PSEUDACTEON SPP. (DIPTERA: PHORIDAE) EFFECT ON FORAGING STRATEGIES OF *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE) AND SPATIOTEMPORAL MONITORING IN URBAN HABITATS

A Dissertation

by

JANIS JOHNSON REED

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Chair of Committee,	Roger E. Gold
Committee Members,	Bastiaan M. Drees
	Robert A. Wharton
	Kim E. Dooley
Head of Department,	David W. Ragsdale

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ABSTRACT

Red imported fire ants (RIFA), *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 RIFA 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.

Laboratory and field experiments were conducted to 1) determine a bait size preference of RIFA exposed to phorid fly attack, 3) determine a bait preference between two candidate baits, 4) investigate worker size abundance in the presence of phorid flies, and 5) determine the presence and distribution of phorid flies in urban environments.

Laboratory experiments were used to determine foraging intensity and resource removal by RIFA foragers exposed to either *P. tricuspis* or *P. curvatus*. Arenas were constructed to allow access to a choice between two candidate baits and foraging RIFA were exposed to phorid flies for a 24 hr period. Results showed daytime foraging was reduced in the presence of *P. tricuspis* while nighttime foraging was increased when compared to RIFA not exposed to phorid fly attack. Additionally, RIFA exposed to *P. curvatus* had increased daytime and nighttime foraging when compared to RIFA not exposed to phorid fly attack. RIFA foragers were attracted to a commercial bait much more than to a laboratory candidate bait, but foragers removed more of the whole particles of the laboratory bait.

Field experiments were conducted to determine grit size selection of RIFA when exposed to phorid populations. Four different grit sizes of two candidate baits were offered to RIFA foragers. Foragers selectively were attracted to, and removed more of the 1-1.4 mm grit 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 the industry provided blank bait, RIFA removed more of the nutrient rich candidate bait from the test vials. There was an abundance of workers in the 0.5-0.75 mm head width category collected from both field sites. This was dissimilar from a previous study where phorid flies were not active and in which large workers were collected in higher abundance at the site where phorids were not active. The implication is that phorid fly activity caused a shift for RIFA colonies to have fewer large foragers.

The population levels of RIFA and *Pseudacteon* species flies were investigated in urban areas of central Texas. The objectives of this study were to determine the presence of phorids, their distribution, and seasonal variability in urban environments. Phorids were found in all types of urban environments examined and during all seasons. There was no difference in the population levels of phorids based on urban environment type and summer and spring were the seasons in which phorids were most abundant.

DEDICATION

This work is dedicated to my husband and partner in life, Thomas W. Reed. I really do not have the words to express what his support of me and this project means. He has been my motivator, my mentor, my counselor, my friend, but most importantly, never waveringly, has given me love. I can never and will never repay him for the sacrifices he made.

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Thank you to my daughter, Faith L. Reed. She sacrificed so much so I could make this crazy dream a reality, and she will never know how much it means to me. I am proud to be her mom; I am proud to be her friend; I am proud she is part of my Aggie family. Gig ‘em!

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction of *Solenopsis invicta* to the U.S.

Red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), hereafter referred to as RIFA, are eusocial insects assigned to the taxonomic order Hymenoptera, family Formicidae. Native to South America, RIFA were first detected in the southern United States in the 1930's. Believed to have been transported as a part of shipping dunnage from their native South American range to Mobile, Alabama, RIFA were then inadvertently moved throughout a considerable portion of the southern United States to eventually infest over 100 million hectares of land (Vinson 1997). Primarily moved about by human activities and nursery stock commerce in these early years (Markin et al. 1971), RIFA were officially recognized as problematic in the 1950's by the United States Department of Agriculture (Vinson 1997, Davidson and Stone 1989). Currently, RIFA have invaded 13 states, primarily in the south, but also in some unexpected states including Maryland and Oregon (Korzukhin et al. 2001). A lack of natural enemies and an affinity for disturbed habitats have allowed RIFA to expand their range and dominate landscapes, especially in urban systems (Klotz et al. 1995). Overall, fire ants are five to ten times more abundant in non-native areas as they are in their native South America, primarily due to their release from natural enemies and lack of native competition (Porter et al. 1992, Porter et al. 1997). RIFA invasion can have an effect on overall ant diversity because competition greatly favors

RIFA over native species. This competitive advantage is due to RIFA preference and inclination to invade disturbed, as opposed to undisturbed, habitats, aggressive foraging habits as well as forcibly displacing other ants (Wuellner and Saunders 2003). Livestock, ground nesting birds, vegetation, machinery, electronics, and humans have all been adversely affected by RIFA activity (Lofgren et al. 1975, Slowik et al. 1996).

Biology and behavior of *S. invicta*

RIFA construct and live in subterranean colonies where they rely on external foraging to obtain resources for the colony. Large, polymorphic colonies are common in Texas for RIFA, with a maximum estimated size of about 200,000 workers, and as the colony size increases, so does the major worker size (Tschinkel 1988). Polygynous colonies tend to have smaller workers compared to monogynous colonies; these colonies contain large workers just in lower abundance than monogyne ones (Greenberg et al. 1985).Workers vary greatly in size, with the largest being 15 to 20 times the weight of the smallest. This size differential contributes to the division of labor within the colony and smaller ants are responsible for brood care while larger workers forager more (Mirenda and Vinson 1981). Age of the worker also determines the role within the colony, older workers forage outside the colony, while brood care and other tasks within the colony are completed by younger workers. Foraging is driven by several factors including needs of the colony, available food resources, and the environment in which the ants live (Wallis 1962). RIFA are opportunistic omnivores, feeding on a range of live and dead insects, vertebrates, honeydew, and plants (Lofgren et al. 1975). Brood production is a primary driver of foraging, and if brood is being produced then protein sources are exploited more heavily (Markin et al. 1974b). Other factors can influence RIFA foraging including colony age colony size, caste composition, season and weather (Porter 1989, Stein et al. 1990, Drees et al. 2009, Cassill and Tschinkel 1999). Additionally, workers forage equally at night and during the day under normal conditions, but seasonality and soil temperature dominate the overall impact on foraging (Porter and Tschinkel 1987). Numerous control strategies have been implemented due to public health concerns, environmental impacts, and agricultural concerns, including quarantines, eradication programs, educational programs and more recently, biological control programs (Tschinkel 2006).

Control strategies for *S. invicta*

In the last several decades, control of RIFA in urban areas as well as rangeland has relied heavily on granular bait products incorporated with a toxicant (Williams et al. 2001). Generally, the bait material is applied to the RIFA infested area and is then gathered by foraging workers, returned to the colony where the solid component is processed by the fourth instar larvae and consumed as food for the colony (Tschinkel 1988). Food attractant, size of grit and active ingredient choice varies considerably between manufacturers and products. As a general rule the formulated product must be attractive to the target species, but be shelf stable. The toxicant must be palatable, slow to act, non-repellent, and must work at very low concentrations. The formulated bait needs to be easy to apply, and as target specific as possible (Banks 1990).

Commercially available baits generally have a range of particle sizes and particle size preference research has shown that RIFA prefer particle sizes of >2 mm which was correlated with ant head width (Hooper- Bùi et al. 2002). Additionally, nutritional content of bait can change how ants forage and recruit to it, and generally protein and carbohydrates are considered the most important of the macronutrients that drive foraging behavior (Cassill and Tschinkel 1999). Recently, research completed by Puckett and Harris (2010) and Chirino et al. (2009) showed evidence of a change in RIFA forager size ratios, with a greater proportion of smaller workers being present in the presence of phorid flies. Potential implications of this shift in forager size are far reaching. Smaller workers are important for brood care and maintenance but can accomplish any needed task, while larger workers generally perform defense, predation and mound maintenance tasks (Mirenda and Vinson 1981). Neff et al. (2011) showed smaller worker ants are more likely forage to smaller bait particles. This can impact RIFA preferences of baits selected, possibly affecting bait acceptance and effectiveness.

Biology of *Pseudacteon* spp. and potential for biological control of RIFA

Phorid flies, in general, are the most common myrmecophiles currently known to impact RIFA. *Pseudacteon* spp. are native to South America, also the native range of RIFA. Inconspicuous in size at approximately 1 mm in length, they are difficult to observe in nature without the presence of their host, and tend to be spatially and temporally rare (Morrison et al. 1999b, Porter 1998a). These solitary parasitoid flies locate their ant hosts by using a variety of visual and chemical cues, such as alarm pheromone, trail pheromone, or chemical cues from midden piles (Morrison and King 2004, Puckett et al. 2007, Mathis and Philpott 2012). *Pseudacteon* flies are only active during daylight hours and when air temperatures are above 20° C. Additionally, these flies can remain active at temperatures greater than 37° C, so ants foraging during night time hours or when temperatures become harsh are less likely to be attacked (Morrison 1999, Pesquero et al. 1996, Morrison et al. 1999b). Pesquero et al. (1996) presented evidence that *Pseudacteon* spp. do not actively parasitize ants until two to three hours after sunrise, reducing their effect on foraging ants early in the morning. By the second or third hour after sunrise, flies were active and could parasitize ants foraging above ground, and would continue to be active for as long as 12 hours after sunrise.

Once hosts are located the female flies oviposit their egg, singly, into the thorax of the fire ant worker. The egg is inserted into thorax, where the coxal region of the prothoracic leg joins the thorax, using a needle like sclerotized ovipositor and the insertion process is completed in 0.1 to 0.8 seconds (Consoli et al. 2001, Porter et al. 1995c). Females may attack many workers during one oviposition activity period, and may have multiple periods of activity (Morrison et al. 1997, Williams et al. 1973). After attack, ant will appear stunned, and will occasionally stand straight up on their legs for several seconds (Porter 1998b, Porter et al. 1995b). Once the fly egg hatches, the larva migrates internally from thorax to the head, usually during the first or second instar, where it completes its development, consuming the contents of the ant head capsule. Eventually, the fly reaches adulthood utilizing the head capsule as a puparium

(Consoli et al. 2001, Porter et al. 1995b). During pupation the parasitized ant head often detaches from the body, earning the flies the common name of the decapitating fly (Disney 1994). This is due to the phorid fly larva excreting an enzyme which degrades intercuticular membranes around the head and thorax of its host ant (Consoli et al. 2001, Porter et al. 1995b). RIFA workers show little effects of parasitization until the fly forms its puparium within the head capsule, at which time the ant falls to its side, crippled by the loss of integument (Porter 1998a). Colony maintenance include removal of dead ants (midden) and head capsules containing puparia are most likely removed and placed outside the colony (Howard and Tschinkel 1976) where flies complete their development and emerge as adults.

Interaction between *Solenopsis* spp. and *Pseudacteon* spp.

The *Solenopsis saevissima* complex, which includes RIFA, hosts a very large number of phorid fly parasitoids. This diversity is largely linked to the size differential worker ants show, which range from very small (<0.5 mm) to large head widths of up to 1.5 mm (Wood and Tschinkel 1981, Tschinkel 1988). Considering *Pseudacteon* flies develop in the head capsule of host ants, it is understandable that the size of the flies directly correlates to the size of the chosen host (Morrison et al. 1997). This distinct size preference that drives host selection has allowed various *Pseudacteon* species to partition a specific niche, in this case a single species or colony of ants (Wuellner et al. 2002, Orr et al. 1997, Morrison et al. 1997). For example, smaller workers are more likely to be parasitized by *P. curvatus* and *P. obtusus,* while medium sized workers are preferred by *P. tricuspis*, and larger workers tend to be selected by *P. litoralis* (Wuellner et al. 2002, Orr et al. 1997, Gilbert and Morrison 1997, Morrison et al. 1997, Pesquero et al. 1996, Porter et al. 1995a, and Morrison and Gilbert 1998). To some extent, this size differential is directly correlated to the fly body size (Morrison and Gilbert 1998). In other flies, host size can drive more than just body size; it can also affect sex ratios, such as with *P. tricuspis*, where larger host ants produce female offspring and smaller host ants yield males (Morrison et al. 1999a).

Phorid flies are highly specific to their hosts and this has helped facilitate their use as classical biological control agents (Porter and Gilbert 2004, Porter et al. 1995a, Gilbert and Morrison 1997, Porter and Alonso 1999, Morrison and Gilbert 1999, Folgarait et al. 2002). Numerous releases of *Pseudacteon* flies have been made in the US to date, with *P.obtusus*, *P. tricuspis* and *P. curvatus* becoming the most established species in Texas. Overall, attempts at releasing populations that then become self-sustaining have been successful with fly populations active in most of central and east Texas (Fig. 1.1, 1.2, and 1.3). Releases of *P. tricuspis* in Texas have been conducted a total of 44 times from 1995 through 2008 in 29 counties, with at least 25 of those releases considered successful. Beginning in 2004, *P. curvatus* was released a total of 22 times in 18 counties, and has been successful in all but one instance (Gilbert et al. 2008, Calcott et al. 2011). *P. obtusus* was released in Texas beginning in 2006 through 2010, a total of 32 times in 26 counties. In the case of *P. obtusus*, establishment has been successful in only 14 of those instances in 11 counties (Plowes et al 2011). Generally, it is believed that a suite of biological control organisms will be needed to see significant control effects. The communities of the decapitating flies alone in the native range of South America can number up to 10 different species acting simultaneously (Orr et al. 1997, Folgarait et al. 2005).

RIFA exhibit a natural and predictable response to phorid fly activity. The foraging ants assume a defensive posture of curling their abdomen under the thorax, raising their head, and the mandibles and antennae are extended outward (Feener 1987). A single *Pseudacteon* fly hovering over a foraging trail is enough stimuli to cause up to 100 ants to assume the defensive posture. This defensive posture obviously reduces foraging efficiency because the ants cease moving and foraging. As a result, foraging is reduced and food retrieval is reduced (Feener and Brown 1992, Morrison 2000). Only approximately 1% of a population of RIFA are directly parasitized (Morrison and Porter 2005), but indirect effects cause responses in large numbers of foragers affecting the colony at the community level. Their indirect effects on food resource competition, a reduction in the overall number of foragers, mediation of interspecies competition, overall colony fitness, and an effect on overall worker size (Porter and Tschinkel 1985, Feener 1981, Feener and Brown 1992, Orr et al. 1995, Folgarait and Gilbert 1999, Morrison 1999, Puckett and Harris 2010).

The suite of *Pseudacteon* phorid flies, as self-sustaining biological control agents, are just one set of many natural enemies used to battle the nefarious RIFA. Other biological control include: viruses (Oi et al. 2009), microsporidian pathogens (Williams et al. 1999), nematodes (Drees et al. 1992), fungi (Stimac et al. 1993) and bacteria (Shoemaker 2000).

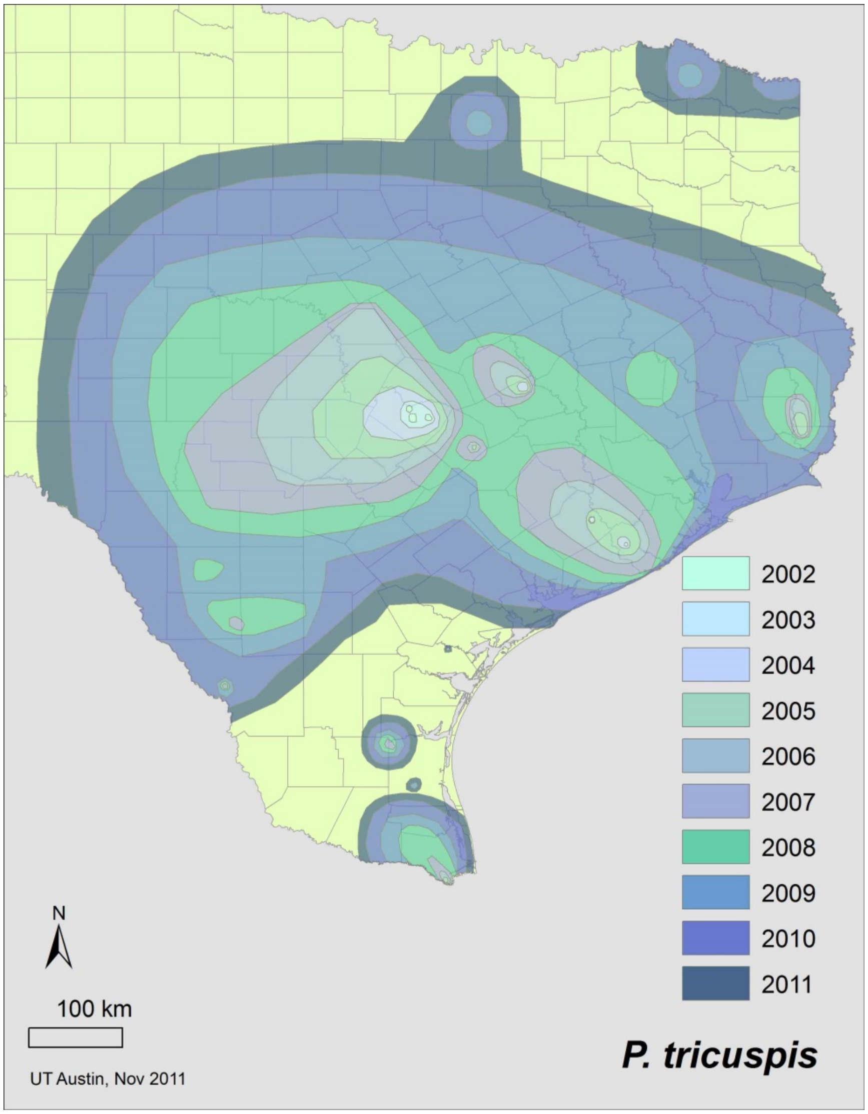

Fig. 1.1. Spread and establishment of *P. tricuspis* in Texas. Map provided by The University of Texas Fire Ant Project (2011).

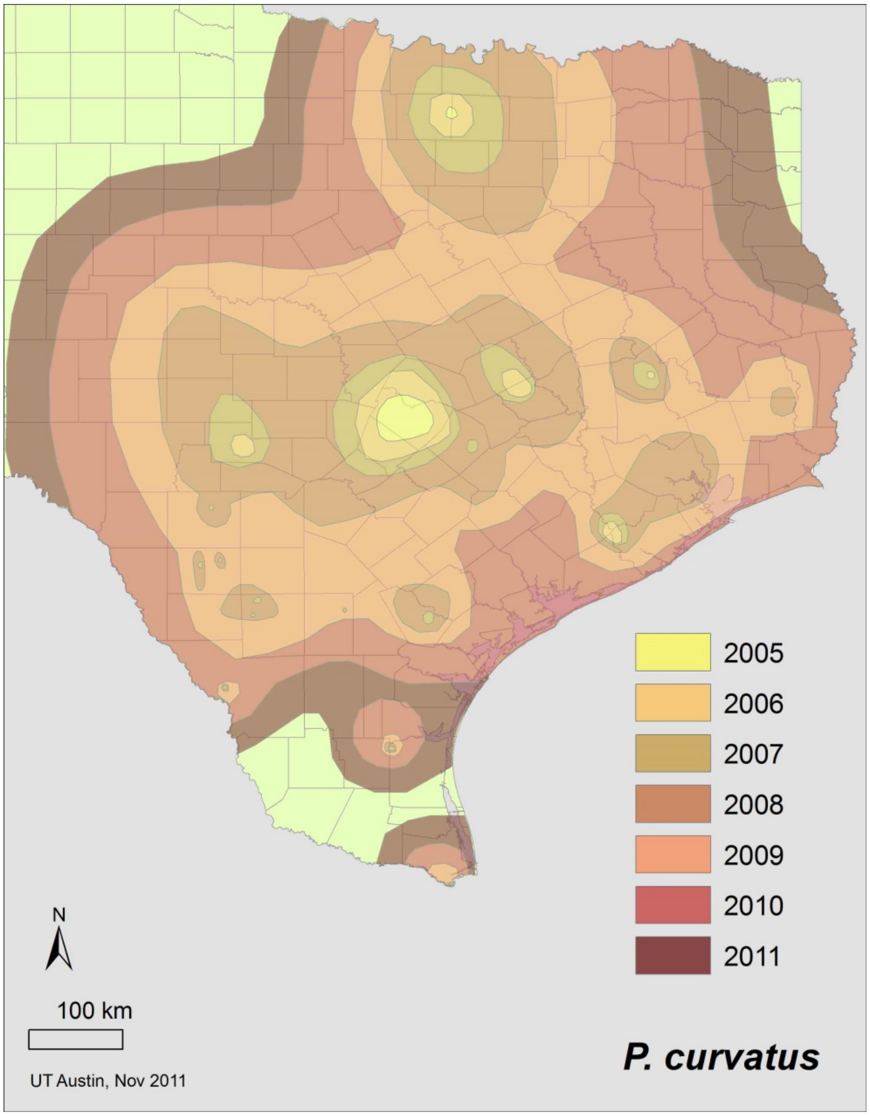

Fig. 1.2. Spread and establishment of *P. curvatus* in Texas. Map provided by The University of Texas Fire Ant Project (2011).

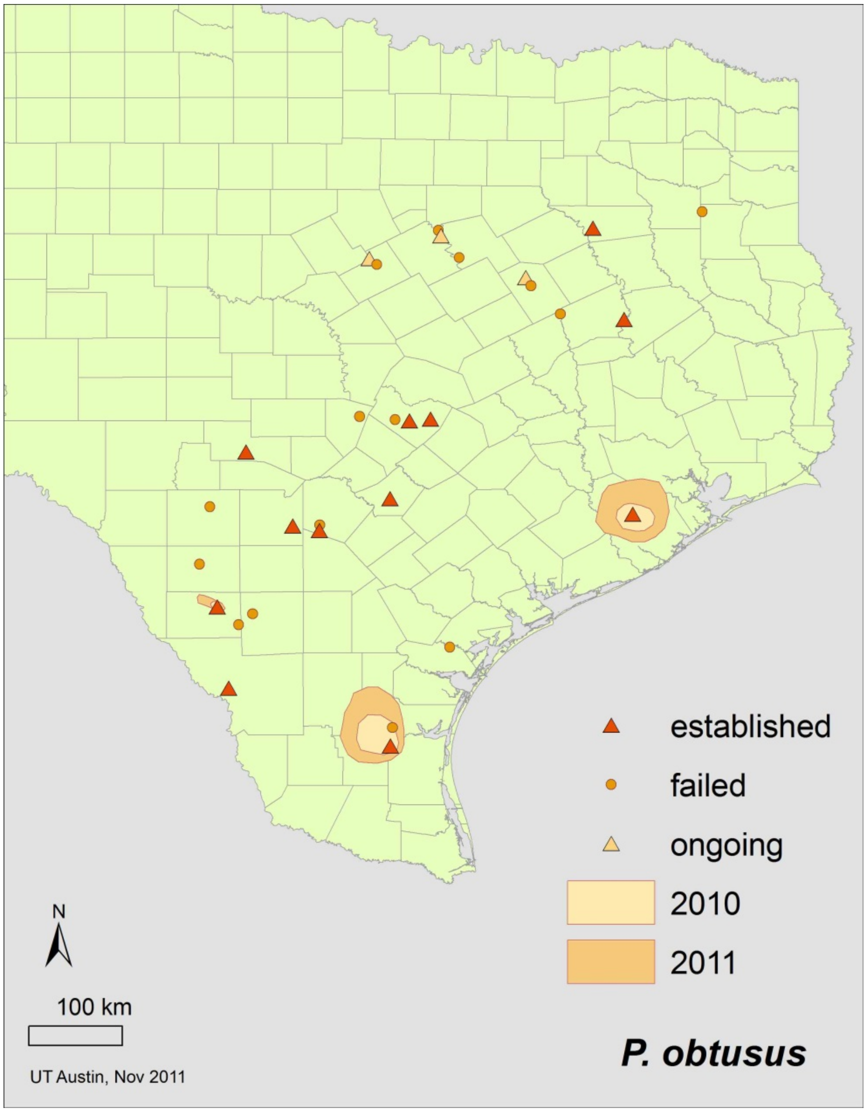

Fig. 1.3. Spread and establishment of *P. obtusus* in Texas. Map provided by The University of Texas Fire Ant Project (2011).

CHAPTER II

INFLUENCE OF *PSEUDACTEON* SPP. ON FORAGING AND FOOD PREFERENCES OF RED IMPORTED FIRE ANTS

Introduction

From the time since it was introduced to the United States in the 1930's, the red imported fire ant (RIFA) *Solenopsis invicta* Buren (Hymenoptera: Formicidae), has invaded most of the southern part of the country. Agricultural, environmental, and public health implications instigated the United States Department of Agriculture to create large quarantine and eradication programs (Williams et al. 2001). Overall, liquid insecticide applications for the control of RIFA have been unsuccessful and in some cases exacerbated the problem (Markin et al. 1974a, Summerlin et al. 1977). Modern baits are used to control populations of pests by enticing them to consume a food attractant that contains a palatable pesticide active ingredient. Conventional ant baits are usually formulated from small food particles that are treated with oil and a pesticide active ingredient. The formulated bait is attractive to workers and the oil component shared via trophallaxis to the worker castes within the colony, and the queen, causing death (Williams et al. 2001, Barr et al. 2005). Large particles are transported to the colony where they are formed into worker buccal pellets and presented to fourth instar larvae (Petralia and Vinson 1978). Baits formulated as or that incorporate oils or liquids can be directly fed to brood as well as adults. Baiting methods of control using conventional ant bait products are much more effective than liquid contact insecticide applications, less expensive, and pose little toxicity hazard to humans and domestic animals (Williams 1983).

Phorid flies in the genus *Pseudacteon* are part of a group of natural enemies which parasitize workers in the native range of RIFA. Several species of flies were imported into the southern United States with the goal of helping to control RIFA. There are numerous studies showing the deleterious effect phorid flies have on foraging ants; foraging is reduced while flies are active and in some cases for up to an hour after the flies departed (Feener 1981, Orr et al. 1995, Morrison 1999). *Pseudacteon* flies are active only during daylight hours so only daytime RIFA foraging is effected (Pesquero et al. 1996). This interaction between RIFA and phorid flies and the resulting behavioral effect could possibly allow native ants to be more competitive by reducing the competitive advantage invasive RIFA has held. Foraging behavior relies on arriving early to a food source and then heavily recruiting to it, essentially out-competing any other ant species. Under normal conditions, RIFA are known to forage throughout the day and nocturnally, consistently overwhelming foraging patterns of native ants in areas in which they are invasive (Porter et al. 1988). Native ants, depending on the species vary in their foraging habits, from diurnal to nocturnal. If phorids are present and active, a shift to nocturnal foraging would allow escape from parasitism. An increase in nocturnal activity might increase interaction with native ants, especially those that rely on nighttime foraging. Unforeseen effects of this type of reduction could lead to increased foraging by native ants on baits meant to control RIFA, causing further reduction in numbers of native ants.

Experiments have shown that RIFA reduce diurnal foraging when phorids are present and show an increase in nocturnal foraging, but no work has been done to show effects on food preference or resource removal over time. This study addressed the following questions via laboratory experiments: 1) is there a shift in resource attraction and removal in the presence of phorid flies, and 2) is there a shift in bait type preferences in the presence of these parasitoids? This experiment was intended to further elucidate foraging pattern changes RIFA displays in the presence of *Pseudacteon* spp. phorid flies.

Experimental design

RIFA adults and brood were collected by removing the soil surrounding the mound with a large shovel and placing it into a plastic 18 L bucket coated with talc to prevent ants from escaping. Ants utilized for this experiment were all collected from 5-Eagle Ranch located in Caldwell, Texas (Burleson County, 30° 54’ 54.57” N; 96 40’ 59.77” W) and transported to the Center for Urban and Structural Entomology. Upon returning to the laboratory, water was slowly dripped into the bucket at a rate of approximately 3 drops per second so ants eventually floated to the surface (Banks et al. 1981). These ants were then collected using slotted spoons and placed in previously prepared plastic trays coated with Fluon® (Northern Products, Inc., Woonsocket RI). Ants were provided food and water until 48 hours prior to the test, at which time the food source was removed.

Ants were treated with carbon dioxide to reduce activity and then sieved very rapidly in small batches to separate brood from workers. Colonoids were created from field collected colonies by separating workers from brood and weighing the groups of ants. Approximately 1 gr of brood and 4 gr of worker ants were used for each replication. There were approximately 4000 ants in each colonoid (Chen et al. 2012). These small colonoids were placed in a plastic tray, 40 cm wide, 33 cm long and 17 cm tall (IRIS USA, Pleasant Prairie, WI). A small Petri dish, containing casted stone (Castone, #99043, Dentsply, York, PA) was placed in the trays to serve as nesting sites for ants (Fig. 2.1A). The stone was moistened and the lid of the Petri dish was painted black (Rust-oleum, Vernon Hills, IL) to provide a dark nesting site. The lid of the Petri dish was modified to contain a small hole to allow ants to enter and exit the nesting site, and provide a means of escape from phorid fly attack. A vial of water stoppered with a moist cotton ball was provided as an additional source of moisture.

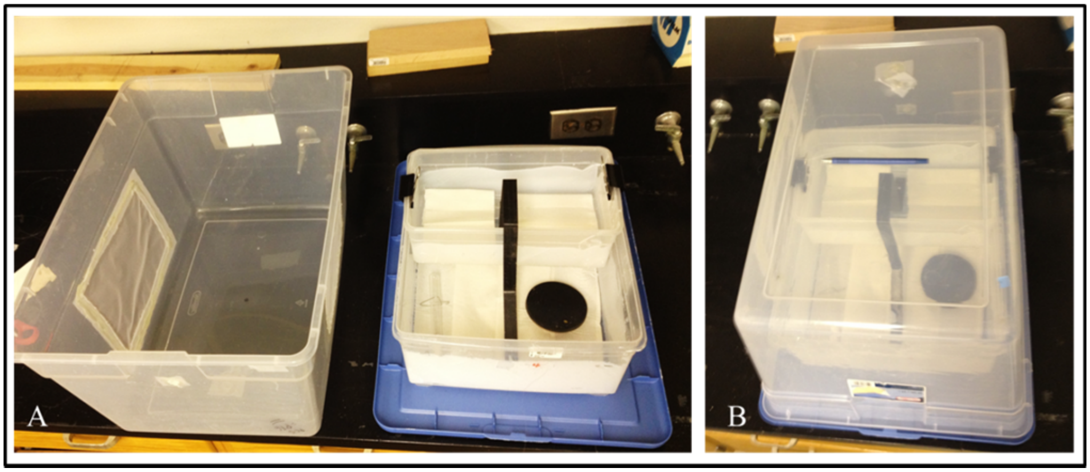

Fig. 2.1. (A) Experimental test arena including large colony box, smaller bait box, metal foraging bridge, and water source; (B) View of the closed experimental arena.

The test arena consisted of a large translucent 53 L storage tub (57 cm H x 42 cm W x 59 cm L) (Sterilite®,Townsend, MA) and was inverted over the nesting box. The storage tub was translucent, allowing natural light to reach the ants and flies contained inside. Additionally, panels were cut from the sides of the box and replaced with fine mesh fabric adhered with hot glue. This design helped to regulate temperature and humidity in the arena. The inverted lid of the storage tub held the nest box and a second Fluon® lined tray 17 cm wide, 31.75 cm long and10 cm tall (IRIS USA, Pleasant Prairie, WI) contained the bait cups containing an experimental lab made bait containing 1:1 protein to carbohydrate ratio (EB1:1) and an industry provided bait (TC-206 Advance Granular Carpenter Ant Scatter Bait, BASF, St. Louis, MO), (CABB) offered in the choice test. This second box containing the candidate baits, was fitted long ways in the larger one and was attached via the use of large binder clips. Ants could access the candidate baits via a 2 cm wide rigid metal ribbon bridge that was bent to act as a liftable foraging bridge between the nest box and the tray containing the candidate baits. The foraging bridge was fastened to the bait box using hot glue, a 2 cm long screw, a nut, and a washer (Fig. 2.1A). The storage tub was also modified to include a method to raise and lower the foraging bridge without removing the lid, thus keeping the phorid flies contained within the arena. This was accomplished by creating a small hole in the plastic tub by inserting a sewing needle through the plastic and then threading fishing line (Rapala® Extra Durable line in 4.5 kg test, 0.3 mm diameter, Minnetonka, MN) through the hole to the exterior of the tub. The fishing line was attached to a small Fluon® coated disk (2 cm diameter) which was then clipped to the foraging bridge with a small piece of wire. The Fluon® coated disk was used to prevent ants from using the fishing line to escape the nest box (Fig. 2.1B) On the outside of the plastic tub, the fishing line was attached to a weight and tied off. This was done to hold the foraging bridge in the up position between observations.

For the duration of the test, a commercial bait without added pesticide active ingredient was used (TC-206 Advance Granular Carpenter Ant Scatter Bait, BASF, St. Louis, MO). This bait will be referred to as CABB, carpenter ant bait blank in this dissertation. This was compared to a 1:1 protein to carbohydrate experimental bait (Cook et al. 2010). This experimental bait will be referred to as EB1:1 for the purposes of this work. The industry provided bait (CABB) was chosen because it has been shown to be highly attractive to RIFA and is used for ant control routinely in the pest management industry (Drees and McDonald 2010). The EB1:1, which primarily consisted of sugar, egg powder and protein powder, was used because manipulation of nutritional content is possible, but this preparation is untested as a possible toxicant carrier. The candidate baits were separated into four size classes: <0.71, 0.72-1.0, 0.9-1.4, and 1.4-2 mm. Different sized particles were used because particle size can impact toxicant bait acceptance (Hooper-Bùi et al. 2002). Candidate baits were hand milled and size classes were separated using US Standard Sieves No. 10, 14, 18, and 25. Each bait type was weighed before introduction into the arena and a total of 4 gr of mixed bait containing all four size classes was used. This included 1 gr from each size class in two separate dishes, one for the EB1:1 bait and one for the CABB bait, in each foraging arena. The candidate baits were weighed prior to exposure to foraging ants and again after the conclusion of the test. Another set of bait dishes was prepared to act as a control, where ants were excluded, but the bait was exposed to the same greenhouse conditions during the test. The average weight gained/lost was added/subtracted from the test bait to obtain a corrected amount of bait removed.

All test arenas were placed in a climate controlled greenhouse located at the Center for Urban and Structural Entomology on the Texas A&M Campus in College Station, Texas in order to provide natural lighting in the arenas. Additionally, arenas were randomized by treatment type in the greenhouse to minimize unforeseen environmental factors. The area directly over the test arenas was covered using landscape fabric to reduce overheating of the nest boxes (Fig. 2.2). Colonies were allowed to acclimate to greenhouse conditions for a minimum of 24 hours prior to testing.

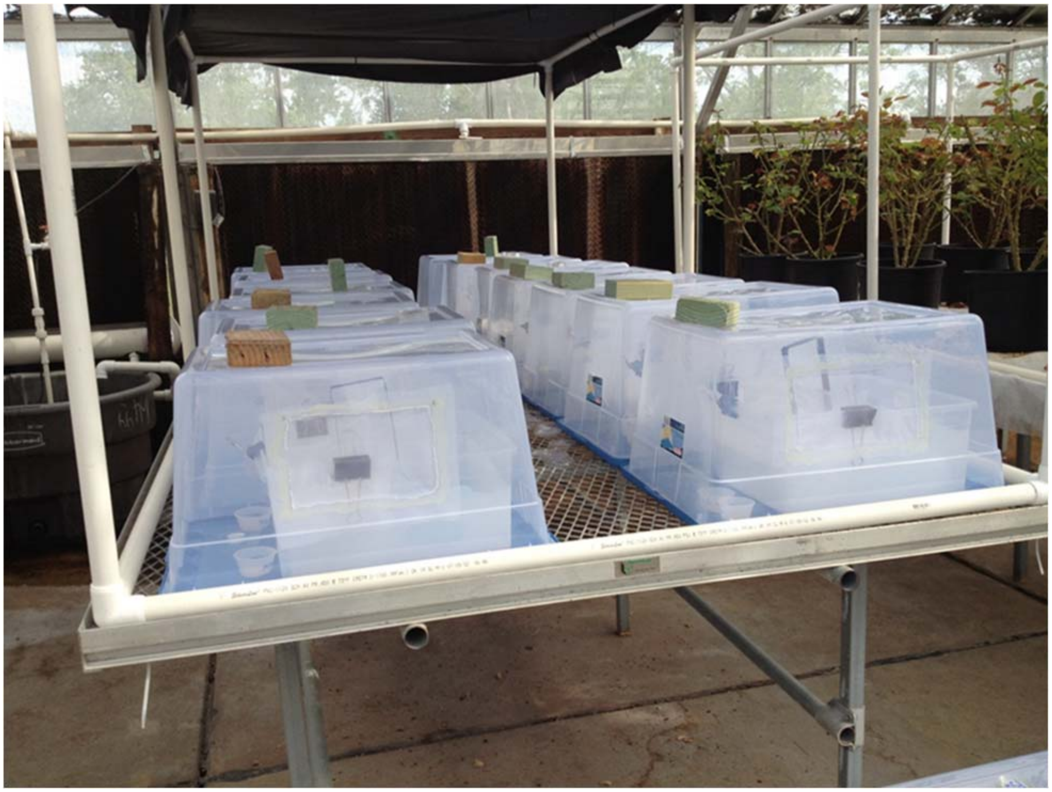

Fig. 2.2. Experimental arena set up in the greenhouse.

Phorid flies used in these experiments were provided by the USDA/APHIS Division of Plant Industry in Gainsville, Florida. The flies were shipped as pupae in cool packed containers. Pupae were received in small condiment cups with plaster in the base and were placed in rearing chambers with organza openings to keep temperatures constant. These rearing containers consisted of a cylindrical container with a lid, approximately 10.3 cm in diameter and 8.9 cm tall (Part #283 C, Pioneer Plastics Inc., Dixon, KY) (Fig. 2.3). Casted stone was placed in the bottom and moistened to provide a source of humidity. Adults were allowed to emerge and were provided a 10% sucrose solution to prolong their lives. Once emerged, flies were chilled on ice packs (CP 28, Air Sea Containers, United Kingdom) that had been wrapped with several layers of paper to reduce condensation. The flies were then sexed and separated into vials by species. Each replication received six females and two males of one species, either *P. curvatus* or *P. tricuspis.*

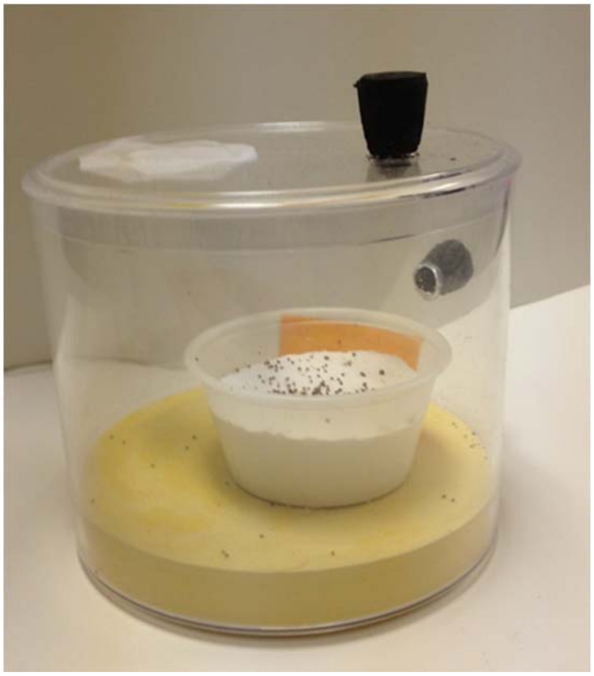

Fig. 2.3. Rearing container for phorid fly pupae.

At the initiations of the test, the two cups containing the pre-weighed EB1:1 and CABB candidate baits were introduced into the testing arena. After introduction of the candidate baits, RIFA were allowed to forage for 1 hr prior to phorid release. Flies were introduced to the test arenas 1 hr after the foraging bridge was lowered. Ants were observed for a period of 24 hrs at 4 hr intervals. At each time interval, the bridge was lowered, the ants foraged for 30 minutes, and then counts of ants crossing the foraging bridge were made for 30 seconds. Only ants travelling towards the next box were counted. Additionally, photographs were taken of each bait dish (Fig. 2.4) at each observation to determine the number of ants on each candidate bait type. After observations were made, the foraging bridge was raised, limiting the access to the food resource until the next observation time. The weight of each candidate bait was taken before and after the test to determine total bait removal. To determine weight removal, the weight change of the bait was calculated from pre-weights and post weights, which were corrected using the change in the control candidate baits.

Fig. 2.4. RIFA foragers on bait cups. The CABB type bait is pictured on the left and EB1:1 bait is pictured on the right.

This experiment was replicated five times for each fly species, *P. curvatus* and *P. tricuspis*. A second, identical set of test arenas were used for the non-phorid exposed ants (control). All replications were carried out in May and June 2012, and temperatures inside the greenhouse were well within optimal foraging temperature thresholds (Porter and Tschinkel 1987 and Drees et al. 2009).

Counts of ants crossing the bridge, counts of ants at each bait type and weight of bait removed were analyzed using ANOVA and means were separated by the use of Tukey's honestly significant difference post hoc analysis. Student's t-tests facilitated separation of means between AM and PM observations and EB1:1 and CABB type baits for ant forager counts as well as weight of candidate baits that was removed. Analysis was facilitated by the use of IBM SPSS Statistics v. 21.

Results

Counts of ants crossing the foraging bridge

There was a significant difference between the treatments during the daytime (light) observations and the nighttime (dark) observations ($F = 6.422$; df = 2,235; $P < 0.005$; Fig. 2.5). There were significantly fewer RIFA crossing the foraging bridge for the *P. tricuspis* treatment than either the control or *P. curvatus* treatments. There was not a significant difference between *P. curvatus* and the control treatments for observations made during the day (light).

There was also a significant difference in the number of RIFA crossing the foraging bridge between the treatment groups during the dark observations ($F = 5.930$; df = 2,172; $P < 0.005$). There were significantly more RIFA during the dark observations for the *P. curvatus* treatment than the control treatment but the mean number was not significantly different from the *P. tricuspis* treatment. Additionally, the control treatment was not significantly different from *P. tricuspis* treatment, nor was the *P. tricuspis* treatment significantly different from either the *P. curvatus* treatment or the control treatment (Fig. 2.5; Tables 2.1 and 2.2).

There were significantly more RIFA crossing the foraging bridge during the dark observations than there was for the light observations for *P. tricuspis* (t = 3.037; df =124) = 3.037; $P < 0.05$). There were significantly more RIFA crossing the foraging bridge during the day observations than the night observations for the control treatments (t = 2.187; df = 151; $P < 0.05$). However, there was not a significant difference between the light and dark observations for *P. curvatus* treatments (t = 0.860; df =124; P = 0.860; Fig. 2.5; Table 2.3).

Table 2.1. Mean number of RIFA crossing the foraging bridge during the daytime (light) observations (n = 72) and 95% confidence intervals. Means are for all replications.

Treatment	**Min/Max**	**Mean ± SE**	**95% confidence intervals**	
			Lower Bound	**Upper Bound**
P. tricuspis	0/18	3.32 ± 0.52^{a}	2.29	4.35
P. curvatus	0/44	7.68 ± 1.11^{b}	5.46	9.90
Control	0/44	6.79 ± 0.87^{b}	5.07	8.51

Means followed by the same letter were not significantly different using Tukey’s HSD Post Hoc Analysis.

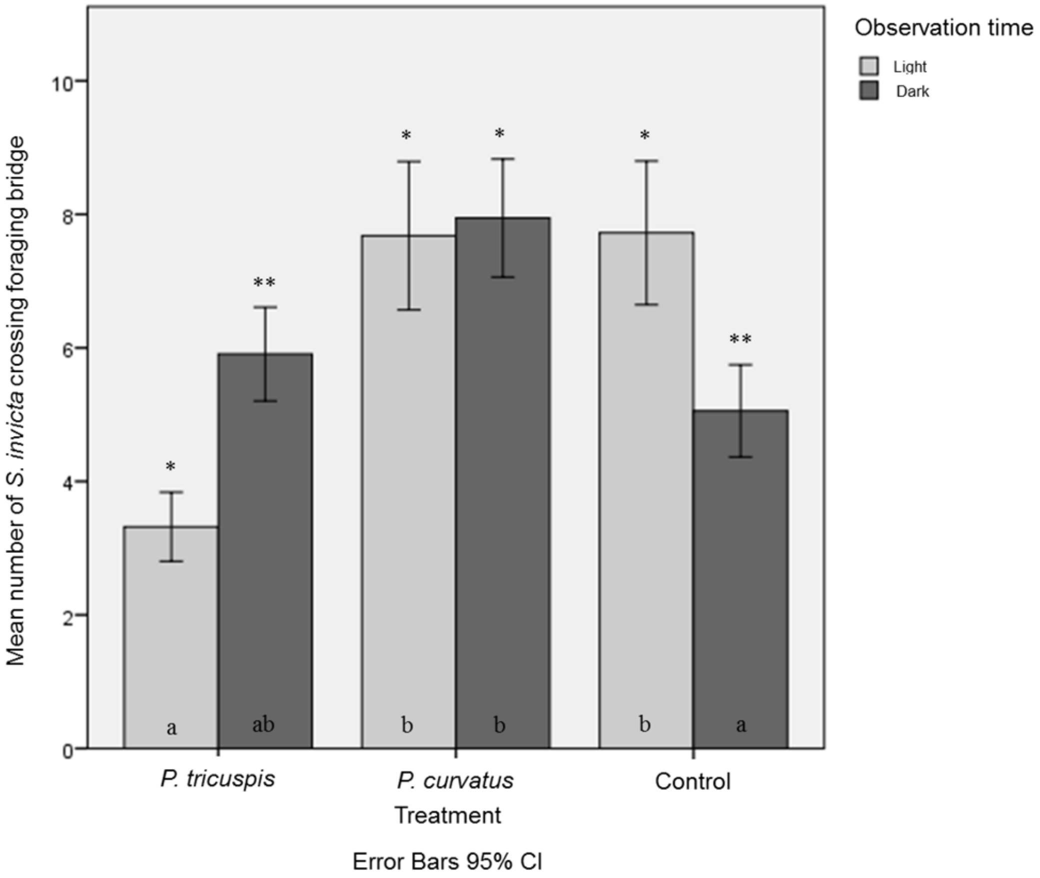

Fig. 2.5. Mean numbers of RIFA crossing the foraging bridge by observation time and treatment. Asterisks represent significant differences between observation times (Light n = 72; Dark n = 54) for each treatment (Tables 2.2 and 2.3) and letters represent significant differences between treatments (*F* = 6.422; df = 2,235; *P* < 0.005) across all observations.

Table 2.2. Mean number of RIFA crossing the foraging bridge during the nighttime (dark) (n = 54) observations and 95% confidence intervals. Means are for all replications.

Treatment	**Min/Max**	**Mean ± SE**	**95% confidence intervals**	
			Lower Bound	**Upper Bound**
P. tricuspis	0/25	5.91 ± 0.70^{ab}	4.50	7.31
P. curvatus	0/25	7.94 ± 0.88^{b}	6.17	9.72
Control	0/21	4.51 ± 0.58^{a}	3.35	5.66

Means followed by the same letter were not significantly different using Tukey's HSD Post Hoc Analysis.

Table 2.3. Results of student's t-test on the mean number of RIFA crossing the foraging bridge during the light and dark observations. Results are shown by treatment (*P. tricuspis* n = 99; *P. curvatus* n = 106; Control n = 106).

Treatment	**Mean # of RIFA ± SE**		**df**	**t**	***P* value**
	Light Obs.	**Dark Obs.**			
P. tricuspis	3.32 ± 0.518^{a}	5.91 ± 0.70^{ab}	124	3.037	< 0.05
P. curvatus	7.68 ± 1.111^{b}	7.94 ± 0.88^{b}	124	0.177	= 0.860
Control	6.79 ± 0.867^{b}	4.51 ± 0.58^{a}	151	2.187	< 0.05

Means followed by the same letter were not significantly different using Tukey's HSD Post Hoc Analysis.

RIFA observed on bait

There was a significant difference between the mean number of ants on the EB1:1 ($F = 3.834$; df = 2,410; $P < 0.05$) and the CABB ($F = 13.978$; df = 2,409; $P < 0.001$) among the three treatments. There were significantly fewer RIFA on bait cups in the *P. tricuspis* treatment compared to all other treatments. The control treatment and *P. curvatus* were statistically equivalent in the mean number of RIFA on the bait grit for both bait types (Fig. 2.6). Mean numbers of ants on bait and 95% confidence intervals are located in Table 2.4 for EB1:1and Table 2.5 for the CABB.

Table 2.4. Mean number of RIFA observed on the EB1:1 by treatment and 95% confidence intervals. Means (*P. tricuspis* n = 126; *P. curvatus* n = 126; Control n = 161) are for all replications.

Treatment	**Min/Max**	**Mean** ± SE	**95% confidence intervals**	
			Lower Bound	**Upper Bound**
P. tricuspis	0/62	3.54 ± 0.63[a]	2.30	4.78
P. curvatus	0/93	6.28 ± 0.94[b]	4.42	8.13
Control	0/57	6.31 ± 0.78[b]	4.78	7.84

Means followed by the same letter were not significantly different using Tukey’s HSD Post Hoc Analysis.

Table 2.5. Mean number of RIFA observed on the CABB by treatment and 95% confidence intervals. Means (*P. tricuspis* n = 126; *P. curvatus* n = 126; Control n = 161) are for all replications.

Treatment	**Min/Max**	**Mean** ± SE	**95% confidence intervals**	
			Lower Bound	**Upper Bound**
P. tricuspis	0/88	20.12 ± 1.803[a]	16.55	23.69
P. curvatus	0/153	39.82 ± 3.226[b]	33.43	46.20
Control	0/116	32.14 ± 2.422[b]	27.36	36.93

Means followed by the same letter were not significantly different using Tukey’s HSD Post Hoc Analysis.

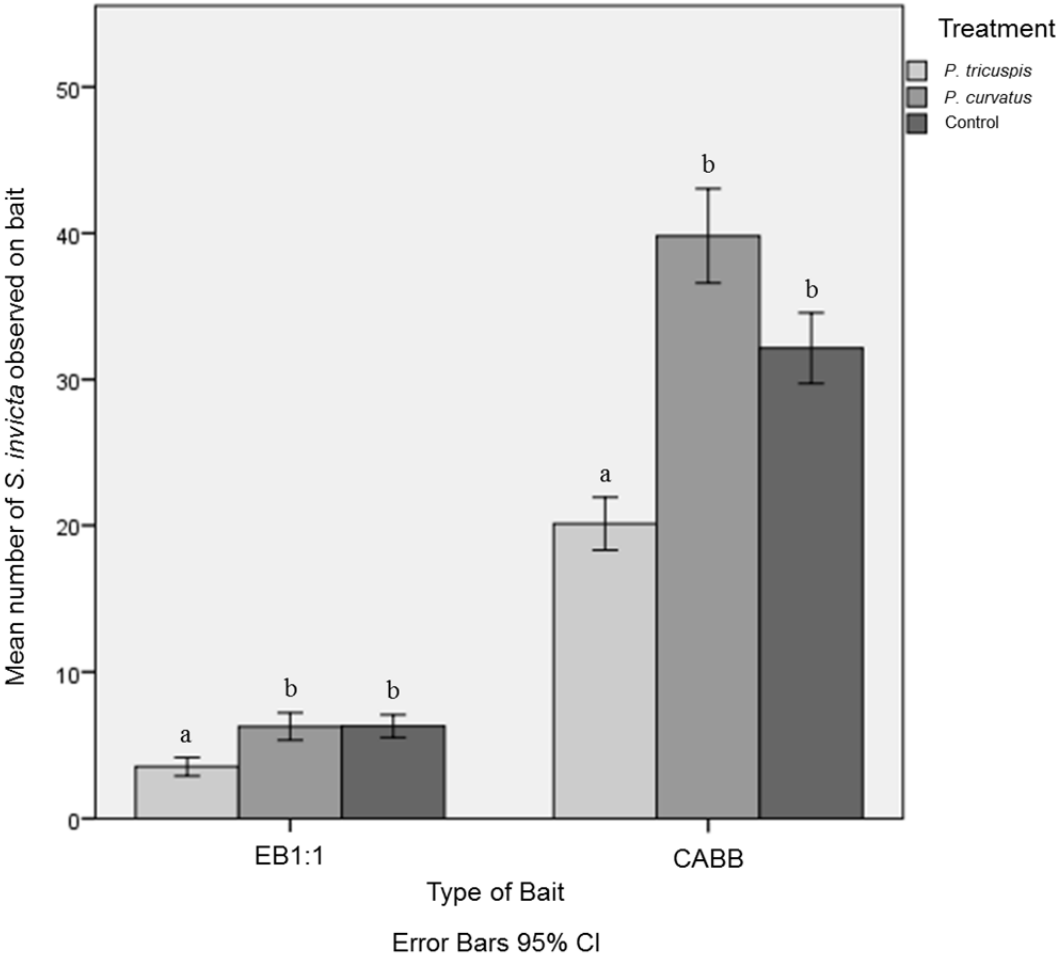

Fig. 2.6. Mean number of RIFA observed on bait cups by bait type and treatment. Letters represent significant differences between each bait type EB1:1 ($F = 3.834$; df = 2,410; $P < 0.05$) and CABB ($F = 13.978$; df = 2,409; $P < 0.001$; *P. tricuspis* n = 252; *P. curvatus* n = 252; Control n = 321).

For all daytime (light) observations, the mean number of ants observed on bait cups was significantly different between the bait types for all the treatments. The EB1:1 had a statistically significant fewer mean number of ants observed on the bait cups than the CABB bait for the three treatment types during the daytime observations ($F = 4.277$; df = 2,246; $P < 0.05$; Fig. 2.7). The *P. curvatus* treatment has significantly more ants than the *P. tricuspis* treatment. The control treatment was statistically equivalent to both the phorid fly treatments for the mean number of ants on the bait cups. The CABB type bait was also statistically different in the mean number of ants observed on the bait cups between the three treatments during the daytime observations ($F = 10.249$; df = 2,242; $P < 0.001$; Fig. 2.7). The control treatment and the *P. curvatus* treatment both has significantly more ants observed on the bait than the *P. tricuspis* treatment for the CABB type bait. There is a statistically significant difference between the EB1:1 and CABB bait types during light observations for the *P. tricuspis* treatment ($t = 6.257$; df = 151; $P < 0.001$), for the *P. curvatus* treatments ($t = 7.334$; df = 144; $P < 0.001$), and for the control treatment ($t = 7.509$; df = 193; $P < 0.001$; Fig. 2.8), with the most RIFA always observed on the CABB bait. Mean numbers of RIFA observed on each bait type are summarized in Tables 2.6 and 2.7.

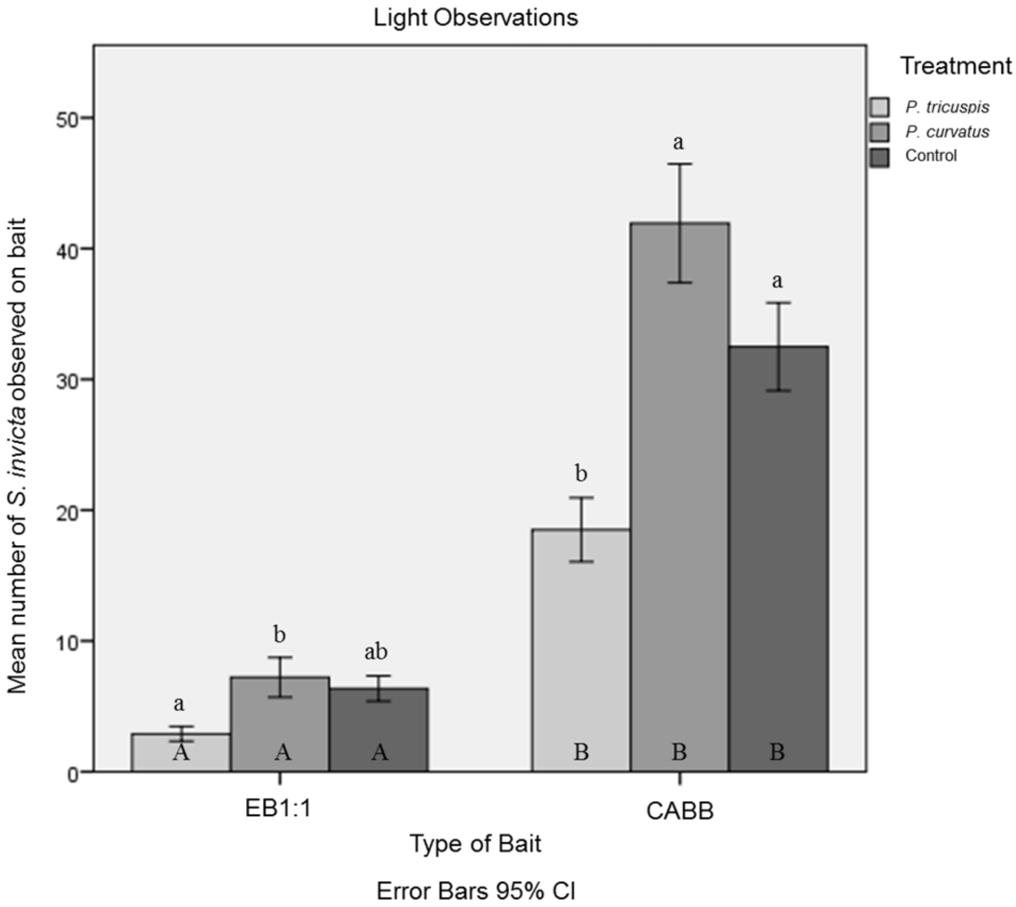

Fig. 2.7. Mean number of RIFA observed on bait cups by treatment type during the daytime (light) observations. There was a statistically significant difference (*P. tricuspis* n = 146; *P. curvatus* n = 146; Control n = 195) (indicated by lowercase letters) between the EB1:1 observations ($F = 4.277$; df = 2,246; $P < 0.05$), as well as the CABB type bait ($F = 10.249$; df = 2,242; $P < 0.001$) (indicated by lowercase letters). There was a significant difference between the number of ants on the EB1:1 and the CABB bait for all treatment types (indicated by uppercase letters) (*P. tricuspis* treatments: $t = 6.257$; df = 151; $P < 0.001$; *P. curvatus* treatments: $t = 7.334$; df = 144; $P < 0.001$; control treatments: $t = 7.509$; df =193; $P < 0.001$).

Table 2.6. Mean number of RIFA observed on the EB1:1 during daytime (light) observations by treatment and 95% confidence intervals. Means (*P. tricuspis* n = 146; *P. curvatus* n = 146; Control n = 195) are for all replications.

Treatment	**Min/Max**	**Mean ± SE**	**95% confidence intervals**	
			Lower Bound	**Upper Bound**
P. tricuspis	0/23	2.90 ± 0.572^{a}	1.76	4.04
P. curvatus	0/93	7.22 ± 1.520^{b}	4.19	10.25
Control	0/47	6.36 ± 0.970^{ab}	4.43	8.28

Means followed by the same letter were not significantly different using Tukey's HSD Post Hoc Analysis.

Table 2.7. Mean number of RIFA observed on the CABB during daytime (light) observations by treatment and 95% confidence intervals. Means (*P. tricuspis* n = 146; *P. curvatus* n = 146; Control n = 195) are for all replications.

Treatment	**Min/Max**	**Mean**	**95% confidence intervals**	
			Lower Bound	**Upper Bound**
P. tricuspis	0/88	18.5 ± 2.442^{b}	13.63	23.37
P. curvatus	0/153	41.93 ± 4.538^{a}	32.88	50.98
Control	0/116	32.49 ± 3.359^{a}	25.83	39.16

Means followed by the same letter were not significantly different using Tukey's HSD Post Hoc Analysis.

For all nighttime (dark) observations, the treatments were statistically equivalent for mean number of ants observed on the EB1:1 candidate bait ($F = 0.601$; df = 2,161; $P = 0.550$; Fig. 2.8). The CABB was statistically different in the mean number of ants observed on the bait cups for the nighttime observations ($F = 3.789$; df = 2,164; $P < 0.05$; Fig. 2.8). The *P. tricuspis* treatment had significantly fewer RIFA observed on the bait than the *P. curvatus*, and was statistically equivalent to the control. The control and *P. curvatus* treatments were statistically equivalent. There was a statistically significant difference between the EB1:1 and CABB during dark observations for the *P. tricuspis* treatment (*t* = 6.143; df =73; *P* < 0.001), for the *P. curvatus* treatments (*t* = 7.045; df = 55; *P* < 0.001), and for the control treatment (*t* = 7.037; df = 80; *P* < 0.001; Fig. 2.9; Tables 2.8 and 2.9), with the most RIFA always observed on the CABB bait.

Table 2.8. Mean number of RIFA observed on the EB1:1 during dark observations by treatment and 95% confidence intervals. Means (*P. tricuspis* n = 99; *P. curvatus* n = 99; Control n = 126) are for all replications.

Treatment	**Min/Max**	**Mean ± SE**	**95% confidence intervals**	
			Lower Bound	**Upper Bound**
P. tricuspis	0/62	4.55 ± 1.341[a]	1.85	7.25
P. curvatus	0/23	4.94 ± 0.678[a]	3.58	6.30
Control	0/57	6.24 ± 1.294[a]	3.65	8.83

Means followed by the same letter were not significantly different using Tukey's HSD Post Hoc Analysis.

Table 2.9. Mean number of RIFA observed on the CABB during dark observations by treatment and 95% confidence intervals. Means (*P. tricuspis* n = 99; *P. curvatus* n = 99; Control n = 126) are for all replications.

Treatment	**Min/Max**	**Mean ± SE**	**95% confidence intervals**	
			Lower Bound	**Upper Bound**
P. tricuspis	0/84	22.58 ± 2.610[b]	17.33	27.83
P. curvatus	0/144	37.00 ± 4.500[a]	27.98	46.02
Control	0/93	31.60 ± 3.364[ab]	24.88	38.33

Means followed by the same letter were not significantly different using Tukey's HSD Post Hoc Analysis.

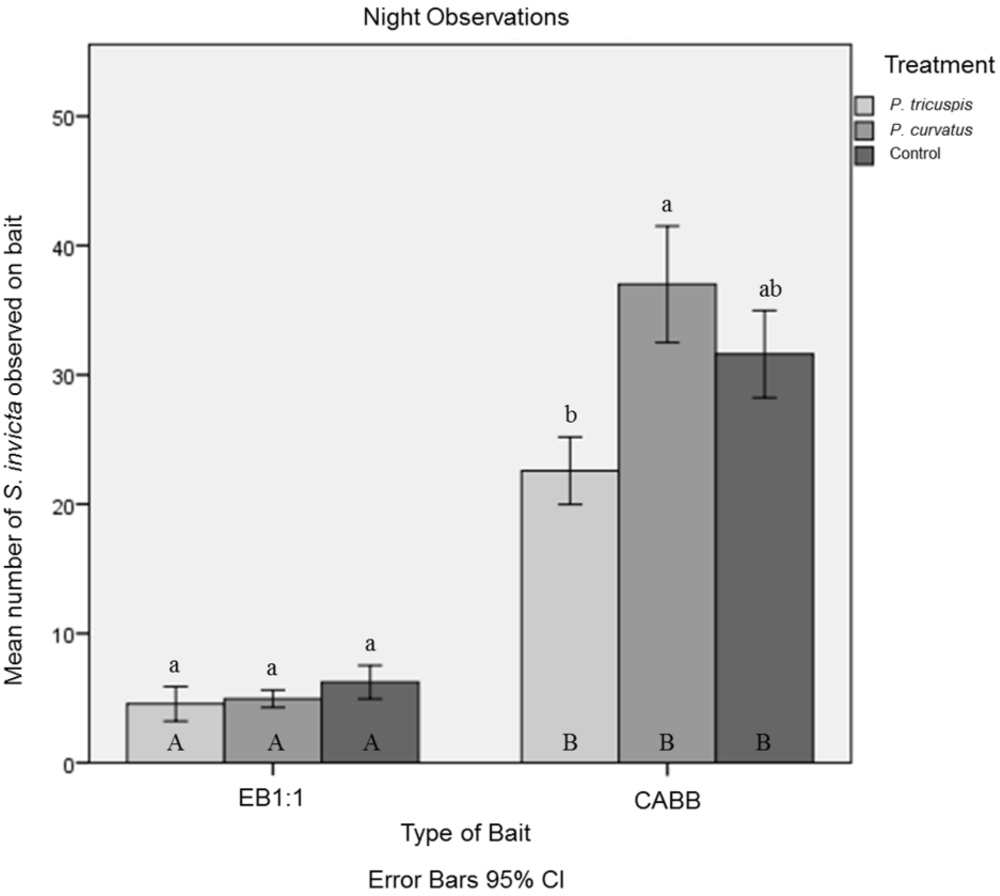

Fig. 2.8. Mean number of RIFA observed on bait cups by treatment type during the nighttime (dark) observations. For all night observations (*P. tricuspis* n = 99; *P. curvatus* n = 99; Control n = 126) the EB1:1 observations were statistically equivalent, but the mean number of ants observed on bait cups for the CABB type bait were significantly different $F = 3.789$; df = 2,164; $P < 0.05$) (indicated by lowercase letters). There was a significant difference between the number of ants on the EB1:1 bait and the CABB bait for all treatment types (indicated by uppercase letters) (*P. tricuspis* treatments: $t = 6.143$; df =73; $P < 0.001$; *P. curvatus* treatments: $t = 7.045$; df = 55; $P < 0.001$; control treatments: $t = 7.037$; df = 80; $P < 0.001$).

Weight of baits removed by RIFA

When considered by bait type, there was also no significant difference between mean amount of total bait removed by RIFA when exposed by the treatment types

(EB1:1 bait: *F* = 0.367; df = 2,57; *P* = 0.694) (CABB bait: *F* = 1.158; df = 2,57; *P* = 0.321) (Fig. 2.9). When analyzing the amount of bait removed by treatment there was no statistically significant difference in the mean amount of bait removed between the EB1:1 and CABB type baits for the *P. curvatus* treatment (*t* = 1.884; df = 30; *P* = 0.069) or for the control treatment (*t* = 2.005; df = 50; *P* = 0.05). There was a statistically significant difference between the EB1:1 and CABB for the *P. tricuspis* treatment (*t* = 3.033; df = 34; *P* < 0.01; Fig. 2.9; Tables 2.10 and 2.11).

Table 2.10. Mean amount of the EB1:1 removed from bait cups by treatment and 95% confidence intervals. Means are for all replications.

Treatment	**Min/Max**	**Mean ± SE**	**95% confidence intervals**	
			Lower Bound	**Upper Bound**
P. tricuspis	-0.63/1.60	0.331 ± 0.146^{a}	0.235	0.638
P. curvatus	-0.62/1.90	0.481 ± 0.218^{a}	0.154	0.946
Control	-0.61/2.43	0.551 ± 0.108^{a}	0.168	0.934

Means followed by the same letter were not significantly different using Tukey's HSD Post Hoc Analysis.

Table 2.11. Mean amount of the CABB removed from bait cups by treatment and 95% confidence intervals. Means are for all replications.

Treatment	**Min/Max**	**Mean ± SE**	**95% confidence intervals**	
			Lower Bound	**Upper Bound**
P. tricuspis	-0.65/0.48	-0.167 ± 0.075^{b}	-0.325	-0.008
P. curvatus	-0.67/1.42	0.124 ± 0.119^{a}	-0.241	0.266
Control	-0.60/2.79	0.089 ± 0.137^{ab}	-0.193	0.370

Means followed by the same letter were not significantly different using Tukey's HSD Post Hoc Analysis.

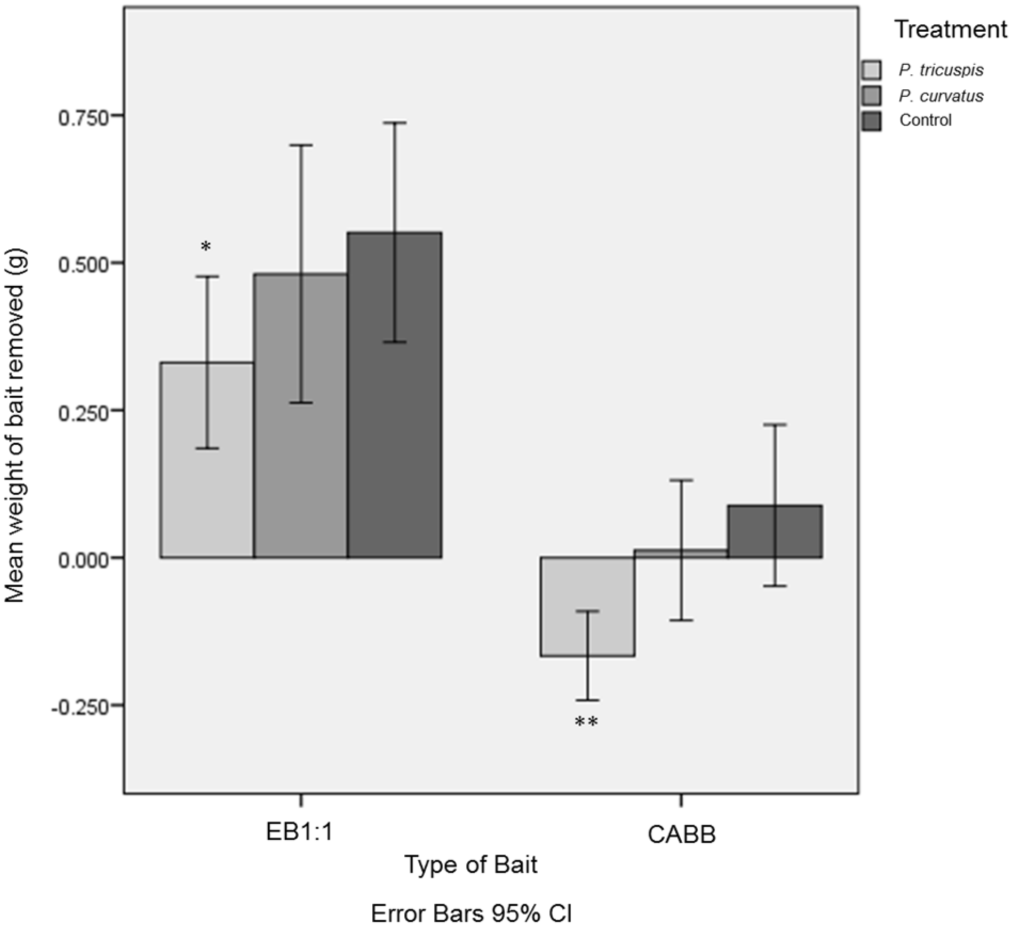

Fig. 2.9. Mean weight of bait removed by RIFA by bait type and treatment. The mean (*P. tricuspis* n = 99; *P. curvatus* n = 99; Control n = 126) amount of bait removed by RIFA for the *P. tricuspis* treatments were statistically significant (indicted with asterisks) between the EB1:1 and CABB baits ($t = 3.033$; df = 34; $P < 0.01$). There was no difference between the EB1:1 and CABB baits for the control or *P. curvatus*.

Discussion

RIFA assume a defensive posture to defend themselves from attack by phorid parasitoids. This defensive posture, and the subsequent cessation of movement, reduces foraging effectiveness by the worker under attack. A single active parasitoid fly can affect up to 100 individual ants by causing them to hold this antiparasitoid defensive posture (Feener and Brown 1992, Morrison 2000).

As shown in this work, the presence of *P. tricuspis* caused a significant drop in the number of ants foraging during the daylight hours and there was also a trend for an increased number of foragers observed during the dark observations as compared to the control. However, no effects were shown by ants exposed to *P. curvatus* as compared to the control. These results were similar to those of Wuellner et al. (2002), where the authors reported various species of phorid flies impacted colony behavior to differing degrees. This work supports their finding that *P. curvatus* attacks isolated ants, reducing the colony level affect it has on RIFA whereas *P. tricuspis* attacks groups of ants increasing the colony level effect (Gilbert and Morrison 1997, Wuellner et al. 2002). Confounding these conclusions is that in this experiment, RIFA exposed to *P. curvatus* showed a significantly higher rate of foraging in the night observations as compared control observations. Also, there was a reduction in daytime foraging, in the presence of *P. tricuspis*, and trend for an increase in nighttime foraging in the presence of both species of phorid flies. This alteration in foraging behavior could result in a reduction of a competitive advantage and possibly a reduction of colony fitness. Mehdiabadi et al. (2004) provided evidence that the presence of *P. tricuspis* helps provide competitive advantage to a native ant species over RIFA, but there was no evidence of colony growth in the native ant after a period of nearly two months. The overall effect of this loss of competitive advantage has yet to be shown unequivocally.

Without exception, RIFA were observed on the CABB more often than the EB1:1. This disparity was seen continuously in each treatment. The total number of ants observed on bait grit in the presence of *P. tricuspis* was, again, significantly lower than the control group coinciding with the results from RIFA crossing the foraging bridge. However, *P. curvatus* treatments showed no difference in the number of ants observed on bait. There also was no difference in the number of RIFA observed on the bait cups between day and night observations.

RIFA from both treatment groups and the controls always were observed on the CABB more than the EB1:1. They were consistently highly attracted to the CABB, often staying on the bait, possibly collecting oil and feeding in situ on small particles from the bait matrix. There was a trend for foragers to transport more of the EB1:1 than the CABB back to their colony space. This is contradictory to the total ants observed on the different candidate baits, where 3-4 times more RIFA were observed on the CABB. Foragers seemed to settle on CABB, taking very little of the solid bait material back to the colonoid nest site, however, solid particles from the EB1:1 bait were physically moved. The EB1:1 candidate bait was not enriched with liquid oils; it only contained solid fats from the egg powder additive.

The CABB controls were inadvertently exposed to high humidity during two of the replications of this experiment. The control weights were considerably higher after the experiment, so corrected weights were lower, sometimes causing negative total removal numbers (Fig. 2.9). However, removal by RIFA foragers of the CABB was very low for all replications.

All colonoids in this study included brood, affecting the overall dietary needs of the colony. Only fourth instar larvae are capable of processing solid food due to their sclerotized mandibles and a lack of a constriction in the digestive system between the thorax and abdomen (Petralia and Vinson 1978). Additionally, fourth instar larvae normally process protein rich foods (Sorensen et al. 1983). The EB1:1contained a 1:1 ratio of proteins to carbohydrates making it very nutrient rich, but it lacked any liquid oil/fat component. Due to feeding limitations of adult RIFA, foragers removed and transported this material to developing brood for processing. The oils/fats and other small particles in the CABB were attractive to the RIFA foragers because the adult workers could gather and process these in situ. Foragers could not process the solid corn cob grit nor is it nutritionally valuable to brood, so foragers did not to transport the solid particles. Additionally, the corn grit component of the CABB is not as nutrient rich as the laboratory made bait, so foragers did not transport the corn grit.

During this experiment the colonoids were small and contained a small amount of brood. Although the adults were actively foraging throughout the study, the colonoids may not have been large enough to accurately predict colony foraging habits. Additionally, the time allowed the foragers to discover and recruit to the two available candidate baits was abbreviated. However, the results in this study were consistent with several other studies showing *P. tricuspis* inhibiting foraging at significant levels

(Porter 1998a) and *P. curvatus* having less of an effect on overall foraging (Wuellner et al. 2002, Porter 2000) due to the host locating habits of the parasitoids.

Implications of this work include that, even in the presence of phorid flies, RIFA foragers were attracted to the CABB and spend a great deal of time antennating and feeding in situ on the material. If the active ingredient incorporated into the bait is lipophilic it is more likely to be retrieved by the foragers as well as absorbed via the wax layer in the integument. Incorporating additional materials into a bait or mixing bait attractants to cover all foraging needs could be successful. Developing a method to measure oil and small particle removal by ants out of a bait would allow for further investigation and insight into this phenomenon.

Ultimately, the analysis failed to reject the null hypotheses for these experiments. There was no significant difference in resource attraction or removal between RIFA exposed to phorid flies and those that were not exposed. There was also no significant difference between the bait type preferences between RIFA exposed to phorid flies and those that were not exposed.

CHAPTER III

PHORID FLY INDUCED EFFECTS ON RIFA FORAGER SIZE RATIOS: IMPLICATIONS OF BAIT SIZE AND COMPOSITION PREFERENCES

Introduction

Red imported fire ants (*Solenopsis invicta* Buren Hymenoptera: Formicidae) (RIFA) are considered a pest not only because they are medically important, non-native 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). Discovered initially in Mobile, Alabama, in the 1930's, they were imported from South America and probably mistakenly gained entrance via shipping dunnage (Buren 1972, Vinson 1997). A lack of a full suite of natural enemies, their propensity to invade disturbed habitats, and highly efficient foraging behavior has allowed RIFA to successfully colonize most of the southern United States (Helms and Vinson 2005).

Phorid flies in the genus *Pseudacteon* spp. attack workers in the *Solenopsis saevissima* complex of fire ants. These flies are also native to South America and have been introduced as biological control agents in several states in the United States (Folgarait et al. 2002, Porter 1998b, 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 RIFA/phorid interactions demonstrated minimal effect on RIFA with a low potential for successful biological control due to parasitism rates of approximately 1-3% (Feener and Brown 1992, Orr et al. 1995, Morrison and Porter 2005). However, later work revealed that *Pseudacteon* spp. activity could negatively affect competitive success of the RIFA colony due to a reduction in foraging (Feener 1981, Porter et al. 1995c, Morrison 1999). This reduction in foraging is an important consideration with respect to the competitive advantages RIFA demonstrate in the absence of the flies, and in the presence of the flies this advantage for the discovery and dominance of available resources could favor native ants.

Modern RIFA control relies primarily on ant baits formulated from a food attractant, usually corn grits, which are impregnated or coated with soybean oil and insecticide dissolved in the oil. The oil acts as a food attractant to foraging ants. 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 RIFA 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 standard, and this work was conducted on RIFA that were not exposed to attack by phorid flies (Hooper-Bùi et al. 2002).

Work conducted by Puckett and Harris (2010) showed that populations of RIFA that were exposed to and attacked by certain *Pseudacteon* spp. showed a change in the ratios of forager sizes compared to those at a site with no phorid activity (Fig. 3.1). This phenomenon has similarly been documented in the native range of RIFA and

Pseudacteon spp. flies in South America (Chirino et al. 2009). This shift in worker size might presumably change the optimal granular bait grit size needed to suppress RIFA populations where phorids are active, which 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.

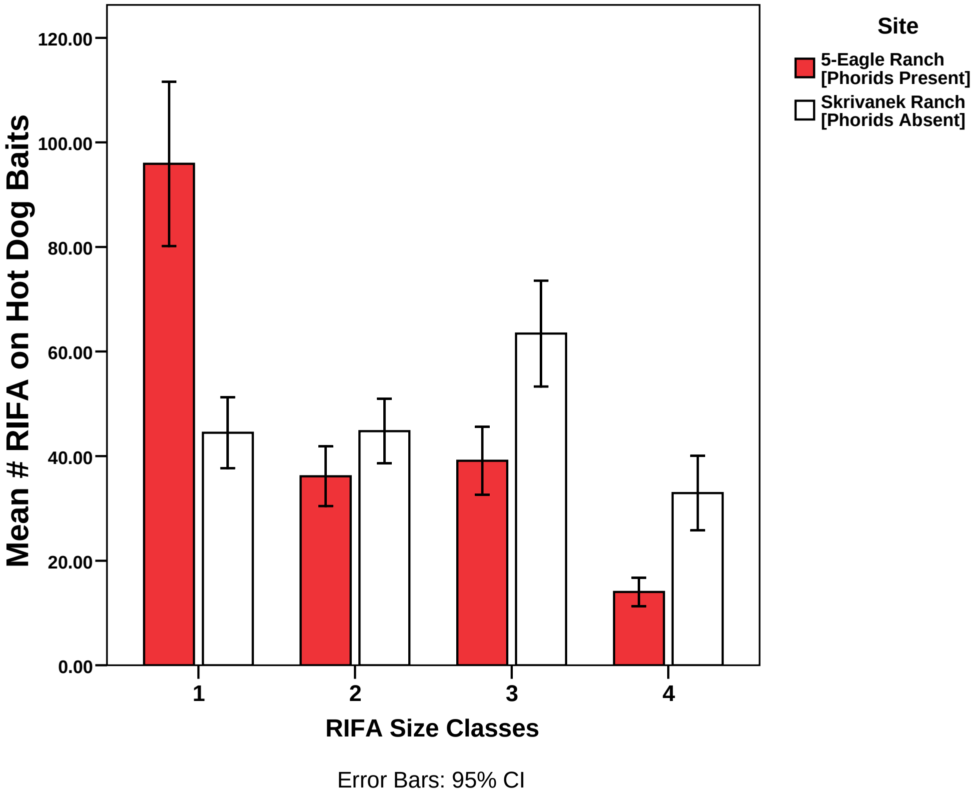

Fig. 3.1. RIFA forager size class differentials collected from hot dog lures. Data shown is from combined AM and PM sampling periods ($\chi^2 = 6811.85$, df = 3, $P < 0.05$). Size classes 1, 2, 3, and 4 correspond with sieve sizes 20, 14, 12, and 10 respectively (Puckett and Harris 2010).

This study addressed three questions through a variety of field experiments, and the null hypotheses of these experiments are: 1) there will be no significant difference in the granular bait grit selected by phorid-impacted RIFA colonies and RIFA colonies that are not phorid impacted; 2) RIFA foragers will equally select two bait types (CABB and EB1:1) in both the phorid impacted and the non-phorid impacted areas; 3) RIFA foragers from phorid impacted colonies will have similar size ratios as foragers where phorids are absent; and 4) RIFA from within phorid impacted colonies will be of similar size ratios as RIFA from within colonies where phorids are absent. This experiment was completed with the goal of forecasting potential effects biological control introductions might have on standard RIFA control efforts and to elucidate changes that might need to be made to those methods where RIFA and *Pseudacteon* spp. phorids occur together.

Experimental design

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, respectively and were established at 5-Eagle Ranch by 2003 and 2005, respectively. While the range of field released *Pseudacteon* phorids is constantly expanding, phorids had not been found within approximately 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.

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 species, presence, and relative abundance; 3) RIFA caste size 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 size selected by RIFA. While the field sites are similar in terms of ecoregion, ant assemblage, and habitat, they are separated by approximately 40 kilometers. As a result, sampling periods were based on civil twilight to ensure that samples relate to RIFA and phorid circadian rhythm, rather than the anthropogenic 24 hour clock. According to the U.S. Naval Observatory (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 normal activities. Sampling was avoided on days when rain was forecasted, but not always successfully. Both sites, Skrivanek Ranch and 5 Eagle Ranch, were sampled simultaneously with the aid of a fellow graduate student and student workers. 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) (Fig. 3.2) were deployed 3 m apart at each location (Fig. 3.3 and 3.4). 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.

Fig. 3.2. Example of a PTS trap (as described by Puckett et al. 2007) used in this study.

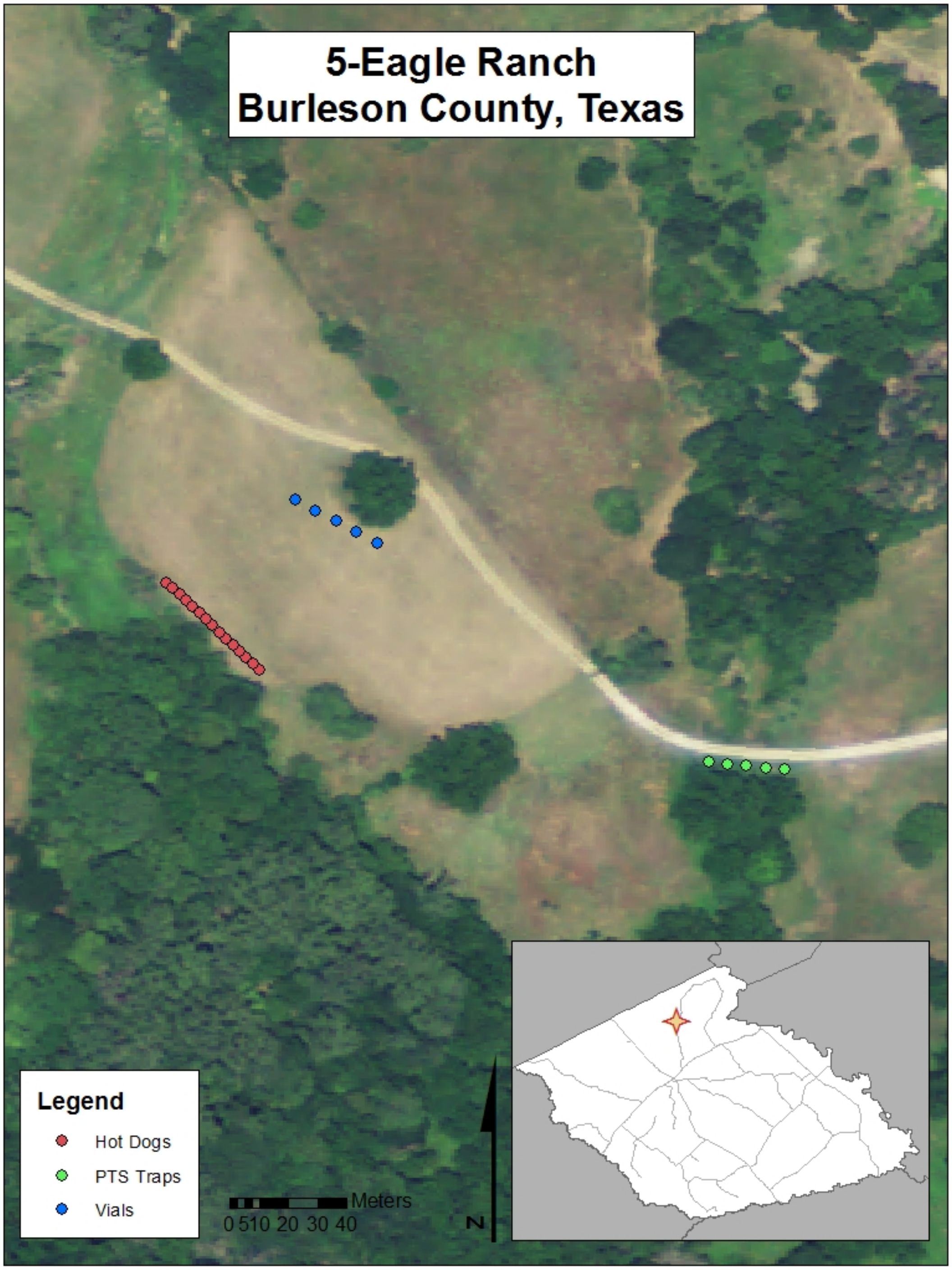

Fig. 3.3. Map of 5-Eagle Ranch in Burleson County, Texas and locations of PTS traps, hot dog traps, and bait vial arrays.

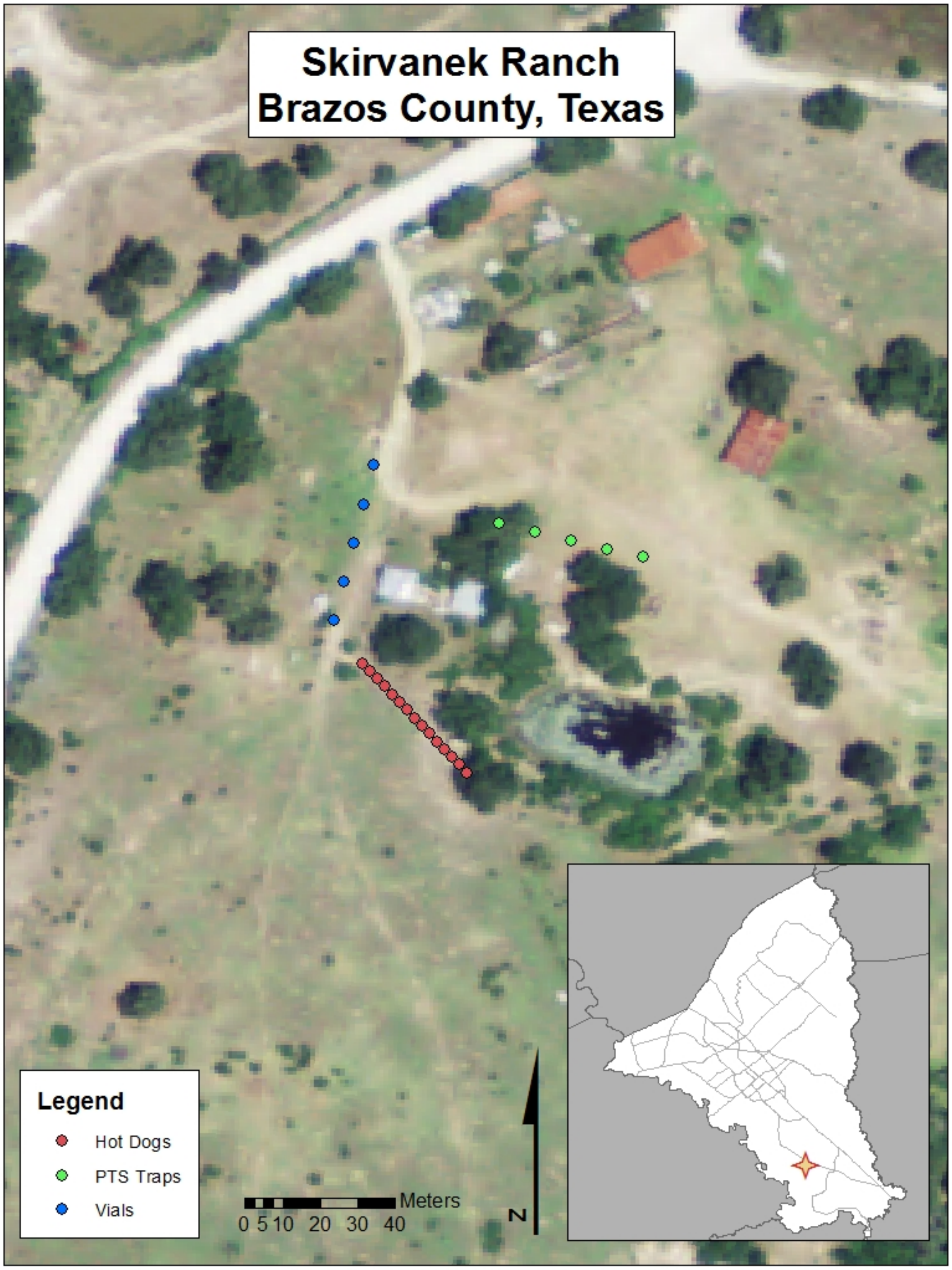

Fig. 3.4. Map of Skrivanek Ranch in Brazos County, Texas and locations of PTS traps, hot dog traps, and bait vial arrays.

A transect of 15 hot dog lures (Bestelmeyer et al. 2000) were deployed at AM civil twilight and 20 minutes prior to PM civil twilight. Slices (approximately 0.6 cm) of hot dogs (Bar S® Franks, Bar S Foods, Phoenix, AZ) were centered on note cards which were placed along the transect at a distance of 3 m apart (Fig. 3.3 and 3.4). Hot dog lures remained available for foraging ants for 20 minutes. 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 small 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. In order to obtain a random sample, the microtubes were inserted into RIFA mounds until the upper rim was flush with the top of the mound. Microtubes were then left in place for 5 minutes or until the tube was full. 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 RIFA head width determination were obtained by measuring RIFA which were collected by two discreet sampling types from the field, the food (hot dog) lures and samples obtained directly from colonies (mounds). 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, and a subsample was obtained by randomly removing approximately 15%, using a large pipette. Individual ant heads were removed and measured using a wedge micrometer method described by Porter (1983) (Fig. 3.5). 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 sizes was used to facilitate measuring head widths of RIFA collected from hot dog samples and colony samples quickly and accurately. Those sizes 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 correspond 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.

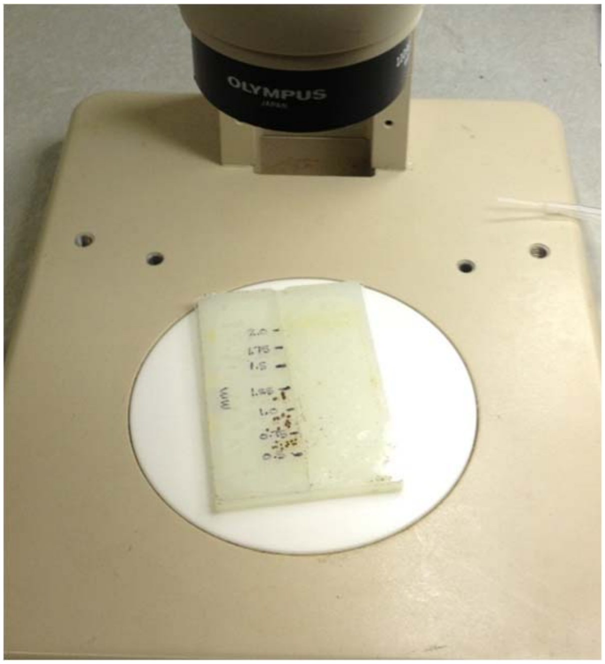

Fig. 3.5. Wedge micrometer used to determine RIFA head widths as described by Porter (1983).

In order to determine grit size and bait type preference of RIFA in phorid and non-phorid infested areas, field bioassays with four grit sizes were conducted. Two different bait types were used for these tests: a 1:1 ratio of carbohydrate to protein bait (EB1:1) described by Cook et al. (2010), and an industry provided blank bait. The industry provided blank bait (TC-206 Advance Granular Carpenter Ant Scatter Bait), was 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 grit 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, and availability of sieves. The baits were coded by type and size in the following manner: CABB signified the industry provided corn grit while “EB1:1” signifies the experimental, 1:1 ratio bait. Prior to sampling days, baits were weighed, recorded, and placed into one of 8 individual vials, with approximately 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 17mm, a width of 38 mm, and length of 200 mm and then drilled with eight, 16 mm holes equidistant apart to accommodate the vials (Fig. 3.6B). 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.

Fig. 3.6. Vial array arrangement (A) and a close up of the test vial array (B) for bait size and nutrition preference studies.

During both the morning and evening on each sampling day, a transect of five vial arrays located 10 m apart were deployed (Fig. 3.3 and 3.4). During morning sampling, bait arrays were deployed 5 minutes prior to civil twilight and during evening sampling; arrays were deployed 1 hour before civil twilight and were available to ant foragers for a total of 65 minutes. Care was taken to ensure the vials were flush with the ground (Fig. 3.6A) 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 RIFA 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. 3.6A). For laboratory analysis, the ending weight of the bait in these control vials was recorded for comparison to the beginning weight with consideration of water gain or loss. The total gain/loss for each vial was calculated and the average weight was added/subtracted from the corresponding test vials to obtain a corrected weight removed by ant foragers for analysis.

Statistical analyses were conducted using IPM SPSS Statistics version 19. Microclimate data and data from PTS traps were analyzed using Mann-Whitney non-parametric test because the data failed to meet the tests for normality. 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 and means were separated by using Tukey's Honestly Significant Difference (HSD) post hoc tests. Ranches were compared by head width using student's t-tests.

Results

Microclimate data

The mean temperatures at 5-Eagle Ranch (19.90±0.478° C) and Skrivanek Ranch (20.69 ± 0.045° C) were significantly different ($Z = 9.987$; $P < 0.001$) over the course of the study. Relative humidity was also very significantly different between 5-Eagle Ranches (73.82 ± 0.106%) and Skrivanek (71.62 ± 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 April through July of each year (Fig. 3.7). Both *P. curvatus* and *P. tricuspis* were present at both sites, but the mean number of flies collected between 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) (Fig. 3.8).

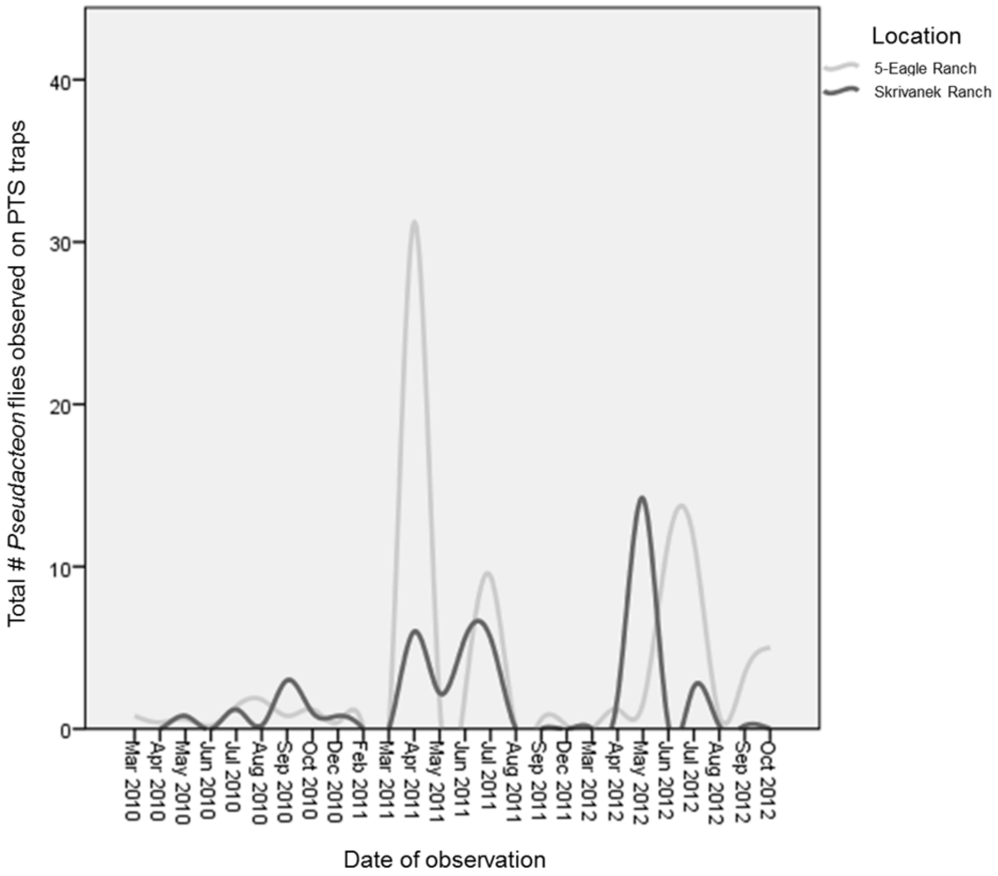

Fig. 3.7. Phenology of the total number flies of both species from PTS traps at 5-Eagle Ranch and Skrivanek Ranch from March 2010 through October 2012. Each sampling date represents five traps at each location. Only months in which sampling occurred are depicted on graph.

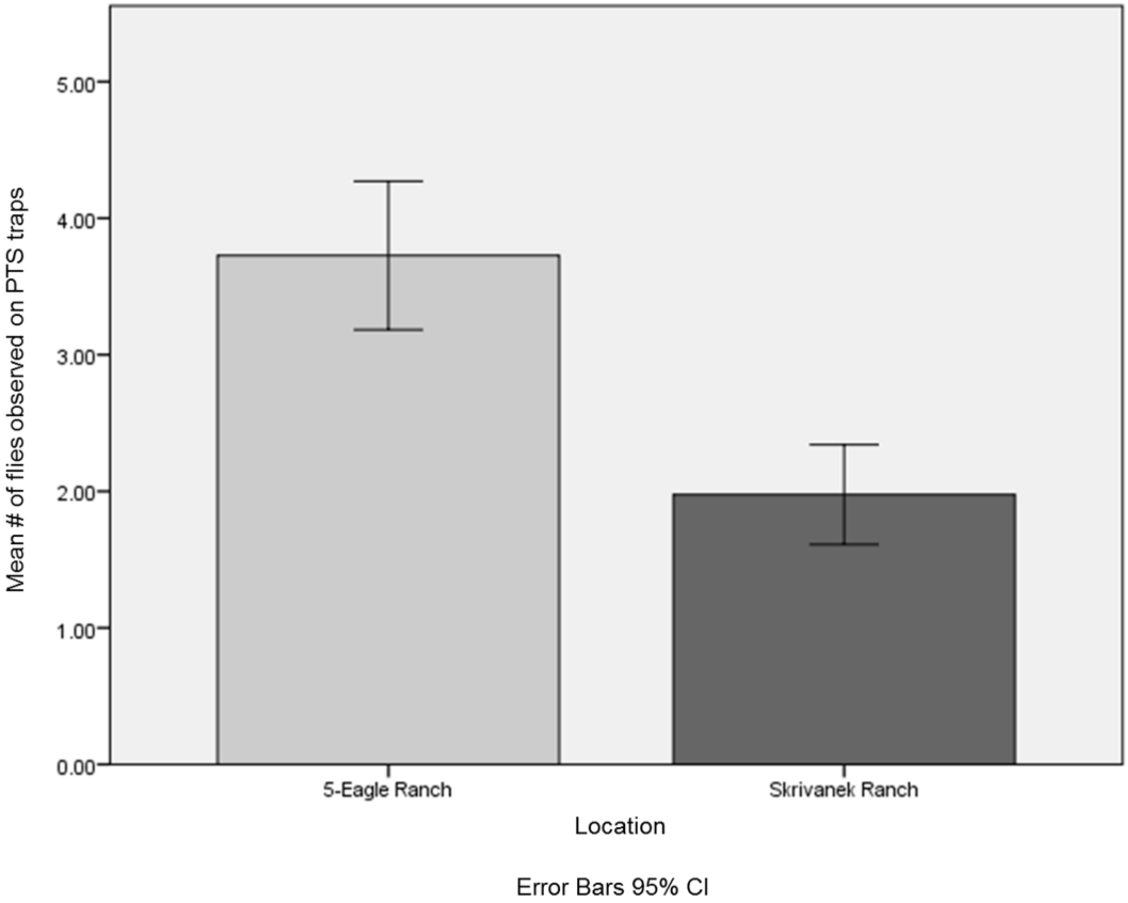

Fig. 3.8. Relative abundance of both species of *Pseudacteon* flies at 5-Eagle Ranch and Skrivanek Ranch for all sampling dates. The number of flies (n = 165) was significantly different between the two ranches (Z = 2.537; $P < 0.05$).

Both sampling sites had *P. curvatus* present (Fig. 3.9), but the mean number of flies collected between 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; $P < 0.01$; Fig. 3.10).

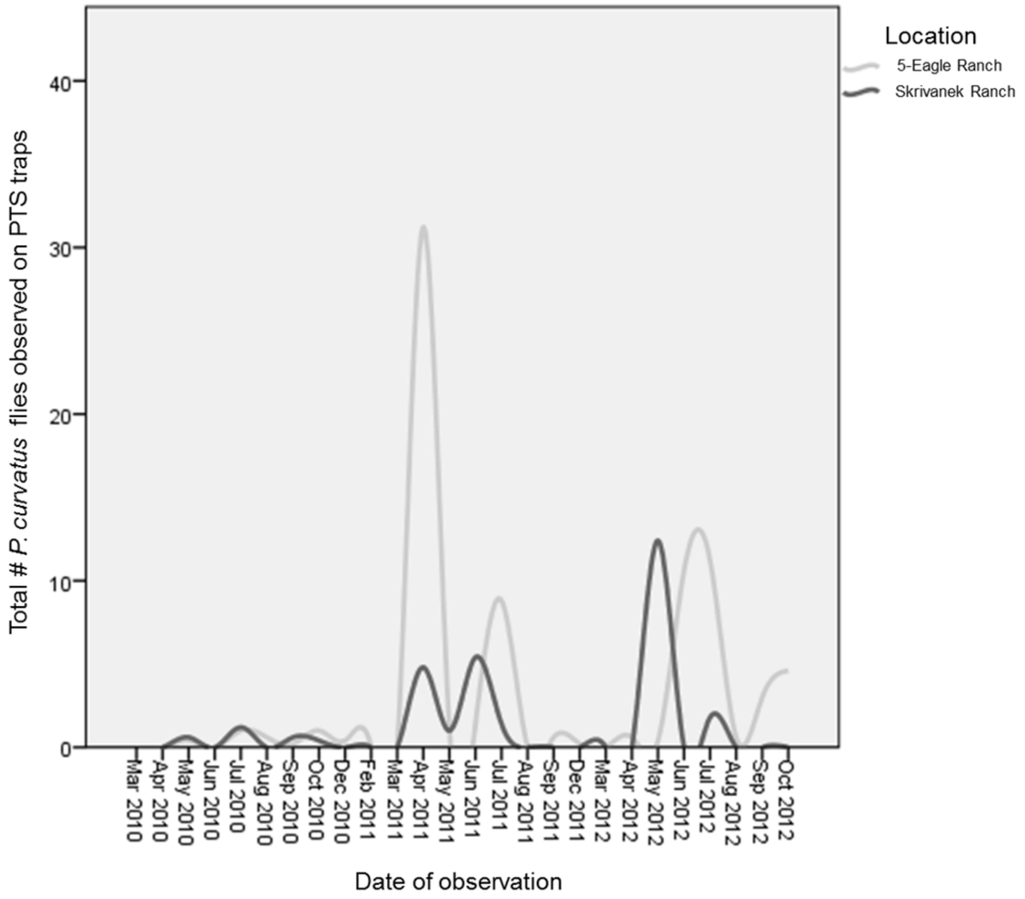

Fig. 3.9. Phenology of *Pseudacteon curvatus* flies from PTS traps at 5-Eagle Ranch and Skrivanek Ranch from March 2010 through October 2012. Each sampling date represents five traps at each location. Only months in which sampling occurred are depicted on graph.

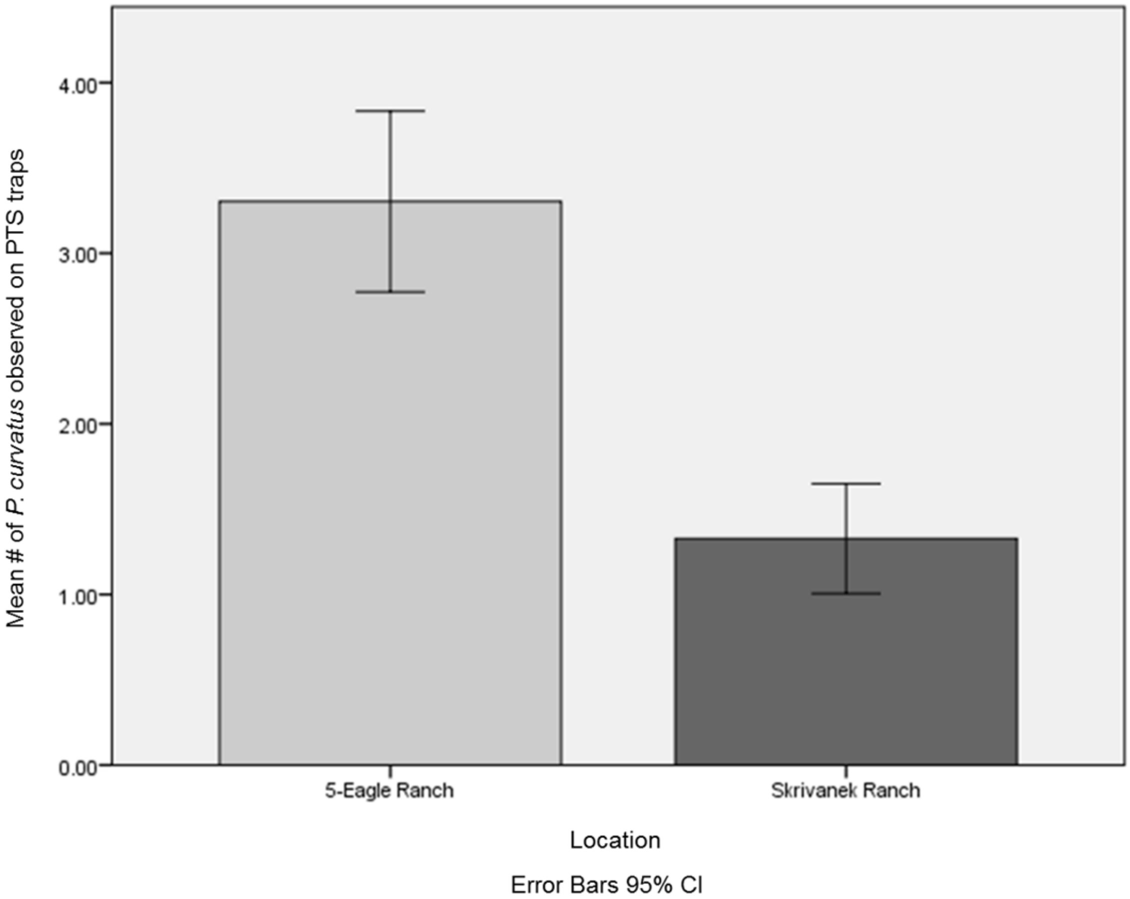

Fig. 3.10. Relative abundance of *P. curvatus* flies at 5-Eagle Ranch and Skrivanek Ranches for all sampling dates. The number of flies (n = 165) was significantly different between the ranches (Z= 2.638; $P < 0.01$).

Both sampling sites yielded *P. tricuspis* on the PTS traps (Fig. 3.11); 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) (Fig. 3.12).

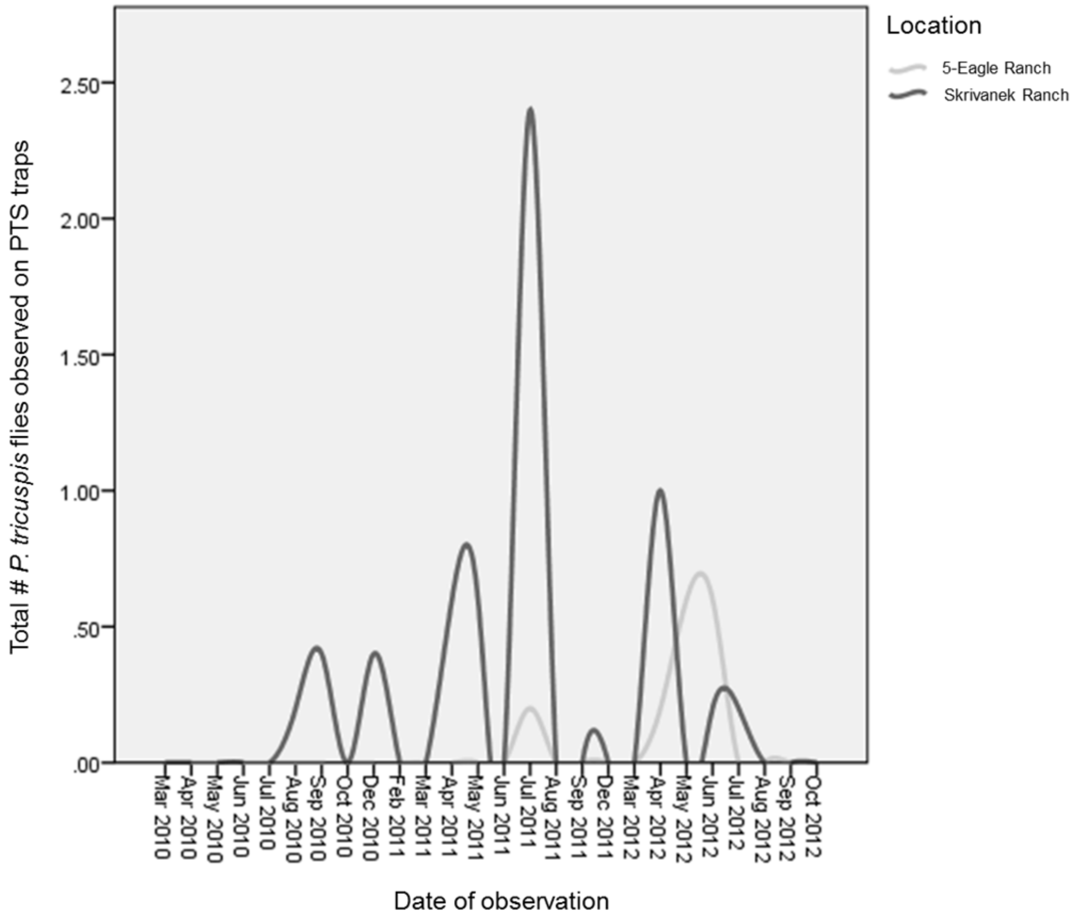

Fig. 3.11. Phenology of *Pseudacteon tricuspis* flies from PTS traps at 5-Eagle Ranch and Skrivanek Ranch from March 2010 through October 2012. Each sampling date represents five traps at each location. Only months in which sampling occurred are depicted on graph.

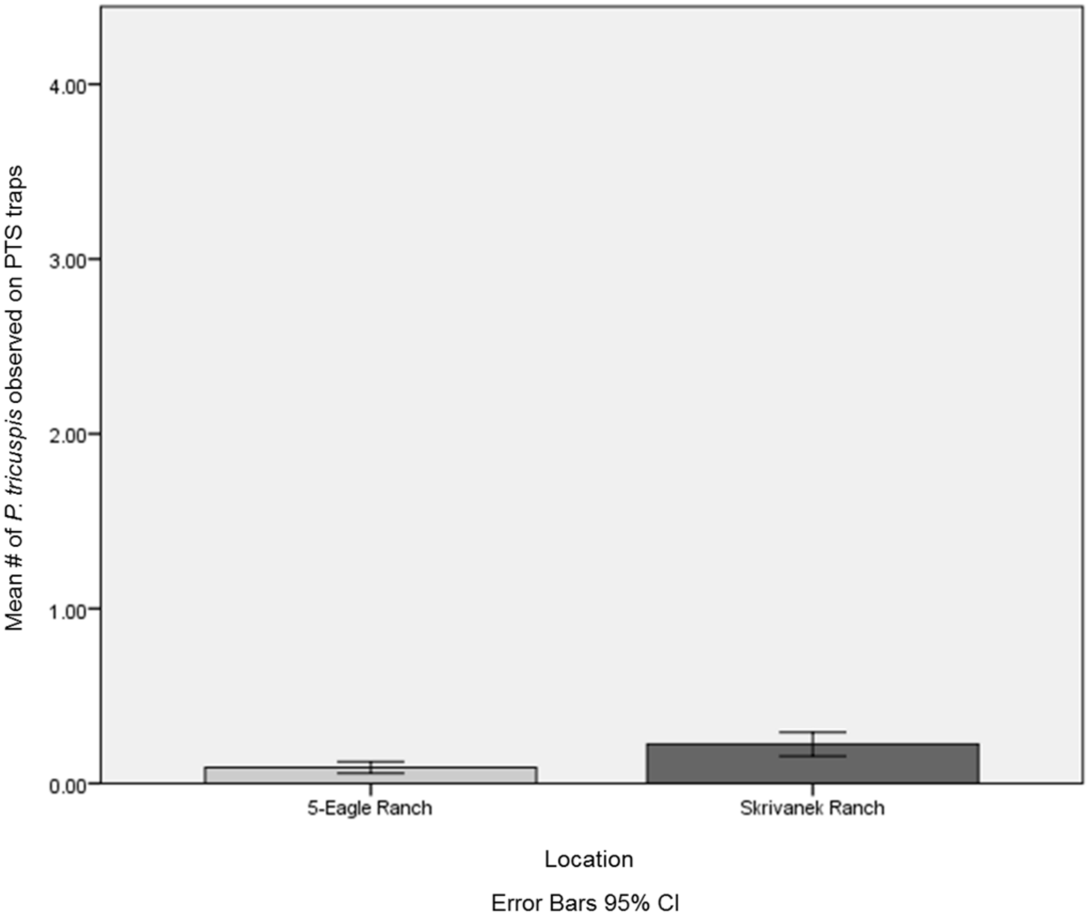

Fig. 3.12. Relative abundance of *P. tricuspis* flies at 5-Eagle Ranch and Skrivanek Ranch for all sampling dates. The number of flies (n = 165) found were significantly different between the ranches (Z = 2.144; $P < 0.05$).

RIFA forager observations on baits

For all data, 5-Eagle Ranch had significantly fewer RIFA than Skrivanek Ranch based on food lure (hot dog) data, colony sample data and forager abundance on bait grit 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. 3.13).

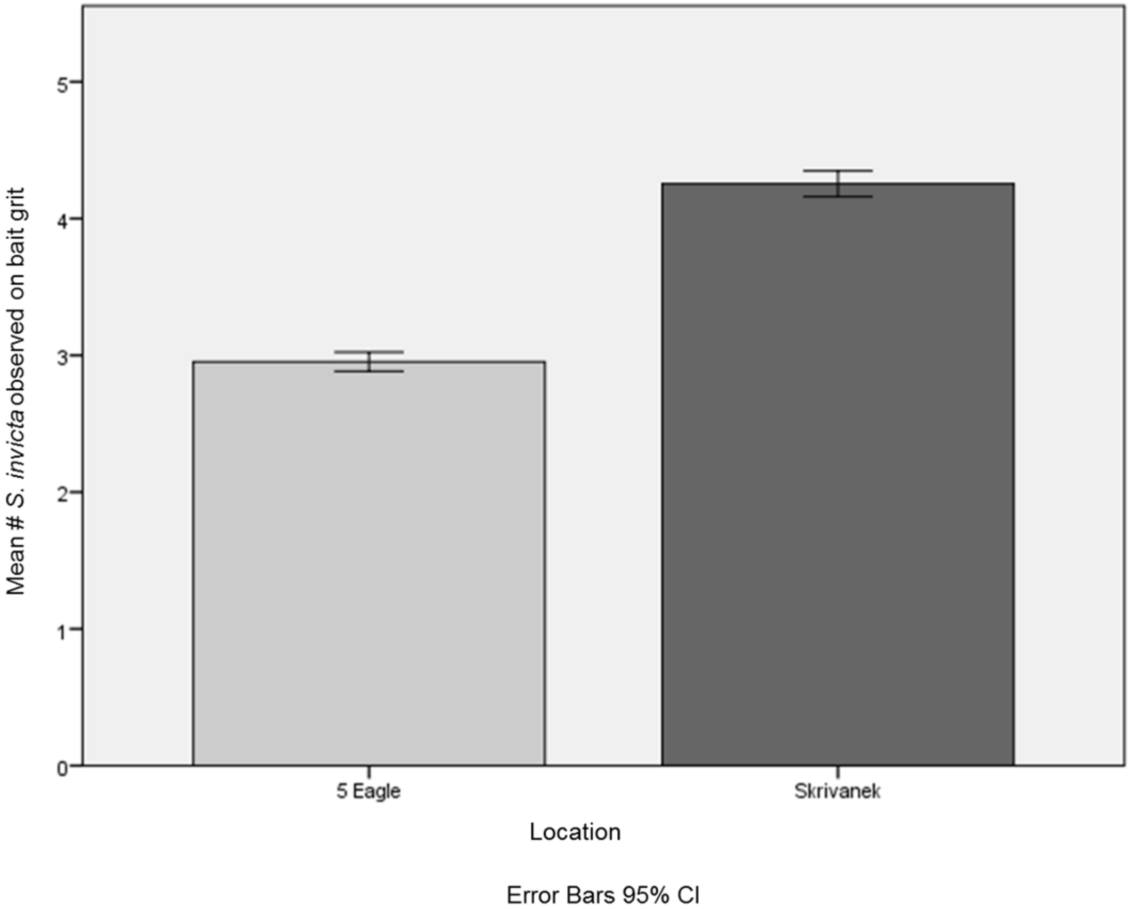

Fig. 3.13. Mean number of RIFA observed on bait grit vials from all observations at 5-Eagle Ranch (n = 8480) and Skrivanek Ranch (n = 8640) for all sampling dates. Mean number of ants were significantly different (*t* = 11.138; df = 17118; *P* < 0.05).

With respect to the number of ants observed on bait, there was a significant difference between the mean number of RIFA foragers in the morning between each ranch ($t = 10.975$; df = 8638; $P < 0.05$; Fig. 3.14) as well as a significant difference between the mean number of RIFA in the evening between each ranch ($t = 5.514$; df = 8478; $P < 0.05$; Fig. 3.14). The 5-Eagle Ranch had fewer RIFA (2.12 ± 0.078) than Skrivanek Ranch (3.66 ± 0.117) during the AM sampling. Similarly, 5-Eagle Ranch had fewer RIFA 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 RIFA foraging intensity during AM and PM observations at 5-Eagle ($t = 12.415$; df =8478; $P < 0.05$; Fig. 3.14). There was a significant difference between the mean number of ants observed on all the bait grit 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. 3.14).

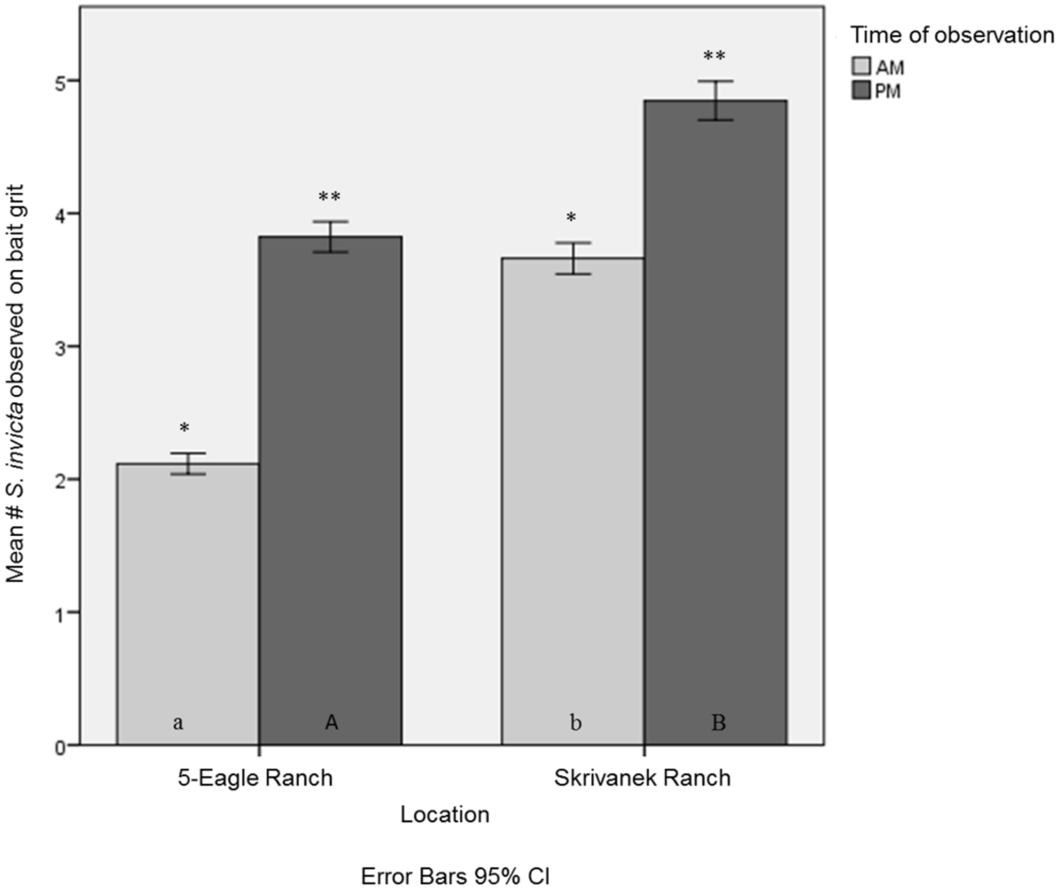

Fig. 3.14. Relative abundance of RIFA on bait grit in vials from morning and evening observations at 5-Eagle Ranch (n = 8480) and Skrivanek Ranch (n = 8640) for all sampling dates. Asterisks represent significant differences between time periods at both ranches [AM ($t = 10.975$; df = 8638; $P < 0.05$) and PM ($t = 5.514$; df = 8478; $P < 0.05$)] and letters represent significant differences between ranches for time periods (5-Eagle ($t = 12.415$; df =8478; $P < 0.05$) and Skrivanek ($t = 6.353$; df = 8638; $P < 0.05$).

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 S2 bait (Fig. 3.15). Foraging activity was higher in the PM in all instances with the greatest differences in C2, C3 and C4 baits

(Table 3.1). There was a significant difference between all the bait types for the AM observations (F = 6.276; df = 7,4312; $P < 0.001$) and PM observations (F = 25.823; df = 7,4152; $P < 0.001$) 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.

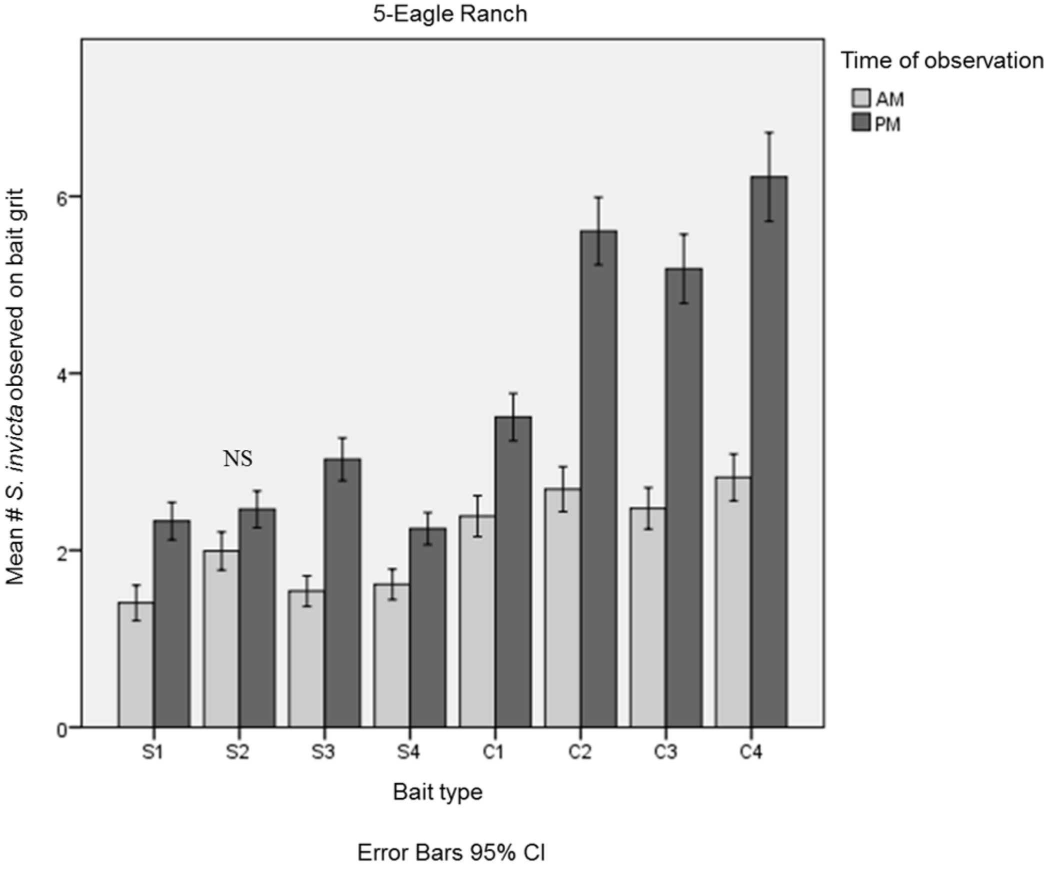

Fig. 3.15. Comparisons of overall mean RIFA foraging intensity on four size classes of two different baits at 5-Eagle Ranch by observation period. Means (n = 1060) noted with "NS" were not significantly different (Table 3.1). S1 – S4 in this graph represent the EB1:1 bait with a 1:1 carbohydrate:protein 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.

Table 3.1. Mean number of RIFA foragers observed at 5-Eagle Ranch (n = 1060) on four sizes of two different baits. EB1:1 represents a laboratory made bait with a 1:1 carbohydrate:protein ratio and CABB represents the industry provided bait. Size classes of 1 – 4 represent US standard sieves No. 25, 18, 14, and 10, respectively.

Bait Type	**Mean # RIFA ± SE at 5-Eagle**		**df**	**t statistic**	***P* value**
	AM	**PM**			
S1	1.41±0.201	2.33±0.212	1058	3.167	< 0.05
S2	1.99±0.216	2.46±0.208	1058	1.571	= 0.116
S3	1.54±0.173	3.03±0.242	1058	5.011	< 0.05
S4	1.61±0.173	2.24±0.181	1058	2.515	< 0.05
C1	2.39±0.231	3.51±0.268	1058	3.164	< 0.05
C2	2.69±0.255	5.61±0.381	1058	6.359	< 0.05
C3	2.47±0.233	5.18±0.390	1058	5.957	< 0.05
C4	2.82±0.263	6.22±0.501	1058	5.999	< 0.05

In all but one case, the mean number of RIFA 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 RIFA with respect to foraging on each bait type between the AM and PM observation periods. Only bait type S2 was statistically equivalent between the AM than the PM observations (Fig. 3.16; Table 3.2). When all bait types were compared for the AM observations at Skrivanek ranch, the baits were statistically equivalent ($F = 1.899$; df = 7,4312; $P = 0.66$). For all bait types in the PM at Skrivanek Ranch there was a significant difference ($F = 7.869$; df = 7,4312; $P < 0.001$) and CABB size class 4 was significantly different from all other bait types.

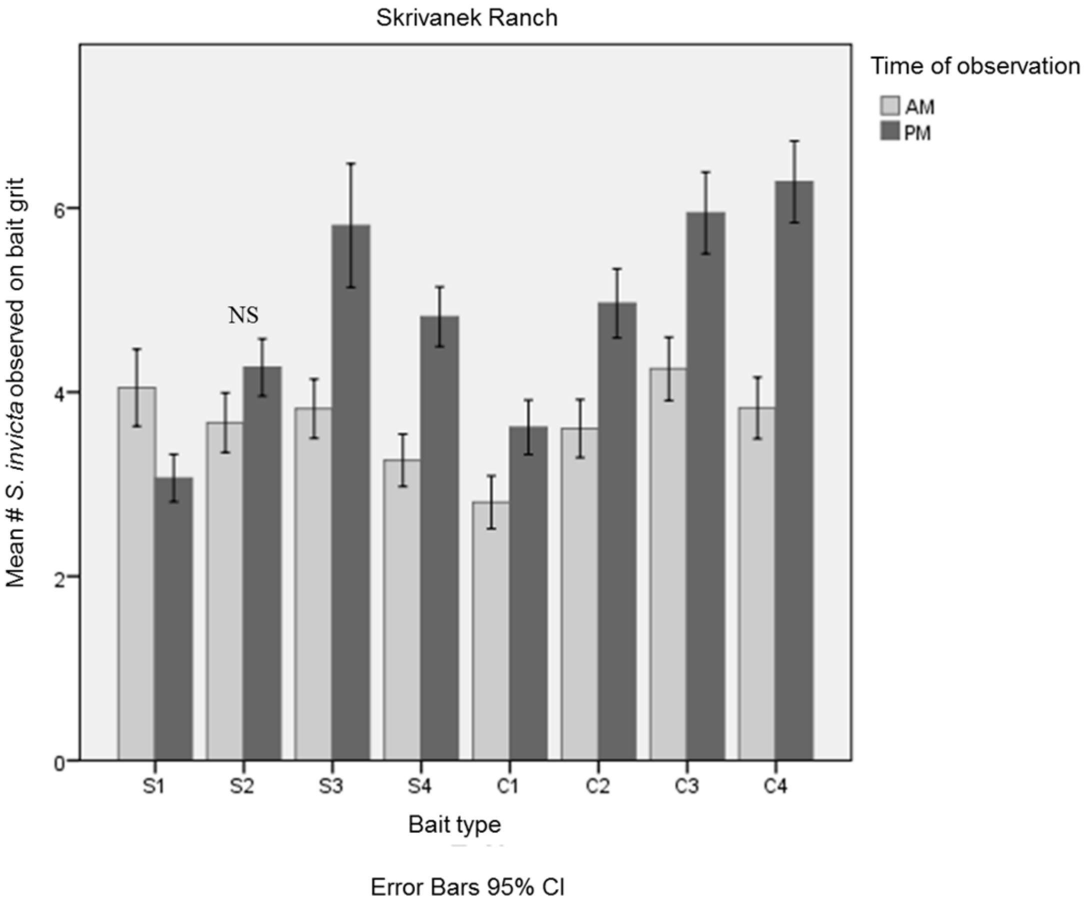

Fig. 3.16. Comparisons of overall mean RIFA foraging intensity on four size classes of two different baits at Skrivanek Ranch by observation period. Means (n = 1080) noted with “NS” were not significantly different EB1:1 represents a laboratory made bait with a 1:1 carbohydrate:protein ratio and CABB represents the industry provided bait. Size classes of 1 – 4 represent US standard sieves No. 25, 18, 14, and 10, respectively. Table 3.2 summarizes student’s t-test results for Skrivanek Ranch.

Table 3.2. Mean number of RIFA foragers observed at Skrivanek Ranch (n = 1080) on four sizes of two different baits. EB1:1 represents a laboratory made bait with a 1:1 carbohydrate:protein ratio and CABB represents the industry provided bait. Size classes of 1 – 4 represent US standard sieves No. 25, 18, 14, and 10, respectively.

Bait Type	**Mean # RIFA ± SE at Skrivanek**		**df**	**t statistic**	***P* value**
	AM	**PM**			
S1	4.05±0.417	3.07±0.258	1078	-2.002	< 0.05
S2	3.67±0.324	4.27±0.312	1078	1.334	= 0.182
S3	3.82±0.321	5.81±0.672	1078	2.670	< 0.05
S4	3.26±0.285	4.82±0.324	1078	3.619	< 0.05
C1	2.80±0.287	3.62±0.295	1078	1.977	< 0.05
C2	3.61±0.315	4.96±0.376	1078	2.772	< 0.05
C3	4.25±0.344	5.95±0.442	1078	3.021	< 0.05
C4	3.83±0.334	6.28±0.443	1078	4.427	< 0.05

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 (Fig. 3.17; Table 3.3). 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) and in the PM (F = 11.463; df = 7,1065; P < 0.001) at 5-Eagle Ranch (Fig. 3.17). The size 3 bait was significantly different from all other baits in the AM and PM in terms of the amount of bait removed from vials.

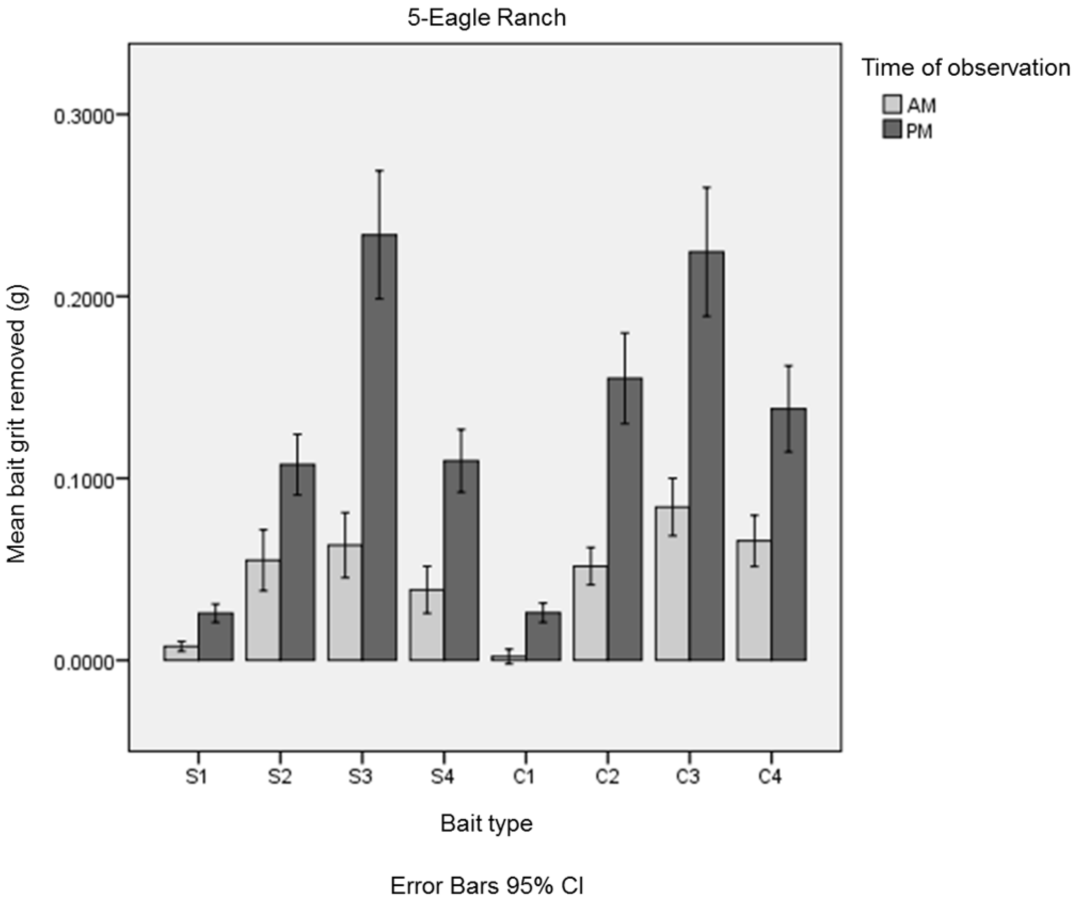

Fig. 3.17. Mean amount of bait grit (g) removed by RIFA at 5-Eagle Ranch at AM and PM observations of four size classes of two different baits. Means (n = 2167) shown are for all observations at 5-Eagle Ranch and all sampling dates. Table 3.3 contains results of the individual t-test results. All pairwise means were significantly different. EB1:1 represents a laboratory made bait with a 1:1 carbohydrate:protein ratio and CABB represents the industry provided bait. Size classes of 1 – 4 represent US standard sieves No. 25, 18, 14, and 10, respectively. Bait types indicated by different letters (lowercase for AM and uppercase for PM) were significantly different.

Table 3.3. Mean amount of bait removed by RIFA foragers at 5-Eagle Ranch at AM and PM observations. Results (n = 2167) are shown on four size classes of two different baits. EB1:1 represents a laboratory made bait with a 1:1 carbohydrate:protein ratio and CABB represents the industry provided bait. Size classes of 1 – 4 represent US standard sieves No. 25, 18, 14, and 10, respectively.

Bait Type and Size	**Mean amount removed± SE**		**t statistic**	***P* value**
	AM	**PM**		
S1	0.008 ± 0.003	0.026 ± 0.005	df (264) = 3.173	< 0.005
S2	0.055 ± 0.017	0.108 ± 0.017	df (268) = 2.224	< 0.05
S3	0.063 ± 0.018	0.234 ± 0.035	df (199) = 4.321	< 0.001
S4	0.039 ± 0.013	0.110 ± 0.017	df (247) = 3.294	< 0.005
C1	0.002 ± 0.004	0.026 ± 0.005	df (268) = 3.639	< 0.001
C2	0.052 ± 0.010	0.155 ± 0.025	df (178) = 3.824	< 0.001
C3	0.084 ± 0.016	0.224 ± 0.035	df (185) = 3.616	< 0.001
C4	0.066 ± 0.014	0.138 ± 0.024	df (208) = 2.634	< 0.01

There was a significant difference in the amount of bait removed (g) for each bait type at Skrivanek Ranch between the AM and the PM observations, with the amount of bait removed during the PM observations always being greater (Fig. 3.18; Table 3.4). 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 ($F = 10.636$; df = 7,1072; $P < 0.001$) and in the PM ($F = 19.104$; df = 7,1079; $P < 0.001$; Fig. 3.18) at Skrivanek Ranch (Fig. 3.17). 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.

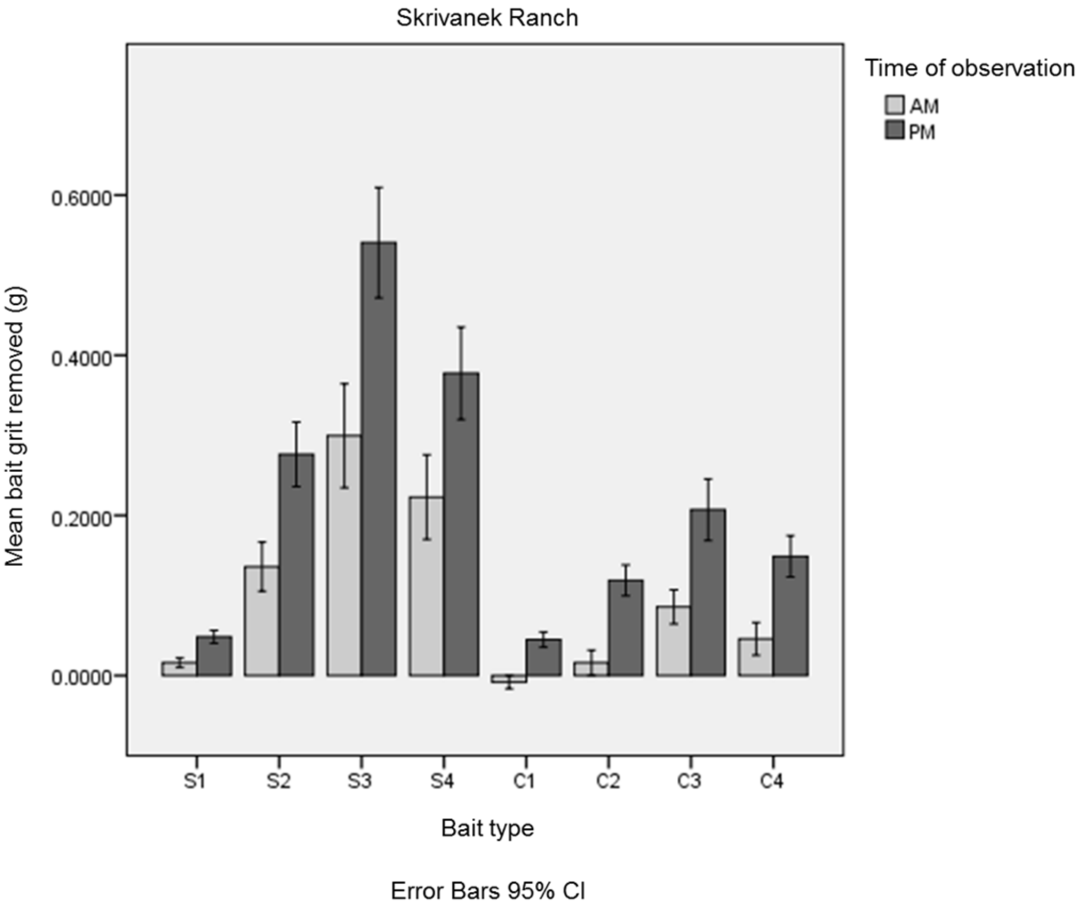

Fig. 3.18. Mean amount of bait grit (g) removed by RIFA at Skrivanek Ranch at AM and PM observations of four size classes of two different baits. Means (n = 2167) are for all observations at Skrivanek Ranch and all sampling dates. Table 3.4 contains results of the individual t-test results. Means noted “NS” were not significantly different. EB1:1 represents a laboratory made bait with a 1:1 carbohydrate:protein ratio and CABB represents the industry provided bait. Size classes of 1 – 4 represent US standard sieves No. 25, 18, 14, and 10, respectively.

Table 3.4. Mean amount of bait removed by RIFA foragers at Skrivanek Ranch at AM and PM observations. Results (n = 2167) are shown on four size classes of two different baits. EB1:1 represents a laboratory made bait with a 1:1 carbohydrate:protein ratio and CABB represents the industry provided bait. Size classes of 1 – 4 represent US standard sieves No. 25, 18, 14, and 10, respectively.

Bait Type and Size	**Mean amount removed± SE**		**t statistic**	***P* value**
	AM	**PM**		
S1	0.016 ± 0.006	0.048 ± 0.008	df (268) = 3.218	< 0.005
S2	0.136 ± 0.031	0.276 ± 0.040	df (251) = 2.770	< 0.01
S3	0.300 ± 0.064	0.541 ± 0.069	df (268) = 2.548	< 0.05
S4	0.223 ± 0.053	0.378 ± 0.058	df (268) = 1.978	< 0.05
C1	-0.008 ± 0.008	0.045 ± 0.010	df (262) = 4.225	< 0.001
C2	0.016 ± 0.016	0.119 ± 0.019	df (257) = 4.123	< 0.001
C3	0.086 ± 0.021	0.207 ± 0.038	df (210) = 2.765	< 0.01
C4	0.046 ± 0.020	0.149 ± 0.026	df (264) = 3.131	< 0.01

Head width measurements – hot dog lures

There was a significant difference in the abundance (n = 405) of RIFA foragers categorized by head width measurement from hot dog lures at 5-Eagle Ranch (F = 41.723; df = 5,4854; $P < 0.001$; Fig. 3.19). Significantly more RIFA with head widths 0.5-0.75 mm (Mean ± SE = 1.073 ± 0.152) was collected than all other head widths.

There was also a significant difference in the number of RIFA foragers collected by head width measurement from hot dog lures at Skrivanek Ranch (F = 171.187; df = 5,4854; $P < 0.001$; Fig. 3.19). The number of RIFA foragers collected in head width 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.

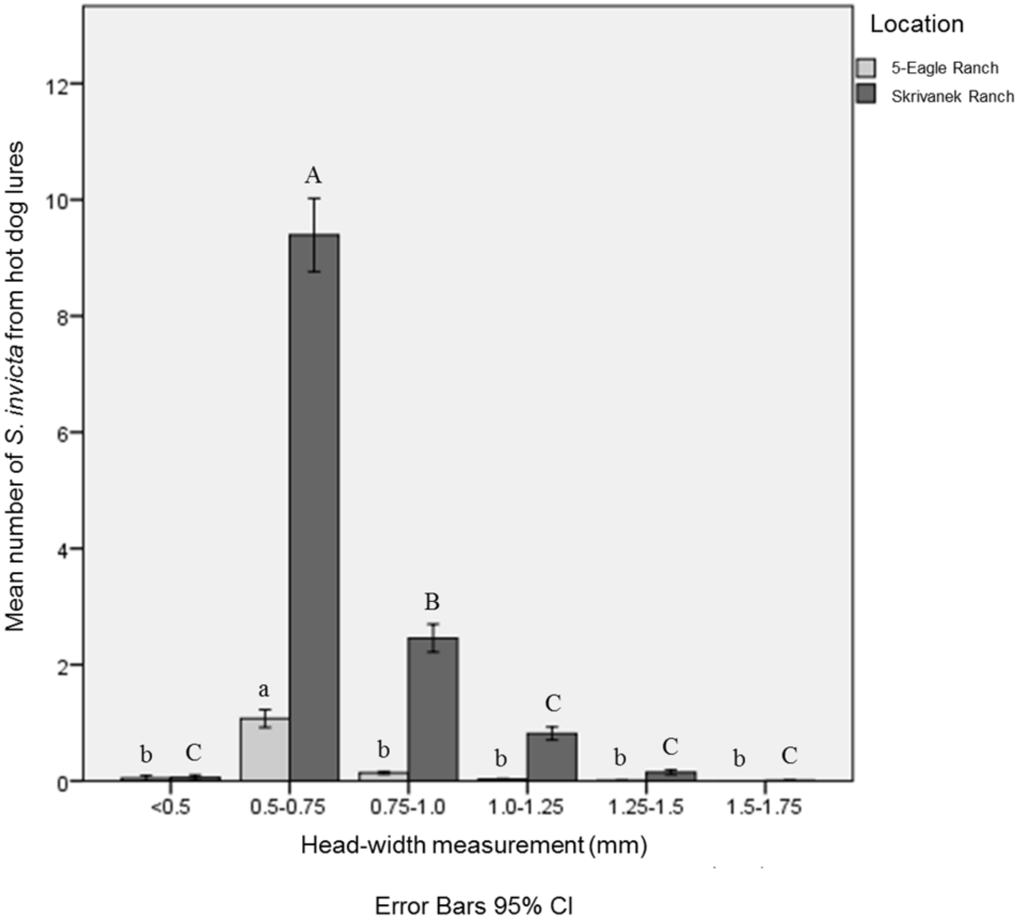

Fig. 3.19. Relative abundance of RIFA by head width (mm) from hot dog lures by location. Both ranches were significantly different [5-Eagle, indicated by lowercase letters (F = 41.723; df = 5,4854; $P < 0.001$) and Skrivanek Ranch, indicated by uppercase letters (F = 171.187; df = 5,4854; $P < 0.001$)]. Head width measurements indicated by different letters were significantly different.

When analyzing the number of RIFA foragers collected during AM and PM observations from Eagle Ranch only, there was a significant difference in the mean number of RIFA in the 0.5-0.75 mm (t = 3.481; df = 577; $P < 0.005$), 0.75-1.0 (t = 2.957; df = 573; $P < 0.005$), and 1.0-1.25 mm (t = 2.362; df = 533; $P < 0.05$) head widths (Fig. 3.20, Table 3.5). There was no significant difference between the <0.5 and (t = 2.362; df = 405; P = 0.318) 1.25-1.5 (t = 0.000; df = 808; P = 1.000) 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.

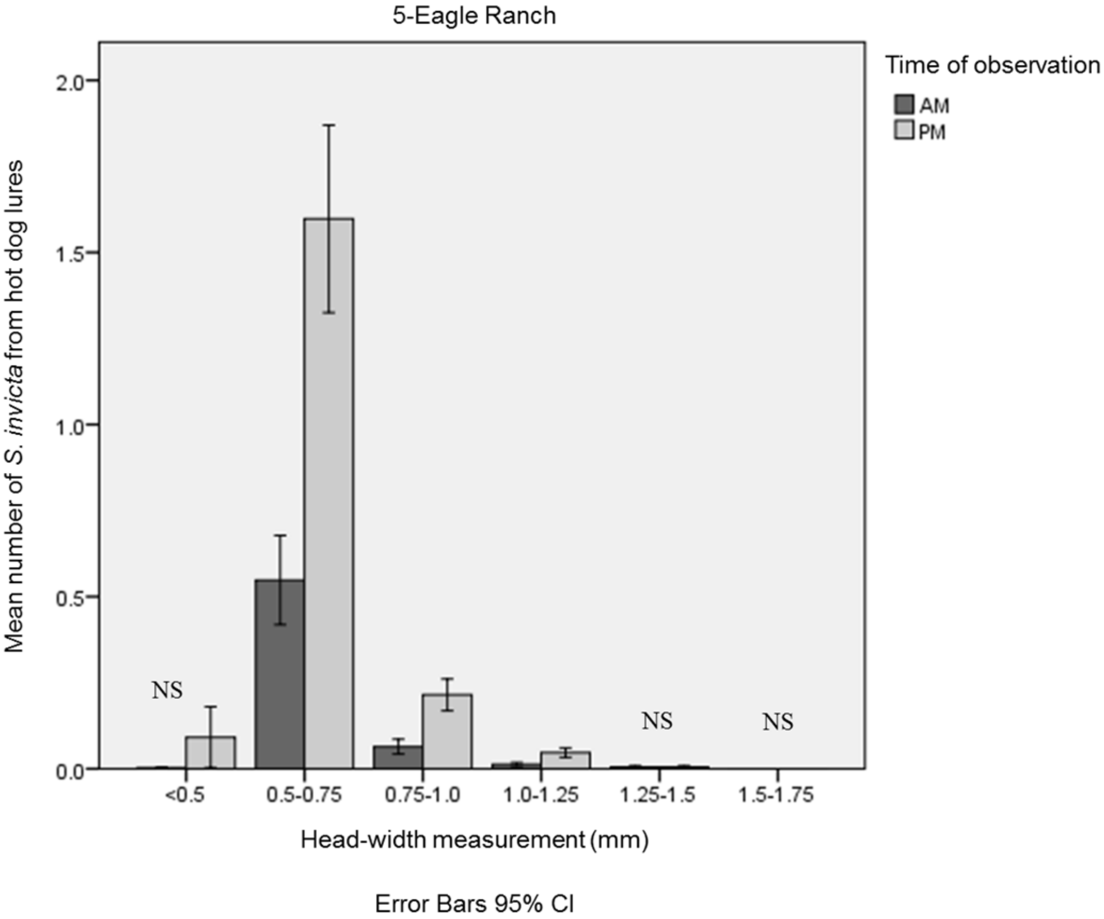

Fig. 3.20. Relative abundance of RIFA by head width (mm) from hot dog lures at 5-Eagle Ranch by AM and PM. Means noted with "NS" were not significantly different. Summary of student's t-test results is located in Table 3.5.

Table 3.5. Results of student’s t-tests on the mean number of foragers on hot dog lures by head widths at 5-Eagle Ranch. Results are shown on six size classes of head widths. No statistics were computed for head width size 1.5-1.75 because standard deviations were both 0.

Head Width (mm)	**Mean # Ants ± SE**		**t statistic**	***P* value**
	AM	**PM**		
<0.5	0.00±0.002	0.09±0.089	df (405) = 0.999	= 0.318
0.5-0.75	0.55±0.129	1.60±0.272	df (577) = 3.481	< 0.05
0.75-1.0	0.06±0.022	0.21±0.046	df (573) = 2.957	< 0.05
1.0-1.25	0.01±0.005	0.05±0.014	df (533) = 2.362	< 0.05
1.25-1.5	0.00±0.003	0.00±0.003	df (808) = 0.000	= 1.00
1.5-1.75	0.00±0.00	0.00±0.00	n/a	n/a

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 (Fig. 3.21; Table 3.6). 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) head widths.

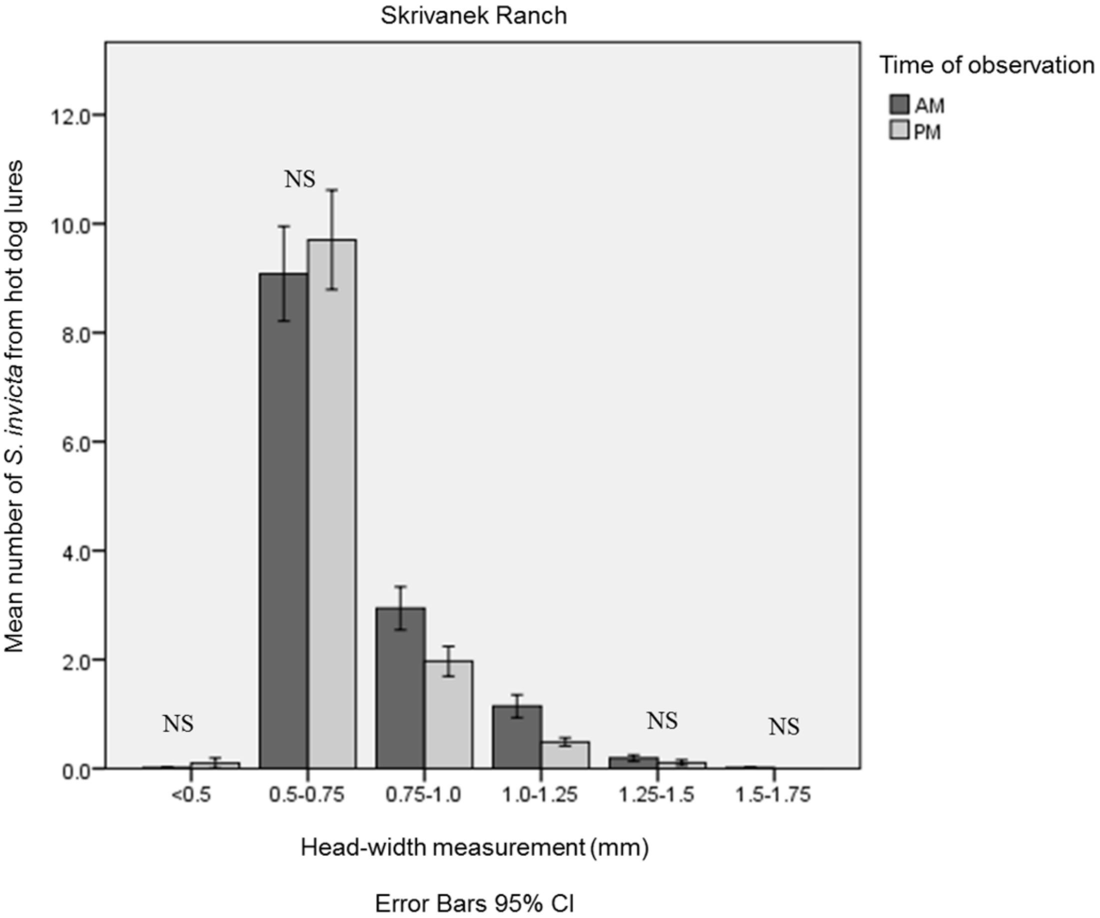

Fig. 3.21. Relative abundance of RIFA foragers by head width size from hot dog lures at Skrivanek ranch by AM and PM. Means noted with “NS” were not significantly different. Summary of student’s t-test results is located in Table 3.6.

Table 3.6. Results of student's t-test on the mean number of foragers by head widths from hot dog lures at Skrivanek Ranch. Results are shown on six size classes of head widths.

Head Width (mm)	**Mean # Ants ± SE**		**t statistic**	***P* value**
	AM	**PM**		
<0.5	0.01±0.007	0.10±0.091	df (808) = 0.915	= 0.360
0.5-0.75	9.08±0.870	9.70±0.914	df (808) = 0.493	= 0.622
0.75-1.0	2.94±0.394	1.97±0.274	df (720) = 2.022	< 0.05
1.0-1.25	1.15±0.210	0.49±0.076	df (508) = -2.954	< 0.005
1.25-1.5	0.19±0.058	0.11±0.051	df (793) = 1.088	= 0.277
1.5-1.75	0.01±0.009	0.00±0.000	df (404) = 1.736	= 0.083

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 RIFA 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 RIFA 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. 3.22). RIFA 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 RIFA foragers collected by head width measurement from colony samples at Skrivanek Ranch (F = 242.146; df = 5,1614; $P < 0.001$; Fig. 3.22). The number of RIFA 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 width 0.75-1.0 mm was also found significantly more than from all head widths, except 0.5-0.75 mm.

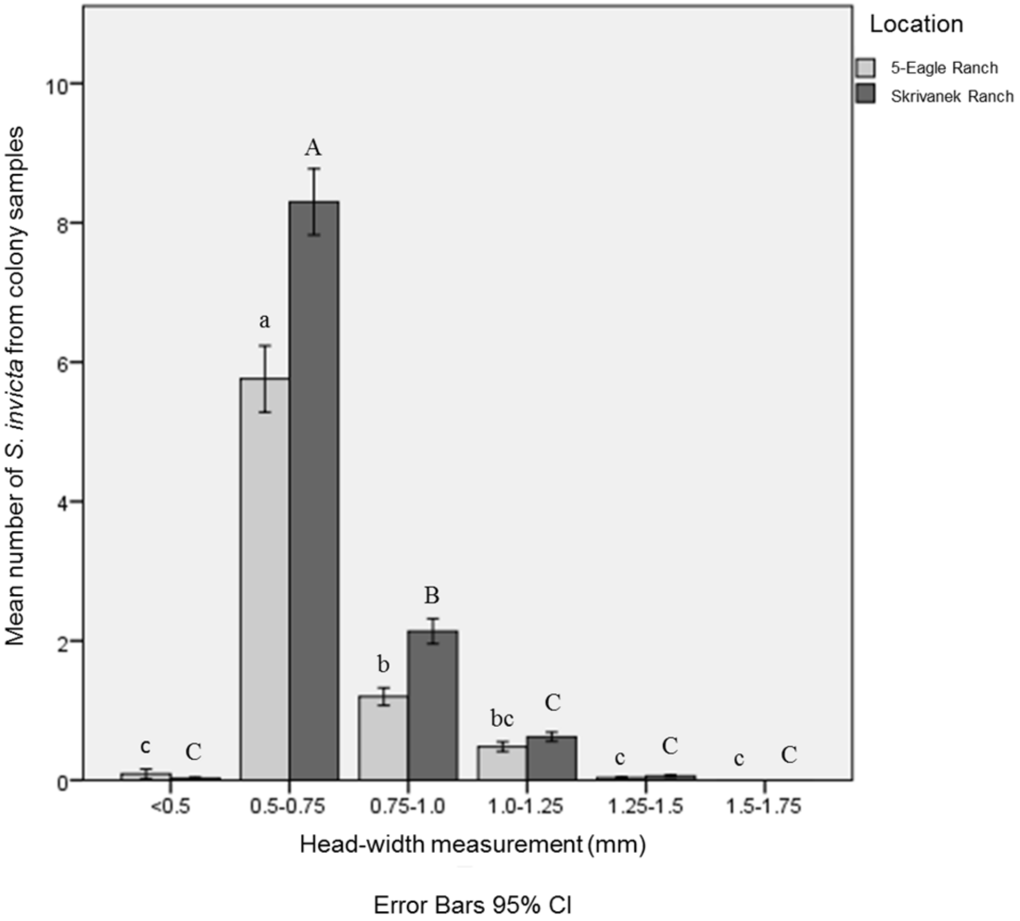

Fig. 3.22. Relative abundance of RIFA by head width (mm) from colony samples by location. Both ranches were significantly different [5-Eagle, indicated by lowercase letters (*F* = 120.509; df = 5,1614; *P* < 0.001) and Skrivanek Ranch, indicated by uppercase letters (*F* = 242.146; df = 5,1614; *P* < 0.001)]. Head width measurements indicated by different letters were significantly different according to Tukey's HSD post hoc analysis.

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. 3.23). 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 (Table 3.7).

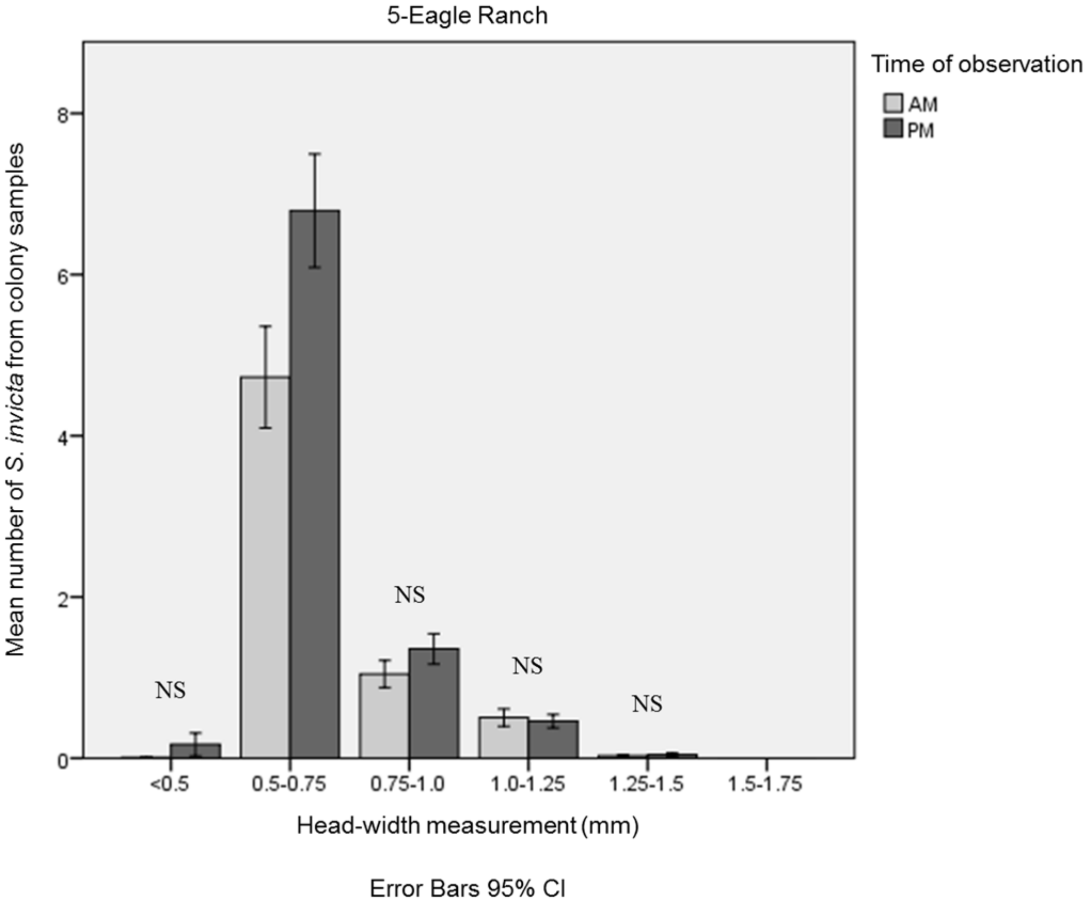

Fig. 3.23. Relative abundance of RIFA by head width from colony samples at 5-Eagle Ranch between AM and PM. Means noted with “NS” were not significantly different (Table 3.7).

Table 3.7. Results of student's t-tests on the mean number ants from colony samples by head widths from 5-Eagle Ranch at AM and PM observations. Results are shown on six size classes of head widths. No statistics were computed for head width size 1.5-1.75 because standard deviations were both 0.

Head Width (mm)	**Mean # Ants ± SE**		**t statistic**	***P* value**
	AM	**PM**		
<0.5	0.01±0.007	0.17±0.142	df (134) = 1.149	= 0.253
0.5-0.75	4.73±0.631	6.79±0.703	df (264) = 2.188	<0.05
0.75-1.0	1.04±0.169	1.36±0.188	df (264) = 1.232	= 0.219
1.0-1.25	0.50±0.108	0.46±0.083	df (251) = 0.327	= 0.744
1.25-1.5	0.03±0.015	0.04±0.021	df (268) = 0.585	= 0.559
1.5-1.75	0.0±0.000	0.00±0.000	n/a	n/a

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. 3.24). 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 (Table 3.8).

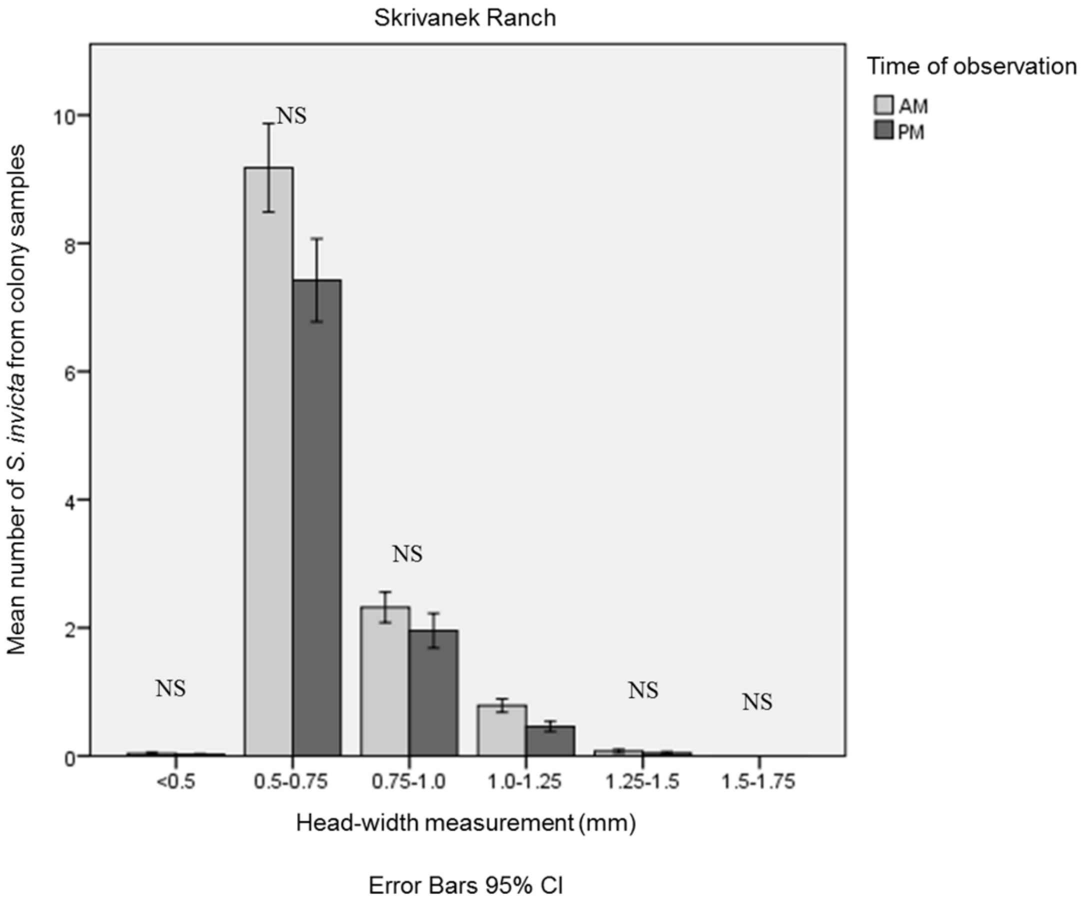

Fig. 3.24. Relative abundance of RIFA by head width from colony samples at Skrivanek Ranch between AM and PM. Means noted with "NS" were not significantly different. Summary of student's t-test results is located in Table 3.8.

Table 3.8. Results of student's t-tests on the mean number ants from colony samples by head widths from Skrivanek Ranch at AM and PM observations. Results are shown on six size classes of head widths. No statistics were computed for head width size 1.5-1.75 because standard deviations were both 0.

Head Width (mm)	**Mean # Ants ± SE**		**t statistic**	***P* value**
	AM	**PM**		
<0.5	0.04±0.022	0.02±0.013	df (268) = 0.581	= 0.561
0.5-0.75	9.18±0.691	7.42±0.647	df (268) = 1.854	= 0.065
0.75-1.0	2.32±0.238	1.96±0.270	df (268) = 1.008	= 0.314
1.0-1.25	0.79±0.104	0.46±0.077	df (247) = 2.521	< 0.05
1.25-1.5	0.07±0.029	0.04±0.021	df (268) = 0.831	= 0.407
1.5-1.75	0.0±0.000	0.00±0.000	n/a	n/a

PCR results

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. 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 completed, 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 (Fig. 3.25).

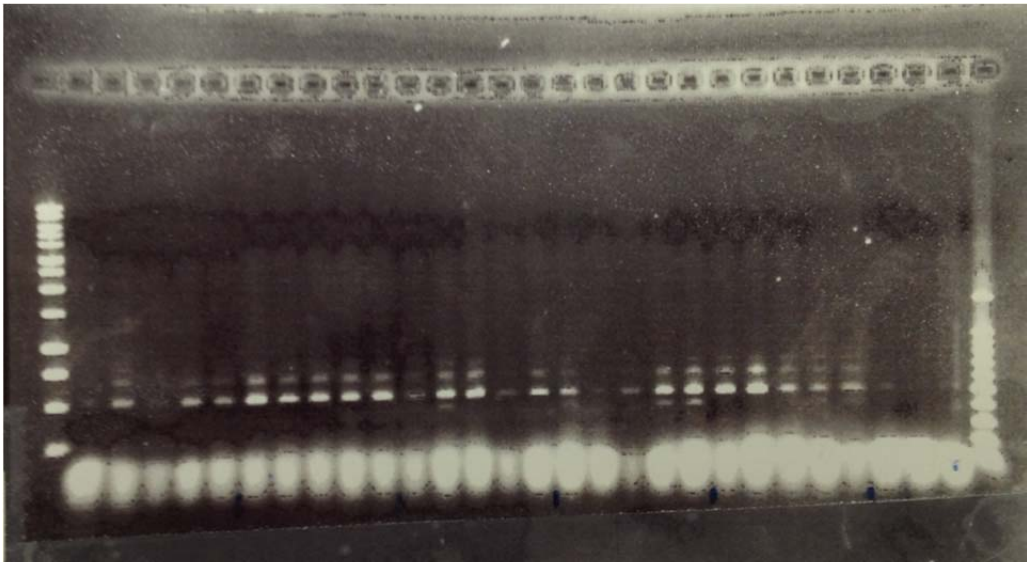

Fig. 3.25. Results of PCR to determine if colonies were monogyne or polygyne.

Discussion

The temperature and relative humidity (RH) data were significantly different from at two ranches, but 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 (Fig. 3.3) containing many more trees than Skrivanek Ranch (Fig. 3.4), 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 space, 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 null hypotheses in this set of experiments were adjusted to reflect 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 (Ferrierre and Cazelles 1999). While *P. tricuspis* was rarely collected, the population levels were steady but not as abundant as *P. curvatus,* but direct competition 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 between 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 RIFA 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, a 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 RIFA populations for up to seven years at 5-Eagle Ranch (Gilbert et al. 2008). RIFA 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 RIFA activity at 5-Eagle Ranch had occurred since 2008, and little has changed other than the continued establishment of populations of phorid flies

Work by Hooper-Bùi et al. (2002) showed that RIFA 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 RIFA 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. These results were similar to the previously discussed laboratory study results (Chapter II). RIFA 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 here was 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 or 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 (Fig. 3.1). 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 RIFA exposed to phorids 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 RIFA 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 RIFA 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, RIFA 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 RIFA 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 approximately 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 RIFA worker size shift to fewer larger workers and the greater presence of smaller workers shown to be present at both ranches (Fig. 3.1, 3.22), 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 RIFA 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 (Fig 3.15, 3.19), smaller than the results shown by Hooper-Bui.

Based on the analysis I reject the first null hypotheses that colonies would select the same granular size in the presence of phorid flies and in the absence of phorid flies. Based on data from Hooper-Bui (2002) and the results of this experiment, ants on these ranches selectively foraged baits smaller than RIFA not exposed to phorid flies. RIFA 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. RIFA 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 stating workers would have similar size ratios in the presence and absence of phorid flies is also rejected.

CHAPTER IV

A SPATIOTEMPORAL SURVEY OF *PSEUDACTEON* SPP. PHORID FLIES IN URBAN ENVIRONMENTS

Introduction

Urban areas are defined by the US Census Bureau as central places and surrounding territory that have a minimum human population of at least 50,000 people, with fringe areas having at least 1,000 people for square mile. These urban areas continue to expand in nearly every geographic region in the United States. Urbanization of a landscape is arguably the most dramatic transformation mankind can make to the natural environment by building infrastructure over a large land area. Urban sprawl changes biodiversity, micro and macro climates, soil structure, and water cycling in the local ecology. Currently, more than half of the total population in the world lives in urban areas, and in the next 20 years it is expected that all population increases will occur in cities (United Nations Population Fund 2007). In Texas, 84% of people live in urban areas and cities and these numbers are continuing to increase (US Census, 2010). Edges of urban areas are vague because boundaries are often set by bodies of water, geopolitical lines, research or other convenient ways to delineate an area. Biological, ecological, and environmental interactions can occur well beyond these synthetic boundaries (Pickett et al. 2001).

The sheer size and scope of these growing urban areas provides ample reason to study the ecological implications that they have on the landscape. Often, urban areas surround and even encompass lands that were previously agricultural or undeveloped making the study of these areas complex and obfuscated. Across even a small urban area, the land can be extremely heterogeneous in terms of topography and use, from community gardens to asphalt parking areas. Shift between these areas of use can occur within relatively short distances and the ecological impacts of these mixed use areas have not been heavily studied in terms of the effect the shifts have on the ecology of insect interactions.

Since its introduction, *Solenopsis invicta* Buren, the red imported fire ant (RIFA) in its invaded areas, has been shown to be one of the most encountered pest ants in urban areas within its range (Klotz et al. 1995). In central Texas, 80% of transplant homeowners, who are new to Texas, encounter and are stung by RIFA within six months of residency (R.E. Gold, personal communication). In addition to being frequently combatted by home owners and pest control professionals as nuisance pests, they are considered medically and economically important pests as well (Banks 1990). In Texas alone, over 40 million people live in environments where RIFA have infested, and annually, 14 million people are stung (Drees 2002). Control of RIFA over the years has run the gamut from area wide pesticide applications, quarantines, to aerial application of bait over large tracts of infested land (Williams et al. 2001). Biological control in urban environments, in general, has been attempted with little success, and compounding the problem is poor public perception of biological control organisms and a lack of understanding and acceptance of this method of pest control (Pereira and Stimac 1997).

Research into interactions between Phorid flies in the genus *Pseudacteon* spp. and RIFA has primarily been conducted in their native range, rural habitats, parts of large ranches or other large, undeveloped tracts of land. Little is known about phorid fly presence/absence, abundance, or temporal distribution in developed or urban environments. Morrison et al. (1999b) showed that *Pseudacteon* spp. that parasitize *Solenopsis geminata* are present in city parks and natural urban areas, but their abundance within these complex areas was not investigated. Distribution of phorids that were introduced for the intended biological control of *S. invicta*, including *P. tricuspis*, *P. curvatus*, and *P. obtusus*, have not been studied in urban areas.

Phorid flies were successfully established throughout central Texas and there was confirmed activity of *P. tricuspis* and *P. curvatus* in Travis county by 2008 and confirmed established populations of *P. obtusus* in 2010 (Plowes et al. 2011). Hays and Comal counties were suspected to contain *P. tricuspis* and *P. curvatus* based on probable distribution maps for 2008 (Calcott et al. 2011) and University of Texas Fire Ant Project maps (2011). Additionally, maps published by the University of Texas Fire Ant Project (2011) show predicted spread and establishment for *P. tricuspis*, *P. curvatus*, and *P. obtusus*. These maps show, at a minimum, *P. tricuspis* and *P. curvatus* active in central Texas. Expansion rates of *P. obtusus* are thought to be similar to *P. tricuspis* and *P. curvatus* so it was expected to move in to areas around release sites also (Plowes et al. 2011).

The main goal of this study was to determine the phenology and seasonal abundance patterns of *Pseudacteon* spp. in urban areas in central Texas. Sampling was done seasonally over the period of one year to determine if phorids were present in urban environments and, if so, the distribution patterns of flies and RIFA. Additionally, GIS technology and remote sensing were used to help document the distribution of phorid population levels in urban environments and the distribution of flies based on diverse habitats. This field work was designed to address the following objectives: 1) Determine the presence/absence of *Pseudacteon* phorid flies in urban systems, 2) Determine if phorids are evenly distributed spatially in defined urban environments, and 3) Determine the seasonal variation of phorid populations in urban environments.

Experimental design

This study was conducted in three urban areas in central Texas, in cities of similar size, total human population, human population density, total land area, rainfall, and ecoregions. Urban areas used for this experiment were also chosen based on nearness to a US or interstate highway, ease of sampling, and proximity to known phorid release sites (Gilbert et al. 2008, Calcott et al. 2011).

The following cities were found to match these criteria: 1) New Braunfels, Texas, located in Comal County (29° 42' 5.86 N; 98° 7' 26.9" W) with a total population of 57,740, a total land area of 113.62 km^2 and average rainfall of 90.78 cm per year; 2) San Marcos, Texas, located in Hays County (29° 52' 54.69" N; -97° 56' 33.21" W) with a total population of 44,894, a total land area of 78.27 km^2 and an average rainfall of 94.46 cm per year; and 3) Cedar Park, Texas, located in Williamson

County (30° 31' 9.36" N; -97° 49' 32.90" W) with a total population of 48,937, a total land area of 59.18 km^2 and an average rainfall of 89.18 cm per year. These cities are all along or near the Interstate 35 corridor, have similar land use patterns, and are located in the geographic region of Texas known as the Hill Country (Fig. 4.1).

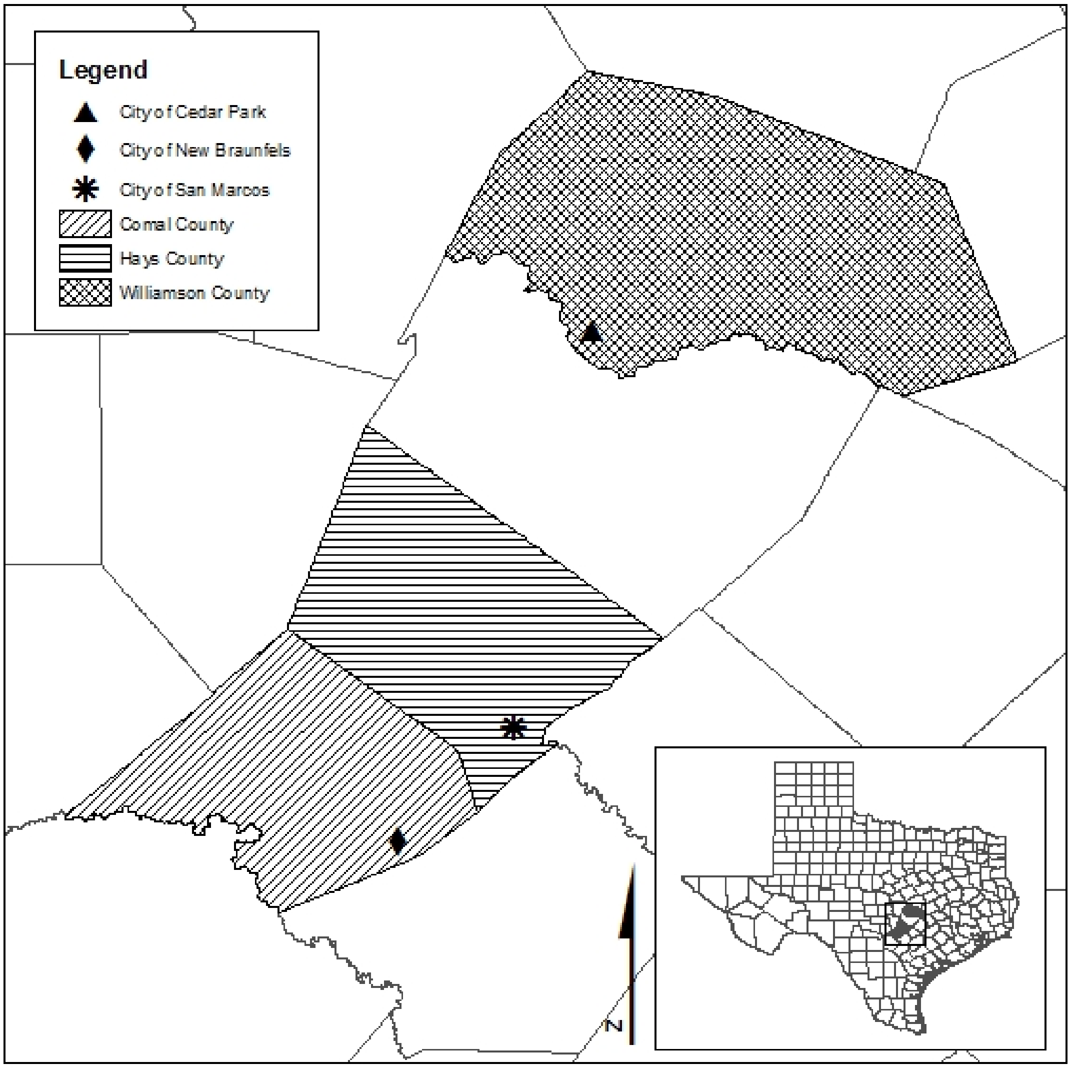

Fig. 4.1. Location of urban areas sampled within Texas.

Sampling sites were selected by using TNRIS (Texas Natural Resources Information Systems) categorization of developed land. Raster files from the TNRIS National Land Cover Data set for Area 10 from 2001, were downloaded and opened using ARCMaps™ 10.0 (ESRI, Redlands, CA). This download consisted of three files: land cover, impervious cover, and canopy cover. The land cover files and impervious cover files were used, but the canopy cover files were not used for this study because there was not a pronounced difference within each urban area. Sampling locations fitting the following five criteria were selected: 1) Developed, Open Space and 6-20% Impervious Cover, 2) Developed, Low Density and 31-45 % Impervious Cover, 3) Developed, Medium Density and 56-70% Impervious Cover, and 4) Developed, High Density and 81-95% Impervious Cover, and a fifth zone was used along roadway right-of-way (ROW) areas. Each city was assigned a 1000 m by 1000 m square with the city center as its midpoint, and all survey sites were located within this square. ArcMaps was used to construct the data sets and maps. For each of the four zones in question, the density of land cover and impervious cover were identified, and the combined transparent layers were used to identify and choose potential sampling sites. In addition to the impervious and land cover layers, city streets, state highways, and railroad tracks were also included into the working maps. Once these layers were all in place, aerial photography of each city was superimposed on each map. Potential trap locations were then identified in areas where these parameters overlapped. For example, for DOS, 6-20% impervious cover, the parameters were set in each data set to only display DOS in the land cover layer and 6-20% in the impervious surface layer. This was repeated for each of the four zones. The ROW trap locations were simply placed alongside a major highway or interstate road way between the edge of the pavement and the end of the property owned by the state.

Possible locations were identified within each zone based on density and impervious cover layers as well as specific site information obtained by using Google Earth®, accessed in June 2012. Areas with limited accessibility via car were not considered, nor were sample locations on private property, or within fenced areas. Placements were chosen in inconspicuous areas away from pedestrian traffic and interference from companion animals, such as landscaped beds. Preference was given to easements, utilities, and government managed property where possible. Numerous possible survey sites were identified and then visited. From these options, ten were selected within each category for use in the study (Fig. 4.2, 4.3, and 4.4). No sampling sites were located closer than 100 m apart to minimize interaction between traps and reduce competition for attraction to flies among traps.

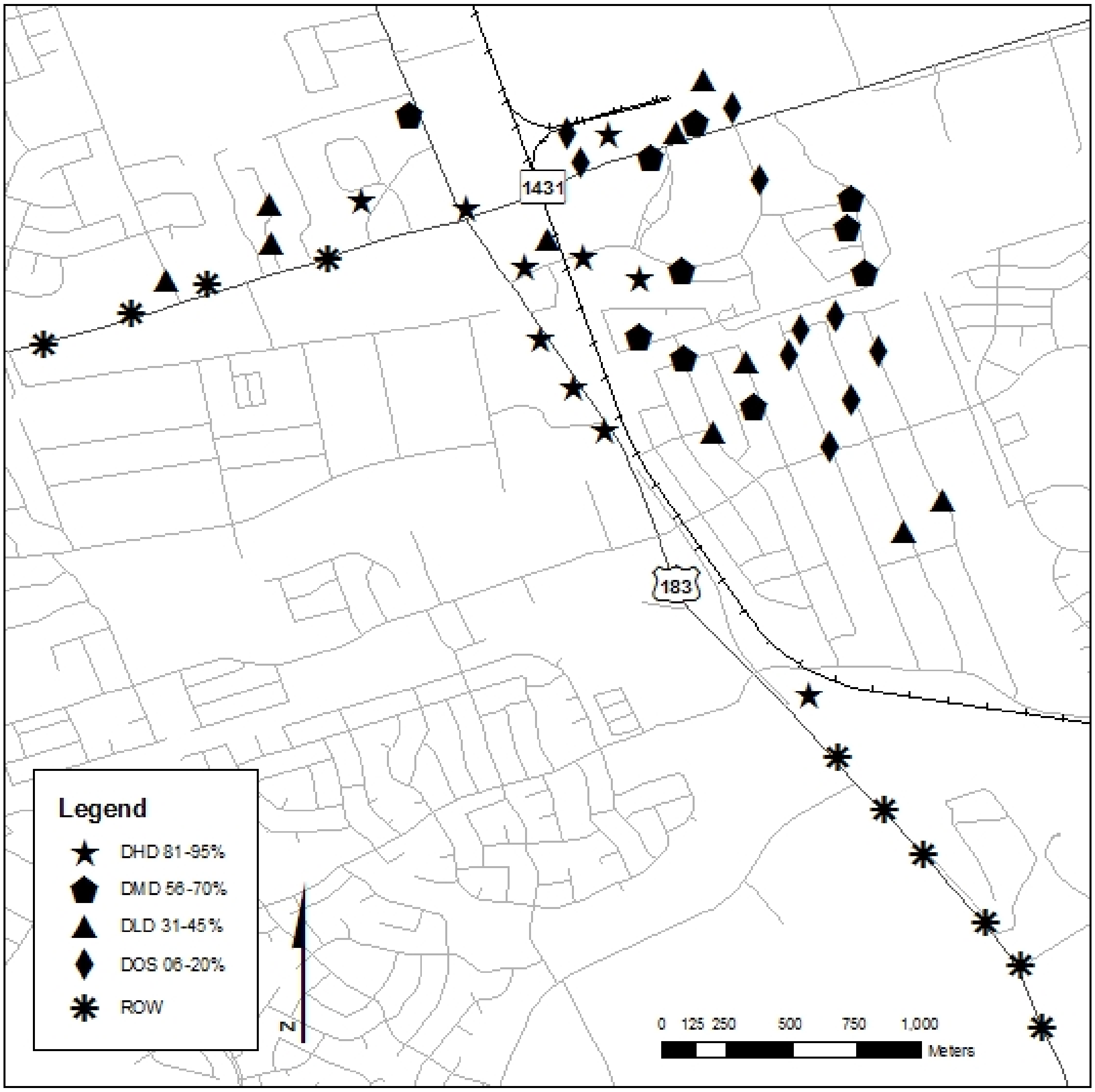

Fig. 4.2. Map of Cedar Park, Texas trap locations by location type. DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

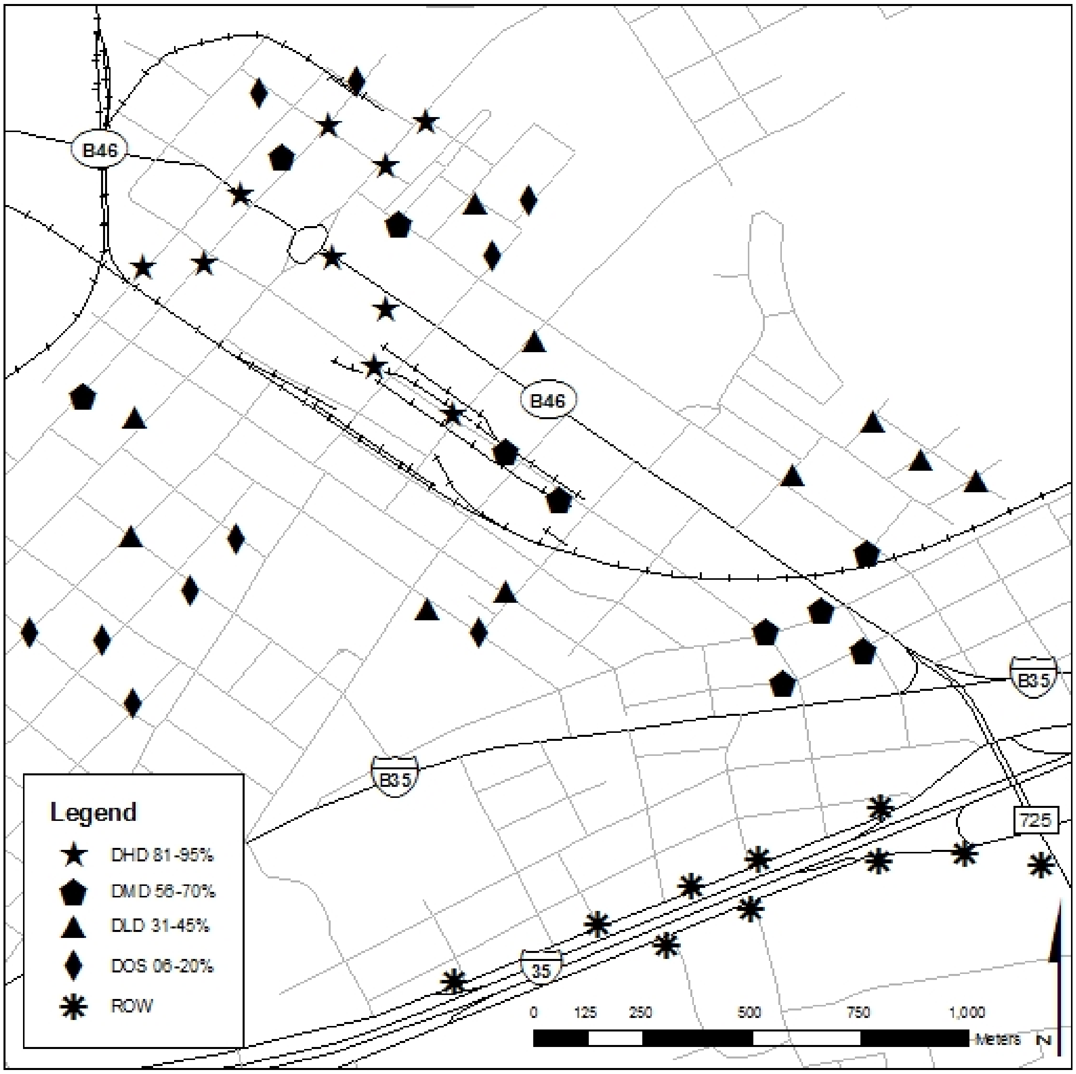

Fig. 4.3. Map of New Braunfels, Texas trap locations by location type. DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

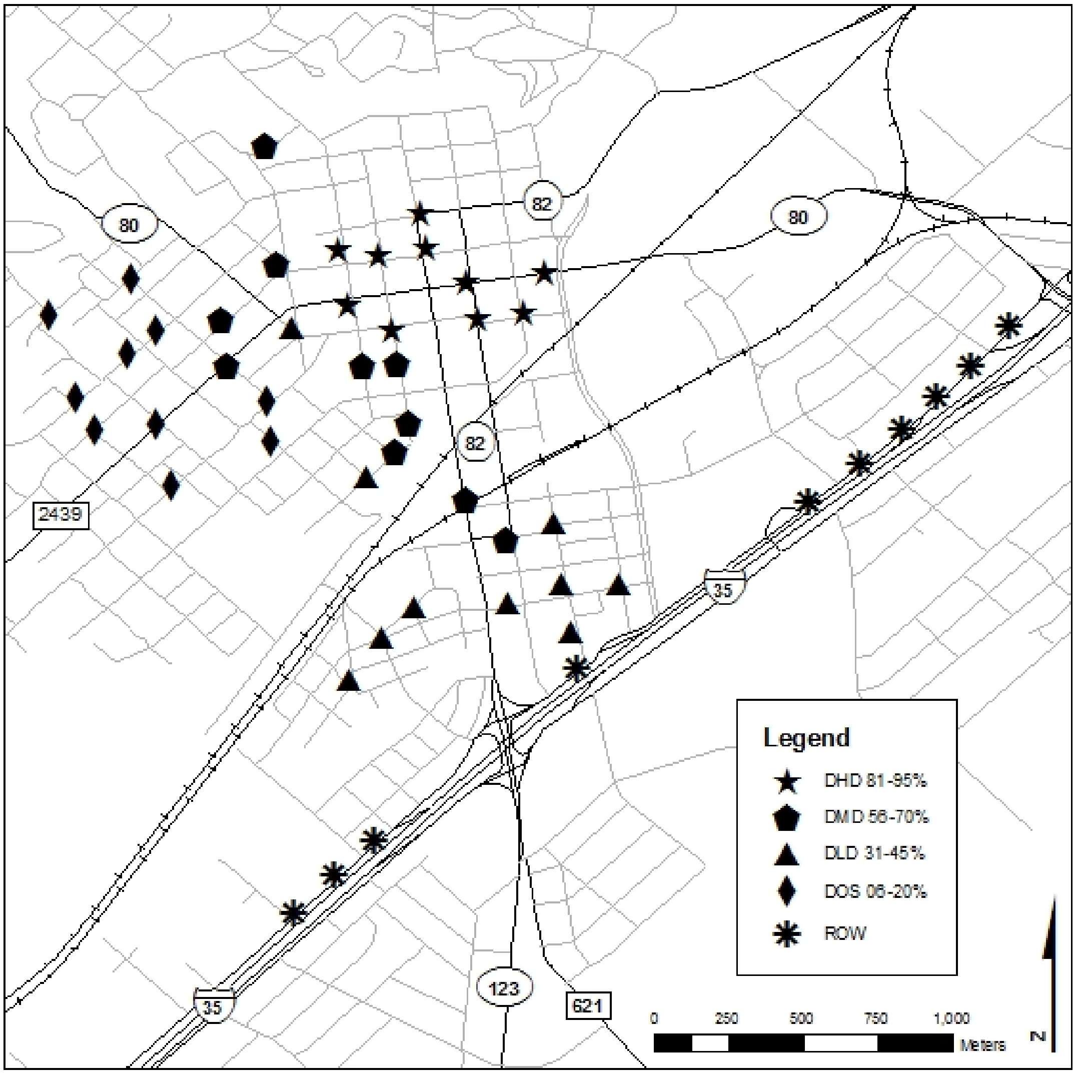

Fig. 4.4. Map of San Marcos, Texas trap locations by location type. DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

Phorid fly traps, (PTS traps) described by Puckett et al. (2007), were selected for use in this study. These traps were chosen based on ease of use, reliability, and effectiveness (Puckett et al. 2013). Approximately, one 5 dr vial of midden (Smith and Gilbert 2003) was pre-measured into plastic portion cups with lids (Bakers & Chefs, Bentonville, AR) to aid in trap placement as well as expedite deployment. Traps were pre-prepared with Tanglefoot® (The Tanglefoot Company, Grand Rapids, MI) and labeled with the city, date, and individually numbered. Corresponding numbers were logged on city maps to track placement as well as maintain data credibility. Traps were deployed, 10 per category, in each city beginning approximately 30 min prior to AM civil twilight. Placing all the traps took approximately 60-75 mins, finishing at 30-45 min after civil twilight, but Pesquero et al. (1996) showed *Pseudacteon* flies are generally not active the first hour after sunrise, so trap effectiveness was assured. Depending on the season, traps were deployed for between 11 and 15 hrs. Deployment and extraction times were chosen to minimize interference by the general public, feral animals, domestic pets, and other unknown factors. Phorid fly traps were retrieved in the same order they were deployed beginning approximately 30 min prior to PM civil twilight. After removal, traps were placed in plastic craft organizer boxes (27 cm X 18 ½ cm x 5 cm) with individual compartments (4.5 cm x 6 cm) to reduce cross contamination between traps (Hobby Lobby®, Oklahoma City, OK). Sampling was conducted on days with little to no expected precipitation and when the air temperature was forecasted to reach above 22.2° C (72° F) so flies would be active (Wuellner and Saunders 2003). All cites were georeferenced and field placement was verified with

GIS data. Surveys were conducted in July (summer), October (fall), January (winter) and April (spring) of 2012 and 2013, respectively.

To determine RIFA presence/absence, foraging intensity, and relative population size, approximately 0.6 cm pre-cut hot dog lures (Bar S® Beef Franks) were deployed within 3 m of each designated trap location. The lures were skewered on landscape flags, placed on the ground, in a shaded area when possible, and then collected after a period of approximately 30-45 minutes. Hot dog lures were retrieved, along with foraging ants, and rapidly placed in numbered zip top bags. The bag number corresponded to the trap number as well as the number on the city map to maintain data credibility. Deployment of hot dog lures occurred in the middle of the day, between 10 AM and 2 PM, between trap deployment and retrieval usually taking approximately 3 hours. At the time of hot dog lure placement, soil temperature at the surface (Traceable ® Infrared thermometer 0666438, Fisher Scientific, Atlanta, GA) as well as at a depth of 3 cm (Cooper probe thermometer; Model DP400W, Middlefield, CT) was recorded. These data were collected to ensure temperature ranges were within acceptable ranges for RIFA foraging of 22 – 36° C (Porter and Tschinkel 1987, Drees et al. 2009).

Collected ants were separated from the lure, and other debris, and counted. Traps were inspected under magnification and collected phorid flies were identified, sexed and recorded.

IBM SPSS Statistics version 19 was used to analyze data collected. Non-parametric Kruskal-Wallis tests were used because the data failed to meet the assumptions of normality. Additionally, Mann-Whitney U pairwise comparisons were used to separate location types when significant results were found.

Results

PTS traps

Only *Pseudacteon curvatus,* was identified on all PTS traps and all locations in this study. Neither *P. tricuspis* nor *P. obtusus* were found on any traps from any the three sampled cities.

Within the three cities sampled, there was a significant difference in the mean number of flies found in New Braunfels (n = 188) (Mean ± SE = 1.25 ± 0.222) versus Cedar Park (n = 182) (Mean ± SE = 0.23 ± 0.045) and San Marcos (n = 183) (Mean ± SE = 0.14 ± 0.035) (df = 2; $x^2 = 24.730$; $P < 0.001$; Fig. 4.5). When independent sample Mann-Whitney U tests were run between the cities, it showed a statistically significant difference between the number of phorids found in Cedar Park -New Braunfels ($Z = 3.065$; $P < 0.05$), and New Braunfels -San Marcos ($Z = 4.723$; $P < 0.05$). Cedar Park and San Marcos were statistically equivalent ($Z = 1.963$; $P = 0.05$; Table 4.1).

Table 4.1. Summary of results of pairwise Mann-Whitney U tests for *Pseudacteon curvatus* flies found in each city at all location types and all sampling dates.

Pairwise Comparison	**Sample Size**	**U value**	***Z* value**	***P* value**
Cedar Park - New Braunfels	182/188	14809	3.065	< 0.01
Cedar Park - San Marcos	182/183	15482.5	1.963	= 0.05
New Braunfels - San Marcos	188/183	13874	4.723	< 0.01

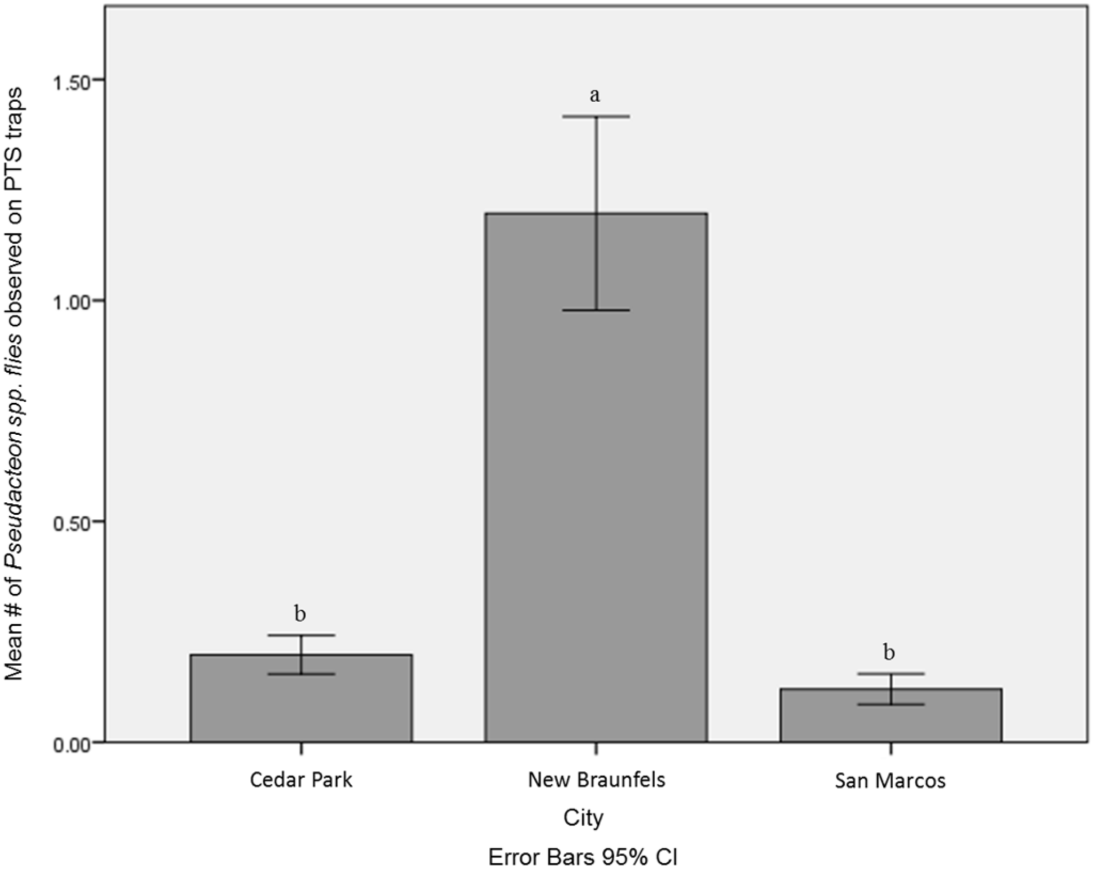

Fig. 4.5. Mean number of *Pseudacteon curvatus* flies found by city from all location types and all sampling dates. There was a statistically significant difference between the cities (df = 2; $x^2 = 24.730$; $P < 0.05$). Cities indicated by different letters were significantly different when compared with Mann-Whitney U pairwise comparison.

When mean number of flies collected by month/season were considered, there was a significant difference in the mean number of flies collected at each month/season (df = 3; $x^2 = 67.163$; $P < 0.01$; January (winter) Mean ± SE = 0.05 ± 0.018; April (spring) Mean ± SE = 0.78 ± 0.202; July (summer) Mean ± SE = 1.26 ± 0.233, October (fall) Mean ± SE = 0.09 ± 0.035; Fig. 4.6). When pairwise tests were completed for each season, there was a significant difference between observations made in January (winter) 2013 and April (spring) 2013 ($Z = 4.977$; $P < 0.05$), April (spring) 2013 and October (fall) 2012 ($Z = 4.481$; $P < 0.05$), July 2012 and October (fall) 2012 ($Z = 6.091$; $P < 0.05$), and January (winter) 2013 and July (summer) 2012 ($Z = 0.540$; $P < 0.05$). July (summer) and April (spring) ($Z = 1.845$; $P = 0.065$) and January (winter) and October (fall) ($Z = 0.571$; $P = 0.568$) were statistically equivalent (Table 4.2).

When individual location types are analyzed by season/month, the winter (January) observations showed no statistically significant difference in the number of flies found on PTS traps between any of the location types (df = 4; $x^2 = 6.945$; $P = 0.139$; Mean DHD ± SE = 0.00 ± 0.00; Mean DMD ± SE = 0.04 ± 0.037; Mean DLD ± SE = 0.14 ± 0.067; Mean DOS ± SE = 0.03 ± 0.033; Mean ROW ± SE = 0.04 ± 0.036; Fig. 4.7).

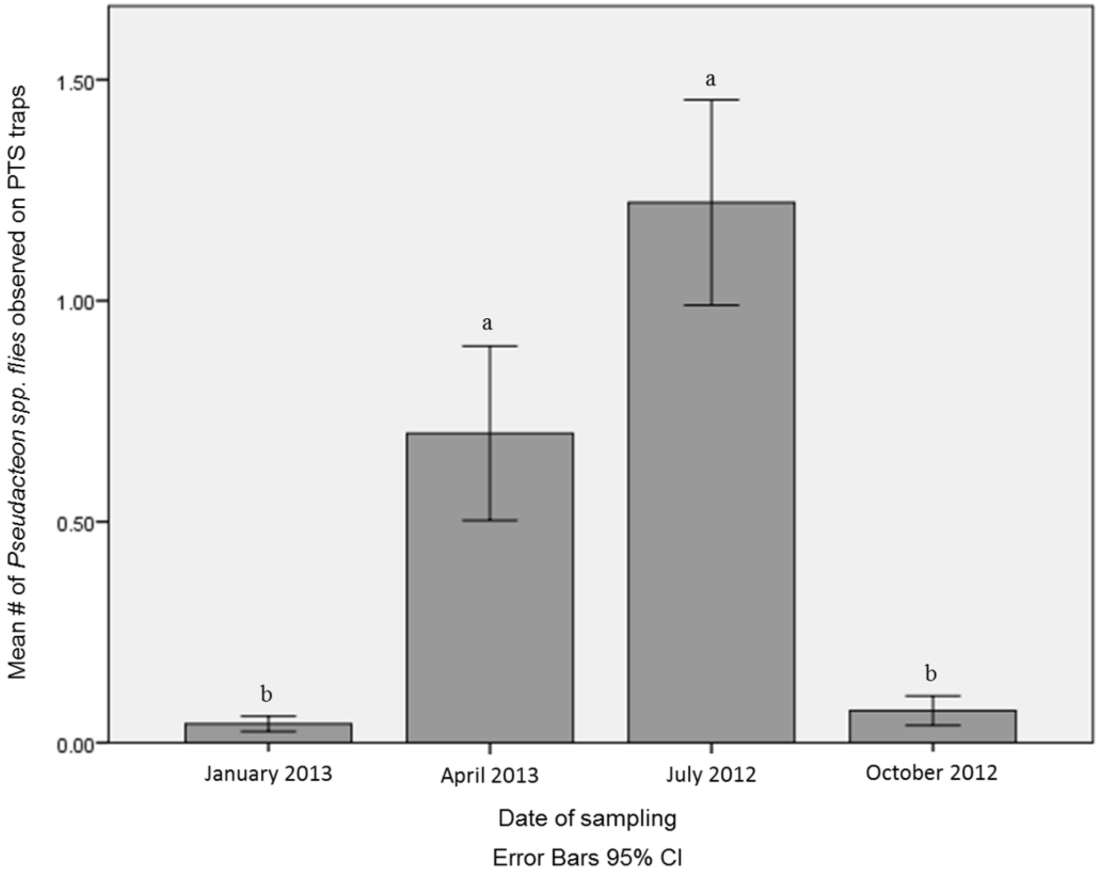

Fig. 4.6. Mean number of *Pseudacteon curvatus* flies found on PTS traps (n = 553) by month sampled in all cities. There was a significant difference in the number of flies found between the months (df = 3; $x^2 = 67.163$; $P < 0.01$). Dates indicated by different letters were significantly different when compared with Mann-Whitney U pairwise comparison.

Table 4.2. Summary of results of pairwise Mann-Whitney U tests for *Pseudacteon curvatus* flies found in each month in all cities and at all location types.

Pairwise Comparison	**Sample Size**	**U value**	***Z* value**	***P* value**
January - April	141/130	7167	4.977	< 0.001
July - January	144/141	6955.5	0.540	< 0.001
October - January	138/141	9574	0.571	= 0.568
July - April	144/130	8372	1.845	= 0.065
July - October	144/138	6955.5	6.091	< 0.001
October - April	138/130	7156.5	4.481	< 0.001

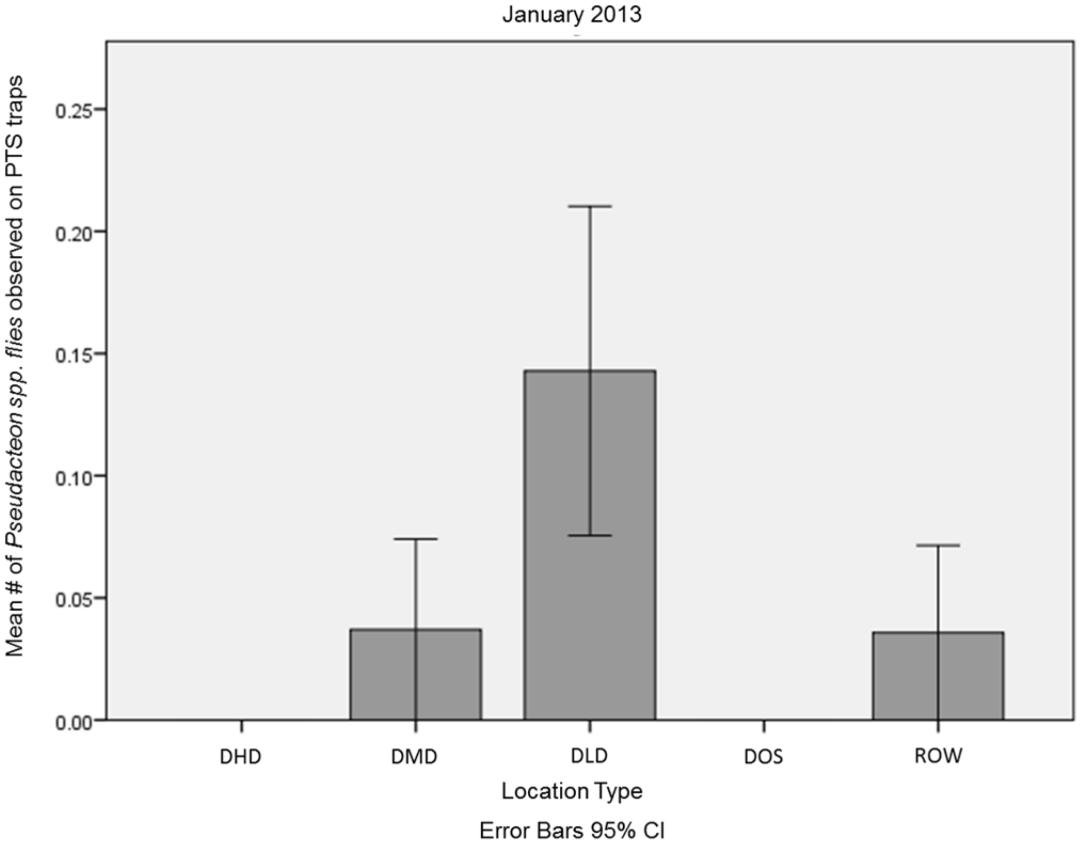

Fig. 4.7. Mean number of phorid flies found at each location type in January (winter) 2013 observations. There was no significant difference between the location types (df = 4; x^2 = 6.945; *P* = 0.139). DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

For April (spring) observations, there was no statistically significant difference between any of the location types in the number of flies found on PTS traps (df = 4; x^2 = 2.822; *P* = 0.588; Mean DHD ± SE = 0.31 ± 0.164; Mean DMD ± SE = 0.77 ± 0.290; Mean DLD ± SE = 0.60 ± 0.327; Mean DOS ± SE = 0.80 ± 0.476; Mean ROW ± SE = 1.48 ± 0.815; Fig. 4.8).

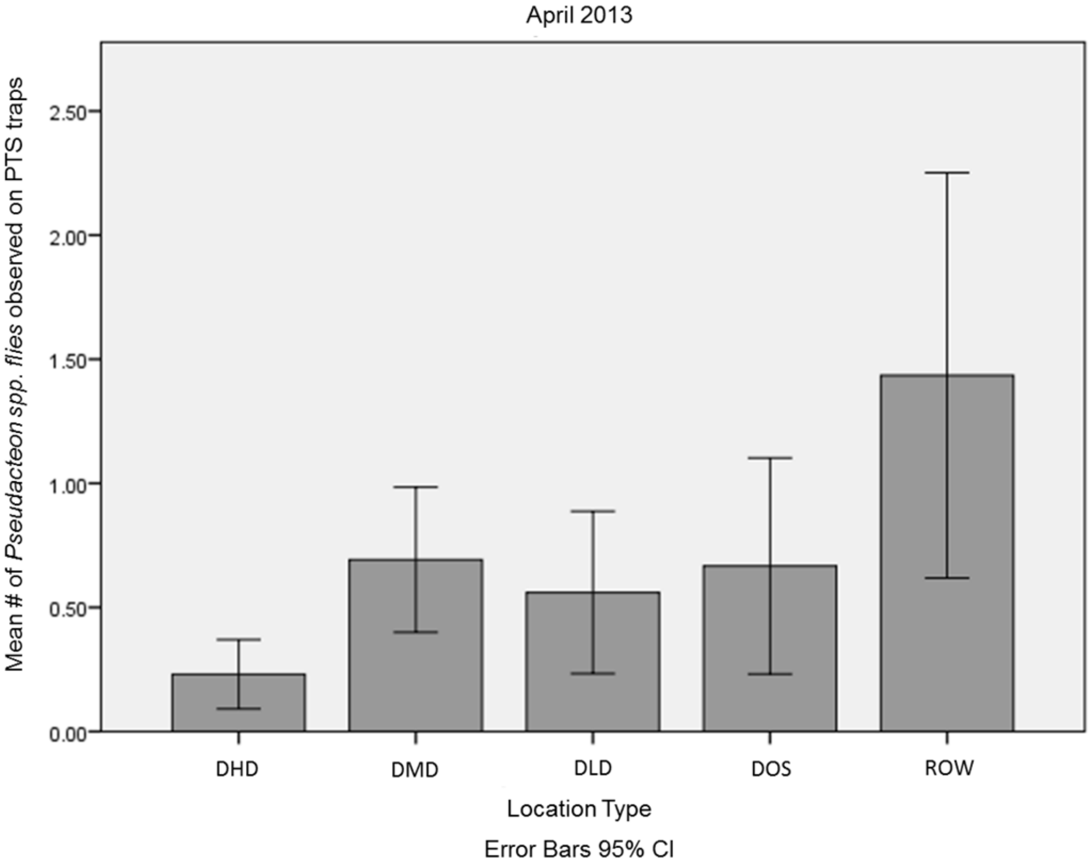

Fig. 4.8. Mean number of phorid flies found at each location type in April (spring) 2013 observations. There was no statistically significant difference between location types (df = 4; $x^2 = 2.822$; $P = 0.588$). DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

For July (summer) observations, there was no significant difference in the number of flies found on PTS traps between any of the location types (df = 4; x^2 = 3.961; *P* = 0.411; Mean DHD ± SE = 0.96 ± 0.533; Mean DMD ± SE = 1.57 ± 0.520; Mean DLD ± SE = 1.24 ± 0.420; Mean DOS ± SE = 1.45 ± 0.646; Mean ROW ± SE = 1.04 ± 0.481; Fig. 4.9).

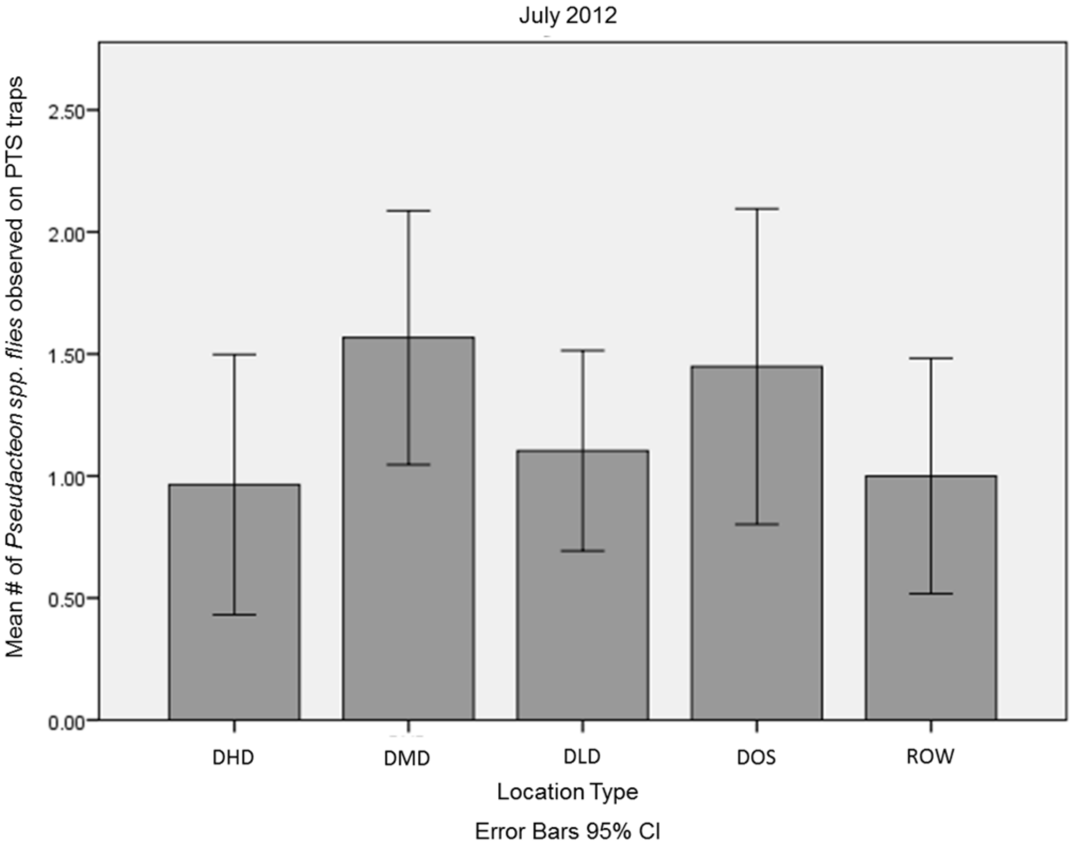

Fig. 4.9. Mean number of phorid flies found at each location type in July (summer) 2012 observations. There was no significant difference between the location types (df = 4; x^2 = 3.961; *P* = 0.411). DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

For October (Fall) observations, there was no statistically significant difference in the number of flies found on PTS traps between any of the location types (df = 4; x^2 = 5.899; $P = 0.207$; Mean DHD ± SE = 0.00 ± 0.000; Mean DMD ± SE = 0.27 ± 0.162; Mean DLD ± SE = 0.04 ± 0.036; Mean DOS ± SE = 0.07 ± 0.048; Mean ROW ± SE = 0.08 ± 0.053; Fig. 4.10).

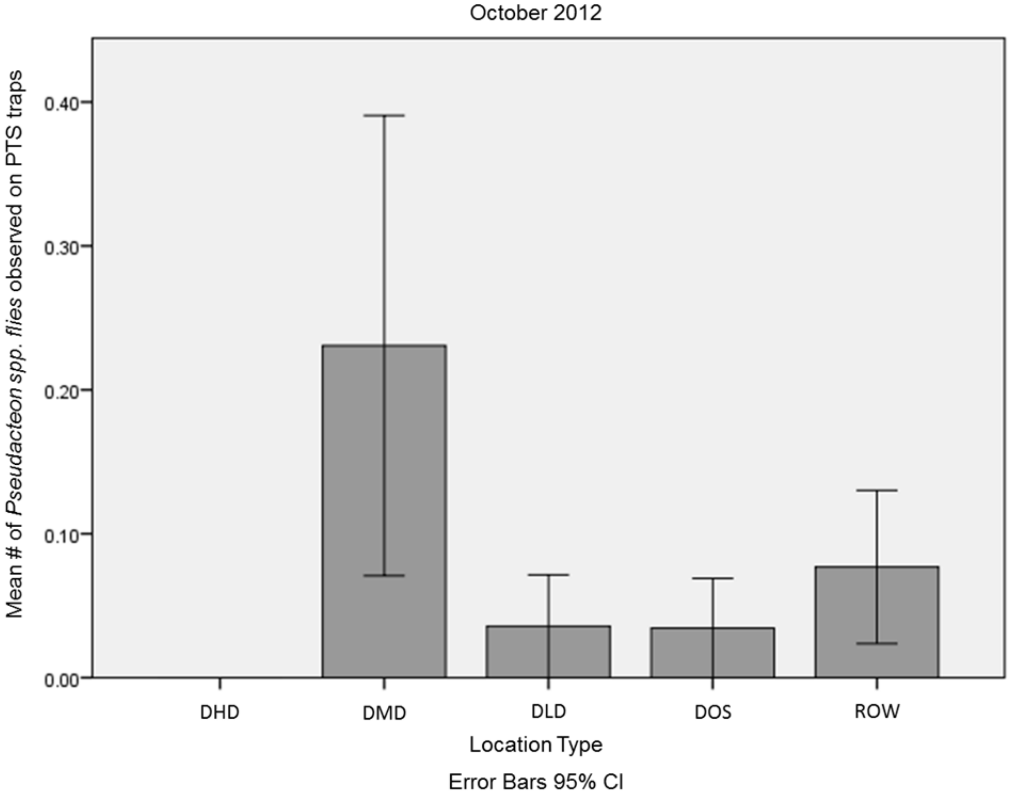

Fig. 4.10. Mean number of phorid flies found at each location type in October (fall) 2012 observations. There was no significant difference between location types (df = 4; x^2 = 5.899; $P = 0.207$). DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

When individual cities are considered, there was no statistical difference in the number of flies found on PTS traps between any of the trap location types in New Braunfels, Texas (df = 4; x^2 = 5.106; *P* = 0.277; Mean DHD ± SE = 0.80 ± 0.386; Mean DMD ± SE = 1.54 ± 0.475; Mean DLD ± SE = 1.11 ± 0.381; Mean DOS ± SE = 1.33 ± 0.590; Mean ROW ± SE = 1.53 ± 0.623; Fig. 4.11).

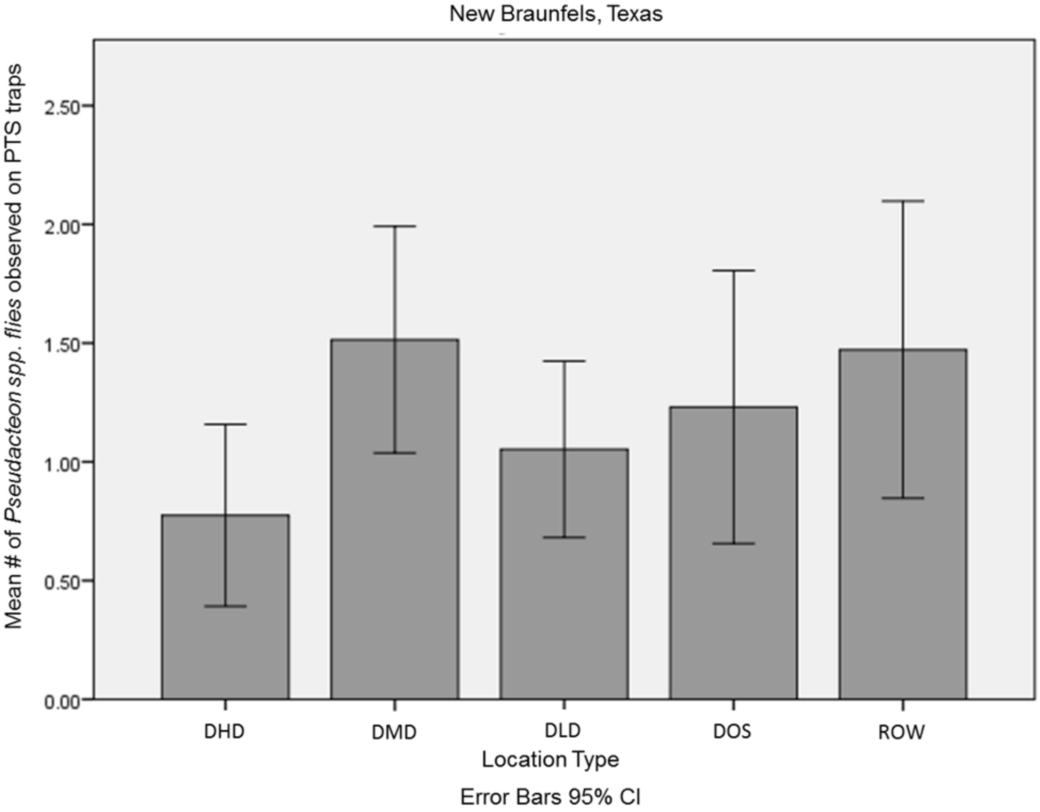

Fig. 4.11. Mean number of phorid flies found in New Braunfels, Texas, at each location type for all sampling dates. There was no significant difference between location types (df = 4; x^2 = 5.106; *P* = 0.277). DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

San Marcos, Texas had no statistically significant difference between the mean number of flies found on PTS traps at each location type (df = 4; $x^2 = 4.849$; $P = 0.303$; Mean DHD ± SE = 0.03 ± 0.026; Mean DMD ± SE = 0.17 ± 0.077; Mean DLD ± SE = 0.16 ± 0.060; Mean DOS ± SE = 0.23 ± 0.124; Mean ROW ± SE = 0.09 ± 0.069; Fig. 4.12).

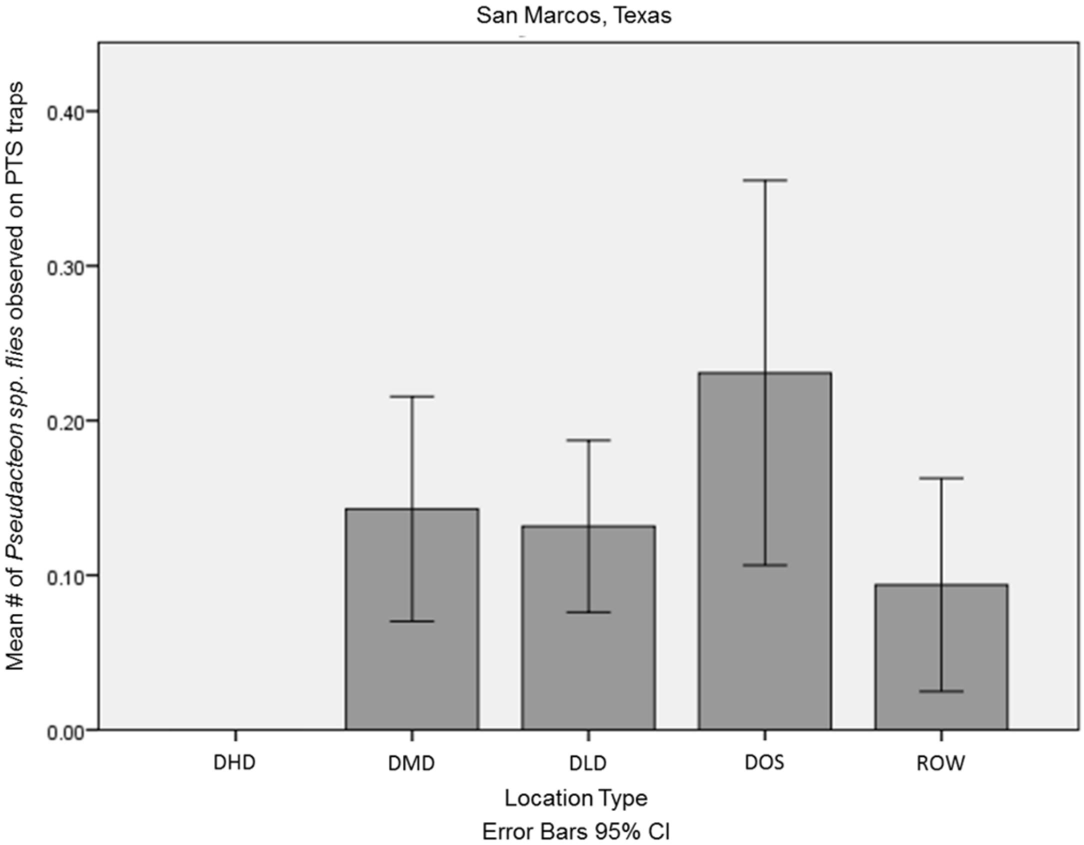

Fig. 4.12. Mean number of phorid flies found in San Marcos, Texas, at each location type for all sampling dates. There was no significant difference between the location types (df = 4; $x^2 = 4.849$; $P = 0.303$). DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

Cedar Park, Texas had no significant difference between the mean number of flies on PTS traps found at each location type (df = 4; x^2 = 4.479; *P* = 0.345; Mean DHD ± SE = 0.06 ± 0.246; Mean DMD ± SE = 0.38 ± 0.150; Mean DLD ± SE = 0.24 ± 0.085; Mean DOS ± SE = 0.20 ± 0.064; Mean ROW ± SE = 0.22 ± 0.104; Fig. 4.13).

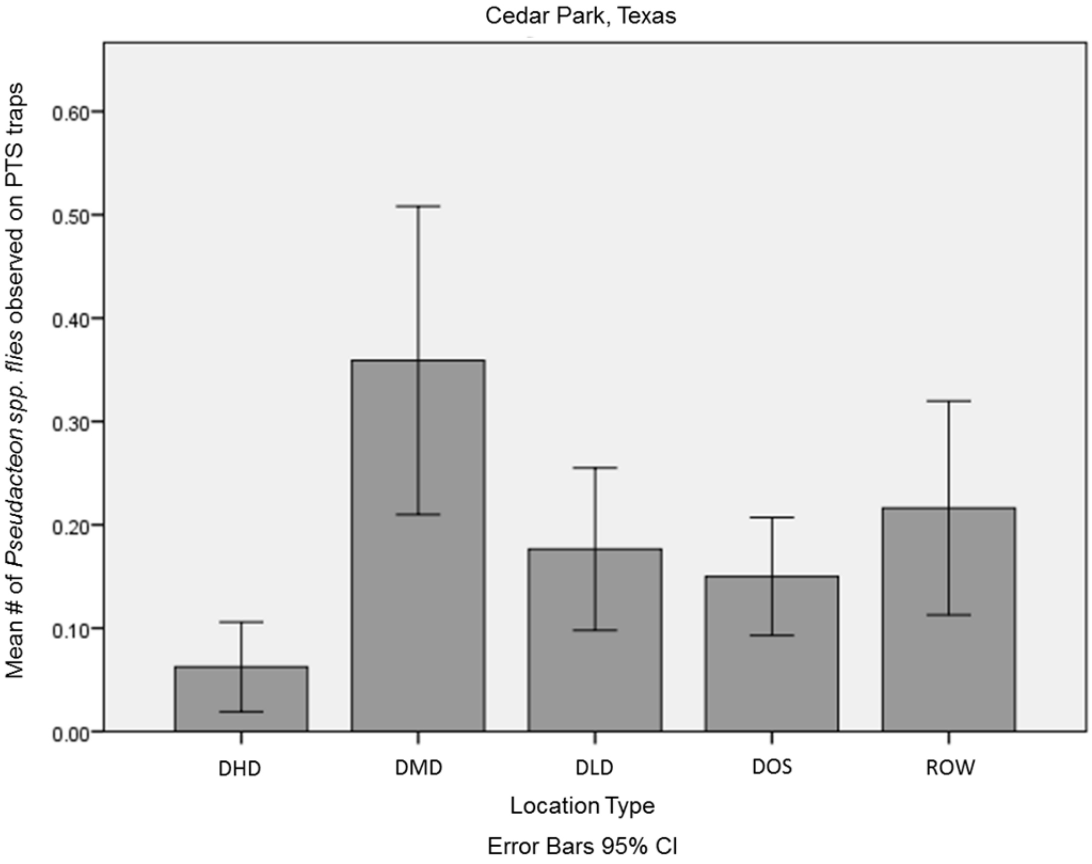

Fig. 4.13. Mean number of phorid flies found in Cedar Park, Texas, at each location type for all sampling dates. There was no significant difference between the location types (df = 4; x^2 = 4.479; *P* = 0.345). DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

Hot dog lure samples

The three sampled cities were statistically equivalent in the number of RIFA that were observed from hot dog lure samples (df = 2; x^2 = 4.001; P = 0.135; Mean Cedar Park ± SE = 125.04 ± 11.085; Mean New Braunfels ± SE = 124.49 ± 13.978; Mean San Marcos ± SE = 121.22 ± 12.879; Fig. 4.14).

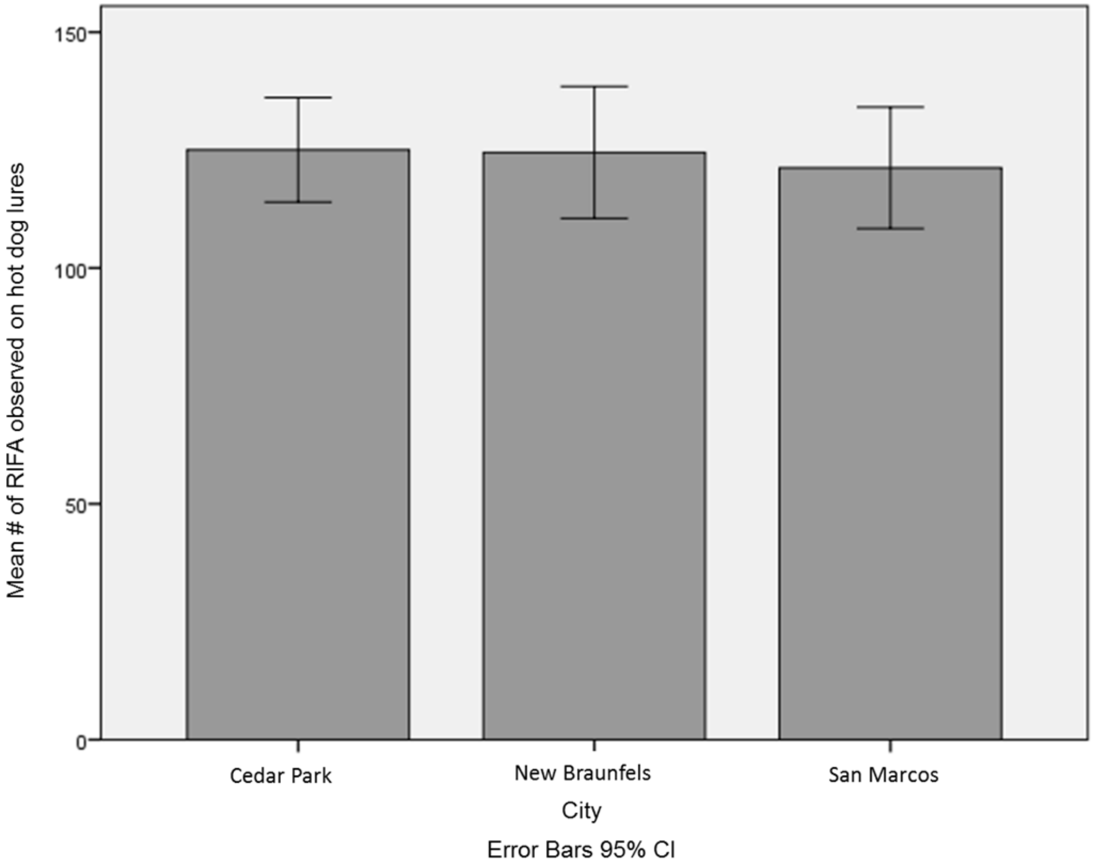

Fig. 4.14. Mean number of RIFA foragers observed on hot dog lures in all sampled cities for all sampling dates. Cities were statistically equivalent (df = 2; x^2 = 4.001; P = 0.135).

Analysis of the number of RIFA found at each location type showed a significant difference between location types (df = 4; $x^2 = 35.348$; $P < 0.001$; Mean DHD ± SE = 145.12 ± 17.861; Mean DMD ± SE = 126.52 ± 16.704; Mean DLD ± SE = 145.32 ± 13.856; Mean DOS ± SE = 80.28 ± 14.818; Mean ROW ± SE = 176.76 ± 17.300; Fig. 4.15). A summary of the paired Mann-Whitney U test results is provided in Table 4.3.

Table 4.3. Summary of results of pairwise Mann-Whitney U tests for RIFA found on hot dog lures from all location types, in all cities and all sampling dates. DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

Pairwise Comparison	**Sample Size**	**U value**	***Z* value**	***P* value**
DHD - DMD	111/109	5714.0	0.734	= .046
DHD - DLD	111/110	5270.5	1.823	=0.07
DHD - DOS	111/118	4948.0	3.389	< 0.05
DHD - ROW	111/105	4890.5	2.069	< 0.05
DMD - DLD	109/110	5522.5	1.052	= 0.29
DMD - DOS	109/118	5180.5	2.702	< 0.05
DMD - ROW	109/105	4469.5	2.813	< 0.05
DLD - DOS	110/118	5674.5	1.765	= 0.08
DLD - ROW	110/105	3911.5	4.169	< 0.05
DOS - ROW	118/105	3578.5	5.636	< 0.05

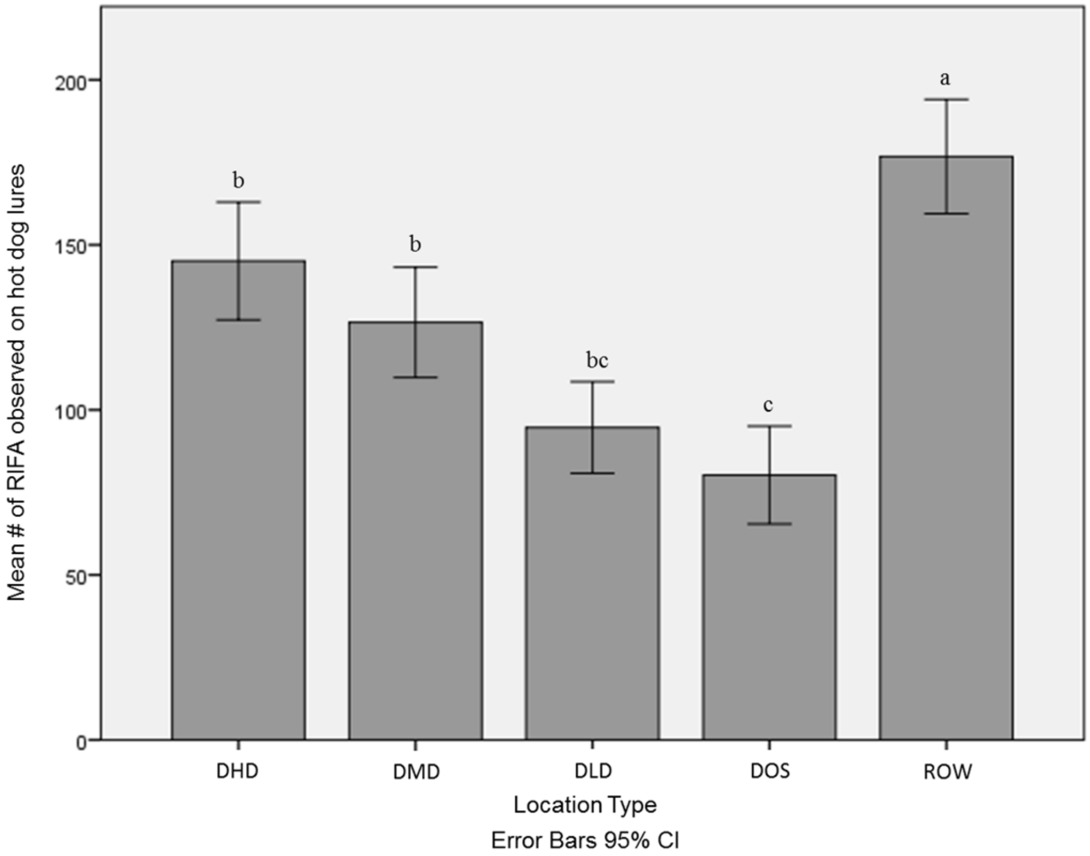

Fig. 4.15. Mean number of RIFA found on hot dog lures at each location type for all cities and all sampling dates. There was a significant difference between the location types (df = 4; $x^2 = 35.348$; $P < 0.001$). Location types indicated by different letters were significantly different when compared with Mann-Whitney U pairwise tests (Table 4.3). DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

The mean numbers of RIFA observed by month/season of sampling was significantly different (df = 3; x^2 = 25.542; P <0.001; Fig. 4.16; January Mean ± SE = 69.21 ± 127.514; April Mean ± SE =165.12 ± 16.226); October Mean ± SE = 147.22 ± 15.983; July Mean ± SE = 116.70 ± 14.277). A summary of paired Mann-Whitney U tests is located in Table 4.4.

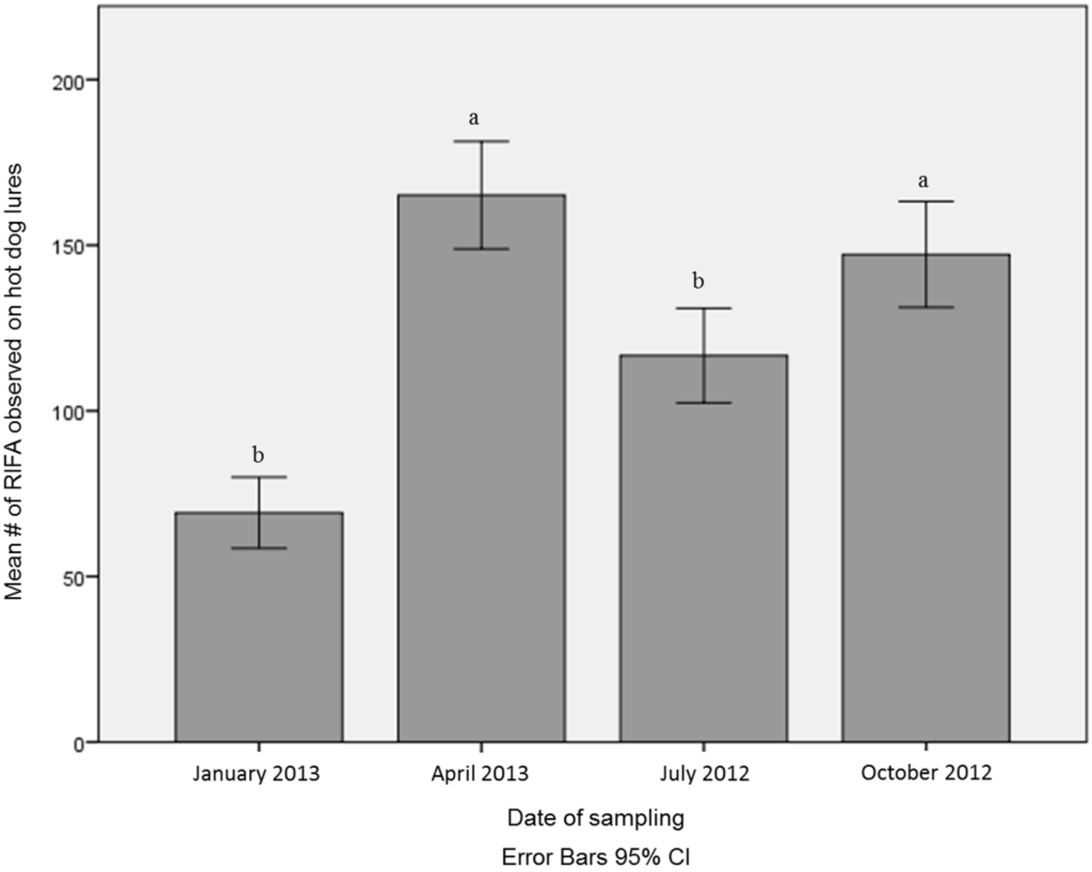

Fig. 4.16. Mean number of RIFA found on hot dog lures by month for all location types in all cities. The number of RIFA was significantly different between the dates sampled (df = 3; x^2 = 25.542; P <0.001). Location types indicated with different letters were statistically different with Mann-Whitney U pairwise comparisons.

Table 4.4. Summary of results of pairwise Mann-Whitney U tests for RIFA foragers found on hot dog lures by month in all cities and all location types. DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

Pairwise Comparison	**Sample Size**	**U value**	***Z* value**	***P* value**
January - April	141/130	6277.5	4.620	< 0.05
January - July	141/144	9515.5	0.975	= 0.33
January - October	141/138	7469.5	3.481	< 0.05
April - July	130/144	7428.5	3.055	< 0.05
April - October	130/138	8289.5	1.093	= 0.28
July - October	144/138	8560.5	2.096	< 0.05

During the January observations, the mean number of RIFA observed from hot dog lures was statistically different between location types (df = 4; x^2 = 14.670; $P < 0.01$; DHD Mean ± SE = 61.25 ± 16.230; DMD Mean ± SE = 26.44 ± 11.494; DLD Mean ± SE = 69.43 ± 19.972; DOS Mean ± SE = 38.63 ± 17.942; ROW Mean ± SE = 150.96 ± 38.773; Fig. 4.17). A summary of paired Mann-Whitney U tests are summarized in Table 4.5

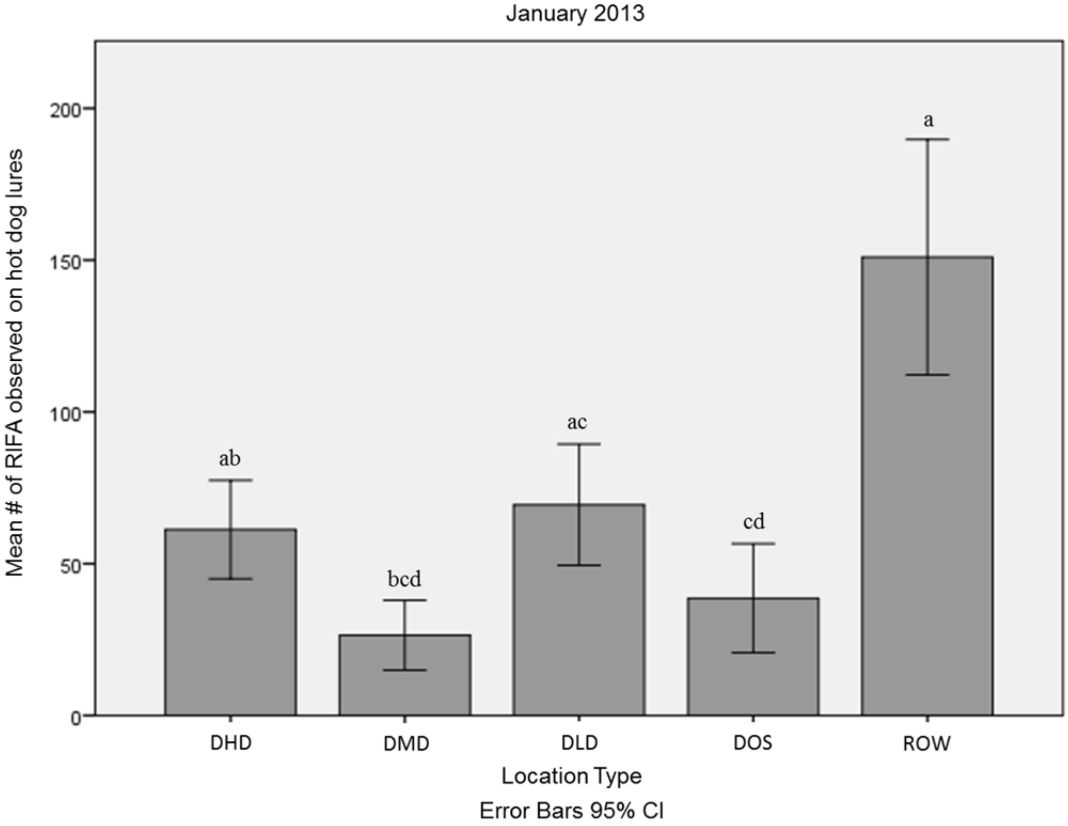

Fig. 4.17. Mean number of RIFA found on hot dog lures at each location type in January 2013. Location types were significantly different (df = 4; $x^2 = 14.670$; $P < 0.01$). Location types indicated with different letters were statistically different with Mann-Whitney U pairwise comparisons. DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways

Table 4.5. Summary of results of pairwise Mann-Whitney U tests for RIFA found on hot dog lures from January observations in all cities and all location types. DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

Pairwise Comparison	**Sample Size**	**U value**	***Z* value**	***P* value**
DHD DMD	28/27	293.5	1.495	= 0.14
DHD - DLD	28/28	381.0	0.188	=0.85
DHD - DOS	28/30	290.5	2.209	< 0.05
DHD - ROW	28/28	303.0	1.488	= 0.14
DMD - DLD	27/28	312.0	1.183	= 0.24
DMD - DOS	27/30	350.0	1.003	= 0.32
DMD - ROW	27/28	218.0	2.784	< 0.01
DLD - DOS	28/30	316.5	1.802	= 0.07
DLD - ROW	28/28	293.5	1.658	= 0.10
DOS - ROW	30/28	220.0	3.327	< 0.01

During the April observations, the mean number of RIFA observed from hot dog lures were statistically different between location types (df =4; x^2 = 10.436; $P < 0.05$; DHD Mean ± SE = 220.50 ± 41.682; DMD Mean ± SE = 108.04 ± 29.320; DLD Mean ± SE = 135.00 ± 32.922; DOS Mean ± SE = 139.37 ± 36.070; ROW Mean ± SE = 233.35 ± 35.306; Fig. 4.18). A summary of paired Mann-Whitney U tests are provided in Table 4.6.

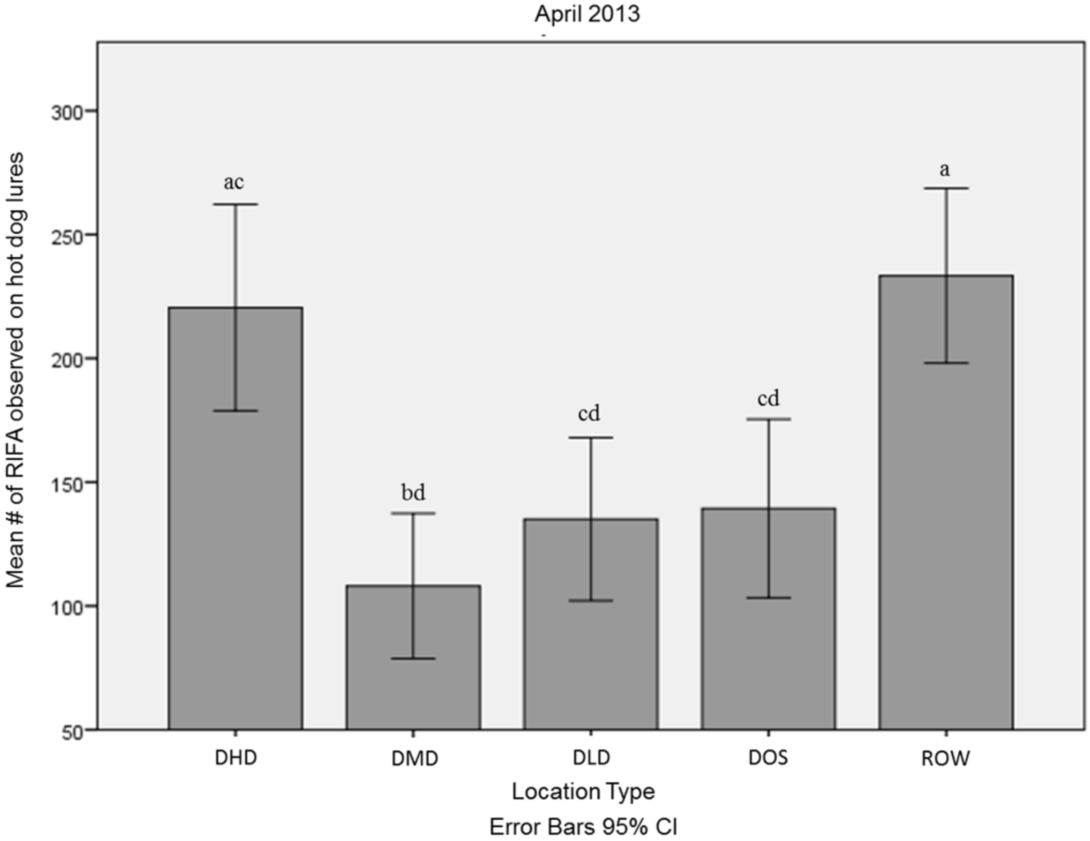

Fig. 4.18. Mean number of RIFA found on hot dog lures at each location type in April 2013. There was a significant difference between the groups (df =4; $x^2 = 10.436$; $P < 0.05$). Location types indicated with different letters were statistically different with Mann-Whitney U pairwise comparisons. Results are shown by location type and for all cities sampled. DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

Table 4.6. Summary of results of pairwise Mann-Whitney U tests for RIFA found on hot dog lures in April observations in all cities and all location types. DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

Pairwise Comparison	**Sample Size**	**U value**	***Z* value**	***P* value**
DHD -DMD	26/26	229.5	2.045	< 0.05
DHD -DLD	26/25	262.5	1.188	=0.24
DHD -DOS	26/30	308.0	1.366	=0.17
DHD -ROW	26/23	279.5	0.392	= 0.70
DMD -DLD	26/25	267.0	1.122	= 0.26
DMD -DOS	26/30	346.5	0.741	= 0.46
DMD -ROW	26/23	163.0	2.773	< 0.01
DLD -DOS	25/30	352.5	0.385	= 0.70
DLD -ROW	25/23	186.0	2.102	< 0.05
DOS -ROW	30/23	221.5	2.233	< 0.05

During the July observations, the mean number of RIFA observed from hot dog lures were statistically equivalent between location types (df =4; $x^2 = 10.680$; $P < 0.05$; DHD Mean ± SE = 126.39 ± 35.748; DMD Mean ± SE = 169.73 ± 37.796; DLD Mean ± SE = 106.62 ± 32.287; DOS (Mean ± SE = 53.76 ± 19.747; ROW Mean ± SE = 125.82 ± 28.590; Fig. 4.19). A summary of paired Mann-Whitney U tests are provided in Table 4.7.

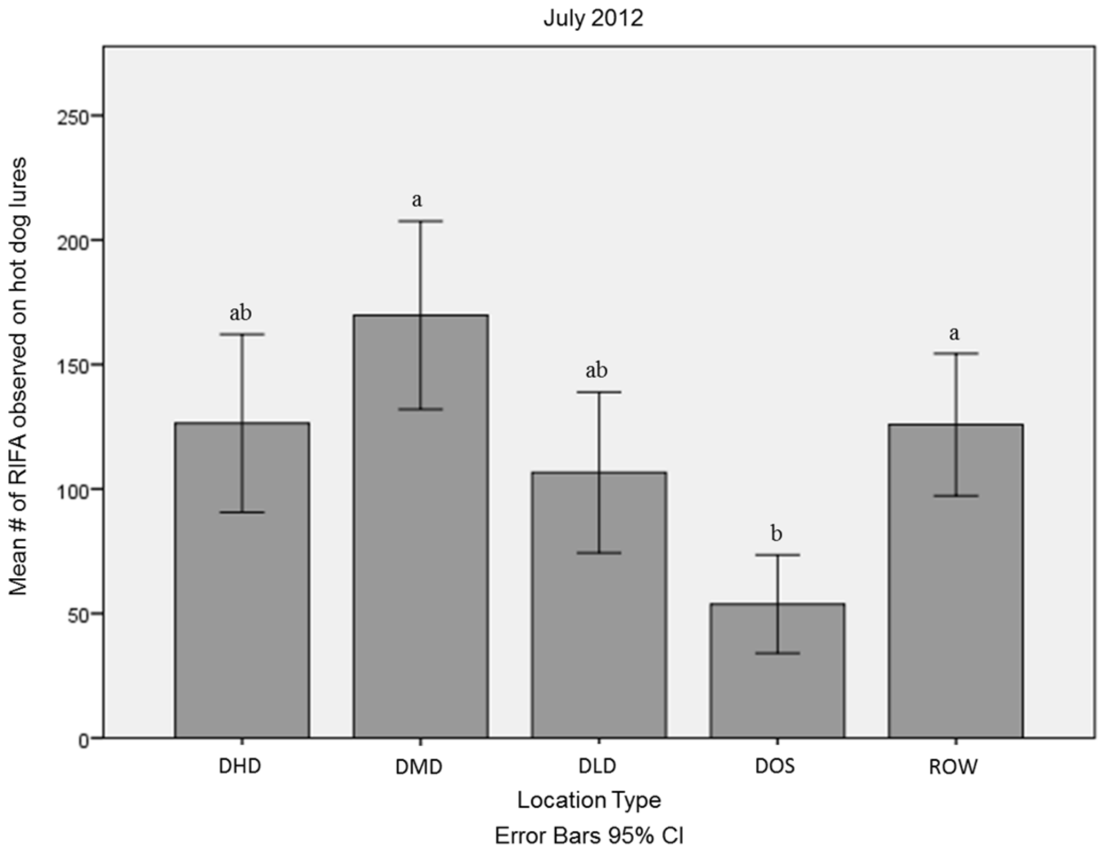

Fig. 4.19. Mean number of RIFA found on hot dog lures at each location type in July 2012. There was a significant difference between the location types (df =4; $x^2 =$ 10.680; $P < 0.05$;). Location types indicated with different letters were statistically different with Mann-Whitney U pairwise comparisons. DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

Table 4.7. Summary of results of pairwise Mann-Whitney U tests for RIFA found on hot dog lures in July observations in all cities and all location types. DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

Pairwise Comparison	**Sample Size**	**U value**	***Z* value**	***P* value**
DHD -DMD	28/30	367.0	0.865	=0.39
DHD -DLD	28/29	368.0	0.669	=0.50
DHD -DOS	28/29	303.0	1.875	=0.06
DHD -ROW	28/28	339.0	0.896	= 0.37
DMD -DLD	30/29	335.5	1.619	= 0.11
DMD -DOS	30/29	273.0	2.704	< 0.05
DMD -ROW	30/28	412.0	0.127	=0.90
DLD -DOS	29/29	365.0	1.034	= 0.30
DLD -ROW	29/28	311.5	1.585	=0.11
DOS -ROW	29/28	238.5	2.869	< 0.05

October observations of RIFA on hot dog lures showed a statistically significant difference between the location types (df = 4; x^2= 19.060; $P < 0.01$; DHD Mean ± SE = 176.59 ± 38.718; DMD Mean ± SE = 199.08 ± 37.581; DLD Mean ± SE = 71.54 ± 23.731; DOS Mean ± SE = 71.54 ± 23.731; ROW Mean ± SE = 209.35 ± 32.592; Fig. 4.20). A summary of paired Mann-Whitney U tests are provided in Table 4.8.

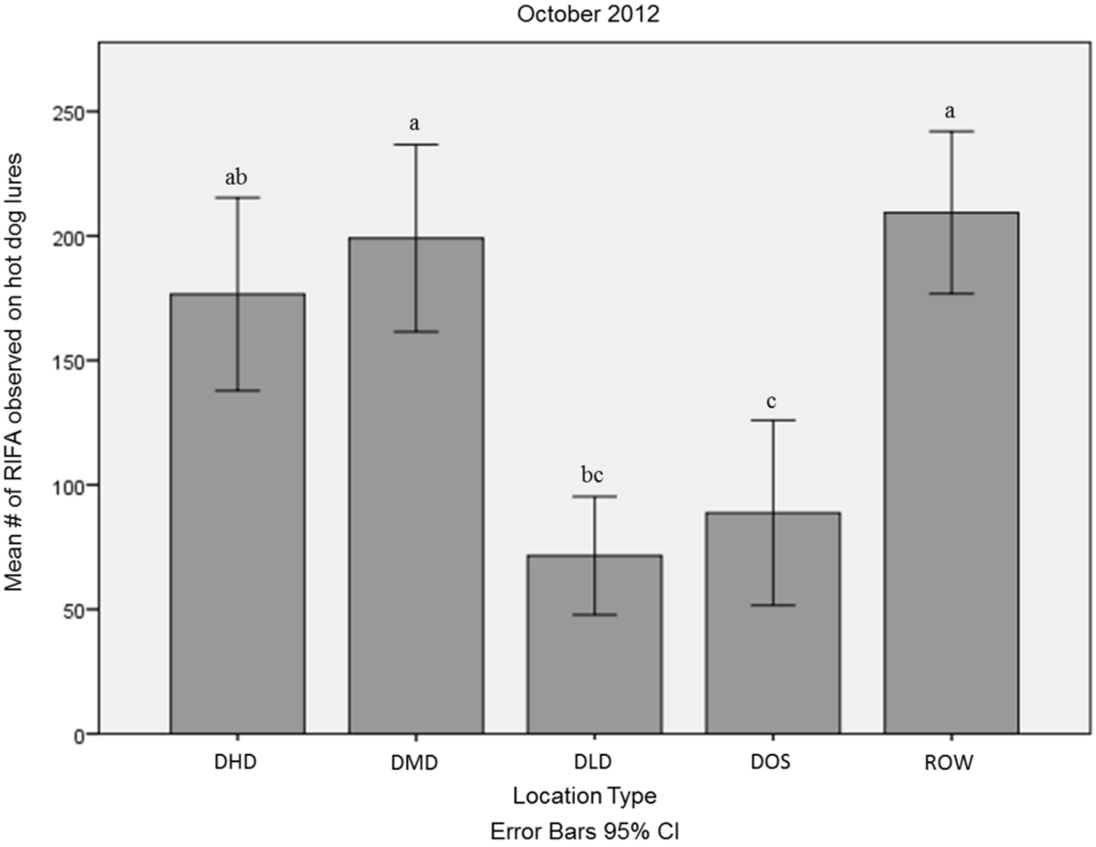

Fig. 4.20. Mean number of RIFA found on hot dog lures at each location type in October 2012. There was a significant difference between the location types (df = 4; x^2= 19.060; *P* < 0.01). Location types indicated with different letters were statistically different with Mann-Whitney U pairwise comparisons. DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

Table 4.8. Summary of results of pairwise Mann-Whitney U tests for RIFA found on hot dog lures in October observations in all cities and all location types. Results are shown on observations by location types and for all cities. DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

Pairwise Comparison	**Sample Size**	**U value**	***Z* value**	***P* value**
DHD -DMD	29/26	334.5	0.725	=0.468
DHD -DLD	29/28	299.0	1.767	=0.077
DHD -DOS	29/29	290.0	2.127	< 0.05
DHD -ROW	29/26	304.5	1.232	= 0.218
DMD -DLD	26/28	219.0	2.567	< 0.05
DMD -DOS	26/29	214.0	2.840	< 0.01
DMD -ROW	26/26	319.0	0.349	=0.727
DLD -DOS	28/29	367.5	0.659	= 0.510
DLD -ROW	28/26	176.0	3.307	< 0.01
DOS -ROW	29/26	177.0	3.456	< 0.01

RIFA presence was analyzed by trap location type in each city and results for New Braunfels showed a significant difference between the groups (df = 4; x^2= 18.505; $P < 0.01$, DHD Mean ± SE = 171.00 ± 27.699; DMD Mean ± SE = 78.13 ± 21.391; DLD Mean ± SE = 106.09 ± 24.340; DOS Mean ± SE = 109.33 ± 23.539; ROW Mean ± SE = 169.16 ± 24.994; Fig. 4.21). A summary of paired Mann-Whitney U tests are provided in Table 4.9.

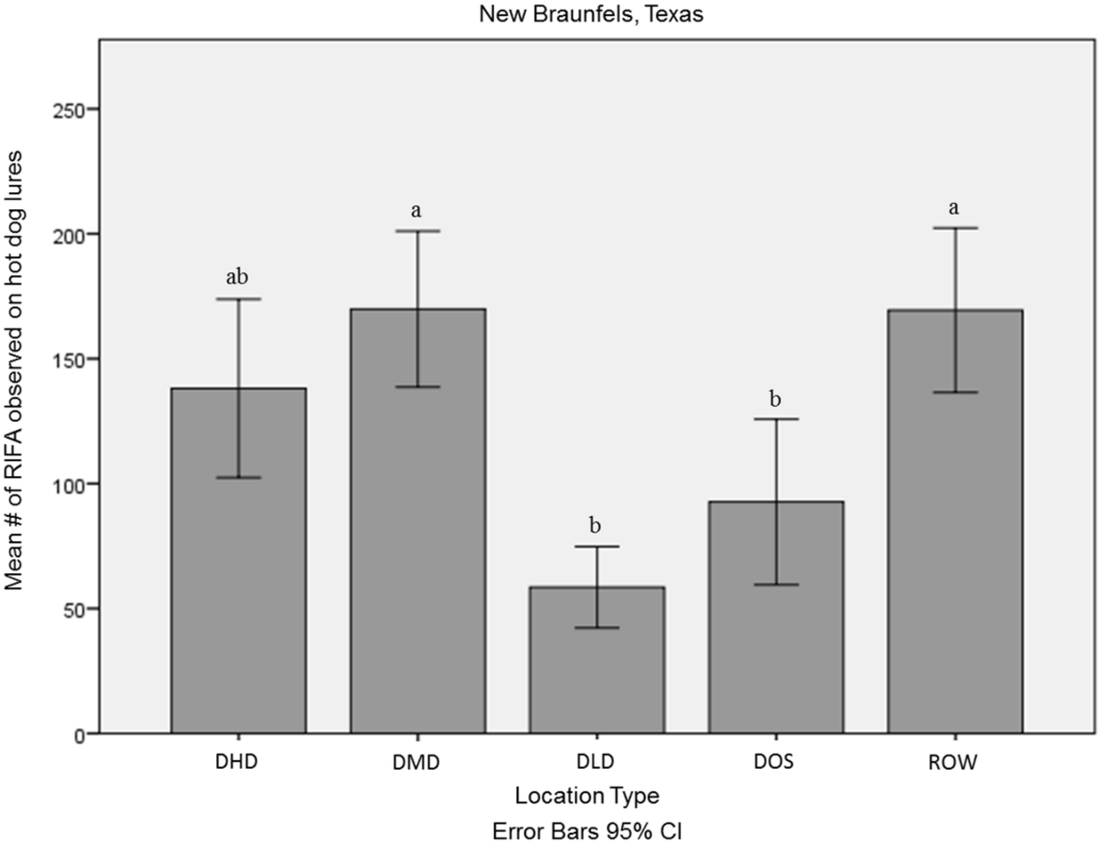

Fig. 4.21. Mean number of RIFA foragers found on hot dog lures from all observations in New Braunfels, Texas, by location type. There was a significant difference between location types (df = 4; x^2= 18.505; $P < 0.01$). Location types indicated with different letters were statistically different with Mann-Whitney U pairwise comparisons (Table 4.10). DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

Table 4.9. Summary of results of pairwise Mann-Whitney U tests by location type for RIFA found on hot dog lures in New Braunfels, Texas. Results are shown on observations by location types and all dates. . DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

Pairwise Comparison	**Sample Size**	**U value**	***Z* value**	***P* value**
DHD -DMD	40/35	547.0	1.692	=0.09
DHD -DLD	40/38	672.0	0.979	=0.33
DHD -DOS	40/39	686.5	1.032	= 0.30
DHD -ROW	40/36	548.5	1.855	= 0.06
DMD -DLD	35/38	396.0	3.118	< 0.05
DMD -DOS	35/39	422.0	2.982	< 0.05
DMD -ROW	35/36	619.0	0.128	=0.90
DLD -DOS	38/39	730.5	0.123	= 0.90
DLD -ROW	38/36	407.5	3.132	< 0.05
DOS -ROW	38/36	430.5	3.038	< 0.05

RIFA presence was analyzed by trap location type in San Marcos and results showed a significant difference between the groups (df =4; x^2=19.969; $P < 0.01$, DHD Mean ± SE = 131.08 ± 27.375; DMD Mean ± SE = 137.14 ± 32.909; DLD Mean ± SE = 120.61 ± 29.040; DOS Mean ± SE = 38.13 ± 17.051; ROW Mean ± SE = 184.764 ± 32.662; Fig. 4.22). A summary of paired Mann-Whitney U tests are provided in Table 4.10.

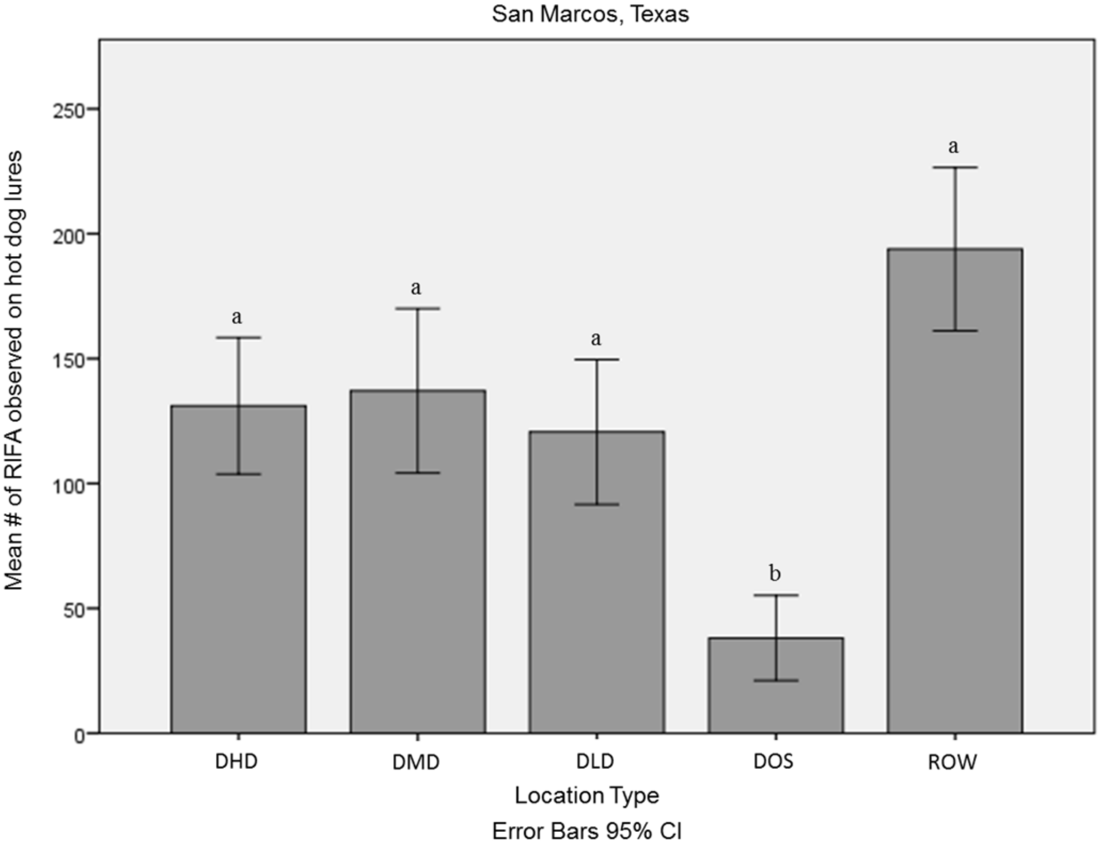

Fig. 4.22. Mean number of RIFA foragers found on hot dog lures from all observations in San Marcos, Texas, by location type. There was a significant difference between the location types (df =4; x^2=19.969; $P < 0.01$). Location types indicated with different letters were statistically different with Mann-Whitney U pairwise comparisons (Table 4.11). DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

Table 4.10. Summary of results of pairwise Mann-Whitney U tests by location type for RIFA found on hot dog lures in San Marcos, Texas. Results are shown for all observations dates. DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

Pairwise Comparison	**Sample Size**	**U value**	***Z* value**	***P* value**
DHD -DMD	40/35	547.0	0.308	=0.758
DHD -DLD	40/38	672.0	0.326	=0.745
DHD -DOS	40/39	686.5	2.956	< 0.01
DHD -ROW	40/36	548.5	1.648	= 0.099
DMD -DLD	35/38	396.0	0.011	= 0.991
DMD -DOS	35/39	422.0	2.502	< 0.05
DMD -ROW	35/36	619.0	1.642	=0.101
DLD -DOS	38/39	730.5	2.775	< 0.01
DLD -ROW	38/36	407.5	1.958	= 0.05
DOS -ROW	38/36	430.5	4.411	< 0.01

RIFA presence was analyzed by trap location type in Cedar Park and results showed a significant difference between the groups (df = 4; x^2=17.179; $P < 0.01$, DHD Mean ± SE = 171.00 ± 27.699; DMD Mean ± SE = 78.13 ± 21.391; DLD Mean ± SE = 106.09 ± 24.340; DOS Mean ± SE = 109.33 ± 23.539; ROW Mean ± SE = 169.16 ± 24.994; Fig. 4.23). A summary of paired Mann-Whitney U tests are provided in Table 4.11.

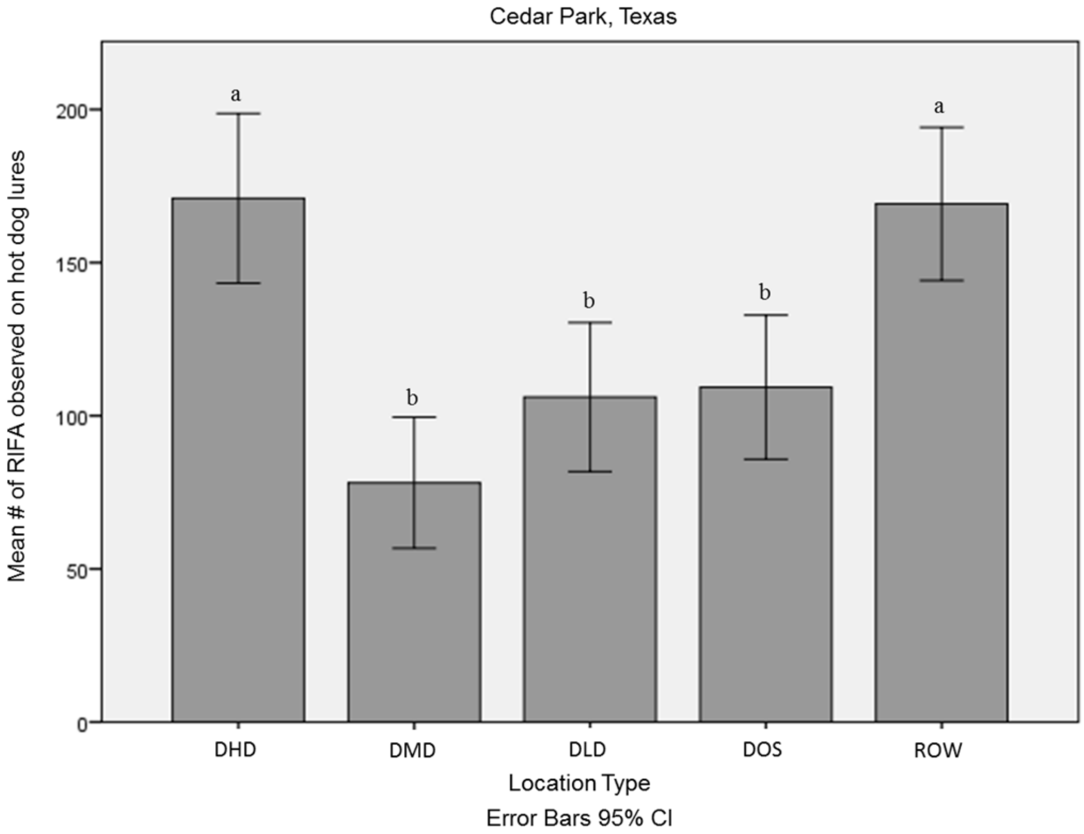

Fig. 4.23. Mean number of RIFA foragers found on hot dog lures from all observations in Cedar Park, Texas, by location type. There was a significant difference between the location types (df = 4; x^2=17.179; $P < 0.01$). Location types indicated with different letters were statistically different with Mann-Whitney U pairwise comparisons (Table 4.12). DHD represents Developed High Density with 81-95% impervious cover, DMD represents Developed Medium Density with 56-70% impervious cover, DLD represents Developed Low Density with 31-45% impervious cover, DOS represents Developed Open Space with 6-20% impervious cover, and ROW represents Right of Way locations along major highways.

Table 4.11. Summary of results of pairwise Mann-Whitney U tests by location type for RIFA found on hot dog lures in Cedar Park, Texas. Results are shown on observations from all dates. DHD is Developed High Density with 81-95% impervious cover, DMD is Developed Medium Density with 56-70% impervious cover, DLD is Developed Low Density with 31-45% impervious cover, DOS is Developed Open Space with 6-20% impervious cover, and ROW is Right of Way locations along major highways.

Pairwise Comparison	**Sample Size**	**U value**	***Z* value**	***P* value**
DHD -DMD	32/39	350.0	3.238	< 0.01
DHD -DLD	32/34	384.5	2.064	< 0.05
DHD -DOS	32/40	453.5	2.144	< 0.05
DHD -ROW	32/37	586.0	0.072	= 0.942
DMD -DLD	39/34	546.5	1.346	= 0.172
DMD -DOS	39/40	681.0	1.025	= 0.305
DMD -ROW	39/37	406.5	3.341	< 0.01
DLD -DOS	34/40	662.0	0.201	= 0.840
DLD -ROW	34/37	445.0	2.135	< 0.05
DOS -ROW	40/37	530.0	2.170	< 0.05

Surface temperature data

There was a significant difference in the surface temperatures recorded in each season (ANOVA; F (3,512) = 116.122 = , $P < 0.001$; Fig. 4.24). July/Summer had the highest average temperature (29.53 ± 0.41°C) and was significantly different from all other seasons/months. April/Spring had the next highest average mean temperature (26.37 ± 0.43°C) and was also significantly different from all other seasons/months. January/Winter (20.31 ± 0.51°C) and October/Fall (20.86 ± 0.28°C) were not significantly different from each other.

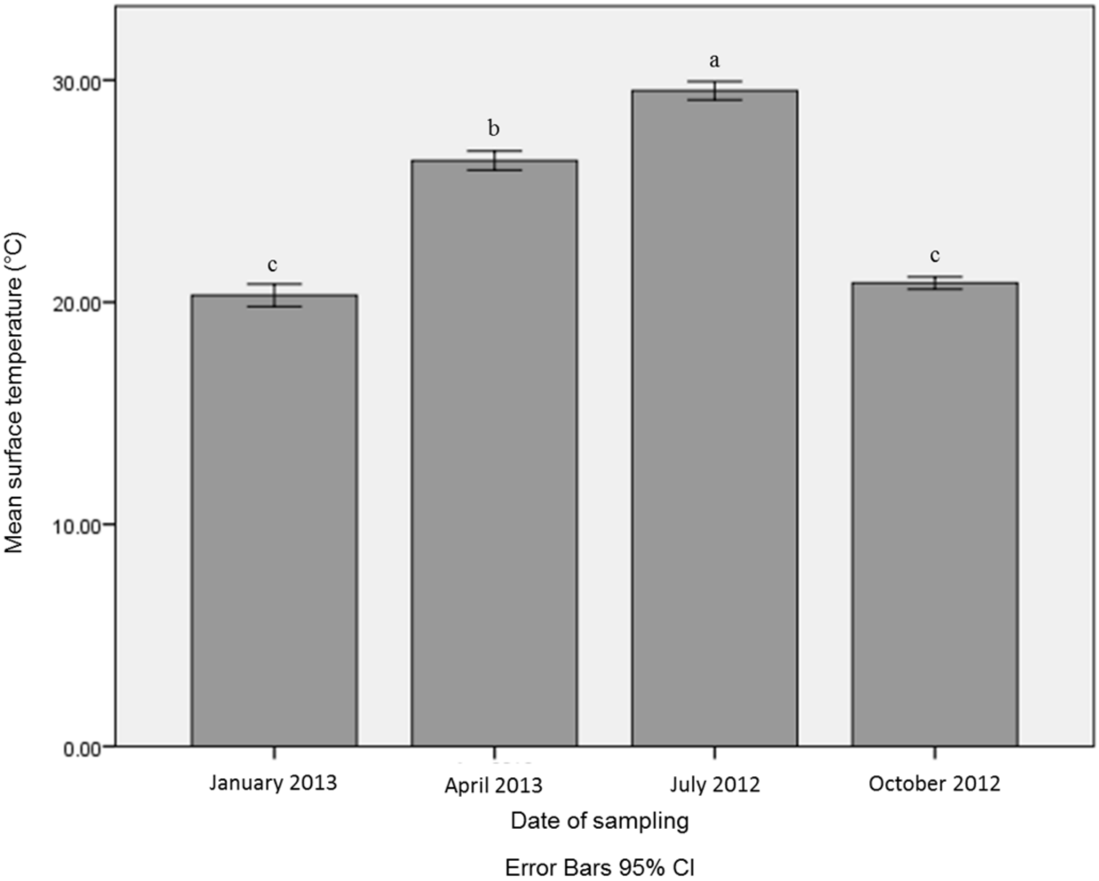

Fig. 4.24. Mean surface temperature (° C) from each month/season, at all location types and all sampling sites.

Discussion

Throughout the experiment, only *Pseudacteon curvatus* was identified from any PTS traps placed in urban areas. Dispersal maps published from 2011 (University of Texas, Fire Ant Project) show that both *P. curvatus* and *P. tricuspis* had been established in all three sampling regions. However, work by LeBrun et al. (2009) showed competitive displacement of *P. tricuspis* by *P. curvatus* in central Texas. This research supports the conclusions by LeBrun et al. (2009) that *P. curvatus* has become the dominant RIFA parasitizing fly, at least in urban areas. Consistently and with rapidity, *P. curvatus* displaced *P. tricuspis* due to, the ability to arrive to disturbed nests first and the advantage of female fecundity in this species. Additionally, the rarity of larger workers required for *P. tricuspis* to successfully develop female flies ensures biased effects, especially once phorids are detected by RIFA.

Phorid flies were found in all location types sampled, with the developed high density, 81-95% impervious cover (DHD) consistently had a trend for the fewest number of flies found, although not significantly different from other location types. Heavy impervious cover in these locations, and therefore little exposed soil space, would limit the available above ground foraging areas for RIFA and inhibit the activity of phorid flies. Foraging RIFA are unlikely to travel across long expanses of concrete or asphalt and flies lacked resting places and harborage. The relative abundance of flies varied by season but little variation was observed in respect to urban location types. Therefore *P. curvatus* were fairly equally distributed across the various location types in urban areas but showed seasonal fluctuation. Regardless of time of year or city sampled, phorids were evenly distributed throughout the urban landscape in relation to the location types investigated in this study.

New Braunfels, Texas in Comal County had the highest abundance of *P. curvatus* as compared to the other sampled cities, while San Marcos had the fewest number of phorid flies. As the temperatures, rainfall, and general climate were similar the explanation for this phenomenon is open for interpretation. We surmised the drought of 2010-2011 played a significant role. Rainfall is a primary influence on not only RIFA population levels (Porter and Tschinkel 1987) but also phorid population levels (Folgarait et al. 2007). If over the two year period, New Braunfels received isolated rain events or if the areas sampled were irrigated, both fly and RIFA abundance could have been less affected by arid conditions.

Phorid flies were most abundant in the spring and summer observation times with the winter observation having the fewest flies observed. Flies were still present in the winter observation, but at very low levels. Winters in Texas are generally very mild, and winter soil temperatures on the sampling day were seasonably mild, with an average temperature of 20.3 ± 0.28 °C (Fig. 4.25). Phorid activity was driven by climactic conditions especially rainfall, so seasons which experience high levels of precipitation would be expected to display an increase in phorid populations if RIFA were present.

Cities sampled were uniform in the total number of RIFA found on hot dog lures; however, the total number of ants observed from the ROW location type was highest while DOS location types had the fewest RIFA. This abundance data did not coincide with phorids found on PTS traps. The numbers of RIFA observed on hot dog lures were highest during spring and fall observations; however, summer was the season in which the most phorids were collected. High temperatures in July may have hindered RIFA foraging on hot dog lures, especially in areas exposed to full sun. Temperature ranges were within acceptable foraging limits (Mean ± SE = 29.53 ± 0.41 °C) for all observation dates (Porter and Tschinkel 1987, Drees et al. 2009).

Factors affecting phorid abundance were complex including climate considerations such as rainfall and temperature, and these same environmental conditions affect RIFA populations as well as phorid flies. Urban systems introduce further complications to being able to predict population fluctuations of either RIFA or phorids. General weather patterns may be considerably reduced in importance due to the influence of irrigation systems, RIFA abatement programs, urban heat islands, and wind pattern changes brought about by structures.

The analysis clearly showed phorid flies were present in urban areas and were evenly distributed within cities. Phorids were found most often on PTS traps during the summer and fall sampling dates. Finally, this work failed to reject the null hypothesis that phorid flies would be evenly distributed throughout the urban landscape. There were trends, but no significant difference between each land use categories, favoring the DMD and DOS land use categories.

CHAPTER V

SUMMARY AND CONCLUSIONS

A great deal of research has been dedicated to understanding the biology and habits of the red imported fire ant, *Solenopsis invicta* Buren (RIFA). Recently, research has been conducted to understand the interaction between RIFA and *Pseudacteon* spp. flies (Diptera: Phoridae), a suite of parasitoid flies that attack RIFA workers. Establishment and expansion of introduced biological control agents is paramount to successful control, and evidence suggested several species of *Pseudacteon* flies are expanding their range in central Texas. Over time, there is evidence that a single species, *P. curvatus*, displaces and outcompetes other species of phorids that attack RIFA (LeBrun et al. 2009). This research further supported the hypothesis that *P. curvatus* is the dominant species in central Texas. The degree to which *P. tricuspis* and other species are displaced by *P. curvatus* and the environmental carrying capacity of these flies is unknown; however, a singularity in sampling shown in this work, which yielded over 30 flies at a single location, demonstrates the system can support many parasitoids at one time.

Host size partitioning is known for many species of phorids, especially for *P. tricuspis*, where large workers are needed for female fly production (Morrison et al. 1999a). Worker size is less important for *P. curvatus* development and sex determination than it is for *P. tricuspis* (Morrison et al. 1997). Introduction and establishment of *P. tricuspis* occurred first, and the response by RIFA to exposure to this species of phorid fly was to reduce the number of large foragers due to parasitism. In a system where large workers were rare due to an abundance of polymorphic colonies, (Greenberg et al. 1985), this further reduction in the abundance of large workers in response to parasitoid activity inhibits the ability of *P. tricuspis* to thrive. When *P. curvatus*, which prefer to oviposit into smaller workers, were introduced, the available forager size composition in central Texas was ideal for their success, possibly explaining their dominance in the landscape, including urban areas. The dominance of *P. curvatus* should continue to be monitored in the field, in urban as well as rural environments, because as these fly densities shift, RIFA response, as it pertains to worker sizes and foraging strategies, could change. RIFA worker head capsule widths could shift back to large workers being more prevalent, as *P. curvatus* is not selective in choosing large workers. Due to the dominant phorid fly species, *P. curvatus*, tendency to parasitize RIFA that are isolated, there is a reduction in the number of encounters with nest mates. This has a lower overall effect on foraging ants and therefore reduces *P. curvatus* effectiveness as a biological control agent. This is unfortunate, as overall biological control effects could decrease as well.

Bait preference experiments presented in this dissertation provided evidence that RIFA are highly attracted to the commercial bait (CABB), most likely due to the small particle or oil components; however, RIFA consistently removed more of the nutrient rich lab made bait (EB1:1) material. The CABB, other than the food attractant component, is not nutritionally valuable, whereas the EB1:1 had a high nutrient profile of equal parts carbohydrates and lipids which might explain the difference in attractiveness and foragers moving the candidate baits. When formulating insecticide baits, active ingredient additions that are lipophilic would be more likely to be consumed by RIFA feeding on the bait in situ. However, hydrophilic insecticides or solid active ingredients could be less effective, especially if the bait matrix is low in nutrients and not transported to the fourth instar larvae for external digestion and distribution throughout the colony via trophallaxis. Implications of this preference could be far reaching due to the reliance on insecticide baits for the control of RIFA in both urban and rural settings. A more detailed set of experiments to elucidate what diet preference is most desirable in the presence/absence of parasitoid flies would help to design custom baits for areas with and without phorid activity.

Bait attractiveness remains important, but formulating baits at the correct particle size for the target species has also become an important issue. RIFA affected by phorid activity, especially *P. tricuspis* may have an abundance of small workers, which selectively forage baits associated with their size (Neff et al. 2011). Ultimately, to be effective, insecticide baits must facilitate delivery of the active ingredient to the target species and cause mortality. The size differential discussed in this dissertation could change the bait particle size preference that is collected by foragers in the field and could render control programs less effective. As species composition of phorid flies affecting RIFA changes, worker size abundance by head width could change, so ongoing research will help determine the best particle size for bait applications for RIFA control.

Increased nocturnal foraging by RIFA in the presence of phorid flies, in order to escape parasitism, could have drastic impacts interactions with native ants. Competition between native ants and RIFA for resources would favor foraging strategies RIFA use for food location and consumption. This temporal shift could have effects on both native ants and RIFA, but the consequences of the shift being positive or negative are unknown. This work should be expanded into natural environments to determine RIFA foraging patterns in the presence of phorid flies. Additionally, work should be done to determine effects these shifts on produce efficacy, if occurring, are having on native an assemblages.

Until this work, it was unknown if phorid flies sustained populations in urban areas. This work shows they survive in all areas of urban environments, including the most highly developed and areas of open space. The presence of phorid fly activity, combined with a high proportion of polygynous colonies in urban areas could cause worker size shifts in these environments. First discussed by Puckett et al. (2007) and confirmed in this dissertation, there is a distinct difference in the abundance of smaller workers, in areas also dominated by polygyne colonies, in the presence of phorid flies. This shift affects bait preferences, specifically causing RIFA to selectively forage smaller bait particles. Highly attractive bait matrices and attractants will entice RIFA foragers, but if the bait formulation and particle size is less than ideal, foragers will be less likely to allay on the bait or transport it.

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