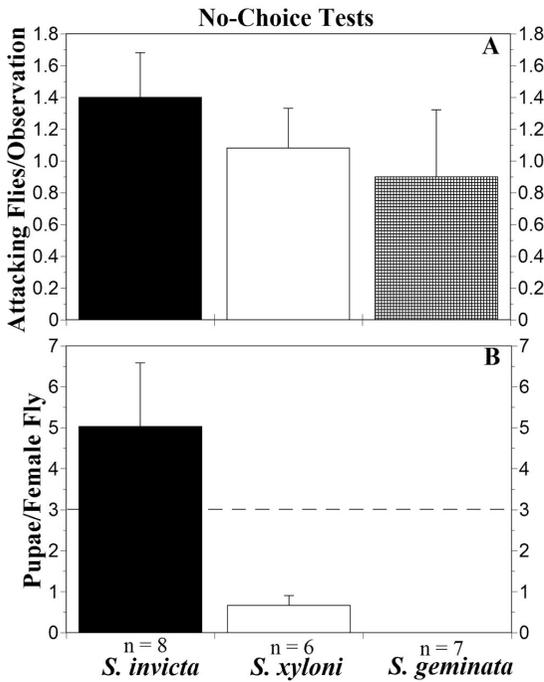


Fig. 1. No-choice trials of the Formosa decapitating fly *P. curvatus* with the fire ant *S. invicta* and the two native fire ants *S. geminata* and *S. xyloni*. (A) Mean number of hovering flies in attack mode per observation. (B) Mean lifetime number of pupae produced per female fly in each trial. The dashed line indicates a conservative estimate of the minimum number of offspring per female necessary to produce a self-sustaining population as calculated by Porter (2000). Error bars show SE calculated from trial means. The number of replicates (*n*) is indicated below each bar.

female flies in attack-mode over *S. invicta* than for female flies in attack-mode over *S. xyloni* (Fig. 2B;  $6.03 \pm 0.82$  [mean  $\pm$  SE] versus  $2.27 \pm 0.68$  attacks per minute,  $t = 5.83$ ,  $df = 6$ ,  $P < 0.001$ , paired *t*-test). The attack rate was 16 times higher for female flies in attack-mode over *S. invicta* than for flies in attack-mode over *S. geminata* (Fig. 2B;  $7.02 \pm 1.41$  [mean  $\pm$  SE] versus  $0.44 \pm 0.28$  attacks per minute,  $t = 4.73$ ,  $df = 4$ ,  $P = 0.009$ , paired *t*-test).

In the paired tests, the mean number of pupae produced per female fly was higher in *S. invicta* than for either native species (Fig. 2C). In the *S. invicta*/*S. xyloni* tests, 4 times more pupae were found in *S. invicta* workers than in *S. xyloni* workers (Fig. 2C;  $1.98 \pm 0.71$  [mean  $\pm$  SE] versus  $0.48 \pm 0.23$  pupae per female fly,  $t = 2.63$ ,  $df = 6$ ,  $P = 0.039$ , paired *t*-test). In the *S. invicta*/*S. geminata* tests, normal numbers of pupae were found in *S. invicta* workers, but no pupae were found in *S. geminata* workers (Fig. 2C;  $1.71 \pm 0.59$  [mean  $\pm$  SE] versus 0 pupae per female fly,  $T = 0$ ,  $N_1 = N_2 = 6$ ,  $P < 0.05$ , Wilcoxon signed-rank test).

**Discussion**

The results of this study indicate that the Formosa biotype displays a high degree of specificity toward

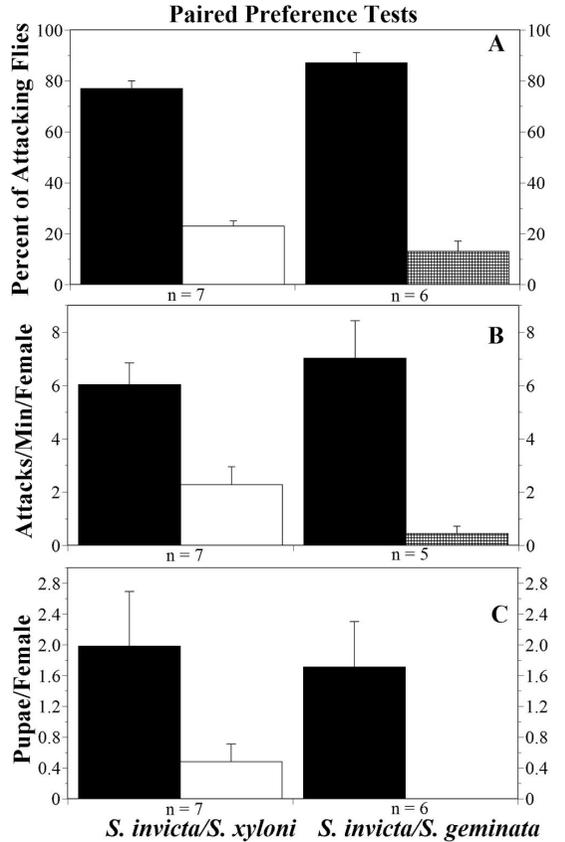


Fig. 2. Paired preference trials of the Formosa decapitating fly *P. curvatus*. (A) Percentage of hovering flies in attack mode over either *S. invicta* or one of the two native fire ant species. (B) Average rate of attack per minute per attacking female fly. (C) Mean number of pupae produced per female fly as a result of the 4-h trials. Error bars show SE calculated from trial means. The number of replicated pairs (*n*) are shown below bars.

red imported fire ants compared with native fire ants. Formosa biotype flies were observed hovering in attack mode over all fire ant species at similar rates during no-choice tests (Fig. 1A). However, rates of pupal production were much lower in *S. xyloni* compared with *S. invicta* and zero in *S. geminata* workers used in the no-choice tests (Fig. 1B). Paired preference tests demonstrated that the Formosa biotype of *P. curvatus* actively prefers to hover over red imported fire ants at significantly higher rates rather than native fire ants (Fig. 2A). Of those flies that chose to actively hover, attack rates were also higher with red imported fire ants compared with native fire ants (Fig. 2B). As in the no-choice tests, rates of pupal production were much lower in *S. xyloni* compared with *S. invicta* and zero in *S. geminata* workers used in the paired preference tests (Fig. 2C).

Results from the no-choice and paired preference tests demonstrate that the Formosa biotype of *P. curvatus* is more host specific to red imported fire ants than the results from a previous study with the Las

**Table 1.** Comparison of percentage of host specificity to red imported fire ants *S. invicta* for two biotypes of the decapitating fly *P. curvatus* (Formosa, Las Flores) when exposed to native fire ants (either *S. xyloni* or *S. geminata*)

| Measures of host specificity    | Ant species                                       |                         |   |                         |
|---------------------------------|---|-------------------------|---|-------------------------|
|                                 | <i>S. invicta</i> / <i>S. xyloni</i> <sup>a</sup> |                         | <i>S. invicta</i> / <i>S. geminata</i> <sup>a</sup> |                         |
|                                 | Formosa   | Las Flores <sup>b</sup> | Formosa   | Las Flores <sup>b</sup> |
| No-choice tests                 |   |                         |   |                         |
| Hovering flies <sup>c</sup>     | 23  | 28                      | 36  | 89                      |
| Pupae/female fly <sup>c</sup>   | 87  | 65                      | 100   | 94                      |
| Paired preference tests         |   |                         |   |                         |
| % Preference <sup>d</sup>       | 77  | 74                      | 87  | 78                      |
| Attacks/min/female <sup>c</sup> | 62  | 2                       | 94  | 86                      |

<sup>a</sup> A value of 100% indicates complete host specificity to red imported fire ants, whereas 0% indicates no host specificity to imported fire ants compared with one of the native fire ants.

<sup>b</sup> Data for Las Flores biotype comes from a previous study conducted by Porter (2000).

<sup>c</sup> Values were calculated by subtracting native fire ant value from imported fire ant value and then dividing by imported fire ant value.

<sup>d</sup> Percentages were directly taken from host preference tests.

Flores biotype (Porter 2000). When considering attacking flies in the no-choice tests with *S. xyloni* (Table 1), the Formosa biotype had about the same percentage of specificity to *S. invicta* as the Las Flores biotype (23 versus 28%). However, host specificity to *S. invicta* as measured by pupal production was much higher in the Formosa biotype than the Las Flores biotype (87 versus 65%; Table 1). Similarly, percentage of preference for *S. invicta* in the paired preference tests, with *S. invicta* and *S. xyloni*, was similar in both biotypes (77 versus 74%) but host specificity as calculated by attack rates was much higher in the Formosa biotype than the Las Flores biotype (62 versus 2%; Table 1). These data indicate that much of the increased host specificity for *S. invicta* exhibited by the Formosa biotype is the result of a higher proclivity to attack or attempt oviposition on *S. invicta* than on *S. xyloni*.

Comparisons between the Formosa and Las Flores biotypes with *S. geminata* demonstrate that hovering flies in the no-choice tests (Table 1) were more host specific to *S. invicta* in the Las Flores biotype than the Formosa biotype (89 versus 36%). However, in regard to pupal production, the Formosa biotype was 100% host specific to *S. invicta*, whereas the Las Flores biotype was 94% host specific (Table 1); in other words, a few of the Las Flores flies were able to develop on *S. geminata*, but none of the Formosa flies were able to develop. In the paired preference tests, percentage of preference for *S. invicta* over *S. geminata* was higher in the Formosa biotype than the Las Flores biotype (87 versus 78%; Table 1). Host specificity as calculated by attack rates was also higher in the Formosa biotype than the Las Flores biotype (94 versus 86%; Table 1). Because the rate of pupal production was zero in both the no-choice and paired preference tests for *S. geminata* (Figs. 1 and 2), we conclude that the Formosa biotype will not be a threat to *S. geminata*. These trials demonstrate that the Formosa and Las Flores biotypes differ substantially in host specificity.

Vink et al. (2003) also observed variability in host specificity between two biotypes of *Microctonus aethioides* Loan (Hymenoptera: Braconidae). Other studies on the host specificity of parasitoid biotypes have shown that geographic variation in host specificity between biotypes was due to the presence of cryptic species (Heimpel et al. 1997, Alvarez and Hoy 2002). Although our results demonstrate that there is geographic variation between the Formosa and Las Flores flies, we cannot rule out the possibility that the variability seen is due to the presence of a cryptic species.

Because the Formosa flies were more host specific than the Las Flores biotype, they were released from quarantine in the spring 2003 under a previous permit from the State of Florida and the Finding of No Significant Impact issued by the USDA-ARS. Trial field releases are underway and initial results look promising.

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