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STATUS OF THE FIRE ANT DECAPITATING FLY *PSEUDACTEON TRICUSPIS* (DIPTERA: PHORIDAE) IN LOUISIANA

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ABSTRACT

Eight releases of the South American fire ant decapitating fly, *Pseudacteon tricuspis* Borgmeier, were conducted in Louisiana from 1999 to 2006. Although *P. tricuspis* was initially recaptured at 88% of the release sites, four of the releases ultimately failed, so long-term establishment was only 43%. Fly populations from two releases have expanded out 50-80 km from their release sites (Fall 2006).

Key Words: red imported fire ant, *Solenopsis invicta*, biological control, establishment

RESUMEN

Se realizaron ocho liberaciones de la mosca que decapita la hormiga de fuego de Sudamérica, *Pseudacteon tricuspsis* Borgmeier, en el estado de Louisisana de 1999 a 2006. Aunque *P. tricuspis* fue inicialmente recapturada en el 88% de los sitios donde fue liberada, 4 de estas liberaciones al final fallaron por lo que su establecimiento al largo plazo fue solamente del 43%. Durante el otoño del 2006, la población de moscas de 2 liberaciones se han extendido 50-80 km fuera del sitio de la liberación inicial.

The Red Imported Fire Ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) was first discovered in Louisiana in the early 1950s (Callcott & Collins 1996) and is now ubiquitous over the entire state. Early efforts to control *S. invicta* with widespread insecticide applications were met with mixed success. Concerns regarding the negative effects of these pesticides on non-target organisms and the environment led to the exploration of alternative, less ecologically disruptive, methods of *S. invicta* control. One such method is biological control with imported natural enemies that attack *S. invicta* in South America, including flies of the genus *Pseudacteon* Coquillet (Diptera: Phoridae).

Pseudacteon flies have been demonstrated to be effective in disrupting foraging activity of ants in the genus *Solenopsis* (e.g., Feener & Brown 1992; Orr et al. 1995), thereby mediating inter- and intraspecific competitive interactions among ants (Feener 2000). This phorid parasitoid could theoretically reduce the ant community dominance currently in favor of *S. invicta* in the United States (Feener 2000) and allow native ant species to better compete with *S. invicta* for resources (Porter 1998).

In 1998, the Southern Legislative Conference and the USDA-ARS created the National Fire Ant Strategy whose mandate was to rear and distribute *P. tricuspis* Borgmeier to cooperators in *S. invicta* infested areas of the southeastern United States. This effort combined with considerable support from the State of Louisiana and a supplement of flies provided by the USDA-APHIS mass-rearing program provided the foundation for decapitating fly releases in Louisiana. The aim of this paper is to describe the methodology employed in releasing *P. tricuspis* in Louisiana, report on the status of releases made in Louisiana thus far, and provide information on current range expansion of established populations of *P. tricuspis* in Louisiana.

MATERIALS AND METHODS

Selection of Release Sites

In association with planned evaluations of *P. tricuspis* effects on *S. invicta* populations in Louisiana, paired release and control study areas were scouted and evaluated for their similarity in habitat and ecotones. Release and control study areas were usually unmaintained pastures that were 10 km to 30 km apart. At least 2 study plots at each site were selected and marked, except for the 2005 and 2006 releases.

Handling of *P. tricuspis* Puparia

Pseudacteon tricuspis were initially mass-reared at the USDA-ARS facilities located in Gainesville, FL. Puparia were shipped to Louisiana State University Department of Entomology

for incubation until adult emergence. *Pseudacteon tricuspis* maggots develop and eventually pupariate inside individual *S. invicta* head capsules (Porter 1998). Head capsules were placed in small plastic cups [Solo® 59 mL Soufflé cup (B200)] containing plaster that was saturated with distilled water. These cups were held inside small plastic emergence cages (22 × 12 × 14 cm) (Fig. 1), and emergence cages were then placed within a larger environmental chamber set at 28°C with a 14:10 L:D photoperiod. Plaster blocks were moistened daily to maintain humidity (approximately 80%) inside emergence cages and minimize desiccation of puparia. We found that most flies eclosed and were capable of flight by 10:00 AM (CDT). To collect flies for releases, emergence cages were placed inside a larger holding cage and the flies were allowed to escape into the larger cage where they flew toward a light source. Flies were then aspirated and counted into 20 cm × 2.5-cm tubes, one end of which contained a slightly moistened cotton wad and the other a small opening over which a mesh screen was glued. Excess moisture was squeezed from the cotton wad to minimize condensation forming on the insides of the tubes. Enough flies to treat at least 10 *S. invicta* mounds per day (usually 300-400) were transported in a cooler from the laboratory for field releases. Releases were done daily in the afternoons over a 7-14 d period, depending on weather conditions and availability of flies. *Pseudacteon tricuspis* were released at seven locations in Louisiana, with two releases done at the same location (Table 1, Fig. 2).

Release Methods

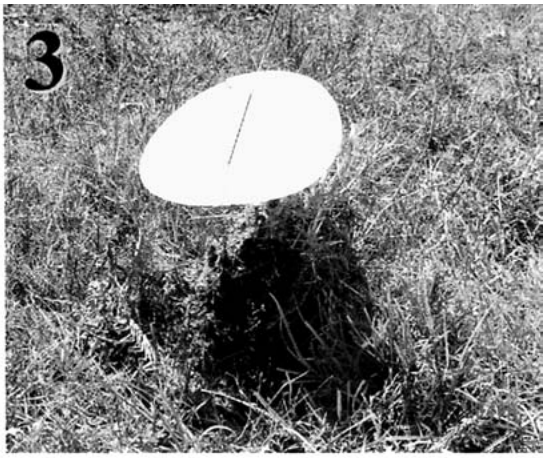
Flies released at disturbed S. invicta *mounds*. Four releases (Covington, the 2 Natchitoches releases, and Pitkin) involved simply liberating *P. tricuspis* adults near disturbed *S. invicta* mounds. Mounds were disturbed by removing approximately a 100-cm^3 portion of the mound with a small spade, inverting, and flattening this portion with the spade for several seconds. Usually 20-50 flies were released at each mound by removing a cap on one end of the tubes, with the open mouth facing the mound within 30 cm and allowing the flies to escape and orient to the disturbed ants. The treatment period was 2 h, during which time *S. invicta* mounds were frequently disturbed to maintain ant availability to attack by *P. tricuspis*.

Styrofoam plate sun shields were employed to shade *S. invicta* mounds from full sun (Fig. 3). This was important to maintain *S. invicta* activity at the soil surface during phorid attacks, particularly when the weather was hot and/or the soil was dry. Soil surface temperatures achieved in full sun would otherwise increase the likelihood that *S. invicta* would retreat below ground to escape heat and desiccation. The upper critical thermal limit of *S. invicta* is reported to be around 40°C (Cokendolpher & Phillips 1990). Applying some water (0.5 liter) to the disturbed mounds during very warm (28-35°C) and dry conditions before releases caused *S. invicta* to remain at the surface and available to attacking *P. tricuspis* females for longer periods (see also Porter et al. 2004). However, when ambient temperatures exceeded 36°C ants were reluctant to remain at the surface, even with the mounds shaded and moistened (see Discussion).

Flies Released in Closed Containers. At Norwood, Montpelier, Lake Arthur and Waverly, Louisiana we released *P. tricuspis* into large plastic storage containers (approx. 86 liters) containing *S. invicta* (Fig. 4), and also at a few disturbed mounds. Sides of containers were lined with fluon to prevent *S. invicta* from escaping. *Solenopsis invicta* from individual mounds were dug up with a shovel and deposited inside 3.8 liter talc-lined pails and the contents then carefully poured into the containers, along with brood and enough moistened mound substrate to cover the bottom (approximately 2 kg). Fire ant mounds were marked by number with wire stake flags, with the pails and storage containers marked by the same number as the source mounds to ensure correct identification of colonies when they were returned to the source mounds at the end of the treatment period. A wooden block was placed inside the storage containers to enable *S. invicta* workers to relocate brood to a protected area. A ventilated lid was placed over the containers and *P. tricuspis* were introduced through a hole of the same width as the tube containing the *P. tricuspis*. The exposure period was 2 h. The lid was slowly opened every 20 min and wooden blocks were repositioned to stimulate ant activity and maintain phorid attack. Treatment containers were kept in a partially shaded area throughout the 2-h treatment period to prevent overheating. After the treatment period had elapsed, treated *S. invicta* workers (and brood) were returned to the same mound from which they were collected and adult *P. tricuspis* were allowed to disperse into the surrounding environment. Treated mounds were watered (0.5 L/mound) after return of ants to reduce stress from desiccation and disturbance. When possible, releases of *P. tricuspis* were limited to warm, sunny days when air temperatures were >21°C, as this has been demonstrated to be the flight threshold for *P. tricuspis* (Morrison et al. 1999).

Determining Status of Releases

Release sites were visited at 3-4-month intervals to determine the status of *P. tricuspis* at release sites. Normally, 10-40 *S. invicta* mounds were disturbed in the late morning and afternoon. Mound disturbances involved removing one-half of the exposed mounds with a shovel, inverting,



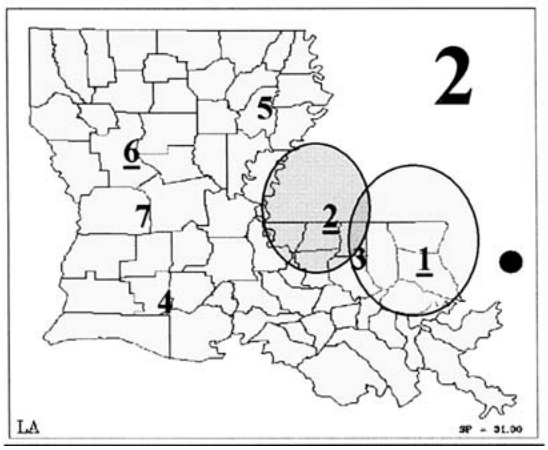


Fig. 1. *Pseudacteon tricuspis* emergence cage containing *S. invicta* head capsules. Fig. 2. Map of Louisiana showing *P. tricuspis* release locations (See Table 1 for details). Established locations are underlined. Shaded ovals are approximate range occupied by Covington (1) and Norwood (2) populations in 2005 and 2006, respectively. Nearest release site at Saucier, MS denoted by •. Louisiana State map courtesy of: http://baby.indstate.edu/gga/gga_cart/basela.gif. Fig. 3. Styrofoam plate shield in combination with a wire flag to shade a disturbed *S. invicta* mound during *P. tricuspis* releases. Fig. 4. Storage boxes containing portion of labeled *S. invicta* mound and wooden blocks. Box lids and pails for transporting mound portions are in the background.

and crushing this removed portion plus ants that appeared for 5-10 s. Disturbed mounds were visited 3 or more times in rotation and all flies seen were counted. Multiple locations at release sites were sampled during each visit to obtain information about the status of *P. tricuspis* populations.

Evaluating Population Expansion

During the fall months (Sep-Nov) we attempted to locate the approximate limits to the populations of *P. tricuspis* in 4 cardinal directions (i.e. north, south, east, and west) of the release sites. At this time of year, *P. tricuspis* population densities are at their peak in Louisiana. Two observers each disturbed 5 *S. invicta* mounds and awaited arrival of *P. tricuspis*. Mound disturbances were the same as above for determining status of *P. tricuspis* releases. If no flies appeared within 30 min of mound disturbance, then the observers moved 1-2 km towards the release area. If flies were observed attacking *S. invicta* at disturbed mounds, then the observers selected another location 1-2 km farther away from the release area. This procedure was continued until the approximate population limits were located. Expansion surveys of the Covington release were conducted from 1999 to 2005 and from 2003 to 2006 for the Norwood release. Flies were not observed at the Norwood release location during 2001 and very few (n = 2 flies at 74 combined mounds surveyed May, Jun and Aug) during 2002.

TABLE 1. LOCATIONS OF *P. TRICUSPIS* RELEASES IN LOUISIANA, 1999-2005.

Location and GPS coordinates	Dates of release	Number released	Method of release	Result
1. 17 km NE of Covington (St. Tammany Parish) 30°36'34"N, 90°01'18"W	8-13 Sep 1999	2,165	At disturbed mounds	Established
2. 9 km E of Norwood (E. Feliciana Parish) 30°59'8"N, 91°00'55"W	27 Apr-8 May 2000	4,714	Containers/disturbed mounds	Established
3. 1.5 km S of Montpelier (St. Helena Parish) 30°40'22"N, 90°38'18"W	25 Sep-5 Oct 2000	3,510	Containers/disturbed mounds	Failed
4. 5 km SE of Lake Arthur (Vermilion Parish) 30°04'08"N, 92°39'25"W	2-11 Apr 2001	2,593	Containers	Failed
5. Waverly (Richland Parish) 32°24'14"N, 91°21'57"W	17-27 Sep 2001	2,052	Containers/disturbed mounds	Failed
6a. 14 km S of Natchitoches (Natchitoches Parish) 31°37'57"N, 93°4'7"W	13-24 May 2002	1,907	At disturbed mounds	Failed
6b. 14 km S of Natchitoches (Natchitoches Parish) 31°37'57"N, 93°4'7"W	2-16 Jun 2005	3,300	At disturbed mounds	Established
7. 4 km NW of Pitkin (Vernon Parish) 30°56'40"N, 92°58'32"W	12-23 Jun 2006	2,300	At disturbed mounds	In progress

RESULTS AND DISCUSSION

Pseudacteon tricuspis Establishment and Population Expansion

1) Covington release. As of Oct 2005 *P. tricuspis* were found at least 60 km north, and 50 km west of the release site. Population spread to the south reached Lake Pontchartrain by Oct 2004 (29 km). We were unable to determine the eastern limit of spread into Mississippi, as populations from the Covington release and a Mississippi release had fused in 2005. However, we did observe declining, then increasing, numbers of flies as we moved from west to east in southwestern Mississippi between the cities of Picayune and Harrison.
3) Norwood release. As of Oct 2006, flies from the Norwood release site were recovered at least 82 km north, 55 km south and 62 km west of the release site. The eastward expanding Norwood population fused with the westward expanding Covington population in 2006.
4) Montpelier release. *Pseudacteon tricuspis* initially established at Montpelier. Flies were observed at disturbed *S. invicta* mounds at least 1 km south and west of the release site during Oct 2001. However, despite subsequent repeated intensive searching from 2002-2005, we failed to observe any *P. tricuspis* at, or near, the Montpelier release site. However, spreading populations of *P. tricuspis* from the Covington release to the east and the Norwood release from the west reached this location in 2006.
5) Lake Arthur release. *Pseudacteon tricuspis* were never recovered from the Lake Arthur release site after repeated visits (May, Jul and Sep 2001; Apr, Jul and Aug 2002; May 2003).
6) Waverly release. A single fly was observed at one mound in 15 in Jun 2002. No flies were observed on subsequent visits in Jul and Sep 2002, Aug 2003, and Aug 2005.
7) Natchitoches releases. A single fly appeared at 2 of 10 mounds in Jul 2002, one of 10 mounds in Sep 2002 at the Natchitoches release site and none thereafter (Jun and Sep 2003). We made a second release in Jun 2005 at the same Natchitoches location as the 2002 release. In Oct 2005, one to 3 flies were collected at 7 of 10 disturbed mounds. Successful overwintering and establishment was confirmed in Sep 2006 when flies were collected at 10 of 20 disturbed mounds. It is unknown at this time how far *P. tricuspis* has spread from this location.
8) Pitkin release. Initial establishment of *P. tricuspis* at the Pitkin release site was confirmed on 31 Oct 2006, when a total of 34 flies were observed at 8 of 10 mounds.

Initial confirmed establishment of our releases was 88% (7 out of 8 releases). However, our long-term establishment rate of *P. tricuspis* in Louisiana (43% for releases up to 2005) is lower compared to Texas releases (60%) (Gilbert & Patrock 2002) and Florida releases (66%) (Porter et al. 2004). The failure of several Louisiana releases to establish, even when first generation flies had been recovered, may be attributed to environmental events. Extreme flooding to the extent that the release sites were almost completely submerged occurred at the Montpelier (Spring 2002) and Natchitoches (Fall 2002) release sites. Prolonged drought in north Louisiana during 2002 could explain the failure of the Waverly release, but weather conditions during the time of the release at this location were often cool, with tempera-

tures frequently near the lower flight threshold temperature for *P. tricuspis*. The Lake Arthur release was often hampered by high winds (>30 km/h) during the entire duration of the release. Additionally, the use of closed containers as a release method was eventually abandoned. Phorid attack rates within the containers did not appear as high as those at disturbed mounds. Each time the lid was lifted to reposition the wooden block, flies would escape.

During our 2005 release at Natchitoches, there were occasions during very hot weather (>36°C) when efforts to maintain *S. invicta* at the surface were ineffective, despite the use of shade and water. We observed that the majority of *P. tricuspis* disappeared soon after release, and *S. invicta* workers immediately retreated into the ground. An infrared thermometer registered unshaded bare soil surface temperatures on these occasions exceeding 55°C. These temperatures are almost certainly lethal to both *S. invicta* and *P. tricuspis* and should be considered when doing releases under extremely hot conditions that can be expected from Jun-Aug in Louisiana.

No *P. tricuspis* releases were made in Louisiana from fall 2002 through 2004. An effort to produce a laboratory colony of *P. tricuspis* at Louisiana State University (LSU) was initiated during the fall of 2002. Sufficient fly production for release was not attained until 2005. The 2005 release of *P. tricuspis* at Natchitoches and the 2006 release near Pitkin, Louisiana used flies produced by our rearing facility at LSU.

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