Occurrence of Phorid Fly (Diptera: Phoridae) Parasitoids of Imported Fire Ants (Hymenoptera: Formicidae) in Georgia[1]

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Abstract Ten releases of *Pseudacteon* decapitating flies (Diptera: Phoridae), a classical biological control agent of imported fire ants (Hymenoptera: Formicidae) in the southern United States, were made at 8 different sites in Georgia (USA) over a period spanning 2000 - 2012. Intensive monitoring and survey activities were initiated in 2008 to delineate the dispersal and occurrence of the *Pseudacteon* spp. in the state. Results show that all 159 counties in Georgia have at least 1 species of *Pseudacteon*; *P. curvatus* Borgmeier is established 129 of those counties, *P. tricuspis* Borgmeier is established in 70 counties, and *P. obtusus* Borgmeier is established in the county in which it was released. Both *P. curvatus* and *P. tricuspis* occur together in 39 counties, and all 3 of the *Pseudacteon* species occur in the 1 county in which all 3 species were released. These results help target supplemental or additional releases of *P. tricuspis, P. curvatus,* and *P. obtusus* as well as releases of other candidate *Pseudacteon* species.

Key Words biological control, classical biological control, *Solenopsis invicta*, *Solenopsis invicta X S. richteri* hybrid, *Pseudacteon tricuspis*, *Pseudacteon curvatus*, *Pseudacteon obtusus*, occurrence, distribution

Buren et al. (1974) estimate that the black imported fire ant, *Solenopsis richteri* Forel, invaded the United States around 1918, whereas the red imported fire ant, *Solenopsis invicta* Buren, likely invaded in the early 1930s. Both species entered the U.S. through the Mobile, AL, port area originating from their native ranges in South America. These imported fire ants currently infest over 1,388,000 km^2 in 14 states and Puerto Rico (www.aphis.usda.gov/plant_health/plant_pest_info/fireants/; last updated 13 December 2011). *Solenopsis invicta* is the predominant species in terms of abundance and area infested, with the U.S. range of *S. richteri* restricted to approximately 30,000 km^2 in northwestern Alabama, northeastern Mississippi, and southern Tennessee (Tschinkel 2006). The 2 species are reproductively isolated in South America, even where their ranges overlap (Ross and Shoemaker 2005). However, they have hybridized in their expanded North American range (VanderMeer and Lofgren 1988) with the resulting *S. richteri* X *S. invicta* hybrid occupying northern Mississippi (Streett et al. 2006), northern Alabama (Bertagnolli et al. 2007), northwestern Georgia (Gardner et al. 2008), and southern Tennessee (Oliver et al. 2009).

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Solenopsis invicta arrived in Georgia in the 1950s. In 1953, they were limited to 12 small localized infestations in commercial nurseries scattered across the state. By 1976, imported fire ants were reported in 106 of Georgia's 159 counties; 11 years later, they were confirmed in 37 additional counties across northern Georgia (Canerday 1988). Gardner et al. (2008) postulated that the spread of imported fire ants into all 159 counties by 2000 was at least partially attributable to the invasion of the *S. richteri* X *S. invicta* hybrid from Alabama.

Williams et al. (2001) provided a historical review of control programs targeting the imported fire ants in their expanded North American range. In summary, control efforts have focused on the development of chemicals and application strategies that could provide relatively quick elimination of fire ant colonies. Yet, imported fire ants remain as a significant pest throughout this expanded range and are 5 - 10 times more abundant in the U.S. than in their native South American range (Porter et al. 1992, Porter et al. 1997). Thus, management strategies must be developed that are aimed at reducing populations of imported fire ants throughout quarantined areas while eliminating them from areas where large numbers of people are likely to come into contact with them. The utilization of natural enemies is but one strategy in such an integrated program aimed at overall reduction of populations.

Porter et al. (1997) eliminated a number of factors that might have explained the unusually high densities of imported fire ants in North America when compared with densities observed in South America and concluded that escape from natural enemies and other competitors occurring in South America was among the most likely explanations for the differences in density between the 2 continents. Porter (1998) surmised that classical biocontrol efforts involving the introduction of natural enemies of *Solenopsis* fire ants from their native range in South America into their expanded North American range might help reduce the density and negative impacts of imported fire ants in the southern United States.

Briano et al. (2012) reviewed efforts to identify and characterize various natural enemies of fire ants in South America. They cited the presence of microsporidia, viruses, a nematode, *Pseudacteon* decapitating flies, parasitoid wasps, a strepsipteran parasitoid, and a parasitic ant in fire ant populations in Argentina and neighboring countries. Of the parasitoids, the *Pseudacteon* flies were the most abundant and occurred most frequently.

Briefly, phorid flies are parasitoids of fire ant workers. Females oviposit individual eggs into the thorax of the worker ant where the larva hatches and feeds. Pupae use the head capsule as a pupal case as the head separates from the thorax, thus, the name decapitating flies. Parasitism rates are reportedly low in the field; however, the important detrimental impact is reduced activity and foraging of the worker ants when even a single phorid female is present. This stresses and weakens the colony to perhaps allow native ant species to successfully compete with the imported fire ants for food and other resources (Callcott et al. 2012).

Fourteen species of *Pseudacteon* parasitize fire ant workers in their native South American range. Many of these species are sympatric. Niche resources are partitioned among the different species by size of worker ants targeted, diel activity patterns, seasonal activity patterns, and mode of attack (Orr et al. 1997). Thus, classical biocontrol approaches using *Pseudacteon* flies should include introduction and establishment of multiple phorid species into the expanded range of fire ants.

As reviewed by Callcott et al. (2012) and Briano et al. (2012), efforts to introduce and successfully establish *Pseudacteon* flies in the southern U.S. range of the imported fire ant were initiated in the mid1990s. The first releases in Texas in 1995 were not successful (Gilbert et al. 2008); however, with improved rearing methods and conducive environmental conditions, *P. tricuspis* was successfully established with several releases made between 1997 and 1999 around Gainesville, FL (Porter et al. 2004). Permits were eventually obtained to expand the release program for additional *Pseudacteon* species and additional quarantined states. Callcott et al. (2012) review these release programs through 2008. Their review included known information on releases and distribution of *Pseudacteon* spp. in Georgia through 2008. However, additional releases have been made with concomitant statewide survey efforts to define the successful establishment and distribution of the *Pseudacteon* flies in Georgia. Our purpose herein is to provide updated information on the occurrence of *Pseudacteon* decapitating flies in Georgia.

Materials and Methods

Pseudacteon releases. Ten releases of *Pseudacteon* spp. decapitating flies were conducted in Georgia from 2000 through 2012. Date, location, and species released are listed in Table 1, and sites are indicated on the distribution map (Fig. 1).

Releases were made by one of two methods. The method of Porter et al. (2004) involved the hand-delivery or shipment of *Pseudacteon* pupae in covered plastic cups to our laboratory by USDA-ARS or USDA-APHIS cooperators. Covers were removed and cups containing pupae were placed in emergence boxes along with water-saturated castone blocks to maintain high levels of humidity in the boxes. The emergence boxes were placed in an environmental chamber at 25 ± 2°C on a photophase of 12 h diffused light and 12 h darkness. Once flies began emerging, the emergence boxes

Table 1. Site information for *Pseudacteon* spp. releases conducted in Georgia, 2000 - 2011.

No.	Date	Species released	County	GPS coordinates
1	October 2000	*P. tricuspis*	Tift	31.295416; –83.331953
2	April 2003	*P. tricuspis*	Spalding	33.240195; –84.475189
3	September 2004	*P. tricuspis*	Spalding	33.199482; –84.169620
4	July 2006	*P. curvatus*	Hall	34.28404; –83.44911
5	April 2007	*P. tricuspis*	Putnam	33.380964; –83.462173
6	October 2007	*P. curvatus*	Putnam	33.380964; –83.462173
7	September 2008	*P. curvatus*	Peach	32.656029; –83.736871
8	June 2009	*P. tricuspis*	Taliaferro	33.31367; –82.75415
9	April 2011	*P. obtusus*	Spalding	33.248648; –84.438881
10	December 2012	*P. curvatus* / *P. obtusus*	Spalding	33.248648; –84.438881

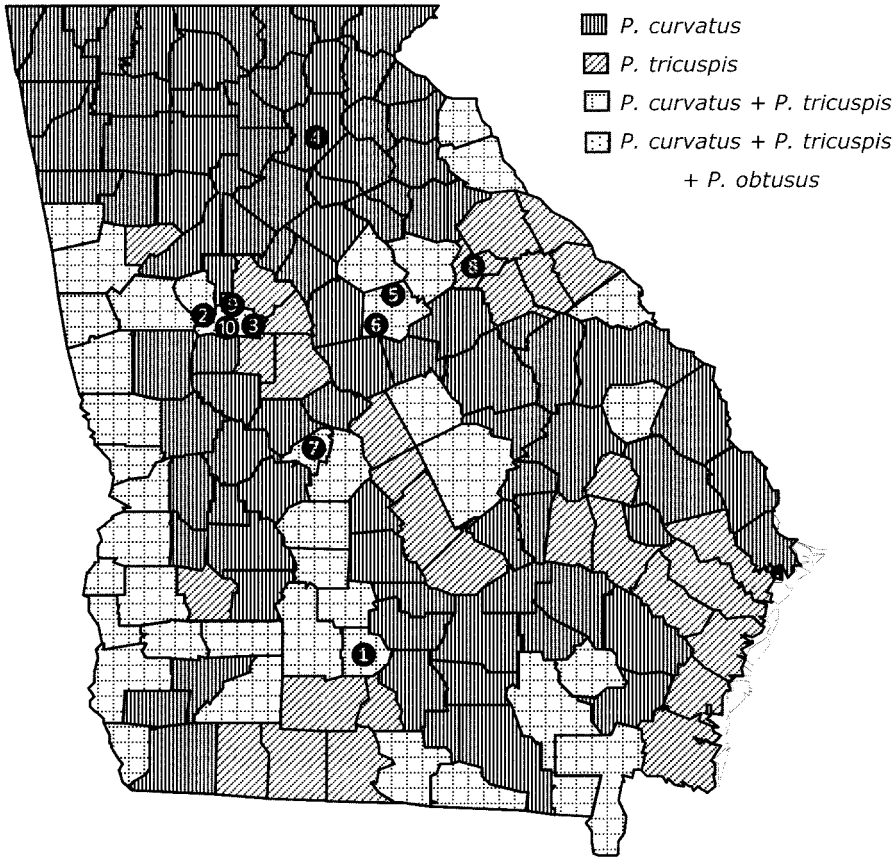

Fig. 1. Occurrence of *Pseudacteon* decapitating flies in Georgia counties with release sites indicated by numbers (*P. tricuspis*: ❶ 2000 in Tift Co., ❷ 2003 in Spalding Co., ❸ 2004 in Spalding Co., ❺ 2007 in Putnam Co., ❽ 2009 in Taliaferro Co.; *P. curvatus:* ❹ 2006 in Hall Co., ❻ 2007 in Putnam Co., ❼ 2008 in Peach Co., ❿ 2012 in Spalding Co.; *P. obtusus:* ❾ 2011 in Spalding Co., ❿ 2012 in Spalding Co.).

were moved to a collection cage each morning. Flies were allowed to escape into the cage and were aspirated into vials for transport to the release site. At the site, flies were released into attack boxes containing worker ants removed from defined colonies. Once all fly activity ceased in the boxes, the parasitized workers were returned to the mound from which they were removed. On-site exposure of worker ants to the flies by this method was an almost daily routine for 7 - 10 days or until fly emergence from the pupae halted.

The second release method was aptly described by Vogt and Streett (2003) and refined by others. It involved the collection and shipment of worker ants of specified

sizes from mounds to the USDA laboratory where they were exposed to flies. Parasitized worker ants were shipped back to us and returned to the mounds from which they were initially removed.

Monitoring. Phorid fly occurrence was monitored using two methods. Initially, we disturbed mounds or placed worker ants in white enamel trays and visually looked for phorid fly activity. We shifted to the use of the trap of Puckett et al. (2007) which was less labor intensive. Using these methods, we monitored areas in the vicinity of release sites for successful establishment of phorid flies. In addition, we conducted statewide surveys for *Pseudacteon* spp. beginning in 2008. In these surveys, imported fire ant mounds were located in each county and monitored for phorid fly occurrence. Sticky traps (Puckett et al. 2007) were placed in close proximity to disturbed mounds for varying periods of time. Flies collected were identified using ovipositor morphology as per Porter and Pesquero (2001).

Results and Discussion

Pseudacteon decapitating flies were released on 10 occasions at 8 sites in Georgia over a period spanning from 2000 through 2012 (Table 1). *Pseudacteon curvatus* Borgmeier was released at 4 sites, *P. tricuspis* Borgmeier was released at 5 sites, and *P. obtusus* Borgmeier was released at 1 site on 2 occasions. The 2000 release of *P. tricuspis* in Tift Co. and the 2006 release of *P. curvatus* in Hall Co. were initially deemed unsuccessful (Callcott et al. 2012) because phorid fly activity was not detected at those release sites 8 - 12 months after the respective releases. Our survey efforts in 2008 and 2009, however, yielded collections of *P. tricuspis* in Tift Co. and *P. curvatus* in Hall Co. indicating that both releases may have been successful but not detected until several years after the releases. The lack of confirmation of established *Pseudacteon* flies at those locations was probably more a function of lack of intensive monitoring activity in those areas rather than population dynamics of the parasitoids. The 2008 release of *P. tricuspis* in Peach Co. is classified as a supplemental release (Callcott et al. 2012) because *P. tricuspis* females were captured in routine trapping conducted while returning parasitized worker ants to their mounds during that release. *Pseudacteon tricuspis* had not been detected at that release site prior to that release. Collections of *Pseudacteon* flies at the remaining 5 release sites (7 releases) indicate that the parasitoid successfully established at those sites.

Survey activities from 2008 through 2012 confirmed that all 159 counties in Georgia have at least one species of *Pseudacteon* decapitating flies (Fig. 1). *Pseudacteon curvatus* was confirmed as occurring in 129 of Georgia's 159 counties, *P. tricuspis* was confirmed in 70 counties, and *P. obtusus* was confirmed in Spalding Co., the only county in which it was released. Both *P. curvatus* and *P. tricuspis* were confirmed in 39 counties, and all 3 *Pseudacteon* species were confirmed in 1 county (Spalding Co.) where all 3 species were released.

The successful establishment and spread of *P. curvatus* and *P. tricuspis* in Georgia can be directly linked to the releases conducted within Georgia (Table 1, Fig. 1) as well as the spread of parasitoids from successful releases conducted at sites along Georgia's borders in northern Florida (Porter et al. 2004), eastern Alabama (Graham et al. 2003a), Tennessee and South Carolina (Callcott et al. 2012). *Pseudacteon curvatus* has expanded its range in Georgia and the southern U.S. more rapidly than predicted (Porter 2010, Callcott et al. 2012). Callcott et al. (2012) reviewed several possible contributing factors. Briefly, *P. curvatus* has successfully established and spread in the northern

areas of the current range of imported fire ants in the U.S. with its adaptation to the cooler temperatures in those areas (Porter 2010) and its ability to successfully parasitize the black imported fire ant, *S. richteri* Forel, and the *S. richteri* x *S. invicta* hybrid that occupy the northern areas of the expanded range (Graham et al. 2003b). Conversely, *P. tricuspis* appears to be best adapted to warm, moist regions of the expanded range (Callcott et al. 2012) and is not well-adapted to *S. richteri* and the hybrid ant (He and Fadamiro 2009). *Pseudacteon curvatus* parasitoids are probably better colonizers than *P. tricupis*, and *P. curvatus* attacks the more abundant minor fire ant workers in colonies yielding higher densities of *P. curvatus* (Callcott et al. 2012). Furthermore, the successful dispersal of *P. tricuspis* into areas with high densities of *P. curvatus* may be limited (LeBrun et al. 2009). Each of these factors could contribute to the observed distribution of these 2 *Pseudacteon* species in Georgia (Fig. 1).

The potential impact that these phorid species are exerting on imported fire ant populations in Georgia has not been assessed. A limited number of previous studies conducted in other states report little to no impact on fire ant densities in the presence of only 1 phorid species (e.g., Morrison and Porter 2005a). Multiple *Pseudacteon* species, especially ones with differing niches (i.e., worker ant size attacked, seasonal and diel activity, etc.), would likely improve the potential impact on imported fire ant populations in their expanded range. Phorids kill a relatively small percentage of worker ants by directly parasitizing the workers (Morrison and Porter 2005b), but their greatest impact is by decreasing colony health and vigor by reducing worker ant foraging activity in the presence of *Pseudateon* female flies (Porter 1998). Oi et al. (2009) also report that *Pseudacteon* flies are carriers of the microsporidian *Kneallhazia solenopsae* Knell, Allen, and Hazard (Microsporidia: Thelohaniidae), an obligate intracellular pathogen of *S. invicta* and *S. richteri,* that has been found in most southern U.S. states.

The majority (75%) of Georgia's counties currently have only 1 species of *Pseudacteon* (Fig. 1). Successful use of *Pseudacteon* flies as a classical biological control management tool for imported fire ants, however, will depend upon establishing multiple species of the parasitoids thereby increasing parasitism rates of worker ants and decreasing foraging activities of worker ants during daylight hours. Thus, dispersal and supplementary releases of *P. tricuspis* and *P. curvatus* should be directed to increasing the coverage of greater areas of the state by both species than currently occurs. Resources should also be focused or diverted to rearing and release of additional *Pseudacteon* species (i.e., *P. obtusus, P. cultellatus* Borgmeier, *P. litoralis* Borgmeier, etc.) to accelerate coverage of the state with multiple species of the parasitoid.

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