FIELD RELEASES OF THE DECAPITATING FLY *PSEUDACTEON CURVATUS* (DIPTERA: PHORIDAE) FOR CONTROL OF IMPORTED FIRE ANTS (HYMENOPTERA: FORMICIDAE) IN ALABAMA, FLORIDA, AND TENNESSEE

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ABSTRACT

The little decapitating fly, *Pseudacteon curvatus* Borgmeier, was released at 11 sites in Alabama, Florida, and Tennessee as a potential self-sustaining biocontrol agent of imported fire ants. We used a biotype from Buenos Aires Province, Argentina that parasitizes black fire ants (*Solenopsis richteri* Forel). Generally, several thousand flies were released as larvae in parasitized ant workers over a 1-2 week period. *Pseudacteon curvatus* flies were successfully established on hybrid fire ants (*Solenopsis invicta* × *Solenopsis richteri*) at a site near Talladega, Alabama where they have persisted more than two years and expanded out 5-20 km from the original release site. Flies failed to establish in Florida and Tennessee although a few 1st-generation field-reared flies were recovered at four sites in Florida. This fly is only the second parasitoid species to be successfully released against imported fire ants or any other pest ant species. Possible reasons for failures at the other sites include insufficient vegetation cover, competition with another *Pseudacteon* species in Florida, severe winter kill of ants at a site in Tennessee, and the possibility that the biotype of *P. curvatus* released was not a viable parasitoid of red imported fire ants.

Key Words: *Solenopsis richteri*, *Solenopsis invicta*, hybrid fire ants, classical biocontrol, parasitoid, southeastern United States

RESUMEN

La mosca pequeña decapitadora *Pseudacteon curvatus* Borgmeier fue liberada en 11 sitios en Alabama, Florida y Tennessee como un agente auto-sostenible de control biológico de la hormigas de fuego importadas. Usamos un biotipo de la Provincia de Buenos Aires, Argentina que parasita las hormigas negras de fuego (*Solenopsis richteri* Forel). Generalmente, varios miles de moscas fueron liberadas como larvas dentro de las hormigas trabajadoras en un periodo de 1-2 semanas. Las moscas *Pseudacteon curvatus* fueron exitosamente establecidas sobre unas hormigas de fuego híbridas (*Solenopsis invicta* × *Solenopsis richteri*) en un sitio cerca de Talladega, Alabama en donde persistieron por más de dos años y se expandierón 12 km del sitio original donde fueron liberadas. Aunque hormigas de la primera generación criadas en el campo fueron colectadas en cuatro sitios en Florida, las moscas no pudieron establecerse en Florida y Tennessee. Esta mosca es solamente el segundo parasitoide de uso en control biológico que há sido exitosamente liberado para el control de las hormigas importadas de fuego o cualquier otra especie de hormiga. Posibles razones por el fallo en otros sitios incluye insuficiente cubrimiento de vegetación, competición con otras especies de *Pseudacteon* en Florida, una muerte severa invernal de las hormigas en un sitio en Tennessee, y la posibilidad de que el biotipo de *P. curvatus* que fue liberado no es un parasitoide viable de las hormigas rojas de fuego importadas.

Translation provided by Demian Kondo.

When the black imported fire ant, *Solenopsis richteri* Forel, and the red imported fire ant, *Solenopsis invicta* Buren, were accidentally introduced into the United States more than sixty years ago, almost all of their natural enemies were left behind in South America (Jouvenaz 1990). The absence of natural enemies is a likely reason why 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). Importation of some of the natural enemies left behind in South America (Williams et al.

2002) will hopefully tilt the ecological balance in favor of our native ants (Porter 1998b). If that happens, imported fire ant populations in the United States may decrease to levels similar to those in South America where fire ants are not considered a major problem.

Phorid flies in the genus *Pseudacteon* are one group of natural enemies that have shown some promise in the battle against fire ants (Porter 1998b; Folgarait & Gilbert 1999). One species, *Pseudacteon tricuspis* Borgmeier, is already permanently established in *S. invicta* populations around Gainesville, FL (Porter et al. 2003), five sites in Alabama (Graham et al. 2001), plus additional sites in at least six other states. *Pseudacteon curvatus* Borgmeier is a second species of particular interest. It has been reared in large enough numbers for inoculative releases (Vogt et al. 2003) and it attacks only small fire ants (Morrison et al. 1997). Consequently, it has the potential of greatly complementing the impacts of *P. tricuspis* which only attacks medium and medium-large fire ants (Morrison et al. 1999).

Pseudacteon flies are very host specific (Porter et al. 1995; Porter 1998a; Porter & Alonso 1999; Gilbert & Morrison 1997; Morrison & Gilbert 1999). *Pseudacteon curvatus* can complete development in several species of fire ants in Argentina (Fogarait et al. 2002) , but Porter (2000) showed that *P. curvatus* flies from south of Buenos Aires, Argentina were entirely specific to *Solenopsis* fire ants. Additional tests demonstrated that these flies could develop in native fire ants, but at a much lower rate than in imported fire ants (Porter 2000). However, Porter (2000) argued that *P. curvatus* are most likely to benefit native fire ants because these flies are a much greater threat to imported fire ants, which are the number one enemy of native fire ants. A series of host preference tests revealed that *P. curvatus* preferred *S. richteri* and hybrid fire ants when tested against *S. invicta* (Porter & Briano 2000). This preference for black fire ants was not surprising because the biotype of flies tested originally came from an area with black fire ants. However, it was interesting that parasitism rates were not significantly different among red, black and hybrid fire ants in no-choice parasitism tests (Porter & Briano 2000).

The objective of this study was to determine if *P. curvatus* could be successfully released as a biological control agent against imported fire ants in the United States. We were particularly interested in determining whether this fly could be established on populations of red imported fire ants, black imported fire ants and/or their hybrid. This information was especially important for Alabama and Tennessee. About half of Alabama is occupied by black and hybrid fire ants and Tennessee appears to be occupied mostly by either hybrid or black imported fire ants.

Materials and Methods

The *P. curvatus* flies used in this study were reared at the USDA-ARS laboratory in Gainesville, FL on red imported fire ants. They were collected from El Toro Ranch east of Las Flores, Buenos Aires Province, Argentina in March of 1997 (Porter 2000). Approximately 1000 parasitized *Solenopsis richteri* ants were originally imported into the Center for Medical, Agricultural and Veterinary Entomology (CMAVE) quarantine facilities in Gainesville, FL.

The flies were released from quarantine in the Fall of 1999 after S.D. Porter obtained permission from the State of Florida and a Finding Of No Significant Impact (FONSI) was issued by the USDA-ARS concerning possible negative impacts on the environment. The FONSI was issued after a select panel of scientists unanimously recommended release. Subsequently, the USF&WS, Region 4 was consulted about the release. State permits were also obtained for field releases in Alabama (K. Flanders) and Tennessee (R. M. Pereira).

Alabama Site

The release site in Alabama was between Atlanta, GA and Birmingham, AL, approximately 3 km east of Talladega, AL (Table 1). Ants at this site were hybrids of *S. invicta* and *S. richteri* as determined by their dark color and cuticular hydrocarbon pattern (Vander Meer et al. 1985). The release site was a 3.6 ha triangular cattle pasture with tall grass and areas of woody shrubs and brambles surrounded on respective sides by a pine forest, a county road, and a small stream. The site gradually sloped down from a well-drained corner to swampy area near the stream.

Mounds at the release site were individually marked with numbered flags. Because cattle tend to pull up flags, a circle was painted on the ground surrounding the mound and a number was painted near the mound. Approximately five grams of workers were collected from each mound and placed into tightly sealed, vented, plastic containers labeled to correspond with the mound numbers. The containers with workers inside were placed into cloth containment bags inside a styrofoam cooler with an ice pack and shipped overnight to the ARS lab in Gainesville, FL on the same day that the ants were collected. Workers were collected from 13 mounds on 8 May 2000, 6 mounds on 10 May, 6 mounds on 17 May, and 13 mounds on 23 May, for a total of 38 mounds over a 15-day period.

Upon receipt in Gainesville, the workers were removed from the containers and allowed to crawl through a number 20 sieve to remove workers too large to be parasitized. One or more groups of 1.0 g of workers were weighed from each colony and each was added to about 1.0 g of brood. Brood was

TABLE 1. RELEASES OF THE DECAPITATING FLY *PSEUDACTEON CURVATUS* AT SITES IN ALABAMA, FLORIDA, AND TENNESSEE.

Site (release dates[1])	Parasitized[2] workers	Colonies used[3] (Gyny[4])	Site description
Alabama			
Talladega (13-28 May 2000)	2000	38 HIFA (mono)	Pasture with tall grass, well drained to wet
Florida			
CMAVE (17-31 Jan 2000)	5000	14 RIFA (both)	Around marshy retention pond
Hogtown Cr. (2-14 Feb 2000)	6000	18 RIFA (mono)	Power line right of way
Sanders Farm (16-28 Feb 2000)	7000	17 RIFA (mono)	Suburban horse and cattle pasture
Morrill Farm (1-13 Mar 2000)	7000	17 RIFA (mono)	Cattle pasture with trees and pond
UF Gardens (5-23 May 2001)	1100[5]	~25 RIFA (poly)	Roadside and drying sinkhole pond
Natural Area (~10-24 Aug 2001)	>6000	~35 RIFA (poly)	Lawn and around large retention pond
UF Beef Res. Unit (7-14 Sept 2001)	1800	25 RIFA (poly)	Edge of cattle pasture
Tennessee			
Ball Farm, Cleveland (15-29 Apr 2000)	4500	35 HIFA (mono)	Hilly cattle pasture, well drained, rocky
Ames Plantation (31 May-20 Jun 2000)	~4000	27 BIFA (mono)	Cattle pasture in woods with pond
Madisonville (26 Sep-11 Oct 2000)	~4000	23 HIFA (mono)	Edge of large corn/pasture rotation

[1]Dates during which parasitized ants were released back into their colonies; these dates are 3-7 days after the ants were collected.
[2]Approximate number of parasitized workers released, estimated from the number of flies produced from red imported fire ant workers that were retained to maintain colony production.
[3]The number and kind of fire ant colonies at each site that were used for releases; RIFA = Red Imported Fire Ant (*Solenopsis invicta*), HIFA = Hybrid Imported Fire Ant (Red × Black fire ants), BIFA = Black Imported Fire Ant (*Solenopsis richteri*)
[4]"Gyny" indicates whether colonies at the release site were monogyne (mono) with a single queen or polygyne (poly) with multiple functional queens.
[5]Adult flies were released at this site.

obtained from hybrid fire ant colonies in the laboratory that had been collected several months earlier just south of Chattanooga TN. The presence of sufficient brood is critical, because without it, the workers 'freeze' during fly attacks, making it difficult for the flies to parasitize large numbers of workers.

The groups of workers and brood were placed into large automatic attack boxes similar to the improved version described by Vogt et al. (2003) where they were exposed to attacks by *P. curvatus* flies for 2-3 days. The newly parasitized workers were collected and most of the brood was removed with sorting sheets (Banks et al. 1981) before the workers were repackaged and shipped overnight to Talladega County, AL for release. Within 24 hours of receipt, these workers were released into the same mound from which they were originally removed. This was done by placing the opened plastic shipping container near the side of the mound that had been disturbed sufficiently to cause workers to emerge. Generally, the parasitized workers were recruited back into the mound in 10-20 min. When conditions were hot and sunny, ants were shaded until the ants rejoined their nestmates inside the mound. On one occasion, we were unable to locate a nest for release of the parasitized workers. Release was attempted in a nearby mound under the assumption that the mound had relocated, but the workers were observed to fight, indicating that it was not the original colony. Most of the workers released into this mound were assumed to have perished.

The weather conditions during the release and throughout the summer were hot and dry. The summer of 2000 was the second summer of a two-year drought in the area. From May until August, mean daily maximum air temperatures averaged 32.1°C and mean daily maximum 10-cm soil temperatures averaged 26.7°C. Total rainfall during these months was only 153 mm compared to 436 mm under normal weather conditions.

Sampling for *P. curvatus* was conducted by disturbing 5-8 mounds in the release area. Several dozen workers from the disturbed mounds were crushed in the disturbed area of the mound in order to attract the flies more quickly. Mounds were observed for up to one hour for the appearance of phorid flies.

Florida Sites

Pseudacteon curvatus flies were released at four sites around Gainesville, FL in the spring of 2000 and another three sites in the spring and fall of 2001 (Table 1). Approximately 2-7 thousand parasitized ants were released at 6 of the sites over a 1-2 week period. Collection and handling procedures at those six sites were similar to methods described above except that ants did not need to be shipped back and forth. We were able to return them to their colonies within 3-4 days

rather than 6-8 days. The other difference is that we were usually able to collect enough brood along with the workers so that we did not need to use brood from other colonies. Adult flies were released over disturbed mounds at the UF Garden site using protocols similar to those described for *P. tricuspis* (Porter et al. 2003).

We chose to release parasitized fire ant workers at most of the sites rather than adult *P. curvatus* (Porter et al. 2003) because we were able to collect sufficient numbers of minor workers without unduly disturbing the mounds and thus, eliminated several hours of release time in the field each day. More importantly, several trials with adult flies, including the release at the UF Garden site, were not particularly successful. Most of the flies tended to drift away from disturbed mounds rather rapidly so we were only able to maintain fly activity at disturbed mounds for 20-30 min rather than the 90-120 min normal for *P. tricuspis*.

Tennessee Sites

Pseudacteon curvatus flies were released at three sites in Tennessee. The westernmost site, at the Ames Plantation in Fayette and Hardeman counties, was infested with black imported fire ants. The two other sites in east Tennessee, one near Cleveland in Bradley Co. and the other near Madisonville in Monroe Co., were both infested with hybrid fire ants (Table 1). All sites consisted of pastures bordered by woods and a creek or pond. Approximately 4-5 thousand parasitized ants were released at each site over a 2-3 week period. Collection and handling procedures at these sites were similar to methods described above for the Alabama site.

Results

Alabama Site

The search for field-reared *P. curvatus* flies was initiated on June 9, 2000, about 30 days after the first flies were parasitized in the lab. Subsequent visits were made to the site on June 13, 15, 20 and 27 and July 5, 10, 11, 14, and 18, but no flies were observed on any of these trips. All visits to the site were made in the afternoon between 1300 and 1500 hours, except for June 9 when the site was visited at 1000 hours. Observations were conducted for up to one hour. The site was not visited again until August 14 when the first field-reared flies were observed and collected (5 total, 3 collected). These flies were positively identified as *P. curvatus*. Flies were observed at the site again on August 28 and September 14 and 27. Flies were numerous at most mounds disturbed during the September observations.

In 2001, flies were first observed on May 4, approximately one year after they were first released (5 flies over 2 mounds). Flies were sighted again on May 7. Sites were visited on May 10 and 24, but flies were not found again until July 5 (3 flies over 1 mound).

On August 16, phorid flies were located at 8 of 12 mounds sampled at the release site. Flies were also found at 5 of 8 mounds about 0.5 km north of the release site and 2 of 10 mounds 1.1 km north. No flies were found 0.3 and 0.5 km further north. Flies were not found at sites 0.2, 0.8 and 1.6 km to the south of the release site.

In July, 2002, flies were numerous at the release site. On July 15, flies were located 3.4 km north of the release site. Sites 3.6 and 4.0 km north of the release were searched, and no flies were found. However, flies were found at these two sites on July 17, but not at sites 6.3 and 7.2 km north of the release. By July 25, the flies were found 11.2 miles north at the Talladega Superspeedway. Flies were located 4.8 km south of the release on July 16, but were not found 5.6 or 7.9 km south of the release. Sites 7.9 and 8.0 km south of the release were devoid of flies on August 5 and September 4. On July 17, flies were found 9.0 km southeast of the release on the edge of the Talladega National Forest and may have spread further in this direction. Gates across the forest roads were locked and we were not able to gain access to search further. One site 7.4 km west-southwest of the release did not have any flies present on this date. Flies were located 19.3 km northeast of the on July 18, but were not seen 21.2 or 21.7 km northeast of the release. Temperatures when flies were active ranged from 26.7°C to 36.6°C and activity was observed from 800 hours until 1600 hours.

Florida and Tennessee Sites

We were able to find several 1st-generation field-reared flies at 4 of 7 Florida sites. However, no additional flies were found in subsequent sample dates even though several of these sites were sampled repeatedly for up to 2 years after the initial releases. In Tennessee, no flies were recovered from the field at all. Almost all of the hybrid fire ant colonies at the Madisonville site were killed over the winter due to record low temperatures in November and December of 2000. This record cold may also have been responsible for the failures at the other two sites.

Discussion

The field release of *P. curvatus* flies in Alabama, where the *Solenopsis* hybrid is the prevalent species, was successful. The flies were released during the second summer of a two-year drought and survived the coldest November and December on record in Alabama. Despite these harsh environmental conditions, *P. curvatus* flies

not only survived but spread to 19.3 km northeast and 9.0 km southeast of the release site. This is the first successful establishment of *P. curvatus* in the United States and only the second successful establishment of a self-sustaining parasitoid species against fire ants (Porter et al. 2002).

Releases of *P. curvatus* in Florida and Tennessee were not successful. The reasons for these failures are not certain, but the success rate (1/11) was much lower than that experienced for *P. tricuspis* in Florida (Porter et al. 2003) and other states (Graham et al. 2001). A likely possibility *P. curvatus* did not establish at the Florida sites is that the fly biotype released was not viable on red imported fire ant populations in Florida because this biotype was adapted to black fire ants in the Buenos Aires region of Argentina where they were originally collected. Laboratory tests showed this fly biotype had a strong preference for black and hybrid fire ants even though they were able to be reared about as well on red fire ants as blacks and hybrids (Porter & Briano 2000). Perhaps in the field, this biotype was unable to locate red imported fire ants in Florida as well as it did hybrids in Alabama. In other words, this biotype may have been functionally too host specific in the field to succeed on red imported fire ants even though it is much less specific with *Solenopsis* species in the laboratory (Porter 2000) than *P. tricuspis* (Gilbert & Morrison 1997; Porter & Alonso 1999). Nevertheless, recapture of several 1st-generation field-reared flies in Florida demonstrated that at least some flies were capable of host location in the field.

Competition with *P. tricuspis* flies already at the Florida sites (Porter et al. 2003) could also have been a factor. However, *P. curvatus* parasitizes smaller ants than *P. tricuspis*. The three sites in Tennessee with hybrid and black fire ants also failed. Heavy winter kill of almost all of the hybrid fire ant colonies likely explains the failure at the Madisonville, TN site. Although no flies had been recovered prior to November 2000, the record cold temperatures may have contributed to failures at the other two sites.

It is also possible that the release techniques used for *P. curvatus* need improvement. Perhaps releasing large numbers of adult flies would be better than releasing parasitized workers, even though *P. curvatus* flies do not stay over disturbed mounds as long as *P. tricuspis* flies do. Curiously, fewer parasitized workers were released at the successful Talladega site than were released at most of the unsuccessful sites. The successful release site near Talladega, AL had areas of very dense waist-high grass and areas of damp soil. The other release sites may have been too hot and exposed for this *Pseudacteon* species.

Apparently, *P. curvatus* is capable of expanding out of its release site at rates similar to the 10-30 km/yr that has been reported for *P. tricuspis* in Florida (Porter et al. 2003). However, impacts of *P. curvatus* on fire ant populations are still unknown. This will be monitored as flies expand out of the successful release site near Talladega, Alabama.

A release of *P. curvatus* was conducted in Mississippi in spring of 2002 and by spring 2003, flies were recovered about 2 km from the release site (Vogt & Street 2003; pers. comm.). *Pseudacteon curvatus* was also released at four sites in Alabama and two sites in Tennessee late summer of 2002 and spring 2003. Flies have been recovered from one site in Alabama and one site in Tennessee (pers. comm.).

Another biotype of *P. curvatus* collected while attacking *S. invicta* in northern Argentina (near Formosa) is currently being reared and evaluated at the USDA-ARS quarantine facility in Gainesville, FL. Hopefully, this new biotype of *P. curvatus* will be more successful on red imported fire ants in Florida than the Buenos Aires biotype.

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