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RELEASE AND ESTABLISHMENT OF THE LITTLE DECAPITATING FLY *PSEUDACTEON CULTELLATUS* (DIPTERA: PHORIDAE) ON IMPORTED FIRE ANTS (HYMENOPTERA: FORMICIDAE) IN FLORIDA

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ABSTRACT

The little decapitating fly *Pseudacteon cultellatus* Borgmeier (Diptera: Phoridae) from Argentina was released as a self-sustaining biological control agent against the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) in Florida to parasitize small fire ant workers associated with multiple-queen colonies. This fly appears to be established at 2 of 5 release sites, but populations still remain very low (≤ 1% of total *Pseudacteon* flies) and localized more than 2 yr after their release. Further releases of *P. cultellatus* in the United States should be limited to a few additional sites in different habitats or climatic regions where this fly might be more successful.

Key Words: *Solenopsis invicta*, biological control, field release

RESUMEN

La mosca pequeña decapitadora *Pseudacteon cultellatus* Borgmeier (Diptera: Phoridae) de Argentina fue liberada como un agente de control biológico autosustentable contra la hormiga de fuego importada, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), en Florida para parasitar obreras pequeñas de esta hormiga asociadas a colonias con múltiples reinas. Esta mosca parece estar establecida en dos de los cinco sitios de liberación, pero la población sigue siendo muy baja (≤ 1% del total de las moscas liberadas) y focalizada más de dos años después de su liberación. Nuevas liberaciones de *P. cultellatus* en los Estados Unidos deberían estar limitadas a unos pocos sitios adicionales en diferentes hábitats o regiones climáticas donde esta mosca podría ser más exitosa.

Palabras Clave: *Solenopsis invicta*, control biológico, liberaciones de campo

Imported fire ants (*Solenopsis* spp.; Hymenoptera: Formicidae) in North America have population densities 5-10 times higher than those normally found in their South American homelands (Porter et al. 1992, 1997). Escape from numerous natural enemies left behind in South America is a likely reason for intercontinental differences in fire ant abundance (Porter et al. 1997).

The little decapitating fly (*Pseudacteon cultellatus* Borgmeier; Diptera: Phoridae; Fig. 1) was selected for release as a self-sustaining biological control agent for imported *Solenopsis* fire ants in the United States because this species parasitizes the smallest sizes of fire ant workers. Small fire ant workers are the most abundant size class in fire ant colonies, especially in young colonies (Porter & Tschinkel 1985a, 1985b) and in multiple-queen or "polygyne" colonies (Greenberg et al. 1985). Polygyne fire ant colonies are found in about 10-15% of the imported fire ant range in the

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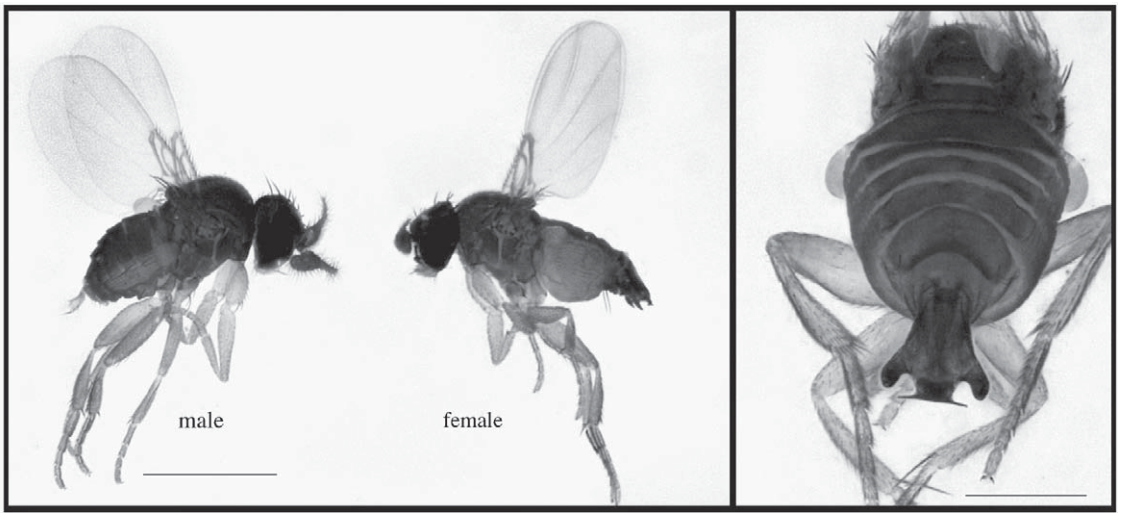


Fig. 1. Male and female *Pseudacteon cultellatus* decapitating flies (left). A posterior view of the ovipositor (right) by which female flies are easily identified (Porter & Pesquero 2001). The male can be distinguished in the United States by its small size, triangular anal tube, and a dark-brown hairy antenna with a narrow curved apical extension. The gray scale line is 0.5 mm (left) and 0.2 mm (right).

United States, except in Texas where 50% of the state has multiple-queen fire ants (Porter et al. 1991; Porter 1992; Porter et al. 1992).

Like other *Pseudacteon* flies, when *P. cultellatus* maggots are ready to pupate, they decapitate their host workers and use the newly emptied host head capsules as pupal cases (Porter 1998). All *Pseudacteon* species that attack fire ants, including *P. cultellatus*, appear to be specific to *Solenopsis* fire ants (Porter & Gilbert 2004; SDP unpublished data).

The natural geographic range of *P. cultellatus* in South America is from Buenos Aires, Argentina, north through Corrientes, Argentina, then northeast into Brazil through the states of Parana and São Paulo to the states of Minas Gerais and Goias (Patrock et al. 2009). In South America, this species is known to attack both red and black fire ants (i.e.; *Solenopsis invicta* Buren and *Solenopsis richteri* Forel) and probably *Solenopsis saevissima* F. Smith as well. The most abundant populations were found in gaps in subtropical gallery forest habitats (Calcaterra et al. 2005). The climatic preferences of *P. cultellatus* flies are rather broad ranging from tropical to temperate; consequently, its potential range in the United States could be anywhere imported fire ants are found.

Five species of phorid decapitating flies have been released and established as fire ant biocontrol agents in the United States: *Pseudacteon tricuspis* Borgmeier (Callcott et al. 2011), *Pseudacteon curvatus* Borgmeier (Callcott et al. 2011), *Pseudacteon litoralis* Borgmeier (Porter et al. 2011), *Pseudacteon obtusus* Borgmeier, (Gilbert et al. 2008; Porter & Calcaterra 2013), and *Pseudacteon nocens* Borgmeier (Plowes et al. 2012).

The objective of this paper is to document the release of *P. cultellatus*, a sixth species of fire ant decapitating fly, at 5 sites in Florida and the establishment of this fly at 2 of these sites.

Materials and Methods

The original *P. cultellatus* flies used in this study were collected in Dec 2006 from a marsh area along the Parana River (~2,700 flies) about 20 km NE of Corrientes, Argentina (S 27.371° W 58.687°) and from a picnic/camping area (~2,000 flies) about 20 km SE of Corrientes (S 27.559° W 58.725°). Most of the host ants at these sites were *S. invicta*, but local biotypes of *S. invicta* were probably not as similar to US biotypes as those found further north along the Paraguay River in the province of Formosa (Ascunce et al. 2011). Immature stages of *P. cultellatus* were carried to a USDA quarantine facility in Gainesville, Florida in parasitized hosts accompanied by an Argentine export permit (DFS N°19517) and a USDA/APHIS import permit (#64943).

Prior to field release in 2010, the flies were mass reared on red imported fire ants from the United States using large attack boxes (1 m wide × 2-2.5 m long × 0.7 m high) with either 8 or 14 smaller attack trays similar to those described by Vogt et al. (2003) except that humidity was controlled by a vaporizer in a chamber mounted under the attack chamber. Every morning, new flies emerged from pupation trays directly into the attack chamber where they could parasitize

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worker ants, which were trailing back and forth between 2 cups as they were automatically raised and lowered in each of the attack trays. Flies were allowed to parasitize workers for 3 or 4 days before the workers were removed. Photographs of attack boxes as well as notes and photographs about rearing procedures can be viewed in Supplementary Material for this article in Florida Entomologist 96(3) (2013) online at http://purl.fcla.edu/fcla/entomologist/browse , as well as at http://www.ars.usda.gov/saa/cmave/ifahi/cultellatus. Extensive care was necessary when rearing *P. cultellatus* to limit contamination from workers already parasitized with *P. curvatus* flies in the field (see Supplementary Material for details).

Fire ant workers used in the attack boxes were obtained from field colonies because workers from laboratory colonies proved unsuitable for fly development if they had been infected by the SINV-3 virus (SDP - unpublished data), which at that time was a major problem in our rearing facility (Porter et al. 2013; Valles & Porter 2013). We used only the smallest fire ant workers for hosts because this improved production efficiency and limited contamination from other *Pseudacteon* species, which parasitize medium- and large-sized workers. A second major rearing problem was that fire ant workers frequently chewed out fire ant head capsules with newly pupated flies, thus killing the pupae and greatly reducing productivity of the fly culture. This problem was mitigated by collecting dead fire ant workers with pupating larvae 6 days a week, using larger holding boxes for parasitized workers, and providing darkened nests for the workers so fewer workers were active in the holding box (see Supplementary Material above).

Field releases were conducted at 5 sites in Florida beginning in the spring of 2010 (Table 1). Sites were selected which contained large populations of fire ants (probably 1500-3000 workers/m2; Macom & Porter 1996) with small workers like those associated with polygyne colonies. The sites were all in open disturbed areas, but releases were conducted using mounds as close as possible to bushes, trees, and water sources to ensure refugia for the fire ants and flies in case of drought or hot weather (Plowes et al. 2011). The 4 releases conducted in 2010 (Table 1) used larvae in parasitized ants as was previously done with *P. curvatus* (for details see: Graham et al. 2003; Vazquez et al. 2006). The 2011 release (Table 1) was done by burying *P. cultellatus* pupae in an insolated box with an exit pipe in the top similar to the method described by Plowes et al. (2012). Our implementation of this method needed improvements because about one quarter of the 13,600 release flies were trapped in the crack between the cooler box and its lid. Also, cool ground temperatures (15-20 °C) in the early spring delayed emergence of the flies longer than was desirable.

TABLE 1. DATA FOR THE FIELD RELEASE OF THE FIRE ANT DECAPITATING FLY *PSEUDACTEON CULTELLATUS* AT FOUR SITES NEAR GAINESVILLE, FLORIDA AND ONE SITE IN MIAMI-DADE COUNTY NEAR MIAMI, FLORIDA.

Site	Coordinates	Release Dates	#Flies Released	Results
UF Organic Gardens	29.6279° N 82.3584° W	19 Apr-2 May 2010 17-30 Sept 2010	14,000	1st Release Failed?
			7,400	Present ≥ 2.5 years
NW of Hawthorne	29.6330° N 82.1492° W	10-27 May 2010	10,000	Several field-reared flies at 5 months
Waldo Ballpark	29.7950° N 82.1703° W	1-18 Oct 2010	12,000	1st generation flies at 5-7 weeks
Miami-Dade Co.	25.5971° N 80.5265° W	23 Nov-13 Dec 2010	12,500	Present > 2 years
Waldo Storage	29.8198° N 82.1691° W	Mar to mid-Apr 2011	8,500	One field-reared fly, ~4 months

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In May 2010, 2 attempts were made to release adult flies over disturbed fire ant mounds as was done with *P. tricuspis* (Porter et al. 2004), but the *P. cultellatus* flies dispersed rapidly (within 5-15 min) without attacking many fire ants so further attempts were discontinued.

Sticky tri-stand traps in 150 × 25 mm Petri dishes were used to monitor the establishment of flies at release sites (Puckett et al. 2007; Porter & Calcaterra 2013). Petri dishes were coated on the inside with a Fluon-like suspension of fluoropolymer resin (Daikin Polyflon PTFE DX-9025; Daikin America, Inc., Orangeburg, New York) and placed in fire ant mounds so disturbed workers would fall into the dish and not be able to escape. Sometimes additional fire ant workers were added from lab colonies if ant numbers were too small to reliably attract phorid flies.

Voucher specimens of *P. cultellatus* and the other *Pseudacteon* species have been deposited in the Florida State Collection of Arthropods, Division of Plant Industries, Florida Department of Agriculture, Gainesville, Florida, and the Museo de Ciencias Naturales de La Plata, Buenos Aires, Argentina.

Results

The little decapitating fly, *P. cultellatus*, was established at 2 of 5 release sites in Florida (Table 1). Flies were first released at the University of Florida Organic Gardens site in the spring of 2010 around the edge of a pond. Several first-generation field-reared flies were recovered in June 2010, but no additional *P. cultellatus* flies were found on 6 subsequent dates in Aug and Sep so an additional fall release was conducted. Several likely first-generation field-reared flies were recovered at the end of Oct 2010. Additional flies have been recovered on 11 of 26 different occasions from Mar 2011 to May 2013, more than 30 months after the fall 2010 release (Table 1).

By the end of the first yr (fall 2011), flies had expanded outward about 200 m from the release site in 3 of 4 directions (E, W, S). No additional expansion was observed at the end of the second yr (fall 2012), but a fly was found out 500 m in the fourth direction (N) in spring 2013. Flies were found on 3 of 8 trap dates in 2012 and 2 of 3 dates for the spring of 2013. The relative abundance of *P. cultellatus* has remained very low at the Organic Garden site from 2010-2013 (0.3-0.7%; Table 2).

Pseudacteon cultellatus was also established at a highly disturbed former mulch site adjacent to a major canal in the Everglades restoration area southwest of Miami, Florida (Table 1). The first *P. cultellatus* fly recovered at this site was in Aug 2011, 8 months after the releases concluded. Extensive trapping in and around this release site on 10 Jan 2012 failed to find either *P. cultellatus* or evidence of its expansion. However, 4 flies were found in Apr 2012 and 6 more flies were found in May 2013 (28 months after the releases). As with the previous site, *P. cultellatus* females only accounted for a small fraction of the total female *Pseudacteon* flies collected (0.2-1.0%; Table 2). We did not attempt to monitor fly expansion out of this release site in 2013 because of logistical constraints.

Releases of *P. cultellatus* appear to have failed at 3 additional sites near Gainesville, Florida (Table 1). Several field-reared flies were trapped at each site 1-5 months after release; however, no additional flies have been found since, despite 13, 9, and 7 additional trapping dates over periods of 29, 21, and 30 months for the Waldo Ballpark, Waldo Storage, and Hawthorn sites, respectively. We were not able to discern any critical habitat differences between the 2 successful and 3 unsuccessful sites. Weather conditions during the releases and monitoring were not unusually extreme.

Trap data from the 4 release sites in the Gainesville area (Table 2) show *P. curvatus* was the most abundant species, accounting for about 91% of total female decapitating flies (range: 83-100% by date) followed by *P. obtusus* with about 8% (range: 0-17%). Like *P. cultellatus, P. tricuspis* was rarely captured at the Gainesville release sites. At the release site near Miami, *P. tricuspis* accounted for about 20% (range 0.6-27%) of total female flies with *P. curvatus* making up most of the remainder (Table 2).

Females accounted for 52% (1,344/2,565) of total *P. obtusus* flies trapped in the Gainesville area while females accounted for only 36% (609/1,674) of total *P. tricuspis* flies trapped at the site near Miami. More than 99% of *P. curvatus* flies trapped were females and all of the *P. cultellatus* flies were females because males of both species do not mate with females while they are ovipositing in fire ant workers.

Discussion

The little decapitating fly *P. cultellatus* appears to be established in Florida because populations have persisted for more than 2 yr at each of 2 release sites (Table 1). The *P. cultellatus* biotype that we released clearly finds *S. invicta* from the United States a suitable host, unlike the *P. curvatus* biotype from black fire ants, which was released 7 times on red fire ants in Florida without any of the releases establishing (Graham et al. 2003). Nevertheless, *P. cultellatus* populations remain very low (Table 2) and expansion rates, so far, appear to be limited to a few hundred m. About 91% of the female flies collected in this study were *P. curvatus*, similar to the results of Porter & Calcaterra (2013) in 2009-2010, while almost all of the remaining females were *P. obtusus*. The once abundant *P. tricuspis* continued to

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TABLE 2. PERCENT SPECIES COMPOSITION OF *PSEUDACTEON* FEMALES TRAPPED DURING MONITORING AT *P. CULTELLATUS* RELEASE SITES SHOWN BY YEAR.

Release Site Year	Sample Dates (Traps)	Total Females Trapped	% of Total Females by *Pseudacteon* Species			
			curvatus	*obtusus*	*tricuspis*	*cultellatus*
UF Organic Gardens						
2010	7 (13)	262	92.0	7.3	0	0.7
2011	11 (63)	3581	91.0	8.5	0	0.5
2012	8 (53)	1835	82.5	17.2	0	0.3
2013	3 (26)	1987	95.8	3.9	0	0.3
NW of Hawthorne						
2010	9 (28)	759	98.0	0.8	0.9	0.3
2011	2 (3)	27	100	0.0	0	0
2012	2 (12)	535	96.8	2.8	0.4	0
2013	3 (24)	1834	97.4	2.6	0	0
Waldo Ballpark						
2010	4 (8)	419	95.0	4.5	0	0.5
2011	8 (22)	1633	96.5	3.5	0	0
2012	1 (3)	144	88.2	11.8	0	0
2013	3 (15)	248	85.1	14.9	0	0
Miami-Dade Co.						
2011	6 (21)	389	82.8	0.0[a]	17.0	0.2
2012	2 (48)	2004	72.9	0.0[a]	26.9	0.2
2013	1(18)	619	98.4	0.0[a]	0.6	1.0
Waldo Storage						
2011	9 (61)	2324	85.2	14.7	0.05	0.05
2012	1 (6)	305	88.5	11.5	0	0
2013	3 (19)	205	82.7	17.3	0	0

[a] *P. obtusus* is not yet established in South Florida

be trapped throughout the study, but only at extremely low levels (Table 2). Curiously, *P. tricuspis* accounted for about 20% of females trapped at the release site near Miami (Table 2), thus demonstrating that, while *P. tricuspis* generally does poorly in competition with *P. curvatus* (LeBrun et al. 2009; Porter & Calcaterra 2013), in some areas and seasons this is not true.

Expansion rates for other *Pseudacteon* species released in the United States have been quite variable with some fly populations showing little or no expansion for 1-3 yr while other populations have expanded several kilometers in the first yr and up to 50 km/yr in subsequent years (Porter et al. 2004; Henne et al. 2007; LeBrun et al. 2008; Porter 2010; Porter et al. 2011). Slow spread of *P. cultellatus* is probably a result of low population densities and perhaps a low natural rate of dispersal. Competition with the very abundant *P. curvatus* is likely a major factor affecting population densities because *P. curvatus* depressed *P. tricuspis* populations in large parts of Texas and Florida (LeBrun et al. 2009; Porter & Calcaterra 2013). Competition with *P. curvatus* and perhaps *P. obtusus* is also a likely reason why we were only able to establish flies at 2 of 5 release sites (Table 1). Plowes et al. (2011) report that success rates of establishing *P. obtusus* in Texas were reduced by the presence of *P. curvatus* and *P. tricuspis* at their release sites.

The ultimate fate of the *P. cultellatus* releases in Florida is unknown. Drought or other extreme weather conditions combined with strong competition from pre-existing species may eventually lead to extinction of what are currently small and localized populations. If *P. cultellatus* is able to disperse from our release sites, it may remain as a rare species in most areas with some locally abundant populations as is currently the case for *P. litoralis* in Alabama (Porter et al. 2011). After passing through a latent phase (Henne et al. 2007), *P. cultellatus* may eventually become a more abundant fly once it adapts to local conditions and fly populations become large enough to ensure easy mating and dispersal. Populations of a *P. curvatus* biotype from red fire ants remained so low at 2 release sites in Florida that none were recovered for almost a yr; however, eventually their numbers increased dramatically and rapid expansion followed (Vazquez et al. 2006; Porter 2010). Similar results have also been reported for *P. tricuspis* in Louisiana (Henne et al. 2007). The prediction was that *P. cultellatus* would do especially well with polygyne fire ant hosts because polygyne colonies have much smaller workers on average than monogyne colonies (Greenberg et al. 1985). Current data is not sufficient to evaluate this prediction; however, the presence of colonies with small workers at each of the release sites did not ensure successful establishment. The release

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site near Miami contains what appear to be mostly polygyne colonies with a predominance of small workers, but the Organic Garden site in Gainesville has become almost entirely monogyne since the original releases.

Around Corrientes, Argentina, *P. cultellatus* is only locally abundant where it was originally collected. Differences in habitat, hosts, or the presence of small polygyne workers are possible explanations. However, the relative abundance of *Pseudacteon* species in South America is not correlated well with the abundance of *Pseudacteon* species, which have been established in the United States (Porter & Calcaterra 2013). *Pseudacteon cultellatus* is currently being reared by USDA/APHIS for release in additional states in hopes that it may do better in other habitats or climates. Nevertheless, only a few more releases are probably justified unless at least some of these releases result in vigorous localized *P. cultellatus* populations.

To date, 6 species of *Pseudacteon* decapitating flies have been released against the red imported fire ant (*S. invicta*) in the United States and each have shown different levels of establishment and dispersal. While additional candidate species remain in South America (Patrock et al. 2009), *P. cultellatus* will probably be the last *Pseudacteon* species our group will release against *S. invicta*. Most of the remaining species are either too rare to be reliably collected, too difficult to rear in quarantine, and/or of questionable host association with the US biotype of *S. invicta*. Several small *Pseudacteon* species, which attack fire ants along trails, may be useful if other research groups can find effective ways to rear and/or release them in the United States. We are still contemplating releasing 1-2 *Pseudacteon* species against black imported fire ants (*S. richteri*) and hybrid fire ants in Tennessee and the northern parts of Mississippi, Alabama, and Georgia, but our research efforts are beginning to shift to other potential groups of agents, especially viruses and microsporidian pathogens (Poinar et al. 2007; Oi et al. 2010; Valles 2012; Porter et al. 2013).

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