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Field release and establishment of the decapitating fly *Pseudacteon curvatus* on red imported fire ants in Florida

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Abstract. Decapitating phorid flies in the genus *Pseudacteon* are being studied as classical biological control agents of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Pseudacteon curvatus* Borgmeier (Diptera: Phoridae) is a small decapitating fly that attacks small fire ant workers. We released a biotype of *P. curvatus* from Formosa, Argentina, at three sites near Gainesville, FL. Field releases were conducted in the spring and summer of 2003 and monitored monthly. Flies were discovered within 5 weeks at the spring site and then monthly thereafter. By late spring 2004, flies released at this site had expanded 1.6 km both north and south and about 0.8 km westward. Initially, we found no flies from the two summer 2003 releases but we were successful at finding them 8 months after release during spring 2004. This paper documents the first successful release(s) of *P. curvatus* on red imported fire ants in the United States.

Key words: biocontrol, biotype, decapitating phorid fly, *Pseudacteon*, *Solenopsis*

Introduction

Solenopsis invicta Buren and *Solenopsis richteri* Forel (Hymenoptera: Formicidae) are two invasive ant species that have been able to thrive in the United States without their natural enemies. Both species of fire ants were accidentally introduced into the United States through Mobile, Alabama around 1918 (*S. richteri*) and in the 1930s (*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, 1997).

Since introduction, *S. invicta* has spread throughout the entire southeastern United States (Callcott and Collins, 1996) while *S. richteri* along with a hybrid (*S. richteri* × *S. invicta*) have been found in Alabama, Georgia, Mississippi, and Tennessee (Vander Meer et al., 1985; 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 (Pereira 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 are costly and generally need to be used several times a year for adequate levels of control (Collins et al., 1992). Classical biological control is a possible alternative strategy that is more environmentally friendly.

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 in South America (Gilbert and Patrock, 2002; Morrison et al., 1997; Porter, 1998, 2000; Vazquez et al., 2004). *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., 1995) and in the United States (Gilbert and Morrison, 1997; Porter and Alonso, 1999; Porter, 2000; Porter and Gilbert, 2004; Vazquez et al., 2004; Vazquez and Porter, 2005) have demonstrated high levels of host specificity in *Pseudacteon* species to *Solenopsis* fire ants.

Pseudacteon tricuspis Borgmeier (Diptera: Phoridae) was the first species to be successfully released. 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 have 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 did not impact fire ant populations sufficiently to be statistically detected (Morrison and Porter, 2005a), indicating that additional species of phorid flies or other natural enemies will be needed.

A second species, *Pseudacteon curvatus* Borgmeier (Diptera: Phoridae), was collected from black fire ants (*S. richteri*) 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 (2000–2001) were successful in Alabama, Mississippi, and Tennessee on hybrid fire ants and black imported fire ants (Vail et al., submitted; Vogt and Streett, 2003), but failed in Florida on red imported fire ants (Graham et al., 2003). A different 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., 2004). 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 (with *S. invicta* fire ants) about 35 km NW of Formosa, Argentina on route 81 (km 1219; 25° 56.139′ S, 58°30.723′ W). 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 (Vazquez et al., 2004). The flies were released under a permit from the Florida Department of Agriculture and the Finding of No Significant Impact (FONSI) issued by the USDA-ARS.

Release methods

Phorid parasitized fire ant workers were transplanted into the field. Fire ant workers were collected from medium to large sized *S. invicta* 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 cm×96 cm×56 cm) similar to the version described by Vogt et al. 2003). Flies were allowed to parasitize the ants for 2–3 days. Parasitized workers were then removed from the attack boxes and retained in small containers (20 cm×12 cm×5 cm) with tight-fitting vented (2 cm by 3 cm) lids. To release the ants, the mounds were first disturbed and small containers (20 cm×12 cm×5 cm) with three 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. On two of 42 occasions, fire ant colonies had moved after we collected the workers. We were able to re-locate one of these colonies. 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 24 km 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 flies per day for 15 days for a total of about 4,500 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 Morrill 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 1.2 ha cow pasture. There is one small pond and mixture of trees where flies were released. We released about 260 flies per day for 3 weeks simultaneously at both the Morrill and Mickle sites for a total of about 5,600 parasitized workers at each site. Releases were conducted from May to June 2003. Colonies of red imported fire ants at these two sites were a mixture of monogyne and polygyne fire ants.

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 (>35 °C ambient temperature), mounds were sprinkled with several liters of water to reduce the heat stress experienced by the ants.

We monitored dispersal from release sites by observing disturbed fire ant mounds at 0.8 km 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 0.8 km in either a north, west, or south direction from last observation site. In the east direction, there was a dense forest. Flies were aspirated with an Allen-type double chamber aspirator, retained in small vials, and knocked down with CO_2 for identification with a hand lens.

Results

The field releases at Whitehurst Ranch were successful. We were able to find first generation flies 5 weeks (April 2003) after the initial release (Table 1). There was a period of intense rainfall following the initial field releases. Initial counts (April–June 2003) of flies for 52 mounds checked were 32 flies (Table 1). In the month of October, large fly population numbers were recorded from Whitehurst Ranch (Table 1). From April through September 2003, we collected many more *P. curvatus* flies than *P. tricuspis* flies (71 vs. 9) even though *P. tricuspis* flies had been at the site for several more years (Porter et al., 2004). *Pseudacteon curvatus* flies successfully over-wintered, as we

Table 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[1]	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)

[1] Flies were not checked on November and December of 2003 due to cold temperatures.

were able to find flies at Whitehurst Ranch from January to May of 2004. We found 169 flies in 52 mounds during this period. Flies were also successfully established at both the Morrill and Mickle sites. In April and May 2004, 5 and 10 flies were found at the Morrill pasture and 20 and 60 flies found at the Mickle site (Table 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 1). In the summer of 2003, we found large numbers of *P. tricuspis* at the Mickle site (80–110 flies). 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, we found a total of 80 *P. curvatus* flies at the Mickle site compared to 12 *P. tricuspis*. At the Morrill site it was 15 *P. curvatus* and zero *P. tricuspis*.

In August 2003, flies had dispersed about 66 m 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 enclosed within a wooden fence line with no entrance for motor vehicles. We found a total of 15 *P. curvatus* flies on these outer

boundaries as well as collected a few *P. tricuspis* flies (6 total). The other two release sites (Morrill and Mickle) were not inspected for dispersal.

Discussion

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 1). Monthly monitoring at the Whitehurst site consistently yielded positive results until cold weather hindered monitoring (Table 1). Collections of flies during the months of January through May 2004 (Table 1) demonstrated that *P. curvatus* had successfully overwintered. *Pseudacteon curvatus* flies collected at the Morrill and Mickle sites in late spring 2004 confirmed overwintering at these sites as well (Table 1). Another later 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). *Pseudacteon curvatus* flies were found in greater abundances than *P. tricuspis* at all release sites indicating that *P. curvatus* flies may be more effective biocontrol agents at least in some sites. Studies on *Pseudacteon* parasitoids have shown considerable seasonal variability in population abundances throughout the year (Folgarait et al., 2003; Fowler et al., 1995; Morrison et al., 1999, 2000; Morrison and Porter, 2005b; Wuellner and Saunders, 2003).

Dispersal of flies outside of the Whitehurst site confirms that these flies are reproducing well and expanding their 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* (1–4 km range, 1.8 km mean, in the first year; Porter et al., 2004).

This paper 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, 2005) with non-*Solenopsis* ants and the native fire ant, *Solenopsis geminata* (Fabricius), confirmed laboratory predictions (Vazquez et al., 2004) that this biotype from Formosa would be specific to imported fire ants.

This fly is another biotype in our arsenal for controlling red imported fire ant populations. 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 popula-

tions in North America as they appear to have on fire ants in South America.

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