



Dispersal and competitive impacts of a third fire ant decapitating fly (*Pseudacteon obtusus*) established in North Central Florida

Sanford D. Porter^{a,*}, Luis A. Calcaterra^b

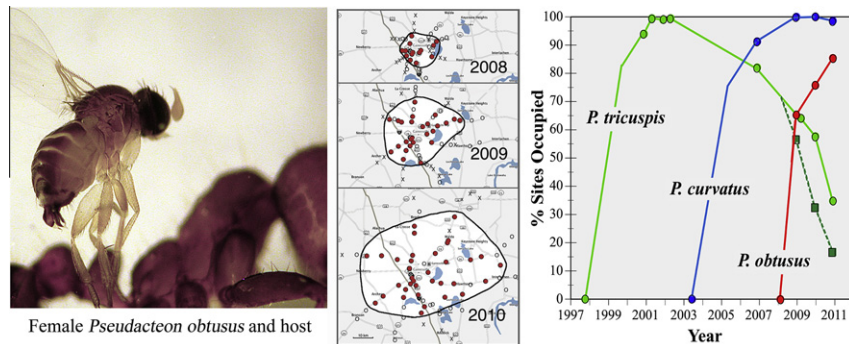
^a Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, 1600 SW 23rd Drive, Gainesville, FL 32608, USA

^b South American Biological Control Laboratory, USDA-ARS, Bolivar 1559 (1686) Hurlingham, Buenos Aires, Argentina

HIGHLIGHTS

- ▶ *Pseudacteon obtusus* is established in Florida and dispersing outward at 8–12 km/yr.
- ▶ *Pseudacteon curvatus* and *P. obtusus* are excluding the previously abundant *Pseudacteon tricuspis*.
- ▶ *P. obtusus* competes well with the very abundant *P. curvatus*, unlike *P. tricuspis*.
- ▶ Despite strong competition, the three flies were positively associated with each other.
- ▶ Release of *P. obtusus* did not measurably increase net parasitoid numbers.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 27 April 2012

Accepted 18 September 2012

Available online 16 October 2012

Keywords:

Competitive exclusion
Competitive displacement
Coexistence
Biological control
Solenopsis invicta
Phoridae
Parasitoid

ABSTRACT

Self-sustaining classical biological control agents offer hope for permanent wide-area control of imported *Solenopsis* fire ants in the United States because escape from abundant natural enemies left behind in Argentina is a likely reason for unusually high fire ant densities in the United States. The fire ant decapitating fly *Pseudacteon obtusus* Borgmeier (Diptera: Phoridae) was released as a biocontrol agent of the red imported fire ant (*Solenopsis invicta* Buren) in Gainesville, FL because it is a common parasitoid of this ant in Argentina and because it has a higher propensity of attacking fire ants along foraging trails than the two *Pseudacteon* species previously released. Field surveys of a rapidly expanding *P. obtusus* population (8–12 km/yr) proved that this fly was capable of thriving and successfully competing with the much more abundant *Pseudacteon curvatus* Borgmeier. However, *Pseudacteon tricuspis* Borgmeier, the first decapitating fly released, was effectively excluded from most sample sites when faced with competition from both *P. curvatus* and the similar-sized *P. obtusus*. Despite clear evidence for competitive exclusion, *P. tricuspis* abundance at sample sites was positively correlated with the abundance of its two competitors—probably because of moderate to strong covariability in the suitability of sample sites for all three congeners. The addition of *P. curvatus*, the second parasitoid released, increased total parasitism pressure on fire ant populations by about 10-fold. The addition of *P. obtusus*, the third species, did not measurably improve total guild parasitism rates on imported fire ants in North Central Florida (as assessed by roadside trap counts), but the performance of this species will likely vary with habitat, region, and climate.

Published by Elsevier Inc.

1. Introduction

Red imported fire ants (*Solenopsis invicta* Buren) were accidentally introduced into the United States from northern Argentina (Caldera et al., 2008) sometime in the 1930s (Lennartz, 1973).

* Corresponding author.

E-mail addresses: sanford.porter@ars.usda.gov (S.D. Porter), luiscalcaterra@hotmail.com (L.A. Calcaterra).

Escape from natural enemies left behind in South America is a likely reason why imported fire ant populations in the United States average 5–10 times higher than in their native homelands (Porter et al., 1997; Wilder et al., 2011). High densities are the primary reason that imported fire ants cause serious agricultural, environmental, and health concerns in the United States (Lofgren, 1986; Vinson, 1997) and other countries recently invaded by these pests (Ascunce et al., 2011).

Several dozen natural enemies have been found in South America (Briano et al., 2012; Williams et al., 2003) including a group of very small decapitating flies (Patrock et al., 2009; Porter and Pesquero, 2001) in the genus *Pseudacteon* (Diptera: Phoridae). Several *Pseudacteon* flies have been selected for release in the United States as potential fire ant biocontrol agents because they are very host specific (Porter and Gilbert, 2004) and they have had sufficient impacts on fire ant populations to have caused the evolution of a suite of defenses against their attacks (Porter, 1998).

Pseudacteon flies have an unusual life history of decapitating their host and using the empty head capsule as a pupal case (Porter, 1998). Almost two dozen species of *Pseudacteon* flies have been discovered in South America attacking *Solenopsis* fire ants in the *saevissima* species group (Patrock et al., 2009). At least 10 species have been recorded attacking *S. invicta* (Calcaterra et al., 2005). Large-sized *Pseudacteon* species parasitize large fire ant workers up to almost 6 mm in length while smaller *Pseudacteon* species parasitize small fire ant workers that are only 2–3 mm long. *Pseudacteon* flies also partition niche space by time-of-day, season, geographic habitat, and a preference for attacking host ants either at mounds or on foraging trails (Calcaterra et al., 2008; Folgarait et al., 2005a, 2007; Patrock et al., 2009; Porter, 1998). Sites often support 2–4 common species as well as an equivalent number of uncommon or rare species. The sex of larger *Pseudacteon* species like *P. tricuspsis* Borgmeier and *P. obtusus* Borgmeier appears to be environmentally determined by the size of the host—with females emerging from large hosts and males emerging from medium to small-sized hosts (Folgarait et al., 2005b; Morrison et al., 1999). However, smaller fly species like *Pseudacteon curvatus* Borgmeier attack only small workers and do not exhibit sexual dimorphism (Chirino et al., 2009; Porter, 2000).

Six species of fire ant decapitating flies in the genus *Pseudacteon* have been successfully released and established in the United States: *P. tricuspsis*, a medium-large species (first released 1997, Porter et al., 2004); *P. curvatus*, a small species (first biotype released 2000, Graham et al., 2003; second biotype released 2003, Vazquez et al., 2006); *P. littoralis* Borgmeier, a large species (2005, Porter et al., 2011); *P. obtusus*, a medium-large species (2006, Plowes et al., 2011b); *P. nocens* Borgmeier, a medium to large species (2006–2010, Plowes et al., 2011a); and *P. cultellatus* Borgmeier, a small species (2010, unpublished data). For comparison, average thorax widths for females of the above species are as follows: 0.51 ± 0.03 , 0.33 ± 0.03 , 0.57 ± 0.02 , 0.53 ± 0.02 , 0.49 ± 0.05 , and 0.33 ± 0.02 mm (mean \pm SD, $n = 10$, from lab colonies; also see Calcaterra et al., 2005; Folgarait et al., 2005b, 2006; Morrison et al., 1997). The first two species released, *P. tricuspsis* and *P. curvatus*, are common and widely distributed across the southeastern United States (Callcott et al., 2010). *Pseudacteon littoralis* and *P. nocens* currently have limited ranges with occasionally abundant populations in Alabama and Texas, respectively. The establishment and expansion of *P. obtusus* in Florida will be detailed in this paper. *Pseudacteon cultellatus* appears to be established at two release sites in Florida where it is in the early stages of expansion.

Studies of the overall effects of this guild of parasitoids on fire ant populations in the United States are in progress, but at this point, impacts do not appear dramatic. Impacts of *P. tricuspsis*, the first species released, did not rise above a 10–30% statistical sensitivity caused by natural host population variability (Morrison and

Porter, 2005). Impacts of *P. curvatus*, the second species released, have yet to be assessed, but their densities are much higher (Porter, 2010). Several studies show that *Pseudacteon* flies have the potential to act as vectors of fire ant pathogens (Oi et al., 2009; Valles et al., 2009), although this possibility has yet to be verified.

The fact that *P. tricuspsis* and *P. curvatus* are congeners restricted to the same host species makes interspecific competition more likely (Reitz and Trumble, 2002), even though they attack mostly different sizes of host workers. LeBrun et al. (2009) reported that *P. curvatus* was displacing the larger *P. tricuspsis* in large parts of Central Texas. The magnitude of *P. tricuspsis* population reductions ranged from 70% to 97% and appeared to be a combination of direct exploitive competition for smaller-sized workers, as evidenced by a sex ratio shift in *P. tricuspsis* from strongly male biased to weakly female biased, and indirect interference competition caused by the more abundant *P. curvatus* suppressing the availability of large fire ant workers required by *P. tricuspsis* to produce female flies. LeBrun et al. (2009) concluded from their tests that indirect competition was likely the more important mechanism driving competitive displacement of *P. tricuspsis* by *P. curvatus*.

Other modes of interspecific competition are also possible but less likely for fire ant decapitating flies. For example, larval competition in jointly parasitized hosts is common with hymenopteran parasitoids (Mills, 2006) and does also occur in some situations with *Pseudacteon* parasitoids (Porter et al., 1995), but probably not commonly based on parasitism rates and differing host-size preferences (Calcaterra et al., 2008; Morrison et al., 1997). Host marking to avoid superparasitism (Nufio and Papaj, 2001) has not been experimentally tested with *Pseudacteon* flies, but observations of attack behavior in the lab indicate that host ants can be attacked multiple times. Disruptive or aggressive behavior is common among competing parasitoids (Boivin and Brodeur, 2006), but does not occur between adult female *Pseudacteon* flies, although it is possible that the frequent mating attempts of *P. tricuspsis* and *P. obtusus* males (Calcaterra et al., 2005) might be disruptive to other species of *Pseudacteon* females which do not mate while ovipositing. Intraguild predation and apparent competition, although important with some parasitoid guilds (Janssen et al., 2006; van Veen et al., 2006), are also not likely scenarios for *Pseudacteon* flies because they have no known hyperparasitoids or specialist predators and they cannot parasitize each other.

Competitive interactions with previously established biocontrol agents often reduce the chances of successfully establishing additional biocontrol agents for the same target host (Mills, 2006). Indeed, Plowes et al. (2011b) reported that the probability of establishing *P. obtusus* at new release sites in Texas declined dramatically when it was released at sites where it was competing with previously released *Pseudacteon* species.

The objectives of this paper are to: (1) document the establishment and expansion of *P. obtusus*, a third species of *Pseudacteon* fly in North Central Florida, (2) examine the competitive impacts of all three *Pseudacteon* species on each other, and (3) determine how the addition of each new species affected the summed population of this parasitoid guild.

2. Materials and methods

2.1. Origin of fly populations

The *P. obtusus* flies in this study were collected at a camping area along a large oxbow of the Paraguay River (26.514° S, 58.284° W) about 3 km SE of Herradura, Formosa Province, Argentina. Most if not all of the host fire ants in the area were *S. invicta*. All of the *P. obtusus* flies in the initial collections were large-form flies (Calcaterra et al., 2007; Kronforst et al., 2007), but there were

initially two biotypes: (1) a standard form with evenly brown abdominal tergites, and (2) a less common patterned form (10–20% of flies) with two light patches on each tergite. Only the standard form survived in a colony established in quarantine facilities (USDA-ARS, Gainesville, FL) from 271 females and 575 males collected October 2002 and 103 females and 86 males collected April 2003. Flies were reared in small rearing boxes for 4–5 months before being transferred to large automatic boxes similar to those described by Vogt et al. (2003). After conducting extensive host specificity and safety tests (Estrada et al., 2006, SDP unpublished; Porter and Gilbert, 2004) and reviews by state, federal, and international organizations, we received permission for field releases early in 2008. Flies from the *P. obtusus* colony described above were also released in Texas (Gilbert et al., 2008; Plowes et al., 2011b).

2.2. Expansion monitoring

Flies were released in the University of Florida Natural Area (29.6347° N, 82.3694° W) and around our laboratory complex about 1 km to the east (29.6365° N, 82.3600° W). Red imported fire ants were abundant in both areas. Flies were released both as adults over disturbed mounds and also as larvae in parasitized ants (see Porter et al., 2004; Vazquez et al., 2006). Several thousand flies were used over a period of about one month.

The expansion of *P. obtusus* flies was monitored in the falls of 2008 (6 Nov–16 Dec), 2009 (23 Oct–8 Dec), and 2010 (22 Oct–23 Nov) using modified Puckett tri-stand sticky traps (Puckett et al., 2007) developed by APHIS personnel in Gulfport, MS. These traps were similar to those described by Porter (2010) except that an inverted 1-oz plastic condiment cup was used to attach the pizza tri-stand to the lid base. The tri-stand was coated with Tangle-Trap® to catch the flies. The outer side of the condiment cup was coated with a Fluon-like suspension of fluoropolymer resin to keep fire ants off the sticky part of the tri-stand. Each trap was placed in a large petri dish (150 × 25 mm), which was placed in a disturbed fire ant mound. The petri dish was coated on the inside with the Fluon-like suspension so that it functioned as pitfall trap for several thousand ants that usually fell in and were then bait for the flies. One or more plastic plates (23 cm) were oriented over the traps with wire flags to act as shades in hot sunny weather. Occasionally, fire ants from the lab were added to traps if ant mounds in the area were not sufficiently large. Two traps were generally set out at each site along road right-of-ways with good fire ant habitat. Traps were left out for 2–6 h between 10 am and 6 pm when air temperatures were above 22 °C. Sites were 1–10 km apart (see Fig. 1) and were generally different year to year. GPS coordinates and photos were obtained for each site along with environmental notes. About 75% of mounds both inside ($n = 56$ mounds) and outside ($n = 36$) the *P. obtusus* range appeared to be monogyne in 2010.

The outer range limit in a particular direction was usually established after finding two negative sites beyond the last positive one. Negative sites with *P. tricuspis* flies were especially valuable because they established that the site was good for larger *Pseudacteon* flies and their presence was often an indication of the absence of *P. obtusus*. The range limit line was estimated by drawing a line between the last positive site and the first negative site, keeping in mind the locations of other nearby positive sites (Fig. 1). The average radius of the range was determined from eight measurements. The first two radii were oriented along the long axis of the estimated distribution and the next six measurements were made at 45° increments.

2.3. Statistics

Linear regressions were used to assess the significance of the relationships between fly abundance and the distance from release

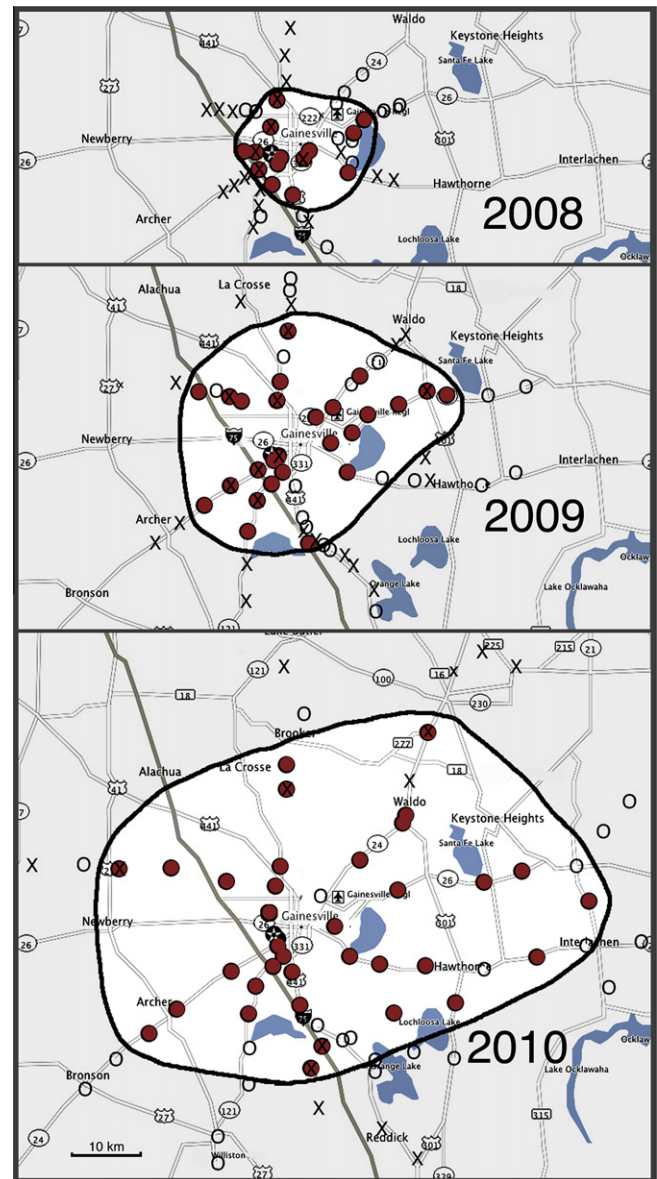


Fig. 1. Annual expanding distribution of *Pseudacteon obtusus* flies (filled dots) for autumn 2008–2010 from release sites (star) in Gainesville, Florida. “X”s indicate sites with *Pseudacteon tricuspis* and “O”s indicate sites with only *Pseudacteon curvatus* (*P. curvatus* was collected at almost 100% of all sites both within and outside of the *P. obtusus* distribution, see Fig. 3).

sites. Data were log-transformed to linearize the relationships. Linear regressions were also used to compare abundances of the different fly species with each other. Analysis of Variance was used to compare trap catch numbers of flies for the survey sites when data could be normalized by log transformations. Nonparametric Kruskal–Wallis tests were used when comparing data from sites where the absence of a species at sample sites created data sets with large numbers of zeros. Two by two chi-square tests were used to compare differences in the frequency occurrence of *P. tricuspis* at sites within and outside of the range of *P. obtusus*.

3. Results

3.1. Expansion monitoring

By the end of 2008, *P. obtusus* had expanded outward 4–16 km (8.4 ± 4.1 , mean \pm SD) from the release sites and occupied an area

of about 275 km² (Fig. 1). By the fall of 2009, *P. obtusus* expanded outward 13–28 km (17.4 ± 5.1) and occupied about 1025 km². By fall 2010, *P. obtusus* had expanded 22–48 km (29.8 ± 9.1) from the release sites and occupied about 2970 km². Expansion rates were faster to the east or a bit north of east (Fig. 1), but expansion rates in the other cardinal directions were similar. The expansion rate increased from 8.9 km/yr in 2009 to 12.4 km/yr in 2010. Within the range boundaries shown in Fig. 1, *P. obtusus* was found at 65% of sites in 2008, 76% in 2009, and 85% in 2010 (see Fig. 3). However, this percentage increases to 92% (12/13) if only 2009 sites within the 2008 range boundary are considered, and 90% (18/20) if only 2010 sites within the 2009 range boundary are considered.

Each fall, the mean number of *P. obtusus* flies collected per trap (within the range of its occurrence) was negatively related to the distance from the release sites (Fig. 2). Linear regressions against the log of fly numbers were highly significant ($P \leq 0.0022$) and accounted for 58% (2008), 44% (2009), and 35% (2010) of variability.

Six-month mean maximum temperature (30.3 ± 1.0 °C) and precipitation (572 ± 61 mm) averages prior to the fall samples were similar for 2006 and 2008–2010. Results were also similar for 3-month averages except that 2010 received only about 30% of the rain as the previous years (48 mm versus 170 ± 3 mm).

3.2. Competitive interactions

Before competition with other *Pseudacteon* species, *P. tricuspis* occupied nearly 100% of roadside and pasture sites (Fig. 3 and 2000–2002). In fall 2006, *P. tricuspis* still occurred at about 80% of sample sites recently occupied by *P. curvatus*. The spatial abundance of *P. tricuspis* progressively declined from fall 2008 until fall 2010, when just over a third of sites contained *P. tricuspis* when in competition with only *P. curvatus*. *Pseudacteon tricuspis* occurrence rates declined even faster when *P. obtusus* was also present: by the fall of 2009, *P. tricuspis* was found at only 32% of sites compared with 59% of sites when in competition with only *P. curvatus* (Fig. 3; χ^2 test, Fisher exact $P = 0.070$, 2-tailed test). By 2010, occurrence rates had fallen to 17% and 35%, respectively (Fig. 3; χ^2 test,

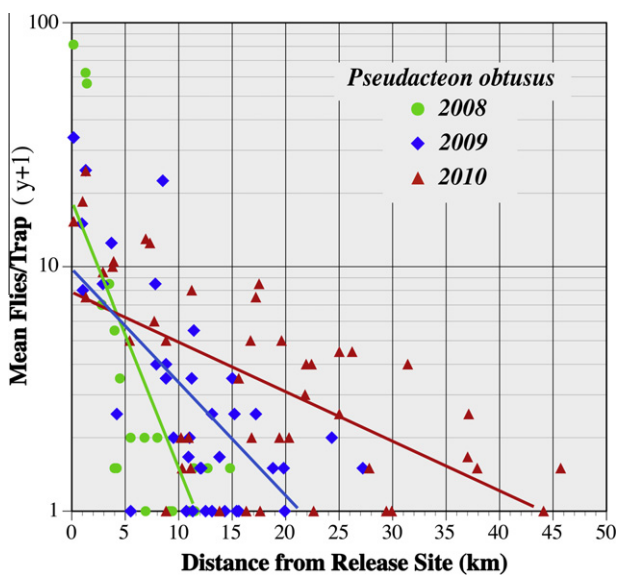


Fig. 2. *Pseudacteon obtusus* fly abundance (both sexes) at sites within the estimated range (see Fig. 1) plotted against distance from release sites for the falls of 2008–2010. Fly numbers are shown on a log scale with a $y + 1$ transformation. Line equations: 2008, $\log(y + 1) = -0.115x + 1.25$, $R^2 = 0.582$, $P = 0.0022$, $n = 23$; 2009, $\log(y + 1) = -0.046x + 0.97$, $R^2 = 0.436$, $P < 0.001$, $n = 37$; 2010, $\log(y + 1) = -0.0205x + 0.876$, $R^2 = 0.351$, $P < 0.001$, $n = 47$.

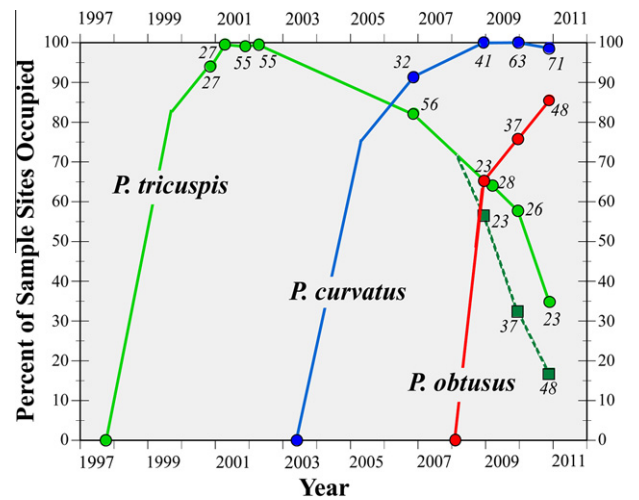


Fig. 3. Spatial abundance of three species of fire ant decapitating flies at sample sites in North Central Florida plotted against time. The number of sample sites is shown by each data point. The round data points on the *P. tricuspis* line show values for sites either before *P. obtusus* was released or before it arrived. The squares indicate *P. tricuspis* values from sites within the expanding range of *P. obtusus*. The 2000–2002 data are from Morrison and Porter (2005) and the fall 2006 data are from Porter (2010).

Fisher exact $P = 0.129$, 2-tailed test). Combining the 2009 and 2010 data above resulted in a more robust statistical difference for the occurrence rates of *P. tricuspis* at sites with and without *P. obtusus* (χ^2 test, Fisher exact $P = 0.0071$, 2-tailed test). In 2010 *P. tricuspis* was not found at any of the survey sites, which had been occupied by both *P. curvatus* and *P. obtusus* for a year or more (Fig. 1; compare 2010 with 2008 and 2009). In contrast, by 2010 *P. obtusus* was present in almost 90% of sites within its range (Figs. 1 and 3); thus making *P. obtusus* much more spatially abundant than *P. tricuspis* when it was in competition with *P. curvatus* alone. After 2006, *P. curvatus* was virtually ubiquitous at sites with fire ant colonies, occurring at 98–100% of sampled sites.

The numerical abundance of the three species of decapitating flies collected on monitoring traps (Table 1) was similar to the pattern reported above for their spatial abundance or occurrence at the survey sites (Fig. 3). The release of *P. curvatus* flies in North Central Florida resulted in trap catch rates for *P. tricuspis* declining from 4–6 flies per trap in 2006 down to less than 1 fly per trap in 2009 and 2010 (Kruskal–Wallis Test $H = 11.32$, $df = 3$, $P < 0.015$, $n = 23, 28, 26$, and 23; Table 1). Significantly fewer *P. tricuspis* flies were collected when both *P. curvatus* and *P. obtusus* were present (2008–2010) compared to when only *P. curvatus* was present (Kruskal–Wallis test, $H = 12.6$, $df = 1$, $P < 0.002$, $n = 108$ and 77; Table 1). By 2010, only about 1 *P. tricuspis* fly was captured for every seven traps when in competition with both *P. curvatus* and *P. obtusus* or about one-fifth the rate for sites where *P. tricuspis* was only in competition with *P. curvatus*.

In 2006, *P. curvatus* trap rates, adjusted to account for males, at recently invaded sites were only one-half to one-third of those for *P. tricuspis* (Table 1, Porter, 2010). However, sites that had been occupied for 2 years or more by *P. curvatus* (2008–2010) had adjusted *P. curvatus* trap rates that were about 10 times higher than they had been for *P. tricuspis* alone. Trap rates for *P. obtusus* (2008–2010) were similar to those of *P. tricuspis* before the introduction of *P. curvatus* (2006). Trap rates for *P. curvatus* from 2008 to 2010 at sites with and without *P. obtusus* were not statistically different (2-way ANOVA, $P = 0.11$, $F_{1,179} = 2.63$, log transformation; Table 1) nor was there a significant interaction between year and the presence or absence of *P. obtusus* (two-way ANOVA, $F_{2,179} = 0.250$, $P = 0.78$). The abundance of *P. obtusus* did not differ significantly

Table 1
Adjusted mean number^b of *Pseudacteon* decapitating flies (both sexes) captured per trap as a function of time and the combination of other *Pseudacteon* species present in the range. Means are shown \pm SE with number of sample sites in parentheses.

<i>Pseudacteon</i> species (other species present)	Fall			
	2006 ^c	2008	2009	2010
<i>P. tricuspidis</i> ^a (only)	4.53 \pm 1.42 (23)	—	—	—
<i>P. tricuspidis</i> ^a (with <i>P. curvatus</i>)	6.45 \pm 1.72 (23)	2.71 \pm 0.86 (28)	0.88 \pm 0.21 (26)	0.80 \pm 0.41 (23)
<i>P. tricuspidis</i> ^a (with <i>P. curvatus</i> and <i>P. obtusus</i>)	—	2.01 \pm 0.64 (23)	0.31 \pm 0.11 (37)	0.14 \pm 0.7 (48)
<i>P. curvatus</i> ^b (with <i>P. tricuspidis</i>)	1.96 \pm 0.57 (23)	62.5 \pm 9.9 (28)	46.2 \pm 7.7 (26)	66.7 \pm 14.4 (23)
<i>P. curvatus</i> ^b (with <i>P. tricuspidis</i> and <i>P. obtusus</i>)	—	77.3 \pm 15.6 (23)	38.2 \pm 6.9 (37)	51.8 \pm 6.7 (48)
<i>P. obtusus</i> ^a (with <i>P. tricuspidis</i> and <i>P. curvatus</i>)	—	9.70 \pm 4.71 (23)	4.23 \pm 1.23 (37)	4.31 \pm 0.80 (48)

^a Both sexes.

^b Unlike *P. tricuspidis* and *P. obtusus*, *P. curvatus* males are not attracted to fire ants so they were not captured in the traps. The primary sex ratio for *P. curvatus* is 1:1 (Chirino et al., 2009; Porter, 2000); therefore the values for *P. curvatus* were doubled to make the numbers of *P. curvatus* trapped comparable with the other two species.

^c Data are from (Porter, 2010).

between 2008, 2009, or 2010 (Kruskal–Wallis test, $H = 3.0$, $df = 2$, $P = 0.23$, $n = 23, 37, 48$; Table 1).

Trap capture rates indicate that the addition of *P. curvatus*, to the pre-existing *P. tricuspidis* population, increased the total parasitism pressure on imported fire ants by about 10-fold (Table 2) when comparing 2006 to the 2008–2010 period (one-way ANOVA, $F_{3,227} = 60.8$, $P < 0.001$, log transformation, Fisher LSD test). When comparing only 2008–2010 data, total capture rates did not vary significantly either by year or with the addition of *P. obtusus* (two-way ANOVA, $P > 0.05$, Table 2).

In the fall of 2009 at sites within the range of *P. obtusus* ($n = 37$ sites), 87.9% of the female flies on the traps were *P. curvatus*, 11.9% were *P. obtusus*, and 0.6% were *P. tricuspidis*. In the fall of 2010, 90.3% of females were *P. curvatus*, 9.4% were *P. obtusus*, and 0.3% were *P. tricuspidis* ($n = 48$ sites).

A series of regressions were conducted to examine whether the number of *P. curvatus*, *P. tricuspidis*, and *P. obtusus* flies trapped were positively or negatively related with each other (Table 3). Outside the expanding *P. obtusus* range, *P. tricuspidis* numbers were positively associated with *P. curvatus* densities for 2008, 2009, and for the three-year combined regression. Inside the *P. obtusus* range, *P. tricuspidis* and *P. curvatus*, flies were positively associated for 2010, close for 2008, and positively associated for the combined regression (Table 3). *Pseudacteon obtusus* flies were positively associated with *P. curvatus* flies for 2008 and for the combined regression (Table 3). *Pseudacteon tricuspidis* numbers were positively associated with *P. obtusus* for 2009, close for 2008, and positively associated for the combined regression (Table 3). None of the regressions were significantly negative or close to being significantly negative.

3.3. Sex percentages

Of the *P. obtusus* flies trapped while monitoring expansion of this species, we found 39% (20/52) were male in 2008, 36% (71/200) were male in 2009, and 34% (118/344) were male in 2010.

Table 2
Adjusted mean number^a of *Pseudacteon* fire ant decapitating flies captured per trap (both sexes, all species present) across years and species combinations. Means are shown \pm SE.

Species combination	Fall			
	2006 ^b	2008	2009	2010
<i>P. tricuspidis</i> only	4.5 \pm 1.4	—	—	—
<i>P. tricuspidis</i> and <i>curvatus</i> ^a	8.4 \pm 1.7	65.2 \pm 10.5	47.1 \pm 7.8	67.5 \pm 14.5
<i>P. tricuspidis</i> , <i>curvatus</i> ^a , <i>obtusus</i>	—	89.0 \pm 19.3	42.8 \pm 7.2	56.3 \pm 6.7

^a Numbers of *P. curvatus* females were doubled to account for males which are not trapped like the other two species (see Table 1).

^b Data for 2006 are from (Porter, 2010).

For *P. tricuspidis*, 56% of flies (140/249) collected outside the range of *P. obtusus* (2008–2010) were male, while 57% were male (90/157) inside the *P. obtusus* range. Only 0.24% of *P. curvatus* flies trapped during the monitoring study were males.

4. Discussion

4.1. Expansion rates

Once established, *P. obtusus* flies expanded rapidly out from the release sites at average rates of 8–12 km/yr or about 1.0–1.5 km per generation (Fig. 1) assuming 7–8 generations per year in North Central Florida. These rates are equivalent to rates reported for *P. tricuspidis* in North Central Florida a decade earlier (Porter et al., 2004), but only about two-thirds the rates reported for *P. curvatus* in the same area (Porter, 2010). Maximum expansion rates for *P. obtusus* at the La Paloma release site in Texas were similar to the maximum rate in Florida, but expansion rates at several other sites in Texas appear to be slower (Plowes et al., 2011b). The expansion rates of *P. obtusus* in Florida will likely continue to increase over time as it has for other *Pseudacteon* species (Henne et al., 2007a; Pereira and Porter, 2006; Porter and Gilbert, 2004), as rare long-distance dispersal events become more and more likely with increasing populations (Henne et al., 2007b; LeBrun et al., 2008).

By the end of 2010, *P. obtusus* in North Central Florida occupied an area of almost 3000 km² with a distribution that was biased toward the east (Fig. 1). Expansion patterns of both *P. tricuspidis* and *P. curvatus* were also biased to the east from releases in the Gainesville area (Porter, 2010; Porter et al., 2004). The reason for this easterly bias is unknown. Strong prevailing winds just to the north of east might explain this bias except that wind patterns in the Gainesville area also blow at about equal rates slightly to the south of west (Porter et al., 2004). Expansion patterns of *P. tricuspidis* flies in Texas followed strong prevailing winds to the NNW (LeBrun et al., 2008). In Louisiana, *P. tricuspidis* showed a northward tendency congruent with prevailing winds and perhaps hurricane storm tracks (Henne et al., 2007a).

Strong correlations between the abundance of *P. obtusus* flies and the distance from the release sites (Fig. 2) indicate that fly populations gradually increased at newly occupied sites over a period of a year or more. Similar relationships were reported for both *P. tricuspidis* (Henne et al., 2007a; Porter et al., 2004) and *P. curvatus* (LeBrun et al., 2009). For year three (2010), we expected to see the number of sites without *P. obtusus* diminish nearer to the release areas and maximum fly numbers level off at distances which had been occupied for longer time periods (Fig. 2). The number of sites without *P. obtusus* did diminish as predicted, but maximum fly numbers continued to increase with no sign of leveling off. Variability associated with yearly weather, different sites, or the

Table 3Pairwise regressions of the abundance of the three *Pseudacteon* species at sample sites both inside and outside the expanding range of *P. obtusus* (2008–2010).

Location Species	Year	No. of sites	R ²	P-value	Relationship
Outside <i>P. obtusus</i> range					
<i>P. tricuspis</i> vs. <i>P. curvatus</i>					
	2008	28	0.42	0.0002	+
	2009	26	0.18	0.030	+
	2010	23	0.07	0.21	0
	2008–2010	77	0.18	0.0001	+
Inside <i>P. obtusus</i> range					
<i>P. tricuspis</i> vs. <i>P. curvatus</i>					
	2008	23	0.12	0.11	+
	2009	37	0.02	0.46	0
	2010	48	0.09	0.035	+
	2008–2010	108	0.11	0.0004	+
<i>P. obtusus</i> vs. <i>P. curvatus</i>					
	2008	23	0.44	0.0005	+
	2009	37	0.15	0.37	0
	2010	48	0.006	0.60	0
	2008–2010	108	0.18	0.00001	+
<i>P. tricuspis</i> vs. <i>P. obtusus</i>					
	2008	18	0.17	0.094	+
	2009	29	0.58	0.00001	+
	2010	40	0.002	0.77	0
	2008–2010	87	0.21	0.00001	+

expanding range may explain this anomaly, but no clear patterns were detected. Clearly, *P. obtusus* was both locally and temporally abundant, occasionally reaching numbers in excess of 60 flies per trap (Fig. 2).

4.2. Intra-guild competition: *P. tricuspis* and *P. curvatus*

Pseudacteon tricuspis, the first fire ant decapitating fly released in the United States, occupied nearly 100% of the sample sites a year or so after moving into an area (Fig. 3, Morrison and Porter, 2005). Porter et al. (2004) reported occurrence rates of 80–90%, but their data included areas only recently occupied by the expanding *P. tricuspis* population. Fly densities were about 1 fly per mound in 30 min of effort with an aspirator at pasture sites in 2000–2002 (Morrison and Porter, 2005) and about 4–6 flies/trap at roadside sites in 2006 (Table 1, Porter, 2010).

Pseudacteon curvatus became very abundant within several years of its release, occupying almost 100% of sample sites at densities about 10 times those previously found for *P. tricuspis* (Fig. 3; Table 1, compare 2006 with 2008–2010). The reasons for these high densities are unknown. One possibility is that smaller fire ant workers, preferred by *P. curvatus*, are 3–6 times more abundant in fire ant colonies than the larger workers preferred by *P. tricuspis*. Another possibility is that the biotype of *P. curvatus* released in the Gainesville area is better matched to its host because it was collected near the presumptive origin of fire ants in northern Argentina (Caldera et al., 2008; Vazquez et al., 2006) while *P. tricuspis* was collected about 1200 km away in Brazil (Porter et al., 2004). The problem with the first explanation is that *P. curvatus* is not particularly abundant along the Parana and Paraguay Rivers in northeastern Argentina where it was collected (Calcaterra et al., 2005) even though smaller fire ant workers are abundant. The problem with the second explanation is that a biotype of *P. tricuspis*, collected from the same site as the *P. curvatus* biotype (Formosa, Argentina), was released and is likely established in Texas (Callcott et al., 2010), but *P. curvatus* is still dominant in this area (Puckett, 2008).

The addition of *P. curvatus* resulted in dramatic declines in both the spatial and numerical abundance of *P. tricuspis* (Fig. 3, Table 1).

After 5–7 years of competition with only *P. curvatus*, *P. tricuspis* was still present in the Gainesville area, but it only occurred at 35% of sites and still appeared to be declining (Fig. 3); furthermore, average trap catch rates had dropped from about 4 flies per trap to under 1 fly per trap (Table 1).

As described in the introduction, LeBrun et al. (2009) also reported that *P. curvatus* had dramatic impacts on *P. tricuspis* populations in Texas due to both indirect and direct competition. LeBrun et al. (2009) proposed that direct scramble or exploitive competition for smaller workers resulted in a sex ratio shift for *P. tricuspis* from strongly male biased (64% males) before the arrival of the smaller more abundant *P. curvatus* flies to weakly female biased (54% *P. tricuspis* females) after their arrival. We found a similar though less dramatic shift. In 2006 before *P. curvatus* invaded sites in North Central Florida, *P. tricuspis* collections were strongly male biased (66% males, $n = 663$) and collections were still strongly male biased at sites with low numbers of *P. curvatus* (65% males, $n = 1007$). However, after *P. curvatus* was firmly established (2008–2010), *P. tricuspis* collections became less male biased both in areas with only *P. curvatus* (56% males, $n = 245$) and in areas with both *P. curvatus* and *P. obtusus* (57%, $n = 157$). Collections of *P. tricuspis* in Louisiana and Mississippi in areas without *P. curvatus* were also strongly male biased (64% males, Henne and Johnson, 2009) as were collections in northeastern Argentina where *P. curvatus* populations are very low (62% males, $n = 384$, Calcaterra et al., 2005).

4.3. Intra-guild competition: *P. curvatus*, *P. tricuspis* and *P. obtusus*

Pseudacteon obtusus did very well in competition with both *P. curvatus* and *P. tricuspis*. A year or so after invading an area, *P. obtusus* had occupied about 90% of the sample sites (Fig. 3, also see interiors of distributions in Fig. 1) and was about as numerically abundant as *P. tricuspis* had been without competition from *P. curvatus* (Table 1). This competitive success may be because *P. obtusus* is more attracted to foraging workers than either *P. curvatus* or *P. tricuspis* (Folgarait et al., 2007). Also, *P. obtusus* attacks slightly larger workers than does *P. tricuspis* (unpublished data, SDP), thus perhaps expanding its resource base and reducing competition

with the other two species. The problem with both of the above arguments is that *P. obtusus* and *P. tricuspsis* are about equally abundant along the Parana and Paraguay Rivers in northeastern Argentina while *P. curvatus* is uncommon (Calcaterra et al., 2005). Clearly, whatever is regulating guild structure in one continent is not functioning the same in the other.

After 2–3 years of competition with *P. obtusus* and *P. curvatus*, *P. tricuspsis* was almost eliminated from sample sites (Fig. 3, Table 1). Presumably, the addition of similar-sized *P. obtusus* flies strongly reduced the survival of the remaining *P. tricuspsis* populations. No *P. tricuspsis* flies were found at 17 sites in 2010 where *P. obtusus* had been present for at least a year; however, several *P. tricuspsis* were still found near the *P. obtusus* release sites in 2010 (2 flies) and 2011 (1 fly) in other sampling efforts. Whether *P. tricuspsis* will remain a rare species in North Central Florida or be extirpated entirely is unknown. It is possible that *P. tricuspsis* may hold out or even thrive in specialized habitats, or other regions of the United States (e.g., Brazos Bend, TX, Plowes et al., 2011b; or 5-Eagle Ranch, TX, Puckett, 2008). What is clear, however, is that if we had been able to predict this outcome, *P. tricuspsis* would not have been released in the Gainesville area.

Unlike *P. tricuspsis*, collections of *P. obtusus* were strongly female biased (65% females, $n = 596$) around Gainesville while collections in South America were moderately female biased (57% females, $n = 251$; Calcaterra et al., 2005). However, the percentage of female flies from field parasitized ants in the Gainesville area was more equitable (48% females, $n = 140$, SDP, unpublished), suggesting that males may not be as attracted to mounds as females or that males may be harder to trap.

4.4. Abundance correlations between competing species

Overall, the data strongly support the conclusion that *P. curvatus* and *P. obtusus* are competitively and progressively excluding *P. tricuspsis* from study sites in North Central Florida (Table 1, Fig. 3). Nevertheless, regressions of *P. tricuspsis* abundance against *P. curvatus* abundance both outside and inside the expanding range of *P. obtusus* were significantly positive two of three years and one of three years, respectively (Table 3). Similarly, correlations between *P. tricuspsis* and *P. obtusus* were significantly positive one of three years and close for another year. Combined data for all three years were significantly positive for all pairwise species combinations (Table 3). In short, despite solid evidence for strong negative impacts on *P. tricuspsis* populations, correlations with the other two *Pseudacteon* species in this study were never significantly negative (2008–2010). The likely explanation for this seemingly counter-intuitive relationship is that sites which were very good for the dominant species were also still good for the remaining populations of the species being displaced. In fact, it seems likely that positive correlations between similar species actively engaged in competitive exclusion should be the expected outcome whenever they have moderate to strong co-variability in site suitability (Levine and D'Antonio, 1999; Morrison and Porter, 2003; Stohlgren et al., 1999).

Even negative correlations between two species may not indicate competition (Bell, 2005; Hastings, 1987; Schluter, 1984). For example, LeBrun et al. (2009) reported a negative correlation between *P. curvatus* and *P. tricuspsis* at 16 locations in Argentina. While this correlation may indeed reflect competitive interactions, it should be noted that a negative relationship only existed for two of the four regions sampled. In particular, *P. curvatus* is rarely abundant in northeastern Argentina along the Parana and Paraguay Rivers (Calcaterra et al., 2005) and also large parts of Brazil (unpublished data) where *P. tricuspsis* and other *Pseudacteon* species are often common. A second concern is that non-competitive factors strongly favoring *P. tricuspsis* over *P. curvatus* or vice versa would

also produce a similar negative relationship. For example, differences in habitat preferences, climatic tolerances, and/or host suitability among at least two sibling species of *P. tricuspsis* (Porter and Pesquero, 2001), several biotypes of *P. curvatus* (Vazquez et al., 2004; 2006) and the 4–5 fire ant species commonly found in different regions of Argentina (Trager, 1991) could all potentially strongly favor one fly over the other.

4.5. Benefit of additional biological control agents

Ideally, each new fire ant biocontrol agent would result in increased parasitism pressure on its host. This clearly happened when *P. curvatus* was added and adjusted trap rates increased by about an order of magnitude (Table 2). While *P. obtusus* has done very well in competition with *P. curvatus*, at least compared to *P. tricuspsis*, the addition of *P. obtusus* did not measurably increase the total parasitoid population (Table 2). It should be noted that *P. obtusus* and *P. tricuspsis* flies parasitize fire ant workers which average about three times the biomass of workers parasitized by *P. curvatus* flies (SDP, unpublished data). Presumably the value of a fire ant worker to its colony is proportional to its biomass. Assuming this to be true, it might be better to compare the cumulative biomass of parasitized fire ant workers represented by the flies on each trap rather than a simple count of the number of flies. Such an adjustment would make the 2009 and 2010 means for the 3-species parasite community about equal to the means for the 2-species community (Table 2) but still would not have resulted in significantly higher estimates for parasitism impacts. In short, it appears that the impacts of adding *P. obtusus* to the parasitoid community were either minimal or sufficiently modest that our sampling methods did not detect a net benefit in the Gainesville area.

Nevertheless, it is still possible that *P. obtusus* may provide important net benefits if impacts were assessed on a yearly basis or with more sensitive methods (e.g., measuring parasitism rates directly). A number of biological control agents are known to perform better in different climates or habitats (Abe and Tokumaru, 2008; Mills, 2006; Sorribas et al., 2010), and this may be true for *P. obtusus* or even *P. tricuspsis* considering the geographic breadth encompassed by imported fire ants in the United States and other countries. For example, *P. obtusus* from northern Argentina (26.5° S) may prove more cold tolerant in the United States than the *P. tricuspsis* biotype from Jaguariuna near São Paulo, Brazil (22.7° S) which is doing poorly in the northern third of *S. invicta*'s US range (Callcott et al., 2010). Finally, because *P. obtusus* is more common at foraging trails than the other species (Folgarait et al., 2007), it may disrupt fire ant foraging to a greater degree than the other two species, thus making a straight comparison of trap numbers misleading when estimating relative impacts on fire ant populations.

5. Conclusions

The successful field release of *P. obtusus* in Gainesville, FL provides clear evidence for both competitive coexistence with *P. curvatus* and competitive exclusion of *P. tricuspsis* (Table 1, Fig. 3). These results, along with those from other biocontrol programs (Reitz and Trumble, 2002), provide strong evidence for the importance of competition in structuring the composition of species in closely related parasitoid guilds. While our sampling efforts did not provide evidence that *P. obtusus* increased net parasitism pressure on fire ant populations in North Central Florida (Table 3), our methods were also insufficient to exclude this possibility, especially in other habitats, climates, or seasons. Overall, we feel that the proclivity of *P. obtusus* flies to parasitize foraging workers

(Folgarait et al., 2007), their ability to parasitize larger workers, and their success in competing with high populations *P. curvatus* (Table 1), merit release attempts in several additional states.

Acknowledgments

We would especially like to thank Juan Briano (USDA-ARS, SABC, Hurlingham, Argentina) for technical and logistical support of this study and phorid fly research generally. Cynthia Vann and Darrell Hall (USDA-ARS, CMAVE, Gainesville, FL) ably assisted with fly rearing, host-specificity testing, and fire ant rearing. Darrell Hall also helped with field monitoring efforts. Field release of the flies used in this paper would not have been possible if Charlie Brown (USDA-APHIS, PPQ, Riverdale, MD) had not personally shepherded a FONSI through regulatory channels. Ed LeBrun (University of Texas, Austin), Colleen Porter (Gainesville, FL), and Lloyd Morrison (Missouri State Univ., Springfield) read the manuscript and provided a number of helpful comments; Lloyd also provided several important references. We also thank those who assisted with reviewing the field release proposal for *P. obtusus* at the state, federal, and international levels as well as the anonymous reviewers of this paper. Funding provided by USDA-APHIS, PPQ assisted substantially with rearing the *Pseudacteon* flies mentioned in this paper.

References

- Abe, Y., Tokumaru, S., 2008. Displacement in two invasive species of leafminer fly in different localities. *Biological Invasions* 10, 951–953.
- Ascunce, M.S., Yang, C.C., Oakey, J., Calcaterra, L., Wu, W.J., Shih, C.J., Goudet, T.J., Ross, K.G., Shoemaker, D., 2011. Global invasion history of the fire ant *Solenopsis invicta*. *Science* 331, 1066–1068.
- Bell, G., 2005. The co-distribution of species in relation to the neutral theory of community ecology. *Ecology* 86, 1757–1770.
- Boivin, G., Brodeur, J., 2006. Intra- and interspecific interaction among parasitoids: mechanisms, outcomes and biological control. In: Boivin, G., Brodeur, J. (Eds.), *Trophic and Guild Interactions in Biological Control*. Springer, Dordrecht, The Netherlands, pp. 123–144.
- Briano, J., Calcaterra, L., Varone, L., 2012. Fire ants (*Solenopsis* spp.) and their natural enemies in southern South America. *Psyche* 2012, 1–19.
- Calcaterra, L.A., Delgado, A., Tsutsui, N.D., 2008. Activity patterns and parasitism rates of fire ant-decapitating flies (Diptera: Phoridae: *Pseudacteon* spp.) in their native Argentina. *Annals of the Entomological Society of America* 101, 539–550.
- Calcaterra, L.A., Porter, S.D., Briano, J.A., 2005. Distribution and abundance of fire ant decapitating flies (Diptera: Phoridae: *Pseudacteon*) in three regions of southern South America. *Annals of the Entomological Society of America* 98, 85–95.
- Calcaterra, L.A., Vander Meer, R.K., Pitts, J.P., Livore, J.P., Tsutsui, N.D., 2007. Survey of *Solenopsis* fire ants and their parasitoid flies (Diptera: Phoridae: *Pseudacteon*) in Central Chile and Central Western Argentina. *Annals of the Entomological Society of America* 100, 512–521.
- Caldera, E.J., Ross, K.G., DeHeer, C., Shoemaker, D.D., 2008. Putative native source of the invasive fire ant *Solenopsis invicta* in the USA. *Biological Invasions* 10, 1457–1479.
- Callcott, A.-M.A., Porter, S.D., Weeks Jr., R.D., Graham, L.C., Johnson, S.J., Gilbert, L.E., 2010. Fire ant decapitating fly cooperative release programs (1994–2008): two *Pseudacteon* species, *P. tricuspidis* and *P. curvatus*, rapidly expand across imported fire ant populations in the southeastern United States. *Journal of Insect Science* 11 (19), 1–25. Available at: <<http://insectscience.org/11.19>>.
- Chirino, M.G., Gilbert, L.E., Folgarait, P.J., 2009. Behavior and development of *Pseudacteon curvatus* (Diptera: Phoridae) varies according to the social form of its host *Solenopsis invicta* (Hymenoptera: Formicidae) in its native range. *Environmental Entomology* 38, 198–206.
- Estrada, C., Patrock, R.J.W., Folgarait, P.J., Gilbert, L.E., 2006. Host specificity of four *Pseudacteon* spp. (Diptera: Phoridae), parasitoids of fire ants in Argentina (Hymenoptera: Formicidae). *Florida Entomologist* 89, 462–468.
- Folgarait, P.J., Bruzzone, O., Porter, S.D., Pesquero, M.A., Gilbert, L.E., 2005a. Biogeography and macroecology of phorid flies that attack fire ants in south-eastern Brazil and Argentina. *Journal of Biogeography* 32, 353–367.
- Folgarait, P.J., Chirino, M.G., Patrock, R.J.W., Gilbert, L.E., 2005b. Development of *Pseudacteon obtusus* (Diptera: Phoridae) on *Solenopsis invicta* and *Solenopsis richteri* fire ants (Hymenoptera: Formicidae). *Environmental Entomology* 34, 308–316.
- Folgarait, P.J., Patrock, R.J., Gilbert, L.E., 2006. Development of *Pseudacteon nocens* (Diptera: Phoridae) on *Solenopsis invicta* and *Solenopsis richteri* fire ants (Hymenoptera: Formicidae). *Journal of Economic Entomology* 99, 295–307.
- Folgarait, P.J., Patrock, R.J.W., Gilbert, L.E., 2007. Associations of fire ant phorids and microhabitats. *Environmental Entomology* 36, 731–742.
- Gilbert, L.E., Barr, C.L., Calixto, A.A., Cook, J.L., Drees, B.M., LeBrun, E.G., Patrock, R.J.W., Plowes, R.M., Porter, S.D., Puckett, R.T., 2008. Introducing phorid fly parasitoids of red imported fire ant workers from South America to Texas: outcomes vary by region and by *Pseudacteon* species released. *Southwestern Entomologist* 33, 15–29.
- Graham, L.C., Porter, S.D., Pereira, R.M., Dorough, H.D., Kelley, A.T., 2003. Field releases of the decapitating fly *Pseudacteon curvatus* (Diptera: Phoridae) for control of imported fire ants (Hymenoptera: Formicidae) in Alabama, Florida, and Tennessee. *Florida Entomologist* 86, 334–339.
- Hastings, A., 1987. Can competition be detected using species co-occurrence data? *Ecology* 68, 117–123.
- Henne, D.C., Johnson, S.J., 2009. Sampling and dynamics of *Pseudacteon tricuspidis* (Diptera: Phoridae) in Louisiana. *Environmental Entomology* 38, 539–550.
- Henne, D.C., Johnson, S.J., Cronin, J.T., 2007a. Population spread of the introduced red-imported fire ant parasitoid, *Pseudacteon tricuspidis* Borgmeier (Diptera: Phoridae), in Louisiana. *Biological Control* 42, 97–104.
- Henne, D.C., Johnson, S.J., Porter, S.D., 2007b. Status of *Pseudacteon tricuspidis* Borgmeier (Diptera: Phoridae) releases in Louisiana. *Florida Entomologist* 90, 565–569.
- Janssen, A., Montserrat, M., HilleRisLambers, R., de Roos, A.M., Pallini, A., Sabelis, M.W., 2006. Intraguild predation usually does not disrupt biological control. In: Brodeur, J., Boivin, G. (Eds.), *Trophic and Guild Interactions in Biological Control*. Springer, Dordrecht, The Netherlands, pp. 191–220.
- Kronforst, M., Folgarait, P.J., Patrock, R.J.W., Gilbert, L.E., 2007. Genetic differentiation between body size biotypes of the parasitoid fly *Pseudacteon obtusus* (Diptera: Phoridae). *Molecular Phylogenetics and Evolution* 43, 1178–1184.
- LeBrun, E.G., Plowes, R.M., Gilbert, L.E., 2008. Dynamic expansion in recently introduced populations of fire ant parasitoids (Diptera: Phoridae). *Biological Invasions* 10, 989–999.
- LeBrun, E.G., Plowes, R.M., Gilbert, L.E., 2009. Indirect competition facilitates widespread displacement of one naturalized parasitoid of imported fire ants by another. *Ecology* 90, 1184–1194.
- Lennartz, F.E., 1973. Modes of Dispersal of *Solenopsis invicta* from Brazil into the Continental United States – A Study in Spatial Diffusion. M.S. Thesis, University of Florida, 242 p.
- Levine, J.M., D'Antonio, C.M., 1999. Elton revisited: a review of evidence linking diversity and invasibility. *Oikos* 87, 15–26.
- Lofgren, C.S., 1986. The economic importance and control of imported fire ants in the United States. In: Vinson, S.B. (Ed.), *Economic Impact and Control of Social Insects*. Praeger, NY, pp. 227–256.
- Mills, N., 2006. Interspecific competition among natural enemies and single versus multiple introductions in biological control. In: Brodeur, J., Boivin, G. (Eds.), *Trophic and Guild Interactions in Biological Control*. Springer, Dordrecht, The Netherlands, pp. 191–220.
- Morrison, L.W., Dall'Aglio-Holvorcem, C.G., Gilbert, L.E., 1997. Oviposition behavior and development of *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). *Environmental Entomology* 26, 716–724.
- Morrison, L.W., Porter, S.D., 2003. Positive association between densities of the red imported fire ant, *Solenopsis invicta*, and generalized ant and arthropod diversity. *Environmental Entomology* 32, 548–554.
- Morrison, L.W., Porter, S.D., 2005. Testing for population-level impacts of introduced *Pseudacteon tricuspidis* flies, phorid parasitoids of *Solenopsis invicta* fire ants. *Biological Control* 33, 9–19.
- Morrison, L.W., Porter, S.D., Gilbert, L.E., 1999. Sex ratio variation as a function of host size in *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). *Biological Journal of the Linnean Society* 66, 257–267.
- Nufio, C.R., Papaj, D.R., 2001. Host marking behavior in phytophagous insects and parasitoids. *Entomologia Experimentalis et Applicata* 99, 273–293.
- Oi, D.H., Porter, S.D., Valles, S.M., Briano, J.A., Calcaterra, L.A., 2009. *Pseudacteon* decapitating flies (Diptera: Phoridae): are they potential vectors of the fire ant pathogens *Kneallhazia* (= *Thelohania*) *solenopsae* (Microsporidia: Thelohaniidae) and *Vairimorpha invictae* (Microsporidia: Burenellidae)? *Biological Control* 48, 310–315.
- Patrock, R.J.W., Porter, S.D., Gilbert, L.E., Folgarait, P.J., 2009. Distributional patterns of *Pseudacteon* associated with the *Solenopsis saevissima* complex in South America. *Journal of Insect Science* 9 (60), 17 pp. Available at: <<http://insectscience.org/9.60>>.
- Pereira, R.M., Porter, S.D., 2006. Range expansion of the fire ant decapitating fly, *Pseudacteon tricuspidis*, eight to nine years after releases in North Florida. *Florida Entomologist* 89, 536–538.
- Plowes, R.M., Folgarait, P.J., Gilbert, L.E., 2011a. The introduction of the fire ant parasitoid *Pseudacteon nocens* in North America: challenges when establishing small populations. *BioControl* 56, 295–304.
- Plowes, R.M., LeBrun, E.G., Gilbert, L.E., 2011b. Introduction of the fire ant decapitating fly *Pseudacteon obtusus* in the United States: factors influencing establishment in Texas. *BioControl* 56, 295–304.
- Porter, S.D., 1998. Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). *Florida Entomologist* 81, 292–309.
- Porter, S.D., 2000. Host specificity and risk assessment of releasing the decapitating fly, *Pseudacteon curvatus*, as a classical biocontrol agent for imported fire ants. *Biological Control* 19, 35–47.
- Porter, S.D., 2010. 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. *Florida Entomologist* 93, 107–112.

- Porter, S.D., Gilbert, L.E., 2004. Assessing host specificity and field release potential of fire ant decapitating flies (Phoridae: *Pseudacteon*). In: Van Driesche, R.G., Murray, T., Reardon, R., (Eds.), *Assessing host ranges for parasitoids and predators used for classical biological control: a guide to best practice*. FHTET-2004-03, USDA Forest Service, Morgantown, West Virginia, pp. 152–176.
- Porter, S.D., Graham, L.C., Johnson, S.J., Thead, L.G., Briano, J.A., 2011. The large decapitating fly *Pseudacteon litoralis* (Diptera: Phoridae): successfully established on fire ant populations in Alabama. *Florida Entomologist* 94, 208–213.
- Porter, S.D., Nogueira de Sá, L.A., Morrison, L.W., 2004. Establishment and dispersal of the fire ant decapitating fly *Pseudacteon tricuspis* in North Florida. *Biological Control* 29, 179–188.
- Porter, S.D., Pesquero, M.A., 2001. Illustrated key to *Pseudacteon* decapitating flies (Diptera: Phoridae) that attack *Solenopsis saevissima* complex fire ants in South America. *Florida Entomologist* 84, 691–699.
- Porter, S.D., Pesquero, M.A., Campiolo, S., Fowler, H.G., 1995. Growth and development of *Pseudacteon* phorid fly maggots (Diptera: Phoridae) in the heads of *Solenopsis* fire ant workers (Hymenoptera: Formicidae). *Environmental Entomology* 24, 475–479.
- Porter, S.D., Williams, D.F., Patterson, R.S., Fowler, H.G., 1997. Intercontinental differences in the abundance of *Solenopsis* fire ants (Hymenoptera: Formicidae): an escape from natural enemies? *Environmental Entomology* 26, 373–384.
- Puckett, R.T., 2008. Decapitating Flies *Pseudacteon* spp. (Diptera: Phoridae) Parasitoids of Red Imported Fire Ants *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Texas A&M University, College Station, Texas, Ph.D. Dissertation, 103 p.
- Puckett, R.T., Calixto, A., Barr, C.L., Harris, M., 2007. Sticky traps for monitoring *Pseudacteon* parasitoids of *Solenopsis* fire ants. *Environmental Entomology* 36, 584–588.
- Reitz, S.R., Trumble, J.T., 2002. Competitive displacement among insects and arachnids. *Annual Review of Entomology* 47, 435–465.
- Schluter, D., 1984. A variance test for detecting species associations, with some example applications. *Ecology* 65, 998–1005.
- Sorribas, J., Rodriguez, R., Garcia-Mari, F., 2010. Parasitoid competitive displacement and coexistence in citrus agroecosystems: linking species distribution with climate. *Ecological Applications* 20, 1101–1113.
- Stohlgren, T.J., Binkley, D., Chong, G.W., Kalkhan, M.A., Schell, L.D., Bull, K.A., Yuka, O., Newman, G., Bashkin, M., Son, Y., 1999. Exotic plant species invade hot spots of native plant diversity. *Ecological Monographs* 69, 25–46.
- Trager, J.C., 1991. A revision of the fire ants, *Solenopsis geminata* group (Hymenoptera: Formicidae: Myrmicinae). *Journal of the New York Entomological Society* 99, 141–198.
- Valles, S.M., Oi, D.H., Porter, S.D., 2009. *Kneallhazia* (= *Thelohania*) *solenopsae* infection rate of *Pseudacteon curvatus* flies determined by multiplex PCR. *Florida Entomologist* 92, 344–349.
- van Veen, F.J., Memmott, J., Godfray, H.C.J., 2006. Indirect effects, apparent competition and biological control. In: Boivin, G., Brodeur, J. (Eds.), *Trophic and Guild Interaction in Biological Control*. Springer, Dordrecht, The Netherlands, pp. 145–169.
- Vazquez, R.J., Porter, S.D., Briano, J.A., 2004. Host specificity of a new biotype of the fire ant decapitating fly *Pseudacteon curvatus* (Diptera: Phoridae) from Northern Argentina. *Environmental Entomology* 33, 1436–1441.
- Vazquez, R.J., Porter, S.D., Briano, J.A., 2006. Field release and establishment of the decapitating fly *Pseudacteon curvatus* on red imported fire ants in Florida. *BioControl* 51, 207–216.
- Vinson, S.B., 1997. Invasion of the red imported fire ant (Hymenoptera: Formicidae): spread, biology, and impact. *American Entomologist* 43, 23–39.
- Vogt, J.T., Porter, S.D., Nordlund, D.A., Smith, R., 2003. A modified rearing system for production of *Pseudacteon curvatus* (Diptera: Phoridae), a parasitoid of imported fire ants. *Biological Control* 28, 346–353.
- Wilder, S.M., Holway, D.A., Suarez, A.V., LeBrun, E.G., Eubanks, M.D., 2011. Intercontinental differences in resource use reveal the importance of mutualisms in fire ant invasions. *Proceedings of the National Academy of Sciences* 108, 20639–20644.
- Williams, D.F., Oi, D.H., Porter, S.D., Pereira, R.M., Briano, J.A., 2003. Biological control of imported fire ants (Hymenoptera: Formicidae). *American Entomologist* 49, 150–163.