Effectiveness Comparison of Multiple Sticky-Trap Configurations for Sampling *Pseudacteon* spp. Phorid Flies (Diptera: Phoridae)

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ABSTRACT A variety of traps have been developed for monitoring introduced populations of *Pseudacteon* spp. phorid flies (Diptera: Phoridae) across their established range in the United States. Such traps typically exploit common aspects of phorid fly biology and behavior, such as their attraction to live or dead red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), as well as the perching behavior of these parasitoids. However, populations of multiple species of phorid flies have been established in the United States to serve as biological control agents against *S. invicta*, and it is unclear if all trap designs are equally effective in sampling this variety of phorid species. This study investigated the effectiveness of six trap designs simultaneously during three sampling events in south–central Texas. Interactions between two species of phorid flies (*Pseudacteon tricuspis* Borgmeier and *P. curvatus* B.) and their hosts have been intensively studied at this location for over eight years. When analyzed independently, there were no significant differences in the mean number of *P. curvatus* or *P. tricuspis* phorids collected by any of the trap designs during any of the sampling events. However, when the total number of phorids collected were combined, significant trap performance differentials were observed during the October 2010 sampling event. Furthermore, there were significant differences among male flies during the September 2012 observation. Additionally, a trap component cost comparison is provided. The consistent and relatively equivalent performance of the phorid traps investigated in these trials suggests that all are appropriate for phorid surveillance, and cost and ease-of-use considerations may be the most important criteria when selecting a trap design.

KEY WORDS phorid flies, fire ants, sticky traps, invasive species

Six *Pseudacteon* phorid fly species have been released in the southern United States to serve as biological control agents against the red imported fire ant, *Solenopsis invicta* Buren. These species include; *Pseudacteon cultellatus* Borgmeier, *Pseudacteon curvatus* Borgmeier, *Pseudacteon litoralis* Borgmeier, *Pseudacteon nocens* Borgmeier, *Pseudacteon obtusus* Borgmeier, and *Pseudacteon tricuspis* Borgmeier (Porter et al. 2004, 2011; Vazquez et al. 2006; Plowes, Folgarait, and Gilbert 2011; Plowes, LeBrun, and Gilbert 2011). In their native South American range, these flies are part of a parasitoid assemblage consisting of >20 species, all known to parasitize workers of the *Solenopsis saevissima* complex. Additional species have been approved, or are being evaluated for release (Callcott et al. 2011). Since 1997, numerous state and federal agencies, as well as personnel from many universities across the southern United States have been involved in efforts to release, monitor, and evaluate phorid fly population expansion and interactions between these parasitoids and their fire ant hosts. During this time period, many phorid fly sampling methodologies and phorid trap designs have been developed, evaluated, reported, and used to detect and monitor introduced phorid fly species (Barr and Calixto 2005, Puckett et al. 2007, Farnum and Loftin 2011). The efficiency provided by phorid traps make them very attractive to researchers involved in such work. However, it is unclear whether the configuration of the wide variety of traps differentially influences their attractiveness and/or effectiveness with regards to different *Pseudacteon* phorid species.

The major advantage of using traps for insect detection and monitoring is the significant increase in sampling efficiency and the reduction of labor and time, as compared with more direct methods (Taylor 1962, Puckett and Harris 2010). However, the components and characteristics of any insect trap contribute to the overall effectiveness and efficiency of the device, and decisions regarding selection of the proper trap for a specific sampling scenario must be made only after trap designs are compared and contrasted. For example, many of the phorid traps that have been developed exploit several behaviors observed in phorid flies (i.e., "perching") by incorporating a variety of sticky surfaces such as Tanglefoot Insect Trap

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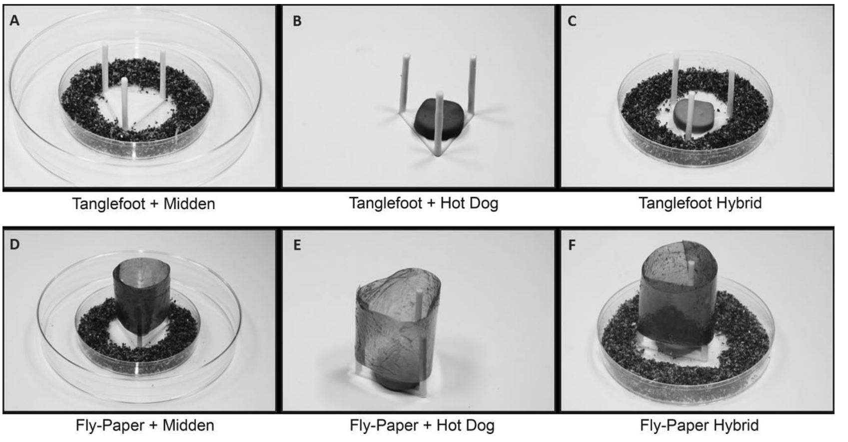

Fig. 1. Phorid trap configurations tested in these trials. (A) Tanglefoot coated PTS with midden attractant, (B) Tanglefoot coated PTS with live *S. invicta* attracted to a hot-dog slice, (C) Tanglefoot coated PTS with midden and live *S. invicta* attracted to a hot-dog slice (Tanglefoot hybrid trap), (D) fly-paper wrapped PTS with midden attractant, E) fly-paper wrapped PTS with live *S. invicta* attracted to a hot-dog slice, and (F) fly-paper wrapped PTS with midden and live *S. invicta* attracted to a hot-dog slice (fly-paper hybrid trap).

Coating or fly-paper to a structure, and a phorid attractant such as dead *S. invicta,* hereafter referred to as 'midden' (Patrock and Gilbert 2005, Puckett et al. 2007), or live *S. invicta* (Gilbert et al. 2008, Farnum and Loftin 2011). Fly-paper can easily be applied to traps in the field, while Tanglefoot typically requires careful application in a laboratory as well as cautious storage of traps during transport to and from sampling sites. Live *S. invicta* colonies are typically abundant in field situations, but environmental conditions affect ant activity. Whereas using midden to attract flies allows researchers to place traps in locations which are not dependent upon the presence of active *S. invicta.* However, midden must either be collected from laboratory colonies or live ants must be collected and killed for this purpose. As a result, midden is not as readily available for some researchers as it is for others.

We conducted a replicated field study to test the effectiveness of six phorid fly trap designs for sampling established field-released populations of *P. tricuspis* and *P. curvatus.* These two species are known to demonstrate niche partitioning by host-size preference in their native range (LeBrun et al. 2009). We also conducted a trap component cost analysis to aid in the selection of traps for sampling and monitoring phorid fly species. The common design component of all traps tested was a Dixie Pizza Tri-Stand (hereafter referred to as PTS) (Puckett et al. 2007), an inexpensive and stable trap platform that is readily available to all researchers.

Materials and Methods

Experimental Field Sites. We conducted this field study at 5-Eagle Ranch (30° 34′ 54.57″ N; 96° 40′ 59.77″ W) located in Caldwell, TX (Burleson Co.). The ranch is located in the East Central Texas Forest ecoregion of south-central Texas (Olson et al. 2001). It is presumed that the ranch first became infested with *S. invicta* during the early portion of the 1970s when these ants are reported to have invaded the region (Vinson 1997). The phorid flies, *P. tricuspis* and *P. curvatus* were released at the ranch in 2002 and 2004, respectively, as part of the U.S. Department of Agriculture–Agriculture Research Service "Area-wide Suppression of Imported Fire Ants in Pastures Project" (Pereira 2003), and are known to have become established by 2003 and 2005, respectively (Vander Meer et al. 2007, Gilbert et al. 2008).

Experimental Design. We used ArcGIS v.9.3 (ESRI 2008) software to establish a grid on an aerial map consisting of 2 by 3 sampling blocks of 100 by 100 m cells. From the grid we randomly selected 20 sampling blocks (replicates), and the centroid of the each of the six contiguous grid cells associated with each sampling block were located and used as sampling locations during trials. Centroids were uploaded as waypoints to hand-held GPS receivers. This system allowed for precise placement of traps at predetermined locations and on multiple days. Combinations of two sticky substances (PTS coated with Tanglefoot Insect Trap Coating or PTS wrapped with Pic fly paper) and three attractants (midden, live ant foragers that were attracted to hot-dog slices, or both [hybrid]) were investigated (Fig. 1). Slices (0.25 cm) of Bar-S beef franks (Phoenix, AZ) were used to attract foraging ants. One of the following phorid trap configurations was placed on one of six sampling points within each of the 20 sampling blocks; 1) Tanglefoot coated PTS with midden attractant, 2) Tanglefoot coated PTS with live *S. invicta* attracted to a hot-dog slice, 3) Tanglefoot coated PTS with midden and live *S. invicta* attracted to a hot-dog slice [Tanglefoot hybrid trap], 4) fly paper wrapped PTS with midden attractant, 5) fly paper wrapped PTS with live *S. invicta* attracted to a hot-dog slice, and 6) fly paper wrapped PTS with midden and live *S. invicta* attracted to a hot-dog slice [fly-paper hybrid trap]. Each trap configuration required one of each of the following components: 150 by 15 mm petri dish, 100 by 15 mm Petri dish (not required for traps that only used hot-dogs as an ant attractant), and PTS. For traps that used midden, or midden and live *S. invicta* attracted to hot-dog slices [hybrid] as the attractant, ≈2 g of dead ants were placed into the 100 by 15 mm dish. The midden was pushed to the outside of the dish while leaving a 'midden-free' zone in the center into which the PTS was placed (prongs upward). For traps using live *S. invicta* that were attracted to hot-dog slices, or midden and hot-dog slices [hybrid] as the attractant, the hot-dog slice was placed directly in the triangular center of the base of the PTS. Tanglefoot was applied to PTS prongs by dipping the first 0.5 cm of the prongs into the insect trap coating, and it was spread evenly by hand along the length of all three prongs and on prong tips. Fly paper (Pic fly ribbon 2.0 by 12.0 cm) was placed on PTS by first wrapping ≈1.0 cm of paper around one prong and then wrapping ≈10 cm of paper (total of 11.0 cm) around the outside of the remaining two prongs and finishing the wrap by meeting the end with the first wrapped prong (Fig. 1). Once PTS were prepared and centered in 100 by 15 mm dishes, all components were centered in the 150 by 15 mm dish. This configuration maintained the position of the attractant in close proximity to the PTS in the smaller dish, while the larger dish served to displace vegetation or other potential perches from the sampling environment.

These field trials were conducted during October 2010, November 2010, and September 2012 within the boundary of the 5-Eagle Ranch. The maximum temperature recorded during the trial was 28.89, 25.55, and 30.55°C (October 2010, November 2010, and September 2012, respectively), all above the minimum threshold of 22°C needed for phorid activity (Wuellner and Saunders 2003). Traps were deployed and retrieved at 1000 hours on successive days. All Tanglefoot coated PTS traps were carefully removed from the 100 by 15 mm dishes with forceps and immediately placed into trays constructed with cardboard partitions to prevent potential transfer of flies resulting from contact between traps. For traps which used fly paper as adhesive, the fly paper strips were carefully removed from the PTS in the field and adhered between two 3.0 by 14.0 cm strips of clear overhead transparency sheets. This method allowed for transport, storage, and assessment of fly paper strips while preventing the transfer of flies between strips. After retrieval, traps were returned to the laboratory for identification of flies.

Data Analysis. We analyzed the data collected using Analysis of Variance (ANOVA). For this analysis we considered "trap type" as the independent variable and "total number of flies," "number of *P. curvatus*," "number of *P. tricuspis*," and "number of males" as dependent variables. The ANOVA procedure was separately conducted for each one of the dependent variables. Duncan post hoc analysis was also conducted for means separation. The statistical package SPSS version 19.0 (SPSS Inc. 2010) was used to perform these analyses (values significantly different when $P < 0.05$). Voucher specimens of *S. invicta* and phorid flies collected in this study were deposited in the Center for Urban and Structural Entomology insect collection at Texas A&M University.

Results

October Sampling 2010 Event. There were significant differences ($F = 2.41$; df = 5,114; $P = 0.04$) in the mean number of total flies collected on the various traps during the October 2010 sampling period (Fig. 2A) with a significantly greater mean number of total flies on the Tanglefoot Hybrid traps than all other trap types except Tanglefoot + Midden traps using Duncan post hoc analysis $P < 0.05$. We found no significant differences in the mean number of *P. curvatus* ($F = 1.03$; df = 5,114; $P = 0.40$), *P. tricuspis* ($F = 2.34$; df = 5,114; $P = 0.05$), or males ($F = 0.73$; df = 5,114; $P = 0.60$) captured by the various traps (Fig. 2B–D). Additionally, we note that no *P. curvatus* were collected on the Tanglefoot + Hot-Dog trap (Fig. 2B) during this sampling period.

November 2010 Sampling Event. We found no significant differences in the mean number of total flies ($F = 0.91$; df = 5,114; $P = 0.49$), *P. curvatus* ($F = 0.93$; df = 5,114; $P = 0.46$), *P. tricuspis* ($F = 1.22$; df = 5,114; $P = 0.30$), and males ($F = 1.49$; df = 5,114; $P = 0.19$) captured during the November 2010 sampling period (Fig. 3A–D). Additionally, it should be noted that no *P. curvatus* were collected on the Tanglefoot Hybrid trap (Fig. 3B) and no *P. tricuspis* were collected on the Tanglefoot + Midden trap during this sampling period.

September 2012 Sampling Event. There were no significant differences with regards to the mean number of total flies ($F = 1.33$; df = 5,114; $P = 0.25$), *P. curvatus* ($F = 1.21$; df = 5,114; $P = 0.31$), or *P. tricuspis* ($F = 1.85$; df = 5,114; $P = 0.15$) collected on the various traps during the September 2012 sampling period (Fig. 4A–C). However, there were significant differences ($F = 4.87$; df = 5,114; $P < 0.01$) in the mean number of male flies collected on traps during the this sampling period (Fig. 4D) with a significantly greater mean number of male flies on the Fly Paper Hybrid traps than all other trap types when analyzed with Duncan post hoc analysis $P < 0.05$.

Trap Component Cost Analysis. The common component of all traps tested was the PTS. We purchased 1,000 PTSs at a cost of US$16.00. The PTS cost per trap was US$0.01. Trap designs that incorporated Petri dishes required one 100 by 15 mm and one 150 by 15 mm dish or dish lid. These were purchased at a cost of US$0.21 and US$0.85 per dish, respectively. A 15 oz container of Tanglefoot Insect Trap Coating was purchased for US$8.00. The amount of Tanglefoot used per trap is difficult to calculate, but we have used one container to construct >1,000 of these traps. Thus, we consider this cost to be of little consequence to the total cost of traps that require Tanglefoot. Pic fly paper is sold in packages of six 35.5 cm rolls (213.36 cm per

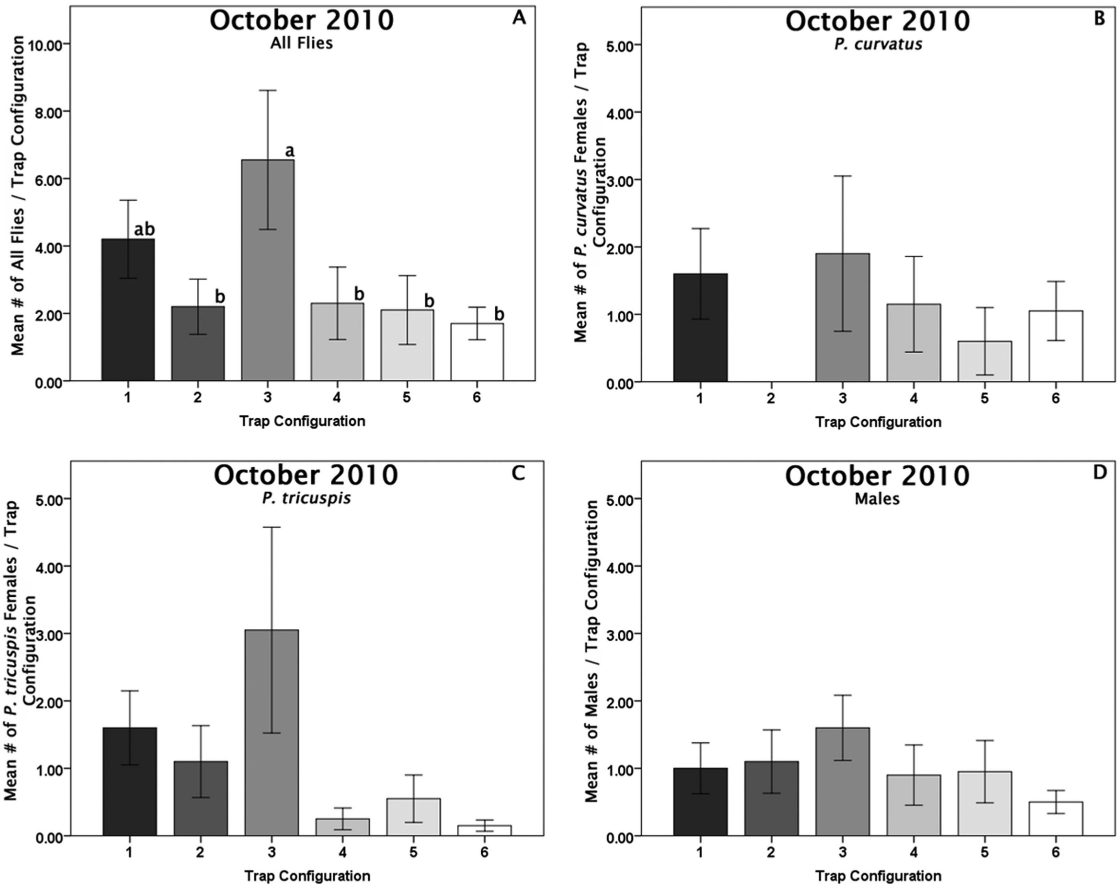

Fig. 2. Mean response of phorids (A, total number of flies; B, *P. curvatus*; C, *P. tricuspis*; and D, males) to various traps during the October 2010 sampling period. Trap Configurations: 1 = Tanglefoot + Midden, 2 = Tanglefoot + Hot-Dog, 3 = Tanglefoot + 'Hybrid', 4 = Fly-Paper + Midden, 5 = Fly-Paper + Hot-Dog, and 6 = Fly-Paper + 'Hybrid'.

package). Each fly paper trap requires 11 cm, which equates to a per trap cost of US$0.03. Transparency paper was purchased for US$23.00 per 100 21.6 by 27.9 cm sheets. Seven pairs of transparency paper strips can be made per sheet when they are cut into fourteen three by 14 cm strips. Each fly paper trap requires one pair of strips at a cost of US$0.04 per trap. Bar S Hot-Dogs were purchased at a cost of US$1.00 per package of eight. Each hot-dog was sliced into 15 pieces. A comparison of the total cost per trap type is included in Table 1.

Discussion

Both *P. tricuspis* and *P. curvatus*, and male flies were collected on all trap configurations evaluated in these field trials; however, mean abundance of phorids collected during the November 2010 and September 2012 sampling period was decreased relative to that of October 2010 sampling. Phorid activity is closely related to ambient temperature (Pesquero et al. 1996, Morrison et al. 1999), and while the reduced fly activity during the November 2010 sampling event was likely related to lower temperature relative to that of the October 2010 sampling, that of the September 2012 observations were likely the result of seasonal phenology of these flies at this location. Additionally, while *P. tricuspis* were numerically more abundant on the Tanglefoot Hybrid traps during the October sampling period (relative to other trap configurations), the effect was not statistically significant. In fact, the only significant performance differentials between trap configurations occurred during the October 2010 sampling event when the total number of flies collected (regardless of species) was considered, and during the September 2012 sampling event (males). This suggests that nuanced differences between trap configurations, such as midden versus live ants and Tanglefoot versus fly paper have only minor impacts on the effectiveness of phorid traps which incorporate these attractants and sticky components. Rather, if the general design of a phorid trap incorporates an attractant and sticky perch, researchers can be quite confident in the ability of the trap to consistently attract and collect phorids. No effort was made in these trials to determine whether traps were capable

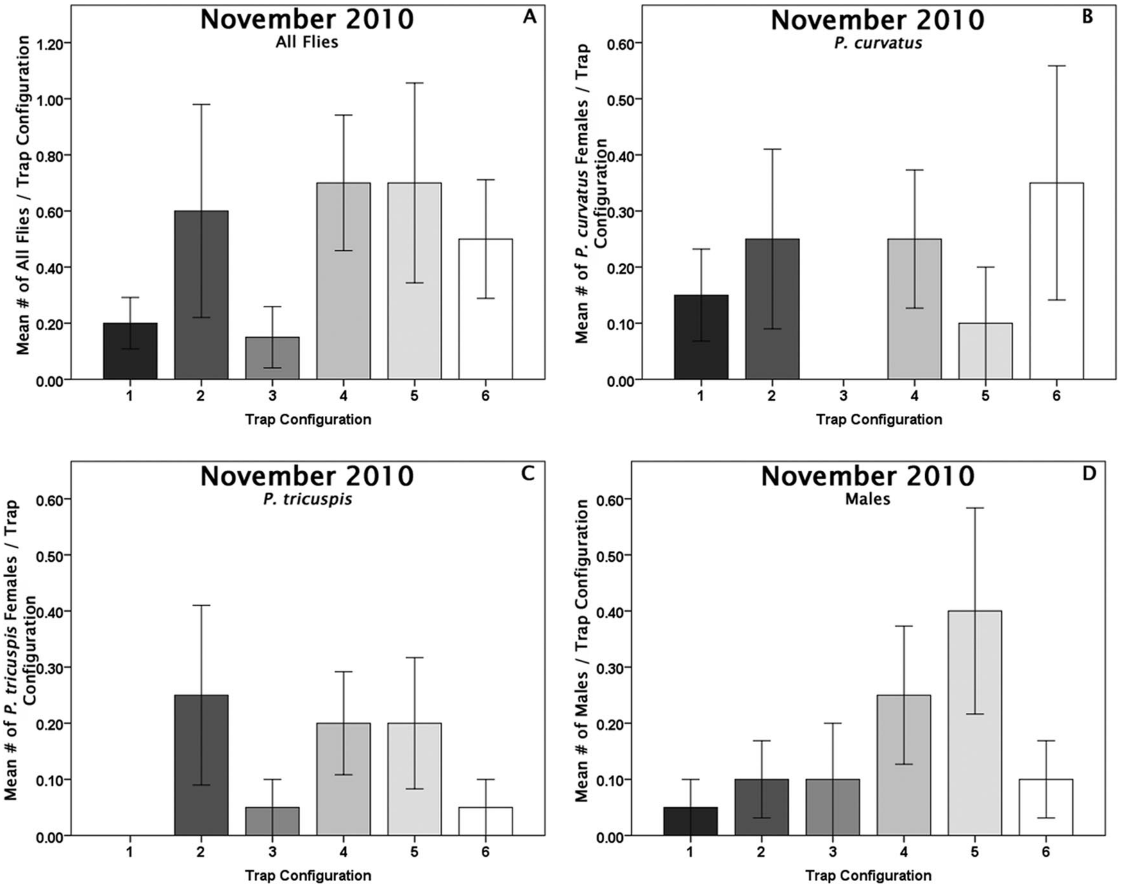

Fig. 3. Mean response of phorids (A, total number of flies; B, *P. curvatus*; 3, *P. tricuspis*; and D, males) to various traps during the November 2010 sampling period. Trap Configurations: 1 = Tanglefoot + Midden, 2 = Tanglefoot + Hot-Dog, 3 = Tanglefoot + 'Hybrid', 4 = Fly-Paper + Midden, 5 = Fly-Paper + Hot-Dog, and 6 = Fly-Paper + 'Hybrid'.

of detecting phorids when population densities are extremely high or low.

Given the consistent performance of each of these trap configurations, the selection criteria for a particular trap need not include a detailed analysis of the aspects of components and design described above. Rather, researchers are more likely to select traps based on monetary costs associated with components and their availability, as well as costs associated with time of construction, time required to deploy and retrieve traps, ease of assessment of phorids collected, and access to midden. The attractant for midden based traps is typically collected from field-collected and laboratory-reared *S. invicta* colonies. As a result, traps that use this material as an attractant are expensive with regards to the time and financial resources required to harvest the midden, but the overall financial expense is difficult to calculate and was not included in the cost/trap in Table 1. However, midden based traps do not require the researcher to locate active *S. invicta* colonies during sampling efforts. This of course allows researchers to deploy traps without the time associated with such activities. Traps that use foraging *S. invicta* on hot-dogs as a phorid attractant are much less expensive, but require traps to be deployed in areas of known *S. invicta* infestations and during periods in which *S. invicta* foraging can be anticipated. Foraging in *S. invicta* is typically curtailed when temperatures are above and below activity thresholds (Drees et al. 2007), and when phorids arrive and begin to parasitize workers. Additionally, hot-dog baits are often dominated by other ant species, disallowing *S. invicta* recruitment and congregation at traps. Hot-dog baits are often totally consumed or removed from the trap arena, whereas midden remains largely unmoved by *S. invicta* and remains attractive for several days (Puckett 2008). Thus, traps that rely on *S. invicta* foraging can limit the activities of researchers both spatially and temporally as compared with midden traps.

Tanglefoot traps are more difficult to assess for the presence of trapped flies after retrieval than are fly paper traps. Typical dissecting microscopes allow for very little space between objectives and stage. As a result, they must often be physically altered to allow for an observer to manipulate the PTS while maintaining focus on the object. Alternatively, once fly paper strips have been removed from the trap and the

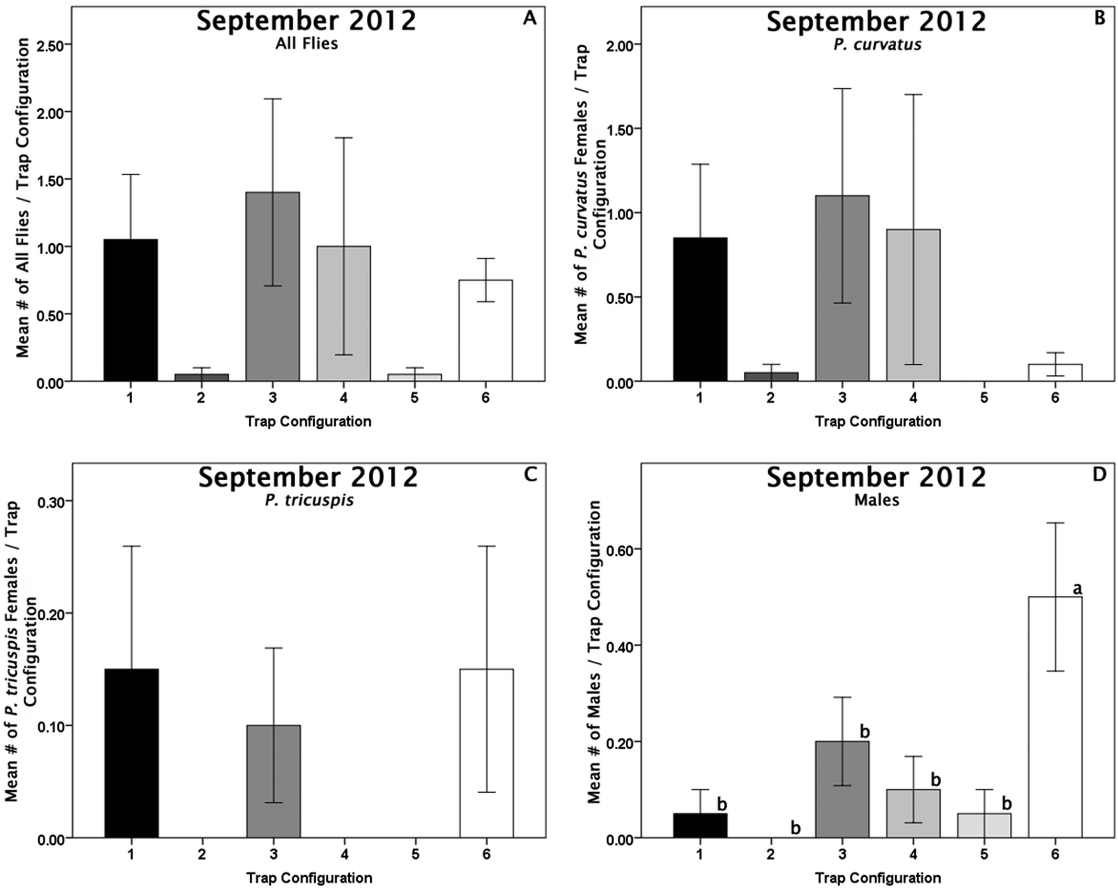

Fig. 4. Mean response of phorids (A, total number of flies; B, *P. curvatus*; C, *P. tricuspis*; and D, males) to various traps during the September 2012 sampling period. Trap Configurations: 1 = Tanglefoot + Midden, 2 = Tanglefoot + Hot-Dog, 3 = Tanglefoot + 'Hybrid', 4 = Fly-Paper + Midden, 5 = Fly-Paper + Hot-Dog, and 6 = Fly-Paper + 'Hybrid'.

transparency paper has been applied to both sides of the strips, a standard dissecting microscope arrangement is sufficient for identifying samples. Additionally, because the transparency paper adheres to the fly paper strips, a permanent ink pen can be used to mark the location of flies. Most importantly, these samples can then be stored efficiently and used as a semipermanent record of activity. While this is possible with Tanglefoot traps, storage becomes spatially inefficient as a result of the size and design of the PTS.

Ultimately, the selection of a sticky trap design for sampling phorids will be guided by the researcher's specific suite of site-specific sampling conditions, balanced against the cost associated with trap design. However, as a result of their relative ease of use, ability to store samples, and cost similarity to Tanglefoot traps, fly paper traps appear to provide the most benefit to those involved in such sampling.

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Table 1. Tabular description of trap-specific selection criteria descriptions

	Tanglefoot + Midden	Tanglefoot + Hot-Dog	Tanglefoot Hybrid	Fly Paper + Midden	Fly Paper + Hot-Dog	Fly Paper Hybrid
Total cost/trap (US$)	1.07	0.02	1.08	1.10	0.05	1.11
Requires live RIFA	No	Yes	No	No	Yes	No
Traps easily storable	No	No	No	Yes	Yes	Yes
Ease of fly assessment	Moderate	Moderate	Moderate	Very	Very	Very

field trials. We thank Laura Nelson for her helpful reviews and comments on earlier drafts of this document.

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