



# Testing for population-level impacts of introduced *Pseudacteon tricuspis* flies, phorid parasitoids of *Solenopsis invicta* fire ants

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## Abstract

Parasitoid phorid flies in the genus *Pseudacteon* are potential biocontrol agents of invasive *Solenopsis* fire ants in the US. One *Pseudacteon* species, *P. tricuspis* Borgmeier, has been released and is now established over a large region of north central Florida. We conducted a multi-year, large-scale field experiment to investigate potential population-level impacts on host *Solenopsis invicta* Buren fire ants and associated arthropods. We established and monitored 67 field sites over a 3.5 year period, documenting *S. invicta* density and activity by three complementary sampling methods: (1) mound counts and measurements, (2) attraction to baits, and (3) pitfall trap catches. *P. tricuspis* abundance was determined by censusing flies at disturbed *S. invicta* mounds. *P. tricuspis* populations increased in abundance over the first two years following colonization and then oscillated seasonally, reaching higher abundances in the fall than in the preceding spring. *S. invicta* populations varied greatly over time, and repeated-measures ANCOVA revealed no effect of phorid parasitism pressure on *S. invicta* density or activity. *P. tricuspis* likely has a relatively small effect on *S. invicta* populations, which may not be consistent over space and time and is difficult to document against the background of high variability in fire ant populations due to various environmental factors (i.e., rainfall, disturbance). *P. tricuspis* parasitism pressure (averaged over the course of the experiment) was positively associated with changes in *S. invicta* density. Additionally, *P. tricuspis* abundance was positively correlated with *S. invicta* density after *P. tricuspis* populations had been established for >1 year. Introduced phorid populations may require many years before a measurable impact is apparent. Alternatively, parasitism pressure of a single phorid species may not be a major factor regulating fire ant populations.

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## 1. Introduction

The red imported fire ant, *Solenopsis invicta* Buren, is one of the worst invasive ant pests (see reviews in Adams, 1986; Allen et al., 1994; Davidson and Stone, 1989; Holway et al., 2002; Lofgren, 1986; Taber, 2000; Vinson, 1997). Introduced to the United States from South America in the 1930s, this species now occupies

>128 million hectares in 13 states and Puerto Rico (Callcott, 2002) and is established on numerous Caribbean islands (Davis et al., 2001). Recently discovered in New Zealand (Harris, 2001; Pascoe, 2001) and Australia (Natrass and Vanderwoude, 2001; Solley et al., 2002), *S. invicta* could potentially invade many regions worldwide (Morrison et al., 2004).

Until recently, attempts to eradicate or control *S. invicta* in the United States have relied heavily upon pesticides (Davidson and Stone, 1989). Despite intense, widespread efforts from the 1950s to the 1970s to eradicate *S. invicta*, this species has continued to expand its range (Callcott and Collins, 1996). Eradication is no

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longer considered a viable objective, and present methods of chemical control are generally not cost effective in rural areas (Allen, 1993; Barr and Drees, 1996).

*Solenopsis invicta* is much more abundant in the United States than in its South American homeland, and one of the hypotheses for this difference is escape from natural enemies (Porter et al., 1997). Recent efforts to control imported fire ants have focused on natural enemies, such as protozoan pathogens (Briano et al., 1995), fungi (Stimac et al., 1993), parasitic ants (Calcaterra et al., 1999), and dipteran parasitoids (Porter, 1998a; Morrison, 2000a).

It has been suggested that parasitoid phorid flies may play an important role in regulating host ant populations (Feener, 2000). Some phorid flies in the genus *Pseudacteon* are solitary parasitoids of *Solenopsis* fire ant workers (Disney, 1994). The basic biology and history of the study of *Pseudacteon* spp. that parasitize *Solenopsis* ants are reviewed by Porter (1998a) and Morrison (2000a). Phorid flies are unique among other biological control agents currently being evaluated because, in addition to the direct effect of mortality, phorid flies may also indirectly effect their host through behavioral modification (Morrison, 1999).

Extensive testing, both in the field in South America (Porter, 1998b; Porter et al., 1995a) and in the laboratory in the United States and South America (Folgarait et al., 2002; Gilbert and Morrison, 1997; Morrison and Gilbert, 1999; Porter, 2000; Porter and Alonso, 1999; Porter and Briano, 2000), has revealed high levels of host specificity in most *Pseudacteon* species that parasitize *Solenopsis* fire ants. Additionally, studies conducted both in the field (Feener and Brown, 1992; Folgarait and Gilbert, 1999; Morrison, 1999; Orr et al., 1995, 1997; Porter et al., 1995b) and in the laboratory (Mehdiabadi and Gilbert, 2002; Morrison, 2000b) have revealed that the presence of *Pseudacteon* spp. may have relatively large, short-term behavioral impacts on host *Solenopsis* spp.

Extrapolation of such short-term behavioral impacts to long-term population regulation in nature is fraught with difficulties, however, given the complexity of *Solenopsis* biology. Ultimately, the only way to determine the population- and community-level effects of phorid flies is to study their impacts in nature over the crucial temporal and spatial scales. Thus, we conducted a multi-year, large-scale field experiment to address this issue.

*Pseudacteon tricuspidis* Borgmeier was released at a number of sites in north Florida and population abundances and potential impacts on *S. invicta* were evaluated over 3.5 years. The following major questions were posed: (1) How abundant will introduced *P. tricuspidis* populations become in north Florida, and will populations vary over time? (2) Do introduced *P. tricuspidis* populations have a measurable impact on *S. invicta* populations? and (3) What is the relationship between *P. tricuspidis* abundance and *S. invicta* density and activity?

## 2. Methods

### 2.1. Selection of study sites

This study was conducted in northern Florida at sites located between 29°38' and 30°32'N latitude and between 82°07' and 83°27'W longitude. Site selection was dependent upon habitat and *S. invicta* social form. Sites were established in pastures and along roadsides—two habitats that are common in northern Florida and typically support high *S. invicta* densities. All pasture sites were located in full sun and grazed by cattle or horses, although grazing intensity varied among sites. All roadside sites were mown at intervals and bordered by wooded areas. No sites had any recent history of pesticide use. We selected sites containing the monogyne (i.e., single queen) form of *S. invicta*, based on field identification of colony characteristics, rejecting sites with the polygyne (i.e., multiple queen) social form. Because both social forms coexist in many areas of this region, and distinguishing between social forms in the field (for large numbers of colonies) is not entirely accurate, a small proportion of polygyne colonies may have been present at some sites. All sites were at least 0.5 km, and usually farther, apart.

At each site, we established a permanent study plot. Plots were standardized by *S. invicta* mound number rather than by size. Plots initially contained 15–20 mounds and varied in size between 467 and 2394 m<sup>2</sup>. Larger plots were used at sites with fewer mounds to reduce sampling error associated with possible non-random distribution of mounds.

Thirty-six treatment sites (24 pasture, 12 roadside) were established between 29°38' and 29°55'N latitude and between 82°16' and 82°40'W longitude, from May 1998 to April 2000. The *P. tricuspidis* population released in this study was originally obtained from São Paulo State, Brazil in 1996, and reared in quarantine facilities at the USDA-ARS in Gainesville, FL (Porter et al., 2004). *P. tricuspidis* was successfully released and established in the field in at least seven locations at or near the study sites from July 1997 to April 2000, and subsequently dispersed to almost all treatment sites by the fall of 2000 (Porter et al., 2004) (Fig. 1).

Thirty-six sites (24 pasture, 12 roadside) originally intended to be controls were established at least 20 km away, between 29°57' and 30°09'N latitude and between 82°07' and 82°43'W longitude in early spring of 2000, under the assumption that *P. tricuspidis* would not colonize these sites over the course of this experiment, based on early expansion rates of 3–4 km/year. As *P. tricuspidis* populations grew, however, this species subsequently dispersed at much higher rates (15–30 km/year; Porter et al., 2004) and this second group of sites became colonized during 2001. Thus, a third set of 12 sites (8 pasture and 4 roadside) was established as controls between

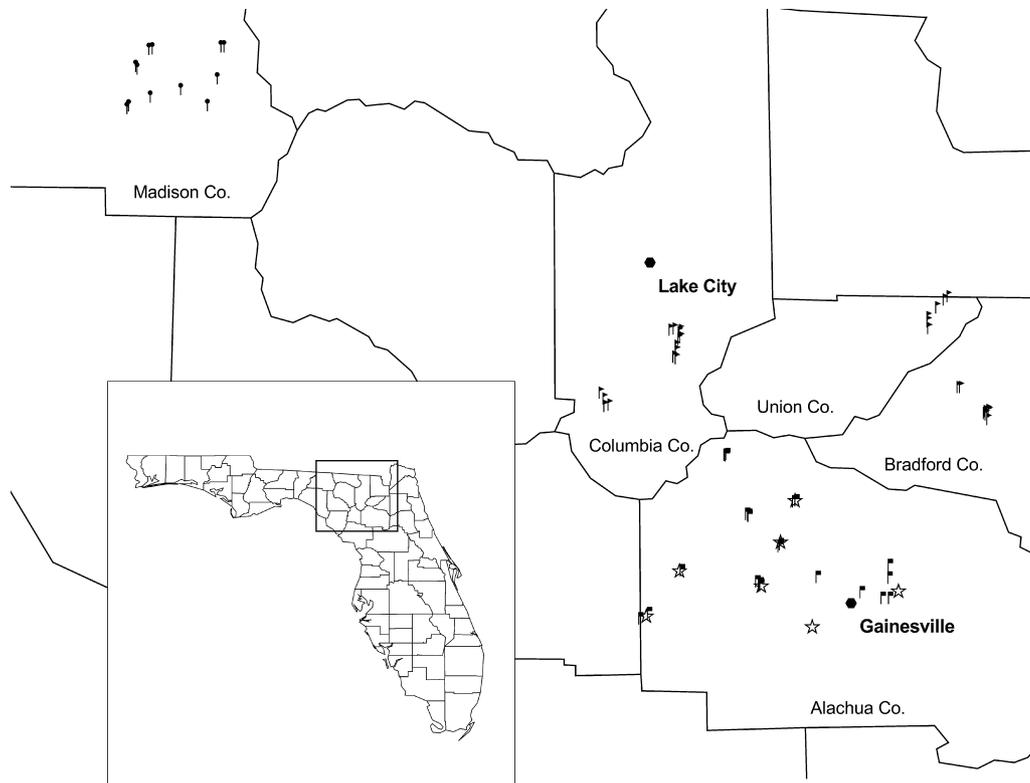


Fig. 1. Map showing location of study sites. Square flags, colonized by *P. tricusps* in 1999–2000 (~3-year sites); triangular flags, colonized in 2001 (~2-year sites); round flags, control sites; and stars, release sites.

30°25' and 30°32'N latitude and between 83°17' and 83°27'W longitude (~70 km away from all other sites) and sampled initially in the spring of 2001.

Seventeen sites were abandoned over the course of the study due to changes in land management practices or high levels of disturbance, and are not included in the analyses of phorid effects. Thus, the three categories of sites and sample sizes included in the analyses were: (1) sites where phorids were established for 2.5–3 years (hereafter referred to as ~3-year sites) ( $n=21$  pasture, 6 roadside), (2) sites where phorids were established for 1.5–2 years (hereafter ~2-year sites) ( $n=19$  pasture, 9 roadside), and (3) sites with no phorids ( $n=8$  pasture, 4 roadside). Each site was sampled twice a year (April and October) from April 2000 to April 2003, except the 12 control sites, which were first sampled in April 2001.

Because sites were colonized at different times and *P. tricusps* populations increased gradually, some variation in phorid abundance was unavoidable among sites within each treatment category. However, ~3-year sites in general supported *P. tricusps* over a longer period than ~2-year sites and at higher phorid abundances, resulting in greater overall parasitism pressure on *S. invicta* ants.

## 2.2. Sampling ants and other arthropods

Within each plot, 25 permanent sample points were established, at least 8 m apart and usually in a 5 × 5 grid.

Occasionally, the sample grid was of a different configuration so that it fit within the plot, and rarely it extended outside the plot at sites where the plot was small. This was acceptable because the boundaries of the plots primarily delimited the region over which mound areas were measured.

We determined *S. invicta* density and activity by three complementary sampling methods: (1) mound counts and measurements, (2) attraction to baits, and (3) pitfall trap catches. *S. invicta* colony size is positively correlated with mound size (Macom and Porter, 1996; Tschinkel, 1993). Baits measured the foraging activity of *S. invicta*. Pitfall trap catches represent a combination of *S. invicta* abundance and activity. Baits also attracted other ant species, and pitfalls captured many different types of arthropods. Data for other species were also recorded and analyzed, but the formal results are not presented here.

To count and measure mounds, observers walked along transects 3 m wide, marking all mounds in the transects. Two or three observers usually walked in tandem. After the entire plot had been searched in this manner, each mound was measured along its long axis and perpendicular to that axis. The two-dimensional shape of most mounds approximated an ellipse, and the area of the mounds was calculated by using the formula for an ellipse ( $A = \pi * a/2 * b/2$ , where  $a$  is the length of the longest axis and  $b$  is the length of the perpendicular axis).

The two-dimensional areas of all mounds in each plot were summed, and this cumulative mound area was divided by the size of the plot to obtain a measure of mound area ( $\text{m}^2/\text{ha}$ ).

Twenty-five baits were set out at each site, at each of the 25 sample points. Baits consisted of  $\sim 0.5$  g of an Oscar Mayer Wiener (pork and turkey) inserted into a  $12 \times 75$  mm glass tube. Baits were placed on the ground and shaded. After 30 min, baits were retrieved. The end of the tube was quickly plugged with cotton and specimens were returned to the laboratory and frozen until they could be identified and counted.

Fifteen pitfall traps were set out at 15 of the 25 sample points. The same 15 points at each site were used in every sampling period. Pitfalls consisted of plastic vials (2.5 cm in diameter and 7 cm in deep) containing propylene glycol as a preservative. A rechargeable battery-powered drill was used to bore holes the size of the diameter of the vials, which were inserted so the lip was flush with the ground surface. This method produced a minimal 'digging in' effect (Greenslade, 1973).

Pitfalls were left out for 72 h. Occasionally pitfalls were disturbed, apparently by small mammals, and these samples were discarded. On rare occasions where  $>4$  pitfalls at the same site were disturbed, all samples from that site were discarded and a new set of pitfalls was set out 1–2 weeks later. All samples were frozen until they could be sorted in the laboratory. Ants were identified to species, and all other invertebrates were identified to at least order (with the exception of classes Chilopoda and Diplopoda) and then sorted as morphospecies. Reference specimens have been deposited in the Florida State Collection of Arthropods in Gainesville, FL.

### 2.3. Sampling phorid flies

Censuses for adult *P. tricuspidis* abundance were conducted in conjunction with pitfall samples. At each site, we disturbed 10 *S. invicta* colonies by digging into them with a small shovel (as in Porter et al., 2004). After 30 min, the number of *P. tricuspidis* at each colony was counted. Two censuses were conducted at each site in each season, the first when pitfalls were placed out and the second three days later when pitfalls were collected. Because adult *P. tricuspidis* in Florida are not active below  $20^\circ\text{C}$  (SDP and LWM, unpublished data), all surveys were conducted on warm days when flight activity would not have been limited by low temperatures. Censuses were conducted between 11:00 and 16:00.

### 2.4. Statistical analyses

To test for impacts of *P. tricuspidis* on *S. invicta*, we used a repeated-measures analysis of covariance (ANCOVA). The between subject factors were (1) treatment (flies present for  $\sim 3$  years, flies present for  $\sim 2$  years,

and flies absent) and (2) habitat (pastures and roadsides). The within-subject factor was time. Three dependent variables associated with *S. invicta* density and activity were evaluated in separate tests: (1) mound area ( $\text{m}^2/\text{ha}$ ), (2) proportion of baits occupied, and (3) abundance in pitfall traps. (See Morrison and Porter, 2003 for the rationale of using multiple response variables associated with *S. invicta* density.) Because pre-release *S. invicta* densities varied among sites, we included the initial measurement of these variables as a covariate.

Treatment  $\times$  habitat interactions were not included in the models because preliminary analyses indicated this interaction was insignificant for all but one comparison and, furthermore, our interest was focused on main effects rather than interactions. *P* values for within-subject factor effects were adjusted by the Greenhouse–Geisser epsilon method (Greenhouse and Geisser, 1959). When multiple comparisons were made within the same data set, the sequential Bonferroni method was used to control the type-I error rate (Rice, 1989).

Since the control sites were not sampled until 2001, we conducted two sets of analyses: The first set evaluated all three treatments, and covered the period from April 2001 to April 2003. The second set covered the period from April 2000 to April 2003, but included only the two treatments with flies present (flies present for  $\sim 3$  years, flies present for  $\sim 2$  years).

Distributions of all variables were examined and transformations were applied when necessary to normalize the data. Mound area and the abundance of *S. invicta* workers in pitfall traps were  $\log_{10}$  transformed; the proportion of baits occupied by *S. invicta* was arcsine square root transformed.

Because *P. tricuspidis* abundances and time since establishment varied among sites within the treatment categories, we regressed changes over time in the three variables associated with *S. invicta* density and activity against phorid parasitism pressure in simple regression analyses. Changes in the *S. invicta* variables were determined by subtracting the average of the first two seasons sampled (spring and fall 2000) from the average of the last two seasons sampled (fall 2002 and spring 2003). This was done to smooth out seasonal variation. 'Phorid parasitism pressure' was determined by averaging the mean number of *P. tricuspidis* found at 10 disturbed mounds over each season for all seven seasons sampled. Thus, sites with higher abundances of phorids, or sites where phorids had been established longer, had higher values of parasitism pressure. 'Phorid parasitism pressure' was square root transformed to normalize the distribution before conducting the regressions. Only sites where *P. tricuspidis* was present ( $n = 55$ ) were included.

To evaluate the static relationship between *P. tricuspidis* abundance and *S. invicta* density and activity, we regressed the abundance of *P. tricuspidis* against each of the three *S. invicta* variables. All variables (including fly

abundance) were averaged over the last two seasons of the study (fall 2002 and spring 2003). Fly abundance was  $\log_{10}$  transformed; the *S. invicta* variables were transformed as in the ANCOVAs (see above).

StatView 5.0.1 (SAS Institute, 1999) and Super ANOVA (Abacus Concepts Inc., 1989) were used for all analyses.

### 3. Results

Conducting a *P. tricuspis* release required several weeks of effort and was dependent upon the availability of flies; not all releases were successful (Porter et al., 2004). Thus, *P. tricuspis* became established at different sites at different times. *P. tricuspis* dispersed from successful release sites to almost all study sites in Alachua County (~3-year sites) by the end of 2000 (Fig. 2). *P. tricuspis* dispersed to all sites in the adjoining counties by the end of 2001 (~2-year sites). The distant control sites had not become colonized, or at least *P. tricuspis* was not present in detectable abundances, by the spring of 2003.

*Pseudacteon tricuspis* populations increased in abundance over the first two years following colonization of a site and then oscillated seasonally (Fig. 3). They were always higher in the fall than in the preceding spring. *P. tricuspis* was more abundant in pastures than at roadsides.

*Solenopsis invicta* density and activity varied greatly over the study period, for all three variables in both pastures and along roadsides (Figs. 4 and 5). In the repeated-measures ANCOVAs, the treatment effect was significant for some comparisons (Tables 1 and 2). The effect, however, was never in the direction predicted by the hypothesis that phorids decrease fire ant density or activity. Habitat was significant for one mound area comparison, but not after correction for multiple comparisons. Time and time  $\times$  treatment interactions were significant for

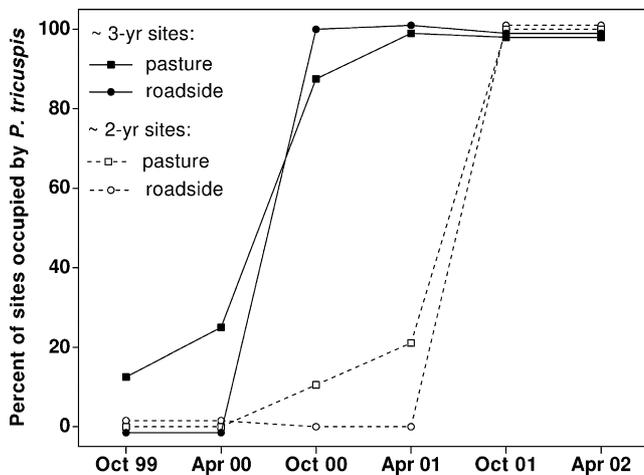


Fig. 2. Cumulative percentage of sites of each treatment category colonized by *P. tricuspis* over time.

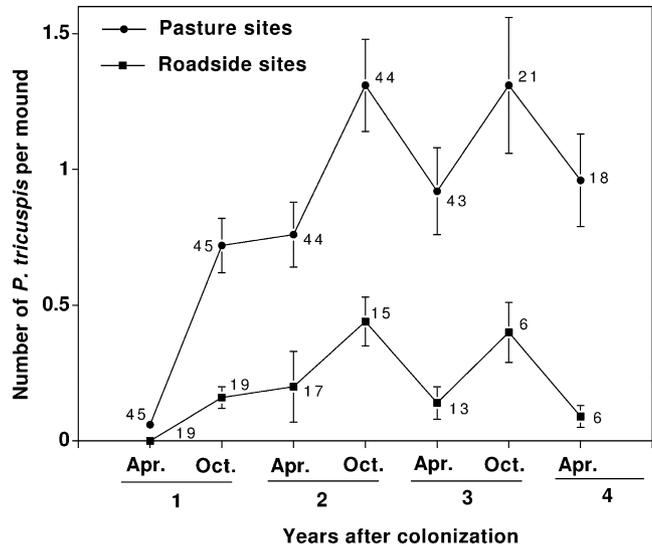


Fig. 3. Mean number of *P. tricuspis* flies per mound at study sites over time. Error bars indicate  $\pm 1$  SE. Numbers refer to the number of sites included in each data point; variation exists because not all sites were colonized at the same time. (Some sites were abandoned due to disturbance events that occurred at some point during the monitoring, but there was no reason to preclude the data prior to those events.)

some comparisons, although there were no robust, long-term trends that would indicate a systematic suppression of fire ant density or activity by the parasitoids.

A significant positive association was observed between overall phorid parasitism pressure over the course of the experiment and change in *S. invicta* density over time as measured by mound area (Fig. 6). Regressions of phorid parasitism pressure against the other *S. invicta* variables were not significant (both  $P > 0.05$ ).

Positive associations were also observed between phorid abundance and *S. invicta* density averaged over the last two seasons of the experiment (Table 3). When all sites were considered, the regressions were marginally significant for mound area and pitfall trap abundance ( $0.05 < P < 0.10$ ). When only pasture sites were considered, however, both these regressions became significant at  $P < 0.05$  (although not after correction for multiple comparisons).

### 4. Discussion

#### 4.1. *Pseudacteon tricuspis* abundance

*Pseudacteon tricuspis* abundances oscillated seasonally around ~1.1 flies/mound in pastures and ~0.2 flies/mound at roadsides. *Pseudacteon* spp. populations may vary greatly throughout the year, often peaking in the fall in the southern United States (Morrison et al., 1999, 2000; Morrison and Porter, 2005). This phenology may be due to greater availability of ant hosts and more rapid development of *P. tricuspis* larvae and pupae (which is

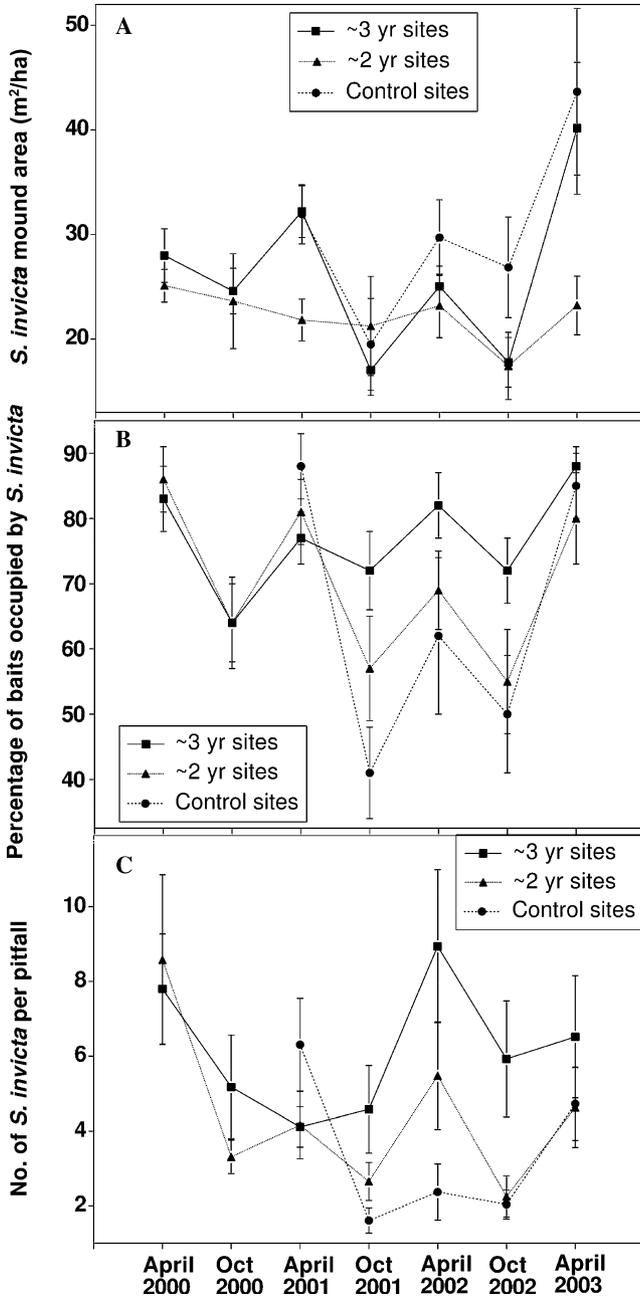


Fig. 4. Density and activity of *S. invicta* over time at pasture sites, as measured by (A) mound area, (B) percentage of baits occupied, and (C) number of workers per pitfall.

temperature dependent; Morrison et al., 1997) during the warmer summer months.

Lower densities of *P. tricuspis* at roadsides may be partly due to higher mortality rates, if flies are struck by passing vehicles. Additionally, turbulent air currents created by passing vehicles could disrupt the precise aerial maneuvering required in mating and injecting eggs into live worker ants. *S. invicta* colonies are often located near the edge of the roadway, particularly when steep ditches are present. Even moderate levels of traffic may reduce the ability of *P. tricuspis* to mate and reproduce in these areas.

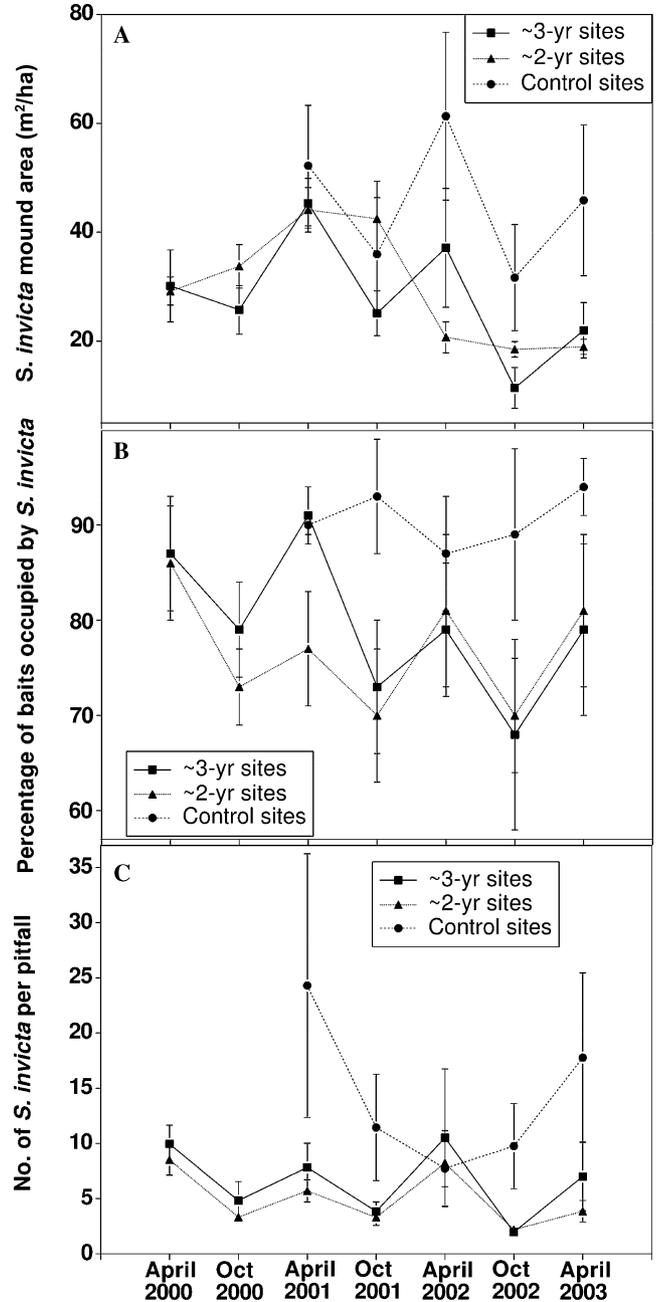


Fig. 5. Density and activity of *S. invicta* over time at roadside sites, as measured by (A) mound area, (B) percentage of baits occupied, and (C) number of workers per pitfall.

While the reported abundances (even in pastures) may seem low in an absolute sense, any measure of *P. tricuspis* abundance must be evaluated relative to the methods used. Our surveys consisted of point observations lasting only a few seconds. In another study (conducted at some of these same sites), we collected flies from disturbed *S. invicta* mounds continuously for 30 min and reported abundances of up to 40 flies/mound (Morrison and Porter, 2005). Because male *P. tricuspis* compete for females at disturbed mounds (Porter, 1998a; Morrison, 2000a), intraspecific interac-

Table 1

Repeated-measures ANCOVA of three *S. invicta* density and activity measures, for two treatments (sites where phorids were established for ~3 years and sites where phorids were established for ~2 years) from April 2000 to April 2003

Effect	<i>F</i>	<i>df</i>	<i>P</i>	Adj. <i>P</i> <sup>b</sup>
<i>S. invicta</i> mound area/ha				
Between subjects (sites)				
Initial level	30.40	1, 51	<0.001 <sup>a</sup>	
Treatment	1.63	1, 51	0.21	
Habitat	1.25	1, 51	0.27	
Within subjects				
Time	0.64	5, 255	0.67	0.63
Time × treatment	3.36	5, 255	0.01	0.01 <sup>a</sup>
<i>Proportion of baits with S. invicta</i>				
Between subjects (sites)				
Initial level	61.95	1, 51	<0.001 <sup>a</sup>	
Treatment	5.91	1, 51	0.02 <sup>a</sup>	
Habitat	0.46	1, 51	0.50	
Within subjects				
Time	1.35	5, 255	0.24	0.25
Time × treatment	3.05	5, 255	0.01	0.02 <sup>a</sup>
<i>Number of S. invicta/pitfall</i>				
Between subjects (sites)				
Initial level	48.08	1, 51	<0.001 <sup>a</sup>	
Treatment	8.03	1, 51	0.01 <sup>a</sup>	
Habitat	0.02	1, 51	0.90	
Within subjects				
Time	4.78	5, 255	<0.001	<0.001 <sup>a</sup>
Time × treatment	1.36	5, 255	0.24	0.24

The covariate (initial level) is the value of each variable based on the April 2000 census.

<sup>a</sup> Effect is significant ( $P < 0.05$ ) by the sequential Bonferroni method (three comparisons).

<sup>b</sup> Significance levels adjusted by the Greenhouse–Geisser epsilon method.

tions may limit the number of flies present at a mound at one time.

#### 4.2. Direct and indirect effects

In general, phorid flies do not parasitize large numbers of host ants. Several studies of different genera and species of phorid flies and their host ants have revealed parasitism rates to be consistently <5% (Feener, 1981, 1988; Morrison et al., 1997; Morrison and Porter, 2005). Thus, the direct effect of mortality due to this type of parasitoid appears to be low. The primary rationale for introducing *Pseudacteon* spp. as biocontrol agents of imported *Solenopsis* fire ants, however, stems from numerous field observations and experiments revealing *Pseudacteon* spp. alter the behavior of *Solenopsis* workers, resulting in decreased foraging efficiency over the short term (i.e., an indirect effect; Feener and Brown, 1992; Folgarait and Gilbert, 1999; Morrison, 1999; Morrison et al., 1997; Orr et al., 1995; Porter et al., 1995b). Such behavioral impacts measured over 1–2 h with newly discovered, rich food resources, however, may not continue over longer periods, or characterize effects on foraging at smaller food

resources. If so, opportunities for the relatively dramatic impacts documented on *S. invicta* foraging in observational and experimental studies may not occur frequently in nature.

The *Pseudacteon* species evaluated here, *P. tricuspis*, does not appear to be attracted to *S. invicta* foraging per se in the field (Morrison and King, 2004). Although *P. tricuspis* is attracted to *S. invicta* while foraging in the lab and decreases foraging efficiency (Mehdiabadi and Gilbert, 2002; Morrison, 2000b), in the field this *Pseudacteon* species is attracted to activity related to the release of alarm pheromones, such as colony disturbance, defense of territory and food resources, and mating flights (Morrison and King, 2004). Thus, the indirect effects of *P. tricuspis* would be mediated through these activities rather than foraging (unless interspecific interactions occurred at food resources). Although other species of phorid flies are known to affect interspecific interactions involving host ants (Feener, 1981, 1988), studies investigating the effect of *Pseudacteon* spp. on direct interspecific interactions (i.e., fighting) of their host with other ants have failed to find an effect of phorids (Morrison, 1999, 2000b; Orr et al., 2003).

Table 2

Repeated-measures ANCOVA of three *S. invicta* density and activity measures, for all three treatments (sites where phorids were established for ~3 years, sites where phorids were established for ~2 years, and sites with no phorids) from April 2001 to April 2003

Effect	<i>F</i>	<i>df</i>	<i>P</i>	Adj. <i>P</i> <sup>b</sup>
<i>S. invicta</i> mound arealha				
Between subjects (sites)				
Initial level	53.51	1, 62	<0.001 <sup>a</sup>	
Treatment	2.33	2, 62	0.11	
Habitat	5.12	1, 62	0.03	
Within subjects				
Time	2.21	3, 186	0.09	0.10
Time × treatment	4.53	6, 186	<0.001	<0.001 <sup>a</sup>
<i>Proportion of baits with S. invicta</i>				
Between subjects (sites)				
Initial level	66.37	1, 62	<0.001 <sup>a</sup>	
Treatment	5.29	2, 62	0.01 <sup>a</sup>	
Habitat	2.19	1, 62	0.14	
Within subjects				
Time	1.78	3, 186	0.15	0.16
Time × treatment	1.54	6, 186	0.17	0.17
<i>Number of S. invicta/pitfall</i>				
Between subjects (sites)				
Initial level	61.08	1, 62	<0.001 <sup>a</sup>	
Treatment	2.52	2, 62	0.09	
Habitat	0.14	1, 62	0.71	
Within subjects				
Time	5.85	3, 186	<0.001	<0.001 <sup>a</sup>
Time × treatment	1.99	6, 186	0.07	0.07

The covariate (initial level) is the value of each variable based on the April 2001 census.

<sup>a</sup> Effect is significant ( $P < 0.05$ ) by the sequential Bonferroni method (three comparisons).

<sup>b</sup> Significance levels adjusted by the Greenhouse–Geisser epsilon method.

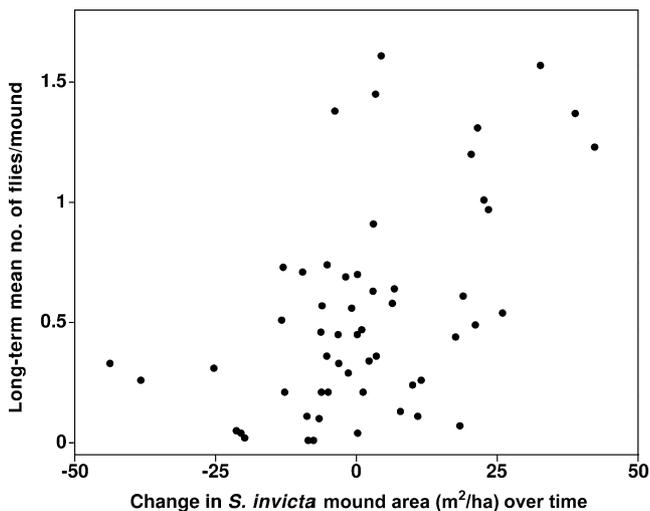


Fig. 6. Relationship between overall parasitism pressure by *P. tricuspis* (see text) and *S. invicta* density, as measured by change in mound area ( $m^2/ha$ ) over time ( $n = 55$ ,  $r = 0.50$ ,  $P < 0.001$ ).

#### 4.3. Positive association of *P. tricuspis* and *S. invicta*

Positive associations were observed between overall parasitism pressure and change in *S. invicta* density over

Table 3

Simple linear regressions of 3 explanatory variables associated with *S. invicta* density and activity against the response variable *P. tricuspis* abundance

	Response variable		
	<i>R</i>	<i>F</i>	<i>P</i>
All sites ( $n = 55$ )			
<i>S. invicta</i> mound area ( $m^2/ha$ )	+0.25	3.51	0.07
% of baits occupied by <i>S. invicta</i>	+0.14	0.98	0.33
No. of <i>S. invicta</i> in pitfalls	+0.24	3.33	0.07
Pasture sites only ( $n = 40$ )			
<i>S. invicta</i> mound area ( $m^2/ha$ )	+0.34	5.06	0.03
% of baits occupied by <i>S. invicta</i>	+0.26	2.73	0.11
No. of <i>S. invicta</i> in pitfalls	+0.37	5.88	0.02

the course of the study, and between *P. tricuspis* abundance and *S. invicta* density in the last season of the study. These associations should not be interpreted as evidence that phorid flies may somehow increase fire ant abundance. There is no known mechanism that would account for this. If phorid parasitism pressure represents a strong force in regulating fire ant populations, we may expect a negative association. However, it may be that, because *P. tricuspis* was only recently introduced, its populations were still increasing in the early stages of

colonization, approaching abundances in proportion to the densities of its hosts.

It is of interest that, in Brazil, where both *S. invicta* and its *Pseudacteon* parasitoids are native, a recent study found no significant association between *Pseudacteon* spp. abundance (as determined by censusing phorids attracted to disturbed mounds) and either *S. invicta* density or mound volume (C. G. Dall'Aglio-Holvorcem, unpublished manuscript). *P. tricuspis*, and *Pseudacteon* parasitoids in general, may in fact not represent a strong force regulating *S. invicta* populations. *S. invicta* populations may vary primarily according to other factors (e.g., rainfall, disturbance), and phorid parasitoids may reach higher abundances where their hosts are more plentiful, rather than exerting a strong influence on host abundance. This scenario would be analogous to a food web with strong bottom-up, rather than top-down, control (at least as far as the flies and ants are concerned; e.g., Boyer et al., 2003; Gratton and Denno, 2003; Hawkins et al., 1999).

#### 4.4. Implications

This study did not detect any impacts of introduced *P. tricuspis* on *S. invicta* populations over the temporal and spatial scales evaluated. Yet parasitoid phorids such as *P. tricuspis* may, and almost certainly do, have some impacts, although of a smaller magnitude than this study was able to detect. Even if the indirect effects of short-term behavioral modification do not translate into long-term population level impacts, the direct effect of mortality will continue to operate: each phorid fly represents the death of a worker ant. Abundances of *P. tricuspis* may at times be relatively high (e.g., several hundred flies captured at 10 *S. invicta* mounds in 30 min, Morrison and Porter, 2005). Yet *S. invicta* densities may be so great that mortality due to *P. tricuspis* parasitism affects a very small proportion of the population (e.g., <1% parasitization rate, Morrison and Porter, 2005).

In nature, *S. invicta* populations are affected by numerous factors, including climate, disturbance, competitors, predators, pathogens, etc. *S. invicta* population fluctuations due to variability in these factors may dwarf the impacts of *P. tricuspis*, at least over some temporal and spatial scales. The measures of *S. invicta* density and activity reported here varied greatly over the 3.5 years of this project. Climatic variables such as precipitation are likely to have much greater impacts on *S. invicta* populations than phorid flies, and large variation in these climatic variables may make relatively small impacts due to other sources (i.e., phorid flies) difficult to detect.

To obtain a crude estimate of the magnitude of effect due to phorid flies likely to be necessary for detection, we calculated standard errors associated with the means for each *S. invicta* variable measured. Division of two stan-

dard errors by the overall mean for each treatment level yields a rough estimate of the size of effect that could be detected, given our levels of variation and sample size. For *S. invicta* mound area, proportion of baits occupied, and abundance in pitfall traps, treatments would need to be roughly 14–27, 9–15, and 26–30% different, respectively. The effects we observed were all smaller than these estimated minimal detectable differences.

#### 4.5. Conclusions

The greater abundance of *S. invicta* in the United States relative to its South American homeland may be due to the absence of natural enemies (Porter et al., 1992, 1997). *P. tricuspis*, however, is only one of a large number of competitors, predators, pathogens, and parasites known to affect *Solenopsis* spp. in South America (Buren, 1983; Jouvenaz, 1983; Jouvenaz and Lofgren, 1981). Interspecific competition is often the major factor limiting ant populations (Hölldobler and Wilson, 1990 and references therein). A recent field study conducted in Brazil found that interspecific competition with other ants appeared to be a much stronger factor regulating *Solenopsis* populations than parasitism pressure from *Pseudacteon* spp. (M.R. Orr, K.M. Wackford, D.H. Feener, Jr., L.E. Gilbert, and W.W. Benson, unpublished manuscript).

Invasive ants are notoriously difficult to control. Biological control has yet to be successfully implemented against any social insect pest. No obvious impacts on *S. invicta* populations were observed from the introduction of a single *Pseudacteon* species over the temporal and spatial scales measured. Introducing additional *Pseudacteon* species, or a combination of different types of natural enemies as biological control agents, may have a greater impact on imported fire ants. Whether such an impact will be measurable in the field against the backdrop of population variation due to fluctuations in numerous environmental variables, however, remains an open question.

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