Diurnal Patterns of Ovipositional Activity of *Pseudacteon tricuspis* and *Pseudacteon curvatus* (Diptera: Phoridae) in Alabama and Host Location Behavior of *Pseudacteon curvatus* (Diptera: Phoridae) in Alabama

by

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Abstract

There are more than 20 species of *Pseudacteon* spp. (Diptera: Phoridae) that parasitize *Solenopsis* spp. (Hymenoptera: Formicidae) in their native South America. In South America, several *Pseudacteon* species coexist and occupy the same niches competing for imported fire ants of different sizes on which to lay eggs in, imported fire ants engaged in different activities, and different periods of diurnal activity. This thesis presents the first documentation of diurnal patterns of ovipositional activity in *Pseudacteon tricuspis* and *Pseudacteon curvatus* in Alabama. Data were collected in Macon and Talladega Counties, Alabama from 2002-2005.

Peak activity of *Pseudacteon tricuspis* in Macon County was during mid-day but *P. tricuspis* extended activity and were still active 12 hours following sunrise. Similar mid-day activity in *P. tricuspis* in South America has been documented. Interestingly, *P. tricuspis* activity 12 hours following sunrise occupies the same time niche that *Pseudacteon litoralis* exhibits in South America.

Pseudacteon curvatus has a bi-modal pattern of activity in Talladega County. The two peak times of activity occurred on either side of the peak activity time for *P. tricuspis* in Macon County. This suggests that *P. tricuspis* and *P. curvatus* may be compatible species in Alabama since they occupy relatively different activity times.

This thesis also presents host location behavior of *P. curvatus* in Alabama. Data from this thesis provide evidence that *P. curvatus* are more attracted to host imported fire ants engaged in an activities such as or dealing with a mound disturbance rather than imported fire ants engaging simply in foraging activity.

At the time that data were collected for this thesis, only *P. tricuspis* and *P. curvatus* were established in Alabama and the populations of the two fly species did not overlap.

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Introduction

Imported fire ants are aggressive, globally invasive ants that are native to the floodplains of Brazil and Argentina, South America. The black imported fire ant and the red imported fire ant made two separate marches into the United States before 1950. The two species and their hybrid have been wreaking havoc and creating mayhem since their independent introductions.

The black imported fire ant, *Solenopsis richteri* Forel, was first reported as *Solenopsis saevissima richteri* by Löding (1929) as an imported fire ant in the United States. Creighton (1930) estimated the black imported fire ant's arrival into the port city of Mobile, Alabama to be around 1918. Chemical analysis indicates that the black imported fire ant originates from about 60 miles north of Buenos Aires, Argentina, South America (Vander Meer and Lofgren 1990).

Felix Santschi named the red South American ant *Solenopsis saevissima* var. *wagneri* in the early part of the twentieth century in honor of its collector, E. R. Wagner. Unaware that Santschi had already named the ant, W. F. Buren named the red introduced ant found in North America, *Solenopsis invicta*. According to the rule of priority, *Solenopsis wagneri* Santschi is the proper name of the red imported fire ant, however, many imported fire ant researchers use *Solenopsis invicta* when referring to the red imported fire ant. This paper will use *Solenopsis invicta* when referring to the red imported fire ant.

E. O. Wilson followed the journey of the imported fire ant from the age of thirteen. Dr. Wilson, a world authority on ants, was one of the first to publish on imported fire ants in the United States (Wilson and Eads 1949). He noticed a difference between the already present black imported fire ant and a different red type in Alabama in the summer of 1942. Determining a definitive entry date for the red imported fire ant into the port of Mobile, Alabama has proved more difficult than the black imported fire ant. The red imported fire ant entered into Mobile sometime between 1933 and 1945 (Callcott and Collins 1996). Chemical analysis indicates that the red imported fire ant comes from the Cáceras regions of southwestern Brazil (Vander Meer and Lofgren 1990).

Both species of imported fire ant are believed to have been accidentally introduced into the United States via infested fruit (George 1958) or soil used as ballast (Vinson 1997) aboard cargo ships bound for North America from South America. Callcott and Collins (1996) estimated that imported fire ants increased their range to over 25,272,706 ha (62,448,000 acres) between 1918 and 1953. The federal fire ant quarantine was established in 1958 and since then, the average range expansion has been 2,400,703 ha per year. The construction and use of the interstate highway system in the 1970's contributed to the rapid spread of imported fire ants eastward and westward. Northern expansion is likely limited due to climate intolerances such as cold temperature, moisture, and winterkill (Morrill et al. 1978, Francke et al. 1986, Porter 1988, Callcott and Collins 1996). The two species do not readily hybridize in South America, but do in North America. The black imported fire ant has been displaced by the red imported fire ant and a hybrid of the black imported and red imported fire ants forcing it into a very limited population in northeastern Mississippi, northwestern Alabama (Lofgren et al.

1975), and southern Tennessee (Oliver et al. 2009). Red imported fire ants were first reported in California in 1998 (Anon. 1999, Code of California Regulations 2000). By 2000, more than 128 million ha in 13 United States and Puerto Rico were infested by the imported fire ant (Callcott 2002). Red imported fire ant infestations were first detected in Brisbane, Queensland, Australia in 2001 (Belton 2002). About 6,000 ha of Taipei, Taiwan and China were reported to be infested by imported fire ants in 2003 (Chen et al. 2006). In 2005, Northern Hong Kong joined the worldwide fight against this formidable adversary (Wong and Yuen 2005).

Numerous natural enemies of the imported fire ant exist. Research is ongoing to explore their effectiveness as biological control agents. Two nematodes in the genera *Steinernema* Travassos (Steinernematidae: Rhabditida) and *Heterorhabditis* Poinar (Rabditidae: Heterorhabditidae) (Miller et al. 1988, Drees et al. 1992) occur in South America and have been found in North American imported fire ant populations. The protozoan *Mattesia*, also known as yellow-head disease, was found in imported fire ant populations in Florida in 2002 (Pereira et al. 2002). The fungus *Beauveria bassiana* (Balsamo) Vuillemin (Moniliales: Monilaceae) has also been found infects imported fire ants (Oi et al. 1994). *Kneallhazia (=Thelohania) solenopsae* (Microsporidia: Thelohaniidae) occurs in South America and was found in a limited number of North American imported fire ant populations. A second microsporidian, *Vairimorpha invictae* Jouvenaz and Ellis (1986) (Microsporidia: Burenellidae), was found in a small portion of the imported fire ant population in north-central Argentina. Researchers are also investigating the parasitic ant *Solenopsis daguerrei* (Santschi) (Hymenoptera:

Formicidae) found in South America for possible biological control (Calcaterra et al. 1999). Several viruses are being evaluated as potential biological control agents (Allen et al. 2011, Valles 2012, Valles et al. 2007). A number of these natural enemies show at least some promise in the fight against the imported fire ant.

Porter et al. (1997) states that the absence of natural enemies can allow exotic pest populations to reach densities that are much higher than occur in their native habitats (van den Bosch et al. 1973, Huffaker and Messenger 1976). Porter et al. (1992) reported that the fire ant mound densities in the United States were almost nine times the average in Mato Grosso do Sul, Brazil. Overall, fire ant densities are about five times higher in the United States then they are in South America (Porter et al. 1992, Porter et al. 1997). Jouvenaz (1990) and Porter et al. (1997) attribute part of the success and rapid spread of the imported fire ant in North America to the fact that their natural enemies were left behind in South America.

Porter et al. (1997) hypothesize that introducing natural enemies into North American imported fire ant populations can result in imported fire ant population levels that are similar to those found in South America. In an attempt to tip the ecological balance in favor of native ants and to reduce imported fire ant abundance, several parasitoid species have been introduced into imported fire ant populations in the United States (Porter 1998; Graham et al. 2003; Vogt et al. 2003; Williams et al. 2003; Porter et al. 2004).

According to Feener and Brown (1997), 78% of the estimated number of parasitoid species are hymenopterans and 20% of the known parasitoids are dipterans

(Morrison and King 2004). Many species in the dipteran family Phoridae are parasitoids of ants (Disney 1990, 1994; Morrison and King 2004). When Coquillett described a third, "new" phorid genus, *Pseudacteon* in 1907, Phoridae only had two described genera, *Apocephalus* from North America and *Melaloncha* from South America. He characterized the females of the species as having a "large, exserted, horny ovipositor." Ten individuals (three males and seven females) of the new species *Pseudacteon crawfordi* were collected June 17, July 19, and October 22, 1906 by J. C. Crawford and W. D. Pierce in Dallas, Texas (Coquillett 1907). Currently, there are over twenty described species of *Pseudacteon* flies in South America that target *Solenopsis* spp. fire ants (Taber 2000). Four species of Brazilian phorid flies (*P. litoralis*, *P. wasmanni*, *P. tricuspis*, and *P. curvatus*) were imported into Texas in the mid-1990s for testing as biological control agents of the red imported fire ant.

Since 1998, five species of the dipteran parasitoid have been released in Alabama: *Pseudacteon tricuspis*, *Pseudacteon curvatus*, *Pseudacteon litoralis*, *Pseudacteon obtusus,* and *Pseudacteon cultellatus*. *Pseudacteon tricuspis* (Figure 1) was released in eight counties in Alabama. *Pseudacteon curvatus* was released in six counties in Alabama. *Pseudacteon litoralis* was released in Wilcox County in 2005, *Pseudacteon obtusus* was released in Lee County in 2008, 2010, and 2013, and *Pseudacteon cultellatus* was released in Lee County in 2011 (Figure 2).

Porter (1998) reports that the attack behavior of *Pseudacteon* flies was first described in detail by Wasmann (1918) in Holland, Borgmeier (1922) in Brazil, and Smith (1928) in the United States. "Larvae of these flies have the unusual habit of decapitating their living hosts and pupating inside the empty head capsule (Porter et al. 1995)." Coquillett (1907) states that a female fly was observed to deposit an egg in the head of *Solenopsis geminata*. Wasmann (1918) marks the beginning of the *Pseudacteon* life cycle as when "a torpedo-shaped egg is injected into the body of a worker ant." The egg hatches and the maggot starts its journey toward the head. The second instar maggot can be found in the ant's head by day four (Porter et al. 1995). The second and third instar maggots consume some tissue, but feed primarily on ant hemolymph. Henne and Johnson (2007) found that *P. tricuspis* third instar larvae controlled the behavior of parasitoidized *S. invicta* creating "zombie" ants. Parasitized ants did not demonstrate overly unusual behavior until the fly had nearly completed larval development. Parasitized ants in the study moved to lateral foraging tunnels near exit holes, but never actually left the nest until just prior to decapitation (Henne and Johnson 2007). The third instar larva releases chemicals that cause the intercuticular membranes of the ant to degenerate, contributing to the loosening of the ant's head, first pair of legs, and sometimes the gaster and other legs (Porter 1998). Porter (1998) further describes that the head contents are consumed by the maggot which typically results in the decapitation of the host in a 6-12 hour process. The resulting loss of the host ant's head leads this group of flies to be referred to as decapitating flies. "The maggot then pushes the ant's mandibles and tongue apparatus" to the side positioning itself for pupation (Porter 1998). Exposed larval segments sclerotize to form a cap that will later serve as the adult fly's emergence portal. Three to four days later, two whisker-like respiratory horns are pushed out of the corners of the oral cavity of the ant head capsule. Depending on temperature, pupal development can take 2-6 weeks (Morrison et al. 1997, Porter et al. 1997; Porter 1998). Porter reports that adult emergence takes only a few seconds and typically occurs around sunrise. Development from egg to adult is 5-12 weeks depending on temperature (Porter 1998). The tiny adult flies can mate and lay eggs within hours of eclosion. The lifespan of an adult fly is 1-3 days in the field and 3-7 days in the lab (Porter 1998).

In South America, several species of *Pseudacteon* are often found at the same site and exhibit at least three behaviors that help explain how the flies partition resources (Porter 1998): 1) they attack different size fire ant workers (Morrison et al. 1997), 2) they attack fire ants engaged in different activities (Orr et al. 1997), and 3) they select different periods of diurnal activity to attack workers (Pesquero et al. 1996). Studies of several *Pseudacteon* species that are parasitoids of *Solenopsis* fire ants suggest that these parasitoids initially locate their hosts from a distance by olfaction and then switch to visual cues at close distances for oviposition (Gilbert and Morrison 1997; Morrison and King 2004; Orr et al. 1997; Porter 1998; Porter and Alonso 1999). Field results by Morrison and King (2004) suggest that *P. tricuspis* in Florida are strongly attracted to host worker alarm pheromones or other defensive compounds but not to recruitment pheromones or chemical signals associated with foraging activities. *Solenopsis* alates engaged in mating flights release excitant pheromones which in turn elicit the release of alarm pheromones from *Solenopsis* workers (Obin and Vander Meer 1994; Alonso and Vander Meer 1997). *Pseudacteon tricuspis* is attracted to mating flights of *Solenopsis* ants (Pesquero et al. 1993). Chen et al. (2009) found that *P. tricuspis* were attracted by venom alkaloids along with alarm pheromone and other non-chemical cues such as visual cues. Orr et al. (1997) found that *P. tricuspis* were more frequently found at disturbed mounds rather than along foraging trails.

Two separate studies were conducted during the summers of 2002-2005. These studies were designed to gather information on the host location behavior of *P. curvatus* and *P. tricuspis* in Alabama. These two particular studies are the first of their kind in Alabama.

The first study was designed to determine the diurnal patterns of ovipositional activity of *P. tricuspis* and *P. curvatus* in Alabama. This study was necessary for three major reasons: 1) to facilitate efficiency for the second field study, 2) to gather data to compare diurnal patterns in Alabama with diurnal patterns in the native South American homeland, and 3) to document diurnal patterns because no previous data of this nature exist for *Pseudacteon* spp. flies in Alabama. I hypothesized that diurnal patterns of *Pseudacteon* spp. in Alabama will vary from patterns in South America due to lack of *Pseudacteon* species diversity.

The second study was designed to determine host location behaviors of *P. curvatus* in Alabama. The following five questions were addressed: 1) Are *P. curvatus* more attracted to workers at a disturbance or to workers at a food source, 2) Are the flies more attracted to workers that are simply foraging or to workers that are competing at a food source, 3) Will increasing the interaction at a food source affect the number of flies attracted to that source, 4) Are flies more attracted to workers at colony disturbances with interaction or to workers at colony disturbances without interaction, and 5) Are the flies differently attracted to the interactions?

Figure 1. Adult *Pseudacteon tricuspis* male, in red circle, on a penny. Photo credit: Vicky E. Bertagnolli

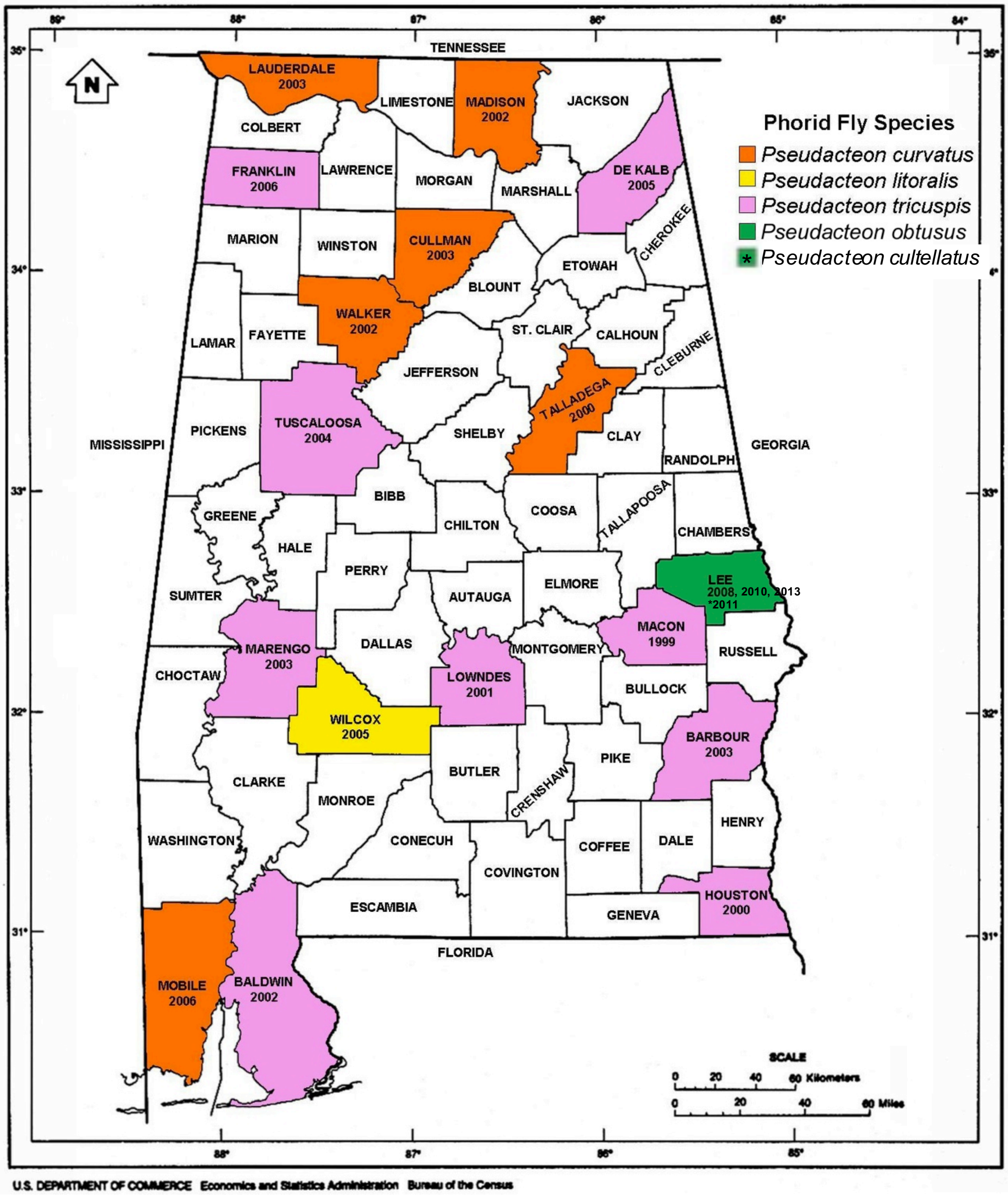

Figure 2. Map of Alabama showing *Pseudacteon* spp. releases. Year release was conducted appears under county name.

I. Diurnal patterns of ovipositional activity in *Pseudacteon tricuspis* and *Pseudacteon curvatus* (Diptera: Phoridae) in Alabama

Background and Significance

According to Feener and Brown (1997), 78% of the estimated number of parasitoid species are hymenopterans and 20% of the known parasitoids are dipterans (Morrison and King 2004). Many species in the dipteran family Phoridae are parasitoids of ants (Disney 1990, 1994; Morrison and King 2004). When Coquillett described a third, "new" phorid genus, *Pseudacteon* in 1907, Phoridae only had two described genera, *Apocephalus* from North America and *Melaloncha* from South America. He characterized the females of the species as having a "large, exserted, horny ovipositor." Ten individuals (three males and seven females) of the new species *Pseudacteon crawfordi* were collected June 17, July 19, and October 22, 1906 by J. C. Crawford and W. D. Pierce in Dallas, Texas. A female was observed depositing an egg in the head of a *Solenopsis geminata* ant (Coquillett 1907). Four species of Brazilian phorid flies (*Pseudacteon litoralis*, *Pseudacteon wasmanni*, *Pseudacteon tricuspis*, and *Pseudacteon curvatus*) were imported into Texas in the mid-1990s for testing as biological control agents of the red imported fire ant. Currently, there are over twenty described species of *Pseudacteon* flies in South America that target *Solenopsis* spp. fire ants (Taber 2000).

In South America, several species of *Pseudacteon* are often found at the same site and exhibit at least three behaviors that help explain how the flies partition resources

(Porter et al. 1997): 1) they attack different size fire ant workers (Morrison et al. 1997), 2) they attack fire ants engaged in different activities (Orr et al. 1997), and 3) they select different periods of diurnal activity (Pesquero et al. 1996). This experiment concentrated on the ability of *P. tricuspis* and *P. curvatus* to utilize different time periods to achieve successful oviposition. The second half of the experiment focused on the number of *P. curvatus* that attacked fire ants engaged in different activities such as foraging at a food source, competing with nonnestmates, or addressing a disturbance to the mound as a measure of the fly's host location behavior.

In Alabama, five species of the dipteran parasitoids have been released: *P. tricuspis*, *P. curvatus*, *P. litoralis*, *Pseudacteon obtusus and Pseudcteon cultellatus* (Figure 2). *Pseudacteon tricuspis* has been released in nine counties in Alabama. *Pseudacteon curvatus* has been released in six counties in Alabama. *Pseudacteon litoralis* was released in Wilcox County in 2005, *Pseudacteon obtusus* was released in one county in 2008, 2010 and 2013 and *Pseudacteon cultellatus* was release in Lee County in 2011.

Pesquero et al. (1996) found that in Brazil, *P. tricuspis* are most active during mid-day and that fly activity is greatly reduced 12 hours following sunrise. *Pseudacteon litoralis* exhibits moderate activity from 4-12 hours following sunrise with two distinctive peaks of activity. The first peak is the lower of the two occurring 2-4 hours following sunrise. The second and higher peak of activity is later in the afternoon, 10-12 hours following sunrise (Pesquero et al. 1996). I hypothesize that periods of fly activity may differ in Alabama from those in the native South America due to lower intraspecies competition during activity times (only one species of phorid fly was present at the sites at the time this study was conducted).

Diurnal activity of two established, introduced species of phorid fly in imported fire ant populations in Alabama were documented in this study in an effort to improve current management strategies.

Materials and Methods

This study was conducted in July, August, and September 2002, June, July, August, and September 2003 and May and July 2004. The two populations of imported fire ants and decapitating flies used in this study were located in Macon and Talladega Counties, Alabama. *Pseudacteon tricuspis* was released at the farm of Tony and Diane Silva (32°34'26.55"N, 85°39'55.03"W), designated Site A (Figure 3), in Macon County in 1999 (Graham et al. 2001) and the flies had spread over 50 km from the initial release site at the time of the study. *Pseudacteon curvatus* was released on Greg Myrick's property (33°28'51.28"N, 86° 2'45.40"W), designated Site B (Figure 4), in Talladega County in 2000 (Graham et al. 2001) and the flies had spread over 24 km from the initial release site. These two sites were chosen because the populations of both imported fire ants and flies were well established and readily accessible. Also, no other species of phorid fly was present at the site at the time of the study, so there was no competition between fly species for imported fire ants.

Imported fire ants used in this study were collected at the original release sites. Four live mounds of similar size were collected using a shovel. Each mound was placed into an individual 5 gallon bucket lined with Fluon® (Asahi Glass Ltd., Chadds Ford, PA). The colonies were transported back to the lab in a climate-controlled vehicle where the ants were separated from the soil using a water drip technique described by Jouvanez et al. (1977) and Banks et al. (1981) that takes advantage of the imported fire ants' tendency to raft during flood conditions. A plastic water tank with a valve to control water flow was retrofitted with a 45.7 cm length of latex tubing and a one mL pipette. The water tank was placed on a table. The 5 gallon bucket of ants and soil was placed on the floor directly beneath the pipette. The distance between the end of the pipette and the top of the bucket was approximately 25 cm. Water was slowly dripped into the bucket until most of the collected ants floated freely on the surface of the water or clung to the side of the bucket. The rafting ants were removed from the bucket with a slotted plastic spoon and placed into a clean 52 x 40 x 13 cm tray lined with Fluon®.

After the clean colony was placed into a clean 52 x 40 x 13 cm tray lined with Fluon®, the colony was supplied with a nest cell, food, water, and a 20% sugar water solution. The nest cell consisted of a 150 x 15 mm Petri dish, the bottom of which was filled with dental plaster, mixed according to label directions. After the dental plaster had hardened, it was soaked in water. Because dental plaster is porous, it holds enough moisture to prevent desiccation of any brood that may be present in the nest cell without leaving standing water. The lid was painted either brown or black to create a favorable dark environment for the colony. The solid food that was provided to each colony consisted of a 4 g section of Gwaltney® chicken hot dog. Two plastic test tubes were filled with water and the 20% sugar solution, respectively, and cotton was inserted in the ends of both tubes to prevent the water and solution from spilling out of the tubes. The nest cell, solid food, water tube, and sugar water solution tube were placed in the bottom of the tray for use by the imported fire ant colony while in the lab.

Each time this experiment was conducted, new colonies were collected and held in the manner described above. Colonies were held no more than two days to prevent any acclimation to the lab environment or adaptation to captivity.

The nest cells, solid food, water tube, and sugar water solution tube were removed from the trays the morning the experiment was to be conducted to prevent the ants from using the Petri dishes as hiding places while in the field. The four trays of ants were transported to the field in a climate-controlled vehicle to control mortality and the trays were hand-placed in shady areas of the release site on one straight transect with the trays spaced approximately 8 m apart from each other. The ants were agitated by shaking the trays to elicit alarm pheromone release. Trays were shaken in succession from tray 1 to tray 4. Thirty minutes after agitation of the first tray, phorid flies were aspirated out of the first tray using an aspirator unit until no flies could be observed in the tray. The aspirator was a double chambered unit with a 2-dram inner vial designed by Terry M. Allen (Item # 1135C, BioQuip® Products, Rancho Dominguez, CA). This aspirator unit was chosen because the suction created within the unit did not to kill the flies on impact allowing for collection of live specimens and live releases, thus having no impact on fly numbers. The flies were transferred from the aspirator to a 14 x 14 x 7 cm plastic

Rubbermaid® holding container via a 2.5 cm hole in the container lid to make the counting of flies easier. Carbon dioxide was then introduced into the holding container to induce fly knockdown. Upon knockdown, the lid was removed from the container and the individual flies were counted. After counting, the open container was placed in the shade within 61 cm of the tray to allow for fly recovery and release. The tray was shaken again and the collection process moved to the next tray.

For the 2002 field season, because the time the flies became active in the field in the mornings in Alabama was unknown, field days began before sunrise. The first collection time was set at 0700 CDT, approximately one half hour following sunrise (HFS). Data were collected every 30 minutes until flies ceased coming to the trays approximately 12-13 hours following sunrise (ca. dusk). Zero *P. tricuspis* were collected in July 2002 in Macon County before 1000 CDT. In August 2002, three flies were collected at the 0800 CDT collection time in Macon County. Zero *P. curvatus* were collected in August 2002 in Talladega County until the 0830 CDT collection time. The September 2002 collections in Talladega County had zero flies until the 1000 CDT sample time. Based on this 2002 data, protocol for the 2003 and 2004 field seasons was changed so that the first collection time was set at 0800 CDT, approximately one and one half hours following sunrise.

Sites were sampled in Macon County on July 23 and August 28, 2002 and June 24, July 23, August 19, and September 26, 2003. Sites were sampled in Talladega County on August 1 and September 11, 2002, June 24, July 28, August 20, and September 19, 2003, and May 24 and July 7, 2004. Air temperature, soil temperature, humidity, an estimation of light intensity, and fly numbers were recorded every thirty minutes. Only fly number data are presented in this thesis.

Results

Pseudacteon tricuspis

On July 23, 2002, a total of 1,235 *Pseudacteon tricuspis* were collected in Macon County (Figure 5). The number of flies collected on August 28, 2002 decreased from the previous month's count with 863 flies collected. In 2003, fly collection in Macon County began June 24; 121 flies were collected that day. Fly numbers were relatively higher on July 23 with 545 flies collected, but dropped to 250 on August 19. The low activity at the August sampling time could be attributed to inclement weather conditions. The weather conditions were overcast with drizzle most of the day. A major rain shower occurred ca. 1300 CDT and lasted approximately one hour. Fly numbers were higher September 26 with a total of 642 collected flies (Figure 5).

Pseudacteon tricuspis in Macon County were active from about 2.5 to 13 hours following sunrise (HFS) (Figure 6). Mean daily activity patterns for *P. tricuspis* in Alabama were similar to those found by Pesquero et al. (1996) in Brazil, with mean peak activity occurring mid-day (Figure 7).

Pseudacteon curvatus

A total of 1,247 *P. curvatus* were collected on August 1, 2002 in Talladega County (Figure 8). The number of flies was low on September 11, 2002 with only 292 flies collected. In 2003 in Talladega County, 123 flies were collected on June 24. Fly numbers increased on the July 28 collection date with 591 flies collected. The August 20 collection day yielded a total of 696 flies collected. Fly numbers decreased on September 19 with 248 flies collected. Only 84 flies were collected on May 24, 2004 in Talladega County. However, by July 7, 2004, fly numbers had increased to 356 flies collected.

Data suggest a cyclic wave of fly activity for *P. curvatus* in Alabama with fly populations peaking in August (Figure 8). Moderate fly activity occurred from 4 to 12 HFS on each sample date in 2002 and 2003 (Figure 9). When data were combined, two noticeable peaks in activity show that *Pseudacteon curvatus* exhibited a bimodal activity pattern where the minor peak of activity occurred about five HFS and the major peak in activity was seen 10-11 HFS (Figure 10).

Discussion

In Alabama, *P. tricuspis* and *P. curvatus* were most active in July and August (Figure 5 and Figure 8, respectively). Mean daily activity patterns for *P. tricuspis* in Alabama were similar to those found by Pesquero et al. (1996) in Brazil, with mean peak activity occurring mid-day (Figure 7). In Brazil, activity was greatly reduced 12 hours following sunrise. However, in Alabama, *P. tricuspis* were still active 12 hours following sunrise (ca. 6 pm), extending ovipositional activity into hours when *Pseudacteon litoralis* is usually active in Brazil (Pesquero et al. 1996). Peak activity for *P. curvatus* occurred later in the afternoon, 10-11 hours following sunrise, with moderate activity observed throughout the day (4-12 hours following sunrise) (Figure 10). This pattern was similar to the pattern *P. litoralis* exhibited in Brazil (Pesquero et al. 1996) where peak activity occurred 10-12 hours following sunrise with slightly lower fly activity two to four hours following sunrise.

The data for this study are important for several reasons. The results reported here support Pesquero et al.'s (1996) observations in South America on the daily patterning of activity of *Pseudacteon litoralis* and *Pseudacteon tricuspis* suggesting that more than one species may be needed for introduction in biological control programs. The data from this study identify the times of day when *P. tricuspis* and *P. curvatus* are most active and available for use in field experiments thus facilitating efficiency in the field in future studies. Note that at the time of this study, each site had only one species of fire ant decapitating fly. Daily fly activity may change over time as additional species are released and fly populations begin to overlap. In South America, several species of *Pseudacteon* are often found at the same site and exhibit at least three behaviors that help explain how the flies partition resources (Porter et al. 1997): 1) they attack different size fire ant workers (Morrison et al. 1997), 2) they attack fire ants engaged in different activities (Orr et al. 1997), and 3) they select different periods of diurnal activity (Pesquero et al. 1996).

Figure 3. Aerial photo of Site A. *Pseudacteon tricuspis* was released in 1999 on the farm of Tony and Diane Silva (32°34'26.55"N, 85°39'55.03"W), in Macon County, Alabama. The orange square highlights the *P. tricuspis* release site where this study was conducted in 2002 and 2003.

Figure 4. Aerial photo of Site B. *Pseudacteon curvatus* was released in 2000 on Greg Myrick's property (33°28'51.28"N, 86° 2'45.40"W), in Talladega County, Alabama. The orange square highlights the *P. curvatus* release site where this study was conducted in 2002-2004.

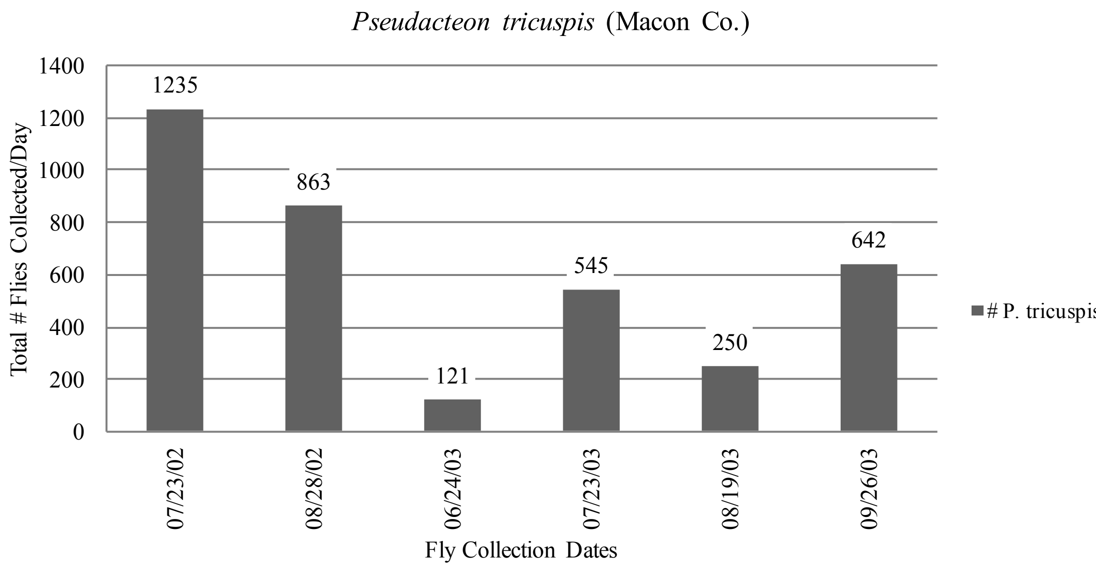

Figure 5. Total number of *Pseudacteon tricuspis* collected in Macon County, Alabama on sample dates in 2002 and 2003.

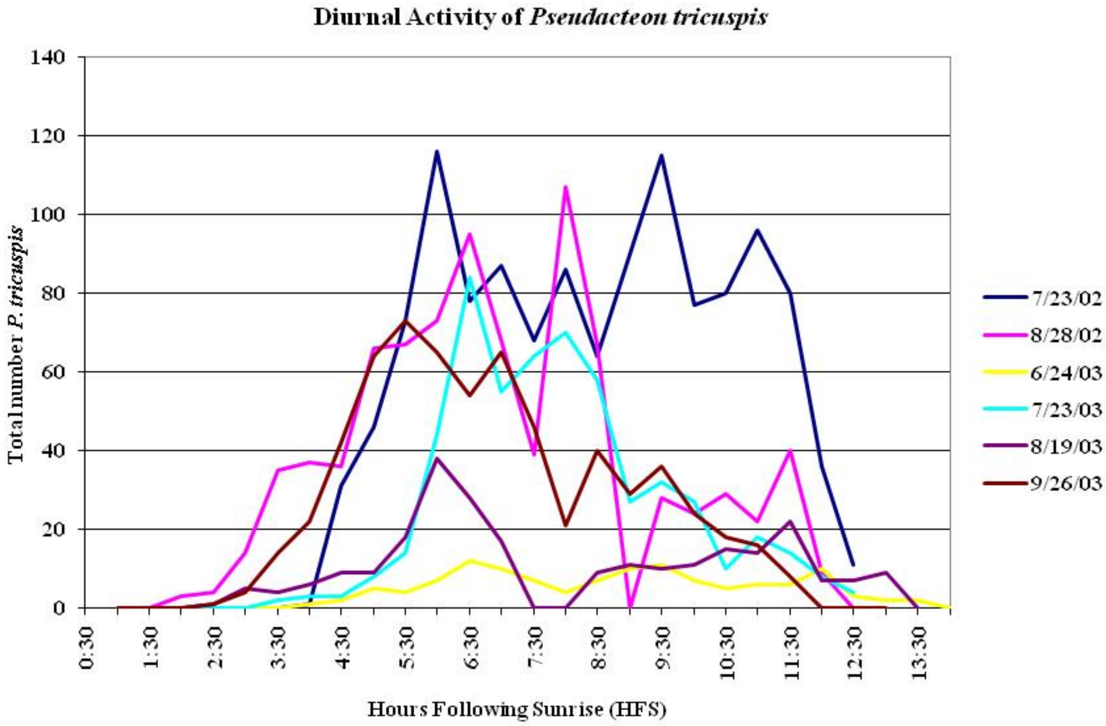

Figure 6. Diurnal activity of *Pseudacteon tricuspis* in Macon County, Alabama on sample dates in 2002 and 2003.

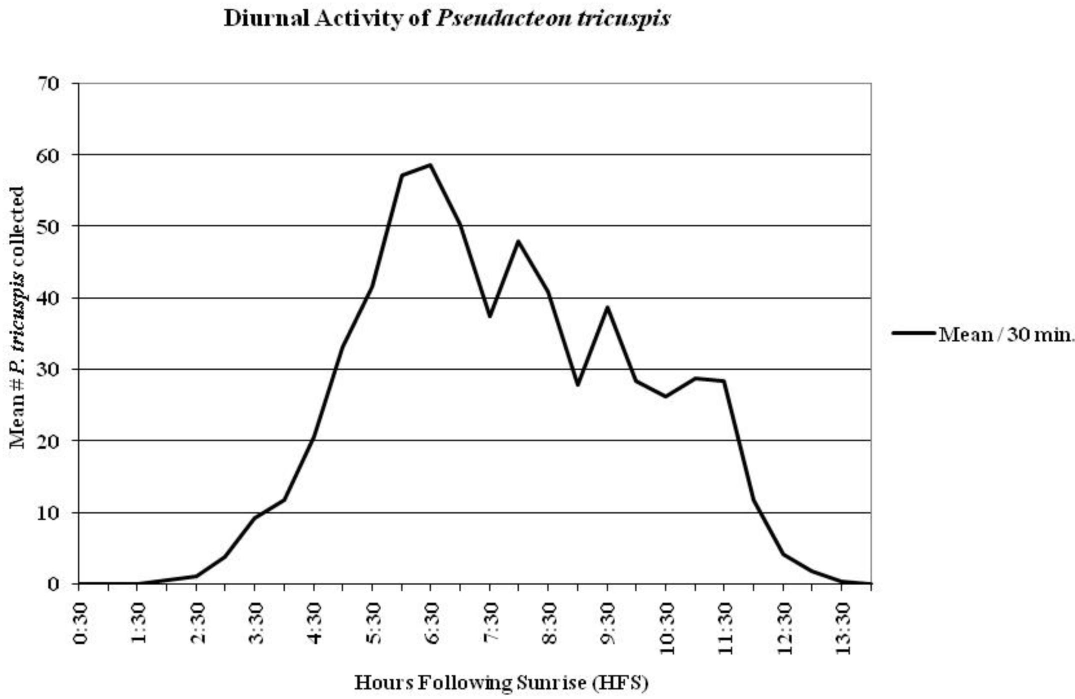

Figure 7. Mean diurnal activity pattern of *Pseudacteon tricuspis* in Macon County, Alabama, 2002-2003.

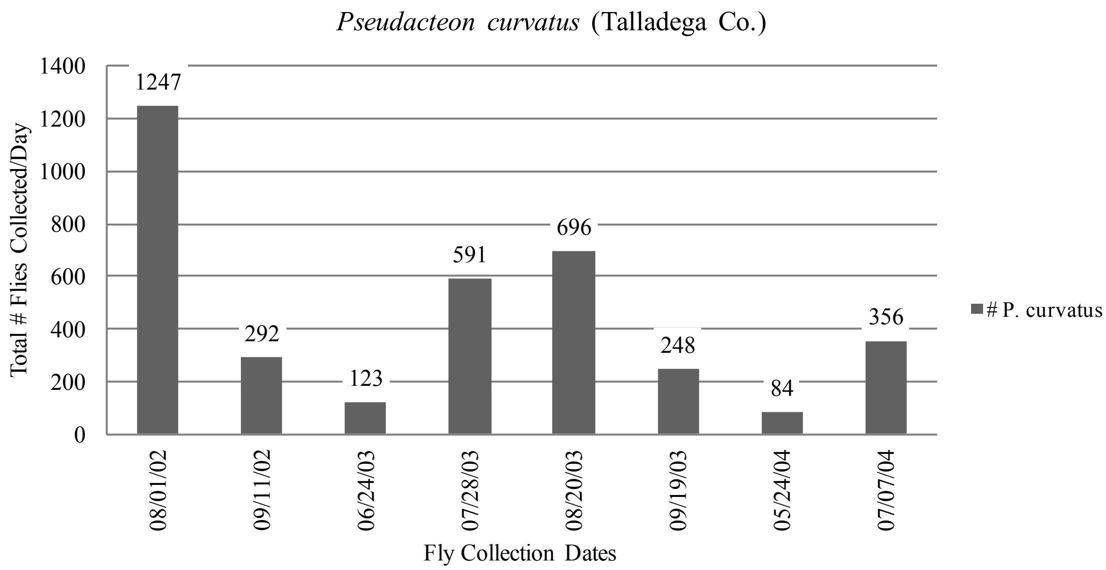

Figure 8. Total number of *Pseudacteon curvatus* collected in Talladega County, Alabama on sample dates in 2002, 2003, and 2004.

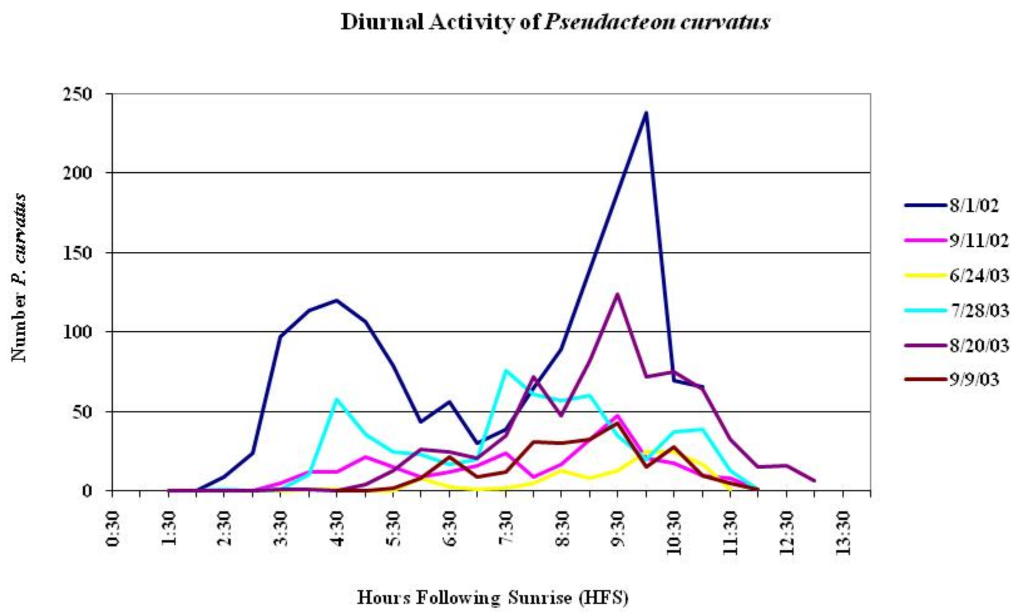

Figure 9. Diurnal activity of *Pseudacteon curvatus* in Talladega County, Alabama on sample dates in 2002 and 2003.

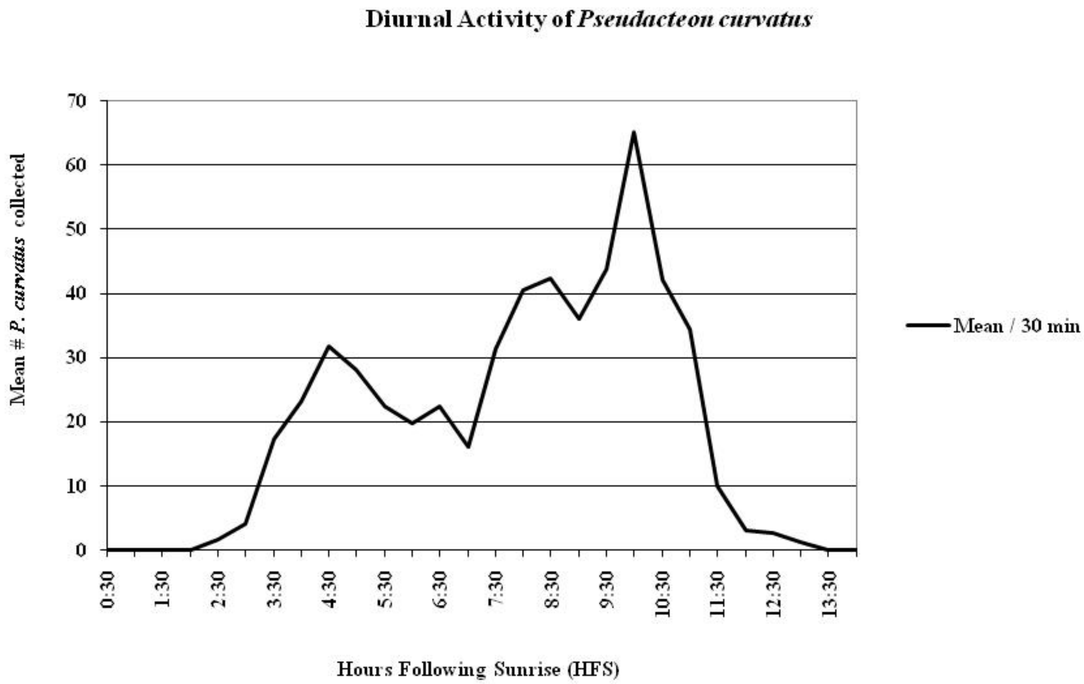

Figure 10. Mean diurnal activity pattern of *Pseudacteon curvatus* in Talladega County, Alabama, 2002-2003.

II. Host location behavior of Pseudacteon curvatus in Alabama

Background and Significance

In South America, several species of *Pseudacteon* are often found at the same site and exhibit at least three behaviors that help explain how flies partition resources (Porter at al. 1997): 1) they attack different size fire ant workers (Morrison et al. 1997), 2) they attack fire ants engaged in different activities (Orr et al. 1997), and 3) they select different periods of diurnal activity (Pesquero et al. 1996). The first half of this experiment, described in chapter I, established that *Pseudacteon tricuspis* and *Pseudacteon curvatus* utilize slightly different time periods in which they have maximum ovipositional activity. This portion of the study focused on the cues that attract *P. curvatus* to fire ant activities by studying the number of *P. curvatus* that attacked fire ants engaged in different activities such as foraging at a food source or competing with nonnestmates. I also looked at the effect a disturbance to the mound had on *P. curvatus* host location behavior.

Experiments on *P. curvatus* were modeled after the methods Morrison and King (2004) used in their experiments on *P. tricuspis* in Florida. Morrison and King (2004) found that *P. tricuspis* in Florida are strongly attracted to host worker alarm pheromones or other defensive compounds but not to recruitment pheromones or chemicals associated with foraging activities. The study was designed to determine cues for the host location behavior of *P. curvatus* in Alabama.

Methods and Materials

The study spanned the summer months of 2004 and 2005. Experiments in 2004 and 2005 were located at the original 2000 *P. curvatus* release site (33°28'51.28"N, 86° 2'45.40"W), designated Site B, in Talladega County, Alabama (Figure 11). The release site was a triangular shaped plot that was formerly a mixed hardwood and pine clear-cut area turned pasture with grazing cattle, but had been fallow for more than five years and used for quail, dove, and turkey hunting at the time this study was conducted. Kelly Creek runs along the Northeast perimeter of the property. A managed pine plantation borders the western perimeter of the property. Eastaboga Road runs the eastern perimeter of the study site. The original plan for 2005 was to duplicate the study in another part of the field at Site B thus having Site B1 and Site B2. However, due to the abundance of and the researcher's sensitivity to poison ivy, *Toxicodendron radicans* (L.) at Site B2, Site B2 was abandoned, and Site C was established approximately 3.69 km from the original release site at landowner Greg Street's Oak Valley Farms (33°30'19.11"N, 86°1'10.51"W), a purebred angus cattle operation in Talladega County, Alabama (Figure 12). Site C was a regularly grazed cattle pasture. A pond was within 100 feet of the study site's northwestern edge and Kelly Creek was within 500 feet of the study site's western edge. From this point forward, Site B is followed by (2004) or (2005) to specify the year data were collected at Site B.

The following is a description of the treatments used for this experiment.

- A “card bait” consisted of a 4 g section of Gwaltney® chicken hot dog placed on a 5 x 5 cm laminated card that was shaded with a wire-staked polystyrene foam plate (generic Styrofoam™). The card baits were spaced 5 m apart on transects.
- A “Petri dish bait” consisted of a 4 g section of Gwaltney® chicken hot dog placed on a 5 x 5 cm laminated card contained within a 100 x 15 mm Fluon®-lined Petri dish that was shaded with a wire-staked polystyrene foam plate. A 3.175 mm hole was drilled into the side of the Petri dishes to allow ants from the field site to find and recruit to the baits and/or disallow the hasty escape of either foraging ants or nonnestmate ants. The Petri dish baits were spaced 5 m apart on transects.
- A nonnestmate (NNM) treatment consisted of a labeled anti-static 96 mL polystyrene cup (Dart Container Corporation, Mason, MI) lined with Fluon® (Asahi Glass Ltd., Chadds Ford, PA) that contained a predetermined number of imported fire ants from a laboratory colony. For nonnestmate treatments of less than 100 workers (5, 25, and 50 NNM treatments), individual worker ants were removed from the collection tray using soft forceps (BioQuip® Products, Rancho Dominguez, CA) and placed into a labeled treatment cup lined with Fluon® until the desired number of individuals was reached. Treatments of greater than 100 individual worker ants were measured using the mass of 100 ants as determined by methods in Appendix 1. Treatment cups were covered with

cup lids until treatment application in the field. A “NNM treatment” was applied to either imported fire ants foraging at a bait card and/or a disturbed mound, as determined by the protocol, when the ants were poured out of the polystyrene cup and the bottom and sides of the cup was tapped to force any stragglers to fall from the cup.

- A crater about 20 x 20 x 20 cm dug into the mound using a small round point shovel (15 x 21 cm head size) created a “disturbance”. As per the protocol, a nonnestmate (NNM) treatment of ants may or may not be applied to the disturbed mound.
- An electrical “shock” treatment was administered to a “disturbed” mound using a modified electric cattle prod similar to one developed by Charles Barr, Texas A&M University (Barr and Calixto 2005). I used a 86.36 cm (34 inch) stock prod (Item # 3449ESP, Springer Magrath, Glencoe, MN) that delivers a 5,500 kV shock. A 5.08 cm (2 inch) length of 6.35 mm (1/4 inch) copper tubing was fitted over each contact probe. The open ends of the copper tubing were crimped closed to keep soil from filling the tubes. Two 1.59 mm (1/16 inch) holes were drilled 6.35 mm (1/4 inch) apart. A 5.08 cm (2 inch) paper clip was cut in half, threaded through the 1.59 mm (1/16 inch) holes, and soldered to each copper tube. The shafts of each half of the paper clips were offset to create an electrical arc and larger area for the ants to come into contact with the electrical charge (Figure 13).

Fire ants used for nonnestmate treatments were collected in Auburn, Alabama prior to the scheduled experiment day. Treatment ants were held no more than one day to prevent mortality, acclimation to the lab environment (i.e., air conditioning, fluorescent lighting, lower relative humidity, artificial habitat), and adaptation to captivity. One live mound that was visually determined to have >50,000 individuals was collected using a shovel and placed into a 52 x 40 x 13 cm collection tray lined with Fluon®. The colony was transported back to the lab in a climate-controlled vehicle where the ants were divided into the various nonnestmate treatment quantities. Ants were counted either individually or by mass and placed into labeled treatment cups lined with Fluon® and covered with a cup lid until application time.

Experiment 1. The first experiment was designed to test whether *P. curvatus* are more attracted to workers at a disturbance or to workers at a food source. It was hypothesized that flies will be more attracted to the workers at the disturbed mounds than to workers foraging at a food source.

One half of the experiment, designated Part 1, had 30 card baits placed 5 m apart on one transect during peak activity time that was determined in the first chapter of this thesis. After 20 minutes, baits were visually monitored for the presence of flies at 20-minute intervals for one hour.

For the other part of the experiment, Part 2, 30 mounds were disturbed by digging a 20 x 20 x 20 cm crater using a small shovel. After 10 minutes, mounds were visually monitored for fly presence at 10-minute intervals for 30 minutes.

The experiment was conducted on July 23, 2004 at Site B. Parts 1 and 2 were both conducted on the same day, but at different times of the day to prevent exposure of flies to two different stimuli. In 2005, Part 1 of the experiment was conducted on July 28 at Site B and Site C. Part 2 of the experiment was executed on July 20, 2005 at Sites B and C.

Individual year data were combined into one data set for analysis across years. Data were analyzed using generalized linear models as implemented in SAS® PROC GLIMMIX (SAS/STAT® Version 9.2, Copyright © 2009 SAS Institute Inc., Cary NC) with a negative binomial distribution function. The full interaction model was reduced to a model that contained only significant fixed effects: date, site, treatment, site* treatment interactions. The ILINK option within the LSMEANS statement was employed to back transform estimated means and 95% confidence intervals to the original scale.

Experiment 2. Experiment two was designed to test whether *P. curvatus* are more attracted to workers that are simply foraging at a food source or to workers that are competing at a food source. My hypothesis was that the *P. curvatus* would be more attracted to the workers competing at a food source than to workers foraging at a food source.

On July 26, 2004 at Site B, 30 Petri dish baits were placed 5 m apart on one transect. After one hour of recruitment time, every other Petri dish bait on the transect received a 200 nonnestmate (by mass) treatment. Baits were monitored for the presence of flies at five-minute intervals for 20 minutes. In 2005, at both Site B and Site C, 40 Petri dish baits were placed 5 m apart on two transects (20 baits per transect). After 30 minutes of recruitment time, baits that had less than 75 workers (determined by visual assessment) were identified and physically removed from the experiment. After one hour of recruitment time, I applied a 200 nonnestmate (by mass) treatment to alternating Petri dish baits on the transects, 32 at Site B and 40 at Site C. Baits were monitored for flies at five-minute intervals for 20 minutes. Experiments were conducted at Site B on July 27 and Site C on July 28.

Individual year data were combined into one data set for analysis across years. The data were analyzed using generalized linear models as implemented in SAS® PROC GLIMMIX (SAS/STAT® Version 9.2, Copyright © 2009 SAS Institute Inc., Cary NC) with a negative binomial distribution function. The full interaction model was reduced to a model that contained only the significant fixed effects: treatment and date*treatment interactions. The ILINK option within the LSMEANS statement was employed to back transform estimated means and 95% confidence interval to the original scale.

Experiment 3. Experiment 3 was designed to test whether or not increasing the foraging ants-nonnestmate interaction at a food source affects the number of *P. curvatus* attracted to the ants at that food source. My hypothesis was that increasing the number of nonnestmates at a food source would result in an increase in the number of *P. curvatus* attracted to the ants at that food source.

In 2004, a total of 30 Petri dish baits were placed 5 m apart on six transects. Treatments consisted of 5, 25, 50, 100, and 250 nonnestmate laboratory workers. Treatments were randomly assigned to six replicates (by pulling different colored marbles out of a hat) in a randomized complete block design. Presence of *Pseudacteon* *curvatus* was monitored at 5 minute intervals for 30 minutes, then at 10-minute intervals for an additional 30 minutes for a total monitoring time of one hour. The experiment was run on July 28, 2004.

In 2005, the design protocol was changed to a 5 x 5 Latin Square to eliminate effect (Steel and Torrie 1980). Changing the design from 6 long transects to a 5 x 5 Latin Square reduced variability by creating a more homogeneous sample population. Twenty five Petri dish baits were set 5 m apart on five transects. Four auxiliary card baits were placed 12 inches away from each central Petri dish bait to increase fire ant recruitment. After 20 minutes, Petri dish baits that had significant recruitment (>75 workers present, determined by visual assessment) were identified and all auxiliary card baits were collected and discarded. Five different treatments, assigned by pulling different colored marbles out of a hat, were administered. Treatments consisted of 5, 25, 50, 100, 250 nonnestmate laboratory workers added to the Petri dish baits. The Petri dish baits were monitored for *P. curvatus* presence at 5-minute intervals for 30 minutes, then at 10-minute intervals for an additional 30 minutes for a total monitoring time of one hour. The experiment was run at Site B on July 21, 2005 and Site C on July 19, 2005.

Because the data set contained many zeros at individual time intervals, indicating that no flies were observed at the particular interval, only the total number of flies collected over the entire 60-minute observation period was analyzed. Data were analyzed using generalized linear mixed models as implemented in SAS® PROC GLIMMIX (SAS/STAT® Version 9.2, Copyright © 2009 SAS Institute Inc., Cary NC) with a negative binomial distribution function. There were two different blocking structures:

simple blocks in 2004, and rows and column blocks in 2005; these entered the model as random effects. The full interaction model was reduced to a model that contained only significant fixed effects: site, treatment, site* treatment interaction. The ILINK option within the LSMEANS statement was employed to back transform estimated means and confidence intervals to the original scale. *P*-values were not adjusted for multiple comparisons because this experiment was preliminary in nature and more experimentation is necessary in the future (Milliken and Johnson 2004).

Experiment 4. Experiment four was designed to test whether *P. curvatus* were more attracted to workers at colony disturbances with nonnestmate interaction or to workers at colony disturbances without nonnestmate interaction and then whether the flies were differentially attracted to the interactions. My hypothesis was that *P. curvatus* would be more attracted to workers at colony disturbances with a nonnestmate interaction than to workers at colony disturbances that had no nonnestmate interaction. At Site B on July 26, 2004 and at Sites B and C on July 20, 2005, 15 mounds at least 5 m apart were disturbed by digging a crater ca. 20 x 15 x 20 cm with a small shovel and shaded. Treatments were assigned (by pulling different colored marbles out of a hat) and each mound received only one treatment. No additional treatment was added to the first set of five mounds. A 15 second shock was administered via the modified cattle prod to the next set of five mounds. One 300 nonnestmate by mass treatment was administered to the last set of five mounds. All 15 mounds were monitored for *P. curvatus* presence at 10-minute intervals for 30 minutes.

The data were analyzed using generalized linear mixed models as implemented in SAS® PROC GLIMMIX (SAS/STAT® Version 9.2, Copyright © 2009 SAS Institute Inc., Cary NC) with a negative binomial distribution function. These numbers were back transformed through ILINK function yielding confidence intervals. The full interaction model was reduced to a model that contained only significant fixed effects: date, site, treatment, site* treatment, treatment*date interaction. The ILINK option within the LSMEANS statement was employed to back transform estimated means and confidence intervals to the original scale. *P*-values were not adjusted for multiple comparisons because this experiment was preliminary in nature and more experimentation is necessary in the future (Milliken and Johnson 2004).

Experiment 5. Experiment five was designed to test whether the number of *P. curvatus* attracted to a colony was related to the size of that particular colony. In 2004, Morrison and King found that the total number of *Solenopsis* workers present in a colony was strongly correlated with mound size. My hypothesis was that *P. curvatus* would be more attracted to colonies that were large and had more workers in comparison to smaller mounds with less workers. Eight *S. invicta* mounds of similar size and shape located at least 5 m apart from each other were selected at both Site B and Site C on July 18, 2005. Measurements of area and volume for each mound were taken and recorded. The eight mounds were disturbed by digging a crater approximately 20 cm in diameter and 20 cm deep with a small shovel and shaded. After 10 minutes of recruitment time, a double-chambered aspirator unit was used to collect all *P. curvatus* individuals for 30 minutes.

Collected flies were counted and the sex determined. The specimens were added to the Auburn University Department of Entomology and Plant Pathology teaching collection.

Area and volume of the mounds were calculated using the following formulas: Two-dimensional area of the mounds were calculated using the formula for an ellipse [$A = \pi \cdot (a/2) \cdot (b/2)$, where a = length (long axis) and b = width (short axis)]. Volume of the mounds were calculated using the formula for half a spheroid [$V = 2/3 \cdot \pi \cdot (a/2) \cdot (b/2) \cdot c$, where c = mound height].

The data were combined over the sites for the analysis. A stepwise poisson regression was performed using SAS® PROC GENMOD (generalized modeling) (SAS/STAT® Version 9.2, Copyright © 2009 SAS Institute Inc., Cary NC). Original terms in the model were length, width, height, area, volume and various 2 and 3 way interactions. The model was reduced keeping only effects significant at $P < 0.1$; therefore the final model contained only width, height, and the interaction between the two.

Results

Experiment 1. At Site B in 2004, a higher number of *P. curvatus* were present at the colony disturbances, with 25 of 30 (83%) mounds having flies present over the total 30 minute monitoring period, than at the card baits with undisturbed foraging ants where only 10 of 30 (30%) card baits had flies present after one hour (Table 1). The ants foraging at card baits attracted a total of 34 flies over the one hour monitoring period, whereas the ants at the disturbed mounds attracted 443 flies over the 30 minute monitoring period (Table 2).

At Site B in 2005, *P. curvatus* were present at 19 of 30 (63%) colony disturbances. Flies were present at only 1 of 40 (2.5%) card baits where there were only foraging ants (Table 1). A total of five flies were counted at the single card bait where flies were present over the one hour monitoring period. The disturbed mounds attracted a total of 74 flies during the 30 minute monitoring period (Table 2).

Pseudacteon curvatus were present at 25 of 30 (83%) colony disturbances at Site C in 2005. Similar to Site B, flies were absent at 39 of 40 (98%) where there were only foraging ants (Table 1). During the one hour monitoring period, a total of three flies were counted at the single bait where flies were present. The colony disturbances attracted a total of 134 flies during the 30 minute monitoring period (Table 2).

Data at Site B and Site C were combined for comparison. The same comparison between card baits and colony disturbance was made for both sites. Significantly more flies were found at the disturbance at both Site B and Site C than were found at the card baits with ants foraging at either site ($P < 0.0001$) (Figure 15).

Experiment 2. At Site B in 2004 and at both Sites in 2005, there were numerically more flies found at the mounds that received nonnestmate treatments than at mounds with no nonnestmate interactions (Table 3). Individual year data were combined into one data set for analysis across years. The full interaction model was reduced to a model that contained only the significant fixed effects: treatment and date*treatment interactions (Figure 16).

Data for the three sites were combined across 2004 and 2005 because there were no significant differences in the site*treatment interactions. However, there was a date*treatment interaction in both 2004 and 2005, therefore, each year was analyzed separately (Figure 16). Although there was a treatment interaction between years the relationship remained the same because the nonnestmate interaction attracted significantly more phorid flies than did the treatment with no nonnestmate interaction. Since there were no statistical differences, data were combined and in the end, the number of phorids found at the nonnestmate disturbance were significantly higher than the number of phorids found at the treatment with no disturbance (Figure 17).

Experiment 3. There was a significant difference observed at the 5, 25, 50, 100, and 250 nonnestmate treatment levels at Site B in 2004 ($P < 0.0318$). The number of flies attracted to the 5 nonnestmate treatment was significantly different from the number of flies attracted to the 25 ($P = 0.032$), 50 ($P = 0.007$), and 250 ($P = 0.013$) nonnestmate treatment, however, it was not significantly different from the number of flies attracted to the 100 nonnestmate treatment ($P = 0.165$). The number of flies attracted to the 25 nonnestmate treatment was not significantly different from any of the other treatments except for the 5 nonnestmate treatment. The number of flies attracted to the 50 nonnestmate treatment was significantly greater than the number of flies attracted to the 100 nonnestmate treatment ($P = 0.029$) (Figure 18).

At Site B in 2005, there was a significant effect of treatment level ($P = 0.004$) on the number of flies attracted to workers engaged in an aggressive interaction with nonnestmates at baits. There was no significant difference between the number of flies attracted to ants at the 5 nonnestmate treatment and the 50 nonnestmate treatment ($P = 0.314$). There was no significant difference between the number of flies attracted to ants at Petri dish baits treated with the 25, 50, or 100 nonnestmate treatments ($P = 0.223$, $P = 0.866$, and $P = 0.063$, respectively). The number of flies attracted to the workers at Petri dish baits with 250 nonnestmate treatments added was significantly different from the number of flies attracted to workers at Petri dish baits with the 50 nonnestmate treatments ($P = 0.004$) (Figure 19).

At Site C in 2005, the number of flies attracted to the workers at the Petri dish baits with 100 nonnestmate treatments was significantly different from all treatments (5 nonnestmate treatment: $P = 0.002$, 25 nonnestmate treatment: $P = 0.040$, 50 nonnestmate treatment: $P = 0.037$, and 250 nonnestmate treatment: $P = 0.010$) (Figure 20). The number of flies attracted to workers at Petri dish baits treated with the 5 nonnestmate treatments was significantly different from the number of flies attracted to workers at Petri dish baits treated with 25, 50, and 100 nonnestmate treatments ($P = 0.041$, $P = 0.033$, and $P = 0.002$, respectively) (Figure 20).

Experiment 4. Data were analyzed for all observation times (10, 20, 30 minutes, and total time) and for all years. The number of flies collected at the 10, 20, and 30 minute sample times yielded similar results (Table 4). However, over the total 30 minute collection time, significant differences were observed. Over the entire 30-minute observation period, the number of *P. curvatus* flies was significantly more abundant at colonies that had the additional shock treatment (89 flies) than colonies that simply had the initial mound disturbance (24 flies) ($P < 0.0001$) (Table 4). Similarly, there were significantly more flies present at colonies that had the additional 300 nonnestmate treatment (101 flies) than colonies with only the initial mound disturbance (24 flies) ($P < 0.0001$). There was no significant difference between the number of flies present at mounds that had the 300 nonnestmate treatment and the number of flies present at mounds that had the 15 second shock ($P = 0.731$).

Experiment 5. The effect of mound width on fly numbers was significant at $P = 0.04$ and the effect of mound height on fly numbers was significant at $P = 0.02$ (Figure 21). However, there was no significant ($P = 0.06$) interaction between mound width and mound height on number of *P. curvatus*. In other words, there were a significant number of *Pseudacteon curvatus* flies present when the mounds were either wide or tall. However, when the mounds were both wide and tall, there was not a significant difference in the number of *P. curvatus* flies observed (Figure 21).

The highest number of flies observed at a single mound was 53 at Site B. The height of the associated mound was 15.24 inches and the width was 30.48 inches (Appendix 6).

Discussion

Five experiments were conducted in the summer months of 2004 and 2005 to measure the number of *Pseudacteon curvatus* attracted to fire ant workers engaged in different activities such as foraging, dealing with a mound disturbance of some sort, and nonnestmate aggression. In both my laboratory and field experiments, *Pseudacteon* *curvatus* flies were found to be “strongly attracted” to imported fire ant workers engaged in activities that warrant the release of alarm pheromone and/or other defensive compounds such as a reaction to an electrical shock, the addition of nonnestmates, and/or a mound disturbance. This result is consistent with data collected by Orr et al. 1997, Morrison and King 2004, Chen et al. 2009, and Vander Meer and Porter, unpublished data. Morrison and King’s 2004 field results suggest that while *P. tricuspis* was strongly attracted to alarm pheromone, the flies were not strongly attracted to recruitment pheromones or chemical signals associated with foraging activities. Field results from this body of work conducted with *Pseudacteon curvatus* in Alabama were similar to Morrison and King’s (2004) field results with *P. tricuspis* in Florida.

I found relatively few *P. curvatus* present at the bait stations where fire ant workers were simply foraging (a total of 34 flies at Site B in 2004, 5 flies at Site B in 2005, and 3 flies at Site C in 2005). In Florida, Morrison and King (2004) never observed *P. tricuspis* to be attracted to fire ant workers foraging between bait stations and foraging tunnel entrances.

Morrison and King (2004) observed *P. tricuspis* to appear at the majority of baits where nonnestmate workers were introduced to foraging workers resulting in aggressive interactions. After nonnestmates were introduced to workers foraging at bait stations, *P. curvatus* were observed to be strongly attracted to the ants at the bait stations where there was nonnestmate aggression. In this study, like that of Morrison and King (2004), I observed few flies at bait stations where there were no nonnestmate interactions. While phorids were attracted to workers engaged in nonnestmate disturbances, attraction was not necessarily enhanced by increasing the number of nonnestmates. Based on these results, I could hypothesize that a saturation threshold of pheromone is met where no more phorids will be attracted despite the amount of pheromone that is in the air. Perhaps the idea of pheromone saturation thresholds can be explored in more detail in the future.

Orr et al. (1997) reported "that mound disturbances, either through the strength or type of cue present, are considerably more attractive than ant foraging trails to *P. litoralis*, *P. tricuspis*, and *P. wasmanni*." Morrison and King (2004) found that *P. tricuspis* were more attracted to imported fire ant workers affected by a mound disturbance with or without a nonnestmate interaction than to workers engaged simply in foraging activities. Similarly, significantly more *P. curvatus* flies were found at colony disturbances (whether it was a simple mound disturbance or a mound disturbance plus a NNM treatment) than at bait stations where workers were foraging undisturbed at a food source at all three sites in Alabama over all years of the study.

In Alabama, three treatments were applied to fire ant workers in mounds to further test the attractiveness of the workers to *P. curvatus*: a mound disturbance alone, a mound disturbance plus a 15 second electrical shock, and a mound disturbance plus a 300 nonnestmate by weight application. When the treatments were compared, *P. curvatus* were least attracted to workers at a mound disturbance alone. More flies were attracted to a colony disturbance where a shock had been applied to the fire ant workers as opposed to a disturbance where there was a conspecific interaction, however, the difference was not significant between these two treatments. Results from this study suggest that *P.*

curvatus were differentially attracted to colony disturbances with and without interactions, but the number of flies attracted may not be significantly different.

The relationship between the number of phorids attracted to fire ant workers at mounds of a certain size yielded mixed results. As expected, statistically, there were significantly more flies present at mounds when the mounds were either wide or when the mounds were tall. The effects of height or width could possibly be due to an increased surface area on which the ants can spread out. However, when the mounds were both wide and tall, it is unclear why a significant number of flies was not observed. Again, I hypothesize that there is a saturation threshold of pheromone where no more phorids will be attracted despite the amount of pheromone in the air.

Understanding the host location behavior of established *Pseudacteon* flies facilitates efficiency in monitoring and survey not only of the established species but for that of future releases and new species. Understanding *Pseudacteon* behavior creates the best possible chance of establishment and distribution. By using these data, we are taking advantage of biology to optimize *Pseudacteon* attack and oviposition behaviors. My results establish baseline methods for determining diurnal patterns and survey methods for future studies.

Table 1. Number of occasions in which *Pseudacteon curvatus* flies were attracted or not attracted to host *Solenopsis invicta* ants at card baits or colony disturbances at Sites B and C, Talladega County, Alabama, 2004 and 2005.

	Baits		Colony disturbances	
Site	Flies present	Flies absent	Flies present	Flies absent
Site B (2004)	10	20	25	5
Site B (2005)	1	39	19	11
Site C (2005)	1	39	25	5

Table 2. Number of *Pseudaceton curvatus* flies attracted to host *Solenopsis invicta* ants at card baits or colony disturbances at Sites B and C at given sample times, Talladega County, 2004 and 2005.

	Baits			Colony disturbances			Baits	Colony disturbances
	Observation time in minutes			Observation time in minutes			Observation time in minutes	
Site	20	40	60	10	20	30	Total	Total
Site B (2004)	2	21	11	124	163	156	34	443
Site B (2005)	0	0	5	21	27	26	5	74
Site C (2005)	0	3	0	31	44	59	3	134

Table 3. Number of *Pseudacteon curvatus* flies attracted to host *Solenopsis invicta* ants at Petri dish baits in the presence or absence of nonnestmate (NNM) treatments at Sites B and C, Talladega County, 2004 and 2005.

	NNM interaction	No NNM interaction
Site	Total number of flies present	
Site B (2004)	75	2
Site B (2005)	26	8
Site C (2005)	85	11

Table 4. Number of flies observed at mounds with only a disturbance, a disturbance plus a nonnestmate (NNM) treatment, and a disturbance plus a 15 second shock treatment at 10 minute, 20 minute, 30 minute after treatment application at Sites B and C, Talladega County, Alabama, 2004 and 2005.

	Observation Time in Minutes			
	10	20	30	Total Time
Treatment	Number of Flies			
300 NNM Treatment	25	35	34	101
15 Second Shock	9	32	30	89
Disturbance	7	9	8	24

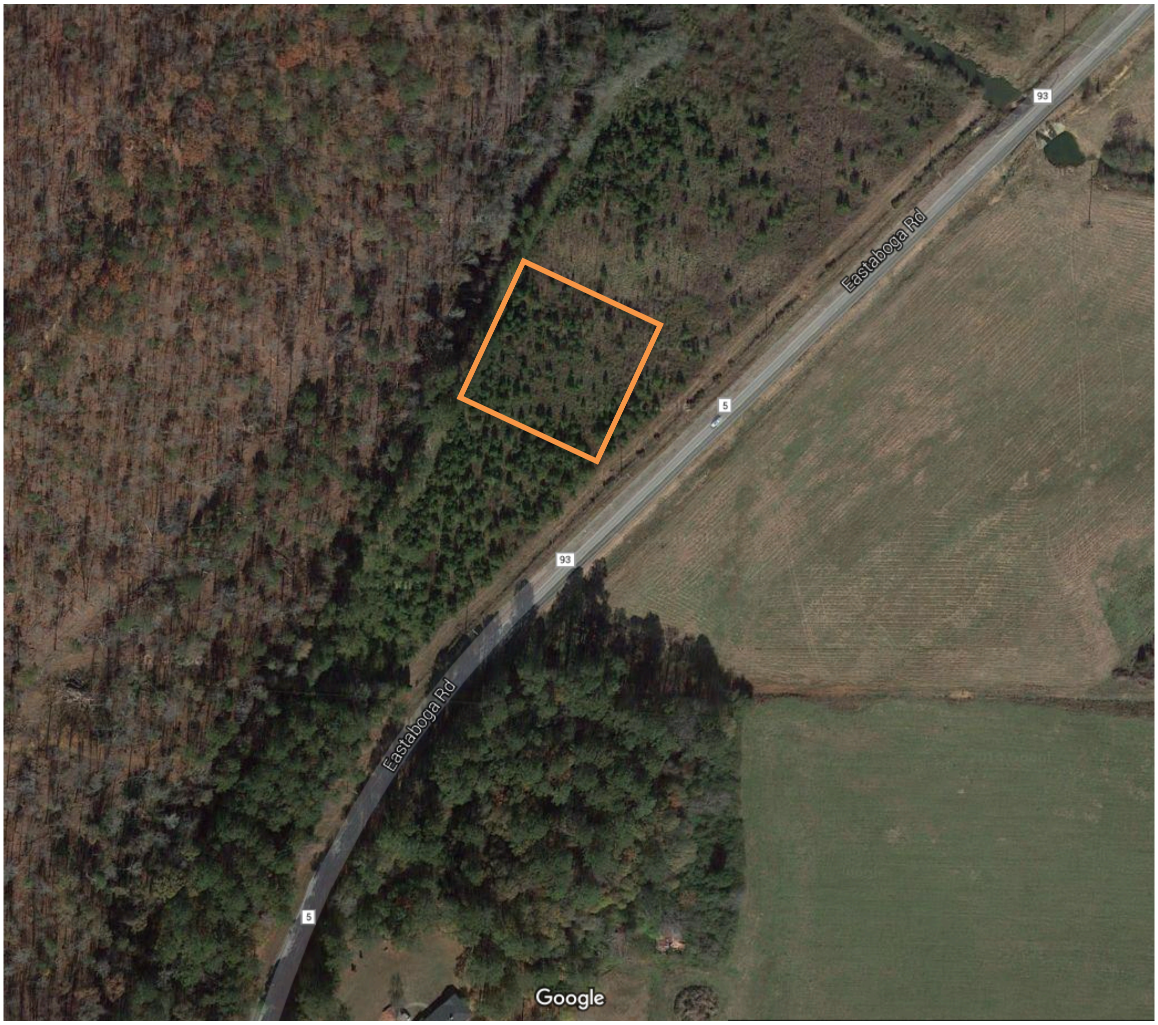

Figure 11. Aerial photo of Site B. *Pseudacteon curvatus* was released in 2000 on Greg Myrick's property (33°28'51.28"N, 86° 2'45.40"W), in Talladega County, Alabama. The orange square highlights the *P. curvatus* release site where this study was conducted in 2004 and 2005.

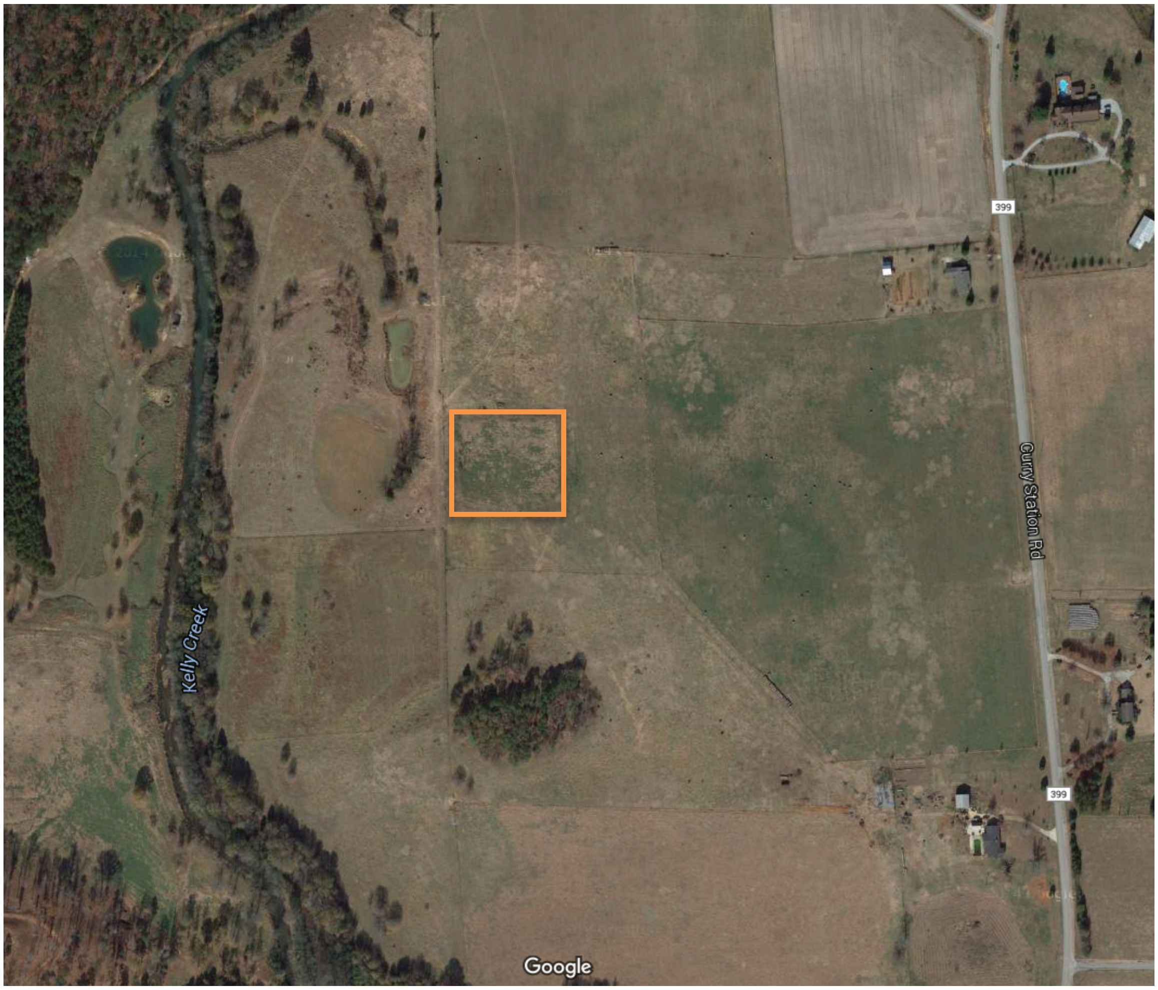

Figure 12. Aerial photo of Site C, Greg Street's property (33°28'51.28"N, 86° 2'45.40"W), in Talladega County, Alabama. The orange square highlights where this study was conducted in 2005.

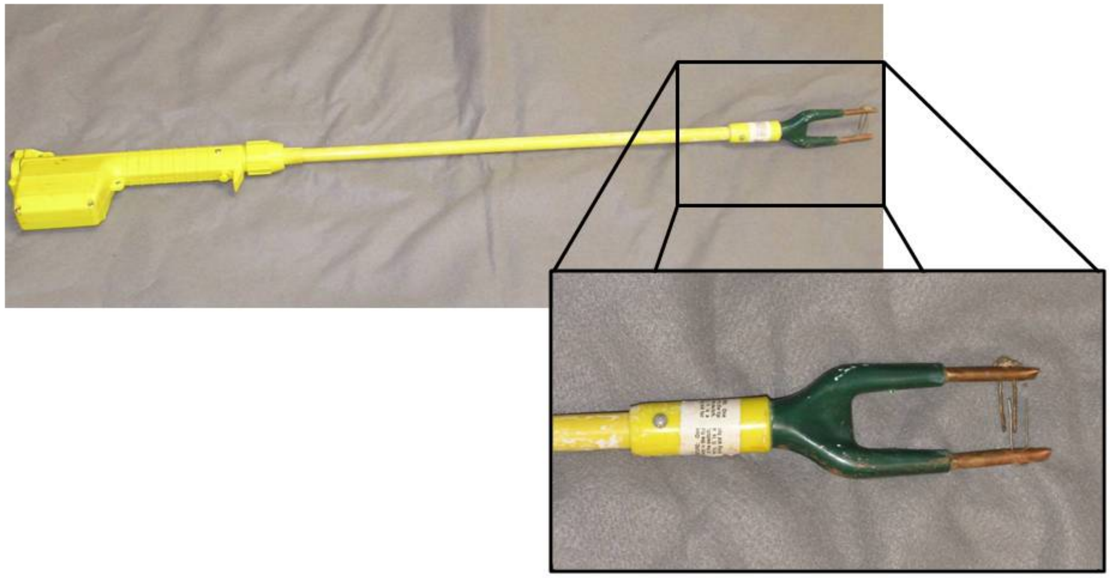

Figure 13. Modified cattle pod used to deliver the electrical "shock" disturbance to imported fire ants. A 86.36 cm (34 inch) stock prod (Item # 3449ESP, Springer Magrath, Glencoe, MN) that delivers a 5,500 kV shock. A 5.08 cm (2 inch) length of 6.35 mm (1/4 inch) copper tubing was fitted over each contact probe. The open ends of the copper tubing were crimped closed to keep soil from filling the tubes. Two 1.59 mm (1/16 inch) holes were drilled 6.35 mm (1/4 inch) apart. A 5.08 cm (2 inch) paper clip was cut in half, threaded through the 1.59 mm (1/16 inch) holes, and soldered to each copper tube. The shafts of each half of the paper clips were offset to create an electrical arc and larger area for the ants to come into contact with the electrical charge.

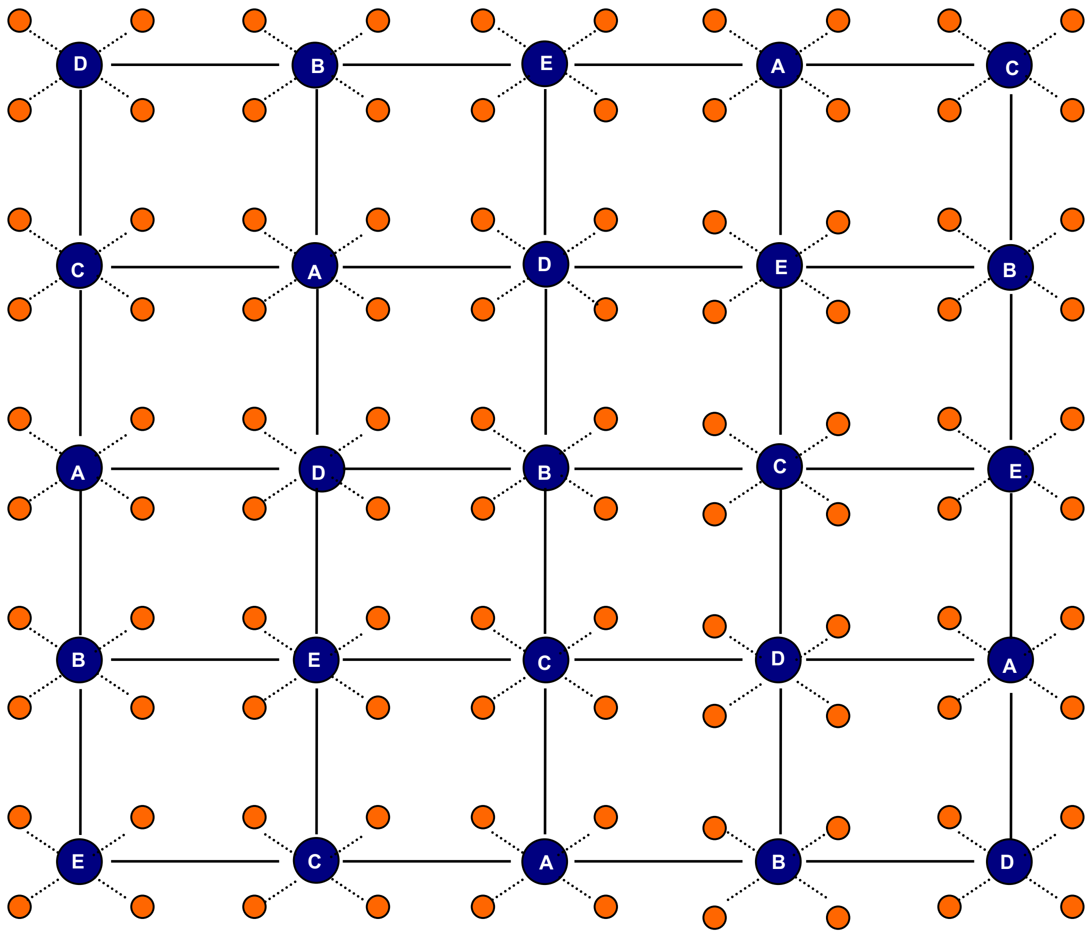

Figure 14. The 2005 design layout was a 5 x 5 Latin Square. Twenty-five Petri dish baits (blue circles) were set 5 m (solid line) apart on 5 transects. Auxiliary card baits (orange circles) were placed 30.48 cm (12 inches) (dotted line) away from the Petri dish bait to increase fire ant recruitment to the Petri dish bait.

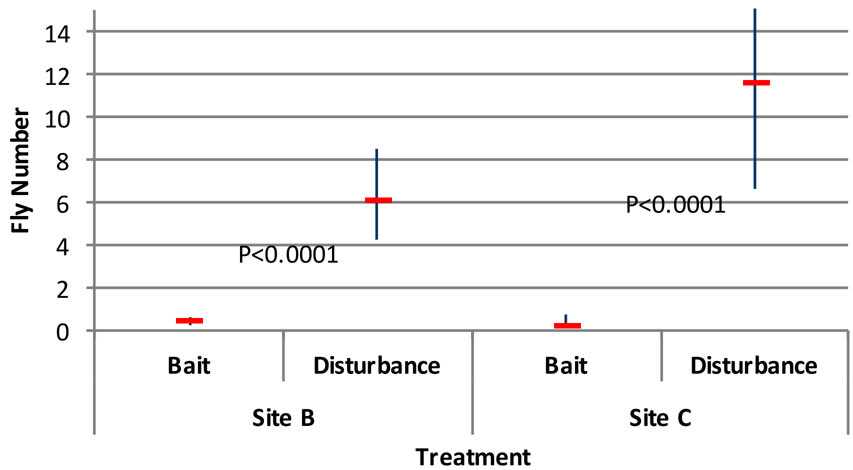

Figure 15. Confidence level comparison for number of *Pseudacteon curvatus* flies present at card bait versus colony disturbance at Sites B and C, Talladega County, Alabama, 2004 and 2005.

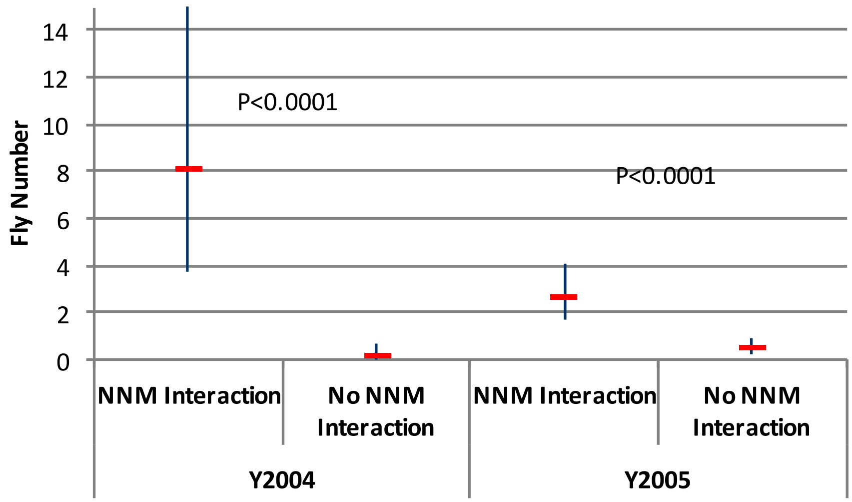

Figure 16. Confidence level comparison of the number of *Pseudacteon curvatus* flies attracted to host *Solenopsis invicta* ants at Petri dish baits with nonnestmate (NNM) interaction versus *Solenopsis invicta* ants at Petri dish baits with no NNM interaction by year at Sites B and C, Talladega County, Alabama, 2004 and 2005.

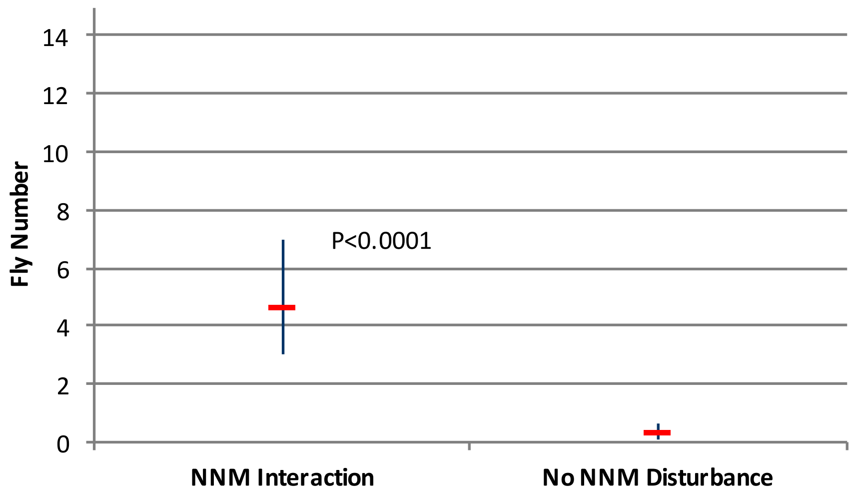

Figure 17. Confidence level comparison of the number of *Pseudacteon curvatus* flies attracted to host *Solenopsis invicta* ants at Petri dish baits with nonnestmate (NNM) interaction versus *Solenopsis invicta* ants at Petri dish baits with no NNM interaction across all years at Sites B and C, Talladega County, Alabama, 2004 and 2005.

Mean Number *Pseudacteon curvatus* at Site B (2004)

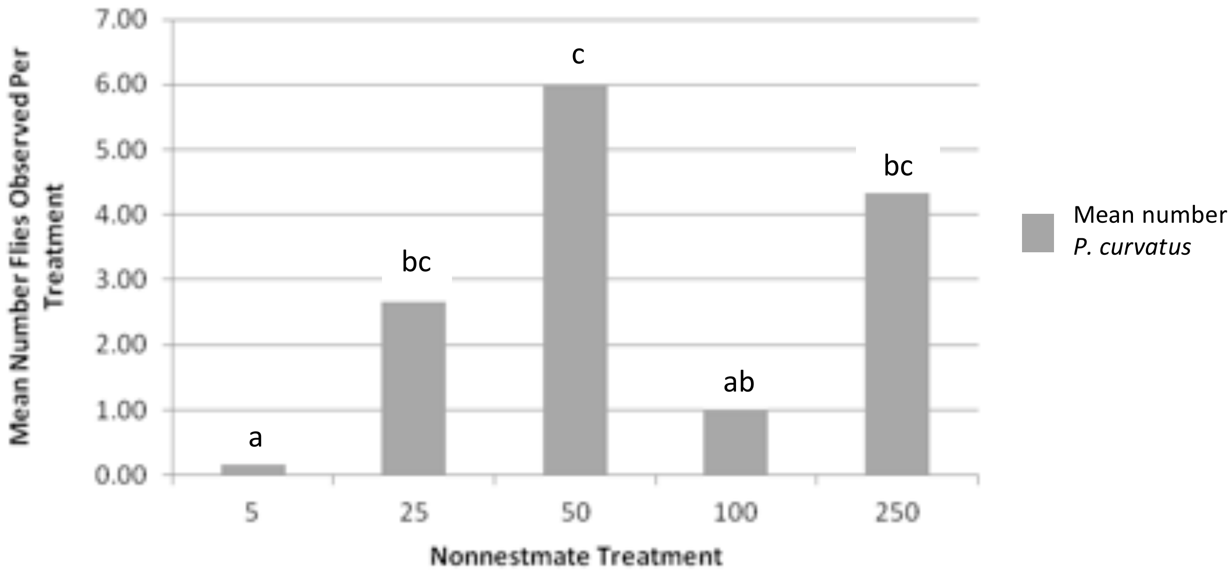

Figure 18. Mean number of *Pseudacteon curvatus* flies attracted to host *Solenopsis invicta* ants at Petri dish baits treated with 5, 25, 50, 100, or 250 nonnestmate (NNM) treatments at Site B, Talladega County, Alabama, 2004.

Mean Number *Pseudacteon curvatus* at Site B (2005)

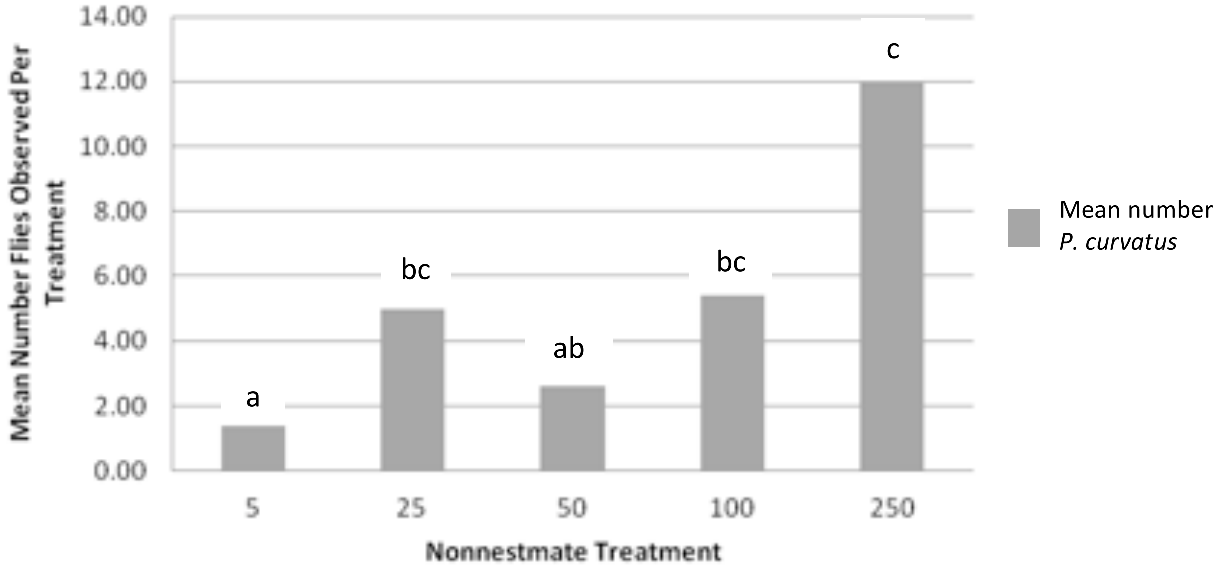

Figure 19. Mean number of *Pseudacteon curvatus* flies attracted to host *Solenopsis invicta* ants at Petri dish baits treated with 5, 25, 50, 100, or 250 nonnestmate (NNM) treatments at Site B, Talladega County, Alabama, 2005.

Mean Number *Pseudacteon curvatus* at Site C (2005)

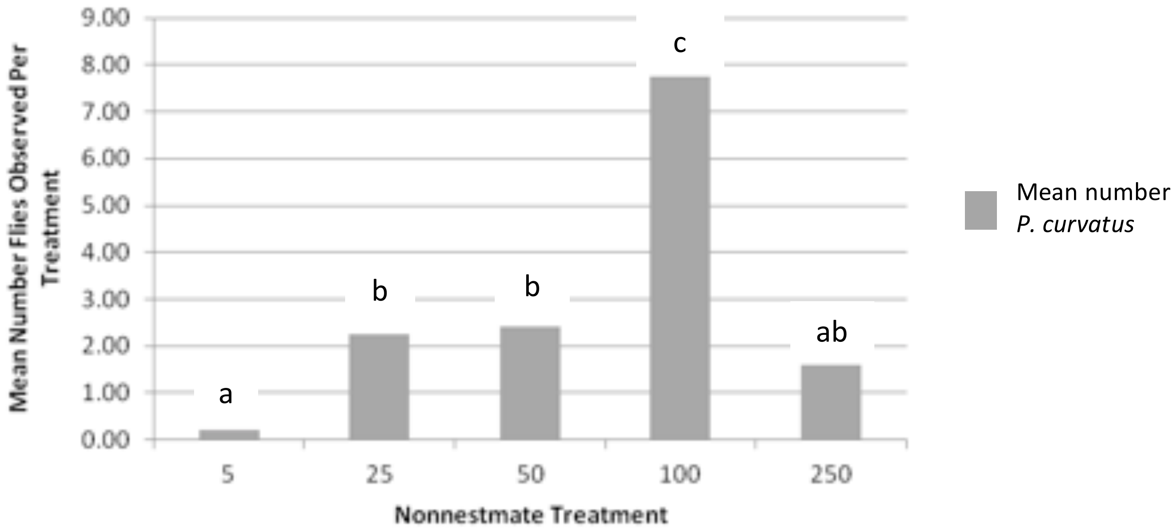

Figure 20. Mean number of *Pseudacteon curvatus* flies attracted to host *Solenopsis invicta* ants at Petri dish baits treated with 5, 25, 50, 100, or 250 nonnestmate (NNM) treatments at Site C, Talladega County, Alabama, 2005.

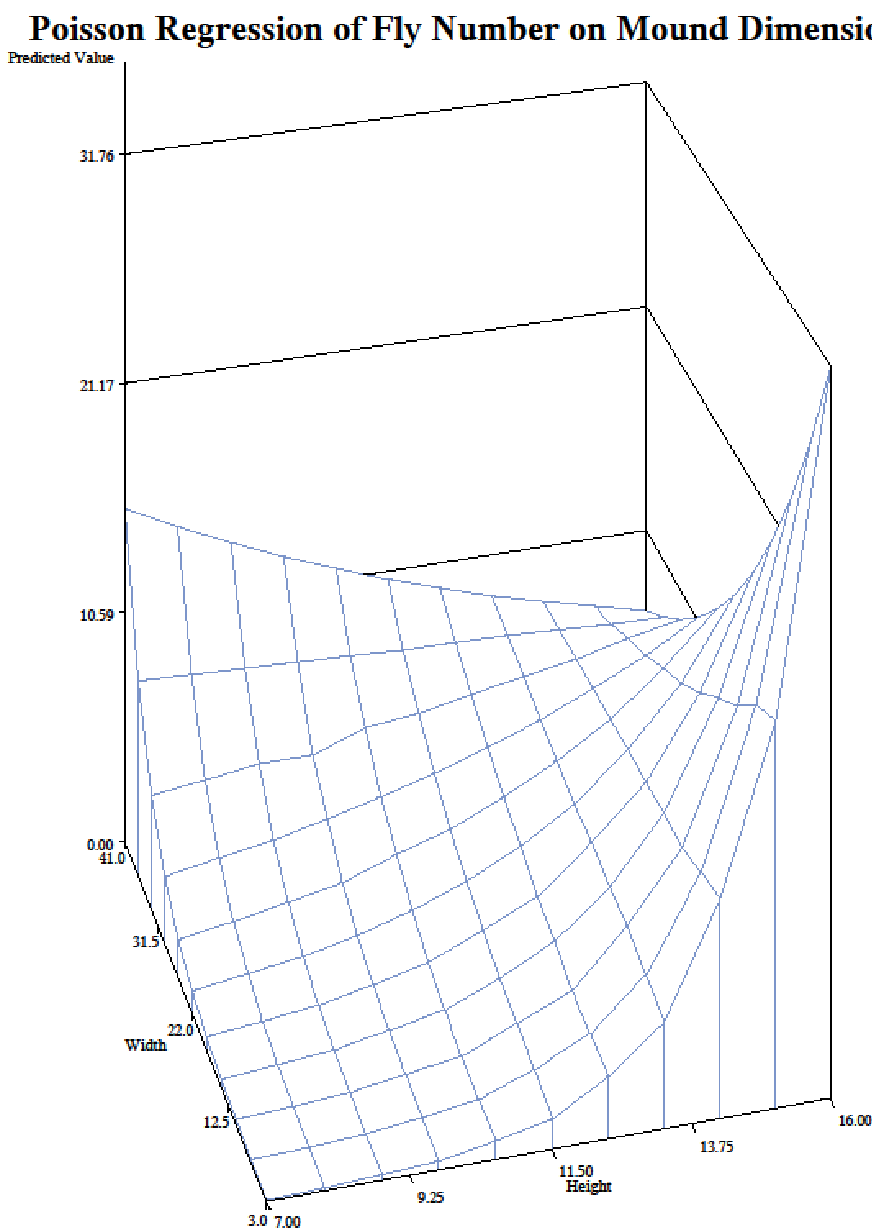

Figure 21. Poisson regression of number of *Pseudacteon curvatus* flies observed at mounds where length, width, and height of each mound were measured, Sites B and C, Talladega County, 2005.

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Appendix

Appendix 1. Fire ants used for mass determination were collected from Auburn, Alabama on July 22, 2004. One large live mound was collected using a shovel and placed into a 52 x 40 x 13 cm tray lined with Fluon®. The colony was transported back to the lab where the ants were separated from the soil using the previously described water drip technique. A Denver Instrument Company XD-1200D electronic balance was used to weigh the ants. An empty 7.62 x 1.905 cm anti-static polystyrene weighing dish lined with Fluon® was placed on the weighing plate and the balance tared. An individual worker ant was removed from the tray using soft forceps and placed into the weighing dish. Ant selection continued until 100 individual ants were in the weighing dish. The mass of the 100 worker ants was recorded. After the mass was recorded, the worker ants were dumped back into the colony tray. The empty weighing dish was returned to the balance and the balance was tared. The process of selecting and weighing worker ants was repeated 16 times. The mass of 100 ants was determined by taking the average mass of the 16 previously recorded individual batch masses. This number was determined to be 0.107 g. From this point forward, 0.107 g/100 worker ants was used to prepare the larger (100, 200, 250, and 300) nonnestmate (NNM) treatments. The following masses were used: 100 NNM = 0.107 g; 200 NNM = 0.213 g; 250 NNM = 0.266 g; 300 NNM = 0.320 g.

Mass of 100 ants
07/22/04
Scale: XD-1200D by
Denver Instrument Co.

sample	**mass (g)**	
1	0.069	
2	0.080	
3	0.100	
4	0.129	
5	0.119	
6	0.098	
7	0.094	
8	0.103	
9	0.088	
10	0.174	
11	0.086	
12	0.098	
13	0.096	
14	0.118	
15	0.101	
16	0.151	
Total	1.704	g
Mean	0.107	g
100	0.107	g
200	0.213	g
250	0.266	g
300	0.3195	g

Appendix 2. Experiment 1, Part 1: Number *Pseudacteon curvatus* flies attracted to host *Solenopsis invicta* ants at card baits at 20, 40, and 60 minute sample times. Experiment 1, Part 2: Number of Pseudacteon curvatus flies attracted to host Solenopsis invicta ants at colony disturbances at 10, 20, and 30 minute sample times. Sites B and C, Talladega County, Alabama, 2004 and 2005 (raw data)

Site B 23 July 2004

Experiment 1 Part 1

	20 min	40 min	60 min	TOTAL
1	0	0	0	0
2	0	0	0	0
3	0	1	0	1
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	1	0	0	1
8	0	3	2	5
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	2	2
15	0	0	0	0
16	0	0	0	0
17	0	0	0	0
18	0	7	1	8
19	0	0	0	0
20	0	2	3	5

Experiment 1 Part 2

	10 min	20 min	30 min	TOTAL
1	1	3	2	6
2	1	4	3	8
3	3	6	2	11
4	7	7	6	20
5	1	0	0	1
6	7	2	4	13
7	3	3	2	8
8	0	0	0	0
9	8	15	20	43
10	2	2	1	5
11	4	8	7	19
12	11	15	15	41
13	7	12	15	34
14	3	2	3	8
15	0	0	0	0
16	0	0	0	0
17	0	0	0	0
18	12	20	20	52
19	12	20	12	44
20	1	1	0	2

Experiment 1 Part 1

	20 min	40 min	60 min	TOTAL
21	0	2	0	2
22	0	0	0	0
23	0	0	0	0
24	0	0	0	0
25	0	1	0	1
26	1	4	2	7
27	0	0	0	0
28	0	1	1	2
29	0	0	0	0
30	0	0	0	0
TOTAL	2	21	11	34

Experiment 1 Part 2

	10 min	20 min	30 min	TOTAL
21	3	2	1	6
22	2	1	2	5
23	1	1	0	2
24	0	4	2	6
25	2	5	7	14
26	1	1	0	2
27	6	4	7	17
28	6	5	5	16
29	0	0	0	0
30	20	20	20	60
TOTAL	124	163	156	443

Site B 29 July 2005
Experiment 1 Part 1

	20 min	40 min	60 min	TOTAL
1	0	0	0	0
2	0	0	5	5
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0
21	0	0	0	0
22	0	0	0	0
23	0	0	0	0
24	0	0	0	0
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0

Site B 20 July 2005
Experiment 1 Part 2

	10 min	20 min	30 min	TOTAL
1	0	0	0	0
2	1	0	0	1
3	0	0	0	0
4	0	0	0	0
5	0	1	2	3
6	0	2	1	3
7	0	0	0	0
8	0	0	0	0
9	1	1	1	3
10	0	1	1	2
11	2	1	0	3
12	1	1	0	2
13	0	0	0	0
14	0	0	1	1
15	1	1	1	3
16	1	2	1	4
17	1	0	1	2
18	0	1	2	3
19	1	1	1	3
20	3	2	2	7
21	1	3	8	12
22	2	3	1	6
23	4	2	1	7
24	1	3	1	5
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	1	2	1	4
29	0	0	0	0

Experiment 1 Part 1

	20 min	40 min	60 min	TOTAL
30	0	0	0	0
31	0	0	0	0
32	0	0	0	0
33	0	0	0	0
34	0	0	0	0
35	0	0	0	0
36	0	0	0	0
37	0	0	0	0
38	0	0	0	0
39	0	0	0	0
40	0	0	0	0
TOTAL	0	0	5	5

Experiment 1 Part 2

	10 min	20 min	30 min	TOTAL
30	0	0	0	0
TOTAL	21	27	26	74

Site C 29 July 2005
Experiment 1 Part 1

	20 min	40 min	60 min	TOTAL
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0
21	0	3	0	3
22	0	0	0	0
23	0	0	0	0
24	0	0	0	0
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0

Site C 20 July 2005
Experiment 1 Part 2

	10 min	20 min	30 min	TOTAL
1	1	0	1	2
2	0	2	3	5
3	2	3	1	6
4	2	1	2	5
5	0	2	2	4
6	0	0	0	0
7	0	0	0	0
8	1	3	2	6
9	3	4	6	13
10	1	2	2	5
11	1	7	4	12
12	1	2	4	7
13	0	0	0	0
14	0	0	0	0
15	0	0	1	1
16	2	0	2	4
17	1	2	0	3
18	0	0	1	1
19	1	1	1	3
20	1	0	2	3
21	3	2	2	7
22	2	1	2	5
23	4	7	10	21
24	1	3	2	6
25	1	0	0	1
26	2	0	2	4
27	0	1	2	3
28	0	0	0	0

Experiment 1 Part 1

	20 min	40 min	60 min	TOTAL
29	0	0	0	0
30	0	0	0	0
31	0	0	0	0
32	0	0	0	0
33	0	0	0	0
34	0	0	0	0
35	0	0	0	0
36	0	0	0	0
37	0	0	0	0
38	0	0	0	0
39	0	0	0	0
40	0	0	0	0
TOTAL	0	3	0	3

Experiment 1 Part 2

	10 min	20 min	30 min	TOTAL
29	0	0	3	3
30	1	1	2	4
TOTAL	31	44	59	134

Appendix 3. Experiment 2: Number of *Pseudacteon curvatus* present at Petri dish bait at 5, 10, 15, 20 minutes after 200 nonnestmate (NNM) treatment, Sites B and C, Talladega County, 2004-2005 (raw data)

	NNM Interaction				No NNM Interaction			
	Observation Time in Minutes				Observation Time in Minutes			
	5	10	15	20	5	10	15	20
Site	Number of Flies Present				Number of Flies Present			
Site B (2004)	5	14	24	32	0	0	2	0
Site B (2005)	3	5	9	10	0	4	3	0
Site C (2005)	5	15	34	31	0	3	3	5

Appendix 4. Experiment 3: Number of *Pseudacteon curvatus* collected when nonnestmate (NNM) treatments (TRT) consisting of 5, 25, 50, 100, and 250 NNM laboratory workers are added to ants foraging at Petri dish baits at 5, 10, 15, 20, 25, 30, 40, 50, and 60 minutes after NNM treatment, Sites B and C, Talladega County, 2005 (raw data)

Date	Site	COL	ROW	BAIT	TRT	FIVE	TEN	FIFTEEN	TWENTY	TWENTY-FIVE	THIRTY	FORTY	FIFTY	SIXTY	TOTAL
2005	B	1	1	1	100	0	0	0	0	0	0	1	2	0	3
2005	B	1	2	2	25	0	2	2	1	1	1	1	0	0	8
2005	B	1	3	3	250	0	1	1	0	1	0	1	0	0	4
2005	B	1	4	4	5	0	0	0	2	1	0	0	0	1	4
2005	B	1	5	5	50	0	0	0	0	1	1	0	0	0	2
2005	B	2	1	6	50	0	0	0	1	1	1	1	0	1	5
2005	B	2	2	7	5	0	0	0	1	1	0	0	1	0	3
2005	B	2	3	8	100	0	0	0	0	1	1	2	0	0	4
2005	B	2	4	9	250	1	2	3	2	2	2	4	2	1	19
2005	B	2	5	10	25	0	0	0	0	1	1	0	0	0	2
2005	B	3	1	11	5	0	0	0	0	0	0	0	0	0	0
2005	B	3	2	12	100	0	1	1	1	1	1	1	1	0	7
2005	B	3	3	13	25	0	0	0	1	0	0	0	0	0	1
2005	B	3	4	14	50	0	1	0	1	0	0	0	0	0	2
2005	B	3	5	15	250	0	0	0	1	1	1	1	0	1	5
2005	B	4	1	16	25	0	1	1	1	1	0	1	1	1	7
2005	B	4	2	17	250	0	1	1	0	0	0	1	0	1	4
2005	B	4	3	18	50	0	0	0	0	0	0	0	1	1	2
2005	B	4	4	19	100	0	1	1	3	1	0	1	2	1	10
2005	B	4	5	20	5	0	0	0	0	0	0	0	0	0	0
2005	B	5	1	21	250	0	3	5	4	3	3	4	4	2	28
2005	B	5	2	22	50	0	0	0	1	1	0	0	0	0	2
2005	B	5	3	23	5	0	0	0	0	0	0	0	0	0	0
2005	B	5	4	24	25	0	2	2	2	1	0	0	0	0	7
2005	B	5	5	25	100	1	0	1	1	0	0	0	0	0	3

Date	Site	COL	ROW	BAIT	TRT	FIVE	TEN	FIFTEEN	TWENTY	TWENTY-FIVE	THIRTY	FORTY	FIFTY	SIXTY	TOTAL
2005	C	1	1	1	5	.	.	.	.	.	.	.	.	.	.
2005	C	1	2	2	25	0	0	0	0	1	0	1	0	0	2
2005	C	1	3	3	250	0	0	0	0	0	0	0	0	0	0
2005	C	1	4	4	5	0	0	0	0	0	0	0	0	0	0
2005	C	1	5	5	50	0	1	1	0	0	0	0	1	0	3
2005	C	2	1	6	50	0	0	0	0	0	0	0	0	0	0
2005	C	2	2	7	5	0	0	0	0	0	0	0	0	0	0
2005	C	2	3	8	100	0	0	0	0	0	0	1	1	0	2
2005	C	2	4	9	250	0	0	0	0	0	0	0	0	0	0
2005	C	2	5	10	25	0	0	0	0	0	0	0	1	1	2
2005	C	3	1	11	5	0	0	0	0	0	0	0	0	0	0
2005	C	3	2	12	100	0	1	0	0	0	0	1	1	1	4
2005	C	3	3	13	25	.	.	.	.	.	.	.	.	.	.
2005	C	3	4	14	50	0	1	1	1	0	0	0	0	1	4
2005	C	3	5	15	250	0	0	0	0	0	0	0	0	1	1
2005	C	4	1	16	25	0	1	0	1	1	1	0	1	0	5
2005	C	4	2	17	250	0	0	1	0	0	2	0	1	2	6
2005	C	4	3	18	50	0	0	0	0	0	0	1	3	1	5
2005	C	4	4	19	100	0	0	1	1	2	1	2	4	3	14
2005	C	4	5	20	5	0	1	0	0	0	0	0	0	0	1
2005	C	5	1	21	250	0	0	0	0	0	0	0	0	1	1
2005	C	5	2	22	50	0	0	0	0	0	0	0	0	0	0
2005	C	5	3	23	5	0	0	0	0	0	0	0	0	0	0
2005	C	5	4	24	25	0	0	0	0	0	0	0	0	0	0
2005	C	5	5	25	100	0	1	1	0	0	1	4	2	2	11

Appendix 5. **Experiment 4: Number of *Pseudacteon curvatus* collected when treatments (TRT) of 300 nonnestmate laboratory workers (ANTS), a 15 second shock (SHOCK), or a mound disturbance (DISTURBANCE) at 10, 20, 30 minutes after treatment, Sites B and C, Talladega County, 2004-2005 (raw data)**

Date	Site	MOUND	TRT	TEN	TWENTY	THIRTY	TOTAL
2004	B	1	ANTS	2	4	3	9
2004	B	2	ANTS	2	2	2	6
2004	B	3	ANTS	0	3	1	4
2004	B	4	ANTS	1	0	1	0
2004	B	5	ANTS	0	0	0	9
2004	B	1	SHOCK	1	4	1	6
2004	B	2	SHOCK	4	3	2	9
2004	B	3	SHOCK	1	1	1	3
2004	B	4	SHOCK	4	4	6	14
2004	B	5	SHOCK	0	0	0	0
2004	B	1	DISTURBANCE	0	0	0	0
2004	B	2	DISTURBANCE	0	0	0	0
2004	B	3	DISTURBANCE	2	1	1	4
2004	B	4	DISTURBANCE	0	0	0	0
2004	B	5	DISTURBANCE	0	0	0	0
2005	B	1	ANTS	0	1	4	5
2005	B	2	ANTS	2	1	1	4
2005	B	3	ANTS	1	5	4	10
2005	B	4	ANTS	0	0	1	1
2005	B	5	ANTS	3	3	3	9
2005	B	1	SHOCK	2	2	3	7
2005	B	2	SHOCK	1	1	0	2
2005	B	3	SHOCK	0	3	6	9
2005	B	4	SHOCK	0	2	2	4
2005	B	5	SHOCK	5	3	1	9
2005	B	1	DISTURBANCE	0	0	0	0
2005	B	2	DISTURBANCE	1	0	0	1
2005	B	3	DISTURBANCE	0	1	1	2
2005	B	4	DISTURBANCE	0	1	0	1
2005	B	5	DISTURBANCE	1	1	1	3
2005	C	1	ANTS	2	3	1	6
2005	C	2	ANTS	6	4	4	14
2005	C	3	ANTS	3	3	2	8
2005	C	4	ANTS	1	4	5	10
2005	C	5	ANTS	2	2	2	6
2005	C	1	SHOCK	3	3	4	10
2005	C	2	SHOCK	2	2	1	5
2005	C	3	SHOCK	1	2	1	4
2005	C	4	SHOCK	1	2	1	4
2005	C	5	SHOCK	2	0	1	3

Date	Site	MOUND	TRT	TEN	TWENTY	THIRTY	TOTAL
2005	C	1	DISTURBANCE	0	0	0	0
2005	C	2	DISTURBANCE	1	1	1	3
2005	C	3	DISTURBANCE	0	1	1	2
2005	C	4	DISTURBANCE	1	2	2	5
2005	C	5	DISTURBANCE	1	1	1	3

Appendix 6. Experiment 5: Number of *Pseudacteon curvatus* collected at 8 mounds where length, width, and height of each mound were measured, Sites B and C, Talladega County, 2005 (raw data)

Date	Site	Mound	Length	Width	Height	Area	Volume	Fly
2005	B	1	33.02	25.4	15.24	658.3858	6689.2	0
2005	B	2	27.94	22.86	10.16	501.3861	3396.055	0
2005	B	3	27.94	25.4	7.62	557.0957	2830.046	6
2005	B	4	30.48	25.4	12.7	607.7407	5145.538	11
2005	B	5	35.56	30.48	15.24	850.837	8644.504	53
2005	B	6	33.02	30.48	15.24	790.0629	8027.039	0
2005	B	7	35.56	35.56	12.7	992.6432	8404.379	17
2005	B	8	38.1	35.56	12.7	1063.546	9004.692	1
2005	C	1	38	33	9	984.39	5906.34	6
2005	C	2	44	32	12	1105.28	8842.24	6
2005	C	3	40.64	30.48	12.7	972.3852	8232.861	2
2005	C	4	5.08	3.81	12.7	15.19352	128.6385	2
2005	C	5	30.48	30.48	15.24	729.2889	7409.575	1
2005	C	6	35.56	30.48	15.24	850.837	8644.504	4
2005	C	7	43.18	30.48	10.16	1033.159	6997.932	1
2005	C	8	55.88	40.64	15.24	1782.706	18112.29	0