

HOST-SPECIFICITY TESTS, FIELD RELEASES, AND ESTABLISHMENT OF THE  
SMALL DECAPITATING FLY, *Pseudacteon curvatus* BORGMEIER (DIPTERA:  
PHORIDAE), IN FLORIDA

By

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This thesis is dedicated to someone who has been very supportive of my endeavors, my mother, Nancy Elena Vázquez-Fuertes and to my father, the late Rodolfo Elias Vázquez-Febles.

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## TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS .....	iii
LIST OF TABLES .....	vii
LIST OF FIGURES .....	viii
ABSTRACT.....	ix
CHAPTER	
1 GENERAL INTRODUCTION .....	1
2 HOST SPECIFICITY OF A BIOTYPE OF THE FIRE ANT DECAPITATING FLY <i>Pseudacteon curvatus</i> (DIPTERA: PHORIDAE) FROM NORTHERN ARGENTINA .....	5
Introduction.....	5
Materials and Methods .....	7
No-choice tests with native fire ants. ....	8
Paired preference tests.....	10
Statistical Analysis. ....	12
Results.....	12
No-Choice tests with native fire ants.....	12
Paired preference tests.....	13
Discussion.....	14
3 FIELD RELEASE AND ESTABLISHMENT OF THE DECAPITATING FLY <i>Pseudacteon curvatus</i> ON RED IMPORTED FIRE ANTS IN FLORIDA .....	20
Introduction.....	20
Release Methods.....	23
Release sites.....	24
Monitoring fly establishment and dispersal. ....	24
Results.....	25
Discussion.....	26

4	RE-CONFIRMING HOST SPECIFICITY OF THE FIRE ANT DECAPITATING FLY <i>Pseudacteon curvatus</i> AFTER FIELD RELEASE IN FLORIDA .....	30
	Introduction.....	30
	Materials and Methods .....	32
	Results.....	34
	Discussion.....	35
	LIST OF REFERENCES.....	37
	BIOGRAPHICAL SKETCH.....	45

## LIST OF TABLES

<u>Table</u>	<u>page</u>
2-1. Comparison of percent host specificity to red imported fire ants ( <i>Solenopsis invicta</i> ) for two biotypes of the decapitating fly <i>Pseudacteon curvatus</i> (Formosa, Las Flores) when exposed to native fire ants (either <i>Solenopsis xyloni</i> or <i>Solenopsis geminata</i> , see footnotes for calculation details). ....	19
3-1. Number of adult <i>Pseudacteon curvatus</i> flies found at Whitehurst Ranch, Morrill Pasture, and Mickle Pasture release sites in Florida from April 2003 to May 2004.	29
4-1. Number of <i>Pseudacteon curvatus</i> flies collected hovering in attack mode over non-host ant species, native fire ants ( <i>Solenopsis geminata</i> ), and red imported fire ants ( <i>Solenopsis invicta</i> ) during sequential series of field trials (see methods). ....	36

## LIST OF FIGURES

<u>Figure</u>	<u>page</u>
2-1. No-choice trials of the Formosa decapitating fly <i>Pseudacteon curvatus</i> with the imported fire ant <i>Solenopsis invicta</i> and the two native fire ants <i>Solenopsis geminata</i> and <i>Solenopsis xyloni</i> . ....	17
2-2. Paired preference trials of the Formosa decapitating fly <i>Pseudacteon curvatus</i> .....	18

Abstract of Thesis Presented to the Graduate School  
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HOST-SPECIFICITY TESTS, FIELD RELEASES, AND ESTABLISHMENT OF THE  
SMALL DECAPITATING FLY, *Pseudacteon curvatus* BORGMEIER (DIPTERA:  
PHORIDAE), IN FLORIDA

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In recent years, decapitating flies in the genus *Pseudacteon* Coquillett have been studied extensively as potential biological control agents because many are common parasitoids of red imported fire ants, *Solenopsis invicta* Buren. The purpose of this study was to evaluate host-specificity of a biotype of *Pseudacteon curvatus* Borgmeier collected from red fire ants in Formosa, Argentina, and determine if it is sufficiently host specific to be released in the field. I also established three field populations and monitored post-release nontarget effects of *P. curvatus*.

I tested the host specificity of *Pseudacteon curvatus* Borgmeier from Formosa, Argentina, on North American colonies of the red imported fire ant, *Solenopsis invicta*, and the native fire ants, *Solenopsis geminata* (Fabricius) and *Solenopsis xyloni* McCook. In no-choice tests, rates of pupal production were 87% lower in *S. xyloni* compared to *S. invicta* and zero in *S. geminata*. In choice tests, the Formosa biotype preferred to hover 77-87% of the time, attack at a ratio of 10 to 1, and produce higher numbers of pupae on

*S. invicta* workers when compared to results observed with the native fire ants. These results indicate that the Formosa biotype displays a high degree of specificity towards red imported fire ants when compared to native fire ants.

The Formosa biotype of *P. curvatus* was released after host-specificity tests demonstrated that this biotype was specific to imported fire ants. Field releases were conducted at 3 sites in Florida in the spring and summer of 2003 and monthly monitoring followed. Field releases were successful in that field reared flies were collected and identified within 5 weeks at the first site and then monthly thereafter. As of May 2004, field populations of *P. curvatus* dispersed one mile North and South from the first release site. Flies from the summer releases were found in April 2004. This was the first successful establishment of *P. curvatus* on red imported fire ants in the United States.

Post-release monitoring confirmed that the Formosa biotype of *P. curvatus* was not attracted to non-*Solenopsis* ants. Flies were attracted to the native fire ant, *S. geminata*, at very low rates (<5% of that with *S. invicta*) but virtually no oviposition attempts were observed. Overall results were consistent with laboratory predictions except attraction rates to nontarget fire ants in the field were much lower than in the small laboratory test chambers.

## CHAPTER 1 GENERAL INTRODUCTION

Biological control may be defined as the use of parasitoid, predator, pathogen, or competitor populations to suppress a pest population, making it less abundant and damaging than it would otherwise be. All living species are attacked by natural enemies- parasites, predators, or pathogens- which feed on them in one way or another and in many cases regulate their population densities (Van Driesche and Bellows 2001). Many potentially injurious pests are kept at very low levels and never reach economic pest proportions due to the effective action of naturally-occurring natural enemies, without deliberate intervention by man.

Natural enemies can be utilized in three major ways: (1) importation of exotic species and their establishment in a new habitat, (2) augmentation of established species through direct manipulation of their populations, as by insect mass production and periodic colonization, and (3) their conservation through manipulation of the environment. When successful, the utilization of natural enemies is an inexpensive, non-hazardous means of reducing pest populations and maintaining them below economic injury levels (Van Driesche and Bellows 2001). Success in biological control is often dependent on a thorough understanding of the organisms involved, both injurious and beneficial, and their intricate interactions.

The modern history of biological control can be dated from the control of the cottony cushion scale, *Icerya purchasi* Maskell, by introduced natural enemies on citrus in California in 1888 (Van Driesche and Bellows 2001). Ever since then, hundreds of

biological control projects have been successfully carried out in many parts of the world. Although biological control was first practiced against insect pests, it is by no means restricted to any particular group of noxious organisms. It is applicable, and has indeed been successfully attempted, against insects and other arthropod pests, other animals as diverse as snails and rabbits, weeds and plant pathogens.

A major ant pest in the United States is the red imported fire ant, *Solenopsis invicta* Buren, which was introduced at Mobile, Alabama, in the 1930s and has over the years spread throughout the southeastern states (Buren et al. 1974, Lofgren et al. 1975, Vinson and Greenberg 1986). Since its introduction, *S. invicta* has spread to more than 316 million acres in several states and Puerto Rico (Callcott 2002) and recently has reached Arizona, California, Caribbean Islands (Davis et al. 2001), New Zealand (Harris 2001), and Australia (Solley et al. 2002). *Solenopsis invicta* has had a substantial impact on humans, wildlife, livestock, and agricultural crops (Adams and Lofgren 1981, Allen et al. 1994, Barr and Drees 1996). It is usually the dominant ant species in areas it infests due to its high reproductive capacity, aggressive foraging behavior, and lack of natural enemies (Porter et al. 1997). Attempts to control *S. invicta* have relied on pesticides and these efforts have been effective but temporary and too expensive for larger area application such as pastures (Brown 1961, Allen et al. 1994, Davidson and Stone 1989). Even though chemical applications temporarily reduce fire ant densities in small areas, fire ants still outcompete native ants and other arthropods, while still expanding their global range (Callcott and Collins 1996, Morrison et al. 2004, Porter and Savignano 1990).

In recent years, research efforts on controlling imported fire ants have focused on natural enemies such as the parasitic ant *Solenopsis daguerrei* (Santschi) (Calcaterra et al. 1999, 2000, Briano et al. 2002), the microsporidium pathogen *Thelohania solenopsae* Knell, Allen & Hazard (Oi et al. 2001, Oi and Williams 2002), and dipteran parasitoids in the genus *Pseudacteon* Coquillett (Feener 2000, Porter 1998a, Morrison 2000). Earlier work noted that *Pseudacteon* species that attack fire ants appear to be specific to fire ants (Borgmeier and Prado 1975; Disney 1994). Field tests in both South America (Porter et al. 1995) and in the United States (Vazquez and Porter 2004) along with laboratory tests in South America (Folgarait et al. 2002) and the United States (Gilbert and Morrison 1997, Porter and Alonso 1999, Porter 2000, Vazquez et al. 2004a) have demonstrated *Pseudacteon* species to be host specific to *Solenopsis* fire ants.

The small decapitating fly, *Pseudacteon curvatus* Borgmeier, normally parasitizes fire ant workers in the *saevissima* complex in South America (Borgmeier 1925, Porter 1998a). *Pseudacteon curvatus* is distributed over a large geographical area from São Paulo, Brazil to Buenos Aires province, Argentina (Folgarait et al. 2004, Porter and Pesquero 2001). Phorid flies have a unique characteristic of decapitating their host during pupation and affecting fire ant behaviour during oviposition attempts (Morrison 1999). The life cycle of a *Pseudacteon* fly begins with a torpedo-shaped egg oviposited into the thorax of a worker ant (Porter 1998a). The egg dramatically increases in size and completes development in about 4 days (Consoli et al. 2001). The first instar spends a short time (about a day) in the thorax and molts into the second instar before it moves into the ant's head (Consoli et al. 2001). During most of the third instar, the maggot probably relies on ant hemolymph for nutrition before pupation (Porter 1998a).

When the maggot is ready to pupate, it releases an enzyme that causes the intercuticular membranes of its host to degenerate which loosens the head and sometimes the legs. The maggot then proceeds to consume the entire contents of the ant head leading to the decapitation of its living host. The ant's mandibles and tongue apparatus are pushed aside by a series of hydraulic extensions by the maggot (Porter et al. 1995b, Porter 1998a). The maggot orients itself under the tentorial arms inside the head capsule where the first three segments compress and harden forming a distinctive sclerotized cap that fills the oral cavity (Porter 1998a). Depending on temperature, pupal development takes 2-6 weeks (Porter 1998a). The sclerotized cap pops open and the adult fly slips out of the ant head capsule within a few seconds. Newly emerged flies mate and lay eggs within several hours of eclosion (Porter 1998a). Female flies contain 100-200 sexually mature eggs in their ovaries upon emergence (Zacaro and Porter 2003) and adult flies can live 3-7 days under laboratory conditions (Porter 1998a).

Classical biological control can be used to control red imported fire ants without the heavy reliance on pesticides. Overall objective of my thesis project was to evaluate the natural enemy *P. curvatus* collected from red fire ants in Formosa, Argentina, for the suppression of *S. invicta* populations in the United States by asking the following questions: 1) is a biotype of *P. curvatus* from Formosa, Argentina, sufficiently host specific to be released in the field, 2) can this biotype successfully establish on red imported fire ants in the field, and 3) do post-released populations of *P. curvatus* confirm quarantine specificity predictions of minimal to non-existent nontarget effects?

CHAPTER 2  
HOST SPECIFICITY OF A BIOTYPE OF THE FIRE ANT DECAPITATING FLY  
*Pseudacteon curvatus* (DIPTERA: PHORIDAE) FROM NORTHERN ARGENTINA<sup>1</sup>

**Introduction**

Before an exotic biological control agent is released in the field, the degree of host specificity must be assessed to determine if that agent will likely parasitize non-target 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 appear 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. 1995a). Furthermore, most *Pseudacteon* flies in Brazil and Argentina appear 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 (*Pseudacteon curvatus* Borgmeier and *Pseudacteon tricuspis* Borgmeier) will parasitize fire ants in the *geminata* complex (Porter et al. 1995a, Gilbert and Morrison 1997, Porter 2000).

*Pseudacteon curvatus* 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. 1995a). In its native habitat, *P. curvatus* is distributed over

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<sup>1</sup> In Press, *Environmental Entomology* 2004

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). *Pseudacteon curvatus* flies reach peak abundances during the summer seasons in South America (January through March; Fowler et al. 1995, Folgarait et al. 2003) and North America (July through September; Fudd Graham, personal communication). Mating occurs on the ground in the morning hours (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 fire ant, *Solenopsis richteri* Forel, in Las Flores, Argentina was able to attack and develop successfully in two native fire ants: *Solenopsis geminata* (Fabricius) 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). Since imported fire ants are the number one enemy of native fire ants and since *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 appears 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 U.S., a new biotype of *P. curvatus* was collected from *S. invicta* fire ants in Formosa, Argentina. The objective of this study was to determine if this new biotype of *P. curvatus* was sufficiently host specific to be released in the field with existing permits obtained from previous tests using the Las Flores biotype. I also compared the Formosa results with the previous Las Flores biotype study.

### **Materials and Methods**

The new biotype of *P. curvatus* flies were collected attacking *S. invicta* fire ants 35 km NW of Formosa, Argentina by Sanford D. Porter and Juan A. Briano (October 2001). Flies were collected by setting up several trays (42 × 28 × 15 cm; Panel Control Corp., Detroit, MI) containing several thousand fire ants. *Pseudacteon 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

the quarantine facility in Gainesville, Florida. 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., Detroit, MI) 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 (US Gypsum Co., Chicago, IL) and plaster wall patch (DAP Inc., Baltimore, MD). Plaster was moistened before each test run. Prior to 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 (10:00 to 18:00 h). A small piece of laboratory tissue (Kimwipes, Kimberly Clark, Roswell, GA) moistened in 1M 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 (CRD) 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, Florida (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 assure 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 sec on a cold table at which point they were placed into vials according to sex. Fifteen to twenty 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 2 consecutive days to increase fly numbers and reduce effects of temporal variation. Trials lasted 4 d to cover the complete lifespan of ovipositing flies (1-2 days). 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 1100-1700 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. *Pseudacteon 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, I 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. I 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 (1998a). The total number of pupae produced in each ant species was divided by the fifteen to twenty 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., Detroit, MI) 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 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 about 3 h during which time we recorded the number of females hovering in attack-mode over each species every 10 min during 1100-1500 EST to produce an average number of females in attack-mode. When possible, I recorded the number of oviposition attempts in 20 sec 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, Florida.

### **Statistical Analysis**

A one-way analysis of variance 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, were 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 (2003).

## **Results**

### **No-Choice tests with native fire ants**

The mean number of flies hovering in attack-mode over *S. invicta* workers was not significantly different than the mean number over either *S. xyloni* or *S. geminata* workers (Fig. 2-1A;  $1.40 \pm 0.28$  (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. 2-1B). 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. 2-1B;  $5.03 \pm 1.55$  (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

*Pseudacteon curvatus* strongly preferred *S. invicta* over either species of native fire ant in the preference tests (Fig. 2-2). Female flies that were hovering in attack-mode preferred to hover over *S. invicta* 77% of the time rather than *S. xyloni* (Fig. 2-2A;  $1.66 \pm 2.6$  (mean  $\pm$  SE) versus  $0.54 \pm 2.0$  flies/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. 2-2A;  $3.00 \pm 4$  (mean  $\pm$  SE) versus  $0.52 \pm 4$  flies/observation,  $t = 3.74$ ,  $df = 4$ ,  $P = 0.02$ , paired  $t$ -test). The attack rate was 2.8 times higher for female flies in attack-mode over *S. invicta* than for female flies in attack-mode over *S. xyloni* (Fig. 2-2B;  $6.03 \pm 0.82$  (mean  $\pm$  SE) versus  $2.27 \pm 0.68$  attacks/min,  $t = 5.83$ ,  $df = 6$ ,  $P < 0.001$ , 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. 2-2B;  $7.02 \pm 1.41$  (mean  $\pm$  SE) versus  $0.44 \pm 0.28$  attacks/min,  $t = 4.73$ ,  $df = 4$ ,  $P = 0.009$ , 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. 2-2C). In the *S. invicta*/*S. xyloni* tests, 4 times more pupae were found in *S. invicta* workers than in *S. xyloni* workers (Fig. 2-2C;  $1.98 \pm 0.71$  (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. 2-2C;  $1.71 \pm 0.59$  (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 towards red imported fire ants when 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. 2-1A). 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. 2-1B). 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. 2-2A). Of those flies that chose to actively hover, attack rates were also higher with red imported fire ants when compared with native fire ants (Fig. 2-2B). As in the no-choice tests, rates of pupal production were much lower in *S. xyloni* compared to *S. invicta* and zero in *S. geminata* workers used in the paired preference tests (Fig. 2-2C).

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 Flores biotype (Porter 2000). When considering attacking flies in the no-choice tests with *S. xyloni* (Table 2-1), the Formosa biotype had about the same percent specificity to *S. invicta* as the Las Flores biotype (23% vs. 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% vs. 65%; Table 2-1). Similarly, percent preference for *S. invicta* in the paired preference tests, with *S. invicta* and *S. xyloni*, was similar in both biotypes (77% vs. 74%) but host specificity as calculated by attack rates was much higher in the Formosa biotype than the Las Flores biotype (62% vs. 2%; Table 2-1). 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 2-1) were more host specific to *S. invicta* in the Las Flores biotype than the Formosa biotype (89% vs. 36%). However, in regard to pupal production, the Formosa biotype was 100% host specific to *S. invicta* while the Las Flores biotype was 94% host specific (Table 2-1); 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, percent preference for *S. invicta* over *S. geminata* was higher in the Formosa biotype than the Las Flores biotype (87% vs. 78%; Table 2-1). Host specificity as calculated by attack rates was also higher in the Formosa biotype than the Las Flores biotype (94% vs. 86%; Table 2-1). Since the rate of pupal production was zero in both the no-choice and paired preference tests for *S. geminata* (Figs. 2-1 and 2-2), I 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 aethiopoidea* (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 my results demonstrate that there is geographic variation between the Formosa and Las Flores flies, I 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 of 2003 under a previous permit from the State of Florida and the Finding of No Significant Impact (FONSI) issued by the USDA-ARS. Trial field releases are underway and initial results look promising.

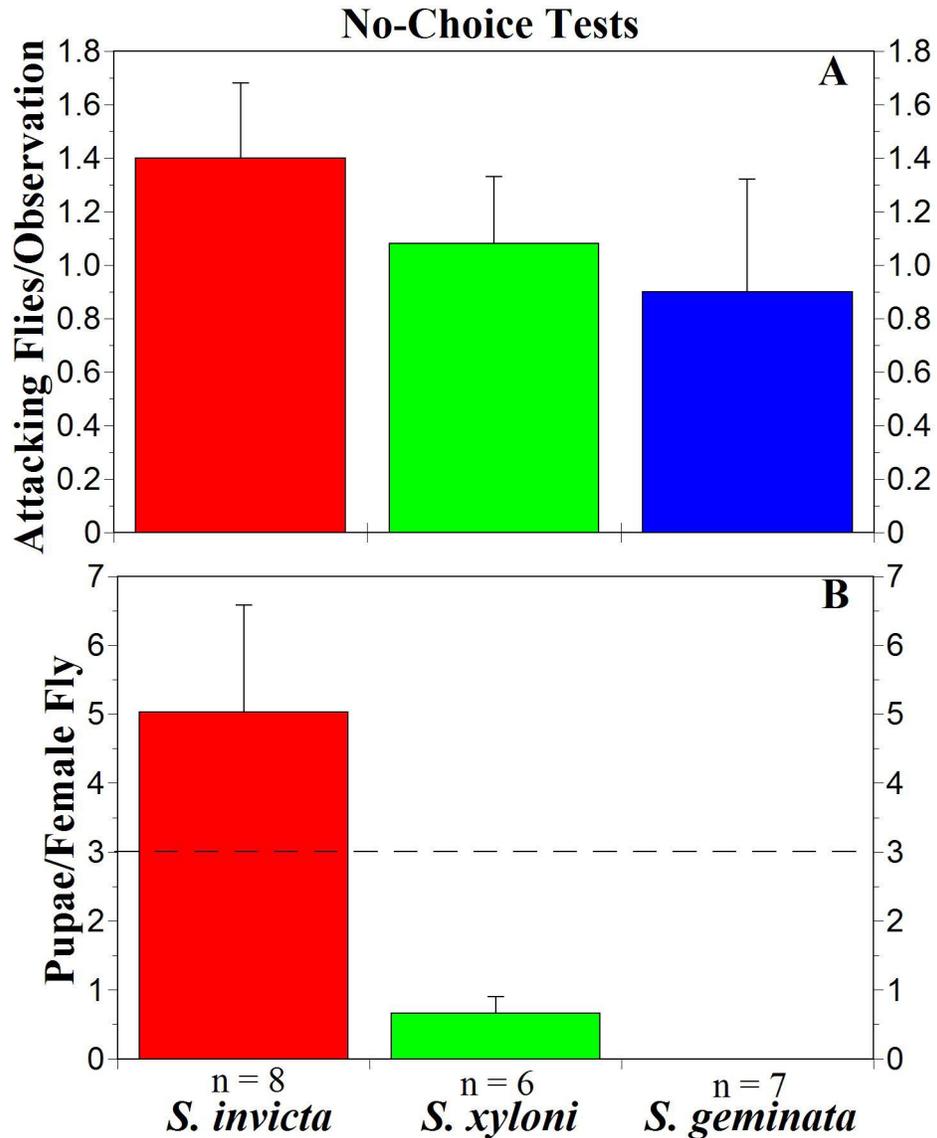


Fig. 2-1. No-choice trials of the Formosa decapitating fly *Pseudacteon curvatus* with the imported fire ant *Solenopsis invicta* and the two native fire ants *Solenopsis geminata* and *Solenopsis xyloni*. (A) Mean number of hovering flies in attack mode per observation. (B) Mean lifetime number of pupae 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.

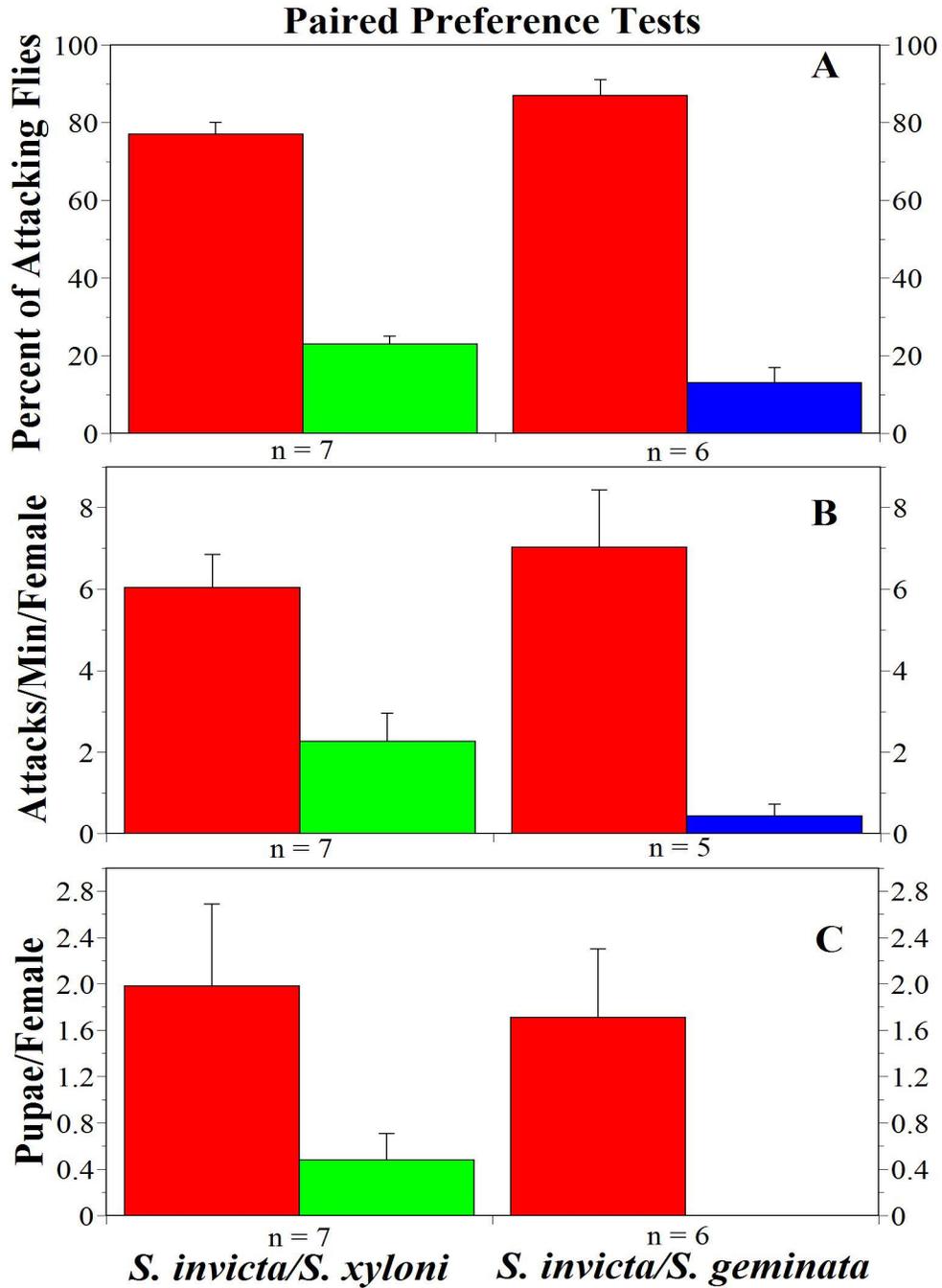


Fig. 2-2. Paired preference trials of the Formosa decapitating fly *Pseudacteon curvatus*. (A) Percentage of hovering flies in attack mode over either *Solenopsis invicta* or one of the two native fire ant species. (B) The average rate of attack per min per attacking female fly. (C) Mean number of pupae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

Table 2-1. Comparison of percent host specificity to red imported fire ants (*Solenopsis invicta*) for two biotypes of the decapitating fly *Pseudacteon curvatus* (Formosa, Las Flores) when exposed to native fire ants (either *Solenopsis xyloni* or *Solenopsis geminata*, see footnotes for calculation details).

Measures of host specificity	Ant Species			
	<i>S. invicta/S. xyloni</i> <sup>a</sup>		<i>S. invicta/S. geminata</i> <sup>a</sup>	
	Formosa	Las Flores <sup>d</sup>	Formosa	Las Flores <sup>d</sup>
No Choice Tests				
Hovering Flies <sup>b</sup>	23%	28%	36%	89%
Pupae/Female Fly <sup>b</sup>	87%	65%	100%	94%
Paired Preference Tests				
% Preference <sup>c</sup>	77%	74%	87%	78%
Attacks/Min/Female <sup>b</sup>	62%	2%	94%	86%

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

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

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

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

CHAPTER 3  
FIELD RELEASE AND ESTABLISHMENT OF THE DECAPITATING FLY  
*Pseudacteon curvatus* ON RED IMPORTED FIRE ANTS IN FLORIDA<sup>2</sup>

**Introduction**

*Solenopsis invicta* Buren and *Solenopsis richteri* Forel are two invasive species that have been able to thrive without their natural enemies. Both species of fire ants were accidentally introduced into the United States through Mobile, Alabama in 1918 (*S. richteri*) and in the 1930's (*S. invicta*), leaving behind most of their natural enemies in South America (Jouvenaz 1990). It is speculated that the absence of natural enemies is the reason fire ant densities are 5-10 times higher in the United States than they are in South America (Porter et al. 1992, Porter et al. 1997).

Since introduction, *S. invicta* has spread throughout the entire southeastern United States (Callcott and Collins 1996) while *S. richteri* along with a hybrid species (*S. richteri* × *S. invicta*) have been found in Alabama, Mississippi, and Tennessee (Shoemaker et al. 1994). *Solenopsis invicta* causes several billion US dollars in damages annually to agricultural crops, electrical equipment, livestock, and human health risks in the United States (Drees et al. 2002). Chemical baits have been used as a means to control imported fire ants in high traffic areas such as playgrounds and residential lawns (Drees et al. 2002). Unfortunately, chemical treatments tend to be costly and generally need to be used several times a year for adequate levels of control (Collins et al. 1992). A

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<sup>2</sup> In Press, *BioControl* 2004

possible alternative to chemicals that is more environmentally friendly is classical biological control.

In recent years, decapitating phorid flies in the genus *Pseudacteon* Coquillett have been studied extensively as potential biological control agents because many are common parasitoids of imported fire ants (Gilbert and Patrock 2002, Morrison et al. 1997, Porter 1998a, 2000, Vazquez et al. 2004a). *Pseudacteon* flies were first reported to be attracted to *Solenopsis* fire ants by Borgmeier (1921) in Brazil with further studies by Williams (1980). About 20 species of *Pseudacteon* are found in South America that attack fire ants (Porter and Pesquero 2001). Extensive specificity tests in both South America (Folgarait et al. 2002, Porter et al. 1995a) and in the United States (Gilbert and Morrison 1997, Porter and Alonso 1999, Porter 2000, Vazquez and Porter 2004, Vazquez et al. 2004a) have demonstrated high levels of host specificity in *Pseudacteon* species to *Solenopsis* fire ants.

*Pseudacteon tricuspis* Borgmeier was the first species to be successfully released (Porter et al. 1999). These flies were initially released in Texas (Gilbert 1996) in early 1995, but attempts failed probably because low numbers of flies were used and weather conditions were hot and dry. The first successful field release occurred in the late summer of 1997 (Porter et al. 1999) in North Florida. Additional releases had been done throughout various southeastern states (Gilbert and Patrock 2002, Porter et al. 2004). A long-term impact study with *P. tricuspis* found that parasitism pressure from this single phorid species was not a significant regulating factor in fire ant populations indicating that additional species of phorid flies or other natural enemies will be needed (L. Morrison, personal communication).

A second species, *Pseudacteon curvatus* Borgmeier, was collected from black fire ants in Las Flores, Argentina (Porter 2000). *Pseudacteon curvatus* is smaller than *P. tricuspis* and studies on oviposition behavior have shown that female *P. curvatus* flies attack fire ant workers that are significantly smaller than the colony mean (Morrison et al. 1997). Field releases of this species (summer 2000 and fall 2001) were successfully established in Alabama and Mississippi on hybrid fire ants and black imported fire ants, but failed in Florida on red imported fire ants (Graham et al. 2003, Vogt and Street 2003). A new biotype of *P. curvatus* was collected from *S. invicta* fire ants in Formosa, Argentina (October 2001). A series of quarantine host-specificity tests demonstrated that this new biotype was sufficiently host specific to be released in the field (Vazquez et al. 2004a). The objective of this study is to document the release, establishment, and dispersal of *P. curvatus* around Gainesville, Florida.

### **Materials and Methods**

The *P. curvatus* flies released in this study were collected by SDP and JAB in October 2001 from a roadside site about 35 km NW of Formosa, Argentina on route 81 (km 1219; 25° 56.139' S, 58° 30.723' W). The collection procedures were similar to those described by Porter (2000). These flies were then imported to quarantine facilities in Gainesville, FL under a permit from USDA-APHIS. We released these flies in the field after conducting host specificity evaluations on nontarget organisms in our quarantine facility. The flies were released under a previous permit from the Florida Department of Agriculture and the Finding of No Significant Impact (FONSI) issued by the USDA-ARS.

## Release Methods

Immature flies were released in parasitized workers. Fire ant workers were collected from medium to large sized fire ant mounds by shoveling dirt with 5-15 g of ants and brood into a bucket. Mounds were individually marked with numbered flags and/or wooden stakes. Numbers were also painted on the ground near the mounds. Workers were collected from mounds over a 2-3 week period. These workers were then separated from the soil in the laboratory by drip flotation (Banks et al. 1981). Workers were separated from brood using sorting sheets and sieved with a U.S. standard 20-mesh sieve to remove sexuals, queens, and excess large workers (>0.9 mm head widths) not normally parasitized by *P. curvatus*. Groups of 1.0-1.5 g of sieved ants from a single colony together with about 1 g of brood were placed in large attack boxes (244 × 96 × 56 cm) similar to those described by Vogt et al. (2003). Flies were allowed to parasitize the ants for 2-3 days. Parasitized workers were then removed from attack boxes and retained in small containers (20 × 12 × 5 cm) with tight-fitting vented (2 by 3 cm) lids. Brood was removed with sorting sheets if it did not originate from the same colony to avoid potential aggressive interaction as they emerged. To release the ants, the mounds were first disturbed and small containers with 3 small holes in one end were placed near the disturbed area so parasitized workers could recruit back into their mother colony, generally within 5-30 min. The ants were returned to their mother colonies 3-4 days after they were collected. Weather conditions during the releases were sunny and dry. On sunny days, ant mounds were drenched with 200 mL of water and shaded with paper plates to prevent desiccation until ants rejoined their nestmates.

**Release sites**

The first release was conducted at Whitehurst Ranch, on the border of Levy and Marion Counties near Williston, FL. The site is a 220 ha well-managed cow pasture, approximately 15 mi SW of Gainesville, FL with a mixture of monogyne and polygyne imported fire ant colonies. The release site was an area of about 2.5 ha bordering a small pond shaded with pine and oak trees. The number of flies released per group of parasitized workers was approximately 300 per day for 15 days for a total of about 4500 parasitized workers. Releases at this site were conducted in March 2003 using workers from 42 mounds.

A second release was done at Morrill Farm and a third at Mickle pasture. Morrill farm was one of the release sites used for *Pseudacteon tricuspis* (Porter et al. 2004). The site is a 16-ha cow pasture with a mixture of trees, bushes, and two small ponds. The Mickle pasture is a private residential home with a small 3 acre cow pasture. There is one small pond and a mixture of trees where flies were released. I released about 260 flies per day for 3 weeks simultaneously at both the Morrill and Mickle sites for a total of about 5600 parasitized workers at each site. Releases were conducted from May-June 2003.

**Monitoring fly establishment and dispersal**

Monitoring for *P. curvatus* establishment was done by disturbing 4-5 mounds in the release area. Disturbed areas were closely inspected for hovering flies. Flies were easily aspirated with an Allen-type double chamber aspirator and identified with a hand lens. Generally, 4-5 mounds were monitored every 5-10 min over a period of up to 30 min. After 30 min, another set of 4-5 mounds was disturbed and the observation cycle was repeated. After each observation, the ants were stirred periodically to keep them active. If no flies were observed, several pinches of ants in each mound were usually macerated

between the fingers to release pheromones that attract the flies (Morrison and King 2004). Monitoring for flies was generally done between 1130-1630 EST, on days with air temperatures greater than 24 °C when adult flies are active. Sun shades were placed over mounds on hot sunny days so that the ants could remain active on the surface during the monitoring period. On severely hot days, mounds were sprinkled with several liters of water to reduce the heat stress experienced by the ants.

I monitored dispersal from release sites by observing disturbed fire ant mounds at 0.5 mi intervals from the release site. As described above, 4-5 mounds were closely inspected for hovering flies over a period of up to 30 min. After 30 min, another set of 4-5 mounds were disturbed 0.5 mi in either a North, West, or South direction from last observation site. Monitoring for dispersal was conducted up to a period of 2 h per d. Flies were aspirated with an Allen-type double chamber aspirator, retained in small vials, and knocked down with CO<sub>2</sub> for identification with a hand lens.

### **Results**

The field releases at Whitehurst Ranch were successful. First generation flies were found 5 weeks (April 2003) after the initial release (Table 3-1). There was a period of intense rainfall following initial field releases. Initial counts (April-June 2003) of flies per 5 mounds were between 7-18 flies (Table 3-1). In the month of October, large fly population numbers were recorded (Table 3-1). During 2003, I collected many more *P. curvatus* flies than *P. tricuspis* flies (71 versus 9) even though *P. tricuspis* flies had been at the site for several more years (Porter et al. 2004). Fly presence was not monitored during the November and December months due to cold temperatures. *Pseudacteon curvatus* flies successfully over-wintered and I was able to find flies at Whitehurst Ranch

from January to May of 2004. Fly abundances in 2004 have ranged from 27-55 flies per 5 mounds (median = 40).

Flies were also successful at establishing at both Morrill and Mickle sites. In April and May 2004, 5-10 flies were found at the Morrill Pasture and 15-25 flies found at the Mickle site (Table 3-1). Earlier attempts to monitor *P. curvatus* presence at these two sites failed to yield positive results from observations made twice weekly for several months (June-October 2003, Table 3-1). In the summer of 2003, I found large numbers of *P. tricuspis* at the Mickle site (80-110 flies) but no *P. tricuspis* flies were found at the Morrill site in spite of the fact that they had been very abundant in previous years. In April and May 2004, I found a total of 75 *P. curvatus* flies at the Mickle site compared to 12 *P. tricuspis*; at the Morrill site it was 15 to 0 respectively.

In August 2003, flies had dispersed about 200 yd from the original release site at Whitehurst Ranch. In April-May 2004, the flies had expanded 1.6 km both North and South and about 0.8 km in a westward direction (May 2004). To the East of the site is a heavily wooded area that is not accessible to motor vehicles. I found a total of 15 flies on these outer boundaries as well as the presence of a few *P. tricuspis* flies. Dispersal rates for the Morrill and Mickle pastures will be conducted in late summer 2004.

### **Discussion**

This study documents the first successful release and establishment of the decapitating fly *P. curvatus* on red imported fire ants in the United States. A post release specificity test in the fall of 2003 (Vazquez and Porter 2004) with non-*Solenopsis* ants and the native fire ant, *Solenopsis geminata* (Fabricius), confirmed laboratory predictions (Vazquez et al. 2004a) that this biotype from Formosa would be specific to red imported fire ants. First generation flies were found within 5 weeks after release at the Whitehurst

Ranch while it took 8 months for flies to be found at both Morrill and Mickle sites (Table 3-1). Monthly monitoring at the Whitehurst site consistently yielded positive results until cold weather conditions hindered monitoring (Table 3-1). Flies have successfully overwintered at the Whitehurst site by the abundance of many *P. curvatus* flies with small numbers of *P. tricuspis* flies during the months of January through May 2004 (Table 3-1). The confirmation of establishment of *P. curvatus* flies at the Morrill and Mickle sites in late spring 2004 are also reassuring (Table 3-1). Another 2003 release of *P. curvatus* on red imported fire ants appears to have been successful at a site near Columbia, SC (T. Davis and M. Horton, personal communication). Monitoring at the Morrill pasture in April 2004 yielded positive results for *P. curvatus* flies. *Pseudacteon curvatus* flies have been found at larger abundances than *P. tricuspis* at all release sites indicating that *P. curvatus* flies may be a more effective biocontrol agent at least in some sites.

Weather conditions at Morrill and Mickle were very dry when flies were first released (May-June 2003); followed by an extended winter season into the month of February (2004). The combination of hot and cold temperature extremes at both Morrill and Mickle could be the attributing factor why flies were not found until 8 months after release. Studies on *Pseudacteon* parasitoids have shown considerable seasonal variability in population abundances according to species throughout the year (Folgarait et al. 2003, Fowler et al. 1995, Morrison et al. 1999, 2000, Wuellner and Saunders 2003).

Dispersal of flies outside of the Whitehurst site is encouraging in that this confirms these flies are reproducing well and expanding its range. The 1.6 km expansion range, in

the first year observed for *P. curvatus* at the Whitehurst site, is similar to initial field release expansion rates for that of *P. tricuspis* (1.5 km in the first year; Porter et al. 2004).

This fly is another species in our arsenal in controlling red imported fire ant populations. It is unlikely that a single new fly will have a substantial impact on imported fire ant densities in the short term. Nevertheless, it is hoped that several species of decapitating flies combined with other natural enemies from South America will have substantial impacts on imported fire ant populations in North America as they appear to have on fire ants in South America.

Table 3-1. Number of adult *Pseudacteon curvatus* flies found at Whitehurst Ranch, Morrill Pasture, and Mickle Pasture release sites in Florida from April 2003 to May 2004.

Month <sup>a</sup>	Number of Flies (Number of Mounds Checked)		
	Whitehurst Ranch	Morrill Pasture	Mickle Pasture
April 2003	7 (15)	-	-
May 2003	7 (26)	-	-
June 2003	18 (11)	0 (8)	0 (7)
July 2003	15 (10)	0 (10)	0 (10)
August 2003	14 (7)	0 (9)	0 (7)
September 2003	10 (21)	0 (10)	0 (8)
October 2003	305 (26)	0 (8)	0 (8)
January 2004	27 (6)	-	-
February 2004	45 (4)	-	-
March 2004	16 (8)	-	-
April 2004	26 (10)	10 (8)	20 (3)
May 2004	55 (20)	5 (4)	60 (6)

<sup>a</sup> Flies were not checked on November and December of 2003 due to cold temperatures.

CHAPTER 4  
RE-CONFIRMING HOST SPECIFICITY OF THE FIRE ANT DECAPITATING FLY  
*Pseudacteon curvatus* AFTER FIELD RELEASE IN FLORIDA<sup>3</sup>

**Introduction**

Critics of biological control programs have argued that there is a lack of post-release monitoring on nontarget effects arising from released exotic insects. Howarth (1991) stated that negative environmental impacts of biological control introductions have not been well documented. Similarly, others have complained that releases of nonindigenous species on target organisms have led to reduction in populations of nontarget species due to inappropriate protocols on host specificity of these nonindigenous species (Barron et al. 2003, Civeyrel and Simberloff 1996, Hopper 2001, Howarth 1991, Secord and Kareiva 1996, Simberloff and Stiling 1996a, b). However, in spite of these criticisms the biocontrol community appears to have a good record of environmental safety (Lindgren 2003, McEvoy et al. 1991). Similarly, Pemberton (2000) analyzed works dealing with 117 natural enemies of 55 weed species and found that only 1 natural enemy completes development in a nontarget plant. A significant problem appears to be that biocontrol practitioners have not always done an adequate job of documenting the post establishment host specificity of organisms that they release.

However, this problem is beginning to be rectified. For example, post-release monitoring has been done for releases of the chrysomelid beetle *Galerucella californiensis* on purple loosestrife *Lythrum salicaria* (in Michigan; Landis et al. 2003, in Canada;

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<sup>3</sup> In Press, *Florida Entomologist* 2004

Lindgren 2003, in Oregon; Schooler et al. 2003), the fungal pathogen *Neozygites floridana* on the cassava green mite *Mononychellus tanajoa* in West Africa (Hountondji et al. 2002), the parasitoid wasp *Trichogramma brassicae* on the European corn borer *Ostrinia nubilalis* in Switzerland (Kuske et al. 2003), a South American mirid *Eccritotarsus catarinensis* on the waterhyacinth *Eichhornia crassipes* in South Africa (Coetzee et al. 2003), the rubber vine moth *Euclasta whalleyi* on the rubber vine *Cryptostegia grandiflora* in Australia (Cruttwell McFadyen et al. 2002), the tephritid fly *Acinia picturata* on the exotic weed *Pluchea odorata* in Hawaii (Alyokhin et al. 2001), and the melaleuca weevil *Oxyops vitiosa* on *Melaleuca quinquenervia* in Florida (Paul Pratt, personal communication). All of these studies have found minimal or no non-target effects.

The host range of phorid decapitating flies in the genus *Pseudacteon* have been studied extensively prior to field releases as self sustaining biocontrol agents of imported fire ants (Folgarait et al. 2002, Gilbert and Morrison 1997, Morrison and Gilbert 1999, Porter 1998, Porter 2000, Porter & Alonso 1999, Vazquez et al. 2004a). *Pseudacteon tricuspis* Borgmeier flies were successfully established on red imported fire ant populations at eight sites in North Florida (1997-1999; Porter et al. 2004). In the fall of 2003, host specificity of *P. tricuspis* was tested in the field and results demonstrated that these phorid flies had no attraction to non-host organisms including native fire ants (Lloyd Morrison, personal communication). These results are consistent with predictions from quarantine laboratory tests (Gilbert and Morrison 1997, Porter and Alonso 1999) and field tests in South America (Porter 1998) prior to its release in the United States.

A second phorid fly species, *Pseudacteon curvatus* Borgmeier from Formosa, Argentina, was released in Florida to control populations of red imported fire ants, *Solenopsis invicta* Buren (Vazquez et al. 2004b). *Pseudacteon curvatus* is a small decapitating fly that normally parasitizes small red imported fire ant workers. Quarantine-based host specificity testing predicted that this Formosa biotype was highly host-specific to *S. invicta* and that nontarget effects to the native fire ants, *Solenopsis geminata* (Fabricius) and *Solenopsis xyloni* McCook would be minimal to non-existent (Vazquez et al. 2004a). The objective of this paper is to document the host specificity of established field populations of the Formosa biotype of *P. curvatus*.

### Materials and Methods

The *P. curvatus* flies were collected attacking *S. invicta* fire ants 35 km NW of Formosa, Argentina by Sanford D. Porter and Juan A. Briano (October 2001). *Pseudacteon curvatus* was first successfully released and established in Florida at Whitehurst Farm, 15 mi SW of Gainesville, FL in the spring of 2003 (Vazquez et al. 2004b). Field observations of host specificity were made in October 2003 between 1300 and 1530 EST, when the temperatures were > 24°C. I tested the attraction of established *P. curvatus* flies to 15 species of non-*Solenopsis* ants: *Aphaenogaster miamiana* Wheeler (0.8-0.9 mm head width, 0.2 g of workers used), *Aphaenogaster* c.f. *carolinensis* Wheeler (0.7 mm, 0.7 g), *Camponotus floridanus* (Buckley)(2.2 mm, 4 g), *Camponotus impressus* (Roger)(0.7-0.8 mm, 0.6 g), *Crematogaster minutissima* Mayr (0.6 mm, 2 g), *Crematogaster pilosa* Emery (0.7-0.9 mm, 2 g), *Cyphomyrmex rimosus* (Spinola)(0.6 mm, 0.2 g), *Dorymyrmex bureni* (Trager)(0.7-0.9 mm, 0.3 g), *Forelius pruinosus* (Roger)(0.5 mm, 0.3 g), *Linepithema humile* Mayr (0.6 mm, 2 g), *Odontomachus brunneus* (Patton)(1.8 mm, 0.4 g), *Pheidole dentata* Mayr (0.6 mm minors, 1.2 mm

majors, 0.6 g), *Pogonomyrmex badius* (Latreille)(2.1-2.4 mm, 1.4 g), *Pseudomyrmex pallidus* (F. Smith)(0.6 mm, 0.1 g), *Trachymyrmex septentrionalis* (McCook)(0.8-1.0 mm, 0.2 g), and 6 colonies of *S. invicta* (0.6-1.4 mm, 1.5 g) workers. In the laboratory, *P. curvatus* successfully parasitizes *Solenopsis* ants with head widths of 0.6-1.1 mm (median of 0.74 mm; Morrison et al. 1997 and SDP unpublished data). All ant species used in these tests were collected near Gainesville, Florida (September 2003).

Trays with the 15 non-*Solenopsis* ants were set out first. Trays were 40 × 26 × 8 cm in size and contained only one species of ant. The non-*Solenopsis* ants were then removed after 30 min and replaced with the 6 trays of *S. invicta*. At the conclusion of 30 min, the *S. invicta* trays were replaced with the 15 trays of non-*Solenopsis* ants to determine if the flies originally attracted from the *S. invicta* trials would exploit the other genera in the absence of its primary host (no-choice). Established *Pseudacteon curvatus* flies observed hovering in attack mode over each tray were collected at 5 min intervals for 30 min. All flies were aspirated with an Allen-type double chamber aspirator and retained in vials until the conclusion of each 30 min trial when they were identified to species using a hand lens. Aspiration of flies normally does not change attack behavior once flies are released (Morrison et al. 1997). Collection and identification for presence of *P. curvatus* flies was necessary since *P. tricuspis* flies were present at the study site from a release in Gainesville, Florida, in the summer and fall of 1997 (Porter et al. 2004). Sampled flies were then released prior to setting up additional trays. These methods were replicated on two consecutive days.

Further tests of *P. curvatus* host specificity were conducted with five trays of *S. invicta* and five trays of the native fire ant, *S. geminata*. Each tray contained 2 g of

workers and 2 g of brood. As described above, the five trays of *S. geminata* were set out first for 30 min. *Solenopsis geminata* trays were then removed and replaced with the *S. invicta* trays and these trays were observed for 30 min. At the conclusion of 30 min, the five trays of *S. invicta* were replaced again with the five trays of *S. geminata* for an additional 30 min. Attacking flies were collected at 5 min intervals as described above. These methods were replicated on two days (five days apart) at the same site mentioned above.

### Results

The *P. curvatus* flies were not attracted to any of the 15 non-*Solenopsis* genera during the sequential series trials over the two days (Table 4-1). However, the flies were readily attracted to *S. invicta* (99 on day 1 and 38 on day 2, Table 4-1). As is normal, these flies hovered above their host, oriented themselves to workers, and readily struck the thorax of workers during oviposition. When the six *S. invicta* trays were removed and replaced again with the 15 trays of non-*Solenopsis* ants, *P. curvatus* flies were not observed hovering over any of the non-*Solenopsis* trays. *Pseudacteon curvatus* flies were present at all six *S. invicta* trays during the trials.

In the *S. invicta* versus *S. geminata* trials, *P. curvatus* flies were not observed hovering or attacking over *S. geminata* during the first day and only 2-4 flies were observed hovering on the second day (Table 4-1). Flies collected above the native fire ants generally hovered briefly without attacking. Only one fly attempted to oviposit, but it flew away immediately after without returning. In quarantine tests, this biotype would occasionally attack *S. geminata* workers but attacks were never successful (Vazquez et al. 2004). *Pseudacteon curvatus* flies were present at all five *S. invicta* trays during the first day and present at four of five trays on the second day. *Pseudacteon curvatus* flies were

present at none of the five *S. geminata* trays during the first day and at 1 of 5 and 3 of 5 trays on the second day (Table 4-1).

### Discussion

Established *P. curvatus* individuals were attracted to *S. invicta* over *S. geminata* by a ratio of about 30 to 1 (119 to 4 total flies, Table 4-1). These results were better than results predicted from quarantine tests where *P. curvatus* hovered over *S. invicta* versus *S. geminata* at a ratio of 1.3 to 1 in no-choice tests (Vazquez et al. 2004). Perhaps this difference was because *P. curvatus* flies in the laboratory tests were confined in small test containers leading to higher rates of hovering. Furthermore, attacks on *S. geminata* were very rare to non-existent in the field confirming laboratory choice tests where attack rates were 16 times higher for females hovering over *S. invicta* than for flies hovering over *S. geminata* ( $7.02 \pm 1.41$  (mean  $\pm$  SE) versus  $0.44 \pm 0.28$  attacks/min, respectively; Vazquez et al. 2004). I demonstrated in quarantine tests (no-choice and choice) that the Formosa biotype of *P. curvatus* does not complete development in *S. geminata* (Vazquez et al. 2004).

Post-release populations of *P. curvatus* were not attracted to any of the 15 non-host ant genera. In host-specificity tests with a biotype from Las Flores, Argentina, *P. curvatus* hovered over most of 19 non-host genera in quarantine conditions (Porter 2000); however, they generally hovered without attacking and no parasitism occurred in any of the 19 non-host genera (Porter 2000). Results from this study demonstrate that host specificity of *P. curvatus* is restricted to *S. invicta* and poses no realistic threat to the congener *S. geminata* or ants in other genera.

Table 4-1. Number of *Pseudacteon curvatus* flies collected hovering in attack mode over non-host ant species, native fire ants (*Solenopsis geminata*), and red imported fire ants (*Solenopsis invicta*) during sequential series of field trials (see methods).

Ant Species	Flies Collected			Total	Trays Attacked
	0-10 min	11-20 min	21-30 min		
<i>S. invicta</i> vs 15 non-host genera (day 1)					
All 15 genera	0	0	0	0	0/15
<i>S. invicta</i>	14	56	29	99	6/6
All 15 genera	0	0	0	0	0/15
<i>S. invicta</i> vs 15 non-host genera (day 2)					
All 15 genera	0	0	0	0	0/15
<i>S. invicta</i>	7	14	17	38	6/6
All 15 genera	0	0	0	0	0/15
<i>S. invicta</i> vs <i>S. geminata</i> (day 1)					
<i>S. geminata</i>	0	0	0	0	0/5
<i>S. invicta</i>	28	20	18	66	5/5
<i>S. geminata</i>	0	0	0	0	0/5
<i>S. invicta</i> vs <i>S. geminata</i> (day 2)					
<i>S. geminata</i> <sup>a</sup>	0	1	1	2	1/5
<i>S. invicta</i>	14	16	23	53	4/5
<i>S. geminata</i> <sup>b</sup>	1	3	0	4	3/5

<sup>a</sup> No oviposition attempts were observed.

<sup>b</sup> Only one oviposition attempt was observed.

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## BIOGRAPHICAL SKETCH

Ricardo José Vázquez was born on January 14, 1971, in Rio Piedras, Puerto Rico. In 1981, he moved with his family to St. Augustine, Florida. He attended St. Augustine High School and enjoyed being a member of the jr. ROTC department. When he graduated high school in 1989, he enrolled in the United States Marine Corps as an enlisted communications center operator. Through the military, Ricky has traveled to distant lands such as Japan, Korea, and Somalia. After 4 years of honorable service, Ricky enrolled at the St. Johns River Community College in St. Augustine, FL, where he earned an A.A. degree with honors. While at SJRCC, Ricky was president of the Phi Theta Kappa Honor Society. He transferred to the University of Florida to pursue an undergraduate degree in entomology. While at the University of Florida, Ricky gained practical experience in research by working for the University of Florida's Entomology and Nematology Department (urban entomology laboratory) and at the Center for Medical, Agricultural & Veterinary Entomology (USDA-ARS) in Gainesville, Florida. During graduate work, Ricky tutored at risk athletes at the University of Florida and did volunteer work for the Alachua County Fire Rescue Reserves as a first responder. He is a member of the Gamma Sigma Delta Honor Society of Agriculture, Blue Chips and Salsa investment club, Entomological Society of America, Florida Entomological Society, Entomology and Nematology Student Organization (ENSO) and the Urban Entomological Society (UES).