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THE LARGE DECAPITATING FLY *PSEUDACTEON LITORALIS* (DIPTERA: PHORIDAE): SUCCESSFULLY ESTABLISHED ON FIRE ANT POPULATIONS IN ALABAMA

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ABSTRACT

The large fire ant decapitating fly, *Pseudacteon litoralis* Borgmeier, from northeastern Argentina was successfully released as a self-sustaining biocontrol agent of imported fire ants in south central Alabama in 2005. Five years later, this fly is firmly established at the original release site and has expanded outward at least 18 km. Nevertheless, populations remain very low considering *P. litoralis* is one of the most abundant fire ant decapitating flies in large areas of its range in South America. The reasons for low densities and why we were only able to establish this fly at 1 of 9 release sites in 4 states (2003-2006) are unknown, but problems with host-matching, release procedures, weather conditions, and competition with previously released decapitating flies are discussed as possible factors.

Key Words: *Solenopsis invicta*, biological control, low population density

RESUMEN

La mosca grande del norte y centro-este de Argentina, *Pseudacteon litoralis* Borgmeier, decapitadora de la hormiga de fuego (hormiga brava), fue liberada exitosamente como agente de control biológico de la hormiga de fuego importada en el sur-centro de Alabama en 2005. Cinco años después, esta mosca se encuentra firmemente establecida en ese sitio y se ha expandido al menos 18 km; sin embargo, las poblaciones permanecen muy bajas considerando que *P. litoralis* es una de las moscas decapitadoras de hormiga de fuego más abundante en su área de América del Sur. Se desconocen las razones de las bajas densidades y el por qué del establecimiento de esta mosca en sólo uno de los nueve sitios de liberación en cuatro estados (2003-2006), pero se discuten como posibles factores los problemas de correspondencia de hospederos, procedimientos de liberación, condiciones climáticas y competencia con moscas decapitadoras liberadas previamente.

Translation provided by the authors.

The decapitating fly *Pseudacteon litoralis* Borgmeier (Fig. 1) is a parasitoid of the red imported fire ant, *Solenopsis invicta* Buren, the black imported fire ant, *Solenopsis richteri* Forel, and 3 other species of *saevissima* complex fire ants in southern Brazil, Paraguay, and northern Argentina (Patrock et al. 2009). *Pseudacteon litoralis* is the largest of the common *Pseudacteon* species that attack fire ants and specializes in parasitizing the largest sizes of fire ant workers (Morrison et al. 1997). It is active throughout the daylight hours, but prefers dawn and especially dusk (Pesquero et al. 1996). As with several other *Pseudacteon* phorids (e.g., *P. tricuspis* and *P. nocens*), sex is probably determined environmentally, primarily by the size of the host, rather than genetically like most other insects (Morrison et al. 1999). Males of *P. litoralis* are not attracted to fire ant mounds like *P. tricuspis* and *P. obtusus* (Porter & Pesquero 2001; Calcaterra et al. 2005). In the lab, mating appeared to occur on and around black objects in the top of the large attack boxes (SDP, unpubl. obs.). This fly is one of the most abundant fire ant decapitating flies throughout much of its range in South America both numerically and spatially (Calcaterra et al. 2005; Patrock et al. 2009, personal observations, SDP). Like other species in the genus, *P. litoralis* is

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Fig. 1. Female *Pseudacteon litoralis* fly preparing to oviposit in the thorax of a fire ant worker.

highly host-specific (Porter & Gilbert 2004; Weissflog et al. 2008) probably because these flies use fire ant alarm pheromones to find their hosts (Vander Meer & Porter 2002) and also because of their highly specialized life history of decapitating fire ant workers and then pupating inside their empty head capsules (Porter et al. 1995).

The characteristics discussed above made *P. litoralis* an attractive target for release as a self-sustaining or classical fire ant biological control agent. The objectives of this paper are to document the release and establishment of *P. litoralis* in south central Alabama and to describe the fate of 8 additional field releases conducted in Florida, Mississippi, and Louisiana from the spring of 2003 to the summer of 2006.

Materials and Methods

The original source population for the *P. litoralis* flies discussed in this paper was from several sites just off Route 11 about 6 kilometers south of San Justo, Santa Fe, Argentina (30.550°S, 60.607°W). About 1,800 fire ant workers parasitized with *P. litoralis* were brought back to Gainesville, FL in Apr 2001. The fire ants at the collection sites were *S. invicta*, although probably not the same biotype as that found in the United States (Ross & Trager 1991; Caldera et al. 2008). By the summer of 2001 the newly established *P. litoralis* laboratory colony had dropped to about 1000 individuals (about 20-30 pupae per day, assuming a 40-d life cycle) and remained at this level through the end of 2001, after which numbers began to gradually increase. In the winter of 2002, 100 or so males were added to the San Justo colony from a collection site on the Paraguay River near Herradura, Formosa, Argentina (26.514°S, 58.284°W). The *S. invicta* ants at this site were probably more similar to the U.S. biotype, but still not quite the same. By the time releases had begun in the spring of 2003 the colony was producing about 500 pupae per day. Maximum production was about 1,000 pupae per day in Jan 2006.

Releases were conducted at sites where fire ants were abundant (Table 1). We selected sites with a large percentage of monogyne colonies because monogyne or single-queen fire ant colonies have a higher percentage of the larger workers preferred by *P. litoralis* females (Morrison et al. 1997). Most sites were near water sources and had patches of tall grass or shrubbery that was assumed to help protect fly pupae from being killed in the sun. All of the sites were pastures except the Florida Ironwood Golf Course (Table 1) which was a mixture of fairways, lake edges, and service roads along drainage canals. The Alabama release site (Table 1) was drenched by Hurricane Dennis just before the final groups of parasitized ants were released in Jul 2005.

Table 1. Field release data for the fire ant decapitating fly *Pseudacteon litoralis*.

Site	County, State	Start Date	Duration (days)	Number Released	Fate
Mickle Farm	Alachua, FL	May 2003	~3	~150[a]	Failed
Morrill Farm	Alachua, FL	15 May 2003	12	2,400[a]	Failed
Whitehurst Farm- A[f]	Marion, FL	15 Sep 2003	21	4,500[b]	Failed[c]
Knox Site[d,f]	Clay, MS	4 Aug 2004	20	6,400[b]	Failed
Whitehurst Farm- B[f]	Levy, FL	25 Apr 2005	27	5,200[b]	Failed
Ironwood Golf Course[f]	Alachua, FL	10 May 2005	32	4,800[b]	Failed[c]
Biddle Farm	Wilcox, AL	21 Jun 2005	18	4,600[b]	Established
Idelwilde Res. Station	E. Feliciana, LA	15 May 2006	20	5,200[b]	Failed
Morrill Farm[f]	Alachua, FL	18 May 2006	44	17,200[e]	Failed

[a]Adult flies released over disturbed mounds.
[b]Estimated parasitized fire ant workers.
[c]First-generation adult flies recovered at release site.
[d]Fire ants at this site were primarily hybrids (black × red); ants at all the other sites were the red imported fire ant, *S. invicta*.
[e]Adult flies emerged from pupae in shaded emergence box in field.
[f]Sites where *P. curvatus* flies were established prior to the release of *P. litoralis*, *P. tricuspis* was previously established at all sites except the Mississippi site.

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Competing *P. tricuspis* flies were present at all of the *P. litoralis* release sites except the Mississippi site where *P. tricuspis* had been unable to establish on the hybrid fire ants (Table 1). At the time *P. litoralis* was released, *P. curvatus* flies were not present at the Mickle and Morrill release sites in Florida, the Louisiana site, or the Alabama site (until 2007).

The *P. litoralis* flies were released at the first 2 sites (Table 1) as adult flies over disturbed fire ant mounds as was the procedure for *P. tricuspis* (Porter et al. 2004). However, only a few of the females were observed to hover over and attempt to oviposit in the disturbed workers. The next 6 releases (Table 1) were conducted by releasing workers parasitized in the laboratory back into their mother colonies as described for *P. curvatus* (Vazquez et al. 2006). The hope was that emerging females would naturally mate with nearby males and then be attracted to attack fire ant workers. At the final site (Table 1), pupae on moist plaster trays were placed inside a large emergence box (61 by 41 by 51 cm; height, width, depth) in the field. This was done several days before the pupae were due to emerge. The box was shaded to prevent overheating and placed on a stand coated with Fluon to limit access for ants and other arthropods. Upon emergence, the flies flew to the light and exited through window screen that protected the pupae from access of larger organisms. Average emergence rates of adult flies from pupae in this box was 84%, a value comparable to that achieved with good rearing procedures in the laboratory.

Initial surveys to determine whether the flies had established were usually conducted in the late afternoon or early evening by disturbing several mounds at or near the release site and aspirating all flies that were attracted to the mounds (Porter et al. 2004; Vazquez et al. 2006). Beginning in 2006, most surveying in Florida was accomplished with sticky traps (baited with live ants) supplemented by aspiration (Puckett et al. 2007; Porter 2010). Sticky traps baited with either live ants or freeze killed ants were also tried in Alabama in 2008. We did not conduct prerelease surveys to detect the presence of *P. litoralis* at our release sites because none of the 20 or so South American *Pseudacteon* species that attack red imported fire ants have ever been found in North America (unless they were intentionally released) despite extensive collections and observations over many years (Porter et al. 2004; Patrock et al. 2009; Porter 2010; Plowes et al. 2011).

Results

The decapitating fly *P. litoralis* only became established at the release site in Alabama (Table 1). This site was a series of small weedy pastures encircled by trees and shrubbery (~7 ha). Releases were conducted in overgrown areas near the tree lines of the pastures. The first *P. litoralis* fly was recovered at this site on 20 Jun 2006. This collection occurred a year after the release even though sampling had been conducted several times previously in both 2005 and 2006. The next flies were detected a year later on 23 Jul (2 flies) and 31 Jul 2007 (7 flies). In 2008 (Jun and Jul) 3 years after the release, *P. litoralis* flies were collected with aspirators at 5 sites: the release site (1 fly), 6 km south (1), 11 km south (2), 6 km west (1), and 18 km west (1). In the summer 2008 (Jun and Jul), sticky traps were placed every half mile along road right-of-ways for 10 miles in each of the 4 cardinal directions (80 total traps) for the sole purpose of monitoring *P. litoralis* expansion. This was repeated 3 times. Many *P. curvatus* and *P. tricuspis* flies were found on the traps, but no *P. litoralis* flies. In Jun 2009, single flies were collected 2, 6, and 14 km north of the release site. In Jul and Aug 2010, a total of 7 flies were collected on 3 different occasions at the release site. Throughout this period, abundance of *P. litoralis* was always low; *P. litoralis* was not collected at most of the sites surveyed, and they were generally found in only a small fraction of disturbed mounds inspected. However, 113 flies were aspirated at the release site in the early morning on 16 Sep 2010, an abundance that is equivalent to high densities of this species in South America. To date, all *P. litoralis* in Alabama have been collected with aspirators.

First generation, field-reared *P. litoralis* females were found about 6 weeks after 2 of the 6 Florida releases (Table 1). Unfortunately, repeated monitoring (2003-2010) failed to detect any additional flies, including in the fall of 2010 when 4 sites near each of the 3 major release areas were checked twice for *P. litoralis* flies (Sep and Oct, 74 total mounds). The Louisiana site was first sampled 4 months after the release (Sep 2006). This release site was rechecked twice in 2009 (Apr and Sep) and twice in 2010 (Apr and Sep) without finding *P. litoralis*. Five other sites were sampled near the release site (1.6-5.2 km away) in 2009 (Apr and Sep) and again in 2010 (Apr and Sep). Ten mounds were inspected at each of the Louisiana sample sites, but no *P. litoralis* flies were collected even though both *P. curvatus* and *P. tricuspis* flies were collected. Flies also were not detected at the Mississippi site which was checked 11 times after the release (Sept-Nov, 2004) and once in Jul 2005, almost a year after the release. Three locations near the Mississippi site were checked in Sep 2010, but only a few dozen *P. curvatus* flies were found.

Discussion

The large decapitating fly, *P. litoralis*, is firmly established on red imported fire ants in south

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central Alabama. Populations of this species are generally low, but they have survived through 5 winters and they have expanded at least 18 km from the release site. This makes *P. litoralis* the third decapitating fly species released and successfully established on imported fire ant populations in the United States. The first 2 *Pseudacteon* species, *P. tricuspis*, and *P. curvatus* were released at numerous sites across the Southeast and currently cover about 65% and 90% of the imported fire ant range in the United States, respectively, (Callcott et al. 2011). A fourth *Pseudacteon* species, *P. obtusus,* has been established in Texas and Florida (Gilbert et al. 2008; SDP) and a fifth very small species, *P. cultellatus,* is currently being released in Florida (SDP). In addition to the flies mentioned above, several other parasitic arthropods (Williams et al. 2003), 2 species of mermithid nematodes (Poinar et al. 2007), 2 species of microsporidian pathogens, and at least 3 kinds of viruses, are being investigated as potential fire ant biocontrol agents (Oi & Valles 2009).

The expansion rate of *P. litoralis* from the release site in Alabama has proven difficult to monitor because low densities make this fly difficult to detect at sample sites. Despite low densities, the rate of expansion for *P. litoralis* in Alabama is similar to expansion rates reported for *P. tricuspis* in Texas and Louisiana, but probably less than the very abundant *P. curvatus* in Florida and Mississippi (Henne et al. 2007; Porter 2010). The low densities of *P. litoralis* at sites in Alabama is curious because *P. litoralis* is consistently one of the most abundant decapitating flies across most of its range in South America both numerically and spatially (Calcaterra et al. 2005; Patrock et al. 2009). The large number of flies recently collected (Sep 2010) from the release site is encouraging, but it is unknown whether this represents a new trend or is just a temporal quirk.

The apparent failure to establish *P. litoralis* at the other 8 sites was disappointing. We made releases at sites with a variety of habitats and climates in hopes that variety would increase the probability of success. The Mississippi site was chosen in hopes that the flies might do better on the *S. invicta* x *S. richteri* hybrid fire ants found at that site.

It is possible that populations have been established at some sites listed in Table 1, but densities are still too low to be easily detected, as has occurred on several occasions with *P. curvatus* (Graham et al. 2003; Vazquez et al. 2006). Nevertheless, this possibility seems unlikely at the Florida, Louisiana, and probably Mississippi sites considering the frequency and duration of the sampling efforts in those areas.

Repeated failures to establish *P. litoralis* in the field is reminiscent of failures to establish *P. curvatus* collected from black fire ants in South America on red fire ants in the United States (Graham et al. 2003; Callcott et al. 2011). Perhaps a biotype of *P. litoralis* better adapted to the biotype of red imported fire ants found in the United States would have been more successful. However, we tried twice to establish additional laboratory colonies of *P. litoralis* from flies collected along the Parana River near Herradura, Formosa, Argentina (Apr 2003, 314 flies; Dec 2005, 1400 flies). Unfortunately, both attempts failed as did other attempts to culture *P. litoralis* flies collected in Saõ Paulo State, Brazil (1997) and the Corrientes area of Argentina (2004-2006). Exactly why we were able to culture the flies collected from San Justo, but not the *P. litoralis* flies collected elsewhere is unknown, although it may be related to problems with mating since the adult females seemed to be attracted normally to the fire ant workers we provided to them in the laboratory attack boxes.

While poor host matching may have been a problem, other factors may also have been important in the failure of *P. litoralis* to establish at some of release sites, especially since they did establish in Alabama and thus should have been able to be established elsewhere on *S. invicta* fire ants. Competition with previously released species is one likely explanation. Our colleagues in Texas provide strong evidence that the presence of *P. curvatus* at their release sites greatly diminished the success rate of establishing *P. obtusus* (Plowes et al. 2011). Similarly in Florida, competition between *P. curvatus, P. tricuspis,* and the recently released *P. obtusus* appears to be greatly reducing *P. tricuspis* populations (SDP and Lu, unpublished). However, competition with *P. curvatus* was not a problem with the first 2 releases in Florida or with the releases in Alabama and Louisiana because *P. litoralis* was released at these sites before *P. curvatus* was present.

Poor weather conditions may have been another factor at some of the failed sites. Examination of release records for *P. tricuspis* (Callcott et al. 2011) indicates that summer releases were about half as successful as releases in the spring or fall. Five of the 9 *P. litoralis* releases, including the successful one in Alabama (Table 1), were at least partly carried out during hot summer months (although rain and clouds from Hurricane Dennis likely reduced negative impacts of summer heat for the Alabama release). Another possible problem is that U.S. fire ant populations may not have enough major workers to sustain large numbers of *P. litoralis*, but intercontinental comparisons of worker polymorphism have not been done to see if this is a real concern. Certainly, U.S. fire ant colonies do have many workers in the size range which *P. litoralis* prefers to parasitize (Porter & Tschinkel 1985; Morrison et al. 1997; Morrison et al. 1999). Poor release technique is another explanation. This would certainly seem to be true for the first 2 releases, because the adult

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flies did not show much interest in the disturbed fire ant mounds and very few flies were used at the first site. The large release box used in the last release was an effort to try something different than what had previously been done. The lack of any first-generation field-reared flies at this release site was disappointing considering the number of flies released and the extended period of the release.

In the fall of 2006, we made the decision to focus on other biocontrol agents with higher probabilities of success. Nevertheless, *P. litoralis* is firmly established in Alabama and will presumably expand into other states. While *P. litoralis* was locally abundant on one occasion in 2010, it failed at most of the release sites and remained rare in Alabama over most of the last 5 years, a curious situation considering *P. litoralis* is one of the most abundant species of fire ant decapitating flies throughout most of its range in South America (Calcaterra et al. 2005; Patrock et al. 2009).

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