Sticky Traps for Monitoring *Pseudacteon* Parasitoids of *Solenopsis* Fire Ants

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ABSTRACT A newly developed method for passively trapping adult *Pseudacteon* phorid flies, which parasitize workers of *Solenopsis saevissima* complex fire ants, is described. Adult flies responding to deployed *Solenopsis invicta* midden were captured when they landed on a Tanglefoot®-coated perch, which is part of the trap. This sampling method provided a uniform, repeatable, and verifiable sample that allowed continuous and simultaneous sampling among locations, which can only be accomplished with other techniques by substantially increasing the number of observers. A field test showed the superior operational efficiency and effectiveness of this method relative to other techniques. These traps have been shown effective in various phorid habitats in Texas and Florida. We expect this trap to also be effective in detecting/monitoring phorid flies in other locations.

KEY WORDS *Solenopsis invicta, Pseudacteon tricuspis, Pseudacteon curvatus*, PTS trap, Phoridae

Many species of *Pseudacteon* phorid flies parasitize workers of the *Solenopsis saevissima* complex fire ants (including *S. invicta*) throughout their native South American range (Disney 1994, Porter and Pesquero 2001, Folgarait et al. 2005); as a result they are considered potential candidates to serve as classical biological controls against *S. invicta* in their non-native distribution in the United States (Porter 1998). Multiple phorid species have been released in the southern United States as a biological control component of the integrated pest management (IPM) approach to suppression of *S. invicta* populations; they are being evaluated in terms of their potential to impact *S. invicta* (Drees and Gold 2003). Documentation of the successful establishment of phorid populations has varied among release sites (Gilbert and Patrock 2002, Graham et al. 2003). A number of factors may influence establishment success, including the landscape mosaic and phorid/habitat associations, as well as the ability to detect and document establishment using current methods. Our new phorid detection method allows rapid and repeatable assessments of establishment and reflects relative densities among habitats with uniform sampling effort.

Methods currently used to attract, observe, and collect field released *Pseudacteon* phorid flies include (1) mound disturbance (Barr and Calixto 2005, Morrison and Porter 2005) and (2) midden attraction (Gilbert and Patrock 2002). Each of these methods requires direct observations of either disturbed fire ant colonies or deployed fire ant midden to monitor phorid activity. Although these methods have been shown effective for detecting and collecting phorids, they are time intensive and require multiple personnel to monitor multiple sites simultaneously. This study documents the efficacy of a new trapping method and compares the efficiency of this trap to standard sampling methods.

Materials and Methods

Study Site

This study was conducted at 5-Eagle Ranch in Burleson County, TX (30°34′54.573″ N; 96°40′59.776′ W); this 2,800-acre ranch is classified as Post Oak Savanna. The ranch supports cattle operations within scattered improved Bermuda grass pastures, a variety of riparian habitats, and dense stands of Post Oaks *Quercus stellata.* The ranch is presumed to have become infested with the red imported fire ants in the early 1970s when this invasive species invaded the region (Vinson 1997) and to have remained infested up to the present. *Pseudacteon tricuspis* and *P. curvatus* were released at this location in 2002 and 2004, respectively, as part of the USDA–ARS "Area-wide Suppression of Imported Fire Ants in Pastures Project." They have since become established at this site and have expanded outside of the boundary of the ranch. The field trial was conducted on 23 May 2006 (PTS Traps retrieved on 24 May 2006) within the boundary of the ranch (Fig. 1). The first replication began at 1005 hours, and the 10th replication began at 1557 hours. The temperature during the trial ranged from 27.8 (1005 hours) to 31.7°C

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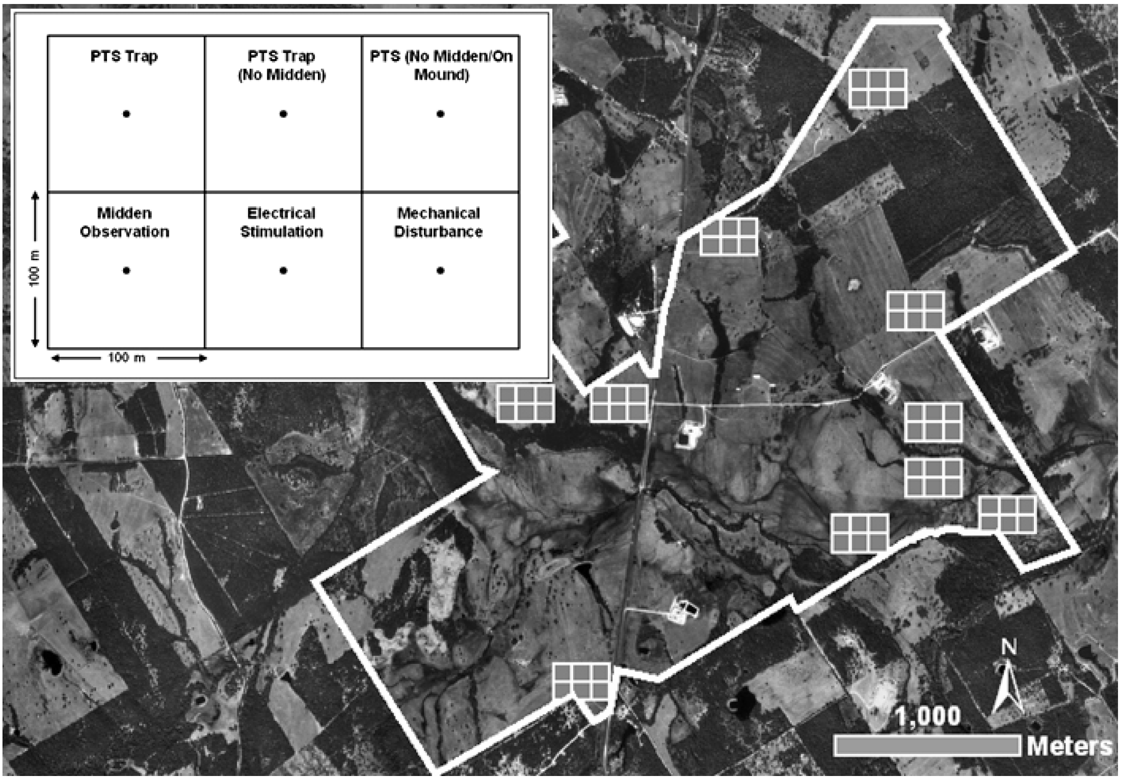

Fig. 1. Map of 5-Eagle Ranch showing location of all 2 by 3 grid blocks (experimental units). Enlarged schematic indicates dimensions of 100 by 100-m cells, cell centroids, and collecting method within each cell.

(1557 hours), well above the minimum threshold of 22°C needed for phorid activity (Wuellner and Saunders 2003).

Experimental Design

A GIS supported by ESRI ArcGIS v.9.0 was used to construct an overlay of the study area that contained 2 by 3 blocks of contiguous 100 by 100-m grid cells at 10 locations (experimental units) within the boundary of the ranch (Fig. 1). The centroid of each grid cell was determined and used as the sampling point within each cell. The grid cell size was sufficiently large to eliminate potential competition for attraction of phorids by multiple methods within a block (S. Porter, personal communication). A Trimble GeoXT handheld GPS receiver was used to locate the sampling points in the field. The minimum distance between the boundaries of any two grid blocks was 100 m, resulting in separation of cell centroids by 200 m. One of each of the following sampling techniques (described in detail below) was used simultaneously at each grid cell centroid. Six phorid collection techniques were compared including (1) the PTS Trap, (2) PTS Trap without midden, (3) PTS on mounds without midden, (4) midden attraction, (5) electrical stimulation of mounds, and (6) mechanical stimulation of mounds.

Solenopsis invicta mound density within the ranch had been assessed before this study and determined at thirty 0.05-ha circular plots within the boundary of the ranch; in May 2005 and December 2005, mean mound densities were 12.9 mounds/0.05 ha and 6.4 mounds/0.05 ha, and the SEs were 1.70 and 0.75, respectively (USDA, unpublished data).

Passive Sampling Methods

PTS Trap. Our newly developed trap exploits both the behavioral response of *Pseudacteon* phorids to red imported fire ant (RIFA) midden and the perching behavior of these flies. The trap consists of *S. invicta* midden for attraction and multiple sticky perches for capturing attracted phorid adults. These features are incorporated into the specific design described below, but traps consisting of other attractants are expected to also be operationally effective. Each trap requires one of each of the following components: 100 by 15-mm petri dish, 150 by 15-mm petri dish, Dixie Pizza Tri-Stand (hereafter referred to as PTS), and 2 g of midden material (from laboratory colonies).

The prongs of the PTS were coated with Tanglefoot insect trap coating and centered (prongs upward) in a 100 by 15-mm petri dish containing 2 g of midden material. This dish was centered within the 150 by 15-mm dish, allowing for the midden to remain in close proximity to the PTS while other potential perches provided by surrounding vegetation (if any) were

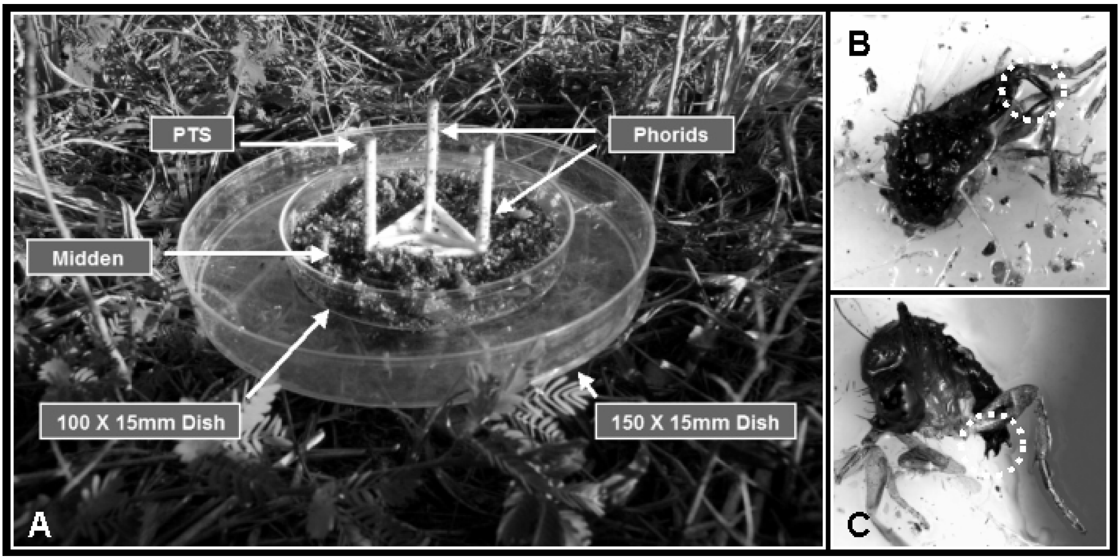

Fig. 2. (A) Illustration of PTS Trap components. (B) *P. curvatus* on trap (insect width = 0.304 mm; length = 0.672 mm). (C) *P. tricuspis* on trap (insect width = 0.496 mm; length = 1.2 mm). Ovipositors, used for species diagnosis, are indicated by dashed circles.

displaced by the larger dish (Fig. 2A). Traps were left in place for 24 h.

PTS Trap Without Midden. Identical to PTS above, but without midden. This provided a control to determine the potential attractiveness of the prongs themselves. Traps were left in place for 24 h.

PTS on Mounds. PTS on RIFA mound without petri dishes or midden. This sampled flies visiting undisturbed RIFA mounds. Traps were left in place for 24 h.

Active Sampling Methods

Midden Attraction. Petri dishes (100 by 15 mm) containing 2 g of midden were observed for 15 min. All observed phorids were collected by aspiration. At ≈2-min intervals, the area above the midden was aspirated regardless of detection of flies.

Electrical Stimulation of Mounds. Mounds were electrically stimulated for 15 min with a Hot Shot LMPLUS cattle prod fitted with near-contact electrodes and continuously observed for responding phorids. All observed phorids were collected by aspiration. At ≈2-min intervals, the area above the electrodes was aspirated regardless of detection of flies.

Mechanical Stimulation of Mounds. Mounds were mechanically disturbed for 15 min. All observed phorids were collected by aspiration. The area around the disturbance was also aspirated at ≈2-min intervals regardless of detection of flies.

Phorid Identification. Phorids were returned to the laboratory, and those collected by aspiration were transferred to 90% EtOH and identified to species. After a period of 24 h, PTS Traps were returned to the laboratory, and flies were identified to species directly on the trap (Fig. 2B and C).

Results

The mean number of flies collected by the PTS Trap was significantly greater than that of all other methods (df = 3,36; $P < 0.05$, $P = 0.02$, and $P < 0.05$ for PTS versus midden, electrical stimulation, and mechanical disturbance, respectively; Fig. 3). The PTS Traps passively collected a total of 142 phorids (*Pseudacteon tricuspis* = 138 and *Pseudacteon curvatus* = 4), whereas no phorids were collected by the PTS Traps without midden or the PTS on mounds without midden. Thirty-two *P. tricuspis* adults were attracted and collected by electrically stimulating mounds, three *P. tricuspis* adults were collected by mechanically disturbing mounds, and two *P. tricuspis* adults were collected over midden piles (Table 1). *P. curvatus* was only collected using the PTS Trap.

The mean amount of time needed for deployment and retrieval of the PTS Traps was significantly less than that of all other methods (df = 3,36; $P < 0.05$ for PTS versus all methods). The total PTS deployment/retrieval time was 170 min (mean = 8.5 min per trap to deploy and 8.5 min to retrieve = 2 h and 50 min). The time needed for active sampling of phorids totaled 288 min (Table 1). This total includes the amount of on site travel/set-up time required for active sampling during the trials (mean = 28.8 min).

The percentage of PTS Traps that successfully collected at least one fly was significantly greater than that of all other methods (df = 3,36; $P < 0.05$, $P = 0.027$, and $P < 0.05$ for PTS versus midden, electrical stimulation, and mechanical disturbance, respectively). All PTS Traps collected phorids (100%). Data regarding PTS without midden and PTS on mounds were not included in statistical analyses because they collected no flies. Phorids were collected at one midden pile

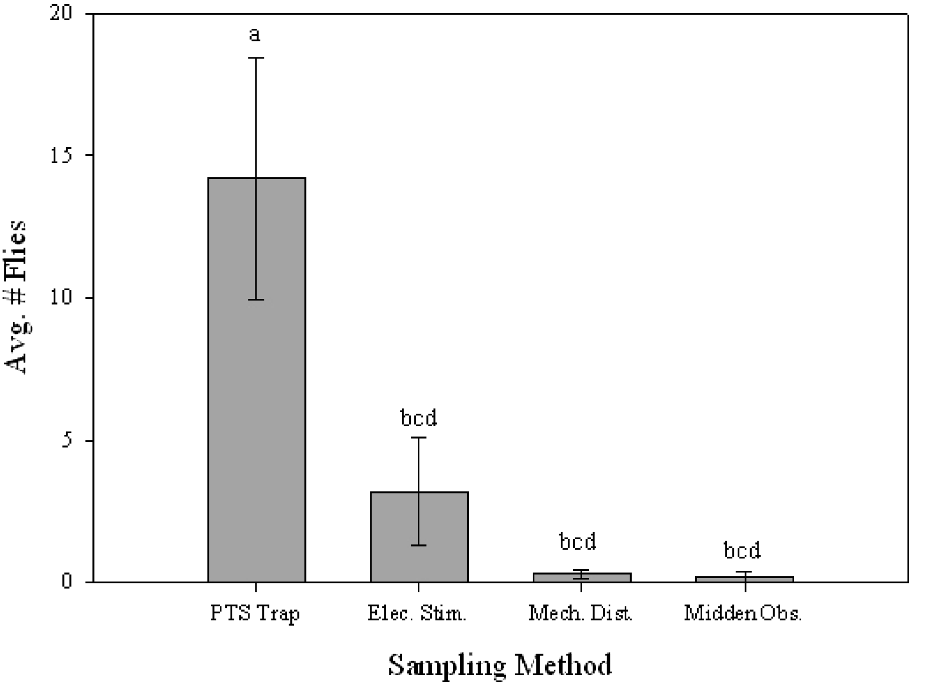

Fig. 3. Mean no. of phorids collected by each method. Bars marked with different letters are significantly different ($P < 0.05$). Note: PTS deployed without midden resulted in zero flies captured and are excluded from the figure.

(10%), six electrically stimulated mounds (60%), and three mechanically disturbed mounds (30%; Table 1). Only the PTS Trap collected *P. curvatus.*

Discussion

These data indicate that the PTS Trap allows for sampling with high resolution of a wide range of densities of both *Pseudacteon* species. This trapping method should allow for studies into habitat selection, dispersal characteristics, phenology, and perhaps density estimates and sex ratio fluctuations, as well as other parameters associated with adult activities of established phorid populations.

A major benefit provided by this trap is the increased efficiency resulting from trap operational effectiveness in the absence of an observer. These trials consisted of 10 replicates and the PTS Trap needed ≈60% of the personnel time needed by active methods (17 min per PTS Trap compared with 28.8 min per active sampling methods). This improvement over active sampling methods provides for greater time efficiency because no observational time is needed. The PTS Trap is quickly deployed and retrieved and allows continuous and simultaneous sampling among locations, which can only be accomplished with other techniques by substantially increasing the number of observers. The PTS Trap provides a uniform, repeatable, and verifiable sampling method, whereas other techniques are more idiosyncratic. We note that the midden for this research was collected in kilogram amounts in a matter of minutes coincident with fire ant rearing operations. As such, midden collection time was not included in the time needed for deployment and retrieval of the PTS Trap. In addition, the trap has become our standard tool to census these flies in various habitats within central Texas (Austin, Caldwell, Lyons, Milano, and Somerville, TX), as well as throughout northern Florida (S. Porter, personal communication), since discovery in late 2005 through 2006. The PTS Trap has been used successfully in all months of the year and at all known fly-infested locations in which it has been deployed.

Table 1. Comparison of a passive sampling method (PTS) with three active methods for census of phorid flies in the field

Method	*N*	Total no. flies (mean ± SEM)	Percent success	Min/max	Range	Total time	Relative efficiency	Time (min)/fly
PTS Trap	10	142 (14.2 ± 4.26)	100	1/39	38	170 min	1 (1.214/1.214)	1.214
Electrical stimulation	10	32 (3.20 ± 1.88)	60	0/19	19	288 min	7.4 (9.0/1.214)	9
Mechanical disturbance	10	3 (0.30 ± 0.15)	30	0/1	1	288 min	79.0 (96/1.214)	96
Midden	10	2 (0.20 ± 0.20)	10	0/2	2	288 min	118.6 (144/1.214)	144

Efficiency of sampling methods based on investigator time invested versus flies captured.

The sampling efficiency of the PTS Trap results in an economy of scale of one to two orders of magnitude compared with active observation methods (Table 1). Methods that need active observation cannot more efficiently monitor for flies because of the fixed time protocol inherent in these techniques (Gilbert and Protrak 2002, Barr and Calixto 2005, Morrison and Porter 2005).

The PTS trapping method detected phorid presence at all sites, whereas the active collecting methods detected phorid presence in 10–60% of the same sites (Table 1). Barr and Calixto (2005) previously showed that electrical stimulation outperformed mechanical stimulation of mounds to detect phorids. The PTS trapping method is more efficient and more sensitive than active sampling methods in detecting and censusing phorids. This suggests that the PTS Trap will allow those involved in such sampling to avoid potential false negatives in terms of phorid presence. These features are particularly important when attempting to delimit range expansion boundaries and in determining relative densities among habitats. *Pseudacteon* spp. phorid flies are being released throughout the southern United States (Graham et al. 2003, Vogt and Streett 2003, Porter et al. 2004), and we expect the PTS Trap to prove useful throughout this region.

The number of *P. curvatus* collected was very low, regardless of method. However, we used PTS Traps to monitor fly density and habitat selection for a period of 6 mo within the boundary of 5-Eagle Ranch, and this work shows that the PTS Trap is effective in the collection of both *P. curvatus* and *P. tricuspis.* The low numbers of *P. curvatus* collected in this trial (relative to *P. tricuspis*) could be reflective of a natural seasonal density trough. However, this requires further study.

Further work is planned to examine attractiveness of various substrates and perches to further improve trap operations. In addition, an attempt will be made to determine the distance to which phorids will respond to these traps and to determine the duration during which the midden remains attractive.

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References Cited

Barr, C. L., and A. Calixto. 2005. Electrical stimulation of *Solenopsis invicta* (Hymenoptera: Formicidae) to enhance phorid fly, *Pseudacteon tricuspis* (Diptera: Phoridae), detection. Southwest. Entomol. 3: 165–168.

Disney, R.H.L. 1994. Scuttle flies: the Phoridae. Chapman & Hall, London, UK.

Drees, B. M., and R. E. Gold. 2003. Development of integrated pest management programs for red imported fire ant (Hymenoptera: Formicidae). J. Entomol. Sci. 38: 170–180.

Folgarait, P. J., O. A. Bruzzone, S. D. Porter, M. A. Pesquero, and M. A. Gilbert. 2005. Biogeography and macroecology of phorid flies that attack fire ants in south-eastern Brazil and Argentina. J. Biogeography 32: 353–367.

Gilbert, L. E., and R.J.W. Patrock. 2002. Phorid flies for the biological suppression of imported fire ant in Texas: region specific challenges, recent advances and future prospects. Southwest. Entomol. Suppl. 25: 7–17.

Graham, L. C., S. D. Porter, R. M. Pereira, H. D. Dorough, and A. T. Kelley. 2003. Field releases of the decapitating fly Psuedacteon curvatus (Diptera: Phoridae) for control of imported fire ants (Hymenoptera: Formicidae) in Alabama, Florida, and Tennessee. Fla. Entomol. 83: 334–339.

Morrison, L. W., and S. D. Porter. 2005. Phenology and parasitism rates in introduced populations of *Pseudacteon tricuspis,* a parasitoid of *Solenopsis invicta.* BioControl 50: 127–141.

Porter, S. D. 1998. Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). Fla. Entomol. 81: 292–309.

Porter, S. D., and M. A. Pesquero. 2001. Illustrated key to *Pseudacteon* decapitating flies (Diptera: Phoridae) that attack *Solenopsis saevissima* complex fire ants in South America. Fla. Entomol. 84: 691–699.

Porter, S. D., L. A. Nogueira de Sá, and L. W. Morrison. 2004. Establishment and dispersal of the fire ant decapitating fly *Pseudacteon tricuspis* in North Florida. Biol. Control 29: 179–188.

Vinson, S. B. 1997. Invasion of the red imported fire ant (Hymenoptera: Formicidae) spread, biology, and impact. Am. Entomol. 43: 23–39.

Vogt, J. T., and D. A. Streett. 2003. Pseudacteon curvatus (Diptera: Phoridae) laboratory parasitism, release, and establishment in Mississippi. J. Entomol. Sci. 38: 317–320.

Wuellner, C. T., and J. B. Saunders. 2003. Circadian and circannual patterns of activity and territory shifts: comparing a native ant (*Solenopsis geminata,* Hymenoptera: Formicidae) with its exotic, invasive congener (*S. invicta*) and its parasitoids (*Pseudacteon spp.,* Diptera: Phoridae) at a central Texas site. Ann. Entomol. Soc. Am. 96: 54–60.

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