Induced Effects on Red Imported Fire Ant (Hymenoptera: Formicidae) Forager Size Ratios by *Pseudacteon* spp. (Diptera: Phoridae): Implications on Bait Size Selection

J. J. REED,[1] R. T. PUCKETT, and R. E. GOLD

Department of Entomology, Texas A&M University, 2143 TAMU, College Station, TX 77845.

Environ. Entomol. 44(5): 1407–1416 (2015); DOI: 10.1093/ee/nvv118

ABSTRACT Red imported fire ants, *Solenopsis invicta* Buren, are adversely affected by phorid flies in the genus *Pseudacteon* by instigating defensive behaviors in their hosts, and in turn reducing the efficiency of *S. invicta* foraging. Multiple *Pseudacteon* species have been released in Texas, and research has been focused on the establishment and spread of these introduced biological control agents.

Field experiments were conducted to determine bait particle size selection of *S. invicta* when exposed to phorid populations. Four different particle sizes of two candidate baits were offered to foragers (one provided by a pesticide manufacturer, and a laboratory-created bait). Foragers selectively were attracted to, and removed more 1–1.4-mm particles than any other bait size. The industry-provided bait is primarily made of particles in the 1.4–2.0 mm size, larger than what was selected by the ants in this study. While there was a preference for foragers to be attracted to and rest on the industry-provided blank bait, *S. invicta* removed more of the laboratory-created bait from the test vials. There was an abundance of workers with head widths ranging from 0.5–0.75 mm collected from baits. This was dissimilar from a previous study wherein phorid flies were not active and in which large workers were collected in higher abundance at the site. This implies that phorid fly activity caused a shift for red imported fire ant colonies to have fewer large foragers.

KEY WORDS phorid fly, fire ant, foraging, bait selection

Red imported fire ants (*Solenopsis invicta* Buren, Hymenoptera: Formicidae) are considered a pest not only because they are medically important, invasive, and displace other arthropod species, but also because they pose a significant threat to the ecology of the areas they invade (Lofgren 1986, Porter et al. 1992). Initially introduced in Mobile, Alabama, in the 1930s, they probably mistakenly gained entrance via shipping dunnage originating in South America (Buren 1972, Vinson 1997). The absence of a full suite of natural enemies, their propensity to invade disturbed habitats, and highly efficient foraging behavior allowed *S. invicta* to successfully colonize most of the southern United States (Helms and Vinson 2005).

Phorid flies in the genus *Pseudacteon* attack workers in the *Solenopsis saevissima* complex of fire ants. These flies are native to South America and have been introduced as biological control agents in several areas in the United States (Porter 1998, Folgarait et al. 2002, and Porter et al. 2004). Flies currently present in Texas have been shown to parasitize only during daylight hours (Pesquero et al. 1996) while their hosts are active during the day and nocturnally throughout much of the year.

Initially, research into *S. invicta*–phorid interactions demonstrated minimal effects on ant populations, with a low potential for successful biological control due to parasitism rates of ∼1–3% (Feener and Brown 1992, Orr et al. 1995, Morrison and Porter 2005). However, additional work revealed that *Pseudacteon* spp. activity could negatively affect competitive success of the *S. invicta* colony due to a reduction in foraging (Feener 1981, Porter et al. 1995, Morrison 1999). This reduction in foraging is an important consideration with respect to the competitive advantages ants demonstrate in the absence of the flies. Conversely, in the presence of the flies this advantage for the discovery and dominance of available resources could favor native ants over red imported fire ants.

Modern red imported fire ant control relies primarily on baits formulated from a food attractant, usually defatted corn grit particles, which are impregnated or coated with soybean oil, and also containing insecticide dissolved in the oil. Particle sizes of baits designed for ant control range from large to small, based on target species and manufacturer (Hooper-Bùi et al. 2002). Smaller *S. invicta* generally select smaller particles and larger ants select large ones (Neff et al. 2011). Size preferences by ant species have been shown to be fairly consistent, and this work was conducted on *S. invicta* that were not exposed to attack by phorid flies (Hooper-Bùi et al. 2002).

Puckett and Harris (2010) showed that populations of red imported fire ants that were exposed to and attacked by *Pseudacteon tricuspis* and *Pseudacteon*

[1] Corresponding author, e-mail: janisreed@tamu.edu.

curvatus demonstrated a change in the abundance of larger foragers compared to those at a site with no phorid activity. This phenomenon has similarly been documented in the native range of *S. invicta* in South America (Chirino et al. 2009). It is presumed that this shift in worker size could affect a change in the optimal bait particle size needed to suppress ant populations where phorids are active. This lead to questions about foraging strategies such as whether or not the presence of phorids results in an alteration in food size selection or if the presence of these flies influence a shift to nocturnal foraging to further avoid parasitism.

This study addressed two questions through field experiments: 1) whether there are significant differences in the bait particle sizes selected by phorid-impacted red imported fire ant colonies and colonies that are not phorid impacted; 2) whether *S. invicta* foragers from phorid-impacted colonies will have similar numbers of large foragers where phorids are absent. These experiments were undertaken with the goal of forecasting potential effects that biological control introductions might have on standard control efforts and to elucidate changes needed for control where *S. invicta* and *Pseudacteon* spp. occur together.

Materials and Methods

Experimental Field Sites. Two field sites were selected for this study, 5-Eagle Ranch in Caldwell, Texas (Burleson County, 30° 54′54.57″ N; 96° 40′59.77″ W), and the Skrivanek Ranch in Wellborn, Texas (Brazos County, 30° 28′49.40″ N; 96° 15′23.00″ W). Two species of phorids, *P. tricuspis* and *P. curvatus*, were released at 5-Eagle Ranch in 2002 and 2004, and were established at 5-Eagle Ranch by 2003 and 2005, respectively (Gilbert et al. 2008). While the range of field released *Pseudacteon* phorids is constantly expanding, phorids had not been found within ~15 km of the Skrivanek Ranch before the start of this study. However, by May 2010, *P. curvatus* had reached the Skrivanek Ranch, and *P. tricuspis* was active there by September of 2010.

Experimental Design. Data collection occurred once per month from February 2010 through October 2012. Due to the physiological inability of phorids to fly when temperatures are lower than 22°C, sampling was not conducted during months when the daytime temperature failed to reach this activity threshold. Additionally, sampling was not conducted in October and November 2011, due a lack of industry provided bait. The following data were recorded at each of the field sites: 1) Temperature and humidity via Hobo data loggers (HOBO U23-001, Onset Computer Corporation, Cape Cod, MA); 2) phorid fly presence, and relative abundance; 3) *S. invicta* head widths from both foraging ants at hot dog lures (Bar S Franks, Bar S Foods, Phoenix, AZ) and from samples taken directly from colonies; and 4) measurements of bait particle size selected by *S. invicta*. While the field sites are similar in terms of ecoregion, ant assemblage, and habitat, they are separated by ~40 km. As a result, sampling periods were based on civil twilight to ensure that samples relate to red imported fire ant and phorid circadian rhythm, rather than the anthropogenic 24-h clock. According to the U.S. Naval Oceanography Portal (2013), civil twilight is defined to begin in the morning when the Sun is geometrically 6 degrees below the horizon, and to end in the evening when the center of the Sun is 6 degrees below the horizon. It is the time of day when there is sufficient light for terrestrial items to be seen, and the horizon is apparent; before morning civil twilight and after evening civil twilight, artificial light sources are needed for data collection. Sampling was conducted when rain probabilities were low, but occasionally rain occurred on sampling days. Both sites were sampled simultaneously with the aid of laboratory personnel. The two field sites were compared based on collected data to ensure microclimates were similar with respect to temperature and humidity.

A transect of five phorid traps, as described by Puckett et al. (2007), were deployed 3 m apart at each location. The traps were deployed 15 min before AM civil twilight and collected 15 min after PM civil twilight to allow for maximum trap effectiveness throughout daylight hours. Upon return to the laboratory, traps were inspected, flies were identified and sexed, and data were recorded.

A transect of 15 hot dog lures (Bestelmeyer et al. 2000) were deployed at AM civil twilight and 20 min prior to PM civil twilight, ~100 m from the phorid traps. Slices (~0.6 cm) of hot dogs (Bar S Franks, Bar S Foods) were centered on note cards which were placed along the transect at a distance of 3 m apart. Hot dog lures remained available for foraging ants for 20 min. After this period of time elapsed, the note card, hot dog lure, and any ants were collected and quickly placed in zip top bags. Upon return to the laboratory, ants were removed from the note card, debris, and lure and stored in 90% ethanol.

Colony (individual mound) samples were obtained using 2 ml microtubes (with lids) coated with talcum powder. Five colony samples were taken for each observation time, morning and evening, at both 5 Eagle Ranch and Skrivanek Ranch to determine colony forager sizes. In order to obtain a random sample, the microtubes were inserted into ant mounds until the upper rim was flush with the top of the mound. Microtubes were then left in place for 5 min or until the tube was full of responding ants. Then the tube was removed, capped, and placed in a zip top bag. Upon return to the laboratory the tubes were placed in a freezer to kill and preserve specimens. Later, ants were removed from any dirt, debris, and talcum powder and placed in 90% ethanol.

Samples for *S. invicta* head width determination were obtained by measuring ants which were collected by the two distinctly different sampling methods from the field described above, food lures and samples obtained directly from colonies. After collection, the ant samples were stored in a vial containing 90% ethanol until they were processed. The total number of ants in each vial from each sample was estimated using known sample vials, and a subsample was obtained by randomly removing ~15%, using a large pipette.

Individual ant heads were removed and measured using the wedge micrometer method described by Porter (1983). Digital calipers (Model DC-122A, Rok International Industry Co., Shenzhen, China) were used to verify measurements on the wedge micrometer. Heads were removed by using fine tipped forceps, grasping the ant between the head and thorax and twisting slightly. The removed heads were then gathered and measured using the wedge micrometer. A range of head size classifications was used to facilitate measuring head widths of red imported fire ants collected from hot dog samples and colony samples quickly and accurately. Those size classifications were: <0.5, 0.5–0.75, 0.75–1.0, 1.0–1.25, 1.25–1.5, and 1.5–1.75 mm. These head width sizes corresponded with Puckett and Harris (2010) in the following way: Class 1 corresponds to head width <0.5–0.75 mm, Class 2 corresponds with head width 0.75–1.0 mm, Class 3 corresponds with head widths 0.75–1.0 mm, and Class 4 corresponds with head widths >1.0 mm.

In order to determine bait particle size and bait type preference of red imported fire ants in phorid and non-phorid infested areas, field bioassays with four particle sizes were conducted. Two different bait types were used for these tests: a 1:1 ratio of carbohydrate to protein laboratory-created bait (EB1:1) described by Cook et al. (2010), and an industry-provided blank bait (TC-206 Advance Granular Carpenter Ant Scatter Bait), provided by BASF (St. Louis, MO) and contained no active ingredient (CABB). As provided by the manufacturer, the blank bait material contains 80% of its mass in size class 4 (1.5–2.0 mm). The candidate baits were sieved to four particle size classes: <0.71, 0.72–1.0, 0.9–1.4, and 1.5–2 mm. Baits were hand milled and size classes were separated using US standard sieves No. 10, 14, 18, and 25. This sieving gave the sizes 1–4, respectively. These sizes were chosen based on the size of bait particles in the standard corn grit bait provided by BASF. The baits were coded by type and size in the following manner: CABB signified the industry-provided corn grit while EB1:1 signifies the laboratory-created, 1:1 ratio bait. Prior to sampling days, baits were weighed, recorded, and placed into one of 8 individual vials, with ~2 g of bait per vial. Four vials were prepared with the CABB and four vials with the EB1:1. Vials were 15-ml clear centrifuge tubes (89004-368, VWR, West Chester, PA). A vial rack was constructed by cutting a piece of pine to a height of 17 mm, a width of 38 mm, and length of 200 mm and then drilling with a 16 mm bit equidistant apart producing 8 holes to accommodate the vials (Fig. 1A). This vial array was used for ease of deployment as well as ordering of the baits in holders in a randomized pattern. Use of this random pattern of bait arrangement remained standard throughout the study and at both sampling locations.

During both the morning and evening on each sampling day, a transect of five vial arrays located 10 m apart was deployed (Fig. 1A). The vial arrays were located ~125 m from the phorid traps and 75 m from the hot dog slices. During morning sampling, arrays were deployed 5 min prior to civil twilight and during evening sampling; arrays were deployed 1 h before civil twilight and were available to ant foragers for a total of 65 min. Care was taken to ensure the vials were flush with the ground by removing surrounding vegetation and manipulating the vial array. Each array contained all four size classes of both baits, for a total of eight vials per array. Visual counts of foragers inside each vial were made four times at 15-min intervals, after which time the vials were capped, removed, and brought back to the laboratory. Debris, ants, and other matter were removed from the bait in each vial and the remaining bait was weighed, and these weights were compared to pre-sample weights. A duplicate transect, offset by 1 m, was used as a control, with vials placed in a plastic container coated with Fluon to exclude ant access (Fig. 1B). For laboratory analysis, the ending weight of the bait in these control vials was recorded for comparison to the beginning weight to determine weight gain or loss that was unrelated to *S. invicta* foraging. The total gain/loss for each vial was calculated and the mean weight was added/subtracted from the corresponding test vials to obtain a corrected weight removed by ant foragers for analysis.

Polymerase chain reaction tests were conducted on colony samples of RIFA from Skrivanek Ranch and 5-Eagle Ranch. A Qiagen DNEasy kit (QIAGEN, Valencia CA) was used to determine monogyne vs. polygyne colonies.

Statistical analyses were conducted using IPM SPSS Statistics version 19. Microclimate data and data from PTS traps were analyzed using Mann–Whitney nonparametric test because the data were not normally distributed (Shapiro–Wilk test). Bait observational data from each ranch, each time period, each bait type and size, and weight of bait removed at each ranch were analyzed using a Student's t-test. Abundance of ants at each ranch by head width from hot dog lures and colony samples was analyzed using an ANOVA. Means were separated by using Tukey's Honestly Significant Difference (HSD) post hoc tests. Ranches were compared by ant head width measurements using Student's t-tests. Voucher specimens of ants and phorid flies are catalogued in the insect collection at the Center for Urban and Structural Entomology at Texas A&M University.

Results

Microclimate Data. The mean temperatures at 5-Eagle Ranch ($19.90 \pm 0.478°C$) and Skrivanek Ranch ($20.69 \pm 0.045°C$) were significantly different ($Z = 9.987$; $P < 0.001$) over the course of the study. Relative humidity was also significantly different between 5-Eagle ($73.82 \pm 0.106\%$) and Skrivanek ranches ($71.62 \pm 0.103\%$) between 2010 and 2012 ($Z = 21.894$; $P < 0.001$).

Phorid Fly (PTS) Traps. Over the course of this study, PTS traps at both locations collected two species of *Pseudacteon* flies including, *P. curvatus* and *P. tricuspis*. The greatest abundance of flies was observed from April through July of each year (Fig. 2). Both *P. curvatus* and *P. tricuspis* were present at both

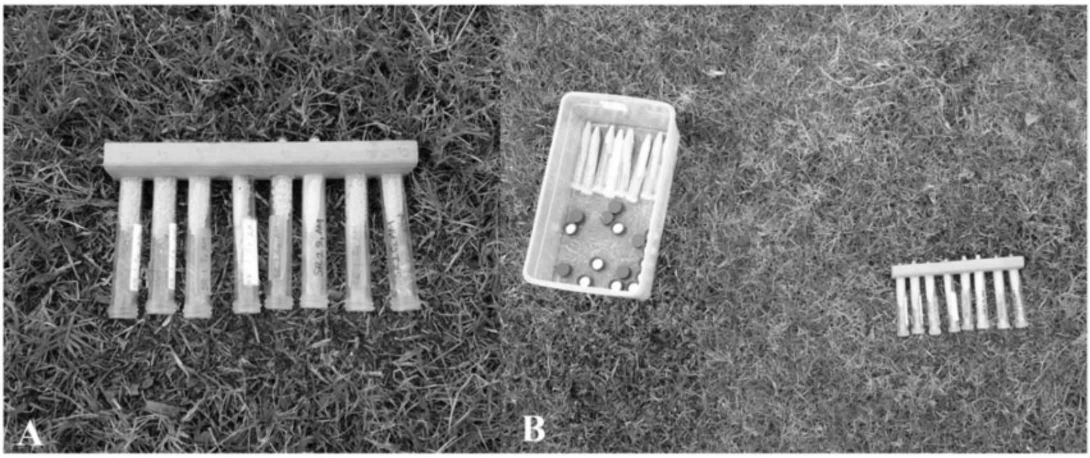

Fig. 1. A close up of the test vial array (A) and vial array arrangement (B) for bait size and nutrition preference studies.

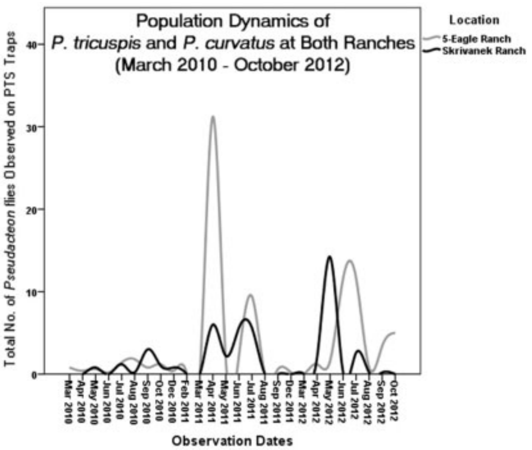

Fig. 2. Phenology of the total number flies of both species from PTS traps at 5-Eagle Ranch and Skrivanek Ranch from March 2010 through October 2012. Each sampling date represents five traps at each location. Only months in which sampling occurred are depicted on graph.

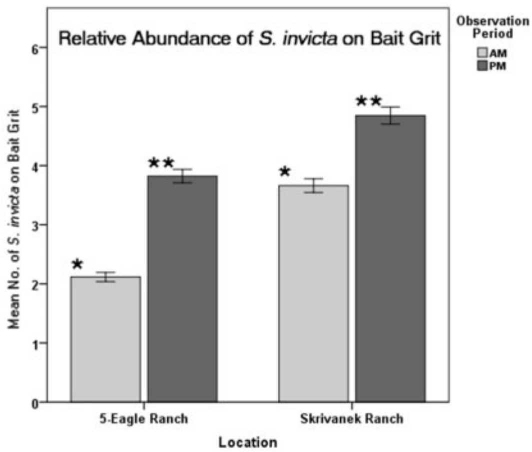

Fig. 3. Relative abundance of *S. invicta* on bait in vials from morning and evening observations at 5-Eagle Ranch ($n = 8480$) and Skrivanek Ranch ($n = 8640$) for all sampling dates. Asterisks represent significant differences between time periods at both ranches.

sites, but the mean number of flies collected from Skrivanek Ranch and 5-Eagle Ranch was significantly different ($Z = 2.537$; $P < 0.05$), with more flies being collected from the 5-Eagle Ranch (3.727 ± 0.544) than Skrivanek Ranch (1.976 ± 0.366).

P. curvatus was present at both sites, but the mean number of flies collected from Skrivanek Ranch (1.33 ± 0.032) and 5-Eagle Ranch (3.30 ± 0.530) was significantly different, with more flies being collected from the 5-Eagle Ranch than Skrivanek Ranch ($Z = 2.638$; $P < 0.01$).

Both sampling sites yielded *P. tricuspis* on the PTS traps; however, the mean number of *P. tricuspis* were significantly different between the two sampling sites ($Z = 2.144$; $P < 0.05$); there were more phorids of this species collected from Skrivanek Ranch (0.22 ± 0.068) than at 5-Eagle Ranch (0.09 ± 0.033).

***S. invicta* Forager Observations on Baits.** 5-Eagle Ranch had significantly fewer red imported fire ants than Skrivanek Ranch based on food lure (hot dog) data, colony sample data, and forager abundance on bait data.

Considering only bait observation data, 5-Eagle Ranch (2.95 ± 0.069) had significantly fewer ants foraging on baits in vials than Skrivanek Ranch (4.25 ± 0.094; $t = 11.138$; $df = 17118$; $P < 0.05$; Fig. 3).

There was a significant difference between the mean number of *S. invicta* in the morning between each ranch ($t = 10.975$; $df = 8638$; $P < 0.05$; Fig. 3), as well as a significant difference between the mean number of foragers in the evening between each ranch ($t = 5.514$; $df = 8478$; $P < 0.05$; Fig. 3). The 5-Eagle Ranch had fewer ants (2.12 ± 0.078) than Skrivanek Ranch (3.66 ± 0.117) during the AM sampling.

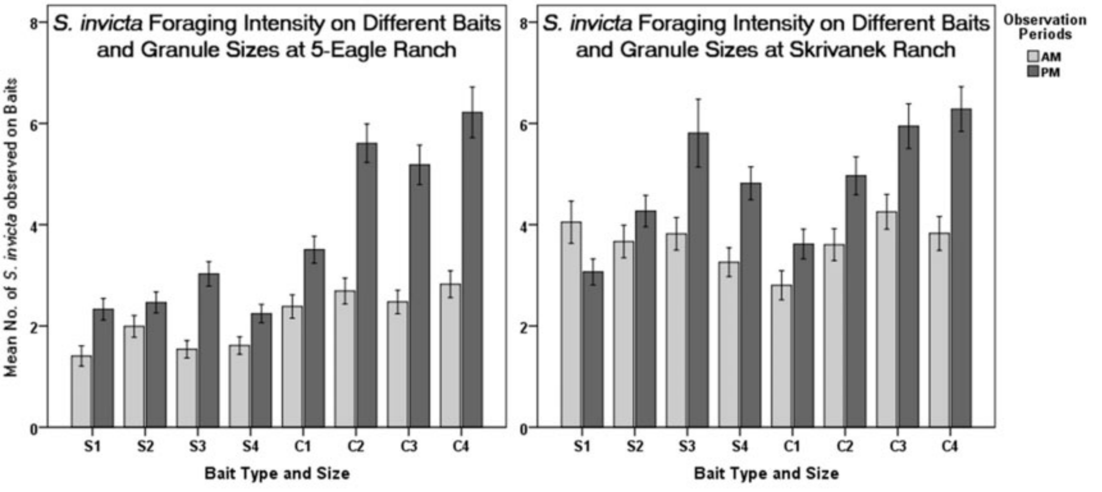

Fig. 4. Comparisons of overall mean *S. invicta* foraging intensity on four size classes of two different baits at 5-Eagle Ranch ($n = 1{,}060$) and Skrivanek Ranch ($n = 1{,}080$) by observation period. Means noted with "NS" were not significantly different. S1–S4 in this graph represent the EB1:1 bait with a 1:1 carbohydrate:protein ratio and C 1–4 represents the CABB bait. Sizes classes of 1–4 represent US standard sieves No. 25, 18, 14, and 10, respectively.

Similarly, 5-Eagle Ranch had fewer foragers (3.82 ± 0.114) than Skrivanek Ranch (4.85 ± 0.145) in the PM.

When comparing AM to PM foraging on baits by ranch, there was a significant difference between *S. invicta* foraging intensity during AM and PM observations at 5-Eagle ($t = 12.415$; df $= 8478$; $P < 0.05$; Fig. 3). There was a significant difference between the mean number of ants observed on all the bait types between the AM (2.12 ± 0.078) and PM (3.82 ± 0.114) observations at 5-Eagle and Skrivanek ranch; when comparing AM (7.690 ± 0.117) to PM (9.563 ± 0.145) mean number of ants on bait, observations also showed a significant difference ($t = 6.353$; df $= 8638$; $P < 0.05$; Fig. 3).

Foragers attracted to bait at each ranch were also analyzed temporally. When only foraging at 5-Eagle Ranch was analyzed, there was a significant difference in the mean number of foragers between the morning observations and the evening observations for all bait types except the EB1:1 bait size category 2. Foraging activity was higher in the PM in all instances with the greatest differences in CABB size 2, 3 and 4 baits. There was a significant difference between all the bait types for the AM observations ($F = 6.276$; df $= 7{,}4312$; $P < 0.001$; Fig. 4) and PM observations ($F = 25.823$; df $= 7{,}4152$; $P < 0.001$; Fig. 4) at 5-Eagle Ranch. For AM observations, CABB sizes 2 and 4 were significantly different from all other bait types and sizes. For the PM observations, CABB sizes 2, 3, and 4 were significantly different from all other sizes.

In all but one case, the mean number of *S. invicta* foragers recorded at Skrivanek Ranch was higher in the PM than during the AM observations, and in every case there was a significant difference between the mean number of ants with respect to foraging on each bait type between the AM and PM observation periods. Only bait type EB1:1 size class 2 was statistically equivalent between the AM and the PM observations. When all bait types were compared for the AM observations at Skrivanek ranch, the number of foragers on baits was statistically equivalent ($F = 1.899$; df $= 7{,}4312$; $P = 0.66$; Fig. 4). For all bait types in the PM at Skrivanek Ranch there was a significant difference in the number of foragers present ($F = 7.869$; df $= 7{,}4312$; $P < 0.001$; Fig. 4), and the number of ant observed foraging on CABB size class 4 was significantly different from all other bait types.

Weight of Bait Removed from Vials. There was a significant difference in the amount of bait removed (g) for each bait type at 5-Eagle Ranch between the AM and the PM observations, with the amount of bait removed during PM observations always being greater. All bait types had more removed during the PM observations than the AM observations. Additionally, there was a significant difference in the amount of bait removed from vials in the AM ($F = 4.815$; df $= 7{,}1068$; $P < 0.001$; Fig. 5) and in the PM ($F = 11.463$; df $= 7{,}1065$; $P < 0.001$; Fig. 5) at 5-Eagle Ranch. The size class 3 bait was significantly different from all other baits in the AM and PM in terms of the amount of bait removed from vials.

There was a significant difference in the amount of bait removed (g) for each bait type at Skrivanek Ranch between the AM ($F = 10.636$; df $= 7{,}1072$; $P < 0.001$; Fig. 5) and the PM ($F = 19.104$; df $= 7{,}1079$; $P < 0.001$; Fig. 5) observations, with the amount of bait removed during the PM observations always being greater. The weight of size EB1:1 size 3 and 4 baits removed from the vials were significantly greater than all other baits in the AM and PM.

Head Width Measurements—Hot Dog Lures. There was a significant difference in the abundance ($n = 405$) of *S. invicta* foragers categorized by

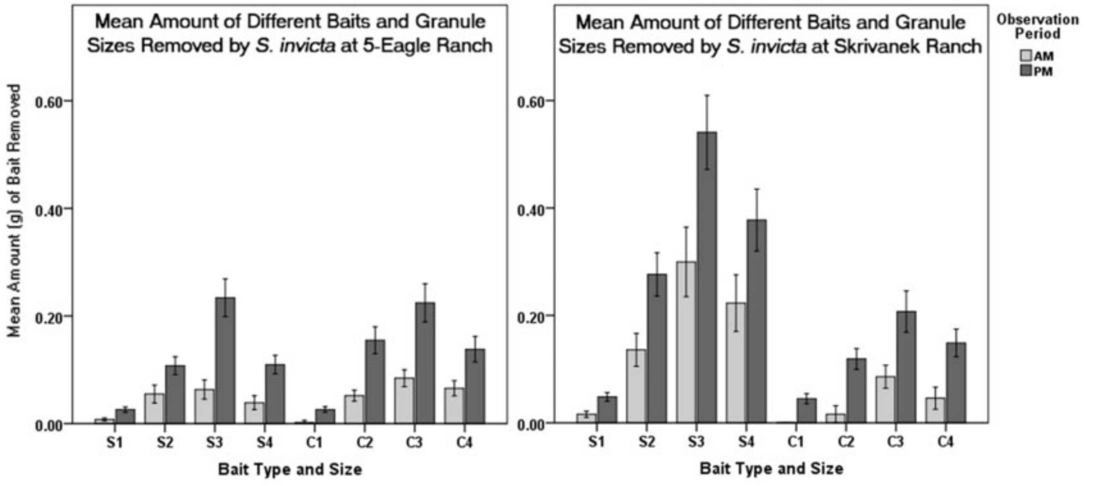

Fig. 5. Mean amount of bait (g) removed by *S. invicta* at 5-Eagle Ranch and Skrivanek Ranch at AM and PM observations of four size classes of two different baits. Means ($n = 2{,}167$) shown are for all observations and all sampling dates. Means noted with "NS" were not significantly different. S1–S4 in this graph represent the EB1:1 bait with a 1:1 carbohydrate:protein ratio and C 1–4 represents the CABB bait. Sizes classes of 1–4 represent US standard sieves No. 25, 18, 14, and 10, respectively.

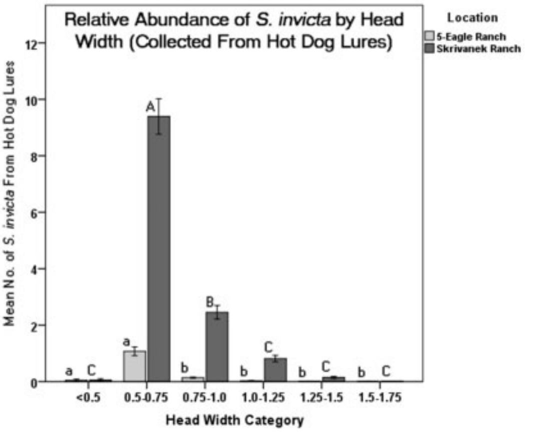

Fig. 6. Relative abundance of *S. invicta* by head width (mm) from hot dog lures by location. Both ranches were significantly different (5-Eagle, indicated by lowercase).

head width measurement from hot dog lures at 5-Eagle Ranch ($F = 41.723$; df = 5,4854; $P < 0.001$; Fig. 6). Significantly more ants with head widths between 0.5–0.75 mm (mean ± SE = 1.073 ± 0.152) were collected than all other head widths.

There was a significant difference in the number of *S. invicta* foragers collected by head width measurement from hot dog lures at Skrivanek Ranch ($F = 171.187$; df = 5,4854; $P < 0.001$; Fig. 6). The number of foragers collected with head width between 0.5–0.75 mm (mean ± SE = 9.393 ± 0.631) was significantly greater than all other head widths, and head size 0.75–1.0 mm was significantly different from all other head widths as well.

When analyzing the number of *S. invicta* foragers collected during AM and PM observations from Eagle Ranch only, there was a significant difference in the mean number of ants in the 0.5–0.75 mm ($t = 3.481$; df = 577; $P < 0.005$), 0.75–1.0 mm ($t = 2.957$; df = 573; $P < 0.005$), and 1.0–1.25 mm ($t = 2.362$; df = 533; $P < 0.05$; Fig 6) head widths. There was no significant difference between the <0.5 ($t = 2.362$; df = 405; $P = 0.318$) and 1.25–1.5 ($t = 0.000$; df = 808; $P = 1.000$; Fig 6) head widths. Statistics were not computed for head width 1.5–1.75 mm from 5-Eagle because standard deviations were zero for both groups.

When analyzing data from Skrivanek Ranch only, there was a significant difference in the mean number of samples in the 0.75–1.0 ($t = 2.022$; df = 720; $P < 0.05$), and 1.0–1.25 mm ($t = 2.954$; df = 508; $P < 0.005$) head widths between the AM and PM observations. There was no significant difference between the <0.5 ($t = 0.915$; df = 808; $P = 0.360$), 0.5–0.75 ($t = 0.493$; df = 808; $P = 0.622$), 1.25–1.5 ($t = 1.088$; df = 793; $P = 0.277$), and the 1.5–1.75 mm ($t = 1.736$; df = 404; $P = 0.083$; Fig. 6) head widths.

Head Width Measurements—Colony Samples. These samples were difficult to obtain during summer months when above ground mound building was limited and at 5-Eagle Ranch where *S. invicta* activity was minimal.

Colony samples taken directly from individual mounds yielded many fewer ants overall than hot dog lures. There was a significant difference in the number ($n = 135$) of red imported fire ant foragers collected and then categorized by head width measurements from colony samples at 5-Eagle Ranch ($F = 120.509$; df = 5,1614; $P < 0.001$; Fig. 7). *S. invicta* with head widths ranging from 0.5–0.75 mm were collected significantly more often than all other head width categories.

There was also a significant difference in the abundance of *S. invicta* foragers collected by head width measurement from colony samples at Skrivanek Ranch

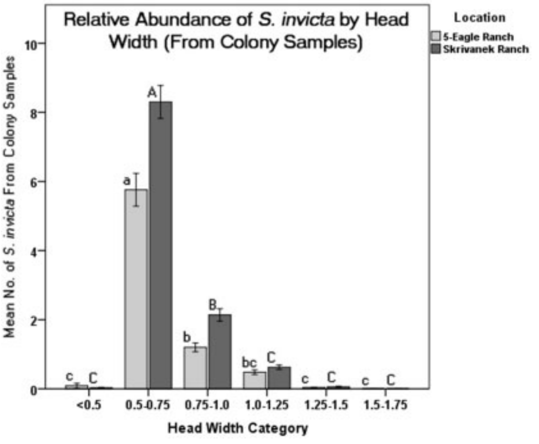

Fig. 7. Relative abundance of *S. invicta* by head width (mm) from colony samples by location. Both ranches were significantly different (5-Eagle, indicated by lowercase letters).

($F = 242.146$; df = 5,1614; $P < 0.001$; Fig. 7). The number of foragers in head width category 0.5–0.75 mm was significantly greater than all other head widths and was the most common head size found. Head widths between 0.75–1.0 mm were also found significantly more than from all head widths, except 0.5–0.75 mm.

The mean number of ants from colony samples at 5-Eagle Ranch only showed a significant difference in the number of ants in head width size 0.5–0.75 mm ($t = 2.188$; df = 264; $P < 0.05$; Fig. 7). There was no significant difference between the mean number of ants from colony samples at 5-Eagle Ranch for head width sizes < 0.5, 0.75–1.0, 1.0–1.25, 1.25–1.5, or 1.5–1.75 mm.

The mean number of ants by head width (mm) from colony samples at Skrivanek ranch showed a significant difference in the number of ants in head width size 1.0–1.25 mm (t = 2.521; df = 247; $P < 0.05$; Fig. 7). There was no significant difference in the mean number of ants in head sizes < 0.5, 0.5–0.75, 0.75–1.0, 1.25–1.5, and 1.5–1.75 mm.

PCR Results. Polymerase chain reaction was performed on colony samples of *S. invicta* from Skrivanek Ranch and 5-Eagle Ranch, to determine the relative abundance of monogyne vs. polygyne colonies at the locations. Once the electrophesis was complete, bands at 517 and 423 were noted. In monogyne colonies, the gene is homozygous, only appearing at 517, while in polygyne colonies the gene is heterozygous, bands at 517 and 423 were present. A total of 28 samples were analyzed, 15 from Skrivanek Ranch and 13 from 5-Eagle Ranch. One sample from each ranch was determined to be from a monogyne colony, 24 samples were polygyne, and 2 samples were inconclusive because no bands were present.

Discussion

The temperature and relative humidity (RH) data were similar from the two ranches, and it is unlikely that the differences were biologically relevant. The overall mean temperature at the study sites differed by less than 1°, and the RH was 2% different for the overall mean. The ranches varied in total plant cover, with 5-Eagle Ranch containing many more trees than Skrivanek Ranch, especially large oaks, which could have influenced microclimates, affecting the daily temperature and humidity fluctuations enough to cause significant differences. Skrivanek Ranch is mainly open pasture, sparsely covered with trees. Neither sampling site was located under shade trees.

Due to the fact that both species of phorid flies were present at both 5-Eagle and Skrivanek Ranches, the design of this set of experiments reflected the encroachment of the fly populations at Skrivanek Ranch. Rather than comparing ranch to ranch, more focus was placed on AM and PM observations as well as previous studies by Puckett and Harris (2010) for overall ant activity and forager sizes, and Hooper-Bui (2002) for particle size preferences.

Phorid fly data from this project supports the conclusions of LeBrun et al. (2009) that *P. curvatus* displaces *P. tricuspis*, possibly due to deleterious interactions in the densities of and competition between these parasitoids that have very similar life strategies (Ferriere and Cazelles 1999). While fewer *P. tricuspis* were collected than *P. curvatus*, the population levels were consistent, but these data do not support that direct competition and was not sufficient to cause the shift in the population levels of the phorid flies shown in this work.

Based on Puckett and Harris (2010), the numbers of foragers on hot dog lures at Skrivanek Ranch and 5-Eagle Ranch were statistically equivalent over the course of their study. In contrast, in the present study there was a significant disparity between the total number of foragers observed, not only on bait in vials but also on hot dog lures, with many more *S. invicta* observed at Skrivanek Ranch than at 5-Eagle. Skrivanek Ranch consistently had more foragers as well as more visible mounds in and around the study site. At the time of their study in 2008 (Puckett and Harris 2010), *Pseudacteon* flies had yet to be recovered from the Skrivanek Ranch. It was not until 2010, and the start of this study, that both *P. tricuspis* and *P. curvatus* were recovered from Skrivanek Ranch. Management practices, weather patterns, and rainfall were similar, and there had been no known releases of biological control agents on the ranch. However, one primary difference was the long term establishment of phorids at 5-Eagle over Skrivanek Ranch. Phorid flies, specifically *P. tricuspis,* were established at 5-Eagle Ranch in the spring of 2003, and by spring of 2005, *P. curvatus* was established. By the start of this study, phorids had been impacting *S. invicta* populations for up to seven years at 5-Eagle Ranch (Gilbert et al. 2008). Red imported fire ant colonies at 5-Eagle had at least five total years of *P. curvatus* exposure and seven years of *P. tricuspis* exposure. The decrease in total ant activity at 5-Eagle Ranch had occurred since 2008, and little has changed other than the persistence of populations of phorid flies

Work by Hooper-Bùi et al. (2002) showed that *S. invicta* foragers, when not exposed to phorid flies prefer to retrieve particle sizes of >2 mm. Additionally, these results were shown from primarily monogyne colonies, which tend to have larger workers than polygyne colonies (Greenberg et al. 1985). However, in this study, the largest particles were 2 mm in size, and there was not a clear delineation for a preference of particle sizes, based on the number of foragers observed, especially as it pertains to the CABB. There was no significant particle size selection difference between three of the four different CABB, with only the smallest size not being preferred. There was a trend for *S. invicta* foragers to select the candidate bait in size 3 (1–1.4 mm) over the other baits but the difference was not always significant. When weight of bait removed was factored into this discussion, a different trend emerged. Significantly more of the EB1:1 was removed from the bait vials and size 3 (1–1.4 mm) was preferred. *S. invicta* foragers spent time antennating and in situ feeding on the CABB, without physically removing it from the bait vial, possibly consuming or collecting the small particles or oils from it. Thus, there were significantly more foragers on the CABB, but very few foragers actually moved particles out of the bait vials. The difference observed was presumably due to the nutritional makeup of the baits; the EB1:1 is nutritionally valuable and contains solid fats only fourth-instar larvae can process while the CABB is lipid and small particle rich. Workers collect the oils or small particles, but do not transport the solid bait matrix because it is less nutritionally rich than the solids in the EB1:1 candidate bait.

Previous work (Puckett and Harris 2010) also showed a difference in the abundance of foragers by class size at Skrivanek and 5-Eagle Ranches. Their data showed that at the Skrivanek Ranch, at which phorids were not active during the study, there were significantly more large foragers (Size Class 3; 0.75–1.0 mm) as compared to 5-Eagle where phorids had been active for several years. Both sites were considered to primarily contain polygynous colonies (R.T. Puckett, personal communication). Our study used a slightly different size classification system and method to separate the caste sizes than the Puckett and Harris (2010) study, but was comparable by size class to head width. Consistent with their findings, we found ants exposed to phorids at both sites had an abundance of small foragers, Class 1, or up to 0.75 mm head widths, present. Very few workers and foragers in the three largest size categories were present. The total number of foragers by head width measurements at both ranches in this study was analogous with findings from 5-Eagle in the previous study (Puckett and Harris 2010). We found Skrivanek Ranch red imported fire ant populations had been selected to contain a worker size composition similar to the one found at 5-Eagle by Puckett and Harris (2010). There was a higher proportion of the smaller worker size classes than all of the large worker sizes, at both ranches in our study; whereas in Puckett and Harris (2010) there was much more variability, and many more larger workers, especially in size classes 3 and 4 (0.75–1.0 and >1.0 mm head widths) at Skrivanek Ranch. This shift occurred in only two to three years after phorid arrival at Skrivanek Ranch. Once flies were introduced or move into a new area, it can take up to four years for fly populations to reach maximum levels (Porter et al. 2004). Based on this data, *P. tricuspis* and *P. curvatus* activity caused *S. invicta* populations to produce fewer large workers as compared to populations that are not exposed to phorid flies. It is reasonable to conclude that from a colony standpoint, ants would not invest resources to develop larger workers and not utilize the larger workers to forage, even as they aged, so fewer larger workers were found in both foraging and colony samples. If larger workers are present, they are more likely to be outside the colony early in the day, after a period of nocturnal recovery from the previous day's phorid activity and before phorids have become active on the day of observation. This research showed no evidence of an abundant number of large workers from any sampling type.

There were also more *S. invicta* foraging in the PM observations than in the AM observations. This was expected due to temperature thresholds, especially in the winter and early spring months. Optimal temperatures for foraging are between 22–36°C, with ∼27–29°C being ideal, and temperatures greater than 50°C inhibit foraging (Porter and Tschinkel 1987, Drees et al. 2009). Many morning observations, and even some evening observations occurred outside the ideal temperature range, therefore foraging was diminished. Summer observations rarely fell outside of optimal temperature ranges due to sampling early in the morning, before temperatures increased, and late in the evening, when temperatures began to cool.

Considering the *S. invicta* worker size shift to fewer larger workers and the greater presence of smaller workers shown to be present at both ranches, it becomes clear this would result in a preference for smaller baits, due to the overall reduction in worker size. This preference of bait size selection based on ant size was demonstrated by Neff et al. (2011). As mentioned, Hooper-Bùi et al. (2002) demonstrated that foragers prefer large sized baits, even larger than were offered in this work, but forager count data as well as removal data show the foragers in this study selected baits in the 0.9–1.4 mm (No. 14 Sieve) and 1.4–2.0 mm (No. 10 Sieve) size ranges, smaller than the results shown by Hooper-Bui.

Based on data from Hooper-Bui (2002) and the results of this experiment, ants on these ranches selectively foraged baits smaller than *S. invicta* not exposed to phorid flies. Foraging ants from both ranches and all time periods were attracted to the CABB bait significantly more than the EB1:1 bait, but removed more of the EB1:1 bait. Therefore these data lead us to reject the null hypothesis that there is no difference in preferences to the CABB or the EB1:1 bait. Foragers from both ranches were shown to have an abundance of smaller foragers and workers, especially when compared to Puckett and Harris (2010), so the null hypothesis that workers would have similar size ratios in the presence and absence of phorid flies is also rejected.

Particle size acceptance of toxicant baits is important for red important fire ant control in urban and rural environments. Understanding biological and environmental effects on ants and how these effects change foraging preferences, including particle size preference will aid in designing better bait products as well as provide better control.

Acknowledgments

Monte Eagleton, Glenn Rutherford, and Sarah Skrivanek allowed this work to be conducted at 5-Eagle Ranch and Skrivanek Ranch. Phillip Shults, Danny McDonald, and Andrew Davitt have our gratitude for their help in executing these field trials. We thank Mrs. Laura Nelson for her helpful reviews and comments on earlier drafts of this document. Finally, BASF provided blank bait for these trials, for which we are grateful. Funding for this work was provided by the Texas Imported Fire Ant Research and Manangement Project (FY 2011–2012).

References Cited

Bestelmeyer, B. T., D. Agosti, L. E. Alonso, C. R. Brandao, W. L. Brown Jr., J. H. C. Delabie, and R. Silvestre. 2000. Field techniques for the study of ground-dwelling ants: an overview, description, and evaluation, pp. 122–144. *In* D. Agosti, J. Majer, E. Alonso, and T. Schultz (eds.), Ants: Standard methods for measuring and monitoring biodiversity. Biological diversity handbook series. Smithsonian Institution Press. Washington, DC.

Buren, W. F. 1972. Revisionary studies on the taxonomy of the imported fire ants. J. Ga. Entomol. Soc. 71: 1–26.

Chirino, M. G., L. E. Gilbert, and P. J. Folgarait. 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. Environ. Entomol. 38: 198–206.

Cook, S. C., M. D. Eubanks, R. E. Gold, and S. T. Behmer. 2010. Colony-level macronutrient regulation in ants: mechanisms, hoarding and associated costs. Anim. Behav. 79: 429–437.

Drees, B. M., B. Summerlin, and S. B. Vinson. 2009. Foraging activity and temperature relationship for the red imported fire ant. Southwest. Entomol. 32: 149–155.

Feener, D. H. 1981. Competition between ant species: outcome controlled by parasitic flies. Science 214: 815–817.

Feener, D. H., and B. V. Brown. 1992. Reduced foraging of *Solenopsis geminata* (Hymenoptera: Formicidae) in the presence of parasitic *Pseudacteon* spp. (Diptera: Phoridae). Ann. Entomol. Soc. Am. 85: 80–84.

Ferriere, R., and B. Cazelles. 1999. Universal power laws govern intermittent rarity in communities of interacting species. Ecology 80: 1505–1521.

Folgarait, P. J., O. A. Bruzzone, R. J. W. Patrock, and L. E. Gilbert. 2002. Developmental rates and host specificity for *Pseudacteon* parasitoids (Diptera: Phoridae) of fire ants (Hymenoptera: Formicidae) in Argentina. J. Econ. Entomol. 95: 1151–1158.

Gilbert, L. E., C. L. Barr, A. A. Calixto, J. L. Cook, B. M. Drees, E. G. Lebrun, R.J.W. Patrock, R. M. Plowes, S. D. Porter, and R. T. Puckett. 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. Southwest. Entomol. 33: 15–29.

Greenberg, L., D.J.C. Fletcher, and S. B. Vinson. 1985. Differences in worker size and mound distribution in monogynous and polygynous colonies of the fire ant *Solenopsis invicta* Buren. J. Kansas Entomol. Soc. 58: 9–18.

Helms, K. R., and S. B. Vinson. 2005. Surface activity of native ants co-occurring with the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Southwest. Entomol. 30: 223–237.

Hooper-Bùi, L. M., A. G. Appel, and M. K. Rust. 2002. Preference of food particle size among several urban ant species. J. Econ. Entomol. 95: 1222–1228.

LeBrun, E. G., R. M. Plowes, and L. E. Gilbert. 2009. Indirect competition facilitates widespread displacement of one naturalized parasitoid of imported fire ants by another. Ecology 90: 1184–1194.

Lofgren, C. S. 1986. The economic importance and control of imported fire ants in the United States, pp. 227–255. *In* S. B. Vinson (ed.), Economic impact and control of social insects. Praeger, New York, NY.

Morrison, L. W. 1999. Indirect effects of phorid fly parasitoids on the mechanisms of interspecific competition among ants. Oecologia 121: 113–122.

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

Neff, R., R. Puckett, and R. Gold. 2011. Particle size and bait preference of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Sociobiology 58:473–488.

Orr, M. R., S. H. Seike, W. W. Benson, and L. E. Gilbert. 1995. Flies suppress fire ants. Nature 373: 292–292.

Pesquero, M. A., S. Campiolo, H. G. Fowler, and S. D. Porter. 1996. Diurnal patterns of ovipositional activity in two *Pseudacteon* fly parasitoids (Diptera: Phoridae) of *Solenopsis* fire ants (Hymenoptera: Formicidae). Fla. Entomol. 79: 455–457.

Porter, S. D. 1983. Fast, accurate method of measuring ant head widths. Ann. Entomol. Soc. Am. 76: 866–867.

Porter, S. D. 1998b. Host-specific attraction of *Pseudacteon* flies (Diptera: Phoridae) to fire ant colonies in Brazil. Fla. Entomol. 81: 423–429.

Porter, S. D., and W. R. Tschinkel. 1987. Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae): effects of weather and season. Environ. Entomol. 16: 802–808.

Porter, S. D., H. G. Fowler, and W. P. Mackay. 1992. Fire ant mound densities in the United States and Brazil (Hymenoptera: Formicidae). J. Econ. Entomol. 85: 1154–1161.

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.

Porter, S. D., R. K. Vander Meer, M. A. Pesquero, S. Campiolo, and H. G. Fowler. 1995. *Solenopsis* (Hymenoptera: Formicidae) fire ant reactions to attacks of *Pseudacteon* flies (Diptera: Phoridae) in Southeastern Brazil. Ann. Entomol. Soc. Am. 88: 570–575.

Puckett, R. T., and M. K. Harris. 2010. Phorid flies, *Pseudacteon* spp. (Diptera: Phoridae), affect forager size ratios of red imported fire ants *Solenopsis invicta* (Hymenoptera: Formicidae) in Texas. Environ. Entomol. 39: 1593–1600.

Puckett, R. T., A. Calixto, C. L. Barr, and M. Harris. 2007. Sticky traps for monitoring *Pseudacteon* parasitoids of *Solenopsis* fire ants. Environ. Entomol. 36: 584–588.

United States Naval Oceanography Portal. 2013. Rise, set and twilight definitions. United States Naval Oceanography portal webpage. (http://aa.usno.navy.mil/faq/docs/RST_defs.php) (accessed 13 July 2015)

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

Received 17 September 2014; accepted 26 June 2015.