BIOLOGICAL AND ECOLOGICAL ASPECTS OF FIELD RELEASED FIRE ANT

DECAPITATING FLIES *PSEUDACTEON SPP.* (DIPTERA: PHORIDAE),

PARASITOIDS OF RED IMPORTED FIRE ANTS

SOLENOPSIS INVICTA BUREN (HYMENOPTERA: FORMICIDAE)

A Dissertation

by

ROBERT TREY PUCKETT

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A Dissertation

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ROBERT TREY PUCKETT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

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December 2008

Major Subject: Entomology

ABSTRACT

Biological and Ecological Aspects of Field Released Fire Ant Decapitating

Flies *Pseudacteon spp*. (Diptera: Phoridae), Parasitoids of Red Imported Fire

Ants *Solenopsis invicta* Buren (Hymenoptera: Formicidae). (December 2008)

Robert Trey Puckett, B.A.; M.S., Sam Houston State University

Chair of Advisory Committee: Dr. Marvin K. Harris

Multiple *Pseudacteon* phorid fly species, including *P. tricuspis and P. curvatus*, have been released in the southern United States beginning in 1997 and 2003 (respectively) to serve as biological control agents against red imported fire ants *Solenopsis invicta* Buren (hereafter referred to as RIFA). Field research in the United States on phorid/RIFA interactions has addressed establishment and spread of released species. Additionally, studies are needed on phorid biology and ecology with respect to spatial distribution and phenology, phorid/habitat associations and phorid-mediated affects on RIFA foraging patterns.

A suite of manipulative laboratory and field experiments/observations were conducted to 1) develop a novel phorid sampling device to provide uniform and repeatable sampling of flies, 2) assess spatial distributions and phenology of *Pseudacteon tricuspis* and *P. curvatus*, 3) assess *P. tricuspis* and *P. curvatus* habitat associations and 4) assess phorid-mediated affects on RIFA foraging patterns and caste ratios.

PTS Traps (developed for this research) are significantly more effective than previous sampling methods in terms of mean number of flies collected, efficiency of use and % trap success. Data collected with these traps allowed for the determination of patterns of species-specific phenology and relative densities through time and species-specific numerical/spatial superiority (Chapter IV). *P. curvatus* was significantly more abundant than *P. tricuspis* in only one of the habitat types sampled (df (160) = 4.57, *P* < 0.005). *P. tricuspis* densities did not differ significantly among habitat type. Llaboratory experiments revealed that phorid-exposed RIFA colonies foraged less intensively diurnally (df = 1,558; *P* < 0.05) and more intensively nocturnally (df = 778; *P* < 0.05) relative to control colonies. Field data regarding this compensatory nocturnal foraging shift did not corroborate those of the laboratory work. In the field there was no significant difference in foraging intensity during nocturnal (df (18) = -0.486, *P* = 0.633) and diurnal (df (18) = 1.375 *P* = 0.186) sampling periods. Lastly, chi-square analysis of RIFA forager size-classes revealed significant differences between phorid-infested (treatment) and phorid-free (control) sites (χ^2 = 6811.85, df = 3, *P* < 0.05) with a significantly greater proportion of small RIFA foragers at the phorid-infested site.

ACKNOWLEDGEMENTS

While the author of a document such as this receives the accolades (and criticisms) that accompany it, such a work is never completed without the care, guidance and patience of others. There are many people whom I wish to thank for allowing me the opportunity to complete this dissertation. Dr. Marvin Harris first recruited me to our department and since my arrival he has offered tremendous wisdom, endless encouragement and (when appropriate) the proper amount of caring criticism. He will forever have my gratitude and my respect as both a scientist and as a man.

Dr. Bastiaan 'Bart' Drees supervised my work as an Extension Assistant during the latter portion of the pursuit of this degree. Bart provided invaluable stability during a time of program uncertainty. I express my gratitude to him, for without his efforts this would have been an immeasurably more difficult process.

My dissertation committee included Dr. Robert Wharton (Entomology), Dr. S. Bradleigh Vinson (Entomology) and Dr. Kirk Winemiller (Wildlife and Fisheries Sciences). These men were instrumental in the development and execution of my research plan and with the preparation of this dissertation. Each provided superb advice during this process and contributed their time and energy to the project and for this I am very grateful.

I am equally grateful to Jessica Honaker, Alejandro Calixto, Allen Dean, Sarah Skrivanek, John Fell, Maggie Toothaker, Johnny Johnson, Julia Smith, Freder Medina and Sherry Ellison, all of whom assisted me with either laboratory experiments, field

experiments or both. Elvis Takow and Maria Tchakerian provided important advice regarding the GIS procedures necessary for this work.

I would also like to thank the United States Department of Agriculture (Area-wide Suppression of Imported Fire Ants in Pastures Project) for providing funding for my position and research.

In addition to providing a tremendous amount of advice regarding research involving *Pseudacteon spp.* phorid flies, Dr. Larry Gilbert (University of Texas – Austin) allowed testing and demonstration of prototypes of PTS-Traps at the Brackenridge Field Laboratory in Austin, TX. The extent to which he and his lab aided in this project was remarkable given the competitive nature of this research arena…and I will never forget it.

Finally, without the love and support of my wife, Jennifer, none of this would have been possible. All of those who attempt a project such as this should hope to do so with a partner such as her. Her patience was boundless, and her support endless.

To our beloved children, Lauren and Cooper… *per aspera ad astra!*

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CHAPTER I

INTRODUCTION: THE ROLE OF *PSEUDACTEON* PHORID FLIES IN

BIOLOGICAL CONTROL OF RED IMPORTED FIRE ANTS *SOLENOPSIS INVICTA*

BUREN: HISTORICAL PERSPECTIVE AND RESEARCH OBJECTIVES

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), hereafter referred to as RIFA, is an invasive species that has become established in the United States and elsewhere (Morrison et al. 2004). These ants are native to South America and were discovered in Mobile, AL in the 1930's (Vinson 1997). RIFA populations have since undergone an explosive dispersal and range expansion across the gulf-coast and eastern seaboard states. Their contiguous range now extends from western Texas east to Florida and north to North Carolina. In addition, disjunct populations have become established in Maryland, New Mexico, Arizona, and California. Like many other species of non-native invasive plants and animals that are liberated from the population regulatory effects of natural enemies in their native range, RIFA pose a significant ecological and economic threat to invaded areas (Lofgren 1986, Porter et al. 1992).

Many species of parasitic flies (*Pseudacteon spp.*; Diptera: Phoridae) parasitize workers of *Solenopsis saevissima* complex fire ants (including *S. invicta*) throughout their native South American range (Folgarait et al. 2000; Calceterra et al. 2005). These

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This dissertation follows the style of Environmental Entomology.

flies are considered potential candidates for biological control of RIFA and are being released in the United States (Porter 1998; Graham et al. 2003; Vogt and Street 2003; Porter et al. 2004). Female phorids oviposit into the thorax of RIFA workers (Morrison et al. 1997). Neonate larvae then travel into the head capsule of the ant where they develop for a period of 2-3 weeks (Consoli et al. 2001; Porter and Pesquero 2001). Before pupation occurs, the intersegmental membranes of the host are chemically dissolved and the ant head capsule becomes separated from the body. The decapitated head serves as a puparium for the developing fly. The sex of developing *Pseudacteon tricuspis* is facultatively determined by host head size (Morrison et al. 1999). Female *P. tricuspis* typically develop in head capsules that are significantly larger than those in which males develop (Morrison et al. 1999).

Initial work with RIFA/*Pseudacteon spp.* interactions in the early 1970's indicated a low potential for biological control success as a result of the extremely low rates of parasitism (1-8%) found in infested laboratory colonies (Gilbert and Patrock 2002). However, Feener's (1981) work renewed interest in the potential utility of phorids as biological control agents after demonstrating that the flies influence a negative shift in competitive success in ant colonies that are routinely attacked. Subsequent field work in this area has supported Feener's observations (Orr et al. 1995, 1997; Porter et al. 1995; Folgarait and Gilbert 1999). This reduction in competitive success is the result of a behavioral response in which ants reduce their foraging effort in the presence of phorids (Porter et al. 1995; Morrison 1999) and is the impetus for a portion of the research reported in this dissertation.

While a significant amount of fundamental research has been conducted with the goal of understanding RIFA/phorid interactions and phorid developmental biology (Feener 1981; Feener and Brown 1992; Folgarait and Gilbert 1999; Morrison 2000; Orr et al. 2003; Consoli et al. 2001; Porter and Pesquero 2001), the development of a successful biological control program against RIFA in their non-native distributions requires the successful establishment and expansion of phorids.
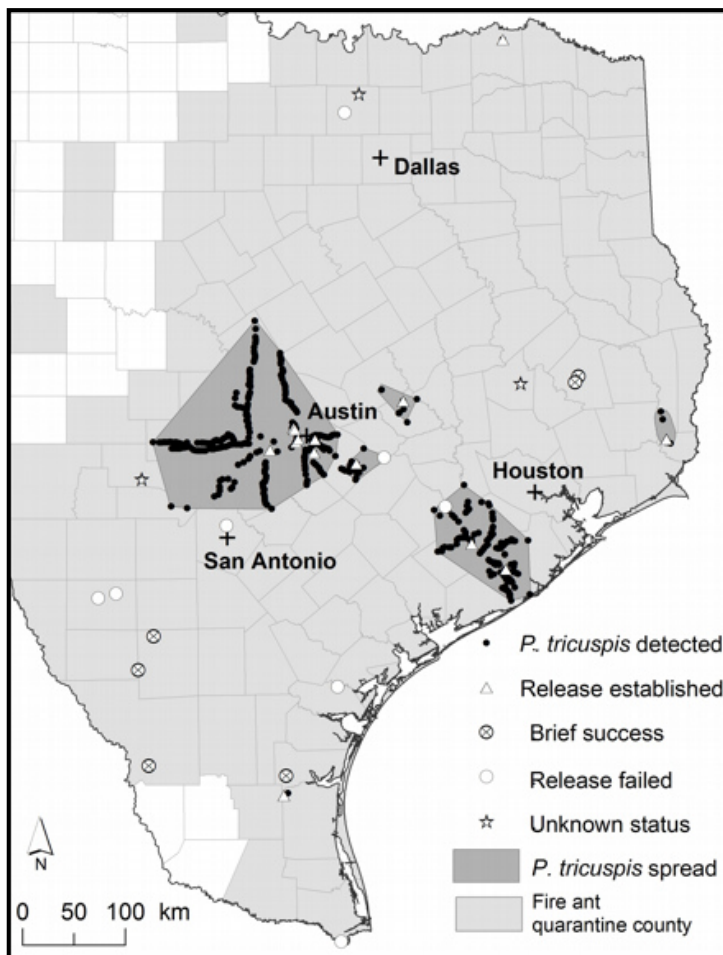
Fig. 1.1. Spread and establishment of *Pseudacteon tricuspis* decapitating flies in Texas as determined by different trap methods (Gilbert et al. 2008).

The first releases of *P. tricuspis* in Texas began in Austin, TX at the Brackenridge Field Laboratory (BFL) in 1995, but releases in earnest throughout the state began in 1999 with releases at 10 sites in 7 counties, and releases of *P. curvatus* began in 2004 at BFL and our field site in Burleson Co., TX (Gilbert et al. 2008). Releases have been inconsistently successful, and at present these populations of flies have expanded to ~7 million acres within Texas (Fig. 1.1 and 1.2) (Gilbert et al. 2008).
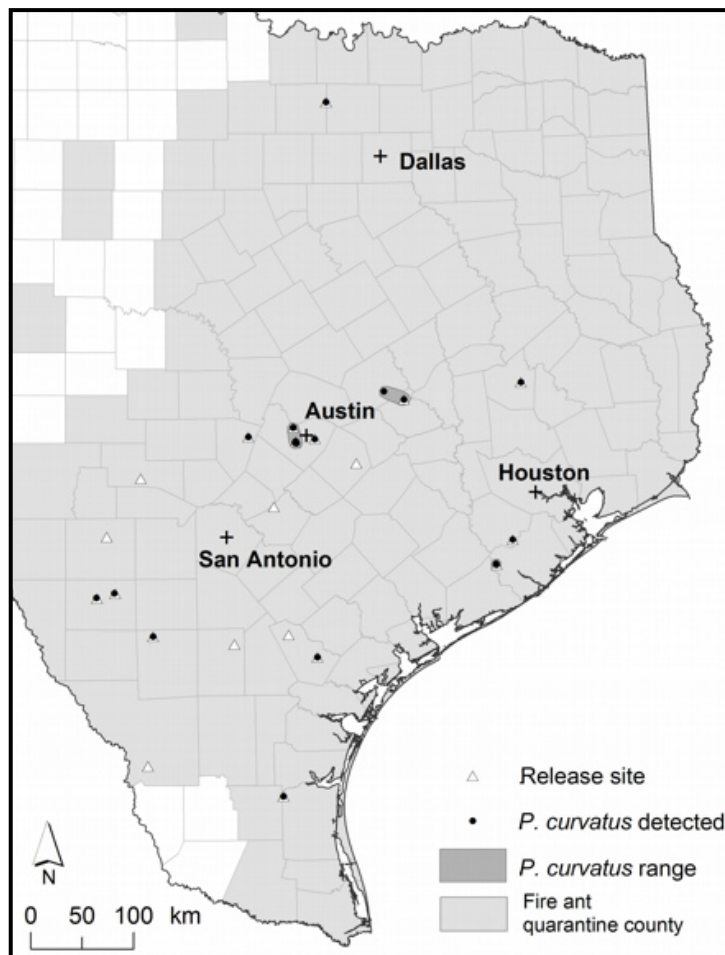
Fig. 1.2. Spread and establishment of *Pseudacteon curvatus* decapitating flies in Texas as determined by different trap methods (Gilbert et al. 2008).

In order to successfully choose appropriate establishment sites, a critical evaluation of the biotic and abiotic factors associated with previously successful establishment sites is necessary. Equally important is an understanding of RIFA/phorid interactions. This project has combined a regime of manipulative laboratory and field experiments/observations to refine our understanding of basic phorid fly biology and ecology, ant/fly interactions, and the potential for these flies to serve as successful biological control agents against RIFA. *Pseudacteon tricuspis* was released and became established at 5-Eagle Ranch in Burleson County, Texas (30º 34' 54.57" N; 96º 40' 59.77" W) in 2002 and has begun to expand its range (Fig 1.3). *Pseudacteon curvatus* was released in the Spring of 2004. *Pseudacteon curvatus* is physically smaller than *P. tricuspis* and was selected for it's purported ability to attack polygyne RIFA colonies, which predominate in most of Texas and are characterized by greater mound density (> triple that of monogyne colonies) with a larger proportion of small worker ants relative to monogyne RIFA colonies (Macom and Porter 1996). The first recovery of adult *P. curvatus* occurred during the Spring of 2005 and flies have been collected during all subsequent sampling periods. Phorids were provided for release by the USDA Center for Medical, Agricultural and Veterinary Entomology in Gainesville, Florida.
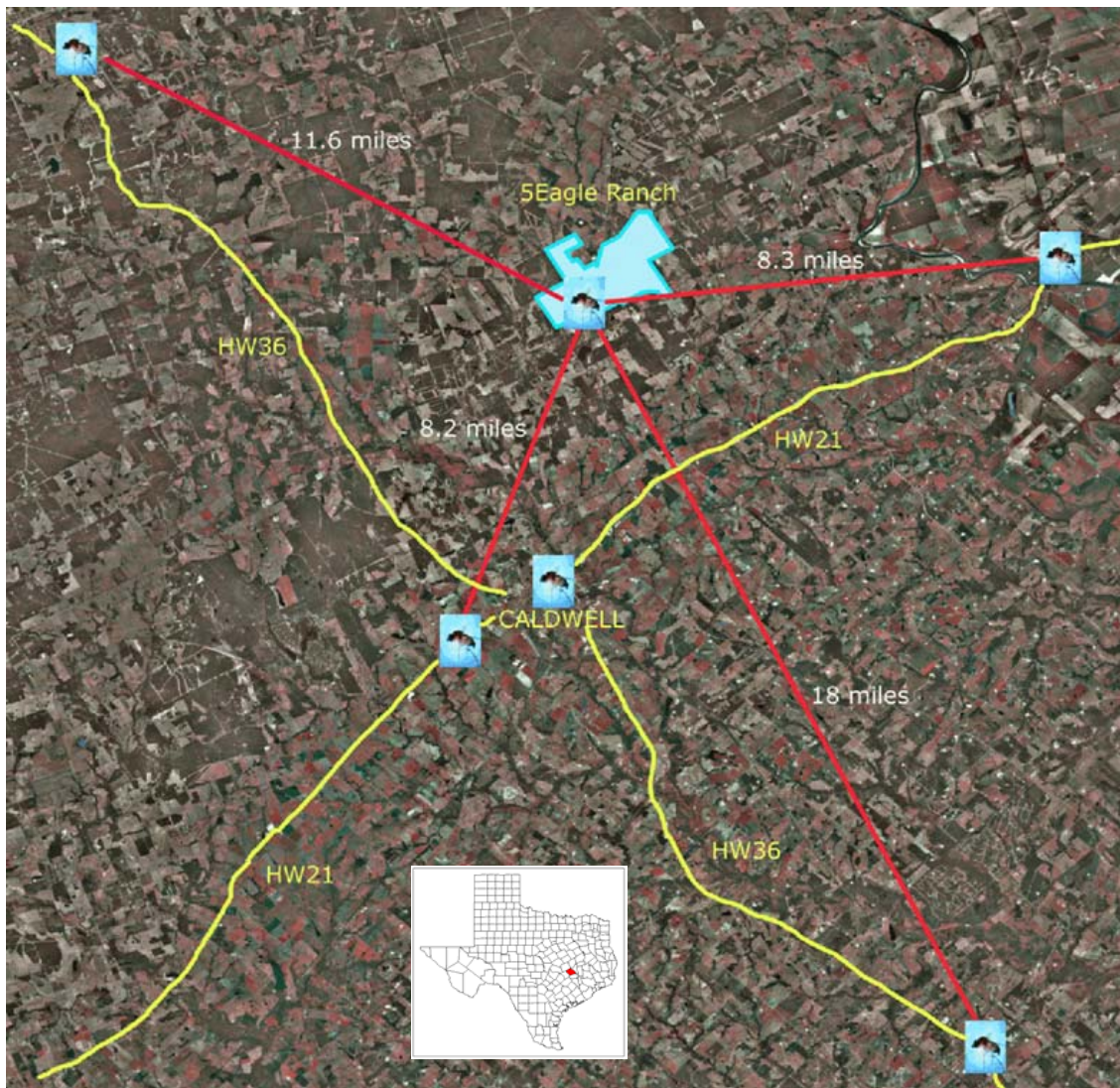
Fig 1.3 Expansion of *Pseudacteon tricuspis* from release site at 5-Eagle Ranch in Burleson County, Texas (2006).

Objectives

Many questions regarding RIFA/phorid interactions have been addressed in laboratory settings. Investigations of phorid-exposed RIFA foraging patterns were conducted in the laboratory and in the field. In addition, experiments regarding phorid

micro-habitat selection and suitability, spatial distribution patterns and phenology were conducted in the field. Understanding of each of these aspects of phorid behavior is critical in predicting appropriate establishment sites.

Following and critically assessing patterns of expansion and dispersal of small (0.6-1.5mm (Porter 1998; Puckett et al. 2007)) insects can be challenging. Development of reliable sampling methods (PTS-Traps - discussed below) and the use of GIS and remote sensing technologies was undertaken to aid in accurate assessment and interpretation of phorid/habitat associations. In addition, field and laboratory experiments were conducted to determine the effects of phorid activity on RIFA diel foraging patterns.

Field and Laboratory Experiments were designed to address the following research objectives:

1) **PTS-Trap development.** Development of a phorid sampling device to be used in field studies was undertaken to improve sampling efficiency. PTS-Traps were the fundamental tool used for sampling phorid flies in all field experiments/observations. H_o: Sampling efficiency of PTS-Traps are equal to conventional methods.

2) **Investigation of the affect of phorids on RIFA foraging patterns.** RIFA are known to forage diurnally and nocturnally; *P. tricuspis* and *P. curvatus* are diurnally active (Pesquero et al. 1996). The hypothesis that RIFA colonies will exhibit a shift towards nocturnal foraging as a result of exposure to phorid parasitism was tested in laboratory and field experiments. H_o: Circadian foraging patterns of RIFA colonies are independent of exposure to phorids.

3) **Phorid influence on RIFA worker size.** Field experiments were designed to determine if phorid host size selection influences the RIFA castes that are

observed in foraging trials. H_o: RIFA worker size is equal between phorid infested and uninfested colonies.

4) **Spatial Distribution and Phorid phenology.** Monthly sampling with PTS-Traps throughout multiple years allowed for a rigorous assessment of *P. tricuspis* and *P. curvatus* spatial distribution and phenology. H_o: Phorid density and spatial/temporal distribution remain the same throughout the year.

5) **Phorid/Habitat Associations**. GIS based investigations of phorid / habitat associations that examine phorid density along a continuum of habitat types. Densities of phorids were measured with PTS-Traps. H_o: Phorid densities are equal among habitat types.

Materials and Methods

Laboratory Research

Laboratory experiments were conducted in a climate-controlled greenhouse (Entomology Research Laboratory (ERL), Texas A&M University, College Station, TX). Flies were provided by the USDA Center for Medical, Agricultural and Veterinary Entomology in Gainesville, Florida.

Compensatory Nocturnal Foraging Shift

Twenty polygyne *S. invicta* colonies of similar size and caste structure were collected from several phorid-free sites within Brazos Co., TX and separated into ten treatment and ten control colonies, that were maintained in two separate climate controlled greenhouse units. Colonies were allowed to acclimate to greenhouse conditions (for approximately 2 weeks) prior to experimentation. *Pseudacteon tricuspis* were shipped from FL as pupae and, upon emergence, were selected and sorted based on

sex. Experimental cages were constructed of inverted 62.5L Rubbermaid® storage tubs. Panels were removed from the tubs and replaced with fine mesh and sealed. Two pans, one containing ants and the other containing diet, were placed on the inverted lid with the tub placed over them and closed. RIFA diet (homogenate containing protein [peanut butter and mealworms] and carbohydrate [honey] components) was provided in a Fluon® (AGC Chemicals) lined pan (fluon-coated surfaces = 90º from horizontal cannot be traversed by apterous RIFA). Ants accessed the diet by traversing a bridge constructed of 2.5cm metal ribbon. Fluon was placed on the bottom surface of the ribbon to ensure that all ants crossing the bridge were observable from above. After a 48 hr. period without food, food of known mass (~1 g) as well as 10 phorids (7 female, 3 male) were introduced into treatment colony cages. Control colonies were left uninfested. Observations (30 sec. in duration) of the number of ants crossing a line drawn midway across the metal bridge were made and recorded at 5 min., 15 min., 30 min., 1 hr., and 2 hr. intervals. These observations began at approximately 9:00am. Additionally, three 30 sec. nocturnal observations were made and recorded at 15min. intervals starting at ~15 hrs. after the initial food offering (midnight). Food was removed and weighed after 24 hrs. This procedure was repeated for a total of 10 trials. Students t-test was performed to compare foraging intensity and 24 hr. consumption rates between phorid affected and control colonies.

Field Research

The field experiments were conducted in the vicinity of 5-Eagle Ranch in Burleson County, Texas (30º 34' 54.57" N; 96º 40' 59.77" W).

Sampling Methodology

Sampling of field released populations of *P. tricuspis* and *P. curvatus* was conducted using PTS-Traps. Adult flies responding to deployed *Solenopsis invicta* midden (Gilbert and Patrock 2002; Smith and Gilbert 2003) are captured when they land on a Tanglefoot®-coated perch, which is part of the trap (Fig. 1.4). This passive method provides a uniform, repeatable and verifiable sample that allows continuous and simultaneous sampling among locations, which can only be accomplished with other techniques by substantially increasing the number of observers. A field test demonstrated the superior operational efficiency and effectiveness of this method relative to other techniques (Puckett et al. 2007). These traps have also been shown effective in various phorid habitats in Texas and Florida.
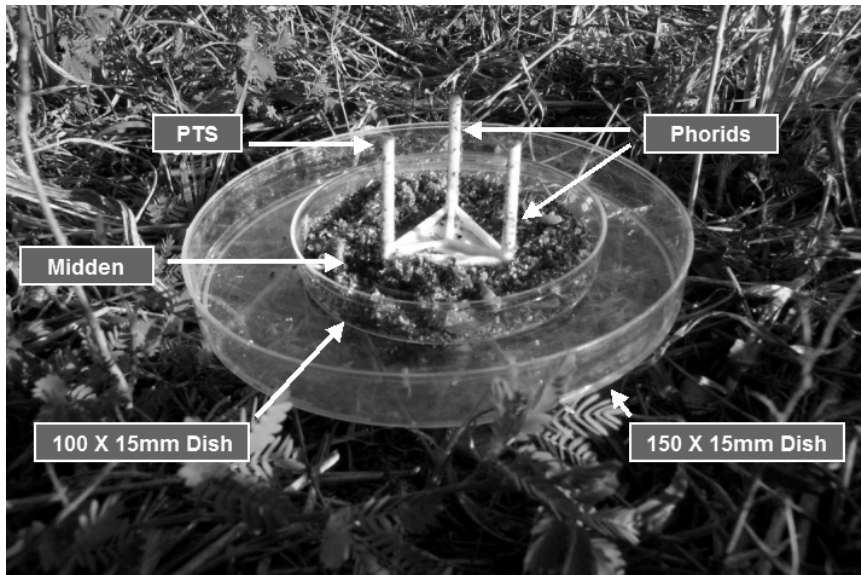
Fig. 1.4. PTS Trap with phorid flies ensnared by Tanglefoot® coated legs.

Nocturnal Foraging Shift

The laboratory experiments described above regarding compensatory nocturnal foraging suggested that RIFA may demonstrate a similar shift under field conditions to achieve a temporal escape from phorid parasitism pressure. Experiments were designed to determine if a foraging shift occurs in RIFA field populations. In Fall 2007, pitfall traps were used to assess nocturnal vs. diurnal fire ant foraging intensity simultaneously at both Caldwell, TX (phorid infested site) and Wellborn, TX (phorid free site). At each site a 3 X 4 grid of 12 pitfall traps (2m apart) was deployed. These traps were randomly divided into 2 sets prior to each replication. The nocturnal trap set was opened at PM civil twilight and closed and collected at the following AM civil twilight. At this time, the diurnal trap set was opened and then collected at the following PM civil twilight. This design allowed the collection of RIFA foraging intensity data over the full diel

cycle simultaneously at both the phorid infested and control sites. As an additional measure of RIFA foraging intensity, transects of 15 hot-dog lures were deployed at both PM and AM civil twilight and at both the phorid infested and control sites. After a period of 20 min. ants responding to these lures were collected. Transects of PTS-Traps were deployed during each replication of these experiments to monitor phorid activity. Hobo® data loggers were used to record temperature and humidity during the sampling period. Students t-test were performed to compare foraging intensity and 24 hr. consumption rates between treatments.

Phorid Influence on RIFA Worker Size Shift

Worker size polymorphism is important to task allocation in RIFA colonies, and experimental manipulation of the composition of RIFA worker sizes within colonies can have important affects on colony success (Porter and Tcshinkel 1984). Small workers are very important to brood production but are able to perform any colony task required of them (Mirenda and Vinson 1981); large workers are important for such tasks as mound maintenance, brood moving, defense and predation (Mirenda and Vinson 1981, O'Neal and Markin 1973, Porter and Tcshinkel 1984, Wilson 1978, Wilson et al. 1971). It is presumed that phorid-mediated manipulated RIFA worker sizes in the field will affect the efficiency with which colonies accomplish these tasks.

Effects of phorid activity on foraging RIFA size class differential were investigated. RIFA workers collected from the hot-dog lure transects in the above experiment were compared for size class composition. Ants were collected, sifted into 4

size classes, and counted. Hobo® data loggers were used to record temperature and humidity at each location during the sampling period. This experiment was replicated 10 times. Analyses of these observations were conducted using ANOVA.

Spatial Distribution, Phenology and Habitat Associations

5-Eagle ranch was mapped using ESRI ArcGIS® v9.0 software. The interior of the ranch was digitally characterized using a grid of 100 m X 100 m cells. Fifty cells were randomly selected using SPSS® software. ESRI software was used to determine the center (centroid) of each selected cell and the coordinates of those points were generated and stored in a Trimble® GeoXT datalogger. PTS Traps were deployed at each cell centroid and retrieved after a period of 24 hrs (1200 total trap-hours) once per month. Once collected, phorids were removed and recorded with respect to species and gender. Spatial distributions were assessed with the ESRI ArcGIS® v.9.0 Spatial Analyst software extension. Assessment of this data provides insight into general habitat selection, spatial distribution patterns, and seasonal phenologies of both fly species.

Phorid/habitat associations were determined by first classifying 5-Eagle Ranch into its constitutive habitat types with ESRI ArcGIS® v9.0 software. Next, 4 habitat types were selected (Hay Pastures, Cattle Pastures, Unmanaged Habitat and full canopy Forest) that represent a continuum of habitat diversity and plant species heterogeneity. Fly densities were measured with grids of 9 PTS-Traps (Puckett et al. 2007) in the abovementioned habitat types. Where possible, grids were placed in a 3 X 3 block formation. The scale and distance between sampling points required an alternative

conformation of sampling points in the Forest and Unmanaged Habitats. This study was conducted during late Summer - early Fall of 2007.

Potential Impact

This project should further our understanding of the potential for phorid flies to serve as biological control agents against one of the most ecologically and economically important invasive insect species in the United Sates. The flies were initially established as part of an investigation into area-wide management of RIFA. The research proposed here is in addition to the objectives of that project.

CHAPTER II

PHORID SAMPLING METHODOLOGY: PTS-TRAPS*

Introduction

Many species of *Pseudacteon* phorid flies parasitize workers of the *Solenopsis saevissima* complex of fire ants (including *S. invicta*) throughout their native South American range (Disney 1994, Porter and Pesquero 2001, Folgarait et al. 2005); as a result they are considered potential candidates to serve as classical biological controls against *S. invicta* in their non-native distribution in the United States (Porter 1998). Multiple phorid species have been released in the southern United States as a biological control component of the Integrated Pest Management (IPM) approach to suppression of *S. invicta* populations; they are being evaluated in terms of their potential to impact *S. invicta* (Drees and Gold 2003). Documentation of the successful establishment of phorid populations has varied among release sites (Gilbert 2002; Gilbert 2008; Graham et al. 2003). A number of factors may influence establishment success, including the landscape mosaic and phorid/habitat associations, as well as the ability to detect and document establishment using current methods. Our new phorid detection method allows rapid and repeatable assessments of establishment, and reflects relative densities among habitats with uniform sampling effort.

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Methods currently used to attract, observe, and collect field released *Pseudacteon* phorid flies include (1) mound disturbance (Barr and Calixto 2005; Morrison and Porter 2005) and (2) midden attraction (Smith and Gilbert 2003; Gilbert 2002). Each of these methods requires direct observations of either disturbed fire ant colonies or deployed fire ant midden to monitor phorid activity. While these methods have been shown effective for detecting and collecting phorids, they are time intensive and require multiple personnel to monitor multiple sites simultaneously. This study documents the efficacy of a new trapping method and compares the efficiency of this trap to standard sampling methods.

Materials and Methods

Study Site

This study was conducted at 5-Eagle Ranch in Burleson County, Texas (30º 34' 54.57" N; 96º 40' 59.77" W); this 2,800 acre ranch is ecologically classified as Post Oak Savanna. The ranch supports cattle operations within scattered improved bermudagrass pastures, a variety of riparian habitats, and dense stands of post oaks, *Quercus stellata*. The ranch is presumed to have become infested with the red imported fire ant in the early 1970's when this invasive species invaded the region (Vinson 1997) and to have remained infested up to the present. *Pseudacteon tricuspis* and *P. curvatus* were released at this location in 2002 and 2004, respectively, as part of the USDA-ARS 'Area-wide Suppression of Imported Fire Ants in Pastures Project'. They have since become established at this site and have expanded outside of the ranch boundary. This

field trial was conducted on 5/23-24/06 within the boundary of the ranch (Fig. 2.1). The

first replication began at 1000hr and the tenth replication at 1600hr. The temperature

during the trial ranged from 27.8ºC (1000 hr) and 31.7ºC (1600 hr), well above the

minimum threshold of 22ºC required for phorid activity (Folgarait et al. 2007 and

Wuellner and Saunders 2003).
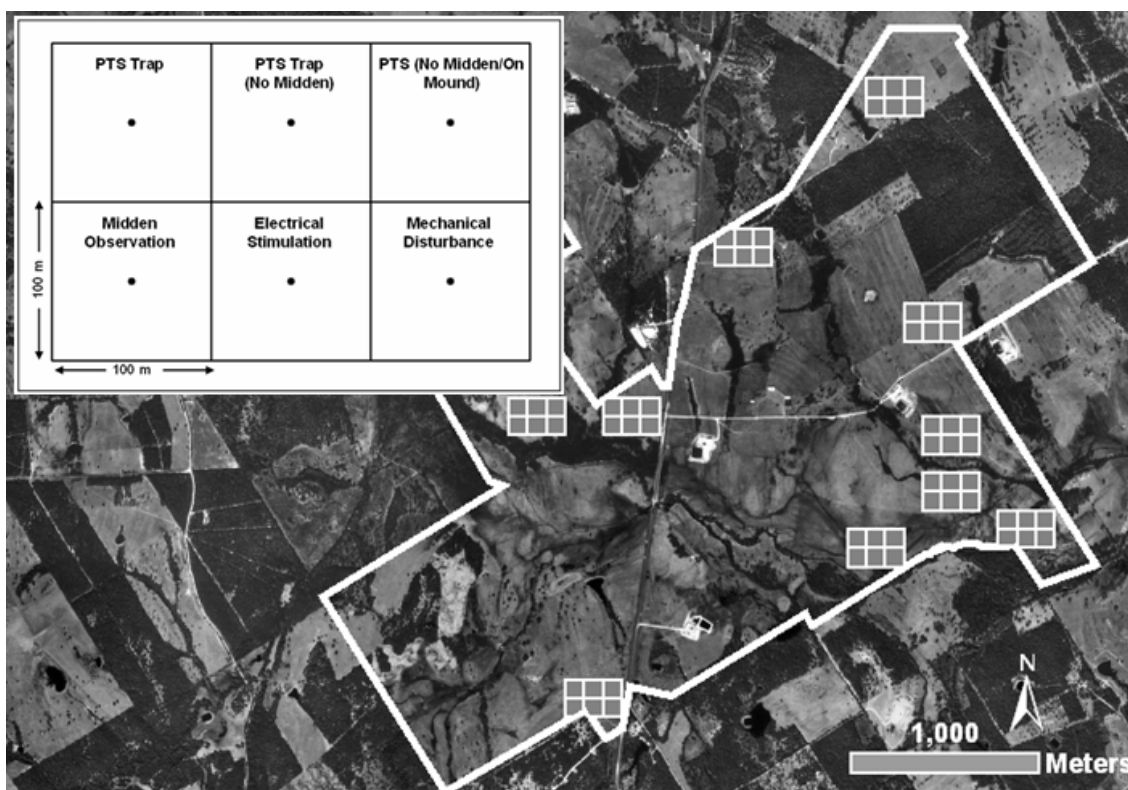
Fig 2.1. Map of 5-Eagle Ranch showing location of all 2 X 3 grid blocks (experimental units). Enlarged schematic indicates dimensions of 100 m X 100 m cells, cell centroids, and collecting method within each cell.

Experimental Design

A GIS supported by ESRI ArcGIS v.9.0 was used to construct an overlay of the study area that contained 2 X 3 blocks of contiguous 100 m X 100 m grid cells at 10 locations (experimental units) within the boundary of the ranch (Fig. 2.1). The centroid of each grid cell was determined and used as the sampling point within each cell. The grid cell size was sufficiently large to eliminate potential competition for attraction of phorids by multiple methods within a block (Sanford Porter *personal communication*). A Trimble® GeoXT™ handheld GPS receiver was used to locate the sampling points in the field. The minimum distance between the boundaries of any two grid blocks was 100m resulting in separation of cell centroids by 200 m. One of each of the following sampling techniques (described in detail below) was employed simultaneously at each grid cell centroid. Six phorid collection techniques were compared including 1) the PTS-Trap, 2) PTS-Trap without midden, 3) PTS on mounds without midden, 4) midden attraction, 5) electrical stimulation of mounds, and 6) mechanical stimulation of mounds.

Solenopsis invicta mound density within the ranch had been assessed prior to this study, and determined at thirty 0.05 ha circular plots within the boundary of the ranch; in May 2005 and December 2005, mean mound densities were 12.9/0.05 ha and 6.4/0.05 ha and the Standard Error of these Means was 1.70 and 0.75 respectively (USDA Area-wide Internal Data, *Unpublished*).

Sampling Techniques

Passive Sampling Methods

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

The prongs of the PTS were coated with Tanglefoot® insect trap coating and centered (prongs upward) in a 100 mm X 15 mm petri dish containing 2g of midden material. This dish was centered within the 150 mm X 15 mm dish, allowing for the midden to remain in close proximity to the PTS while other potential perches provided by surrounding vegetation (if any) were displaced by the larger dish (Fig. 2.2A). Traps were left in place for 24 hrs.
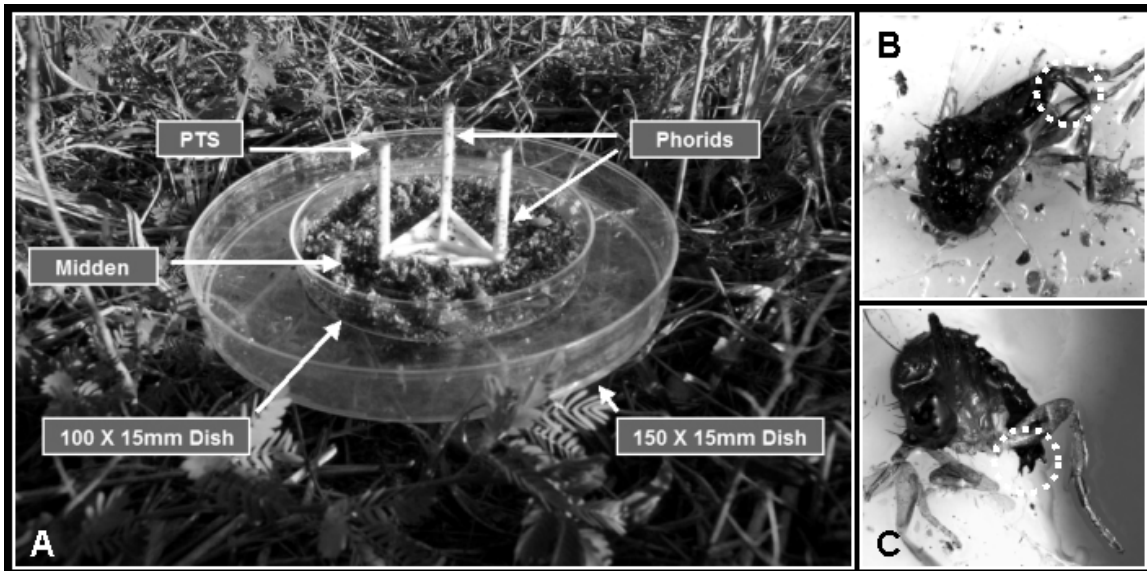
Fig. 2.2. (A) Illustration of PTS-Trap compnonents. (B) *Pseudacteon curvatus* on trap (insect width = 0.304mm; length = 0.672mm). (C) *Pseudacteon tricuspis* on trap (insect width = 0.496 mm; length = 1.2 mm). Ovipositors, used for species diagnosis, are indicated by dashed circles.

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

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

Active Sampling Methods

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

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

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

Phorid Identification

Phorids were returned to the laboratory and those collected by aspiration were transferred to 90% EtOH and identified to species. After a period of 24 hrs PTS-Traps were returned to the laboratory and flies were identified to species directly on the trap (Fig. 2.2B & 2.2C).

Results

Total # Flies per Method

The mean number of flies collected by the PTS-Trap was significantly greater than that of all other methods ($df = 3,36$; $P < 0.05$, $P = 0.02$, $P < 0.05$ {PTS-Trap vs. Midden, Electrical Stimulation, and Mechanical Disturbance respectively}) (Fig. 2.3). The PTS-Traps passively collected a total of 142 phorids (*Pseudacteon tricuspis* = 138

and *Pseudacteon curvatus* = 4), while no phorids were collected by the PTS-Traps without midden or the PTS on mounds without midden. Thirty-two *P. tricuspis* adults were attracted and collected by electrically stimulating mounds, 3 *P. tricuspis* adults were collected by mechanically disturbing mounds and 2 *P. tricuspis* adults were collected over midden piles (Table 2.1). *Pseudacteon curvatus* was only collected using the PTS-Trap.
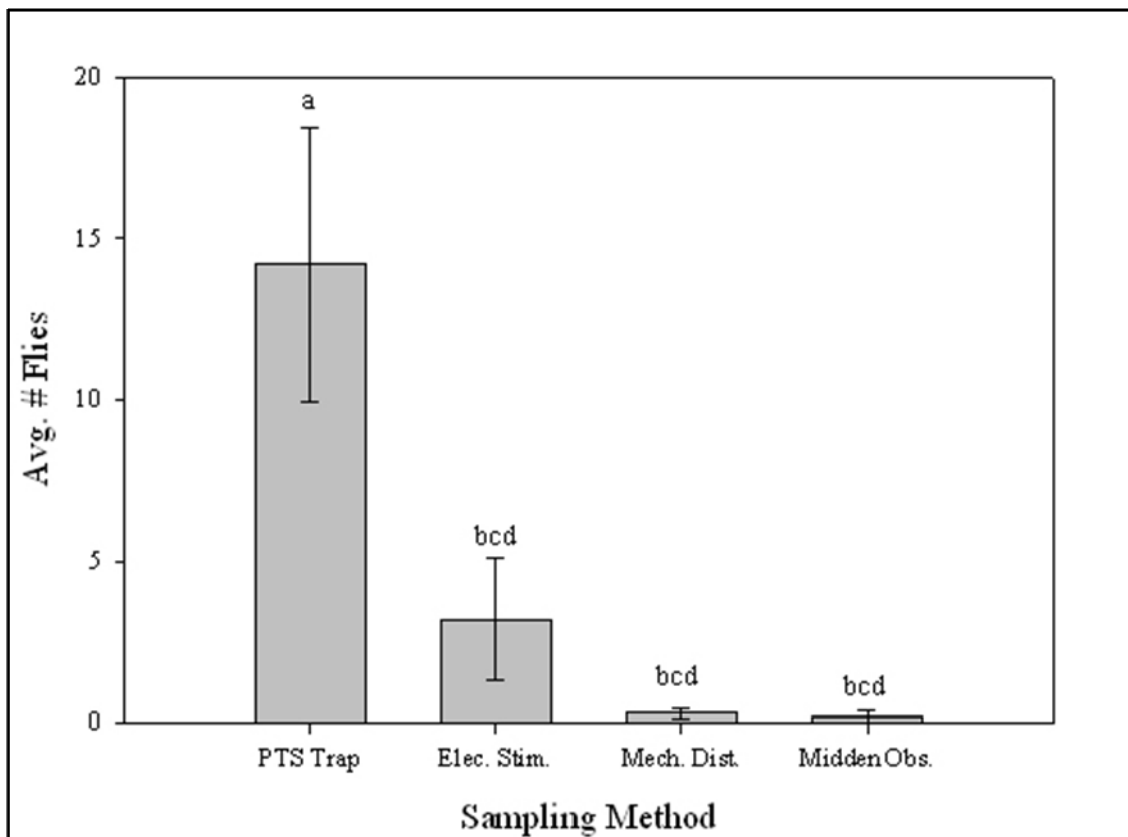
Fig. 2.3. Mean number of phorids collected by each sampling method. Bars marked with different letters are significantly different (p < 0.05). Note: PTS deployed without midden resulted in zero flies captured and are excluded from the figure.

Time Required per Method

The mean amount of time required for deployment and retrieval of the PTS-Traps was significantly less than that of all other methods ($df = 3,36$; $P < 0.05$ {PTS-Trap vs. All Methods}). The total PTS-Trap deployment/retrieval time was 170 min. (avg. = 8.5 min. per trap to deploy and 8.5 min. per trap to retrieve = 2 hrs. 50 min.). The time required for active sampling of phorids totaled 288 min. (4 hrs. 48 min.) (Table 2.1). This total includes the amount of on site travel/set-up time required for active sampling during the trials (avg. = 28.8 min).

Table 2.1 Comparison of a passive sampling method (PTS-Traps) with three active methods for census of phorid flies in the field.

Method	N	Total # Flies (Mean ± SEM)	% Success	Min / Max	Range	Total Time	Relative Efficiency		Time(Min.) /Fly
PTS Trap	10	142 (14.2 ± 4.26)	100	1 / 39	38	170 min.	1	(1.214/1.214)	1.214
Elec. Stimulation	10	32 (3.20 ± 1.88)	60	0 / 19	19	288 min.	7.4	(9.0/1.214)	9
Mech. Disturb.	10	3 (0.30 ± 0.15)	30	0 / 1	1	288 min.	79.0	(96/1.214)	96
Midden	10	2 (0.20 ± 0.20)	10	0 / 2	2	288 min.	118.6	(144/1.214)	144

% Success per Method

The percentage of PTS-Traps that collected at least one fly was significantly greater than that of all other methods ($df = 3,36$; $P < 0.05$, $P = 0.027$, $P < 0.05$ {PTS-Trap vs. Midden, Electrical Stimulation, and Mechanical Disturbance respectively}). All PTS-Traps collected phorids (100%). Data regarding PTS without midden and PTS on mounds were not included in statistical analyses because they collected no flies. Phorids were collected at 1 midden pile (10%), 6 electrically stimulated mounds (60%), and 3 mechanically disturbed mounds (30%) (Table 2.1). Only the PTS-Trap collected *P. curvatus*.

Discussion

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

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

the present. The PTS-Trap has been used successfully in all months of the year and at all known fly-infested locations in which it has been deployed.

The sampling efficiency of the PTS-Trap results in an economy of scale of 1-2 orders of magnitude compared to active observation methods (Table 2.1). Methods that require active observation cannot more efficiently monitor for flies due to the fixed time protocol inherent in these techniques (Barr and Calixto 2005; Gilbert 2002; Morrison and Porter 2005).

The PTS trapping method detected phorid presence at all sites, while the active collecting methods detected phorid presence in 10% to 60% of the same sites (Table 2.1). Barr and Calixto (2005) previously demonstrated that electrical stimulation outperformed mechanical stimulation of mounds to detect phorids. The PTS trapping method is more efficient and more sensitive than active sampling methods in detecting and censusing phorids. This suggests that the PTS-Trap will allow those involved in such sampling to avoid potential false negatives in terms of phorid presence. These features are particularly important when attempting to delimit range expansion boundaries and in determining relative densities among habitats. *Pseudacteon spp.* phorid flies are being released throughout the southern U.S. (Graham et al. 2003, Porter et al. 2004, Vogt and Streett 2003) and we expect the PTS-Trap to prove useful throughout this region.

The number of *P. curvatus* collected was very low regardless of method. However, we have used PTS-Traps to monitor fly density and habitat selection throughout the period of work required for this dissertation and within the boundary of

5-Eagle Ranch, and this work indicates that the PTS-Trap is equally effective in the collection of both *P. curvatus* and *P. tricuspis*. The low numbers of *P. curvatus* collected in this trial (relative to *P. tricuspis*) could be reflective of a natural seasonal density trough. This however requires further study. Further work is planned to examine attractiveness of various substrates and perches to further improve trap operations.

CHAPTER III

INFLUENCE OF *PSEUDACTEON SPP.* (DIPTERA: PHORIDAE) ON FORAGING

PATTERNS OF RED IMPORTED FIRE ANTS *SOLENOPSIS INVICTA* BUREN

Introduction

The red imported fire ant, *Solenopsis invicta* Buren, is a widespread pest insect occurring throughout much of the southern United States. RIFA pose a significant ecological and economic threat to invaded areas (Lofgren 1986, Porter et al. 1992). Native to South America, these ants were discovered in Mobile, AL in the 1930's (Vinson 1997). With the exception of *Thelohania solenopsae* (Jouvenaz et al. 1977), a microsporidian parasite, RIFA arrived in the U.S. with few natural enemies. In addition to the lack of natural enemies in their non-native range, the extremely efficient foraging behavior exhibited by RIFA has contributed to their success in competitive interactions with native ants in invaded territories (Wojcik et al. 2001; Helms and Vinson 2005). RIFA are also differentially more successful in invading disturbed habitats (Tschinkel 2006).

Pseudacteon spp. phorid flies represent an important assemblage of natural enemies of RIFA in their native South American range and are currently being imported and released in the southern United States for biological control of these ants. Numerous studies have documented the reduction of RIFA foraging in the presence of these parasitoids (Feener 1981; Orr et al. 1995; Porter et al. 1995; Morrison 1999). These studies demonstrate that RIFA foraging intensity is diminished during, and for a period

after *Pseudacteon spp.* phorids are present. Competition with other species of ants is an important factor in the ability of an ant colony to obtain the resources necessary to support colony activity (Holldobler and Wilson 1990). Thus, it is hypothesized that RIFA specific phorid-mediated competitive interference will allow native ant assemblages to become more competitive with RIFA for available resources and that this will begin tipping the ecological balance in favor of native ants. The phorid species released at our field site (*P. tricuspis and P. curvatus*) near Caldwell, TX are only active diurnally (Pesquero et al. 1996), whereas RIFA forage both diurnally and nocturnally. Helms and Vinson (2005) showed that nocturnal RIFA foraging is an important component of their foraging strategy. Nocturnal RIFA foraging is especially prevalent in the late summer and fall of the year in central Texas, similar to the activity patterns of native ant species in this study area (Helms and Vinson 2005).

Prior to this research, no attempt has been made to determine the degree of RIFA colony-level behavioral response (nocturnal foraging intensity) to phorid-mediated diurnal foraging reduction. Are RIFA colonies capable of increasing their nocturnal foraging intensity to compensate for diurnal challenge by phorid flies? A RIFA decrease in diurnal foraging and increase in nocturnal foraging in response to parasitoids would provide native ant assemblages increased access to resources during the diurnal period while exposing them to increased competitive interactions nocturnally.

The hypotheses of this study are: 1) phorids will reduce RIFA foraging intensity diurnally and 2) colonies will respond to this challenge by compensating through

increased nocturnal foraging intensity relative to control colonies. These hypotheses were tested under both laboratory and field conditions.

Materials and Methods

Laboratory Experiment

Laboratory experiments were conducted in climate-controlled greenhouses at the Entomology Research Laboratory (ERL) on the Texas A&M University campus in College Station, TX. Flies were provided by the USDA Center for Medical, Agricultural and Veterinary Entomology in Gainesville, Florida.

Twenty polygyne *S. invicta* colonies of similar size and caste structure were collected from several sites within Brazos Co., TX during September of 2004 and separated into ten treatment and ten control colonies, that were maintained in two separate climate controlled greenhouse units. Colonies were allowed to acclimate to greenhouse conditions for 3 weeks prior to experimentation. *Pseudacteon tricuspis* were shipped as pupae and upon emergence were selected and sorted by sex.

Experimental arenas were constructed of inverted 66qt. Rubbermaid® storage tubs (Fig. 3.1). Panels were removed from the tubs and replaced with fine mesh and sealed (Fig. 3.1A&B). Fluon® lined pans containing ant colonies, colony dishes (to provide ants escape from phorid attack) and a water vial (Fig. 3.1A) were placed on the inverted lid. Diet (homogenate containing protein [peanut butter and mealworms] and carbohydrate [honey] components) was provided in a separate Fluon® lined pan. Pans were connected by a bridge constructed of 1" metal ribbon (Fig. 3.1A). Fluon was

placed on the lower surface of the ribbon to ensure that all ants crossing the bridge were observable from above. After a 48 hr. period of food limitation, diet of known mass as well as 10 phorids (7 female, 3 male) were introduced into treatment colony cage. Diet was offered via feeding tubes that consisted of 2dr. vials that that were stoppered with cotton wicks and aluminum foil to create an indentation into which food was placed (Fig. 3.2). The tubs (fitted with a sealed observation window) were placed over the arenas and closed (Fig. 3.1B).
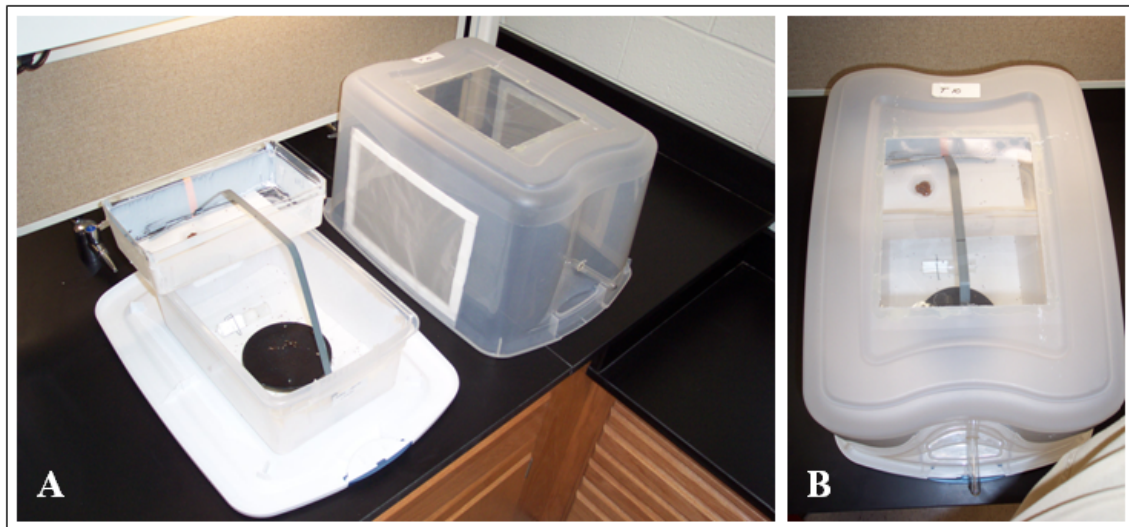
Fig. 3.1 (A) Experimental arenas including lower colony tray (with colony dish, water vial), upper food tray (with diet source) and metal observation bridge. (B) View of closed experimental arena through sealed observation window.

Observations (30 sec. in duration) of the number of ants crossing a line drawn midway across the metal bridge were made and recorded at 5 min., 15 min., 30 min., 1 hr., and 2 hr. intervals. These observations began at approximately 0900 hr. Additionally, a head-lamp was used to make three 30sec. nocturnal observations at

15min. intervals starting at approximately 15 hrs. past the initial food offering (midnight). Diet was removed and weighed after 24 hrs. This procedure was repeated for a total of 10 trials. Student's t-test was performed to statistically compare foraging intensity and consumption rates between phorid affected and control colonies.
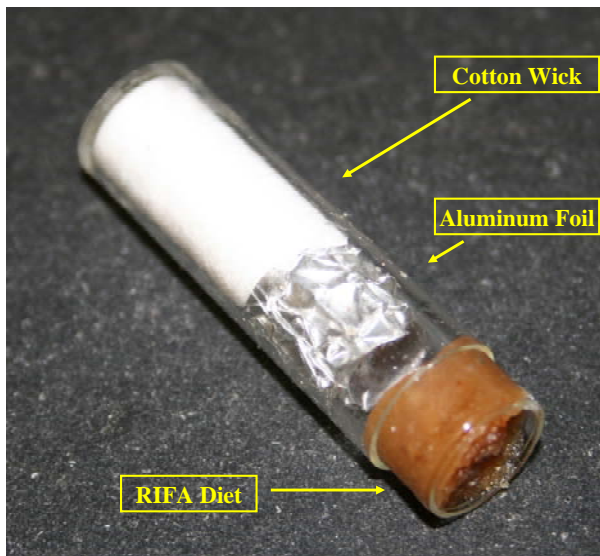
Fig. 3.2. Feeding tubes used to offer RIFA diet to foraging ants.

Field Experiments

In late Summer and Fall of 2007, pitfall traps were used to assess nocturnal vs. diurnal fire ant foraging intensity simultaneously at both 5-Eagle Ranch (30º 34' 54.57" N; 96º 40' 59.77" W) in Caldwell, TX (phorid infested site) and the Skrivanek Ranch in Wellborn, TX (30º 28' 49.40" N; 96º 15' 23.00" W) (phorid-free site). At each site a 3 X 4 grid of 12 pitfall traps (2 m apart) was deployed (Fig. 3.3). These traps were randomly divided into two sets prior to each replication. The nocturnal trap set was

opened at PM civil twilight and closed and collected the following AM civil twilight, at which time the diurnal trap set was opened and then closed and collected at the following PM civil twilight. This design allowed the collection of RIFA foraging intensity data over the full diel cycle simultaneously at both the phorid infested and control sites. Transects of 5 PTS-Traps were deployed for the duration of each replication of these experiments to monitor phorid activity. Hobo® data loggers were used to record temperature and humidity during the sampling period. Student's t-test was used to compare foraging intensity and consumption rates between treatments.

Concurrent with the sampling dates of the above experiment, transects of 15 hot-dog baits (Bestelmeyer et al. 2000) were placed on 3" X 5" index cards and were deployed for 20 min. prior to PM civil twilight and for 20 min. after AM civil twilight at both the phorid-infested and phorid-free site. Transect locations were 50 m away from the pitfall trap grids and were maintained throughout the duration of the study. After 20 min., the index cards with ants were collected by quickly placing them into 1 gal. Ziploc® bags. These samples were returned to the laboratory where they were frozen until analysis. Prior to analysis the ants were separated from hot-dogs and index cards. Ants were then processed through a KECK Instruments® SS SandShaker Mechanical Graduated Sieve Field Analysis Kit. Samples were separated into 4 size classes by filtering them through US Standard Sieve No. 20, 14, 12, and 10 (respective mesh openings= 0.76 mm, 1.29 mm, 1.54 & 1.82 mm). Throughout the remainder of this document size classes will be notated as Size Class 1, 2, 3, & 4 which relate to US Standard Sieve No. 20, 14, 12, and 10, respectively. All surfaces of the sieve equipment

were coated with talcum powder to reduce friction, and each sample was sieved for 3 min. The purpose of these procedures was two-fold. The hot and dry conditions prior to the initiation of these experiments made it difficult to compare RIFA mound densities between sites, and the hot-dog baits allowed for a measure of RIFA density comparisons between sites. Also, this design allowed for a direct comparison of overall as well as size-specific foraging rates between sites. Students t-test was performed to compare foraging rates and RIFA size class separation was assessed via chi-square analysis.
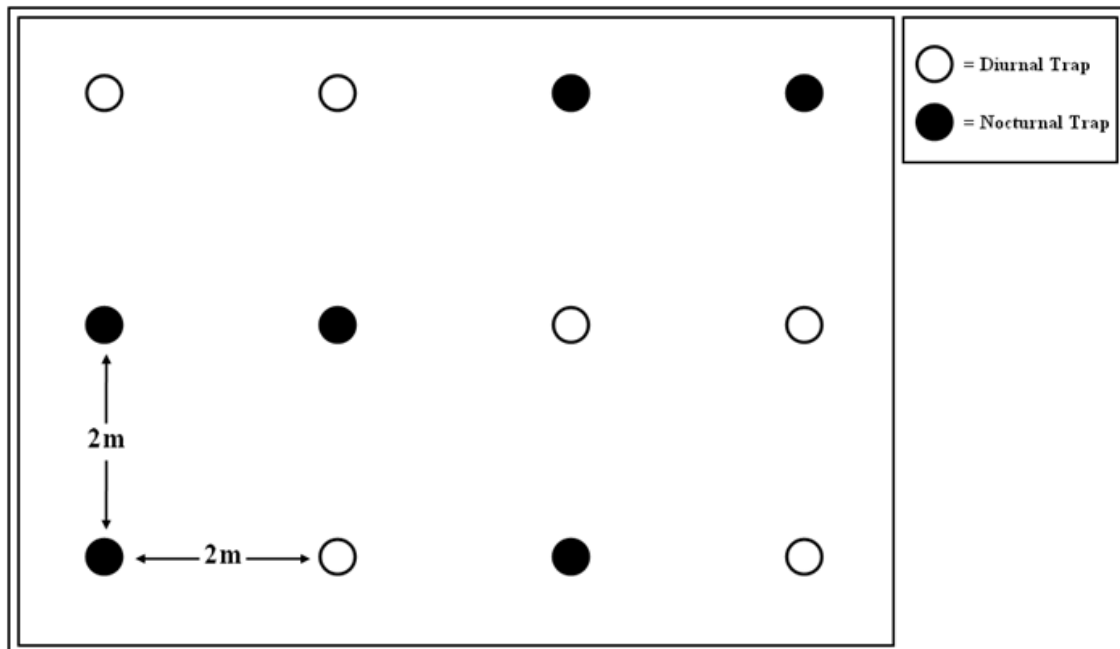
Fig. 3.3. Schematic of pitfall trap grid demonstrating diurnal and nocturnal traps.

Results

Laboratory Experiment

There was no statistically significant difference between the amount of food consumed by treatment and control colonies (Student's t-test: t = 1.585; df = 238; P = 0.114) (Fig. 3.4).
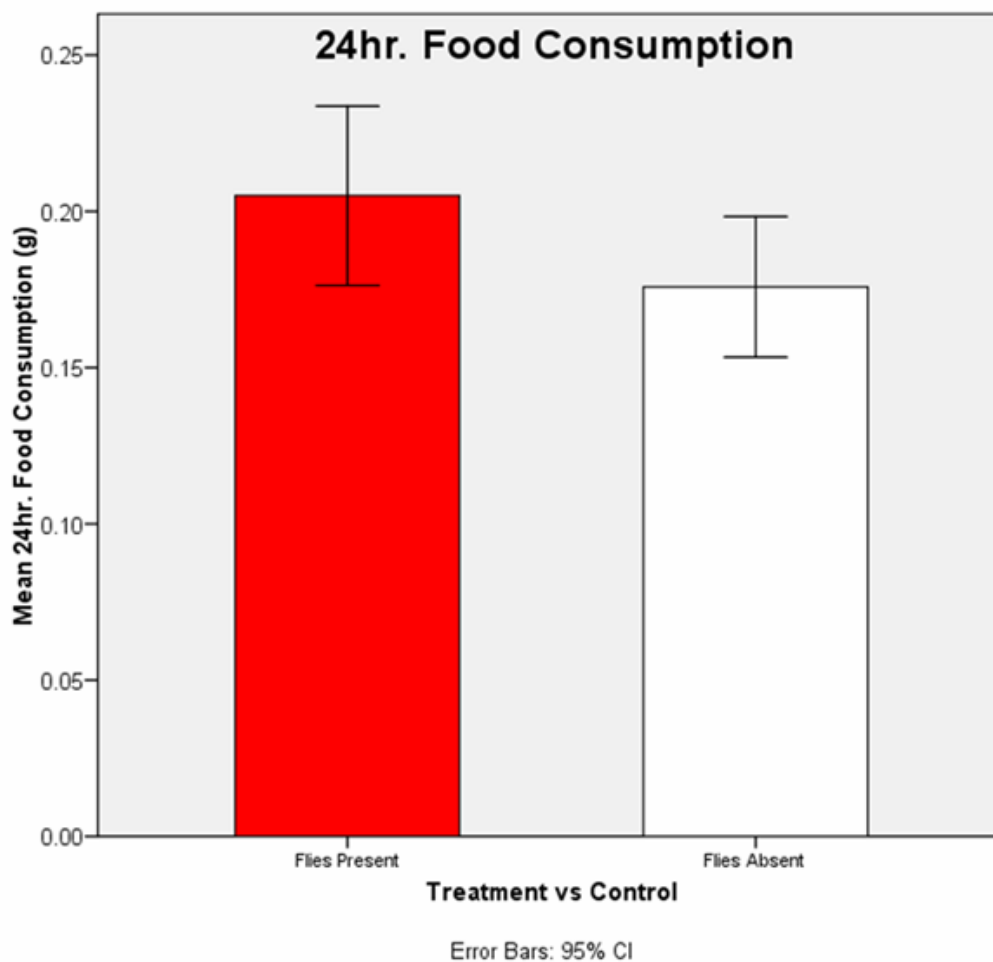
Fig. 3.4. Mean food consumption rates over 24 hrs. of treatment and control colonies. No statistically significant difference between treatment and control colonies (Student's t-test: t = 1.585; df = 238; P = 0.114).

The average amount of food consumed by treatment colonies was slightly greater than that of control colonies, despite the fact that during diurnal observations, treatment colonies foraged less intensively than control colonies (Fig. 3.5).
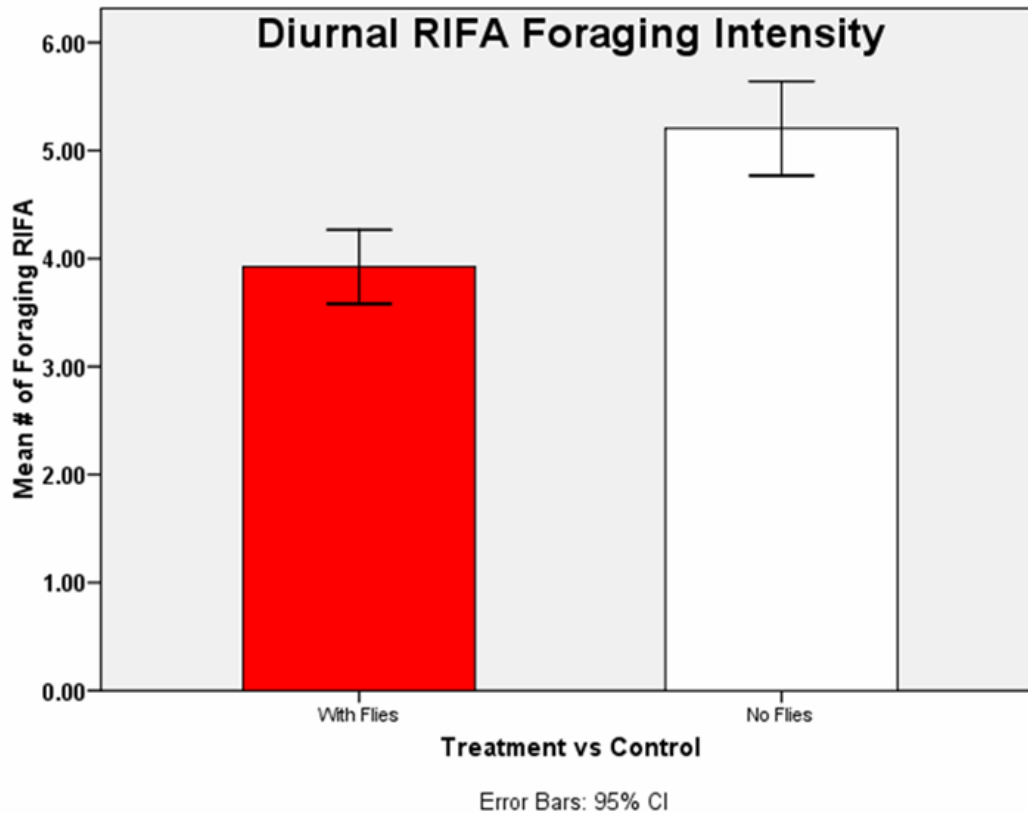
Fig. 3.5. Mean diurnal foraging intensity rates of treatment and control colonies. Treatment colonies foraged significantly less intensively than control colonies (Mann-Whitney U: df = 1,558; $P < 0.05$).

Phorid attacks resulted in diminished foraging intensity during diurnal observations, and diurnal foraging was significantly reduced in treatment colonies (Mann-Whitney U: df = 1,558; $P < 0.05$) (Figs. 3.5 & 3.6).
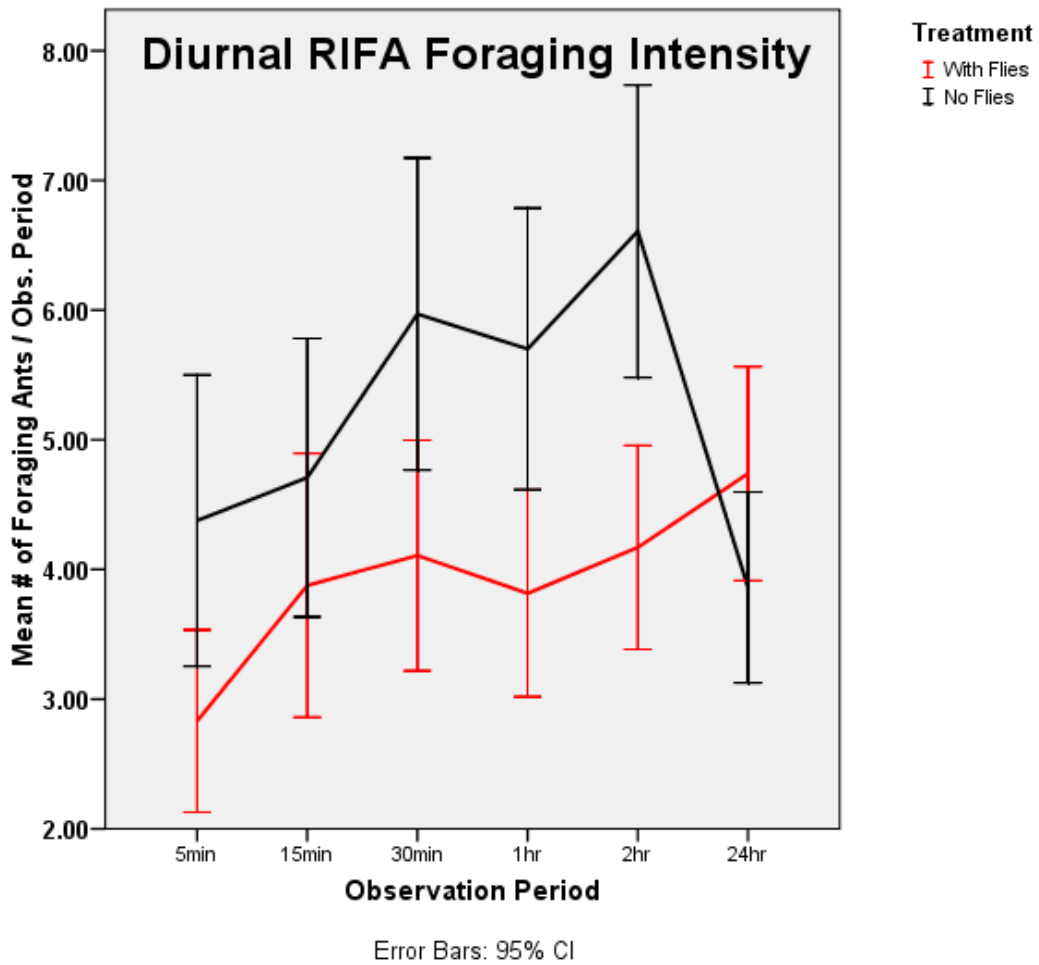
Fig. 3.6. Mean diurnal foraging intensity at each of 6 observation periods.

Treatment colonies demonstrated increased foraging intensity during nocturnal observations and nocturnal foraging was significantly greater in these colonies (Mann-Whitney U: df = 778; $P < 0.05$) (Figs. 3.7 & 3.8).
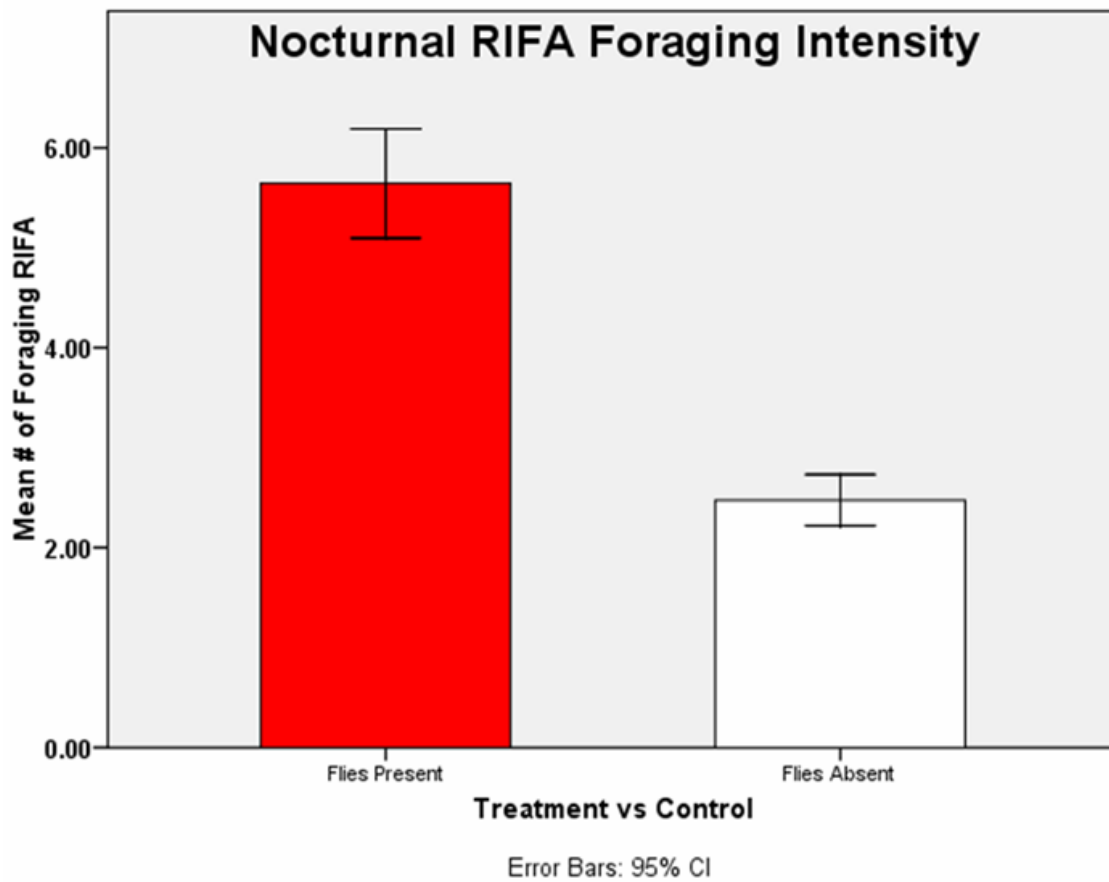
Fig. 3.7. Mean nocturnal foraging intensity rates of treatment and control colonies. Treatment colonies foraged significantly more intensively than control colonies (Mann-Whitney U: df = 778; $P < 0.05$).
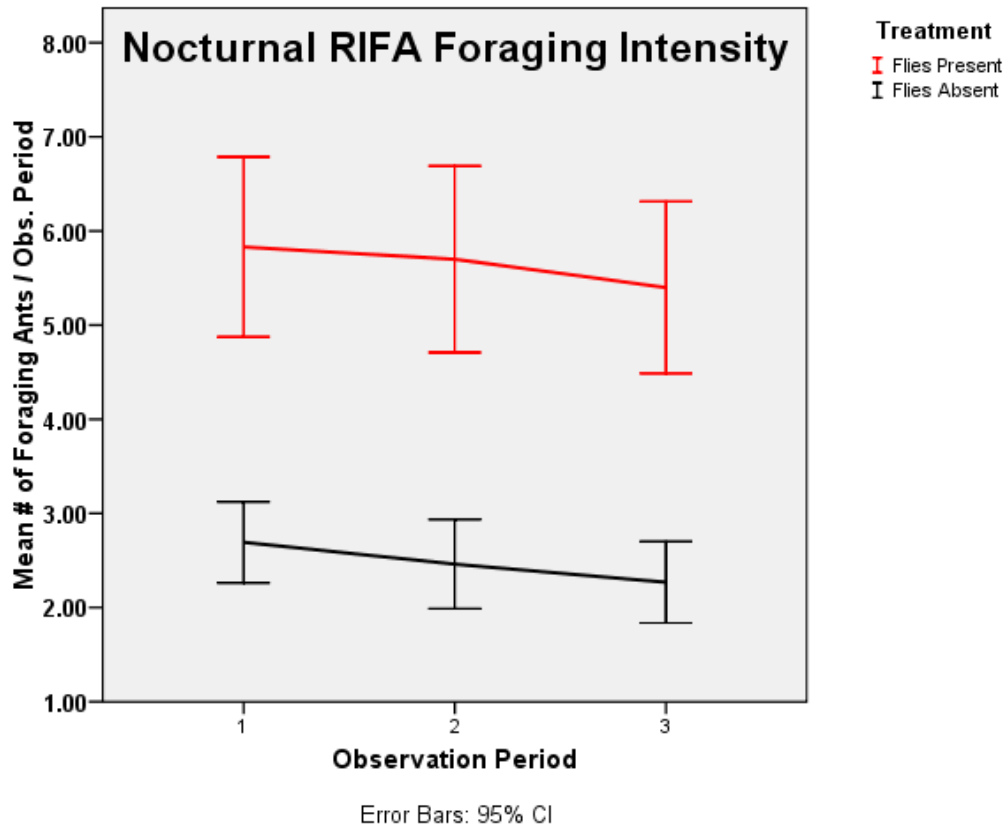
Fig. 3.8. Mean nocturnal foraging intensity trends at each of 3 observation periods.

Field Experiments

PitFall Traps

Both *P. tricuspis* and *P. curvatus* were present at the treatment site during each of 10 replications and were always absent at the control site. There were no statistically significant differences in foraging intensities during diurnal and nocturnal sampling periods at or between the treatment and control sites. Diurnal foraging intensity was not significantly different between sites (Student's t-test: t = -0.486; df = 18; P = 0.633; Fig.

3.9). Nocturnal foraging intensity was not significantly different between sites (Student's t-test: t = 1.375; df = 18; P = 0.186; Fig. 3.9).
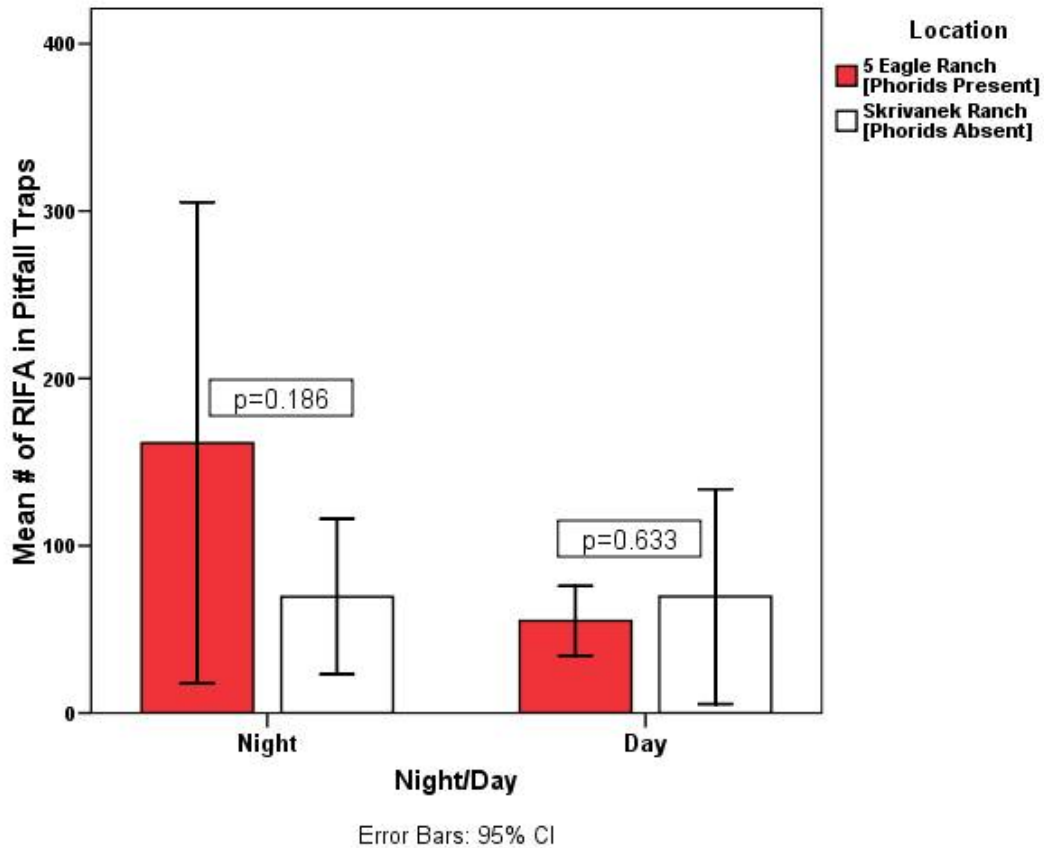
Fig. 3.9. Mean nocturnal and diurnal foraging intensity rates at treatment and control sites. There were no statistically significant differences in foraging intensities during diurnal and nocturnal sampling periods at or between the treatment and control sites. Nocturnal foraging intensity = (Student's t-test: t = -0.486; df = 18; P = 0.633); Diurnal foraging intensity = (Student's t-test: t = 1.375; df = 18; P = 0.186).

Hot-Dog Bait Transects

     Cumulative RIFA Samples (All Size Classes)-   There was no significant difference in RIFA foraging intensities on hot-dog baits at the treatment and control sites (Student's t-test: df = 418; $P = 0.985$) (Fig. 3.10). In addition, there was no significant

Error Bars: 95% CI
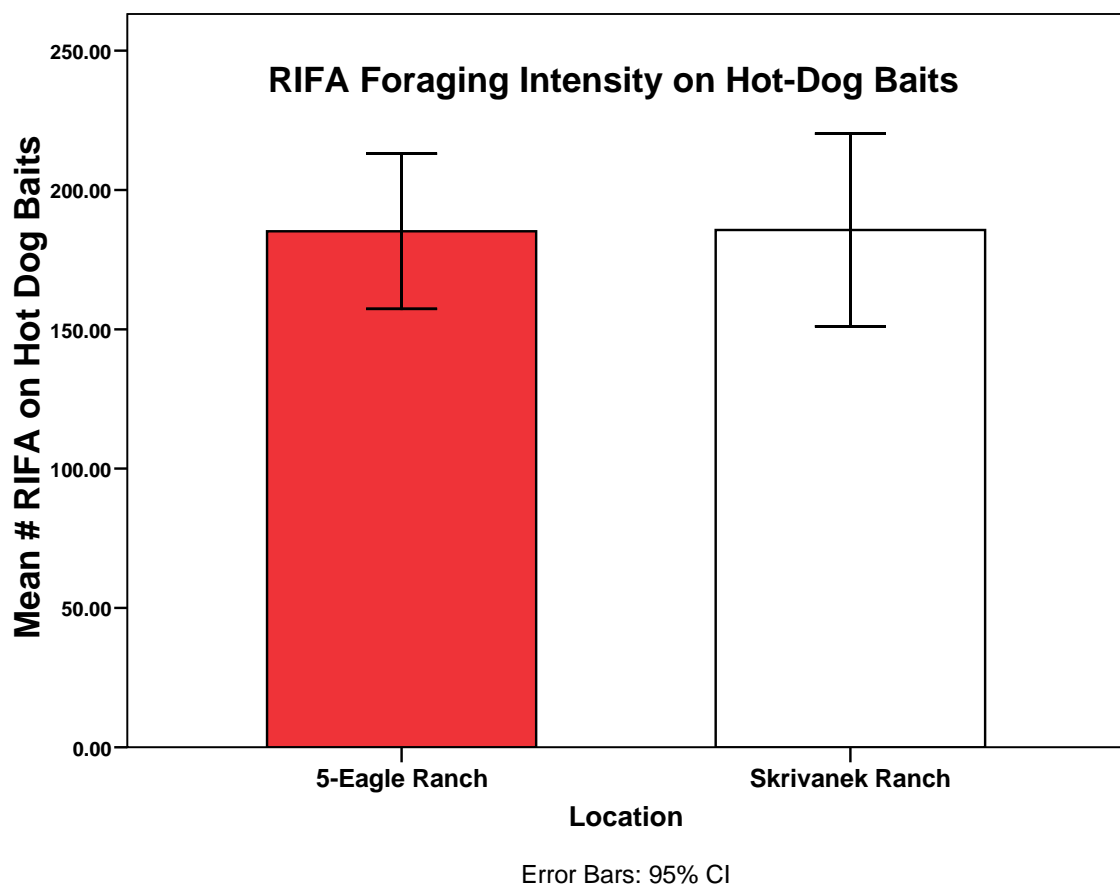
Fig. 3.10. Mean RIFA (all size classes) foraging intensity on hot-dog baits at treatment and control sites. No significant difference between sites (Student's t-test: df = 418; $P = 0.985$).

difference in foraging intensities of cumulative samples (all size classes) at treatment and control sites when PM and AM civil twilight sampling periods were analyzed independently; PM civil twilight - (Student's t-test: df = 208; P = 0.104) (Fig. 3.11) and AM civil twilight - (Student's t-test: df = 208; P = 0.128) (Fig. 3.12).
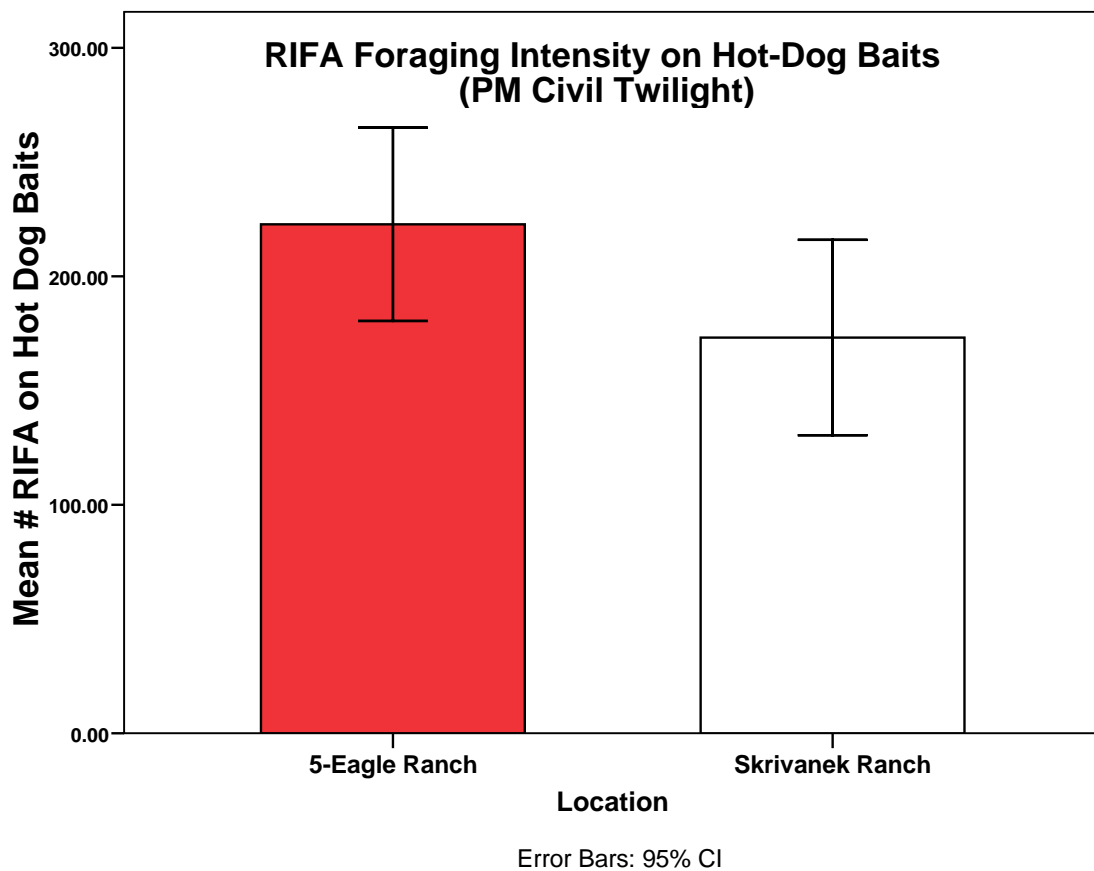
Fig. 3.11. Mean PM twilight RIFA (all classes) foraging intensity on hot-dog baits at treatment and control sites. No significant difference between sites (Student's t-test: df = 208; P = 0.104).
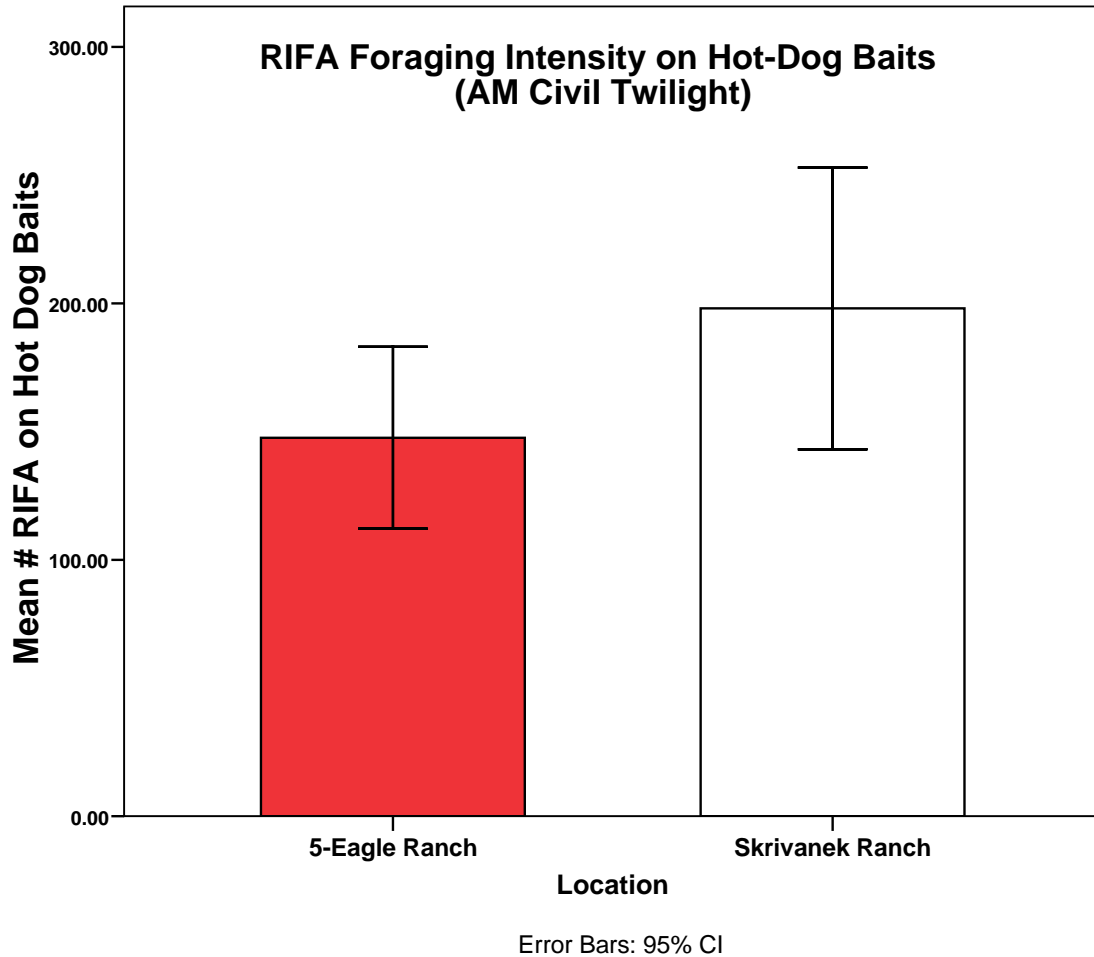
Fig. 3.12. Mean AM twilight RIFA (all classes) foraging intensity on hot-dog baits at treatment and control sites. No significant difference between sites (Student's t-test: df = 208; *P* = 0.128).

Separated RIFA Size Classes (Cumulative)- Once separated, Chi-square analysis revealed statistically significant differences among size classes between sites (x^2 = 6811.85, df = 3, P < 0.05; Fig. 3.13). Means and 95% Confidence Intervals are reported in Table 3.1.
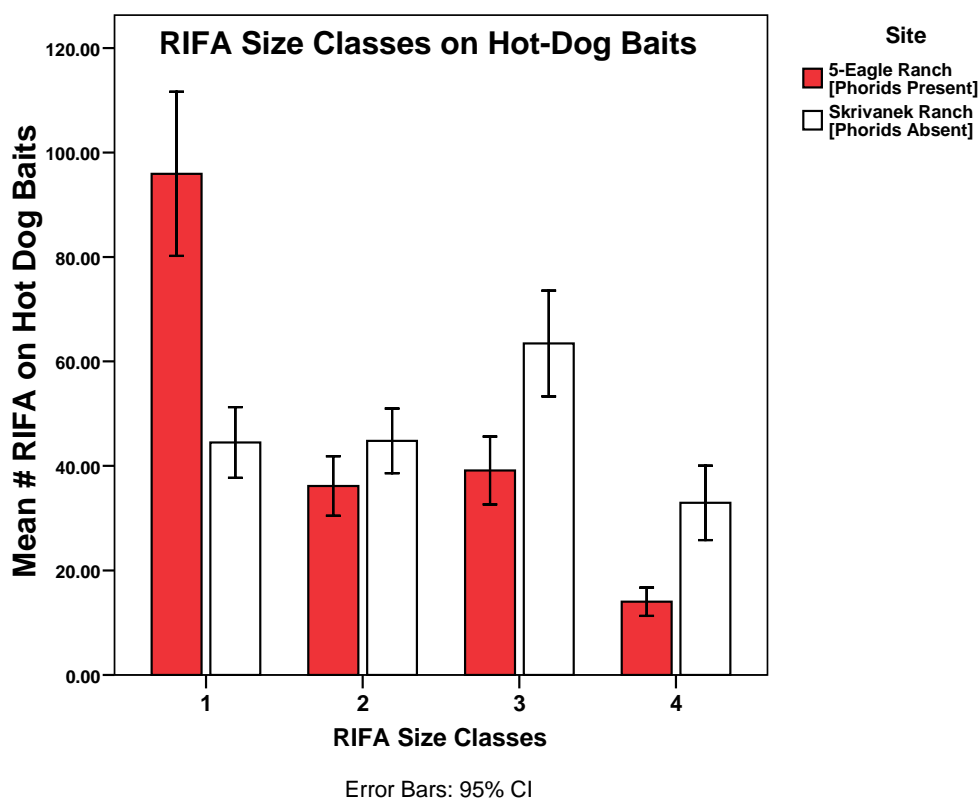
Fig. 3.13. RIFA forager size class differentials at combined AM and PM sampling periods ($x^2 = 6811.85$, df = 3, P < 0.05).

Table 3.1. Means and 95% Confidence Intervals of RIFA size classes at treatment and control sites (* indicates statistically significant differences). RIFA workers were collected via hot-dog lures 20 min. prior to and 20 min. after PM and AM civil twilight respectively.

Size Class	Site	Mean	95% Confidence Intervals	
			Lower Bound	Upper Bound
1 *	5-Eagle Ranch (**Treatment**)	95.905	85.909	105.900
	Skrivanek Ranch (**Control**)	44.476	34.481	54.472
2	5-Eagle Ranch (**Treatment**)	36.152	26.157	46.148
	Skrivanek Ranch (**Control**)	44.790	34.795	54.786
3 *	5-Eagle Ranch (**Treatment**)	39.119	29.124	49.115
	Skrivanek Ranch (**Control**)	63.429	53.433	73.424
4	5-Eagle Ranch (**Treatment**)	14.014	4.019	24.010
	Skrivanek Ranch (**Control**)	32.929	22.933	42.924

Separated RIFA Size Classes (PM Twilight Only)- Chi-square analysis of PM twilight samples revealed statistically significant differences between sites among size classes ($x^2 = 5352.79$, df = 3, P < 0.05; Fig. 3.14). Means and 95% Confidence Intervals are reported in Table 3.2.
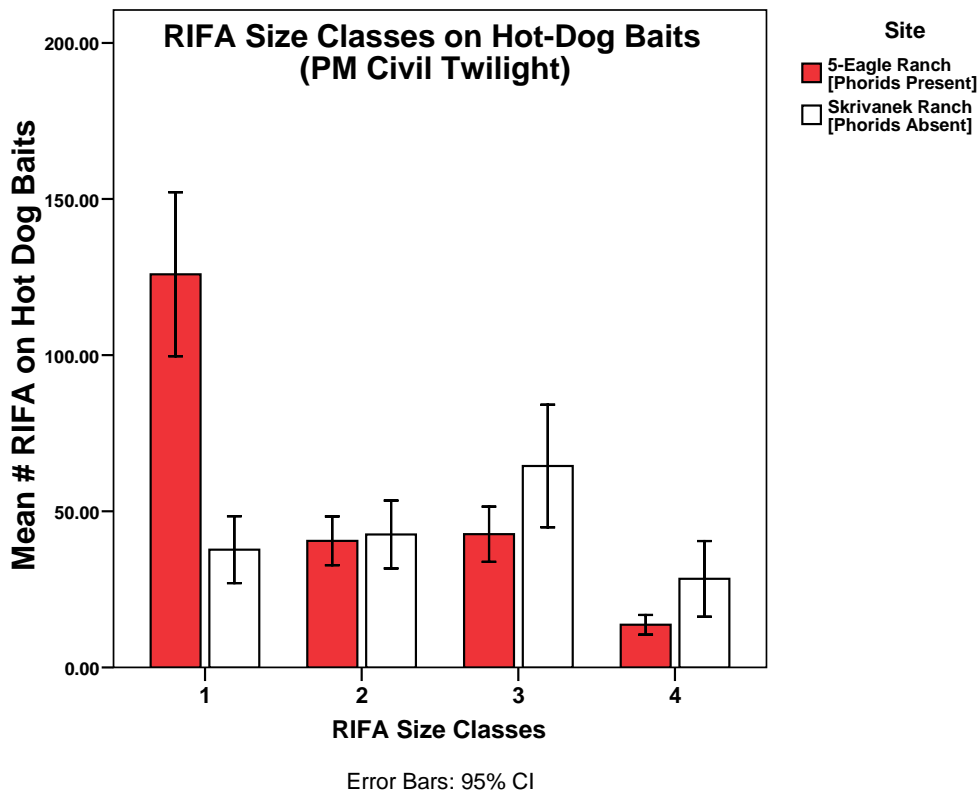
Fig. 3.14. RIFA forager size class differentials at PM sampling periods only ($x^2 = 5352.79$, df = 3, P < 0.05).

Table 3.2. Means and 95% Confidence Intervals of RIFA size classes at treatment and control sites (* indicates statistically significant differences). RIFA workers were collected via hot-dog lures 20 min. prior to PM civil twilight.

Size Class	Site	Mean	95% Confidence Intervals	
			Lower Bound	Upper Bound
1 *	5-Eagle Ranch (**Treatment**)	125.914	111.903	139.926
	Skrivanek Ranch (**Control**)	37.724	23.712	51.736
2	5-Eagle Ranch (**Treatment**)	40.533	26.522	54.545
	Skrivanek Ranch (**Control**)	42.590	28.579	56.602
3	5-Eagle Ranch (**Treatment**)	42.676	28.664	56.688
	Skrivanek Ranch (**Control**)	64.512	50.503	78.526
4	5-Eagle Ranch (**Treatment**)	13.667	-0.345	27.678
	Skrivanek Ranch (**Control**)	28.390	14.379	42.402

Separated RIFA Size Classes (AM Twilight Only)- Chi-square analysis of AM twilight samples revealed statistically significant differences between sites among size classes ($x^2 = 1562.24$, df = 3, $P < 0.05$; Fig. 3.15). Means and 95% Confidence Intervals are reported in Table 3.3.
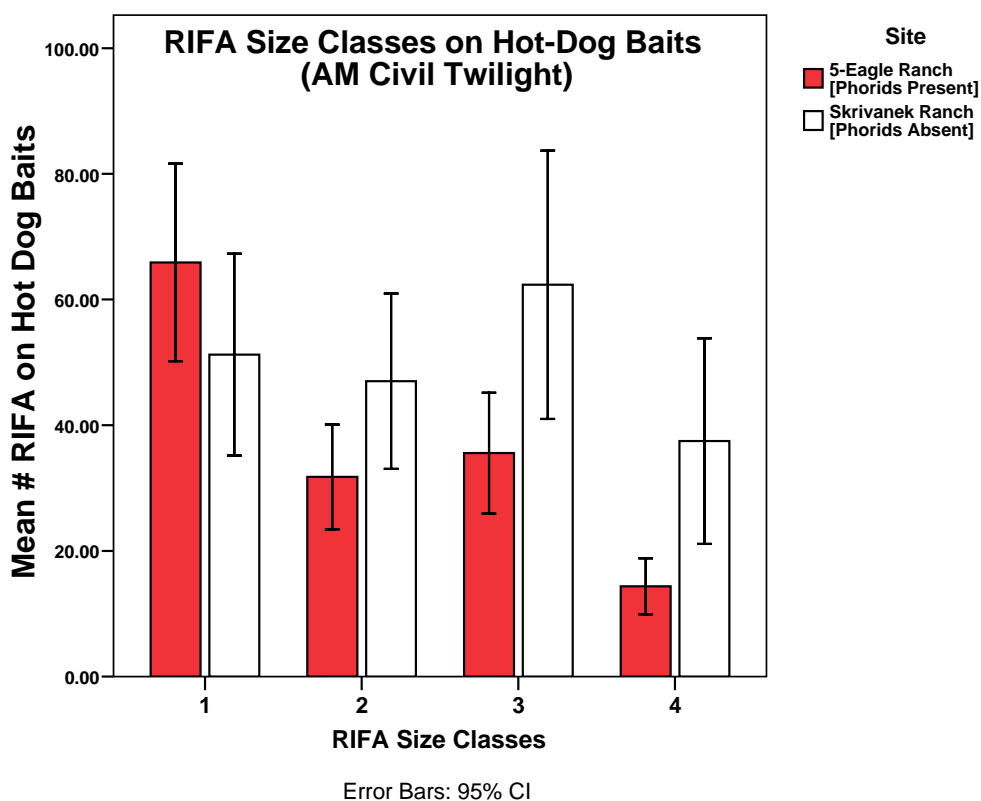
Fig. 3.15. RIFA forager size class differentials at PM sampling periods only ($x^2 =$ 1562.24, df = 3, P < 0.05).

Table 3.3. Means and 95% Confidence Intervals of RIFA size classes at treatment and control sites (no statistically significant differences observed within a size class).

Size Class	Site	Mean	95% Confidence Intervals	
			Lower Bound	Upper Bound
1	5-Eagle Ranch (**Treatment**)	65.895	51.877	79.914
	Skrivanek Ranch (**Control**)	51.229	37.210	65.247
2	5-Eagle Ranch (**Treatment**)	31.771	17.753	45.790
	Skrivanek Ranch (**Control**)	46.990	32.972	61.009
3	5-Eagle Ranch (**Treatment**)	35.562	21.543	49.580
	Skrivanek Ranch (**Control**)	62.343	48.324	76.361
4	5-Eagle Ranch (**Treatment**)	14.362	0.343	28.380
	Skrivanek Ranch (**Control**)	37.467	23.448	51.485

Discussion

Laboratory Experiment

The results of our laboratory experiments suggest that in the absence of either inter- or intraspecific competition, RIFA respond rapidly to phorid parasitism by curtailing foraging during diurnal periods when phorids are active, and in doing so, RIFA also appear capable of obtaining the amount of resources necessary to support colony activities by compensating for this decreased diurnal foraging with increased nocturnal foraging.

This reduction of diurnal RIFA foraging after exposure to phorid flies in these trials is similar to that seen in previous experiments (Feener 1981; Orr et al. 1995; Porter et al. 1995; Morrison 1999). This response is encouraging with respect to the anticipated effect of field-released phorids. However, the compensatory nocturnal foraging response serves as a source of some concern regarding unanticipated affects of the release of these flies. Should RIFA be capable of a similar compensatory foraging strategy in field situations, they would largely escape parasitism; the effects that this nocturnal foraging increase may have on nocturnal competitive interactions between RIFA and native ant assemblages are also of concern. Helms and Vinson (2005) demonstrated that nocturnal foraging activity overlap occurs among RIFA and native ant assemblages in central Texas. This overlap was particularly significant during late summer and fall months (coinciding with our field experiment). Logic suggests that any temporal shift in RIFA foraging due to parasitism would be detrimental to RIFA, but this remains to be shown.

Field Experiments

Pitfall Trap Samples

Fig. 3.9 shows a non-significant trend toward greater mean RIFA nocturnal foraging intensity at the treatment site relative to that of both the diurnal period at the same site as well as RIFA nocturnal foraging intensity at the control site. In addition, mean nocturnal and diurnal foraging intensity at the control site were identical with only slightly greater variation during the diurnal period relative to that of the nocturnal period. This suggests greater stability in RIFA foraging patterns without phorids and could signal a trend toward greater nocturnal RIFA foraging intensity at the phorid infested site. Phorid densities continue to increase at this site and it is unknown if a critical phorid density/activity threshold exists, above which RIFA would shift to a primarily nocturnal foraging pattern as they did in the laboratory experiment.

Hot-Dog Samples

While overall RIFA foraging intensity rates are statistically similar at 5-Eagle (phorids present) and Skrivanek (phorids absent) ranches, there appears to have been a phorid-mediated affect on the composition of foraging RIFA with regards to the ratios of size specific RIFA sub-classes. Large RIFA foragers are less abundant and small RIFA foragers are more abundant from samples at 5-Eagle compared to Skrivanek Ranch; this may be a result of increased phorid-mediated mortality or this may be a colony-level phorid avoidance behavior, etc. This effect (forager size skewed towards small ants at 5-Eagle Ranch) is not completely unexpected given the anticipated selection effect on

large RIFA from *P. tricuspis*.   Female *P. tricuspis* prefer large hosts and the sex of

developing larvae is facultatively determined by host size (Morrison et al. 1999).

Pseudacteon tricuspis was first released at 5-Eagle Ranch in Spring 2002 and recovered

during the Fall of 2002.

Confounding these results is the fact that, as a result of their preference for small

RIFA hosts (Morrison and Porter 1998), *P. curvatus* was selected for release in this

system.   *P. curvatus* is physically smaller than *P. tricuspis* and was selected for it's

ability to attack polygyne RIFA colonies, that predominate in most of Texas and are

characterized by greater mound density with a larger proportion of small worker ants

relative to monogyne RIFA colonies.   This species was first released at 5-Eagle Ranch

in Spring 2004 and first recovered by the author in Spring 2005.

Since an initial lag phase in *P. curvatus* density at the release site, the density of

these flies has increased to almost five-fold that of *P. tricuspis* (June 2007) (Puckett and

Harris, *Unpublished Data*).   This scenario is repeated at other sites where *P. curvatus*

was released after *P. tricuspis* had become established (Larry Gilbert – *Personal*

Communication).   Presumably, the higher percentage of small RIFA foragers has

contributed to the success and dramatic density increases of *P. curvatus* at our field site

(and potentially others) by predisposing the system to the successful establishment and

expansion of populations of these flies.   It is unclear if the mortality of small RIFA

foragers resulting from selective parasitism from *P. curvatus* will contribute to 1) a

decrease in overall foraging, 2) a decrease in small RIFA foragers, 3) no affect on the

present RIFA forager distribution, 4) homeostasis or 5) maintenance of stochastic conditions.

Worker size polymorphism is important to task allocation in RIFA colonies, and experimental manipulation of the composition of RIFA worker sizes within colonies can have important effects on colony success (Porter and Tcshinkel 1984). Small workers are very important to brood production but are able to perform any colony task required of them (Mirenda and Vinson 1981), whereas large workers are important for such tasks as mound maintenance, brood moving, defense and predation (Mirenda and Vinson 1981, O'Neal and Markin 1973, Porter and Tschinkel 1984, Wilson 1978, Wilson et al. 1971). It is presumed that phorid-mediated manipulated RIFA worker sizes in the field would influence the efficiency with which colonies accomplish these tasks. However, further field experimentation to measure such colony dynamics will be necessary to determine if and to what degree colonies are affected by caste size manipulation.

CHAPTER IV

SPATIAL DISTRIBUTION, PHENOLOGY AND HABITAT ASSOCIATIONS OF

INTRODUCED POPULATIONS OF *PSEUDACTEON TRICUSPIS* AND

P. CURVATUS

Introduction

Many species of parasitic flies in the family Phoridae (*Pseudacteon spp.*) are known to parasitize workers of the *Solenopsis saevissima* complex of fire ants (including *S. invicta*) throughout their native South American range (Folgarait et al. 2000; Calceterra et al. 2005). These flies are being investigated to determine their potential to serve as biological control agents against RIFA and are being released in their non-native distribution within the United States (Porter 1998; Graham et al. 2003; Vogt and Street 2003; Porter et al. 2004). Female phorids oviposit into the thorax of RIFA workers (Morrison et al. 1997).

Initial work with RIFA/*Pseudacteon spp.* interactions in the early 1970's indicated a low potential for biological control success as a result of the extremely low rates of parasitism (1-8%) found in infested laboratory colonies (Gilbert and Patrock 2002). However, Feener's (1981) work renewed interest in the potential utility of phorids as biological control agents after demonstrating that the flies induce a negative shift in competitive success in ant colonies that are routinely attacked. Subsequent field work in this area has supported Feeners observations (Orr et al. 1995,1997; Porter et al. 1995; Folgarait and Gilbert 1999). This reduction in competitive success is the result of

a behavioral response in which ants reduce their foraging effort in the presence of phorids (Porter et al. 1995; Morrison 1999).

A significant amount of fundamental research has been conducted with the goal of understanding RIFA/phorid interactions and phorid developmental biology (Feener 1981; Feener and Brown 1992; Folgarait and Gilbert 1999; Morrison 2000; Orr et al. 2003; Consoli et al. 2001; Porter and Pesquero 2001). Nonetheless, development of a successful biological control program against RIFA throughout their non-native distributions requires first 1) the successful establishment, and then 2) expansion of phorids. In order to successfully choose appropriate release sites, a critical evaluation of the biotic and abiotic factors associated with previously successful establishment sites is necessary. *Pseudacteon tricuspis* was released and became established at 5-Eagle Ranch in Burleson County, Texas (30º 34' 54.573" N; 96º 40' 59.776" W) in 2002 and has begun to expand its range. *Pseudacteon curvatus* was released in the Spring of 2004 at the same site. *Pseudacteon curvatus* is physically smaller than *P. tricuspis* and was selected for it's ability to attack polygyne RIFA colonies, that predominate in most of Texas and are characterized by greater mound densities and a larger proportion of small worker ants relative to monogyne RIFA colonies (Macom and Porter 1996). The first recovery of adult *P. curvatus* in Texas occurred at 5-Eagle Ranch by the author during the Spring of 2005, and flies have been collected during all subsequent sampling periods.

The establishment of these 2 species afforded an opportunity to examine *P. tricuspis* and *P. curvatus* spatial distributions and phenologies in a sympatric situation. Monthly sampling with PTS-Traps throughout multiple years allowed for a rigorous

GIS-based assessment of these aspects of the biology and ecology of these flies. In addition, GIS-based investigations of phorid / habitat associations that examine phorid density along a continuum of habitat types were conducted. In both investigations, densities of phorids were measured with PTS-Traps (Puckett et al. 2007).

Materials and Methods

Spatial Distribution and Phenology

Phorids were provided for release at 5-Eagle Ranch by the USDA Center for Medical, Agricultural and Veterinary Entomology in Gainesville, Florida. 5-Eagle ranch was mapped using ESRI ArcGIS® v9.0 software and the interior of the ranch was digitally characterized using a grid of 100 m X 100 m cells. All cells were assigned a unique numerical I.D. and fifty cells were randomly selected using SPSS® software. ESRI ArcGIS® v9.2 software was used to determine the center (centroid) of each selected cell (Fig. 4.1), and the coordinates of those points were generated and stored in a Trimble® GeoXT datalogger. PTS Traps were deployed at each cell centroid and retrieved after a period of 24 hrs. (1200 trap-hours/sampling period) once per month. Monthly sampling began in September 2005 and ceased in September 2007 (totaling 21,600 trap-hours). Traps were returned to the laboratory where phorids were identified to species and counted.

Species-specific spatial distribution of phorids was assessed with ESRI ArcGIS® v.9.2 and Spatial Analyst software extension. Densities of both phorid species were assessed monthly and mapped with Inverse Distance Weighted (IDW) Interpolation,

resulting in two species-specific raster layers for each monthly sample (1 *P. curvatus* and 1 *P. tricuspis*). IDW Interpolation assumes a greater degree of similarity in objects that are closer together than those that are further apart so that, in predicting values, IDW Interpolation values closest to the prediction location have greater influence on the predicted value (Shi et al. 2007). Next, within the ESRI ArcGIS® v.9.2 Spatial Analyst software extension, 'Raster Calculator' was used to create raster layers that demonstrate which species was numerically superior spatially within the boundary of 5-Eagle Ranch on each sampling date. The 'Raster Calculator' equation used was ([*P. cruvatus* IDW – P. tricuspis IDW] = 'Output Layer') for each monthly sample. Each calculation resulted in a raster layer output in which cell values ranged from positive to negative integers that related to species-specific abundance. Each raster layer was then classified into positive and negative classifications whereby a positive value indicates *P. curvatus* numerical superiority spatially, and a negative value indicates *P. tricuspis* numerical superiority spatially.
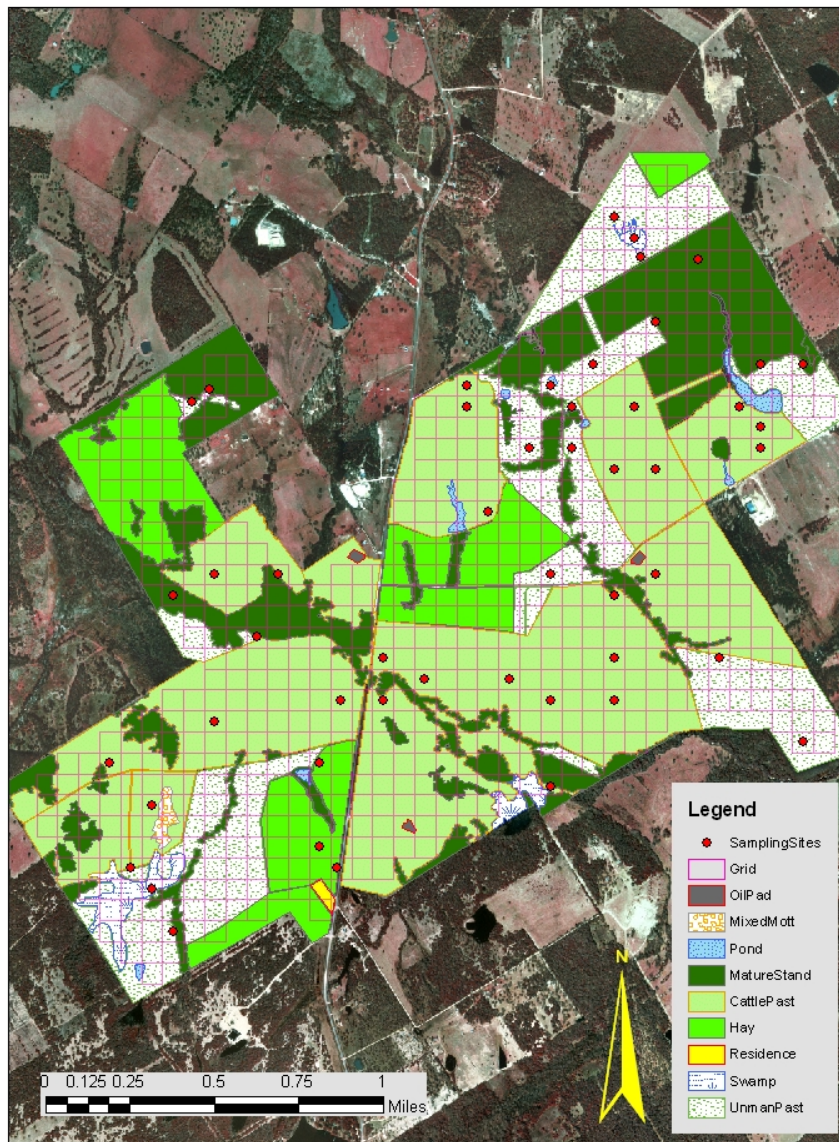
Fig. 4.1 Map of 5-Eagle Ranch showing habitat classifications and PTS-Trap sampling sites.

Habitat Associations

Phorid/habitat associations were determined by first classifying 5-Eagle Ranch into its constituent habitat types with ESRI ArcGIS® v9.0 software. Next, 4 habitat types were selected (Hay Pastures, Cattle Pastures, Unmanaged Habitat and full canopy Forest; Figs. 4.2-4.5 respectively) that represent a continuum of habitat diversity and plant species heterogeneity.

Fig. 4.2 Typical Hay Pasture at 5-Eagle Ranch. These pastures consist of monoculture of Coastal Bermudagrass *Cynodon dactylon* (L.) Pers. (Bogdan). These pastures receive 1 herbicide treatment/year and are fertilized twice yearly with nitrogen supplements only. Cattle are not permitted to graze in these pastures and hay harvesting occurs 2-3 times annually between May and September.

Fig. 4.3 Typical Cattle Pasture at 5-Eagle Ranch. These pastures consist of a mixture of Coastal Bermudagrass *Cynodon dactylon* (L.) Pers. (Bogdan) and a variety of native grasses and shrubs. These pastures receive 1 herbicide treatment/year and are fertilized once yearly with nitrogen supplements only. Cattle grazing occurs on a rotational basis and require occasional (< once/year) shredding.

Unmanaged Habitat

Fig. 4.4 Typical Unmanaged Habitat at 5-Eagle Ranch. These pastures were created by removing trees ~25 yrs ago and exist in a transitional ecological state that, if unmanaged further, would presumably revert to the original Post Oak Savanna ecotype: they currently consist of many grasses and shrubs including Goldenrod *Solidago sp.*, Croton *Croton sp.*, Milkweed *Asclepias sp.* and Senna Beans *Senna obtusifolia* (L.) Irwin & Barneby. Cattle grazing occurs on a rotational basis.

Fig.4.5 Typical Forest at 5-Eagle Ranch. Full canopy of Oaks *Quercus spp.* with dense Yaupon *Ilex sp.* understory. In addition to cattle, feral hogs, deer and other wildlife have access to much of this habitat, it is not considered part of the managed portion of 5-Eagle ranch and is not manipulated in any way.

Fly densities were measured with grids of 9 PTS-Traps (Puckett et al. 2007) within the abovementioned habitat types. Where possible, grids were placed in a 3 X 3 block formation. The scale and distance between sampling points in the Forest and Unmanaged Habitats required an alternative conformation of sampling points than in Hay and Cattle Pastures (Fig. 4.6). The sampling points were a minimum of 100 m from each other and from alternative habitat types. This is considered a sufficient distance to eliminate potential competition for attraction of phorids between traps and among habitat types (Sanford Porter *personal communication*). Traps were deployed and then retrieved after a period of 24 hrs weekly for 9 weeks. These traps were returned to the laboratory

where phorids were identified to species and counted. In addition, RIFA densities were monitored by mound counts (0.05 ha at each sampling site) and hot-dog lures (3 at each sampling site) at the beginning and end of the study. This study was conducted during late summer - early fall of 2007.
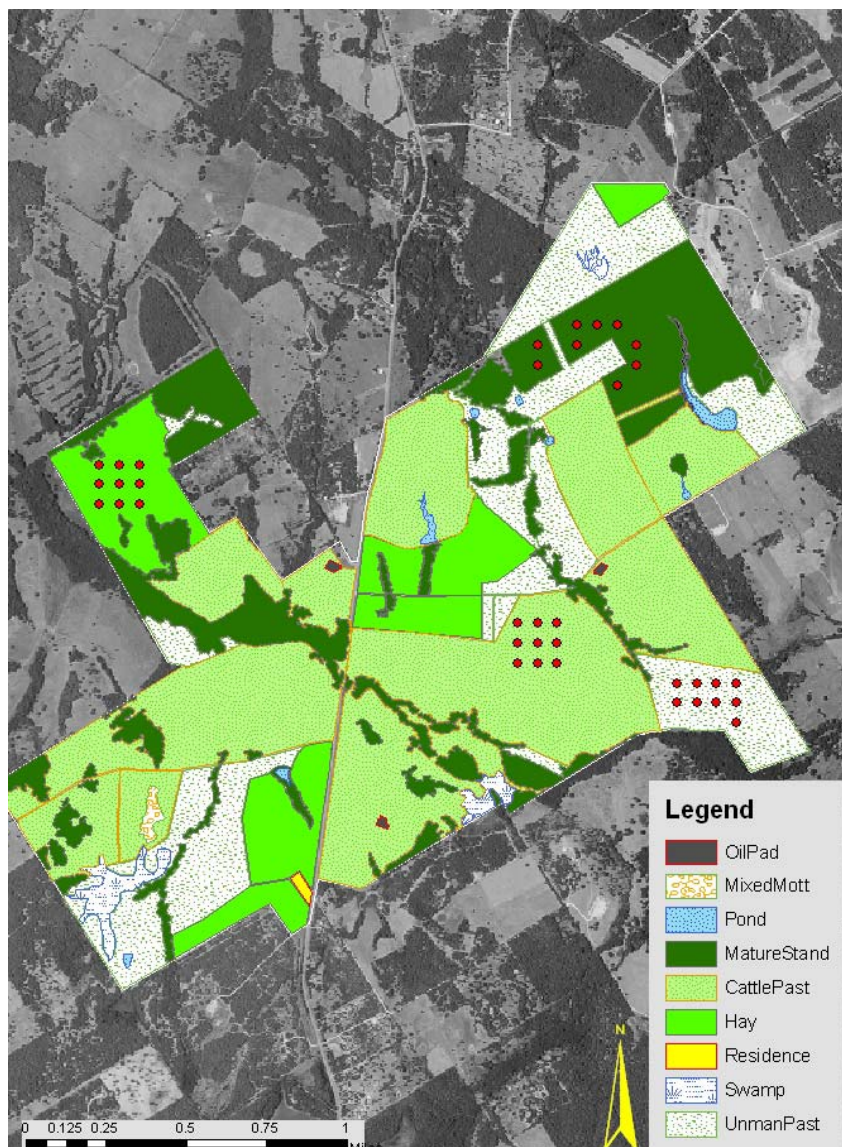
Fig. 4.6 Map of 5-Eagle Ranch showing habitat classifications and PTS-Trap grid conformation within sampled habitats.

Results

Spatial Distribution and Phenology

The first post-release collection of *P. tricuspis* and *P. curvatus* at 5-Eagle Ranch occurred in Spring 2003 and 2005, respectively. *Pseudacteon curvatus* was predictably less numerically/spatially superior than *P. tricuspis* during Summer and Fall 2005 (Fig. 4.7 and Table 4.1). However, as densities of both fly species increased in 2006, mean densities of *P. curvatus* quickly increased to level greater than that of *P. tricuspis* (Fig. 4.7) and remained throughout most of 2006. This trend continued through 2007. During spring, summer and fall of 2007, *P. curvatus* occurred at significantly higher densities compared to *P. tricuspis* (Fig. 4.7), despite *P. tricuspis* densities increasing from 2006 to 2007. The consistency of *P. tricuspis* densities throughout the study period (concurrent with *P. curvatus* density increase) indicates that the phorid fly carrying capacity in this environment accommodates both species rather than an increase in one species resulting in displacement of the other. Population densities of both phorid species in this study followed similar seasonal trends. The trend demonstrated in Fig.4.7 shows typical seasonal 'peaks' in phorid abundance (Spring and Fall) that are similar to previously published data (Folgarait et al. 2003; Morrison and Porter 2005). However, Summer densities of *P. tricuspis* tended to be greater than those reported by others using less sensitive sampling methods (Morrison and Porter 2005).

Pseudacteon tricuspis was numerically/spatially superior at our study site during Fall 2005 (Table 4.1 and APPENDIX A-1 & A-2). However, as densities of *P. curvatus* increased in 2006 their numerical/spatial superiority increased as well (APPENDIX A-3

– A-12). This trend continued through Summer and Fall of 2007 when *P. curvatus* consistently dominated *P. tricuspis* spatially with a peak % area occupied of 99.82 in June 2007 (Table 4.1 & APPENDIX A-15 – A-18).
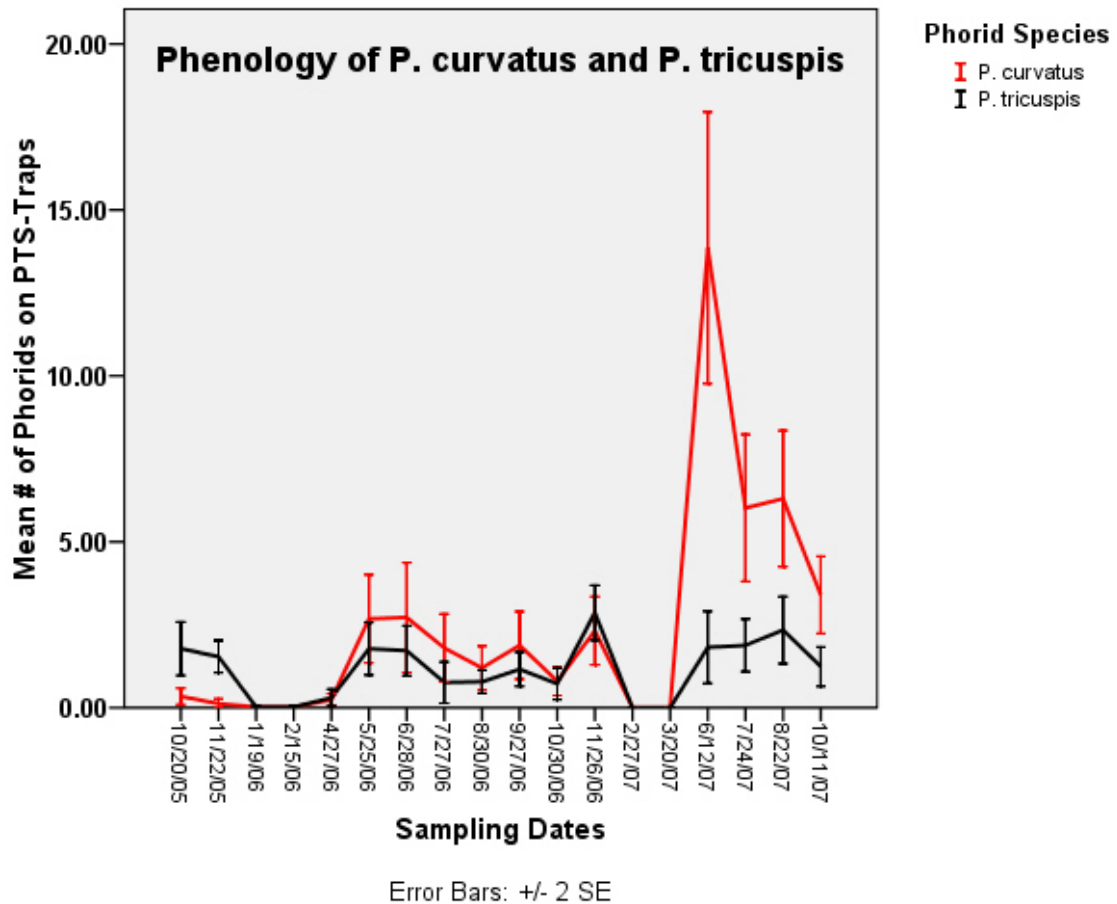
Fig. 4.7 Mean seasonal phenology of *Pseudacteon curvatus* and *Pseudacteon tricuspis* from 50 PTS-Trap sampling sites within 5-Eagle Ranch (Burleson Co., TX).

Table 4.1.   Relative numerical/spatial superiority of *Pseudacteon curvatus* and *Pseudacteon tricuspis* during each sampling date at 5-Eagle Ranch in Burleson Co., TX.

Sample Date	% Area of Study Site in which *P. curvatus* was numerically superior	% Area of Study Site in which *P. tricuspis* was numerically superior
10/20/2005	19.92	80.08
11/22/2005	0	100
1/19/2006	47.62	52.38
2/15/2006	11.05	88.95
4/27/2006	37.43	62.57
5/25/2006	55.86	44.14
6/28/2006	48.05	51.95
7/27/2006	68.39	31.61
8/30/2006	56.60	43.40
9/27/2006	68.19	31.81
10/30/2006	50.57	49.43
11/26/2006	26.47	73.53
2/27/2007	0	0
3/20/2007	0	0
6/12/2007	99.82	0.18
7/24/2007	93.38	6.62
8/22/2007	92.22	7.78
10/11/2007	92.47	7.53

Habitat Associations

Pseudacteon curvatus was significantly more abundant in Unmanaged Habitat (Mean $\pm$ SE = 5.76 $\pm$ 0.97) than in any other habitat type (ANOVA; $F (3,320) = 19.96$, $P < 0.005$; Tukey's-b Post Hoc Analysis, $P < 0.005$, Mean $\pm$ SE = 1.72 $\pm$ 0.27; $P < 0.005$, Mean $\pm$ SE = 1.99 $\pm$ 0.34; and $P < 0.005$, Mean $\pm$ SE = 0.13 $\pm$ 0.07; for Unmanaged Habitat vs. Hay Pasture, Cattle Pasture and Forest respectively; Fig. 4.8).

Pseudacteon tricuspis was significantly more abundant in Cattle Pasture (Mean $\pm$ SE = 2.15 $\pm$ 0.29) compared to densities observed in Unmanaged Habitat and Forest

(ANOVA; F (3;320) = 17.44, $P < 0.005$; Tukey's HSD Post Hoc Analysis, $P < 0.05$,

Mean $\pm$ SE = 1.19 $\pm$ 0.22; $P < 0.005$, Mean $\pm$ SE = 0.01 $\pm$ 0.01, respectively; Fig. 4.8)

and *P. tricuspis* abundance was not significantly different in Hay and Cattle Pasture

(Tukey's HSD Post Hoc Analysis, $P = 0.07$, Mean $\pm$ SE = 1.42 $\pm$ 0.21; Fig. 4.8).
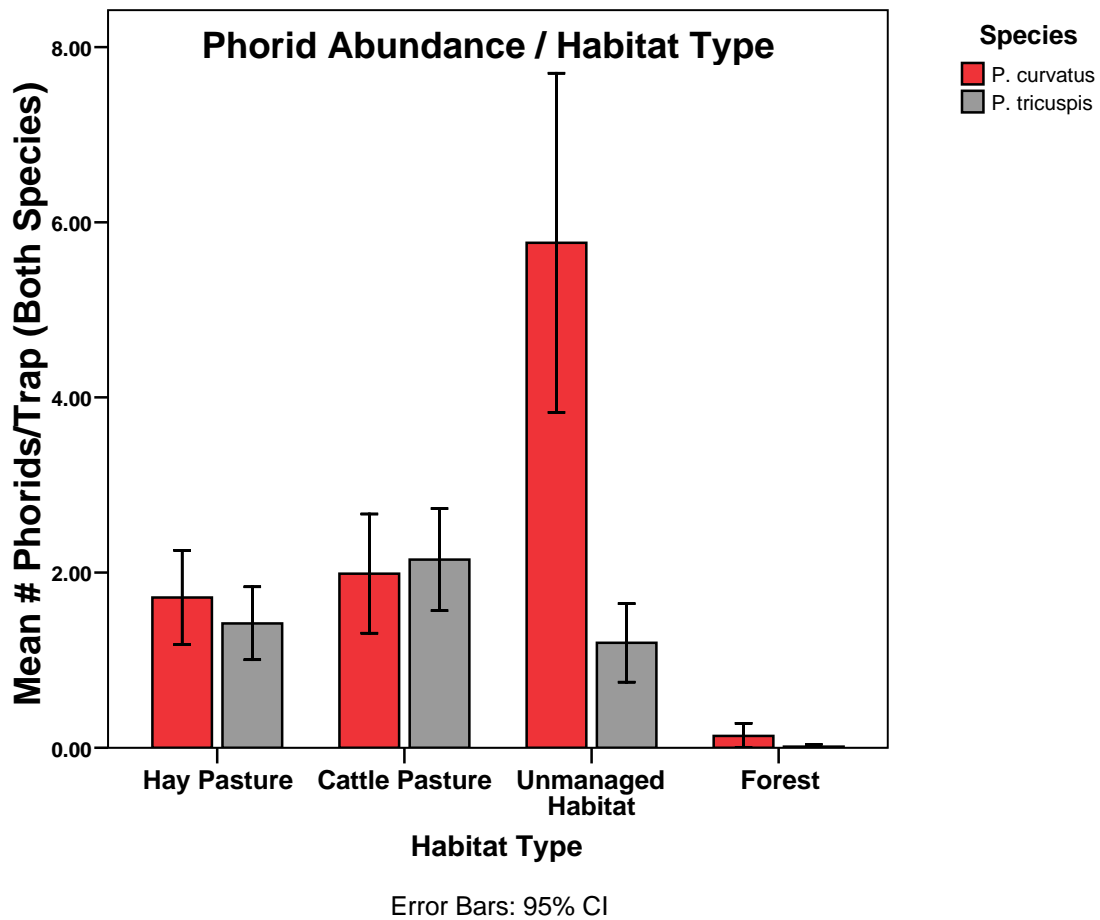
Fig. 4.8 Relative abundance of *Pseudacteon curvatus* and *Pseudacteon tricuspis* in four habitat types.

Pseudacteon curvatus was significantly more abundant than *P. tricuspis* in Unmanaged Habitat (Student's t-test - df (160) = 4.57, $P < 0.005$; Fig. 4.9). There was
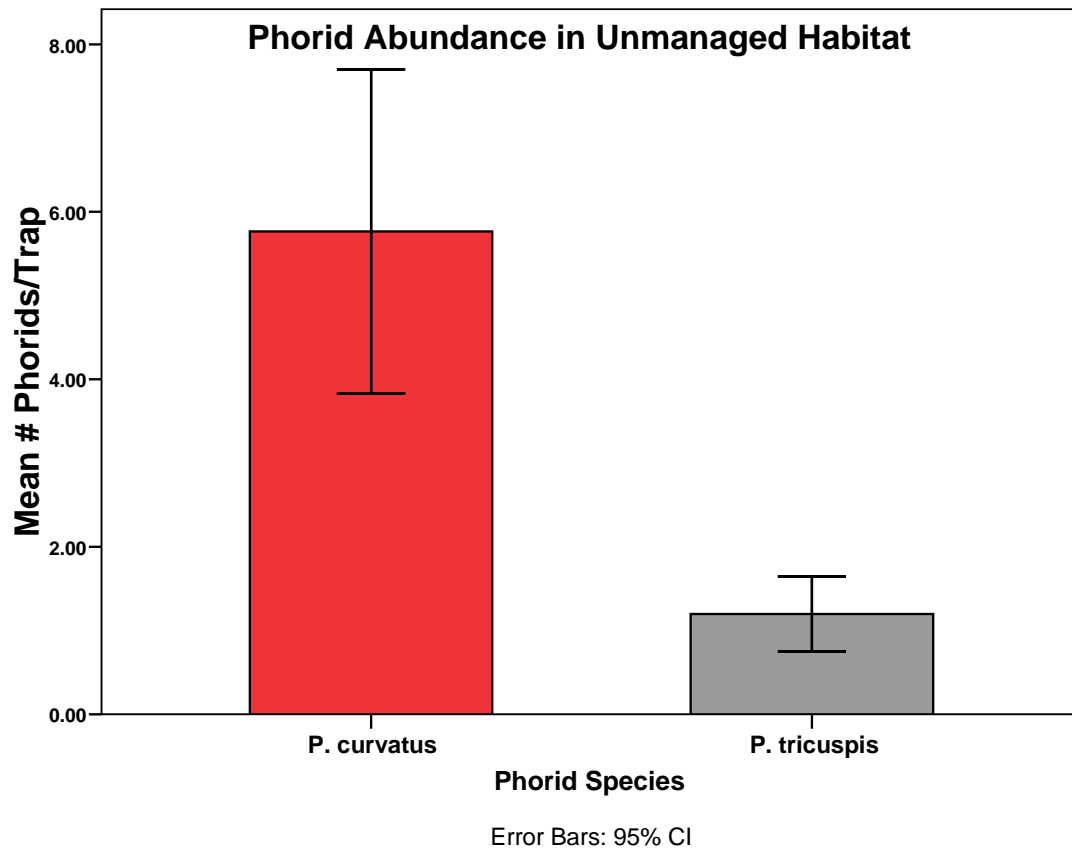
Fig. 4.9 Relative abundance of *Pseudacteon curvatus* and *Pseudacteon tricuspis* in Unmanaged Habitat. *Pseudacteon curvatus* abundance was significantly greater than *Pseudacteon tricuspis* (Student's t-test - df (160) = 4.57, $P < 0.005$).
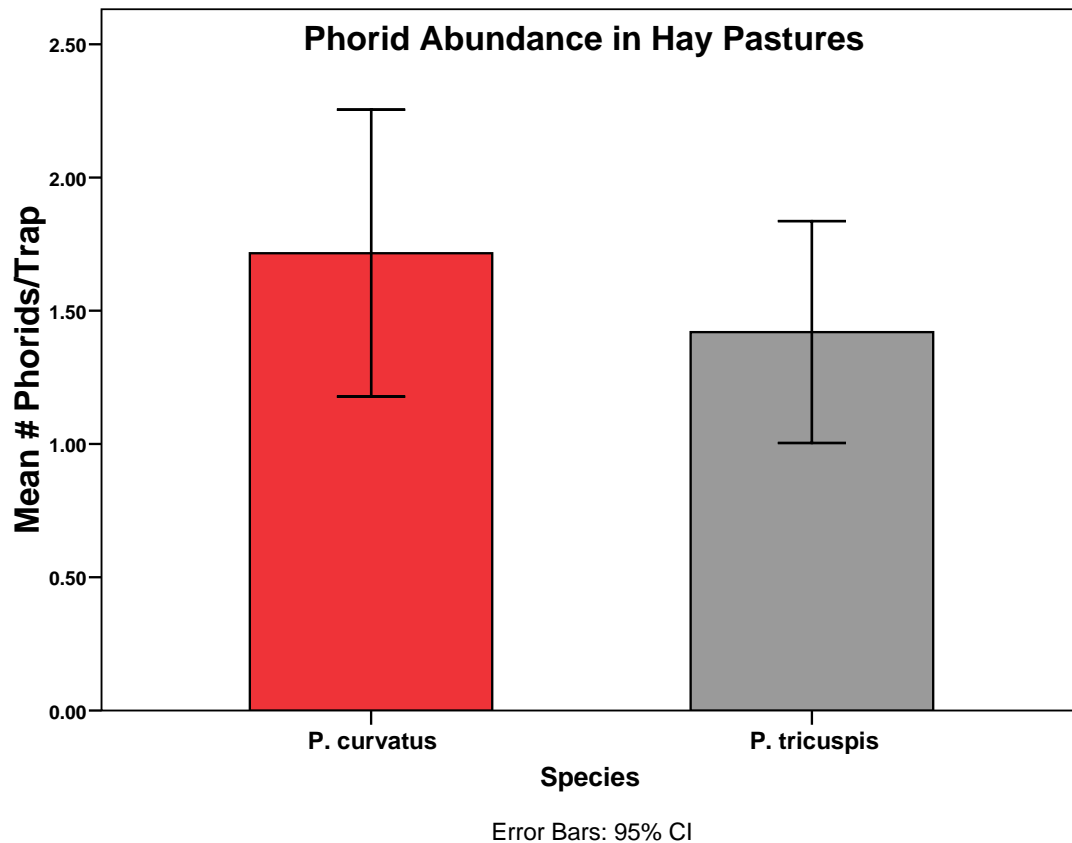
Fig. 4.10 Relative abundance of *Pseudacteon curvatus* and *Pseudacteon tricuspis* in Hay Pastures. There was no significant difference in the relative abundances of *Pseudacteon curvatus* and *Pseudacteon tricuspis* (Student's t-test - df (160) = 0.86, *P* = 0.39).

no significant difference in the relative abundance of *P. curvatus* and *P. tricuspis* in Hay Pastures (Student's t-test - df (160) = 0.86, *P* = 0.39; Fig. 4.10), Cattle Pastures (Student's t-test - df (160) = 0.35, *P* = 0.72; Fig. 4.11) or Forest (Student's t-test - df (160) = 1.69, *P* = 0.09; Fig. 4.12).
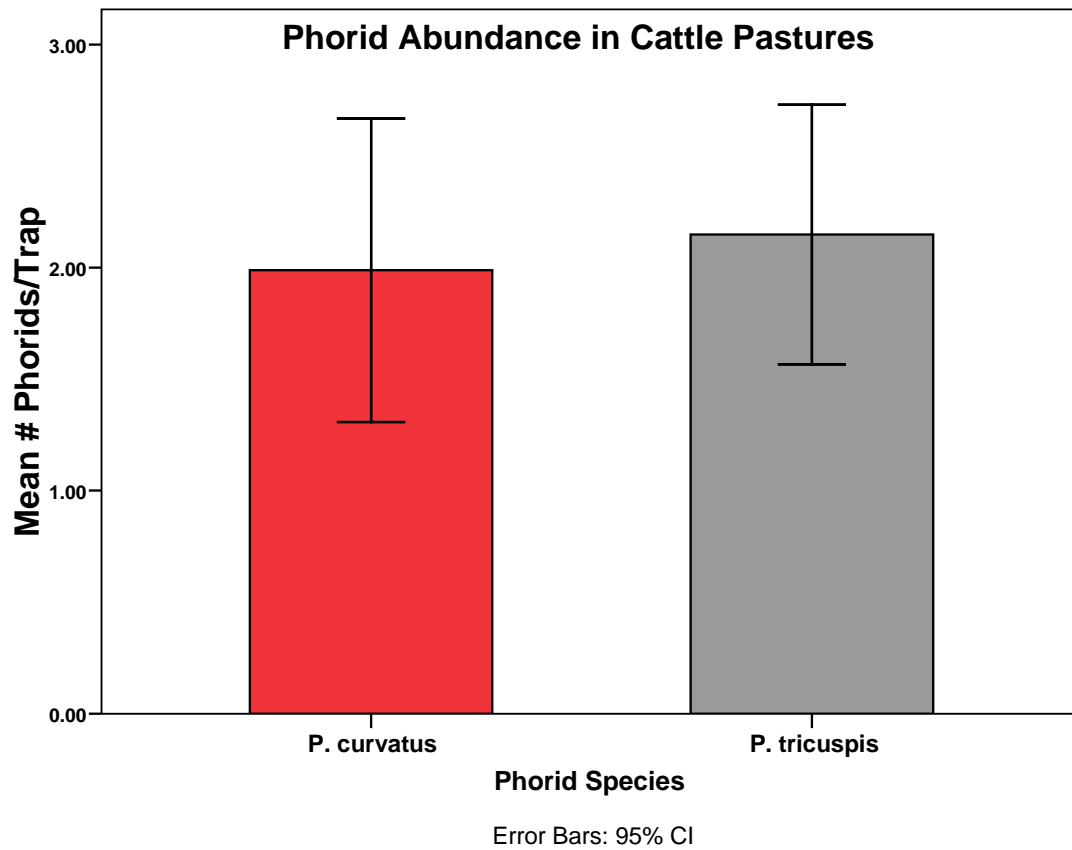
Fig. 4.11 Relative abundance of *Pseudacteon curvatus* and *Pseudacteon tricuspis* in Cattle Pastures. There was no significant difference in the relative abundances of *Pseudacteon curvatus* and *Pseudacteon tricuspis* (Student's t-test - df (160) = 0.35, *P* = 0.72).
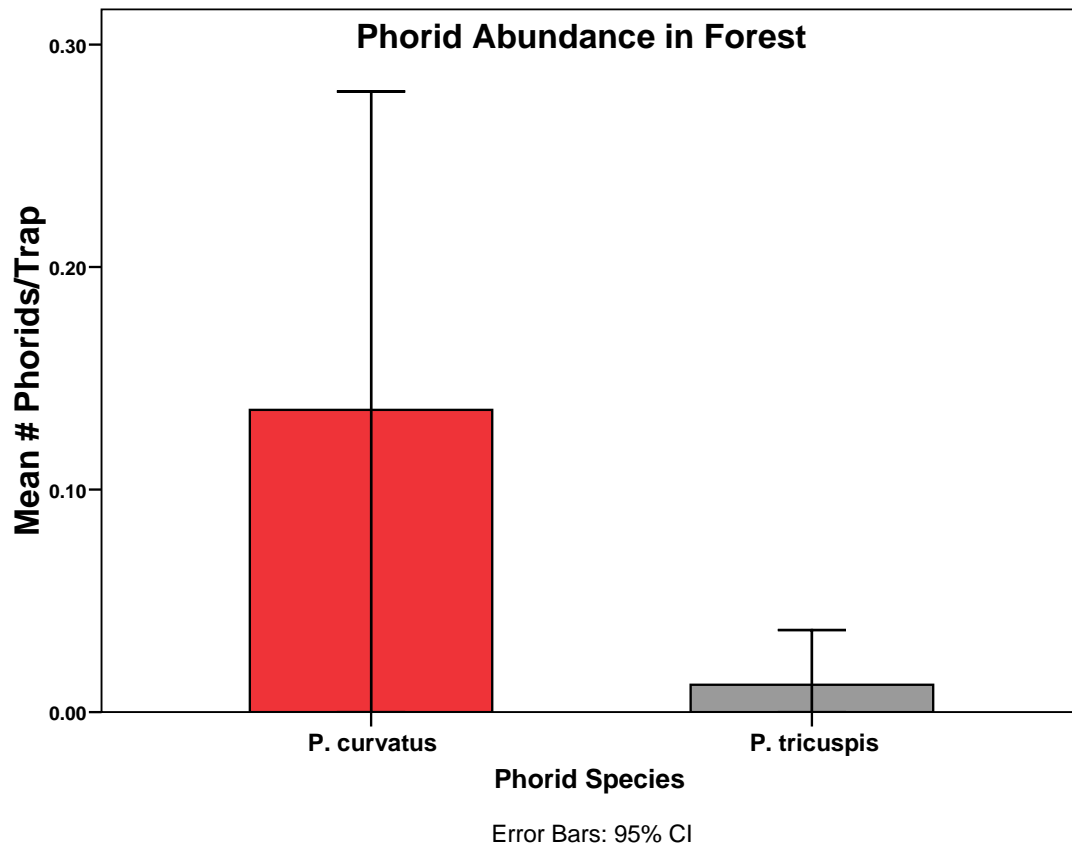
Fig. 4.12 Relative abundance of *Pseudacteon curvatus* and *Pseudacteon tricuspis* in Forest. There was no significant difference in the relative abundances of *Pseudacteon curvatus* and *Pseudacteon tricuspis* (Student's t-test - df (160) = 1.69, *P* = 0.09).

Hot-Dog Bait Samples demonstrated that RIFA were significantly less abundant in Forest (Mean $\pm$ SE = 0.00 $\pm$ 0.00) than in any other habitat types (ANOVA; *F* (3,32) = 5.58, *P* < 0.005; Tukey's HSD Post Hoc Analysis, *P* < 0.05, Mean $\pm$ SE = 1.55 $\pm$ 0.29; *P* < 0.05, Mean $\pm$ SE = 1.44 $\pm$ 0.38; and *P* < 0.05, Mean $\pm$ SE = 1.67 $\pm$ 0.37; for Forest vs. Hay Pasture, Cattle Pasture and Unmanaged Habitat, respectively; Fig. 4.13).

RIFA Hot-Dog Samples / Habitat Type

Error Bars: 95% CI
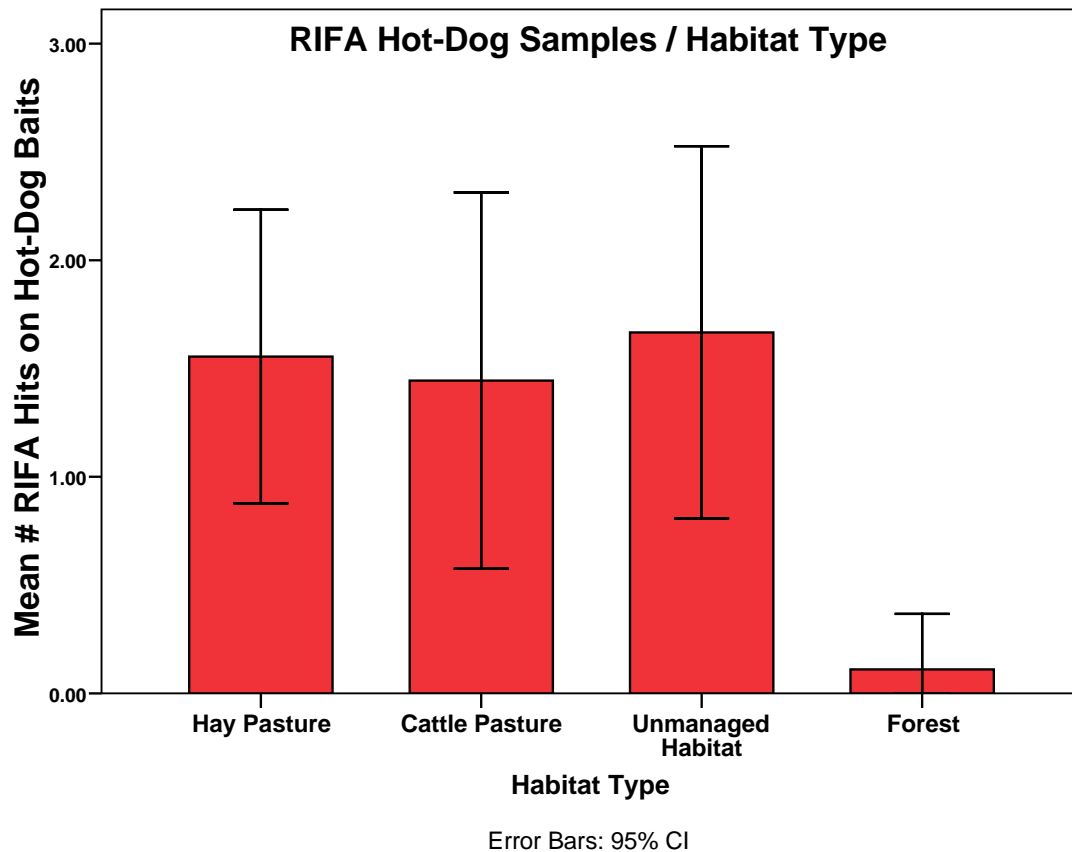
Fig. 4.13 Mean # of RIFA 'hits' on hot-dog baits in all habitat types. (ANOVA; F (3,32) = 5.58, $P < 0.005$; Tukey's HSD Post Hoc Analysis, $P < 0.05$, Mean $\pm$ SE = 1.55 $\pm$ 0.29; $P < 0.05$, Mean $\pm$ SE = 1.44 $\pm$ 0.38; and $P < 0.05$, Mean $\pm$ SE = 1.67 $\pm$ 0.37; for Forest vs. Hay Pasture, Cattle Pasture and Unmanaged Habitat, respectively).

RIFA Mound-Counts demonstrated that mounds were significantly less abundant in Forest habitat (ANOVA; F (3,32) = 20.22, $P < 0.005$; Tukey's HSD Post Hoc Analysis, $P < 0.005$, Mean $\pm$ SE = 12.32 $\pm$ 2.59; $P < 0.005$, Mean $\pm$ SE = 16.78 $\pm$ 1.49; and $P < 0.005$, Mean $\pm$ SE = 7.33 $\pm$ 1.12; for Forest vs. Hay Pasture, Cattle Pasture and Unmanaged Habitat respectively; Fig. 4.14), RIFA mounds were significantly less abundant in Unmanaged Habitat than in Cattle Pastures (Tukey's HSD Post Hoc

Analysis, $P < 0.005$; for Unmanaged Habitat vs. Cattle Pasture; Fig. 4.14) and mound counts were statistically equivalent in Hay and Cattle Pastures (Tukey's HSD Post Hoc Analysis, $P = 0.22$; Fig 4.14).

Error Bars: 95% CI
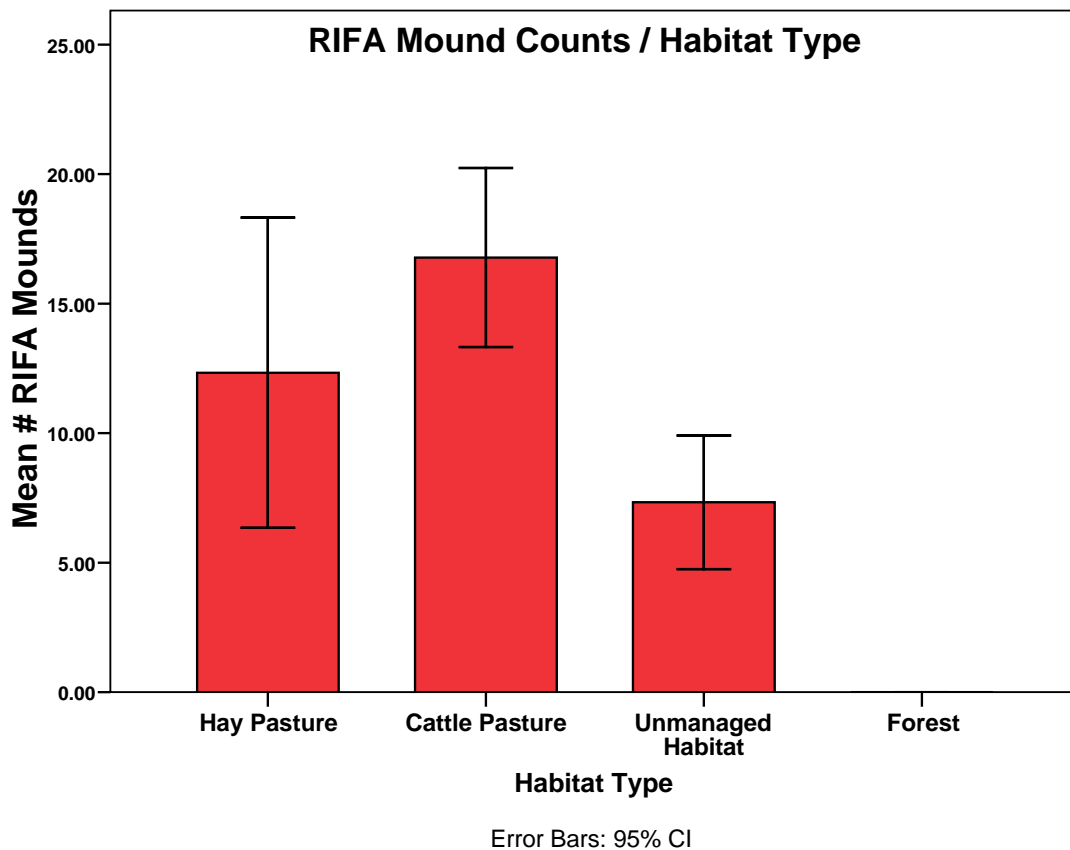
Fig. 4.14 Mean # of RIFA mounds in all habitat types. RIFA mounds were statistically least abundant in Forest habitat (ANOVA; $F (3,32) = 20.22$, $P < 0.005$; Tukey's HSD Post Hoc Analysis, $P < 0.005$, Mean $\pm$ SE = 12.32 $\pm$ 2.59; $P < 0.005$, Mean $\pm$ SE = 16.78 $\pm$ 1.49; and $P < 0.005$, Mean $\pm$ SE = 7.33 $\pm$ 1.12; for Forest vs. Hay Pasture, Cattle Pasture and Unmanaged Habitat, respectively), RIFA mounds were statistically less abundant in Unmanaged Habitat than in Cattle Pastures (Tukey's HSD Post Hoc Analysis, $P < 0.005$; for Unmanaged Habitat vs. Cattle Pasture) and mound counts were statistically equivalent in Hay and Cattle Pastures (Tukey's HSD Post Hoc Analysis, $P = 0.22$).

Discussion

Since its release and subsequent establishment in this system, *P. curvatus* has rapidly increased in density to a level that was measured to be five-fold that of it's *P. tricuspis* congener (Fig. 4.7) in June 2007. However, this increase does not appear to have been at the expense of the *P. tricuspis* population that was established several years prior to the release of *P. curvatus*. The data presented herein demonstrate that *P. tricuspis* densities are oscillating around seasonal system equilibria that appear to be independent of the explosive increase in *P. curvatus*. These species exhibit host body-size niche partitioning in their native South American range, and they may be partitioning the RIFA resource in our study such that interspecific competition is minimized or absent. In addition, in Fall 2005 *P. tricuspis* was the dominant phorid species in terms of the % area of the study site that it dominated spatially relative to *P. curvatus* (as revealed by IDW Interpolation of phorid densities) (Table 4.1 and APPENDIX A-1 & A-2). Concurrent with an increase in density, in 2006 *P. curvatus* began to routinely demonstrate numerical/spatial superiority over *P. tricuspis* (Table 4.1 and APPENDIX A-3 – A-12). While this superiority was only marginal during most of 2006, 2007 samples showed a dramatic shift towards *P. curvatus* superiority, which was maintained throughout Summer and Fall 2007 with a % area occupied peak of 98.92. Again, this increase in *P. curvatus* spatial superiority was not at the expense of *P. tricuspis* densities which appeared to be oscillating around system equilibrium. LeBrun (*In Review*) has shown similar interactions between *P. curvatus* and *P. tricuspis* that resulted in the eventual displacement of *P. tricuspis*. It is unknown whether *P. curvatus*

densities will continue to increase relative to *P. tricuspis* at this site, and it is unclear

what the carrying capacity of this system is with regards to densities of *Pseudacteon spp.*

phorid flies.   In addition to host-size partitioning, these flies appear to be partitioning at

least one habitat-specific niche (Fig. 4.8) despite the fact that host densities are similar in

those habitats sampled. *Pseudacteon curvatus* is numerically superior in the Unmanaged

Habitat at our study site. This indicates that, in this system, phorids are capable of co-

existence in most habitat types without displacement of either species. In another study

(Unpublished Data), we determined that at our study site there is a different composition

of foraging RIFA size classes, with small ants making up a statistically larger portion of

the foragers. It is presumed that this is a RIFA behavioral response to avoid parasitism

by *P. tricuspis* that were released several years prior to *P. curvatus* at this site. The

composition of habitat and the available RIFA forager size distribution seems to have

contributed to the rapid increase and expansion of *P. curvatus*, which again, prefer to

oviposit in smaller hosts than *P. tricuspis*. It is unclear whether this scenario will change

as *P. curvatus* densities and incidence of attack increases, which will present a

concomitant increase in selective pressure against smaller RIFA forager size. Further

monitoring of this system will provide insight into these unanswered questions regarding

future phorid competitive success, and will allow for more accurate strategic planning as

it pertains to release and establishment of additional species of *Pseudacteon* phorid

parasitoids in the U.S. and elsewhere.

CHAPTER V

CONCLUSIONS AND DISCUSSION

Red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) demonstrated a nocturnal compensatory foraging strategy in the laboratory experiments detailed in Chapter III. These results were of particular concern with regards to the potential of a similar RIFA strategy occurring in the field where phorids and RIFA interact. This parasitism-avoidance strategy would presumably shift the RIFA circadian behavior and may increase the frequency and intensity of competitive interactions between RIFA and native ant assemblages.

Data from our field experiments did not show statistically significant differences in RIFA foraging intensities during diurnal or nocturnal sampling periods at or between the treatment and control sites. However, the field data demonstrate similar mean-separation trends as found in the laboratory study (greater mean nocturnal foraging intensity and lower mean diurnal foraging intensity in phorid-exposed RIFA relative to those at the control site). This trend may intensify as phorid fly densities increase and researchers continue to release new phorid species in the southern United States.

Phorid-exposed RIFA at 5-Eagle Ranch (treatment site) demonstrated a significantly different ratio of worker sizes relative to those at the Skrivanek Ranch (control site). The proportion of small workers at 5-Eagle Ranch was significantly greater than that of the Skrivanek Ranch and the proportion of larger RIFA workers was significantly lower during PM sampling periods. These ratios were not significantly

different during AM samples, suggesting that this RIFA colony-level response fluctuates with daily fluctuations in parasitism pressure.

PTS-Traps developed for this dissertation research have greatly enhanced the ability of *Pseudacteon spp.* phorid fly researchers to study these parasitoids and their behavior in natural settings. Data demonstrated that these traps dramatically reduce the amount of time required by researchers to study these flies while providing an equally impressive increase in the resolution, reliability and repeatability of the data collected relative to other phorid sampling techniques. PTS-Traps have provided a method for determining phorid phenology and habitat associations, and have provided increased confidence in assessing presence and absence of flies at research sites, which is critical when selecting treatment and control sites for field experiments such as those described in Chapter III. These traps and others based on the general PTS-Trap design are already being employed by other researchers in the United States to address questions regarding phorid range expansion rates (Gilbert et al. 2008).

Phorid sampling (2005-2007) revealed distinct trends in the phenology of both *P. curvatus* and *P. tricuspis*. The initiation of this sampling appears to have occurred during the lag-phase of *P. curvatus* density increase. *P. curvatus* densities in 2005 were lower than those of *P. tricuspis*. However, throughout most of 2006 *P. curvatus* densities were greater than those of *P. tricuspis* and both species demonstrated similar density trends. In summer 2006 *P. curvatus* densities increased dramatically relative to those of *P. tricuspis* and *P. curvatus* remained numerically superior through the end of sampling in 2007. *P. tricuspis* densities remained consistent throughout this sampling

period. Also, species-specific numerical/spatial superiority followed similar trends to those of species-specific density fluctuations. In addition, phorid species are significantly more abundant in only 1 habitat type of those sampled. These results indicate that this system appears capable of supporting both of these phorid species in sympatry, but homeostasis may not yet have occurred in the system and further monitoring is warranted.

The success of *P. curvatus* in this system may be due to both the timing of releases of these phorid species as well as the differences in their preferred host sizes. *P. tricuspis* prefer large RIFA workers, and were established in this system 2 years prior to *P. curvatus*, which prefer smaller hosts. *P. tricuspis* presumably shifted RIFA towards a greater proportion of small workers at 5-Eagle ranch. The mechanism for this is unclear (differential removal of large workers through parasitism or by influencing caste-specific RIFA foraging behavior?) but the data in Chapter III clearly show this colony level affect. Thus, as *P. curvatus* became established, its preferred host was disproportionately abundant, that may account for their rapid density increase and range expansion.

Many recommendations result from this work. First, it is of critical importance that the issue of compensatory nocturnal foraging in RIFA be monitored throughout the current and future *Pseudacteon spp.* phorid fly range. In addition, while this system appears capable of supporting both phorid species at present, it is unclear whether future interactions among fly species and RIFA will result in the displacement of one or more of the fly species studied as populations continue toward population equilibria.

Continual monitoring of this situation will provide further information regarding the fate of these phorids in the field. Studies of phorid/habitat associations yielded insight regarding selection of future *P. curvatus* and *P. tricuspis* release sites. *P. curvatus* was significantly more abundant in Unmanaged Habitats and it is assumed that releasing this species in either analogous habitats or those with a significant proportion of such habitats in close proximity to the release site will provide this species with appropriate 'nursery habitat', resulting in a greater level of establishment success. Alternatively, *P. tricuspis* abundance was not significantly different among the habitat types studied and no such statement can be made regarding this species.

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APPENDIX
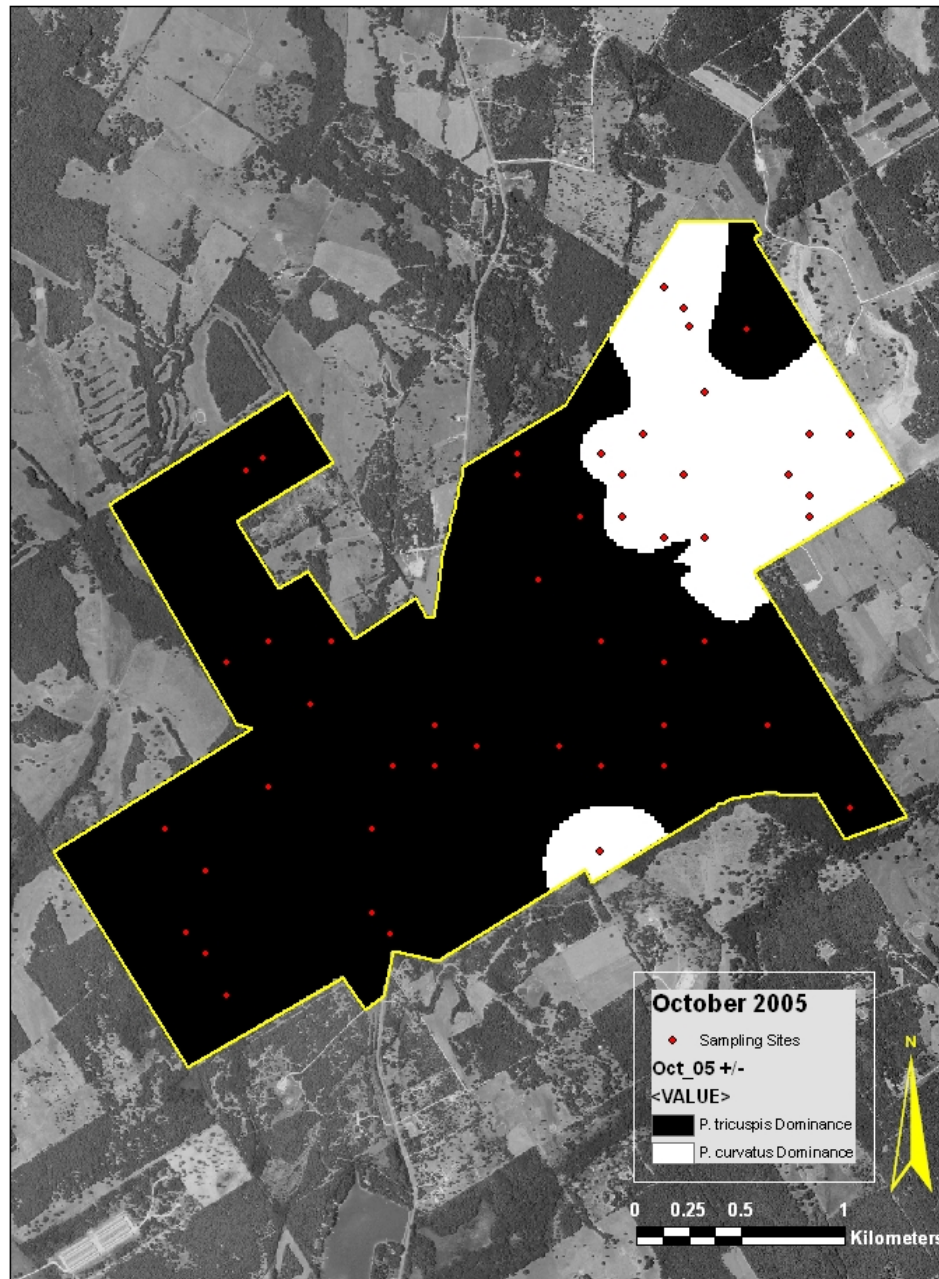
A-1. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis*
species-specific numerical/spatial superiority during October 2005 (% area dominated by
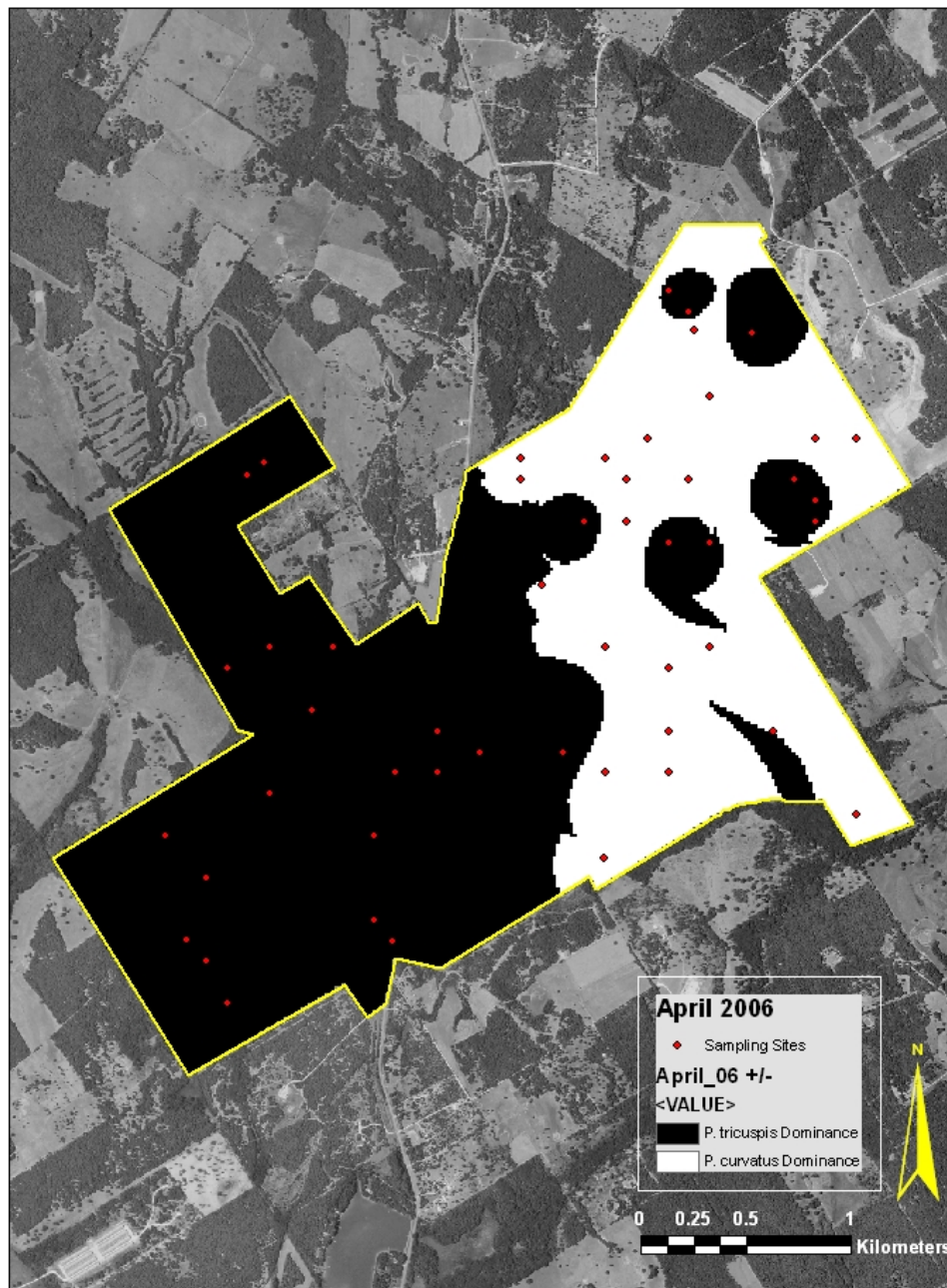*P. curvatus* = 19.92 and *P. tricuspis* = 80.08).
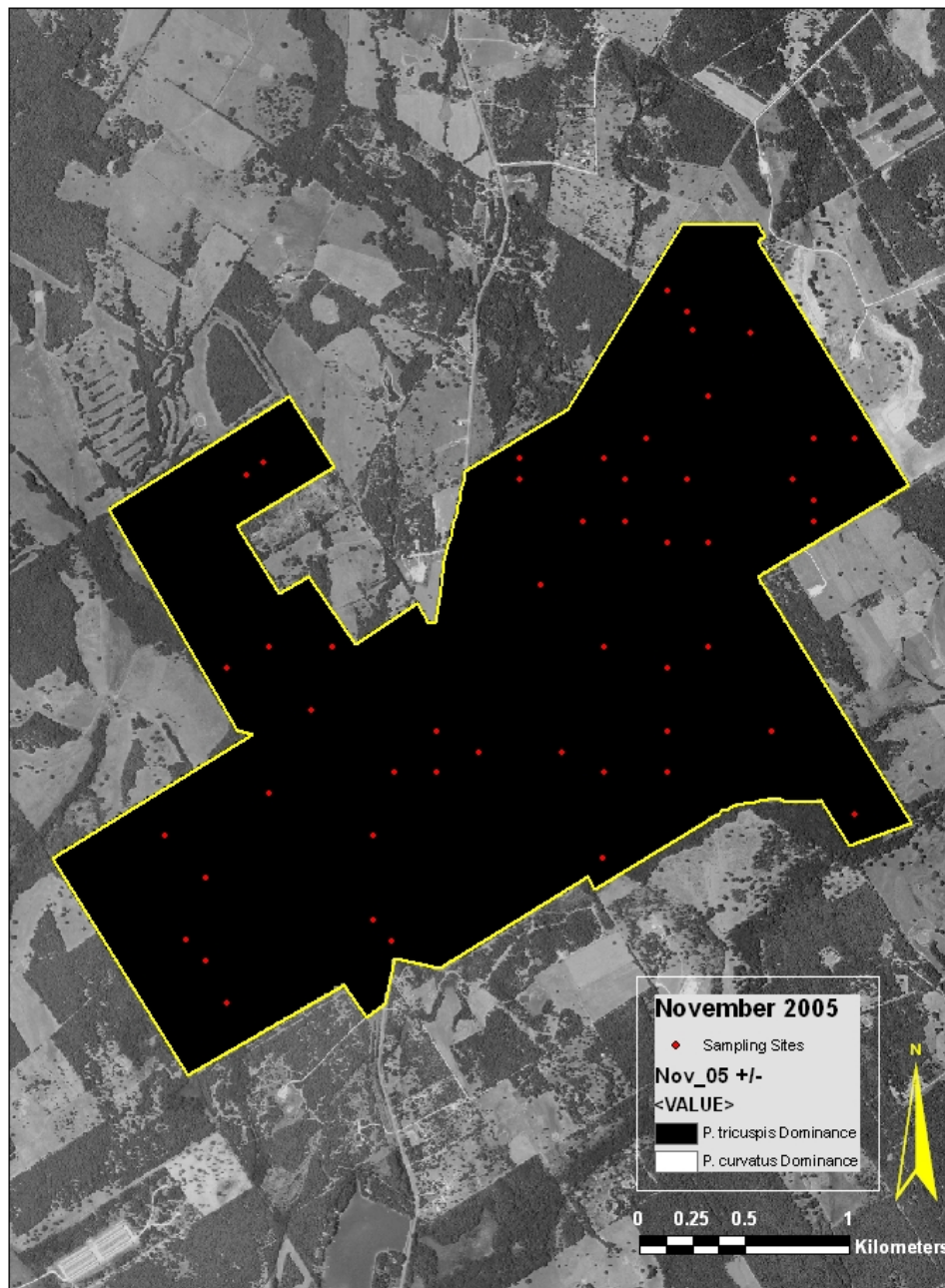
A-2. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during November 2005 (% area dominated by *P. curvatus* = 0.00 and *P. tricuspis* = 100).
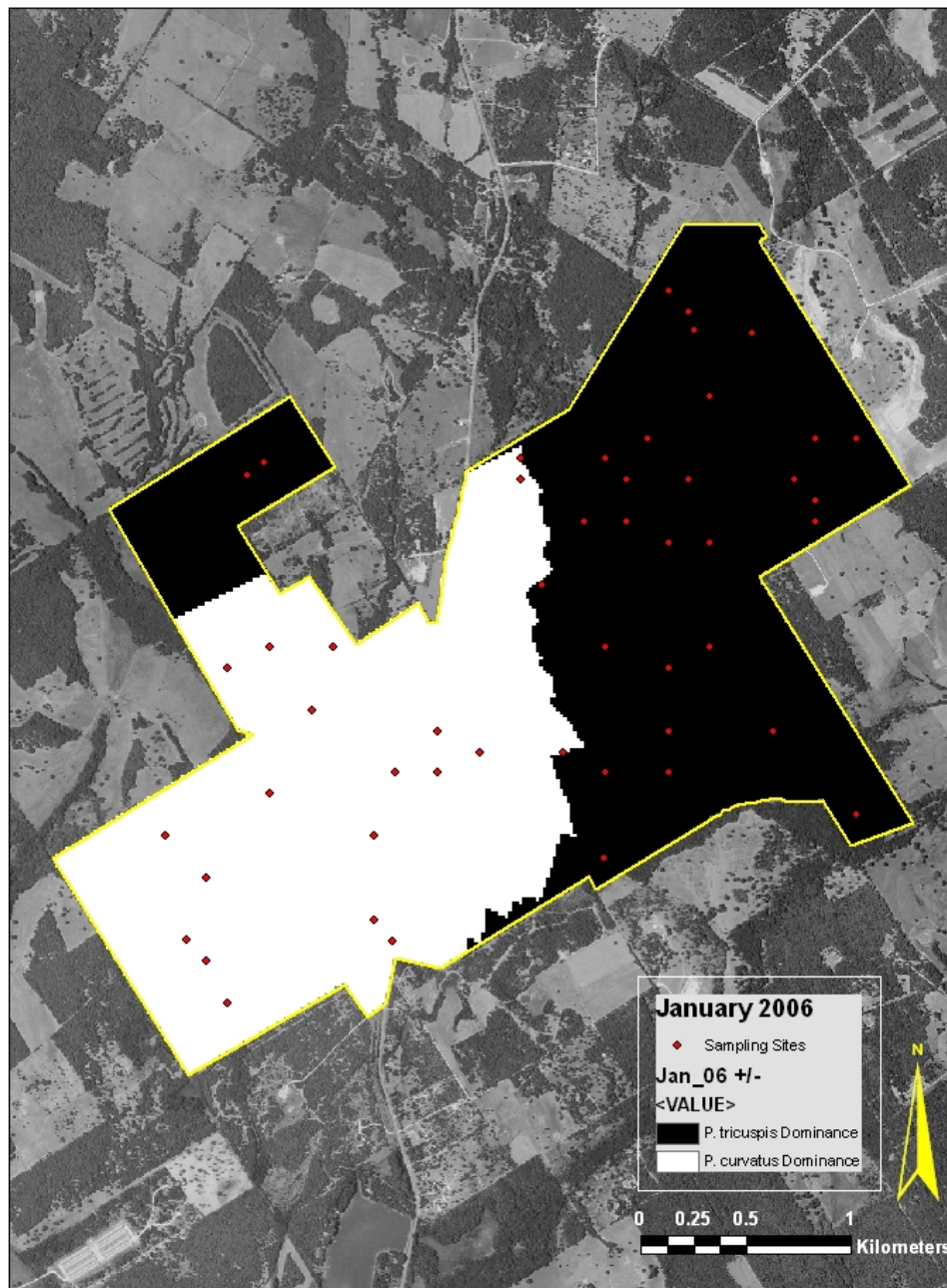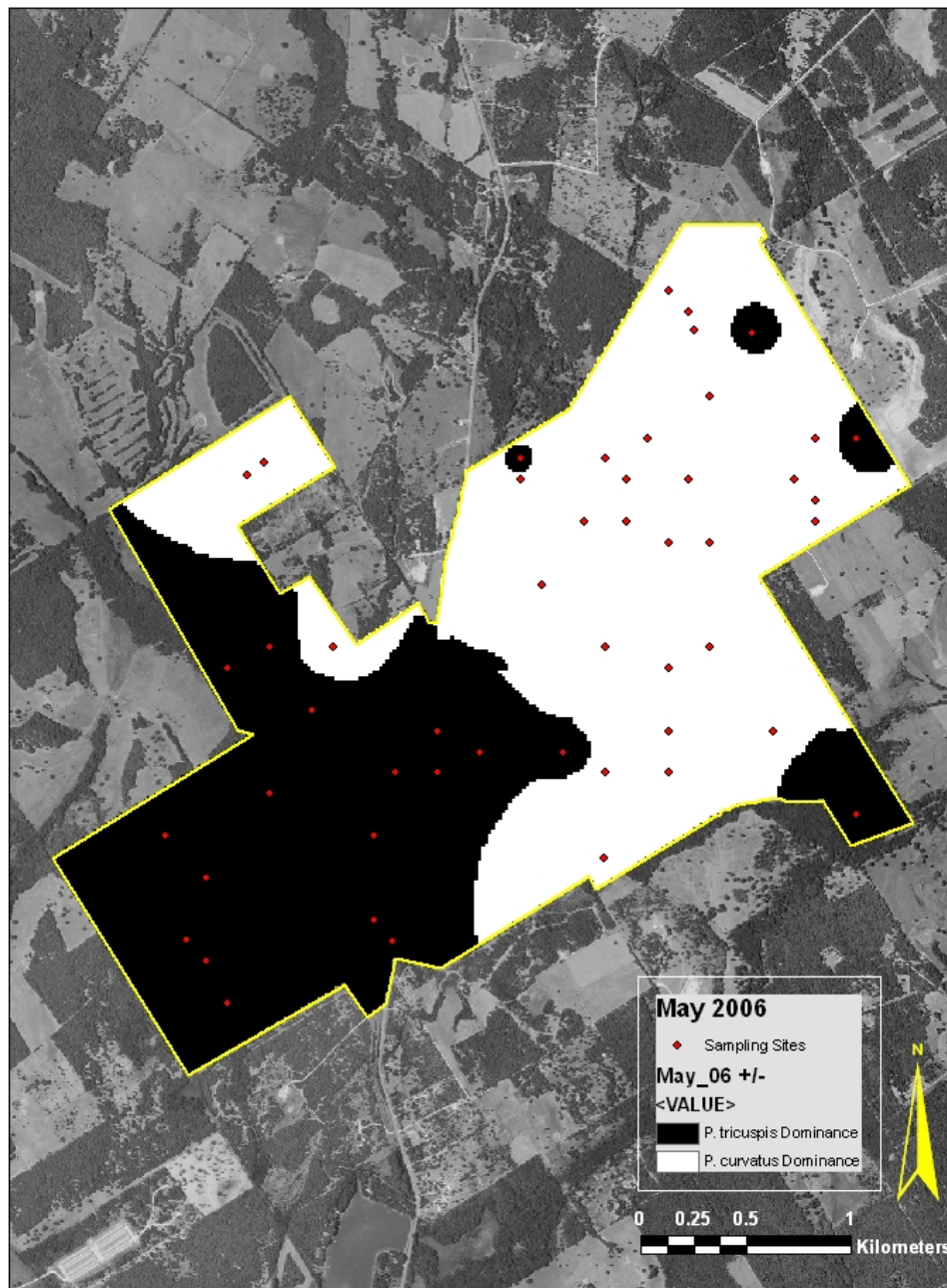
A-3. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during January 2006 (% area dominated by *P. curvatus* = 47.62and *P. tricuspis* = 52.38).
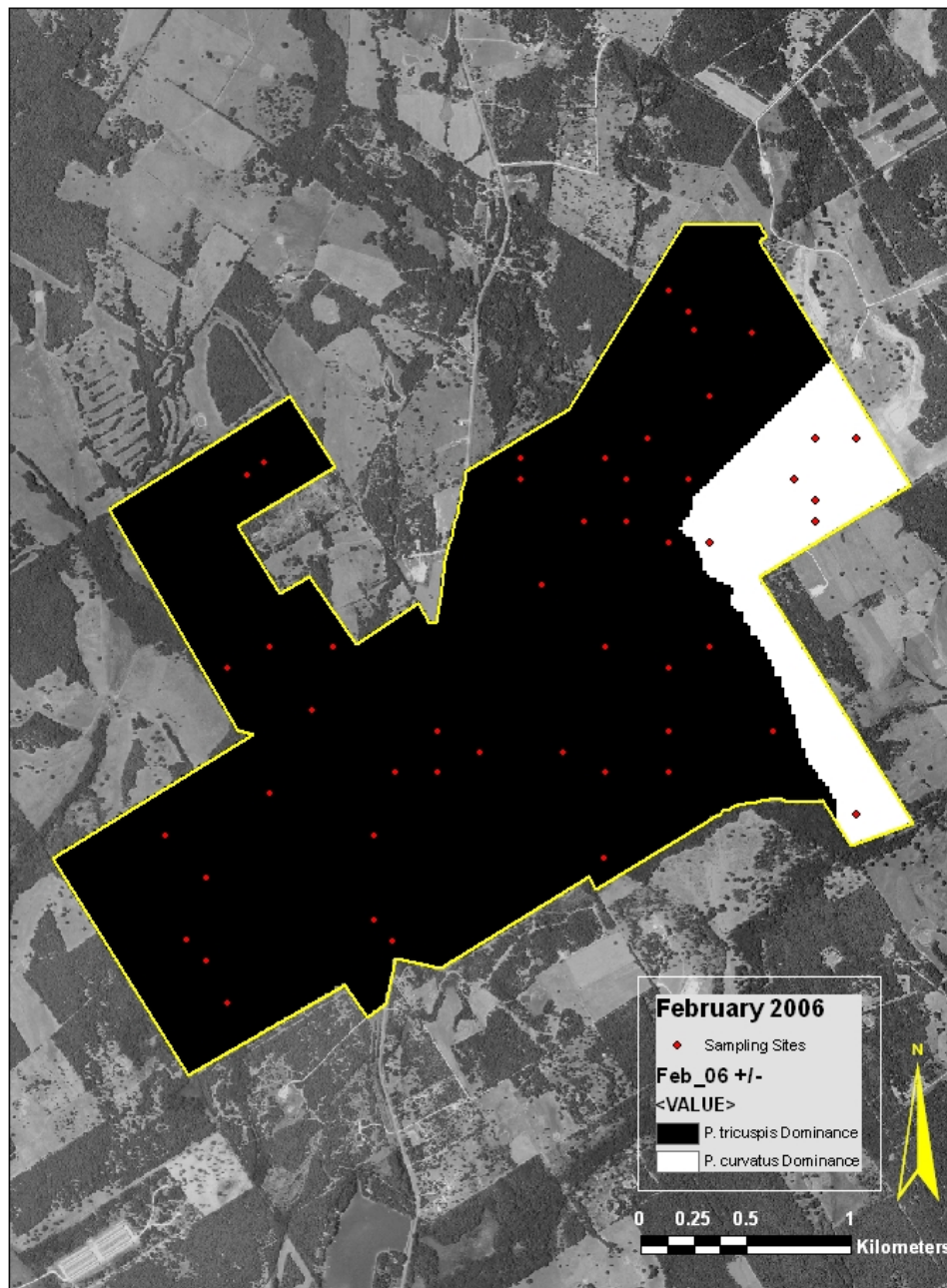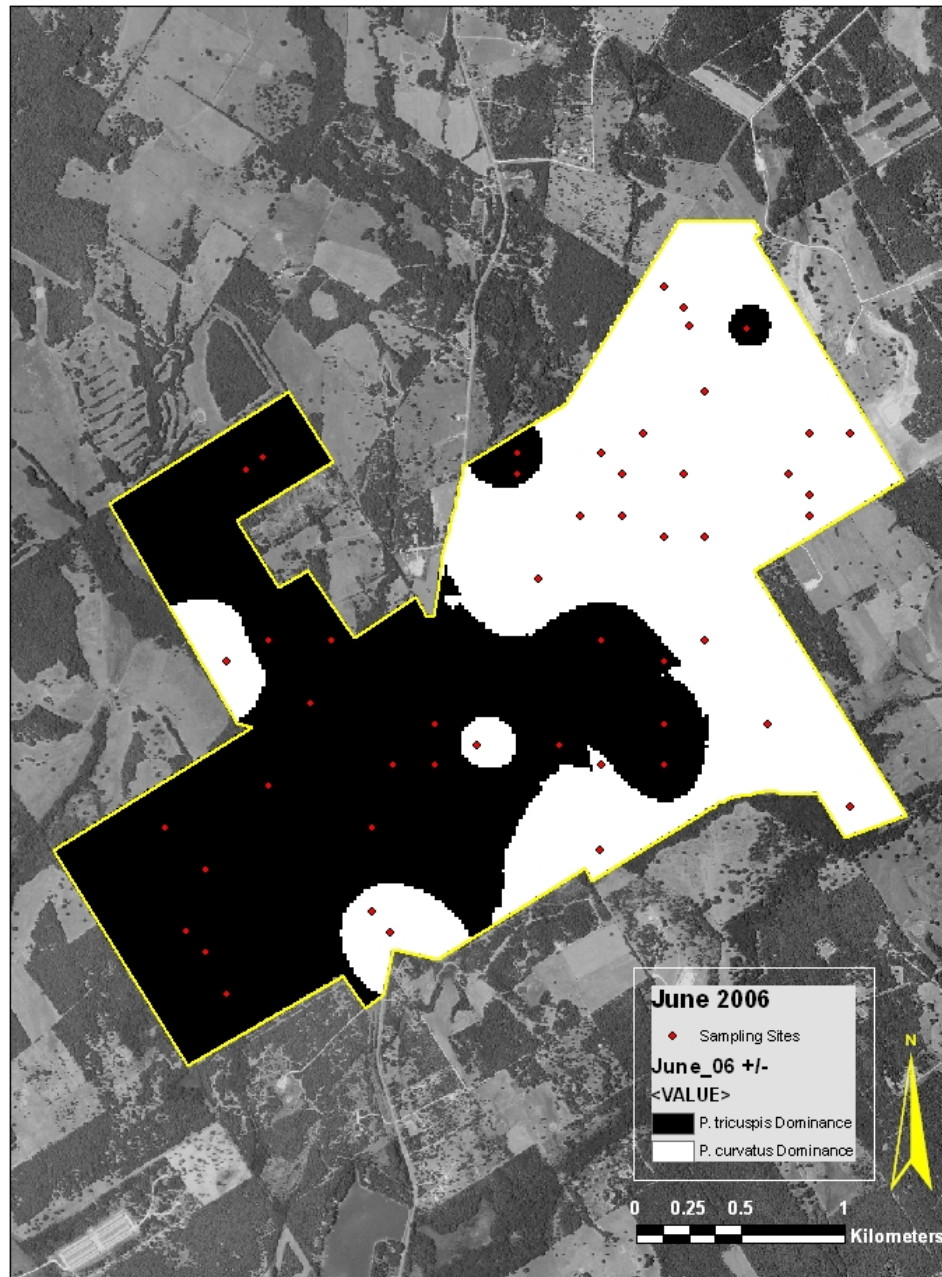
A-4. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during February 2006 (% area dominated by *P. curvatus* = 11.05 and *P. tricuspis* = 88.95).
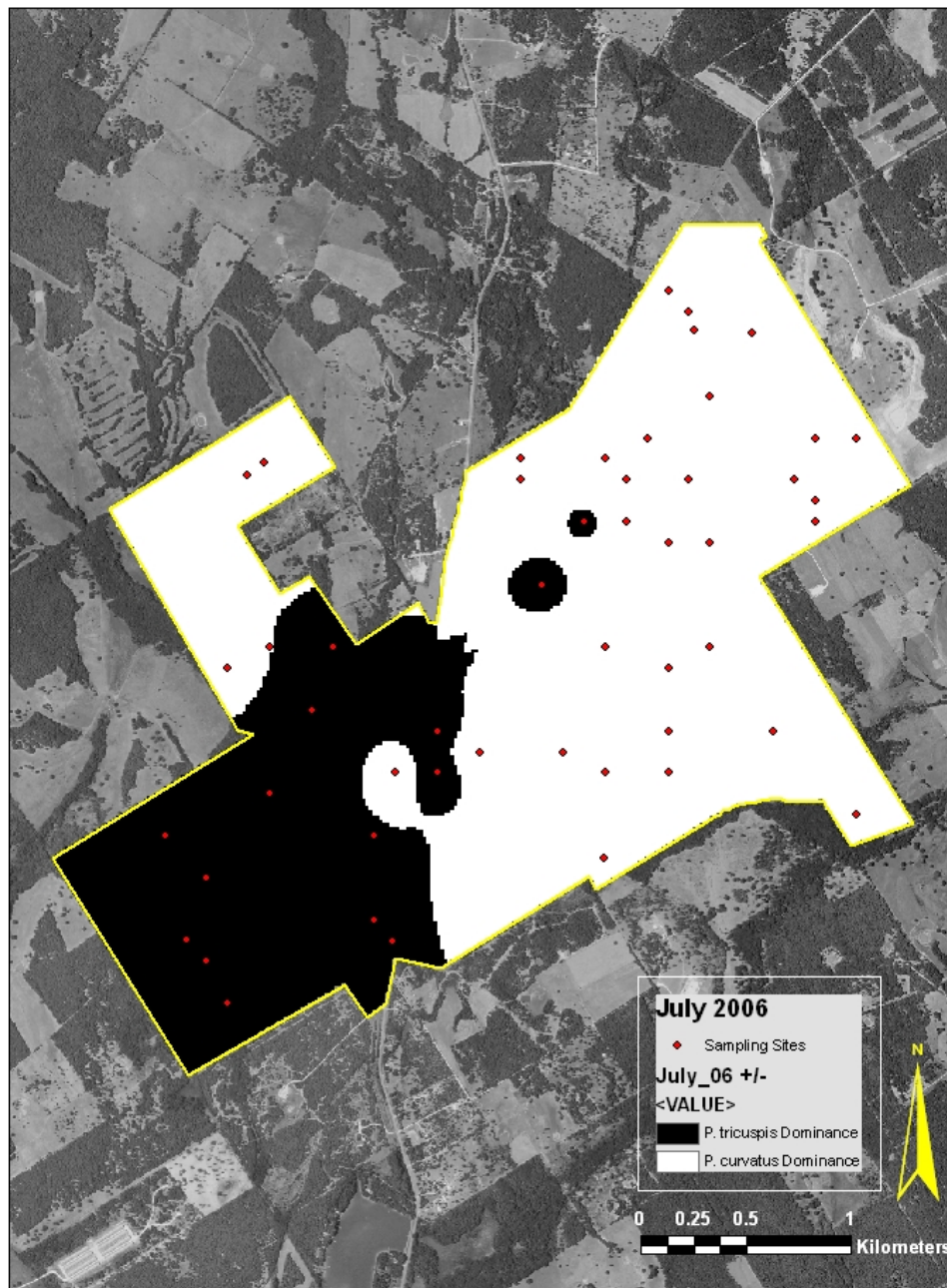
A-5. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during April 2006 (% area dominated by *P. curvatus* = 37.43 and *P. tricuspis* = 62.57).
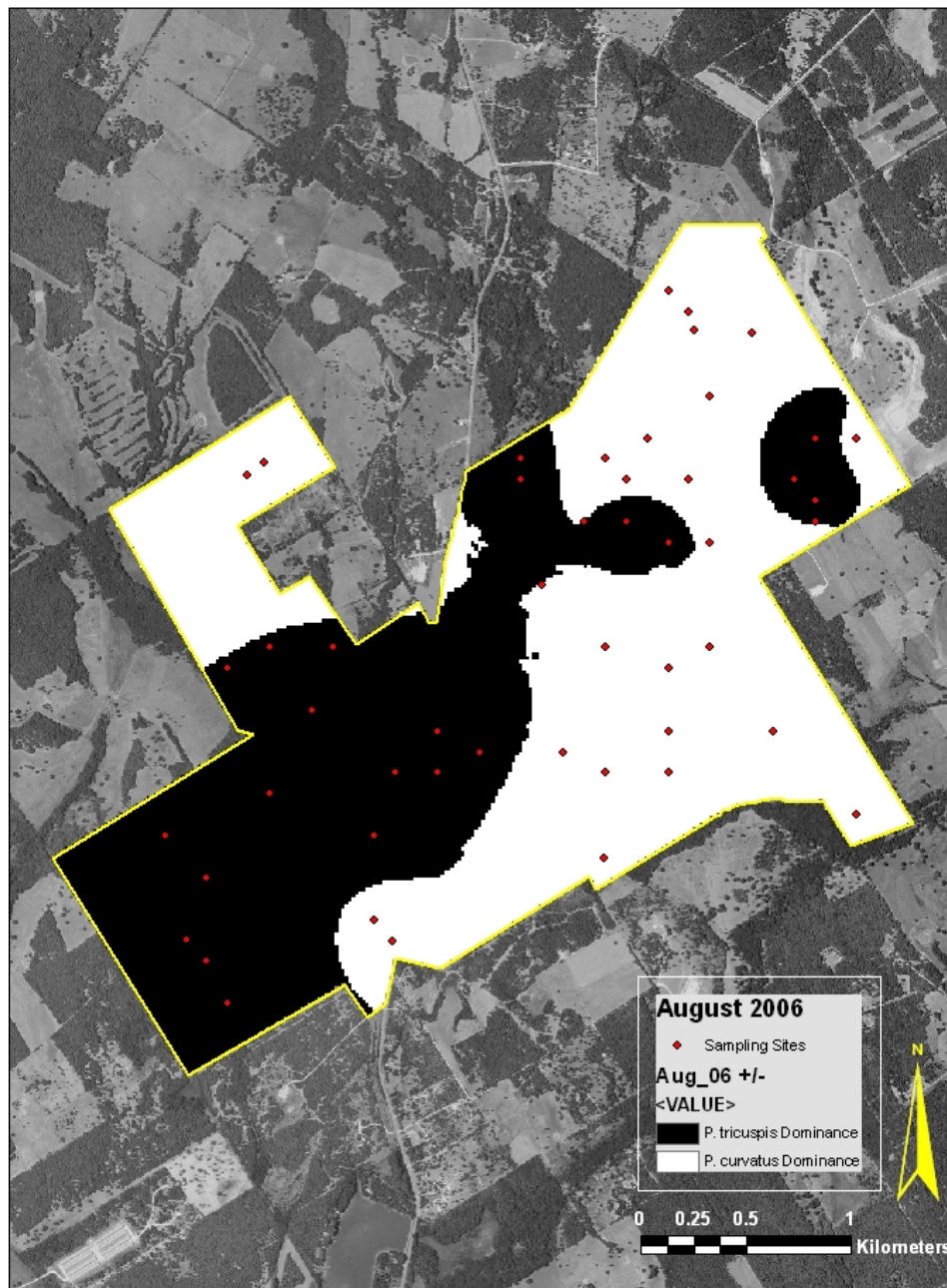
A-6. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis*
species-specific numerical/spatial superiority during May 2006 (% area dominated by *P.
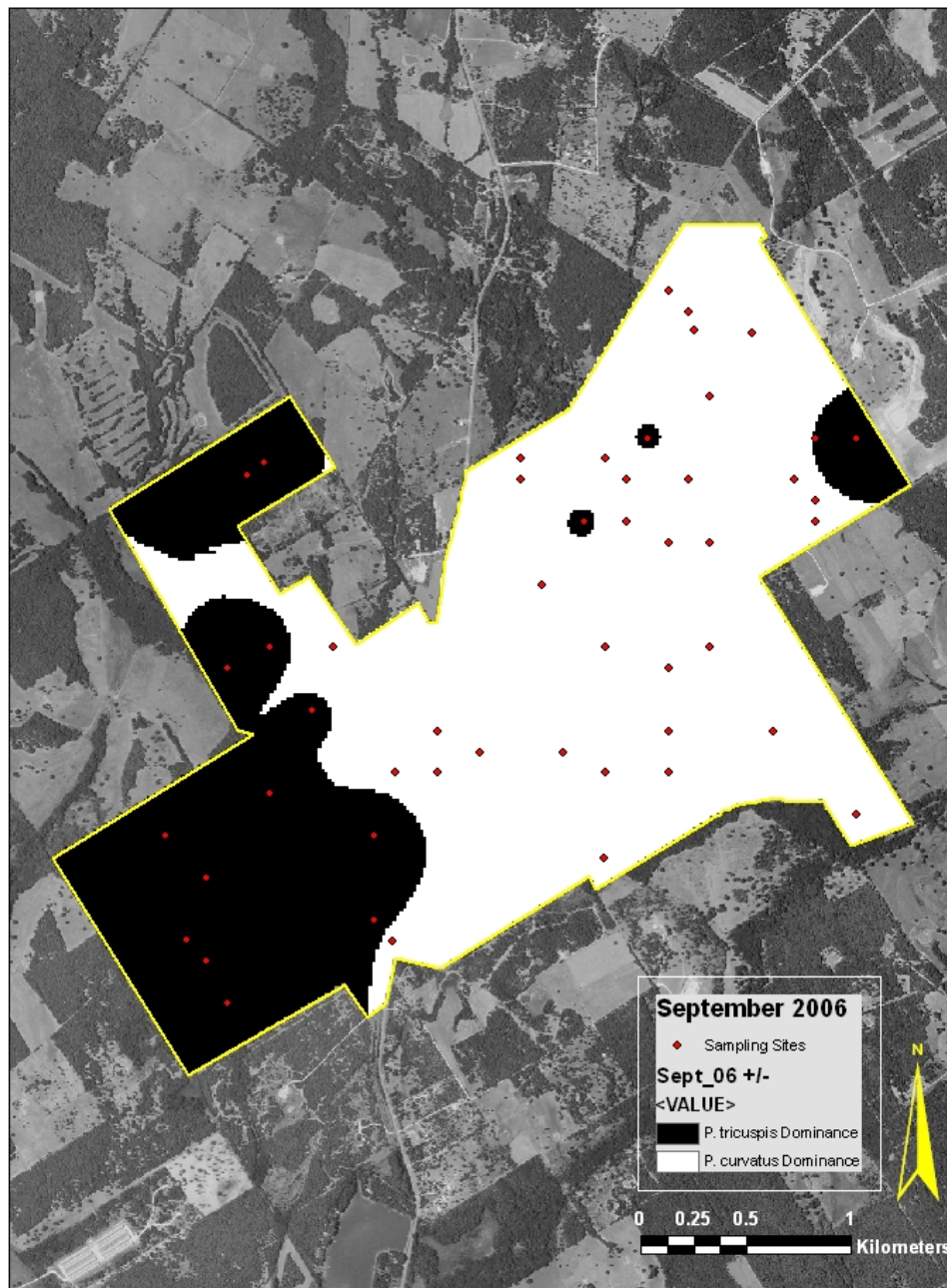curvatus* = 55.86 and *P. tricuspis* = 44.14).

A-7. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during June 2006 (% area dominated by *P. curvatus* = 48.05 and *P. tricuspis* = 51.95).

A-8. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during July 2006 (% area dominated by *P. curvatus* = 68.39 and *P. tricuspis* = 31.61).

A-9. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during August 2006 (% area dominated by *P. curvatus* = 56.60 and *P. tricuspis* = 43.40).
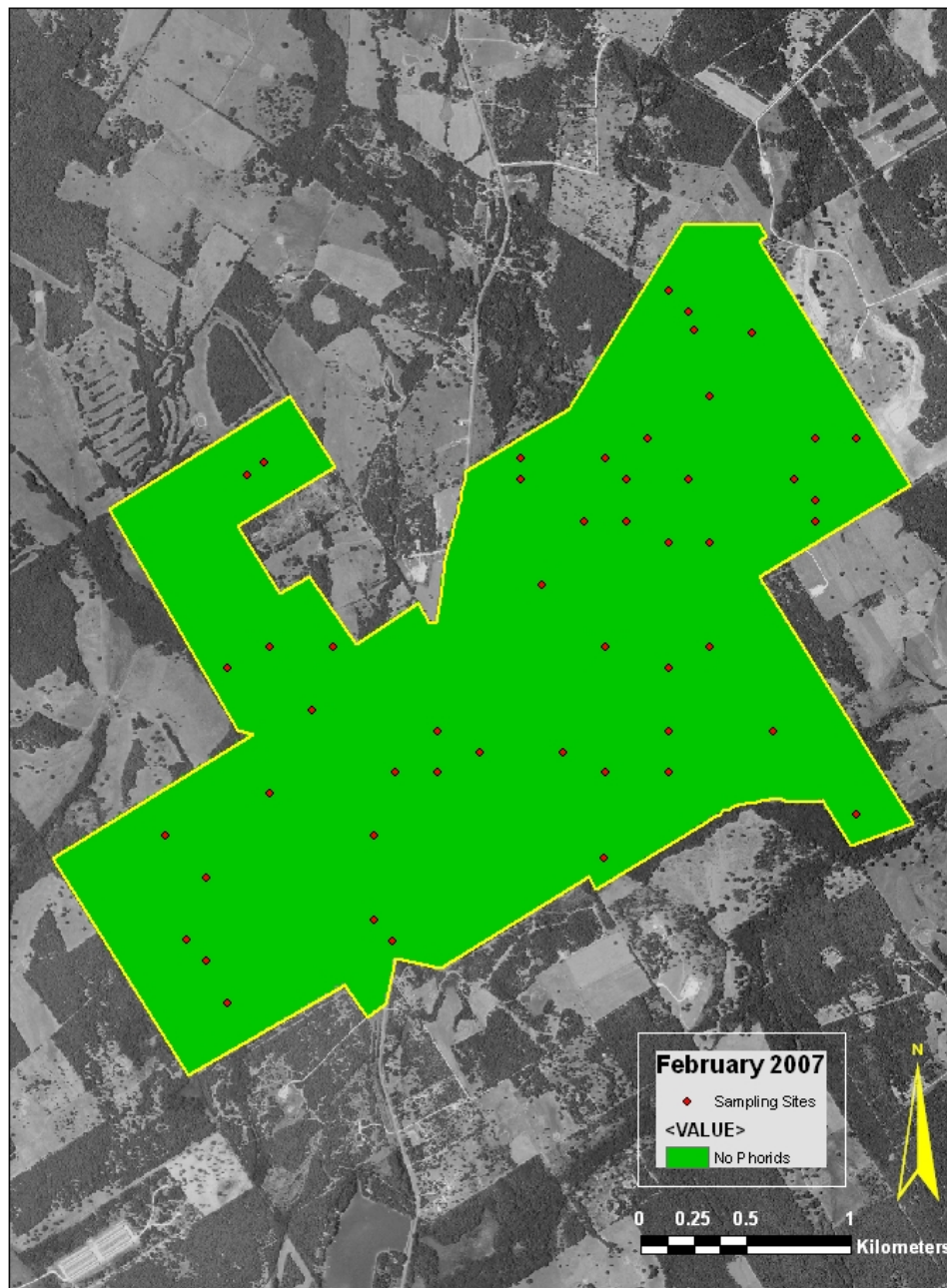
A-10. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during September 2006 (% area dominated by *P. curvatus* = 68.19 and *P. tricuspis* = 31.81).
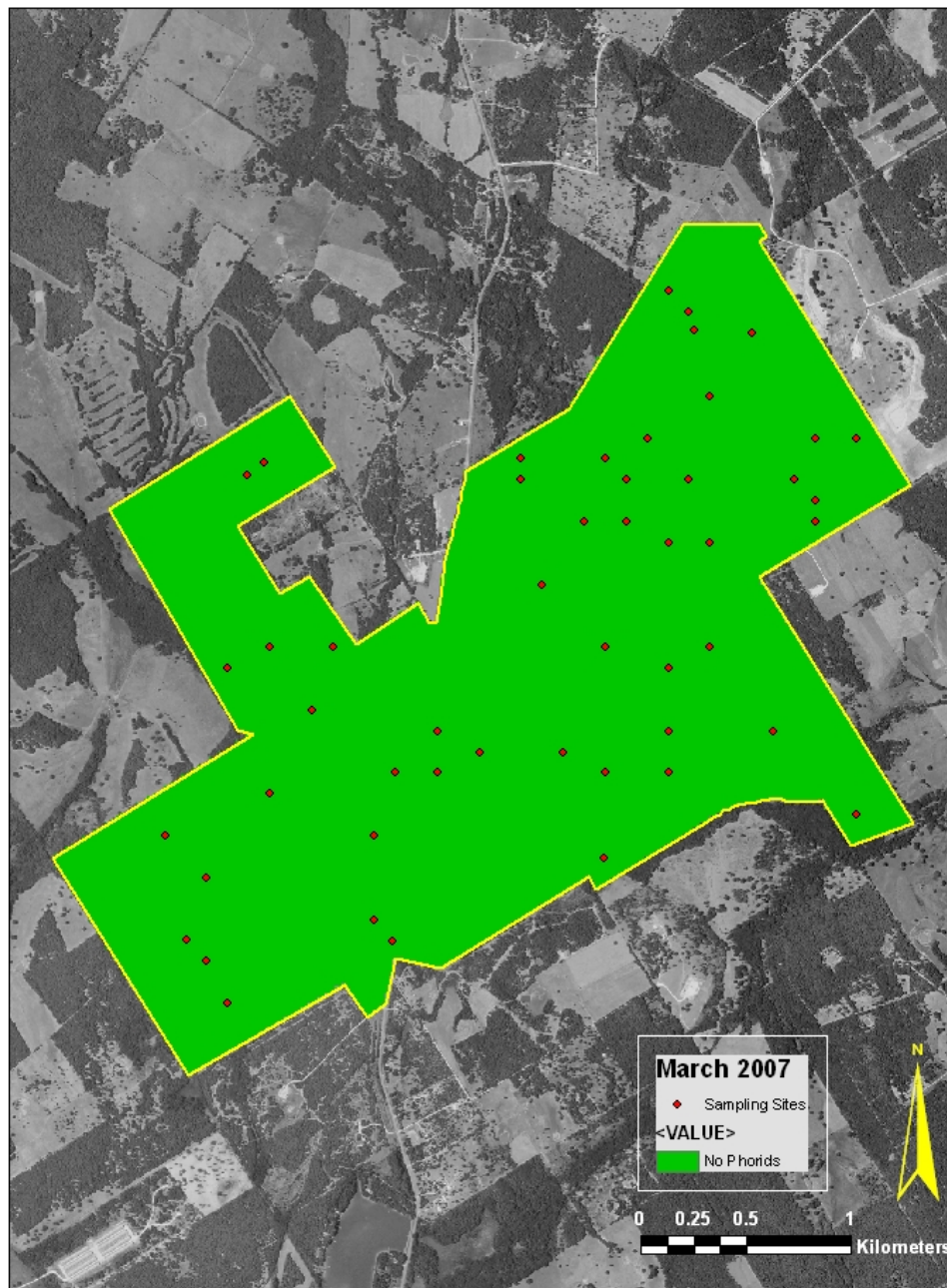
A-11. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during October 2006 (% area dominated by *P. curvatus* = 50.57 and *P. tricuspis* = 49.43).
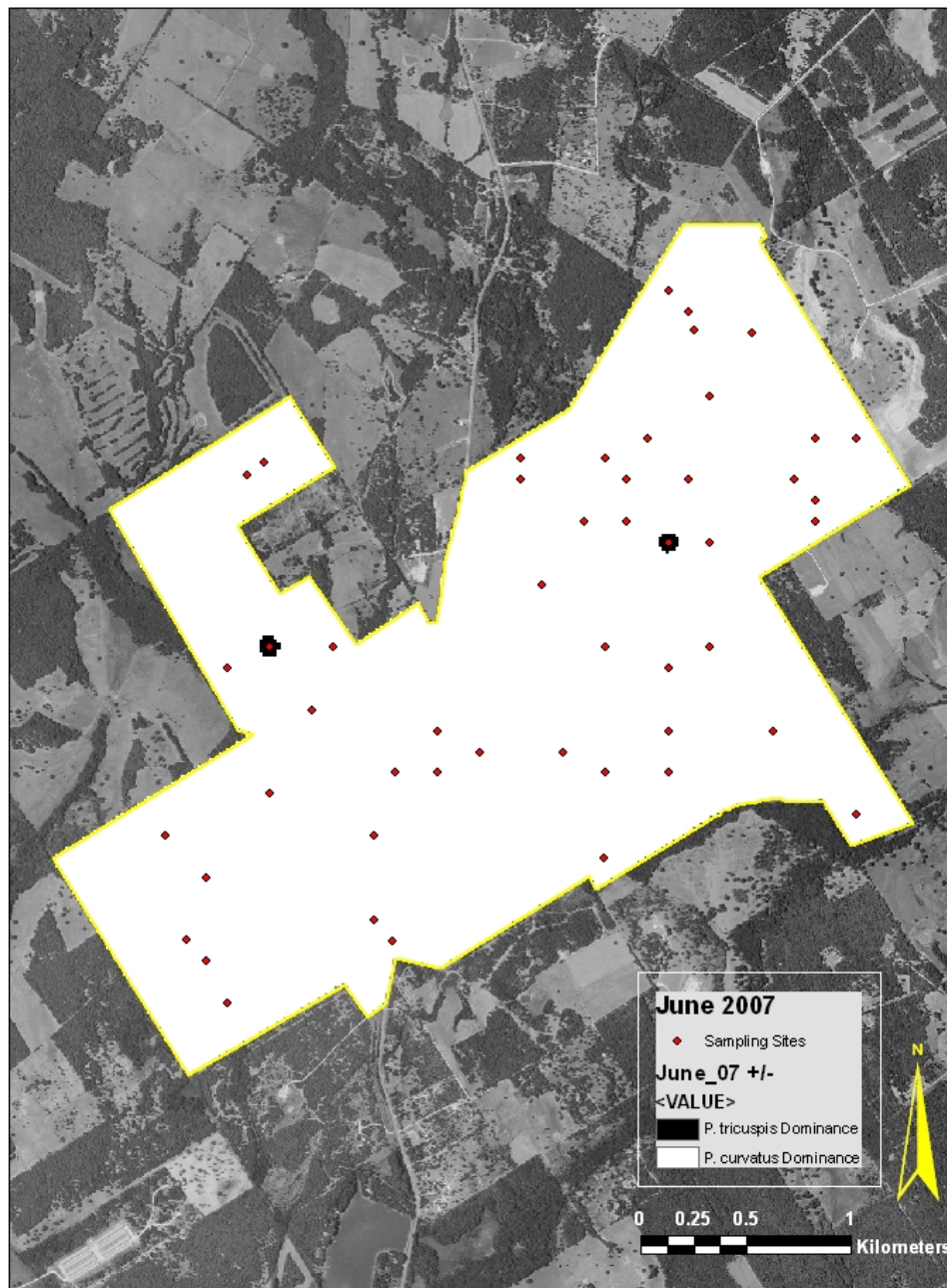
A-12. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during November 2006 (% area dominated by *P. curvatus* = 26.47 and *P. tricuspis* = 73.53).
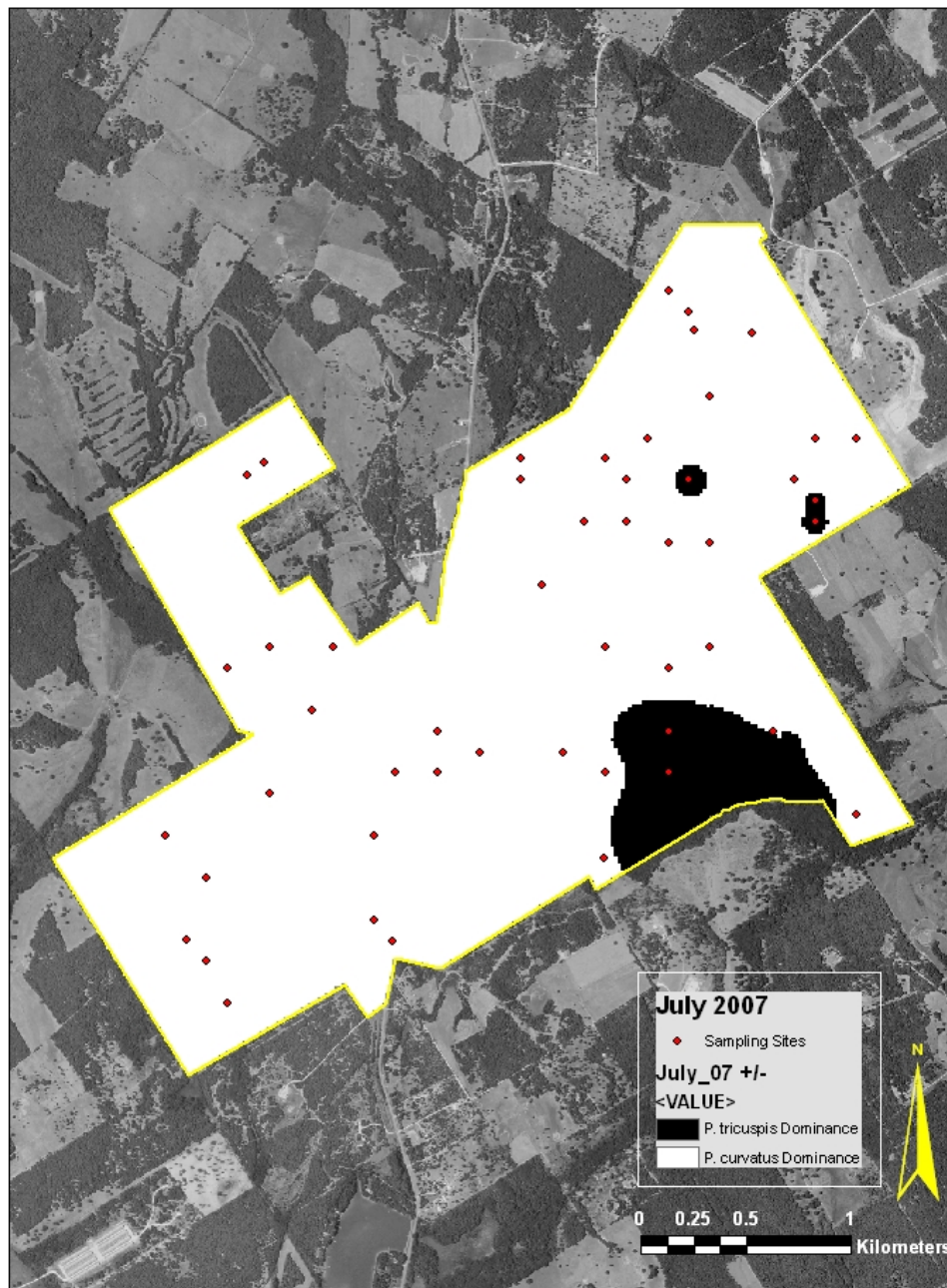
A-13. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during February 2007. No phorids were collected during this sampling period.

A-14. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during March 2007. No phorids were collected during this sampling period.

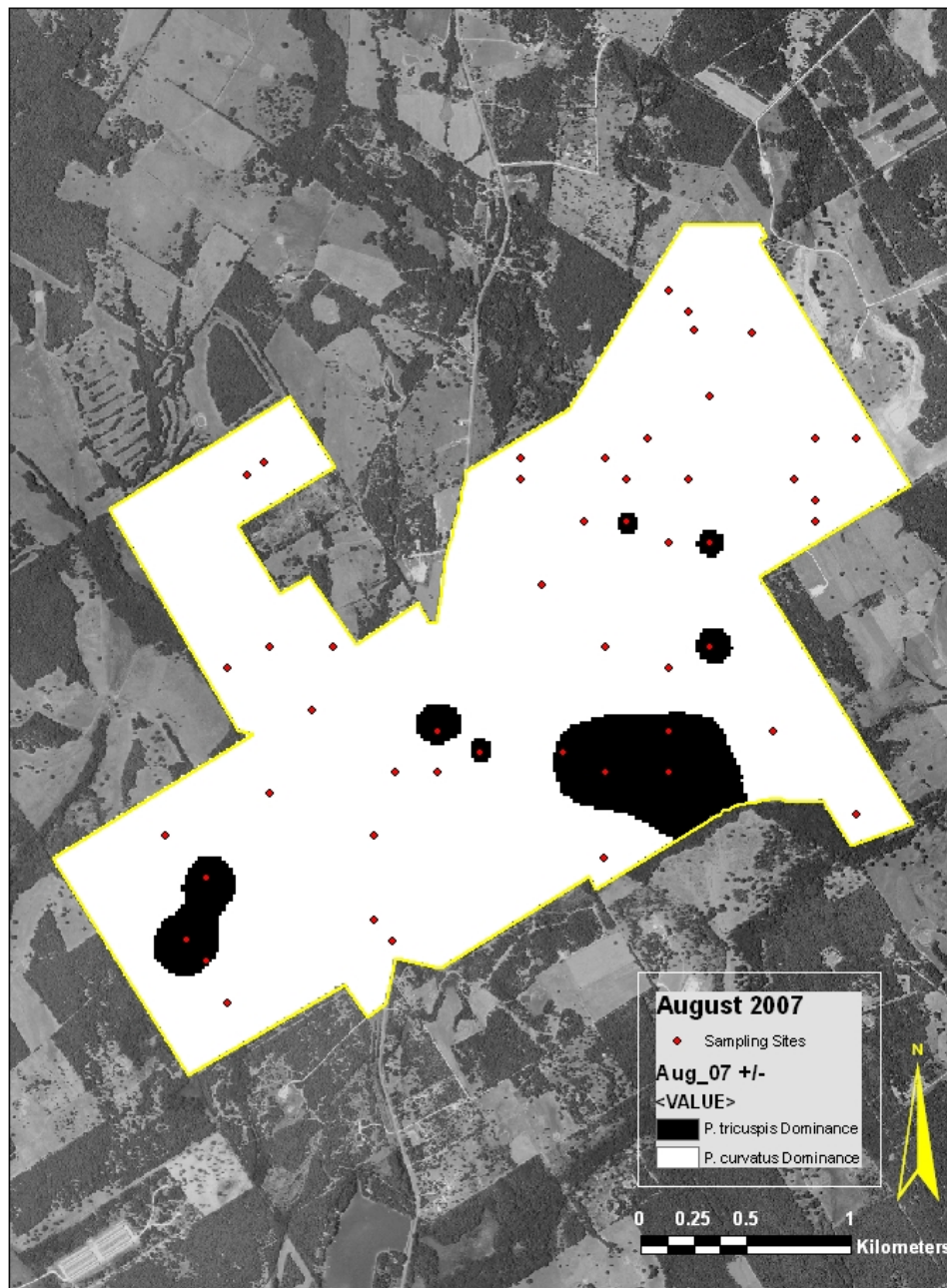A-15. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during June 2007 (% area dominated by *P. curvatus* = 99.82 and *P. tricuspis* = 0.18).

A-16. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during July 2007 (% area dominated by *P. curvatus* = 93.38 and *P. tricuspis* = 6.62).

A-17. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during August 2007 (% area dominated by *P. curvatus* = 92.22 and *P. tricuspis* = 7.78).

A-18. Inverse Distance Weighted (IDW) Interpolation of *P. curvatus* and *P. tricuspis* species-specific numerical/spatial superiority during October 2007 (% area dominated by *P. curvatus* = 92.47 and *P. tricuspis* = 7.53).

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Selected Publications

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