

## **Distribution of the Formosa Strain of the Fire Ant Decapitating fly *Pseudacteon curvatus* (Diptera: Phoridae) Three and a Half Years after Releases in North Florida**

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# DISTRIBUTION OF THE FORMOSA STRAIN OF THE FIRE ANT DECAPITATING FLY *PSEUDACTEON CURVATUS* (DIPTERA: PHORIDAE) THREE AND A HALF YEARS AFTER RELEASES IN NORTH FLORIDA

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## ABSTRACT

The Formosa biotype of the decapitating fly *Pseudacteon curvatus* Borgmeier was released and successfully established as a self-sustaining biocontrol agent of the red imported fire ant *Solenopsis invicta* Buren at several sites around Gainesville, FL in 2003. In order to determine the status of these releases, 59 sites were surveyed for flies in the fall of 2006 with sticky traps and aspirators. Results of this survey showed that the flies had expanded outward an average of 74 km and occupied more than 30,000 km<sup>2</sup> in North Central Florida. This rate of expansion was faster than rates reported for *Pseudacteon tricuspsis* Borgmeier in Florida, Louisiana, and Texas. The rapid rates of expansion and high densities reported for the Formosa biotype of *P. curvatus* indicate that it is a vigorous parasitoid which will require relatively fewer field releases to establish it as biocontrol agent of red imported fire ants in the United States.

Key Words: *Solenopsis invicta*, red imported fire ant, phorid fly, biological control, parasite

## RESUMEN

El biotipo de Formosa de la mosca decapitadora *Pseudacteon curvatus* Borgmeier fue liberado y se estableció con éxito como un agente de control biológico auto-sostenible de la hormiga de fuego roja importada *Solenopsis invicta* Buren en varios sitios en los alrededores de Gainesville, FL, en 2003. Para determinar el estatus de estas liberaciones, 59 sitios fueron relevados para detectar la presencia de moscas en el otoño de 2006 usando trampas pegajosas y aspiradores. Los resultados de este relevamiento revelaron que las moscas se expandieron en un promedio de 74 km y ocuparon más de 30.000 km<sup>2</sup> en el centro norte de Florida. Esta tasa de expansión fue más rápida que las tasas registradas para *Pseudacteon tricuspsis* Borgmeier en Florida, Louisiana y Texas. Las rápidas tasas de expansión y las altas densidades registradas para el biotipo formoso de *P. curvatus* indican que éste es un parasitoide vigoroso y que requerirá un número relativamente menor de liberaciones para que se establezca como un agente de control biológico de la hormiga de fuego roja importada en los Estados Unidos.

Several species of decapitating flies have been released in the United States as self-sustaining biocontrol agents of imported fire ants. The first species was *Pseudacteon tricuspsis* Borgmeier in 1997 (Porter et al. 2004). This species is now widely distributed in 9 states and Puerto Rico as a result of cooperative release programs between USDA-APHIS, USDA-ARS, and state cooperators (Callcott et al. 2007).

A second decapitating fly *Pseudacteon curvatus* Borgmeier was released in Florida at 7 sites between 2000 and 2001 (Graham et al. 2003). The flies used in these releases were collected in Las Flores, Argentina from the black fire ant *Solenopsis richteri* Forel in 1997 and reared on the red fire ant *Solenopsis invicta* Buren for 3 years in the laboratory. These flies preferred black fire ants over red fire ants in paired choice tests but produced an equal number offspring in no-choice laboratory tests (Porter & Briano 2000). However, when these flies were released in the field they

failed to establish at all 7 release sites in Florida with *S. invicta* fire ants (Graham et al. 2003). Nevertheless, these flies were successfully established at multiple sites with black and hybrid fire ants (red X black) in Alabama, Mississippi, and Tennessee (Graham et al. 2003; Vogt & Streett 2003; Parkman et al. 2005).

In order to establish *P. curvatus* on red imported fire ants in the United States, a second biotype of *P. curvatus* was collected from *S. invicta* ants near Formosa, Argentina in 2001 (Vazquez et al. 2006). The Formosa biotype was somewhat more host specific in laboratory tests than the Las Flores biotype (Porter 2000; Vazquez et al. 2004). This new biotype was released on red imported fire ants at 3 sites near Gainesville, FL during the spring, summer, and fall of 2003. By spring 2004, we were able to confirm that flies had established at all 3 release sites (Vazquez et al. 2006). After 1 year, the flies at the first site, had expanded outward 1-2 km. Post-release host specificity tests re-

confirmed predictions that these flies would not pose a threat to native ants (Vazquez & Porter 2005). The Formosa biotype is currently established on red imported fire ants in 10 states as a result of the rearing and release program of USDA-APHIS (Callcott et al. 2007) and also the efforts of Gilbert et al. (Gilbert et al. 2008) in Texas.

The objectives of this paper are to document the expansion of *P. curvatus* (Formosa biotype) from release sites near Gainesville, FL several years after their field release and to compare the observed expansion and populations with data from other studies.

#### MATERIALS AND METHODS

A total of 59 sites were sampled for *P. curvatus* in the fall of 2006. Sampling at the first 23 sites was conducted by aspirating as many flies as possible while they attacked fire ant workers in disturbed mounds (see Porter et al. 2004). Generally, we remained at each site for 30-40 min unless *P. curvatus* flies were discovered sooner. Additionally, I experimented with several trap designs based on those developed by Puckett et al. (2007)

and LeBrun et al. (2008). I settled on a design (Fig. 1) similar to that used by Puckett et al. (2007) except rather than using dead ants as bait for the flies, I used live ants like LeBrun et al. (2008).

The trap itself was a white pizza tri-stand, a small plastic device about 40 mm in diameter and 40 mm high used to keep the center of a pizza box from sticking to the pizza. The tri-stand was oriented with the 3 arms up and coated with Tanglefoot® on the arms and the edges. The underside of the tri-stand was coated with Fluon® to exclude the ants and screwed onto a 30-mm bolt held up by a 65-mm plastic base (a lid for 2-oz. condiment cup). The base was wider than the tri-stand top so the traps could be carried in a tray without sticking together (Fig. 1).

The trap was placed in the bottom half of a Petri dish (25 by 150 mm). The inside edges of this dish were coated with Fluon® and the Petri dish was settled into a disturbed fire ant mound so it acted like a pitfall trap rapidly collecting 1-10 gm of agitated ants as they swarmed out in defense of their mound. Once trapped in the Petri dish, the ants could no longer retreat into the mound. The ants usually remained in the Petri dish for 2-8 h



Fig. 1. The fly trap used in this survey was based on traps developed by Puckett et al. (2007) and LeBrun et al. (2008). It consisted of a white tri-stand top coated with Tanglefoot® attached to a slightly larger base by a bolt. The trap was placed inside a large fluon coated Petri dish filed with ants that swarmed into it after being placed into a disturbed mound. Flies were attracted to the disturbed ants and trapped when they landed to rest on the sticky tri-stand.

until they died from exposure or escaped during the evening hours as dew inactivated the slippery Fluon coating.

The *Pseudacteon* flies were attracted to the alarmed ants by their smell (Vander Meer & Porter 2002; Morrison & King 2004; Chen & Fadamiro 2007). When the flies grew tired of attacking and hovering over the ants, they often landed on the pizza tri-stand and were trapped in the sticky Tanglefoot® coating. I used ants from disturbed colonies because they seemed to be more attractive than dead ants (LeBrun et al. 2008) and because I only wanted to sample at sites where fire ants were common enough to find 3-4 large colonies in 5-10 min of searching. Once I found the colonies, it was easy to disturb a colony and place Petri dish into the mound (Fig. 1).

On sunny days, we shaded the traps with a plastic plate held at an angle over the trap with a wire flag (Fig. 1). A label stating "Please Do Not Disturb, Fire Ant Biocontrol Trap" with my name and phone number reduced the number of traps disturbed by curious passersby. A 1.5-cm hole was usually drilled into the bottom of the Petri dish and covered with fine-mesh screen so that if it rained, the water could drain out and I would not end up retrieving traps from a soup of mud and dead ants (Porter 2005).

After the first 23 sites, aspirating flies was mostly discontinued. At 33 of the 36 remaining sites, we set out 3-5 traps and then simply retrieved them after 24 h. The traps were then taken to the lab where they were examined under a dissecting microscope to identify the flies. Sampling was done between 20 Sep and 18 Oct 2006 along the edges of roads radiating out from the release sites (Fig. 2).

## RESULTS

Aspirating flies while they attacked ants was a problem on the edge of the range where *P. tricuspidis* densities were high and *P. curvatus* densities were still very low. Determining how many decapitating flies to collect to ensure that *P. curvatus* was collected, if present, was difficult. For example, at 7 of the first sites, we collected about 326 decapitating flies by aspiration; only 1 of which was a *P. curvatus*. In contrast we collected 925 flies on traps; 6 of which were *P. curvatus*. As the survey progressed, we were able to detect *P. curvatus* at 55% more sites by using traps than by using aspiration alone (17/20 vs. 11/20 sites within ellipse of occurrence, Fig. 2). At this point, I concluded that it would be more efficient and effective to largely abandon attempts to aspirate the flies and rely on the traps. Consequently, aspirators were only used intermittently for the final 36 sites.

The results of our survey showed that 3.5 years after releasing the Formosa strain of *P. curvatus*

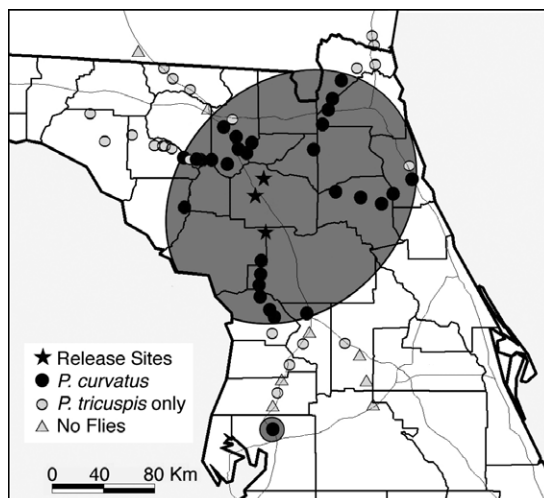


Fig. 2. Distribution of the fire ant decapitating fly *Pseudacteon curvatus* in North Central Florida (dark shading) in the fall of 2006 about 3.5 years after field releases near Gainesville, FL. Also, note the disjunct collection near Tampa Bay. Most sites with *P. curvatus* also had *P. tricuspidis*.

they had expanded outward an average of  $74 \pm 10$  km ( $n = 5$ ; SE) from the release sites (range: 47-100 km) (Fig. 3). The ellipse covering their distribution extended from the Gulf of Mexico to the Atlantic Ocean and from the Georgia boarder south into Citrus county, an area of about 30,000 km<sup>2</sup> (Fig. 2). Slightly more than 90% of the sites (28/31) encompassed by the ellipse contained *P. curvatus* flies. The 3 sites without *P. curvatus* were all near the periphery of the estimated distribution where populations were still likely low. Surprisingly, *P. curvatus* flies were also found at a single outlying site just to the east of Tampa.

Densities of *P. curvatus* at our sample sites (Fig. 2) were usually lower than densities of *P. tricuspidis*. We found *P. curvatus* on 47% of traps at sites where it occurred (Fig. 2, black dots,  $n = 29$  sites) compared to 69% of traps for *P. tricuspidis* at sites where it occurred ( $n = 46$  sites, most of the gray and black dots, Fig. 2). The mean number of *P. curvatus* females per trap was  $1.2 \pm 1.5$  compared to  $2.3 \pm 2.3$  for *P. tricuspidis* females. Almost 2 *P. tricuspidis* males were collected for every female (1.9:1.0,  $n = 1677$ ). The sex ratio for *P. tricuspidis* flies collected in aspirators and on traps was equivalent (1.8:1.0 vs. 1.9:1.0). Males of *P. curvatus* were rarely found on traps because they are not attracted to fire ant workers for mating (Wuellner et al. 2002).

In contrast to the survey sites which were mostly on the perimeter of the range, the densities of *P. curvatus* in the core of the range near the original release sites was much higher. At the Morrill Release site in Oct 2006 (Fig. 2, northern

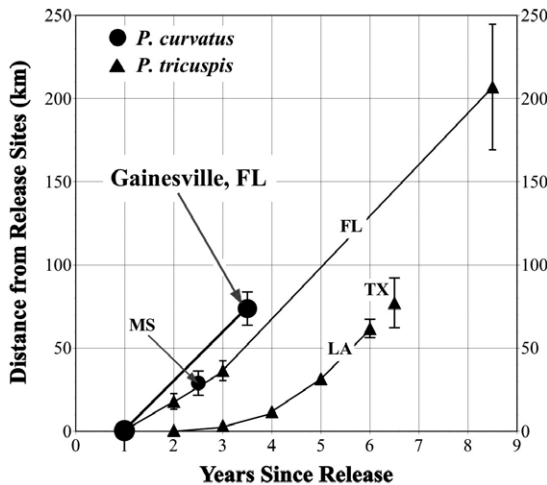


Fig. 3. Expansion rates of 2 species of *Pseudacteon* decapitating flies from field release sites in the southeastern United States. The large black dots shows data for *P. curvatus* flies (Formosa Strain) in the Gainesville, Florida area (see Fig. 2 and Vazquez et al. 2006). The smaller black dot shows data for *P. curvatus* (Las Flores Strain) released in Mississippi on hybrid fire ants, *S. invicta* X *S. richteri* (Thead et al. 2005). The black triangles show data for *P. tricuspsis* flies released on red imported fire ants in Florida (Porter et al. 2004; Pereira & Porter 2006), Louisiana (Henne et al. 2007), and Austin, Texas (Gilbert et al. 2008; LeBrun et al. 2008). Error bars show standard errors of the mean.

most star), we collected an average of 11.0 *P. curvatus* females on each of 5 traps. The next month in Nov we collected 20.0 *P. curvatus* females per trap at the same site. Similarly, a sample in early Dec 2007 from 3 sites near our lab in Gainesville, FL resulted in an average of 33.9 *P. curvatus* flies per trap (Porter & Lui, unpublished data). The relative density of *P. curvatus* compared to *P. tricuspsis* was much higher in the 2 core areas just mentioned. At both the Morrill and the Gainesville sites, 98% of female flies were *P. curvatus* ( $n = 174$ ,  $n = 346$ ; respectively).

#### DISCUSSION

The decapitating fly *P. curvatus* is vigorously expanding outward and firmly established on red imported fire ant populations in North Central Florida. The survey data from this paper indicate that *P. curvatus* will eventually occupy virtually every site where its host is common. Our sampling effort was very effective in detecting *P. curvatus* flies within the body of the distribution. The only uncertainty was within 10-15 km of the periphery where *P. curvatus* densities are likely relatively low because introduced *Pseudacteon* populations can take a year or more to reach carrying capacity after occupying a new area (Porter et al.

2004; LeBrun et al. 2008, 2009). The 90% success rate of finding *P. curvatus* at sites within the estimated area of occupation was slightly better than the 80-85% rates reported for *P. tricuspsis* (Porter et al. 2004). This higher success rate may have been due partly to the use of sticky traps and partly to higher *P. curvatus* densities in the core of the range.

The major benefit of the traps was that they caught considerably more flies than we were able to collect by aspiration alone (also see Puckett et al. 2007). Consequently we were able to find flies at 6 more of the first 20 sites than we would have without the traps. The traps also saved time in collecting the flies although much of this benefit was lost by having to drive back to pick them up the next day. Another benefit of the traps is that reliable collections could be made by assistants without the training needed to identify the flies. Traps are also useful in simultaneously monitoring a number of sites with standard effort for studies of competition and population dynamics (LeBrun et al. 2008, 2009).

The 8 triangles on the map (Fig. 2) indicate sites where neither *P. tricuspsis* nor *P. curvatus* were found. The absence of both species from a site is an indication of a poor habitat or poor climatic conditions. Six of these sites were in the southern part of the survey region which was experiencing a drought. However, it is also likely that the 3 negative sites near Orlando may have been out of the range of *P. tricuspsis*, which was expanding to the south more slowly than *P. curvatus* (Pereira & Porter 2006).

By the fall of 2006, we found that *P. curvatus* had expanded coast to coast in Florida, up to the Georgia boarder and down toward Tampa. The distribution pattern we observed in Fig. 2 is a poor match for wind patterns in the Gainesville area, which are strongly oriented NEE and SWW (Porter et al. 2004). Different wind patterns in other parts of North Florida and chaotic events like droughts may have obscured a correlative pattern. Wind patterns did not appear to influence the direction and speed of *P. tricuspsis* expansion in North Florida either (Porter et al. 2004), but wind drift did appear to promote jump dispersal of *P. tricuspsis* in Texas (LeBrun et al. 2008). Henne et al. (2007) reported a possible association between the more rapid northerly expansion of *P. tricuspsis* in Louisiana and local wind patterns including 2 hurricanes that tracked to the north during their study.

*Pseudacteon curvatus* has expanded more rapidly out of its release sites in North Florida than *P. tricuspsis* did. This is especially true to the south where after only 3.5 years *P. curvatus* was nearing the southern limits reached by *P. tricuspsis* in 8 years (Pereira & Porter 2006). Expansion rates for the Las Flores strain of *P. curvatus* in Mississippi were about the same as for *P. tricuspsis* in

Florida but more than *P. tricusps* in other states. Expansion rates of *P. tricusps* in Louisiana and Texas were much slower than either *P. curvatus* or *P. tricusps* in Florida, primarily because populations in Louisiana and probably also Texas (LeBrun et al. 2008) did not begin expanding until 3-4 years after the field releases. Possible explanations for this delay or 'latent phase' include drought, floods, and the need to adapt to local conditions. Small release populations may also make it difficult for males and females to find each other to mate (Hopper & Roush 1993; Henne et al. 2007).

Shortly after release and along the leading front of the expanding population, fly densities may enter an 'eclipse phase' where densities are too low to be reliably measured (Hopper & Roush 1993; Henne et al. 2007). As populations grow, rare events become more common and very rare events become possible. The increasing occurrence of rare jump-dispersal events probably explains the accelerating rates of dispersal seen in Fig. 3 (also see LeBrun et al. 2008). Eventually, one would expect the flies to reach a constant rate of expansion; however, it is not clear that this has happened with the *Pseudacteon* flies which have been released so far. A constant rate of expansion is difficult to document because expansion rates are variable depending on habitat, wind direction, host type, and chaotic factors such as droughts and hurricanes (LeBrun et al. 2008). Also, merging populations and the time commitment to monitor the phenomenon are complicating factors.

We found one outlier site about 16 km north-east of Tampa (Fig. 2). The origin of this site is a somewhat of a mystery. This site was 80 km, approximately 2 years beyond the main wave front to the north. USDA-APHIS and their state cooperators released *P. curvatus* in Sarasota County in Nov 2004 about 80 km to the south. They also released *P. curvatus* in St. Lucie county in 2005 about 200 km to the southeast. Because of the distance and because expansion is usually only a few kilometers in the first year, neither of these sites would seem likely sources. An inspection of hurricane tracks for 2004-2006 also does not show any that would likely have blown the flies from the north or from the south. Perhaps this site is an example of a rare long-distance dispersal event mediated by human activities or unknown weather events.

The high densities of *P. curvatus* in the core of the range indicate that this species may be a much more effective biocontrol agent than *P. tricusps*. It is also likely that *P. curvatus* is reducing the densities of *P. tricusps* in North Florida as it has done in Texas (LeBrun et al. 2009). The most likely explanation of why *P. curvatus* is so much more abundant than *P. tricusps* is that *P. curvatus* attacks smaller fire ant workers. About 80-

90% of fire ant workers are subject to *P. curvatus* parasitism compared to only 15-30% for *P. tricusps* (Porter & Tschinkel 1985; Greenberg et al. 1992; Morrison et al. 1997; Morrison et al. 1999). However, *P. tricusps* is often more abundant than *P. curvatus* in Argentina along the Paraguay and Parana rivers where *S. invicta* and these flies are native (Calcaterra et al. 2005), so available host sizes are not the only factor influencing the relative abundance of *P. curvatus* and *P. tricusps* flies.

In conclusion, the high rates of expansion observed for *P. curvatus* in North Florida indicate that fewer releases will be necessary to establish this species in Florida and other states infested with fire ants. The high densities observed for *P. curvatus* indicate that it may be a more effective biocontrol agent than *P. tricusps* (Morrison & Porter 2005). We are currently monitoring the seasonality of 3 species of decapitating flies (*P. curvatus*, *P. tricusps*, *P. obtusus*) which have been released and established as biocontrol agents against red imported fire ants in the Gainesville area in order to better assess their impacts on fire ant populations.

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