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Population ecology of Pseudacteon tricuspis Borgmeier (Diptera: Phoridae), an introduced parasitoid of the red imported fire ant Solenopsis invicta Buren (Hymenoptera: formicidae) in Louisiana

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POPULATION ECOLOGY OF *PSEUDACTEON TRICUSPIS* BORGMEIER
(DIPTERA: PHORIDAE), AN INTRODUCED PARASITOID OF THE RED
IMPORTED FIRE ANT *SOLENOPSIS INVICTA* BUREN (HYMENOPTERA:
FORMICIDAE) IN LOUISIANA

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In pursuing this Ph.D., I was well aware that I was faced with an enormous challenge. The study described herein required an exploration of many diverse areas of science to obtain explanations and solutions to understand the patterns that I found, and to comprehend the mysterious quantitative ecological literature. These digressions were the source of focused study of mathematics, statistics, ecology, insect behavior, meteorology, and other esoteric topics.

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ABSTRACT

Aspects of the population ecology of a parasitoid (*Pseudacteon tricuspis*) of the red imported fire ant (*Solenopsis invicta*) in Louisiana were studied. The spatio-temporal abundance patterns, dispersal, population spread, aggregation, direct mutual interference and functional response characteristics of this parasitoid were studied to address deficiencies in our knowledge about phorid flies, particularly *Pseudacteon* parasitoids. This endoparasitoid was discovered to manipulate host ant behavior in ways that benefit its own survival. Laboratory experiments to gain insights into behavioral and functional responses revealed that fly aggregations were density-dependent and interference was not significant when 1-3 females were simultaneously confined with hosts, although per capita oviposition success appeared to decline. Searching efficiency of 2-3 simultaneously ovipositing females was not significantly different than solitary females. Solitary females parasitized a constant proportion of hosts according to a Type 1 functional response. Modelling of the local spatial population structure of *P. tricuspis*, and relationship of abundances to host social form and pathogen-infected colonies, revealed no significant spatial associations between fly counts and infected host colonies. When fly populations peaked, significant count clusters were associated with polygyne colonies. Fly counts reflected a random spatial and temporal distribution, as count patterns were not stable. Dispersal experiments were conducted to quantify local fly movement. Diffusion rates tended to decline over time after release and most dispersal density-distributions did not conform to a simple diffusion model, implying heterogeneous population dispersal. Long-term population spread was monitored for two expanding populations of *P. tricuspis*. Range expansion accelerated the first four years

post release, contrasting with a linear pattern expected with simple diffusion. Annual rates of spread were low in the first two years, increased rapidly years 3-4, and leveled off years 5-6, peaking at 15-25 km/yr. Finally, daily and seasonal dynamics of *P. tricuspis* were studied. Findings resulted in a protocol for sampling *P. tricuspis* populations in Louisiana. In addition to providing essential information about *P. tricuspis* population ecology, results of this study will be useful in conservation, augmentation, sampling and management of *P. tricuspis* and other species of *Pseudacteon* that have been released in the United States.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

A population is frequently defined as a group of organisms of the same species occupying a particular place at a particular time, and these individuals have the potential to interbreed and interact (Krebs 1994). Population ecology addresses population densities, dynamics, spatial distributions, movement and how and why population numbers change spatially and temporally (Turchin 2003, Vandermeer and Goldberg 2003). Biological control of invasive organisms relies on theory and principles that are grounded in population ecology.

Populations of the red imported fire ant, *Solenopsis invicta* Buren, are 5-10 times higher in the United States than in their native South America, and are a ubiquitous and significant economic pest in the southeastern United States (Lofgren 1986, Porter et al. 1992). Additionally, two species of North American fire ants, *S. geminata* (F.) and *S. xyloni* (McCook) have been largely displaced by *S. invicta* (Wilson 1951, Wilson and Brown 1958, Porter et al. 1988, Porter and Savignano1990). Earlier efforts to eradicate *S. invicta* with chemical control were ineffective and ultimately abandoned because of concerns that large-scale applications of broad-spectrum insecticides were harmful to non-target organisms and the environment (Taber 2000, Tschinkel 2006). Current efforts have shifted toward biological control of *S. invicta* by importing natural enemies from the indigenous range of *S. invicta* in South America, including parasitoid flies of the dipteran family Phoridae. This endeavor is promising because phorid flies are thought to be an important contributor to low abundances of *S. invicta* in South America (Porter 1998) and may similarly suppress *S. invicta* populations in the United States.

Phorid flies of the genus *Pseudacteon* Coquillet exert a powerful influence on host ant behavior. Much research has been directed at understanding how phorid flies influence competitive interactions between various ant species (see Feener 1981; Feener and Brown 1992; Folgarait and Gilbert 1999; Morrison 2000a, Orr et al. 1995, 2003). In the presence of phorid flies *Solenopsis* workers often curtail or terminate foraging activity (Feener and Brown 1992, Orr et al. 1995). Resource retrieval rates decline by as much as 84% when under attack by *Pseudacteon* (Feener and Brown 1992, Morrison 1999). A single attacking *P. tricuspis* Borgmeier female per 200 foraging *S. invicta* workers can decrease colony protein consumption almost 2X and significantly reduce numbers of large-sized workers 50 days later (Mehdiabadi and Gilbert 2002).

Female *Pseudacteon* are solitary endoparasitoids and hover over their host before penetrating the intersegmental membrane and inserting a single egg into the hosts' thorax (Porter et al. 1995). After the egg hatches, the maggot moves into the head where it feeds on internal head structures, and eventually pupates inside the decapitated host's empty head capsule (Porter et al. 1995, 1997; Porter 1998; Cônsoli et al. 2001). Development from egg to adult occurs in 5-6 weeks, depending on temperature (Porter et al. 1995, Folgarait et al. 2002 a, b).

Despite the research that has accumulated on *Pseudacteon*, our understanding of phorid fly population ecology remains weak (Morrison 2000b), particularly in the United States. In fact, little information is available concerning the spatial and temporal dynamics of the Phoridae in general (Disney 1994). Fundamental information about *P. tricuspis* biology and ecology, and its associations with *S. invicta* are still unknown or are inadequate, particularly under climatic conditions unique to Louisiana.

REVIEW OF THE LITERATURE

Phorid Flies

The Phoridae are commonly referred to as scuttle, humpbacked and manure flies. The common name 'scuttle flies' is probably a reference to the habit of adults to engage in short swift runs. Very little is known about this family, and most of what is known was compiled by Disney (1994) (but see Morrison (2000b) for a review of the biology of *Pseudacteon* parasitoids). Adult phorid flies are thought to comprise nearly 2% of all animal species, while larvae have diverse habits but are mainly saprophagous or parasitic on other insects, primarily ants (Disney 1994). However, the interaction between phorid flies and ants has attracted the most attention because of their important influence on ant behavior.

Behavior and Fate of Parasitized Fire Ant Hosts

Parasitism rates of *S. invicta* by *P. tricuspis* were estimated by Morrison and Porter (2005) from field colonies that were collected and monitored in the laboratory. In contrast to expectations, *P. tricuspis* puparia did not appear until approximately eight days after field collection, although they were expected to have appeared at least within the first few days. Morrison and Porter (2005) hypothesized behavioral changes in older parasitized ants were responsible for their exclusion from collection.

In earlier laboratory studies of *S. invicta* and *Pseudacteon* spp., *S. invicta* workers removed remains of parasitized colony members and deposited them in nearby middens (Porter et al. 1995, 1997) as a function of their necrophoric behavior (Howard and Tschinkel 1976). However, Porter et al. (1995) posed several questions about the behavior and fate of parasitized *S. invicta* under natural conditions, and the effect of the

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environment on the phorid puparium. If the necrophoric behavior observed in the laboratory also occurs under natural conditions, what happens to phorid puparia if they are discarded in middens? The upper lethal thermal limit of *S. invicta* is approximately 40º C (Cokendolpher and Phillips 1990). Consequently, *P. tricuspis* pupae may be killed from lethal temperatures and desiccation if they are placed in middens piles along with other trash. Furthermore, under laboratory conditions *S. invicta* chew open head capsules containing the parasitoid and kill it (Porter et al. 1997). These hostile conditions imply that these parasitoids have an alternative strategy for the successful transition from inhabiting a host ant inside an ant colony into free-living adult flies.

Aggregation and Mutual Interference

It is known that *Pseudacteon* parasitoids are attracted to host ant aggregations along foraging trails, disturbed mounds, alate flights and aggressive intraspecific interactions (Williams et al. 1973, Orr et al. 1995, Pesquero et al. 1993, Morrison and King 2004). These parasitoids detect ant semiochemicals, and exploit these cues to locate their hosts (Porter 1998, Morrison and King 2004). The only information regarding aggregative responses of *Pseudacteon* under field conditions is from Morrison and King (2004), who found that increasing the number of non-nestmate *S. invicta* workers at baits already occupied by *S. invicta* led to enhanced numbers of *P. tricuspis.* This is presumably because increased alarm pheromone production by fighting non-nestmates attracted more flies.

Males in phorid aggregations are aggressive towards conspecifics (Feener and Brown 1992, Porter et al. 1995, Morrison et al. 1999). However, aggressive interactions also occur between *P. tricuspis* females that are attacking *S. invicta*, i.e. females have

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been observed bumping into and chasing other females (Pers. Obs). Searching parasitoids that encounter other searching parasitoids may react by temporarily ceasing to search or will otherwise disperse from the area (e.g. Hassell and Varley 1969, Hassell 1971, Hassell et al. 1976). This type of interaction is known as direct mutual interference, where a decrease in parasitoid searching efficiency occurs with increasing parasitoid density, due to increased intraspecific interactions (Free et al. 1977).

No research has been published that has quantified interference or determined the functional response curve of any *Pseudacteon*. Most laboratory research on *Pseudacteon* has been directed at oviposition behavior (Porter et al. 1995, Morrison et al. 1997, Porter 1998, Folgarait et al. 2002a, Wuellner et al. 2002). *Pseudacteon* females have been observed attacking host ants in the laboratory for up to an hour or more, with several attacks per minute, and can make >100 oviposition attempts (Morrison et al. 1997). Actual rates of oviposition success of *Pseudacteon* are between 11 and 35% (Porter et al. 1995, 1997; Morrison et al. 1997).

<div align="center">Spatial and Temporal Abundance Patterns</div>

Study of the spatial structure of *P. tricuspis* populations may facilitate identification of microclimates and other landscape features that could potentially influence the distribution of these species in a spatial context. Populations of *Pseudacteon* parasitoids of *S. geminata* in central Texas were characterized as having significant variations in abundance, both spatially and temporally (Morrison et al. 1999). Wuellner and Saunders (2003) discovered that *S. geminata* and its phorid parasitoids co-exist under similar conditions of temperature and humidity, but not light intensities. Morrison and King (2004) determined that *P. tricuspis* abundances were not uniform at

disturbed fire ant mounds, and abundances were high at some colony locations and rare or absent at nearby colony locations. However, these abundance patterns of *P. tricuspis* were not modeled spatially or temporally. Also, no studies relating *P. tricuspis* spatial distribution to that of their host have been attempted. Current spatial software (S-Plus, SADIE) allows modeling of spatial features and attributes, including those derived from data describing soil features, temperatures, population densities, etc.

Dispersal and Spread

Quantifying dispersal of insects is an integral part of understanding insect population dynamics (Osborne et al. 2002). Data from dispersal studies are vital in understanding animal movement behavior, and are needed to build predictive models of species spread (Turchin 1998). No detailed studies of phorid dispersal have been attempted (Disney 1994), and no methodology for quantifying and modeling dispersal of *Pseudacteon* have been developed. Only a few studies have given some insight into *Pseudacteon* dispersal and spread. Morrison et al. (1999) studied dispersal of *Pseudacteon* parasitoids of *S. geminata* in central Texas and determined that *Pseudacteon* parasitoids dispersed up to 650 meters from the nearest *S. geminata* colonies. In terms of population spread, Porter et al. (2004) documented *P. tricuspis* population rates of spread in north-central Florida of 10-30km/year, and spread rates increased over time. With an additional two years of data, Pereria and Porter (2006) reported revised expansion rates approaching 57 km/year, with expansion rates faster to the north of release areas.

Daily and Seasonal Dynamics

Diurnal activity patterns of *P. tricuspis* and *P. litoralis* were studied in Brazil by Pesquero et al. (1996). In Brazil, activity of *P. tricuspis* peaked during mid-day, and

abundances were significantly related to air temperature, soil temperature and humidity. At warmer temperatures *Pseudacteon* parasitoids of *S. geminata* in Texas appear earlier in the morning and remain active later in the day (Wuellner and Saunders 2003). Adult *Pseudacteon* are not active when air temperatures fall below 20° C (Morrison et al. 1999), but are active at temperatures exceeding 35° C (Henne et al. 2007).

Abundances of *Pseudacteon* parasitoids of *S. geminata* were studied in relation to biotic and abiotic variables by Morrison et al. (2000). No single abiotic variable accounted for more than 23% of the variation in *Pseudacteon* activity, and abundances were only weakly correlated with host ant activity. In an arid region of Argentina, daily flight periods of *P. tricuspis* were associated with hotter, drier conditions (Folgarait et al. 2007).

Fowler et al. (1995) evaluated seasonal activity of *Pseudacteon* in Brazil and found *P. tricuspis* to be the seasonally most abundant species. Folgarait et al. (2003) studied the seasonal activity patterns of adult *Pseudacteon* that attack *S. richteri* Forel in Argentina, with *P. tricuspis* among the species studied. It was determined that *P. tricuspis* was most abundant during months having greater rainfall and fewer days with frosts, mainly those in the fall. In north-central Florida, *P. tricuspis* is present all months of the year, but abundances are highest during November (Morrison and Porter 2005). Morrison et al. (1999, 2000) studied the phenology of *Pseudacteon* parasitoids of *S. geminata* in central Texas and found that phorid abundances varied seasonally, with rainfall patterns possibly linked to these abundances. Morrison et al. (2000) also determined that soil moisture levels were often a good predictor of phorid abundance. As an indication that adults have limited life spans under natural conditions, considerable

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weekly variations in population abundances of *Pseudacteon* have been observed (Morrison et al. 2000). Abundances of *Pseudacteon* at three sites separated by 8-16 km in north-central Florida were positively correlated over time (Morrison and Porter 2005).

Sex ratios of *Pseudacteon* parasitoids that appear at disturbed colonies and along foraging trails are often male-biased (Pesquero et al. 1993, Morrison et al. 2000, Wuellner and Saunders 2003). For example, Calcaterra et al. (2005) found that *P. tricuspis* male-female sex ratios at fire ant mounds at multiple locations in three regions of southern South America were approximately 2:1, and Morrison and Porter (2005) found male to female sex ratios of 2.65:1 in north-central Florida.

STUDIES IN LOUISIANA

Here, studies of *P. tricuspis* population ecology were conducted as a vital step towards addressing gaps in our knowledge about this parasitoid, and to supplement existing knowledge and test theory of host-parasitoid biology and ecology. The release and establishment of *P. tricuspis* in Louisiana (see Henne et al. 2007) provided the opportunity to study the population ecology of this species. In Chapter 2, laboratory studies were conducted to gain insights into the behavior of parasitized *S. invicta* workers in the hours leading up to their decapitation, and to determine possible parasitoid pupariation sites. In Chapter 3 laboratory experiments were conducted to quantify aggregative responses of *P. tricuspis* adults to variable host densities, determine effect of direct mutual interference between pairs of ovipositing *P. tricuspis* females confined with host *S. invicta*, elucidate the effect of confining one or two additional males with already mated females on progeny sex ratios, and, finally, determine the form of the functional response of individual ovipositing *P. tricuspis* to varying host densities. In Chapter 4, field

studies were conducted to characterize the spatial and temporal abundances of *P. tricuspis* populations at three study sites over five weeks, and attempt to relate the abundances of *P. tricuspis* to host social form and presence/absence of the microsporidian parasite *Thelohania solenopsae* Knell, Allen and Hazard. In Chapter 5, dispersal of *P. tricuspis* was studied by performing mass-release-recapture experiments. This was done to obtain information about *P. tricuspis* redistribution away from the release point at 30 minute intervals, up to two hours after release. Another objective in Chapter 5 was to determine the redistribution patterns of *P. tricuspis* dispersers, and to fit the data to a simple diffusion model. The aim of Chapter 6 was to describe and model the spread of two established *P. tricuspis* populations in Louisiana, and determine if spread rates were consistent with simple linear models of species spread. In Chapter 7, the daily and seasonal dynamics of *P. tricuspis* were studied at two locations in south Louisiana. The objectives were to determine the following: daily activity pattern of *P. tricuspis*, and relate these patterns to various abiotic variables, the dynamic behavior of *P. tricuspis* populations over an extended time, if populations are synchronized over small and large spatial scales, and if they are correlated with various abiotic variables, the sex ratios and frequency distributions of *P. tricuspis* that appear at disturbed *S. invicta* mounds, and determine the minimum sample size and sampling methodology that will provide an estimate of the true relative population mean of *P. tricuspis* at any location.

SIGNIFICANCE OF STUDY

In addition to providing critical information about *P. tricuspis* population biology and ecology, results of this study will be useful in conservation, augmentation, sampling and management of *P. tricuspis*, and important contributions will be made towards

understanding host-parasitoid interactions. In South America, 20 species of *Pseudacteon*

attack *S. invicta* (Porter and Pesquero 2001). At least three species of *Pseudacteon* have

been imported and released in the United States: *P. tricuspis* Borgmeier (Graham et al.

2001, Porter et al. 2004), *P. curvatus* Borgmeier (Graham et al. 2003), and *P. litoralis*

Borgmeier (Porter and Alonso 1999). Other *Pseudacteon* species are being evaluated for

release in the United States in the next few years: *P. borgmeieri* Schmitz (Folgarait et al.

2002a), *P. cultellatus* Borgmeier (Folgarait et al. 2002b), *P. obtusus* Borgmeier (Folgarait

et al. 2005), and *P. nocens* Borgmeier (Folgarait et al. 2006). The first species of

Pseudacteon introduced into the United States for biological control of *S. invicta* was *P.

tricuspis*, released in Texas in 1995 (Gilbert 1996) and Florida in 1997 (Porter et al.

1999). Valuable knowledge about phorid flies is obtained by studying the population

ecology of *P. tricuspis*. Additionally, information obtained here may be extended to

evaluating other species of parasitic phorids as well.

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CHAPTER 2

ZOMBIE FIRE ANT WORKERS: BEHAVIOR CONTROLLED BY DECAPITATING FLY PARASITES[1]

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INTRODUCTION

Few ecological associations are as intimate as the host-parasite (or parasitoid) interaction (Poulin 1995). The ability of parasites to influence host behavior is an important feature of host-parasite biology (Price 1980), because reproductive success of the parasitoid is dependent on the behavior of its host. Parasitoid survival relies on aspects of host growth, development and survival. If the host dies before the parasitoid reaches a critical point of development, then the parasitoid also dies (Fritz 1982). Consequently, changes in host behavior that minimize premature host mortality during parasitoid development ultimately benefit the parasitoid.

There are many examples reported in the literature of parasitoids that induce behavioral changes in their hosts towards the end of their development. For example, *Chelonus inanitus* (L.) (Hymenoptera: Braconidae) causes its host caterpillar, *Spodoptera litoralis* (Boisduval) (Lepidoptera: Noctuidae) to dig into the soil at its fourth instar rather than the sixth instar (Rechav and Orion 1975). Another *Chelonus* sp. causes its host, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) to prematurely initiate metamorphosis by spinning a cocoon but not actually pupating; this way the protective structure of the cocoon is provided to the developing parasitoid (Jones 1985). Ants that are parasitized by nematodes will drown themselves in water so that the nematodes can emerge (Kaiser 1986, Maeyama et al. 1994).

In recent years, several species of parasitoids in the genus *Pseudacteon* Coquillet (Diptera: Phoridae), collectively referred to as 'decapitating flies,' have been introduced in the United States as biological control agents of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). These parasitoids oviposit in host ants that

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are engaged in various activities outside of the nest and eventually pupariate inside the decapitated host's empty head capsule (Porter et al. 1995, 1997).

Morrison and Porter (2005) estimated *P. tricuspis* Borgmeier parasitism rates in *S. invicta* field colonies that were collected and monitored in the laboratory. No *P. tricuspis* puparia were found until approximately eight days after field collection, although they were expected to have appeared within the first few days. Behavioral changes in parasitized ants were hypothesized as being responsible for this effect.

In previous laboratory studies of *S. invicta* and *Pseudacteon* spp., *S. invicta* workers removed the remains of parasitized colony members and deposited them in nearby middens (Porter et al. 1995, 1997) during the course of normal *S. invicta* necrophoric behavior (Howard and Tschinkel 1976). Porter et al. (1995) posed several questions about the behavior and fate of parasitized *S. invicta* under natural conditions, and the resulting effect of the environment on the phorid puparium. For example, if this necrophoric behavior also occurs under natural conditions, what would happen to phorid puparia that are exposed to high soil surface temperatures? The upper critical thermal limit of *S. invicta* is reported to be approximately 40º C (Cokendolpher and Phillips 1990). Our field measurements of exposed soil surface temperatures in the summer showed that thermal limits that are lethal to *S. invicta* are commonly exceeded. In many cases, soil surface temperatures exceeding 55º C were recorded (Henne and Johnson, unpubl. data). Consequently, *P. tricuspis* puparia that are inside *S. invicta* head capsules could be vulnerable to lethal temperatures and desiccation if they are discarded in a middens pile. Moreover, under laboratory conditions *S. invicta* will chew open head capsules containing the parasitoid and kill it (Porter et al. 1997, pers. obs.). These hostile

conditions would imply that an alternative strategy exists for these parasitoids to successfully develop into adult flies.

Our observations of *P. tricuspis* parasitized *S. invicta* colonies in a large laboratory arena revealed that these parasitized ants were exhibiting behaviors that appeared consistent with host manipulation to benefit survival of the parasitoid. The objective of this study was to describe the behavior of parasitized *S. invicta* workers in the hours leading up to their decapitation and to determine possible parasitoid pupariation sites in the laboratory.

MATERIALS AND METHODS

Four monogyne *S. invicta* colonies were collected at the Louisiana State University Agricultural Experiment Station in St. Gabriel, Louisiana (30° 16′ N, 91° 05′ W) (two in February 2006, two in July 2006). As of 2006, expanding populations of *P. tricuspis* in Louisiana had not yet reached this location. Colonies were separated from soil by the drip flotation method (Banks et al. 1981). Ants from each colony were then sieved to yield 5-6 grams (approximately 600-1,000 ants · gram^{-1}) of individuals that were within the preferred size class for *P. tricuspis* females (approximately 1 mm head width (see Morrison et al. 1997)). Ants plus a small amount (approximately 1 gram) of brood were placed inside a open plastic container (Glad® 1.89 L) lined with Fluon® to prevent ants from escaping. Ants were subjected to continuous oviposition attack by 50-100 *P. tricuspis* females for four days at a temperature of 28° C and 80% relative humidity.

To establish that parasitoid-induced behavior consistently occurred among several unrelated colonies, two initial set-ups were done consecutively during the spring of 2006.

These set-ups involved placing a mound of moist potting soil (approximately 50 cm^3) in the middle of the arena. After exposure to *P. tricuspis*, ants entered this mound and constructed a nest. Two subsequent set-ups were also done consecutively in the summer of 2006 and involved the placement of inverted plastic containers (Ziploc® 236 ml snap lid containers) in the middle of the arena so that internal observations of the colony could be made (Figure 2.1).
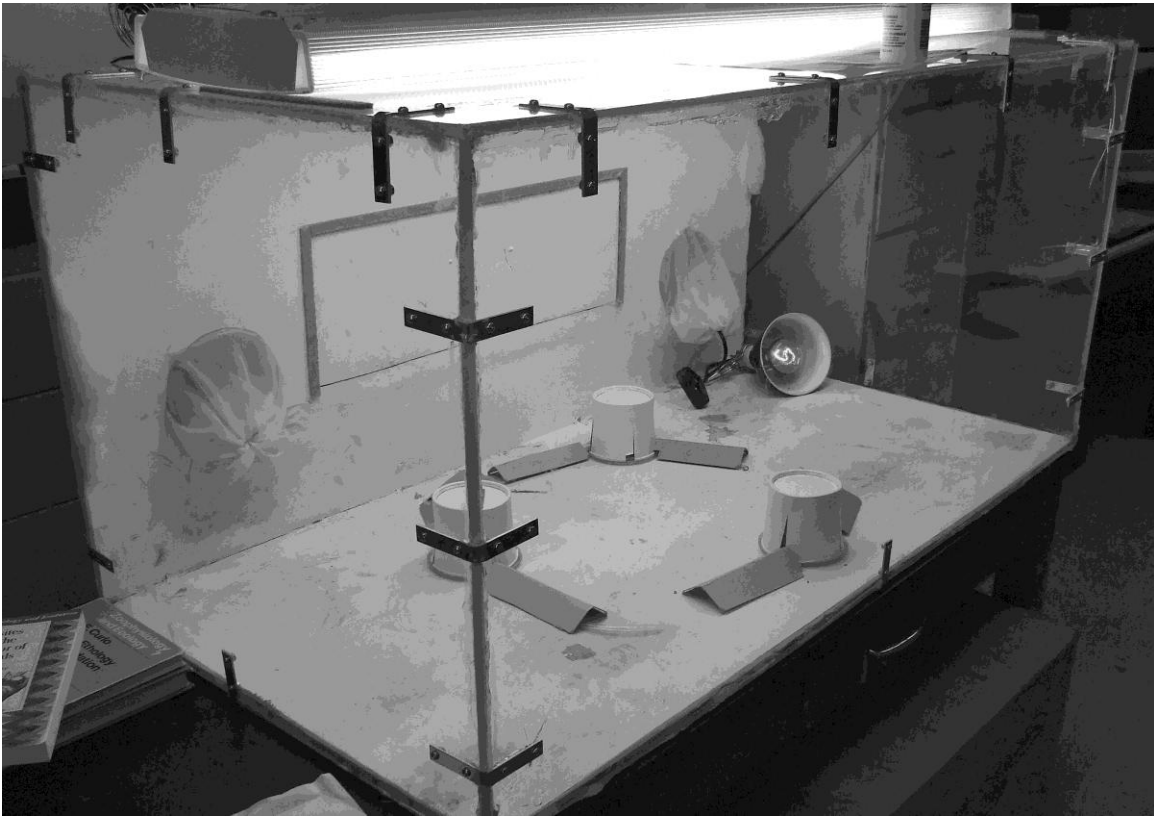
Figure 2.1: Experimental arena, containing three plastic observation nest units and lateral PVC foraging tubes.

A moistened plaster block was placed inside each container. Two 15 cm PVC tubes were inserted into opposite ends of each container to imitate foraging tunnels associated with *S. invicta* colonies under natural conditions (Markin et al. 1975). The entire container was covered with a removable cardboard sleeve. The PVC tubes were

also covered with cardboard to block light. After exposure to *P. tricuspis*, ants were placed on the floor inside the arena, and they quickly moved into all three containers. Temperature inside the arena was maintained at approximately 25 ± 2^0 C and 60% relative humidity. Water and sugar water were provided for ants *ad libitum*.

Observations were made through a large enclosed Plexiglas® arena (60 cm x 120 cm x 60 cm) that was illuminated by an overhead fluorescent lamp and heated by a 75 W infrared lamp (Figure 2.1). While foraging ants were observed daily inside the arena, parasitized ants did not appear outside of the nest until approximately 15 days after *P. tricuspis* oviposition. Observations continued daily between 0700 h and 1600 h for two subsequent weeks. More than two-thousand ants were randomly collected inside the arena from all four trials combined and examined under a stereo microscope to determine their status as parasitized or unparasitized. The late third-instar maggot was always observed moving around inside the ventral portion of the parasitized ants' head capsule, and the maggot's cephalopharyngeal skeleton could be seen moving as well.

To determine possible *P. tricuspis* pupariation sites, ants (n=100 - 120) that were confirmed to be parasitized were placed in a Fluon®-lined 31.4 cm x 25.6 cm x 9.7 cm plastic container (Pioneer Plastics, model 395C, Dixon, KY) with a 5 cm thick layer of sod containing grass and thatch. Moist sand and moist potting soil were also placed between two vertical 5mm thick sheets of clear plastic, with 1 cm between sheets, and the top and sides plugged with cotton. Parasitized ants were placed on the substrate surface to determine if parasitized ants burrowed into these substrates.

To determine the insulating properties of the soil thatch layer, temperature measurements were made on 10 cm x 10 cm x 5 cm pieces of sod and bare soil obtained

from a pasture and placed under an infrared lamp. A thermoprobe was placed on the surface of bare soil, and the distance between the soil surface and infrared lamp was adjusted until the temperature stabilized at $40 \pm 0.2°$ C (approximately 5 cm). Then the infrared lamp was placed over sod with the grass and thatch layer intact, with 5 cm between the infrared lamp and the top of the thatch layer. The thatch layer was approximately 2 cm thick, and the thermoprobe was placed at the soil-thatch layer interface. Measurements were repeated 10 times.

RESULTS

Parasitized ants routinely left their nest approximately 8-10 hours prior to decapitation (n>500 observations from four replicate colonies). Initially, their behavior was indistinguishable from unparasitized ants. Unlike unparasitized foragers that were also collected in the arena, parasitized ants never returned to the nest after leaving. After exiting, parasitized ants were observed walking around the arena floor for 2-4 hours before collapsing. They would then sit motionless for several more hours, sometimes twitching their legs. Parasitized and unparasitized ants were frequently observed inside the PVC tubes and would mass near the exit holes before exiting (Figure 2.2).

Parasitized ants examined under a stereo microscope were found capable of some degree of defense, since they attempted to sting the forceps being used to hold them. Additionally, droplets of venom were frequently observed exuding from the stinger, and the ants repeatedly rubbed this venom on their legs and the forceps. However, parasitized ants were unable to bite, since damage to the mandibular muscles by the parasitoid was evident. In all cases (n>500), positive identification of the maggot inside the head capsule was made.

Figure 2.2: Ants inside PVC foraging tubes. These ants left the PVC tubes shortly after this photo was taken. They were later confirmed to contain *P. tricuspis* maggots inside their heads

More than 100 parasitized ants that were placed in a container with sod were later found in the sod thatch layer, generally within approximately 5 mm of the surface. Results of the plastic vertical sheets observations showed that at least some parasitized ants burrowed into moist sand to a depth of 21 ± 4.2 mm (Mean ± SE, n=6) and in moist potting soil to a depth of 5 ± 0.52 mm (Mean ± SE, n=9), but if no structure was available to hide in most (n=100-120) would collapse on the surface, or make feeble attempts to burrow but unable to because their mandibles were no longer functional. Temperatures at the bottom of the thatch layer were approximately 15° C lower ($25 \pm 0.51^{\circ}$ C, mean ± SE, n=10) than bare soil temperatures 5 cm under an infrared lamp.

DISCUSSION

This study revealed that ants parasitized by *P. tricuspis* probably do not die inside the nest, but instead leave the nest shortly before their decapitation. Other studies reporting that phorid pupae are deposited in the middens pile involved colonies in small containerized environments, which restricted parasitized ants from wandering away to die.

Parasitized ants seem to be under the control of the parasitoid larva in a way that benefits the survival of the parasitoid and ultimately the adult fly. Once parasitized, ants never leave the nest until the parasitoid has virtually completed larval development. A possible explanation is that the maggot is exploiting the host as a vehicle to locate a suitable microclimate for pupariation. The host's brain is evidently still intact when the ants leave the colony. The brain is reported to be the last structure in the head to be consumed by the parasitoid (Porter et al. 1995, 1997; Cônsoli et al. 2001). Presumably the maggot is exploiting the host sensory system to seek out a suitable location for pupariation. Whether other species of *Pseudacteon* affect their hosts in a similar manner is presently unknown.

In our laboratory colonies, parasitized workers remained inside their nests and were among the other ants and brood in a cluster surrounding the moistened plaster blocks. Dead parasitized ants were never observed inside the inverted plastic containers. Unparasitized foragers (25-50 per day) were observed walking around the arena during the first two weeks after exposure to *P. tricuspis*, but the majority of ants remained inside the nest (see Mirenda and Vinson 1981). Parasitized hosts in social species suffer greater mortality if they behave differently (Curio 1976, Morse 1980). Thus, parasitoids should

not cause their social insect hosts to elicit unusual behaviors (Fritz 1982). It has been reported that in laboratory colonies, parasitized workers tend brood, are less aggressive and seldom forage, the last a feature that would contribute to the fitness of the parasitoid (Cônsoli et al. 2001) since the host would escape environmental hazards outside the nest. Parasitized ants in our study were never observed outside of their nests until they left just prior to decapitation. Cônsoli et al. (2001) are correct that, during advanced parasitoid development, these ants are less aggressive. However, our study found that they react to being handled by vigorously attempting to escape and expelling venom.

Precisely where parasitized workers are to be found under natural conditions for the eight days prior to decapitation is still unknown. The setup in the study reported here was not an exact replication of natural conditions and may have constrained some behaviors. Tracking individual parasitized ants with visually detectable markers, such as paint, are not practical, as these marks are scraped off (Mirenda and Vinson 1979. As Morrison and Porter (2005) hypothesized, it is likely that parasitized ants move into lateral foraging tunnels and, thus, escape collection. Furthermore, it is suggested that behavioral changes in host ants likely begin shortly after injection of the egg into the host's thorax. Cônsoli et al. (2001) discuss the role of possible chemicals injected with the egg and/or changes in host hormones or physiology as a consequence of parasitoid development.

In our study, parasitized ants were often observed in the lateral PVC 'foraging' tunnels provided. This behavior, if it also occurs in the field, would seem to ultimately benefit the parasitoid, since it not only reduces the risk of mortality to its host but it also positions the ants near exit holes when it is time to leave the nest. The fact that

parasitoids must leave the host at some time to complete their life cycle implies that survival of the parasitoid depends on its location in the environment when it leaves the host (Poulin 1995). By moving into the thatch layer, a suitable incubation microclimate is achieved for *P. tricuspis* pupariation. Multiple measurements of the temperature at the soil surface thatch layer interface confirmed that the thatch layer is a good insulator against high temperatures that would otherwise be lethal to *P. tricuspis* puparia.

We do not know how far parasitized ants travel once they leave the nest, but it could be up to several meters. *Pseudacteon tricuspis* adults frequently appear at *S. invicta* mounds almost immediately after disturbance (pers. obs.), suggesting that they were already in the vicinity of the disturbed mound. Cônsoli et al. (2001) reported that the cuticle of parasitized ants darken slightly during the time when the parasitoid is approaching pupariation. This could be interpreted as a precursor to a form of crypsis that enables the parasitoid to avoid detection when the parasitized ant leaves the colony. Fritz (1982) discusses the implications of host behavioral manipulation by parasitoids and suggested that the degree of parasitoid benefit from this is proportional to the intensity of host predation. By remaining in the nest until it is time for parasitoid pupation, the host of *P. tricuspis* escapes superparasitization and predation.

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CHAPTER 3

LABORATORY EVALUATION OF AGGREGATION, DIRECT MUTUAL INTERFERENCE AND FUNCTIONAL RESPONSE CHARACTERISTICS OF THE DECAPITATING FLY, *PSEUDACTEON TRICUSPIS* BORGMEIER (DIPTERA: PHORIDAE)

INTRODUCTION

The study of host-parasitoid interactions has produced a wealth of theory. Ever since the development of simple theoretical models by Thompson (1924) and Nicholson (1933), a proliferation of research has revealed that many factors interact to determine how many hosts a parasitoid can successfully parasitize. These factors include host density, parasitoid density and the spatial distribution and density of hosts (Hassell and May 1973, Beddington 1975, Cook and Hubbard 1977). The study of insect pests and their biological control have benefited from these theoretical insights, as there is intense interest in establishing the mechanisms by which parasitoids control host densities (Stiling 1987). However, more empirical research is needed to supplement theory (May 1978).

One prediction of optimal foraging theory is that parasitoids should aggregate in higher density host patches in a density-dependent way in order to achieve maximal oviposition rates (Charnov 1976, Cook and Hubbard 1977). This has long been suggested as an important stabilizing factor allowing the persistence of discrete time host-parasitoid interactions, because parasitism risk is spatially heterogeneous (Hassell and May 1973, Chesson and Murdoch 1986, Godfray and Pacala 1992). Small and/or sparsely distributed host populations can therefore escape parasitism spatially and/or temporally in refugia because they are at low risk to parasitism. Conversely, in a continuous time framework density-dependent host mortality theoretically destabilizes the interaction (Murdoch and Stewart-Oaten 1989). However, certain other factors are important when parasitoids aggregate that can stabilize host-parasitoid interactions.

Hassell and Varley (1969) and Hassell and May (1973) recognized the importance of behavioral interactions between multiple searching conspecifics that encounter one

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another, also known as direct mutual interference. Multiple simultaneously ovipositing females may engage in aggressive interactions with conspecifics resulting in delayed searching, and resulting in more time wasted (Visser and Driessen 1991, Visser et al. 1999, Hassell 2000), thereby leading to declining rates of host parasitism as parasitoid density increases (Free et al. 1977). These interactions present unique problems for individual parasitoids when faced with optimal foraging decisions (Maynard Smith 1974), such as maximizing host parasitism rates. The resulting contribution of these interactions, if sufficiently strong, can lead to the long-term stability of host-parasitoid interactions (Hassell 2000).

The study of insect predation rates at variable host densities led to the derivation of the well-known type I, II and III functional response curves (Holling 1966). Solomon (1949) defined the functional response as the density-dependent rate of attack of a single natural enemy to changes in the number of hosts available. Therefore, the functional response describes the relationship between per capita predation (parasitization) rate of a predator (parasitoid) and prey density (Holling 1959, 1961, 1966), and is a fundamental basis of all trophic (consumer-victim) interactions (Mills and Lacan 2004). The three kinds of functional responses were derived according to the relative shape of the curve. The type I functional response characterizes arthropod predators (and parasitoids) that search for hosts randomly in a patch and attack at an increasingly linear rate to a maximum level, and attack rates become independent of increasing prey density (a combination of density-dependent and density-independent responses (Chong and Oetting 2006, Parajulee et al. (2006)). The type II functional response, or 'disk' equation, describes the nonlinear predation rate as a function of prey density. As host density

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increases, the number of hosts that can be attacked in a fixed period of time hyperbolically reaches an asymptote, as the predator is spending all its time handling prey (Holling 1961, Parajulee et al. 2006). However, as host density increases the proportion of hosts parasitized by a type II parasitoid decreases exponentially (inverse density dependence) (Chong and Oetting 2006, Parajulee et al. 2006). The type III functional response applies when the number of prey killed reaches an asymptote as a sigmoid function, where prey killed increases in proportion up to an inflection point and then decreases in proportion (Parajulee et al. 2006). Therefore, functional responses are critical to descriptions of predation and parasitism (Hassell 2000), and can also be useful for parasitoid conservation (Parajulee et al. 2006).

Beginning in the late 1990's, several species of parasitoids in the genus *Pseudacteon* Coquillet (Diptera: Phoridae), collectively referred to as 'decapitating flies,' have been introduced in the United States as biological control agents of the exotic red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Parasitic phorid flies strongly mediate interspecific competitive interactions among ant species (Feener 1981; Feener and Brown 1992; Folgarait and Gilbert 1999; Morrison 1999, 2000; Orr et al. 1995, 2003). *Solenopsis* spp. workers will reduce or terminate foraging activity in response to attacks by *Pseudacteon* flies (Feener and Brown 1992, Orr et al. 1995, Morrison 1999). Females of these solitary endoparasitoids insert a single egg into host ants that are engaged in various activities outside of the nest. The maggot feeds on internal head structures and eventually pupariates inside the decapitated host's empty head capsule (Porter et al. 1995, 1997). *Pseudacteon* phorid flies are considered an

important factor in maintaining lower abundances of *S. invicta* in South America (Porter et al. 1992), and may be useful in suppressing *S. invicta* populations in the United States.

Under field conditions, aggregations of > 5 (but occasionally >50) *P. tricuspis* Borgmeier can be observed at individual disturbed *S. invicta* mounds (D.C. Henne and S.J. Johnson, unpublished data). Both sexes are attracted to host aggregations and mating occurs while females are actively searching for hosts (Porter et al. 1997, Porter 1998). Additionally, aggressive interactions between conspecific males and females can be commonly observed under both laboratory and field conditions (Morrison and Porter 2005a, Pers. Obs.). Males are promiscuous and will mate with the same female multiple times (Porter et al. 1997, Pers. Obs.). However, nothing is known about *P. tricuspis* aggregation, direct mutual interference and the functional response of individual females, necessitating exploratory research into these areas. The objectives of this study were to: 1) quantify aggregative responses of *P. tricuspis* adults to variable host densities, 2) determine effect of direct mutual interference between pairs of ovipositing *P. tricuspis* females confined with host *S. invicta*, 3) elucidate the effect of confining 1 or 2 additional males with already mated females on progeny sex ratios, and 4) determine the form of the functional response of individual ovipositing *P. tricuspis* to varying host densities.

MATERIALS AND METHODS

Monogyne *S. invicta* colonies were collected at the Louisiana Agricultural Experiment Station in St. Gabriel, Louisiana (30⁰ 16′ N, 91⁰ 05′ W). As of 2006, expanding populations of *P. tricuspis* in Louisiana had not yet reached this location. Colonies were separated from soil in the laboratory by the drip flotation method (Banks et al. 1981). Ants from each colony were then sieved to yield host ants that were within

the preferred size class for *P. tricuspis* females (approximately 1 mm head width (see Morrison et al. 1997)). Except where otherwise indicated, all statistical analyses were conducted using Prism® 4.03 (GraphPad Software, Inc., San Diego, CA). All statistical analyses (described below) were conducted at a significance level of $\alpha=0.05$.

A large enclosed Plexiglas® cage (120 cm x 60 cm x 60 cm) that was illuminated by an overhead fluorescent lamp that was modified to eliminate flicker, and heated by a 75 W infrared lamp was used to conduct laboratory trials. Plaster blocks saturated with water were placed on the middle and corners of the cage floor to provide humidity. Trials were conducted when temperatures inside the cage was approximately 26-28° C and had 80-90% RH. At least 200-300 newly emerged *P. tricuspis* were released inside the cage prior to the trials. To minimize variance in performance of *P. tricuspis*, only flies less than 1 day old were used. While many *S. invicta* colonies were used, in order to reduce variation, individual trials used ants from the same colony.

A) Aggregative Responses

Laboratory experiments were conducted to examine the relationship between host density and numbers of attacking *P. tricuspis*. Ant densities used were typical of densities found under field conditions at hot dog bait stations (Henne and Johnson, unpublished data). Prior to experiments, ants were weighed and placed in 90 mm x 15 mm Petri dishes. The inner walls of the Petri dishes were coated with Fluon® to prevent ants from escaping. A weighed 0.10 g random sample contained 108 ants. First, five trials were conducted with three host densities (1.0 g (1,080 ants), 0.25g (270 ants), and 0.06g (65 ants) - each successive density ¼ of the previous highest density). Next, eight trials were conducted with four host densities (0.5g (540 ants), 0.25g (270 ants), 0.12g (135 ants), and 0.06g (65

ants) - each successive density ½ of the previous highest density). Ant density treatments were grouped inside the cage according to a completely randomized design. Finally, seven trials were conducted with five host densities (0.5g, (540 ants) 0.25g (270 ants), 0.125g (135 ants), 0.06g (65 ants), and 0.03g (32 ants) - each successive density ½ of the previous highest density). During each trial, flies were counted at Petri dishes at 1, 5 and 10 minutes after lids were removed from the dishes. In all trials, Petri dishes were separated by at least 5 cm. A preliminary experiment revealed no differences in attractiveness of hosts attacked for several hours vs. hosts that were never attacked. After each trial, all Petri dishes were covered and then haphazardly rearranged within the arena.

Statistical Analysis

Data consisting of counts are frequently Poisson rather than normally distributed, with the result that the mean and variance will not be independent but will tend to vary together (Sokal and Rohlf 1995). Therefore, *P. tricuspis* counts were ln x+1-transformed before analysis to achieve normality and stabilize variances.   A profile ANOVA was conducted on treatment effect (i.e. host density) and time (1, 5 and 10 minutes) (PROC GLM, SAS Institute 2002). A profile ANOVA is a multivariate test (similar to repeated measures analysis; Simms and Burdick 1988), but allows for the sample trials to be non-independent in time.

B) Direct Mutual Interference

Two laboratory trials were conducted to determine the effect of increasing the density of *P. tricuspis* females when confined with a constant density of *S. invicta* workers. In both trials 1, 2 or 3 female *P. tricuspis* were confined with 0.5 g (ca. 500 ants) of *S. invicta* workers. In both trials, each parasitoid density was replicated four times Ants were

weighed and placed into individually labelled plastic containers (Ziploc® 236 ml snap lid containers) lined with Fluon® to prevent ants from escaping. After newly emerged flies were released inside the cage, several hours were allowed to elapse before experiments were initiated. We found that newly emerged *P. tricuspis* are not, or are only weakly, responsive to host *S. invicta* pheromones for 1-2 hours (Pers. Obs). They also appear to undergo an obligate dispersal phase after emergence. Males would actively fly along the top of the cage near the lights while females would sit on the walls of the cage.

After this post emergence phase, a single container with approximately 1.0 g (ca. 1,000 ants) of *S. invicta* was placed in the center of the cage to prime the flies to begin attacking hosts, and to allow the two sexes to mate. Adults of *P. tricuspis* mate while females are attacking hosts (Porter 1998). Ants were lightly probed to elicit alarm behavior and production of alarm pheromones, which attract both male and female *P. tricuspis*. Phorid parasitoids locate their hosts by detecting ant semiochemicals (Porter 1998, Morrison and King 2004).

After 15 minutes, the primer container was removed and experimental containers were placed into the cage with their lids on. Individual containers were randomly chosen among the replicates and the lid was opened, allowing flies access to host *S. invicta*. The two sexes have different hovering behaviors; females hover a few mm from their hosts, while males tend to maintain a larger distance between themselves and *S. invicta*, and can also be distinguished from females by their searching behavior. When searching for females, males tend to hover in place and turn from side to side at approximately 45-90° angles from center (see also Porter 1998). They also tend to spend more time searching

among hosts that are more densely aggregated (Pers. Obs.). When males descended into experimental containers, an aspirator was used to remove them.

When the predetermined numbers of female *P. tricuspis* descended into individual containers and were confirmed to be attacking hosts, the lid was snapped into place and set aside in the cage. The time that the lid was closed was written on a label on the lid and the next container was opened. This process was continued until all replicates were done. However, at the same time that flies were being confined with *S. invicta*, previously completed replicates were tapped lightly to induce ant alarm behavior and maintain fly activity. When under attack by *P. tricuspis*, *S. invicta* tended to cluster and required occasional disturbance to disperse them. Flies were confined with hosts for two hours after which the lids were removed, the flies allowed to escape or aspirated and the containers removed from the cage.

After all flies were removed, a small (1 cm^3) plaster block that was saturated with water was placed in the containers to provide humidity. A 1 ml drop each of water and sugar water was also deposited on the bottom of the containers and the lids closed. Numerous small pinholes were made in the lids to provide ventilation. Containers were placed in an environmental chamber (Percival Intellus 136 VL) with temperature set at a constant 28° C and a 14:10 photo/scotoperiod. Every two days, the containers were cleaned of middens, and fresh water and sugar water provided. Containers were randomly rearranged in the chamber daily. Parasitized ants began to die approximately 10 days after exposure to female *P. tricuspis*. For two subsequent weeks, decapitated heads from individual replicates were carefully removed daily with soft forceps and placed onto moistened filter paper inside individual 90 mm x 10 mm Petri dishes bearing

the same information as the source containers. The dishes were sealed with paraffin laboratory film to maintain humidity and held in an environmental chamber under the same conditions as described above.

Male Interference

Another experiment was conducted to determine if individual female parasitism rates were affected by confining males with mated females. Mated solitary females were captured from an aggregation of flies that were attacking *S. invicta* inside the cage. Females were confined with zero, one and two males. Each treatment was replicated eight times. The procedure was similar to that described above, except that the combination of males and females that entered the containers was manipulated with an aspirator. The post experiment handling of *S. invicta* and puparia was the same as described above.

Statistical Analysis

Numbers of hosts parasitized were ln - transformed prior to analyses, as above. Replicates with zero hosts parasitized were omitted from analyses, as female *P. tricuspis* in these replicates either failed to successfully parasitize at least one host, were captured and killed by *S. invicta* inside the container, or were otherwise defective. A one-way ANOVA and Tukey's HSD tests were conducted on numbers of hosts parasitized by 1, 2 or 3 females.

The proportion of total hosts encountered by parasitoids per unit time (per capita searching efficiency) can be quantified in terms of the rate of decline in searching efficiency as parasitoid density increases (Hassell 2000). Changes in per capita searching

efficiency (*s*) of *P. tricuspis* in relation to parasitoid density were estimated from the following equation (Visser and Driessen 1991):

$$s = \frac{1}{p_t} \ln[\frac{N_t}{N_t - N_a}]$$ (1)

Where P_t is density of *P. tricuspis*, N_t is the number of hosts and N_a is the number of hosts killed. Searching efficiency was regressed against ln host density using least squares regression.

Finally, the effect of confining additional males with solitary females on progeny sex ratios was tested with a Pearson chi-square test. The null hypothesis was that progeny sex ratios were not different among treatments. Progeny sex ratios were also tested against a hypothesized 1:1 ratio with a Pearson chi-square test.

C) Functional Response

Four laboratory trials were conducted to determine the shape of the functional response when confining a single female *P. tricuspis* with variable densities of *S. invicta*. Ants were placed in plastic containers (Ziploc[®] 236 ml snap lid containers) that were lined with Fluon[®] to prevent ants from escaping. The procedure of confining female *P. tricuspis* with host *S. invicta* and maintenance of ants was the same as described above for the interference trials. In trials 1 -3, individual female *P. tricuspis* were confined with 135, 270, 540, 810 and 1080 ants, with each host density replicated four times. In trial 4, individual female *P. tricuspis* were confined with 25, 50, 100 and 200 ants, with each host density replicated eight times. Post experiment maintenance of exposed ants was the same as described above for the interference experiment. Sex ratios of progeny adults were determined every second day when emergence began.

Statistical Analysis

In order to distinguish among the three types of host dependence in the functional response, a two-step approach recommended by Juliano (2001) was followed. First, the shape of the functional response curve on the percentage of ant hosts successfully parasitized by *P. tricuspis* as a function of ant density was determined by logistic maximum likelihood regression (PROC CATMOD, SAS Institute 2002). The logistic model is as follows:

$$\frac{N_a}{N_o} = \frac{\exp(P_o + P_1 N_o + P_2 N_o{}^2 + P_3 N_o{}^3)}{1 + \exp(P_o + P_1 N_o + P_2 N_o{}^2 + P_3 N_o{}^3)} \tag{2}$$

Where the parameter N_o is the host density, N_a is the number of hosts parasitized, and P_0, P_1, P_2, and P_3 are the logistic regression parameters associated with the slope of the curve. The null hypothesis is that the linear parameters are not significantly different from zero. A type I functional response is indicated by linear terms not significantly different from zero (i.e. zero slope), a type II functional response by a significant negative value of P_0, and a type III functional response by a positive P_0 parameter and a negative P_1 (quadratic) parameter. If the linear parameter computed from the logistic regression is not significantly different from zero, it indicates no effect of increased host density on the proportion of hosts parasitized and the type I functional response is fitted to the data by the following linear equation (Parajulee et al. 2006):

$$N_a = \alpha + \beta N_0 \tag{3}$$

Where N_a is the number of hosts parasitized, N_0 is the host density, and α and β are the intercept and slope of the attack rate prediction line, respectively.

Second, if the appropriate functional response form is determined to be type II or III, parameter estimation of a (attack constant) and b (functional response asymptote) are achieved by fitting the numbers of ants parasitized at variable host densities to the appropriate functional response selected by the logistic procedure using a non-linear least squares procedure (PROC NLIN, SAS Institute 2002). Equations for Type II and III functional responses are given in Juliano (2001). Since the results indicated *P. tricuspis* females attack according to a Type I functional response (see results), the slopes and intercepts of the mean number of hosts parasitized in relation to host density, and the proportion parasitized in relation to host density for trials 1-3 were compared with an ANCOVA (Sokal and Rohlf 1995). Replicates where no puparia were produced (i.e. no successful attacks occurred) were excluded from the analysis, as these females either were defective or otherwise were captured and killed by *S. invicta*.

RESULTS

Aggregative Responses of *P. tricuspis*

Flies quickly recruited to Petri dishes containing host *S. invicta*. In general, proportional fly abundances among treatment levels were invariant over the 10-minute time interval, but the overall total number of flies that recruited slightly increased over time. No significant effects of time or time x treatment effects on fly abundances were found in any of the experiments (p>0.05).

With three host density levels (65, 270 and 1080 ants), more flies aggregated at the highest host density (1080 ants) than the other densities (Figure 3.1A). With four host density levels (65, 135, 270 and 540 ants) more flies aggregated at host density 540 than the other three host densities, and host density 270 attracted higher numbers of flies than

densities 135 and 65 (Figure 3.1B). With five host density levels (32, 65, 135, 270 and 540 ants), more flies aggregated at host densities 540 and 270 than the other host densities (Figure 3.1C).

(A)

(B)
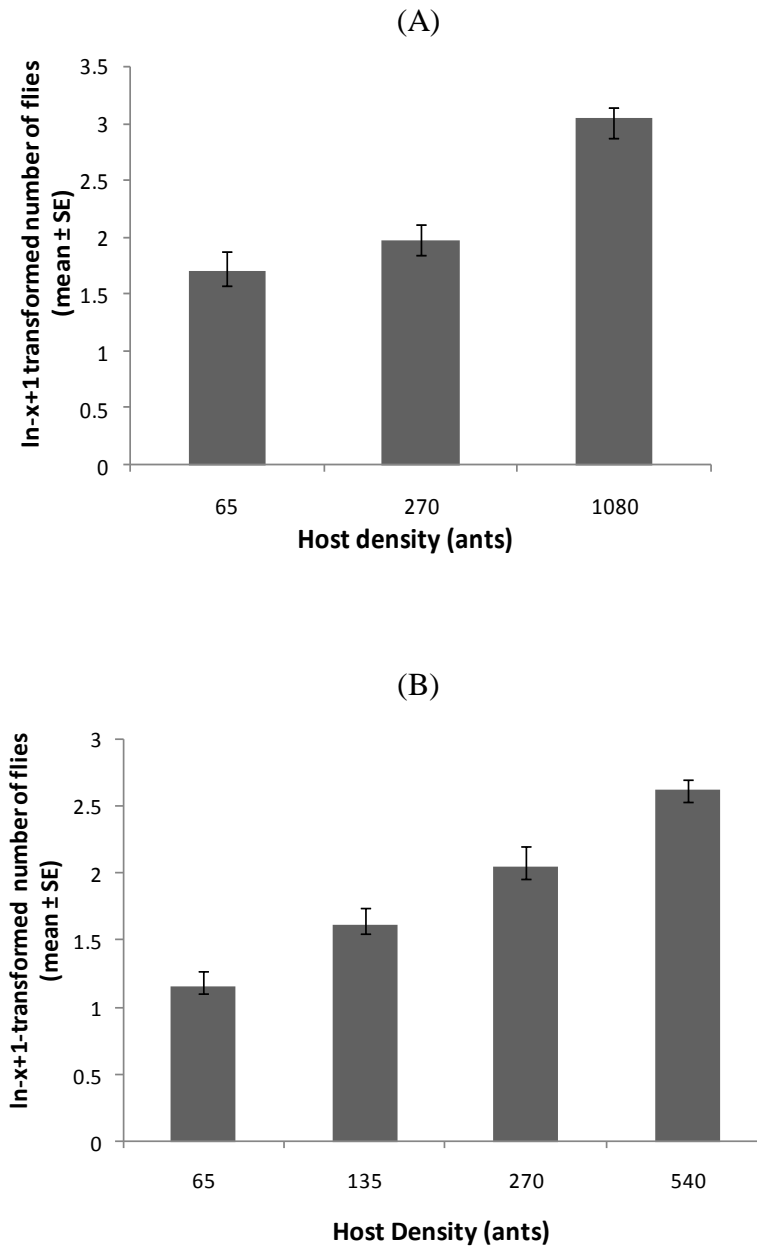
Figure 3.1: Aggregation responses of *P. tricuspis* (mean ± SE) to (A) three levels of host density, (B) four levels of host density and (C) five levels of host density.

Figure 3.1 (con't)
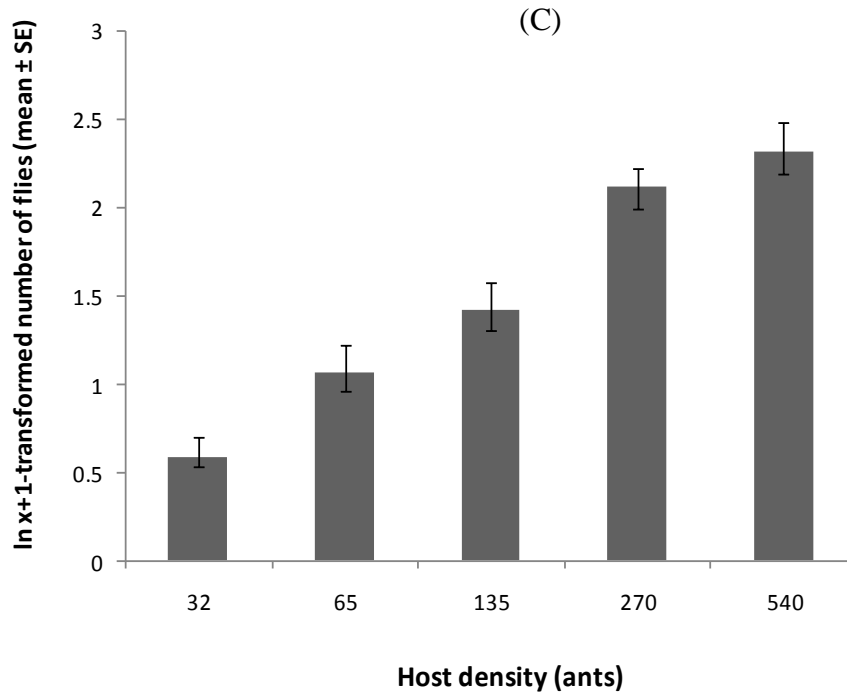
Direct Mutual Interference

Total Hosts Parasitized

Tthe total number of *S. invicta* hosts that were successfully parasitized by *P. tricuspis* was not significantly different over the limited range of female densities evaluated ($P>0.05$) (Figure 3.2A). No significant ($P<0.05$) reductions in the number of hosts successfully parasitized/female was found in both trials when more than one female was confined. However, a trend toward lower numbers of successful parasitism per female was evident when the number of female conspecifics was increased (Figure 3.2B).

(A)

(B)
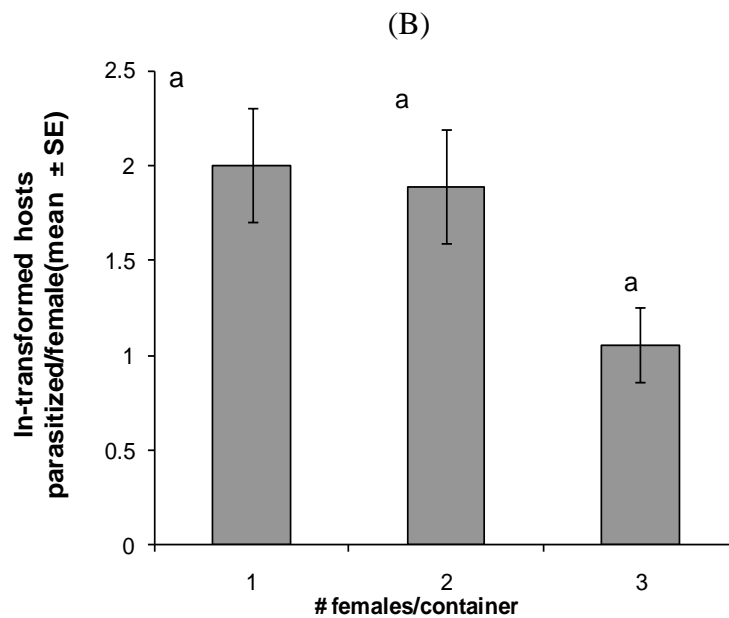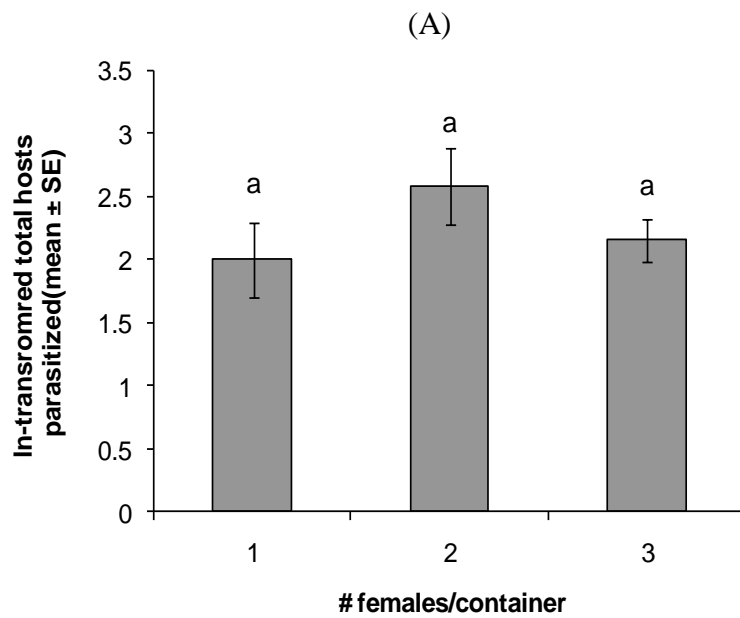
Figure 3.2: Results of laboratory trials evaluating total host *S. invicta* parasitized (A) and total host *S. invicta* parasitized per female (B) (Mean ± SE) at three levels of female *P. tricuspis* density. Bars with the same letters are not significantly different at α=0.05.

Searching Efficiency

Although there was a declining trend in per capita searching efficiency, no correlation was found between the log searching efficiency and the log number of *P. tricuspis* (Figure 3.3) [(Trial 1: R^2=0.22; *df*=1,10; *F*=2.883; *P*=0.12), (Trial 2: R^2=0.31; *df*=1,11; *F*=4.835; *P*=0.05)]. Therefore, interference among several ovipositing *P. tricuspis* females does not appear to be significant at low female densities.
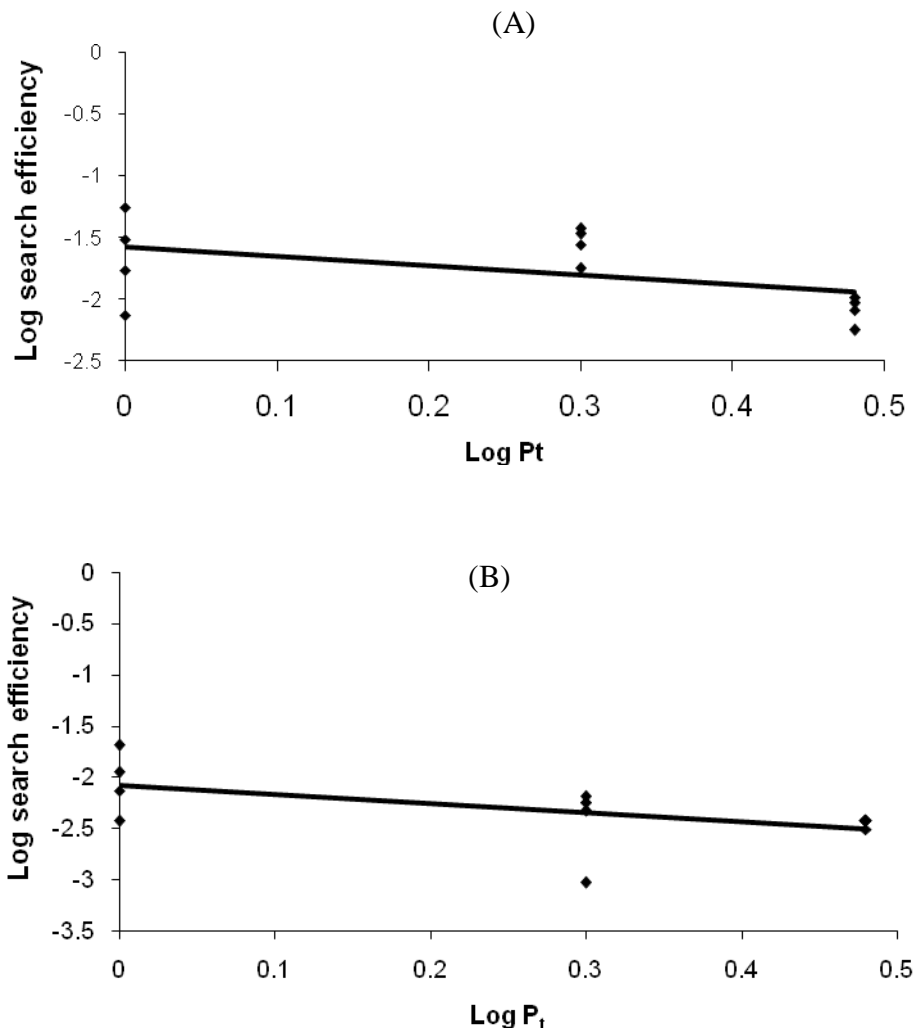
Figure 3.3: Results of direct mutual interference experiments, showing per capita searching efficiency of *P. tricuspis* in relation to female density: (A)=trial 1, (B)=trial 2.

Male Interference

The presence of additional males confined with mated solitary females did not have a significant effect on the total number of successfully parasitized hosts ($P<0.05$). The chi-square analysis showed no significant effect on progeny sex ratios from having additional males confined with already mated females ($df=2$, 132; $X^2=0.3$; $P=0.86$). Male to female sex ratios shifted over the range of treatments, 3.6:1 (0 males), 3:1 (1 male), 2.8:1 (2 males). The progeny sex ratios deviated significantly from a hypothetical 1:1 ratio (no males: $df=1$, $X^2=14.7$, $P=0.0001$; 1 male: $df=1$, $X^2=8.0$, $P=0.005$; 2 males: $df=1$, $X^2=12.8$, $P=0.0003$).

Functional Response

None of the linear parameters in the logistic models were significantly different from zero ($P>0.05$), suggesting that *P. tricuspis* parasitism rates follow a type I functional response under laboratory conditions (Table 3.1, Figure 3.4). Therefore, attack rates are host density-independent. The results of the ANCOVA for comparing slopes and intercepts of mean number of hosts parasitized in relation to host density, and the mean proportion parasitized in relation to host density for trials 1-3 indicated no significant differences between either slopes or intercepts [mean hosts parasitized (slopes: $dfn=2$, $dfd=9$; $F=0.49$; $P=0.63$) (intercepts: $dfn=2$, $dfd=11$; $F=0.30$; $P=0.74$); calculated pooled slope for trials 1-3 is 0.002 and the pooled intercept is 2.45], [mean proportion hosts parasitized (slopes: $dfn=2$, $dfd=9$; $F=0.64$; $P=0.55$) (intercepts: $dfn=2$, $dfd=11$; $F=0.12$; $P=0.89$); calculated pooled slope for trials 1-3 is -1.18e-005 and the pooled intercept is 0.015].

Table 3.1: Results of the maximum likelihood estimates by PROC CATMOD for the functional response of *P. tricuspis* to varying host densities.

Trial	Logistic regression parameters	Estimate ± SE	X^2	d.f.	P-value
1	Intercept	-3.71 ± 1.10	11.38	1	0.0007
	P_0	-0.005 ± 0.007	0.60	1	0.44
	P_0^2	$8.9 \times 10^{-6} \pm 1.2 \times 10^{-5}$	0.58	1	0.44
	P_0^3	$-5.2 \times 10^{-9} \pm 5.9 \times 10^{-9}$	0.77	1	0.38
	Likelihood ratio		24.00	13	0.03
2	Intercept	-2.49 ± 0.96	6.80	1	0.009
	P_0	-0.01 ± 0.007	2.31	1	0.13
	P_0^2	$1.4 \times 10^{-5} \pm 1.3 \times 10^{-5}$	1.08	1	0.30
	P_0^3	$6.04 \times 10^{-9} \pm 7.1 \times 10^{-9}$	0.72	1	0.40
	Likelihood ratio		7.37	11	0.77
3	Intercept	-4.89 ± 1.06	21.23	1	<0.0001
	P_0	0.005 ± 0.007	0.56	1	0.45
	P_0^2	$1.0 \times 10^{-5} \pm 1.2 \times 10^{-5}$	0.95	1	0.33
	P_0^3	$6.3 \times 10^{-9} \pm 6.6 \times 10^{-9}$	0.91	1	0.34
	Likelihood ratio		16.37	13	0.23
1-3	Intercept	-3.60 ± 0.57	39.73	1	<0.0001
	P_0	-0.004 ± 0.004	1.15	1	0.28
	P_0^2	$4.5 \times 10^{-6} \pm 6.8 \times 10^{-6}$	0.43	1	0.51
	P_0^3	$2.2 \times 10^{-9} \pm 3.6 \times 10^{-9}$	0.39	1	0.53
	Likelihood ratio		55.90	48	0.15
4	Intercept	-0.17 ± 0.67	0.06	1	0.80
	P_0	-0.05 ± 0.03	2.77	1	0.10
	P_0^2	$4.1 \times 10^{-4} \pm 3.3 \times 10^{-4}$	1.53	1	0.22
	P_0^3	$1.1 \times 10^{-6} \pm 1.0 \times 10^{-6}$	1.19	1	0.28
	Likelihood ratio			24	<0.0001
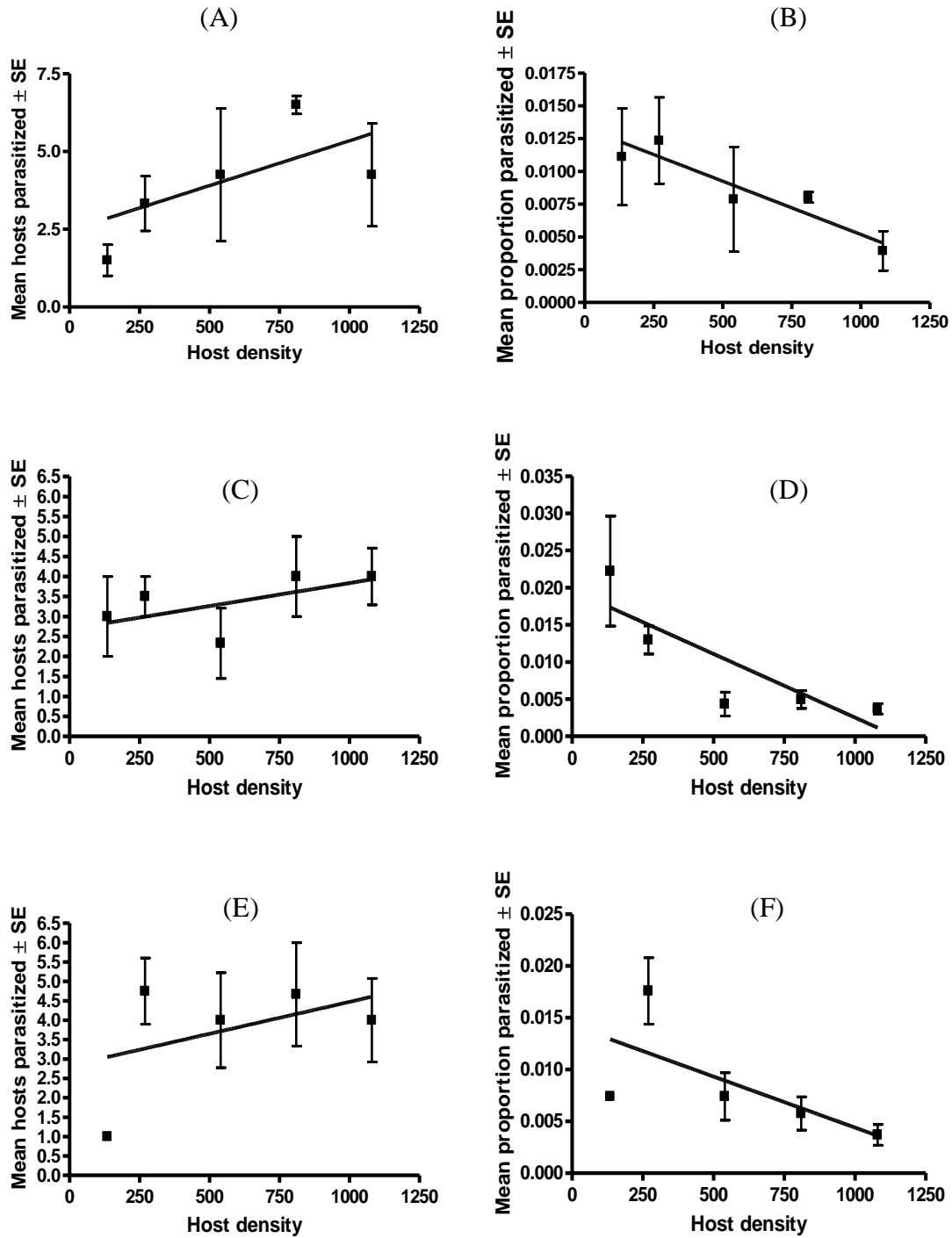
Figure 3.4: Results of functional response trials: A, C, E, G, I are mean hosts parasitized (mean ± SE) at varying levels of host density; B, D, F, H, J are mean proportion of hosts parasitized at varying levels of host density: A,B=Trial 1; C,D=Trial 2; E,F=Trial 3; G,H=Trials 1-3 pooled; I,J=Trial 4.

Figure 3.4 (con't)

(G)

(H)

(I)

(J)
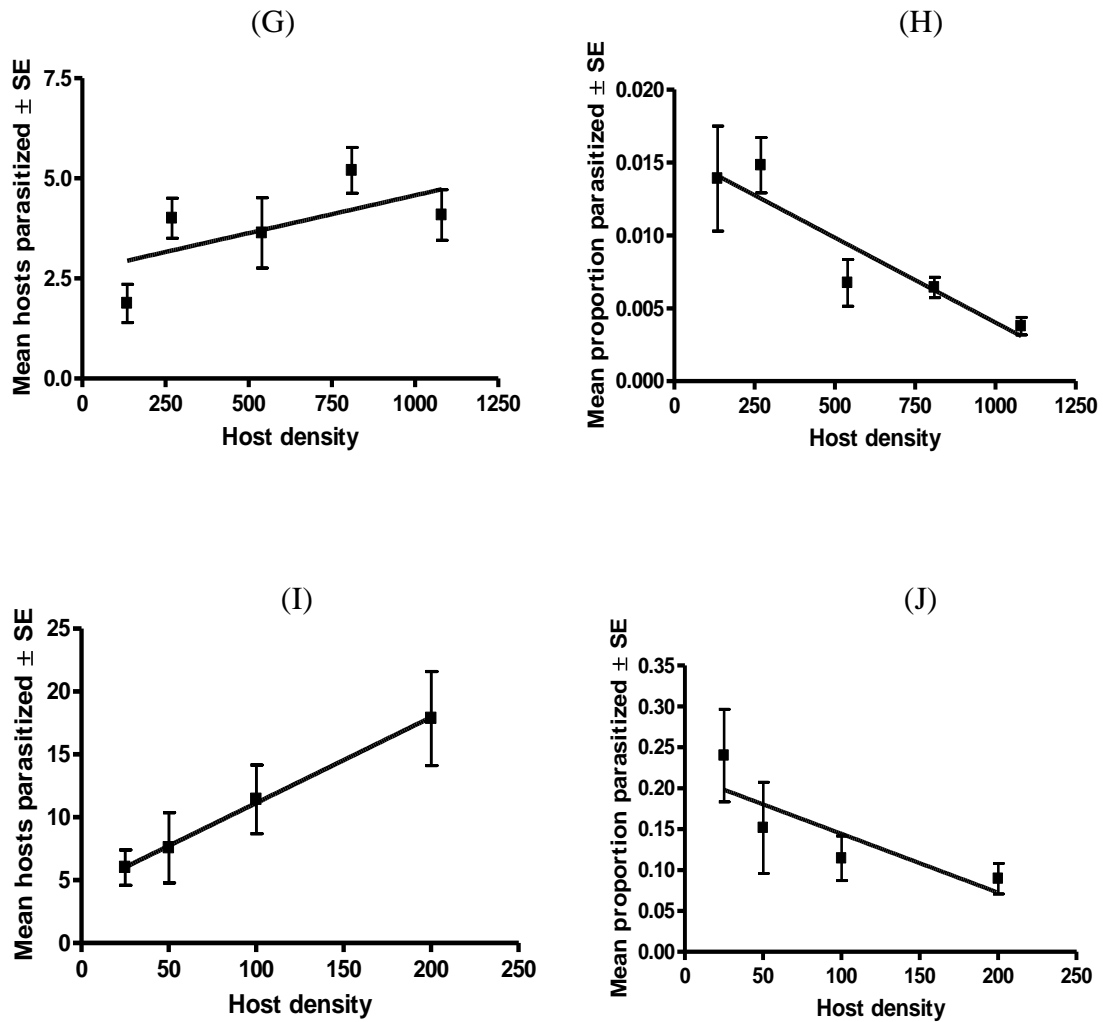
DISCUSSION

Aggregative Responses

A density dependent aggregative response of *P. tricuspis* to host density was found under laboratory conditions. Similarly, under field conditions Morrison and King (2004) found that increasing the number of nonnestmate *S. invicta* workers at baits already occupied by *S. invicta* led to enhanced numbers of *P. tricuspis*, presumably because increased alarm pheromone production by fighting nonnestmates attracted more flies. Furthermore, Morrison and Porter (2005b) established a positive correlation between *P.*

tricuspis abundance and *S. invicta* density in north-central Florida. In the laboratory experiments described in this paper, *P. tricuspis* continued to aggregate at the higher host densities, even when Petri dishes were covered and the dishes rearranged. Perhaps *P. tricuspis* are able to learn and distinguish between relative patch sizes in space. Parasitoids can profit most when they aggregate and spend most of their time in patches where host densities are highest (Free et al. 1977).

A lot of frenzied activity occurs when adults of *P. tricuspis* aggregate at patches of host *S. invicta*. Not only are females competing with one another for access to hosts, males also aggressively compete with other males for access to females (Pers. Obs.). A similar pattern of activity occurs with *Scatophaga stercoraria* L. (Diptera: Scatophagidae), where intra-male competition for females at dung pats is strong, as males outnumber females by 4:1 (Parker 1974). Male *P. tricuspis* are variable in size, with larger males often as large as some females (Pers. Obs.). Presumably these larger males live longer and have an advantage in competing with smaller males for mates (Morrison et al. 1999).

Pseudacteon tricuspis females are probably pro-ovigenic and egg-limited parasitoids. No information on fecundity of *P. tricuspis* is available. However, fecundity of the related *P. wasmanni* Schmitz ranges from 30 to nearly 300 eggs (Zacaro and Porter 2003). Egg-limited parasitoids characteristically have short handling times (Getz and Mills 1996, Mills and Lacan 2004). Handling time in *P. tricuspis* is very short (<1 s) in relation to overall time spent searching. A small ratio of handling to search time in parasitoids that are confined to a single patch for a longer time than by choice could result in a linear functional response (Hassell 2000, but see Mills and Lacan 2004). Therefore, the problem for parasitoid females, such as *P. tricuspis*, is to parasitize as many hosts in its short

lifespan (Wajnberg 2006). Similarly, males should maximize their fitness by mating with as many females as possible. Natural selection will enhance strategies that optimize not only female reproductive success, but mating success as well (Cook and Hubbard 1977, Parker 1978). In the laboratory, males were often observed to appear at higher density host patches before females, a strategy that would increase probability of ultimate mating success when females appear at high density host patches also.

<div align="center">Direct Mutual Interference</div>

No evidence of direct mutual interference was found when two or three female *P. tricuspis* were confined in small laboratory containers, although per capita oviposition success (measured as number of hosts killed) appeared to decline when more than two females were confined. This study did not demonstrate any reductions in estimates of searching efficiency of at least 2 or 3 simultaneously ovipositing *P. tricuspis* females. However, Visser and Driessen (1991) warn that it is important to consider population *and* generation level effects of mutual interference on estimates of searching efficiency, as dispersal from patches containing high densities of conspecifics can lead to enhanced searching efficiency if hosts are uniformly distributed. However, this study did not evaluate nor allow dispersal of females between host patches.

Field studies of *P. tricuspis* populations in Louisiana revealed that approximately 50% of *P. tricuspis* aggregations at disturbed *S. invicta* mounds include 1-3 females (Henne and Johnson, unpublished data). Moreover, it was extremely difficult to consistently confine more than three *P. tricuspis* females together in small containers in the laboratory experiments described in this paper because some females tended to leave the container when too many females were present. Therefore, direct mutual interference may

become more important when higher densities of ovipositing *P. tricuspis* are simultaneously present than those evaluated in this study. The intensity of interactions usually increases at higher parasitoid densities, leading to greater mutual interference and overall suppressed searching efficiency of the parasitoid population (Visser and Driessen 1991). In contrast, indirect mutual interference is a reduction in searching efficiency at the population level due to superparasitism (Visser and Driessen 1991). Superparasitism of individual *S. invicta* workers by *P. tricuspis* has been observed in the laboratory on numerous occasions, suggesting that *P. tricuspis* females are unable to discriminate between parasitized and unparasitized hosts. A laboratory experiment comparing attractiveness of hosts exposed to *P. tricuspis* parasitism for four days versus non parasitized hosts showed no apparent differences in attractiveness, as equal numbers of flies were attracted (Henne and Johnson, unpublished data). Superparasitism is probably rare under natural conditions, given that natural parasitism rates of *S. invicta* by *P. tricuspis* are very low (see Morrison and Porter 2005a).

Male Interference

This study did not reveal any significant effect of having additional males confined with single females. However, the sex ratios trended downward toward a 1:1 ratio when the number of males confined with a single female was increased from zero to two. It is unclear what mechanism(s) is (are) responsible for shifts in sex ratio allocation in *Pseudacteon* spp. Consistent with host size-dependent-sex allocation theory (Charnov et al. 1981), sex ratios of *Pseudacteon* spp. have been linked to host size, with more females arising from larger hosts (Morrison et al. 1999). Secondary sex ratios may simply be an artifact of the size range of available host ants (Morrison and Porter 2005a).

Sex ratio shifts have been documented in other parasitoid species. For example, Wylie (1965) found that increasing the ratio of *Nasonia vitripennis* (Walk.) (Hymenoptera: Pteromalidae) females to host *Musca domestica* L. (Diptera: Muscidae) resulted in a reduction in the proportion of progeny females. Perhaps female *P. tricuspis* are able to adjust sex ratio allocation of progeny by differential selection of host sizes in response to interference by conspecifics. The theory of local mate competition (Hamilton 1967) has been proposed as an explanation for sex ratio shifts among Hymenopteran parasitoids, where an increase in the number of female conspecifics at a host patch results in an increase in the proportion of male progeny produced. Unfortunately, a similar evaluation of sex ratio shifts when multiple females were confined was not conducted in this study, but this would be an interesting research direction to pursue. Nevertheless, a *P. tricuspis* 3:1 sex ratio (males to females) has been consistently found under field conditions, and was reproduced under laboratory conditions.

Functional Response

None of the linear parameters in the logistic models were significantly different from zero suggesting that *P. tricuspis* had constant attack rates regardless of host density under the laboratory experimental design. It is possible that host density levels evaluated in this study were too high, and therefore *P. tricuspis* attack rates were at an asymptotic level. It should also be pointed out that these females were confined with their hosts and not allowed to disperse freely between host patches. A different functional response curve may be relevant under more natural situations or laboratory settings where females are allowed unrestricted movement (Chong and Oetting 2006).

Type I functional responses among insect parasitoids are rare (see Mills and Lacan 2004 for examples). However, Turchin (2003) argues that differences between Type I and Type II functional responses are minor. It is important to mention that the functional response is not independent of host density alone and should account for the reality that parasitoids rarely exist as single individuals and more likely interact with conspecifics, necessitating the need for ratio-dependent functional response studies (Arditi and Ginzburg 1989, Mills and Lacan 2004, Chong and Oetting 2006). Furthermore, effects of temperature on functional response can be important (see Parajulee et al. 2006, Zamani et al. 2006) but were not evaluated in this study.

CONCLUSIONS

The studies conducted in this paper have provided some insights into *P. tricuspis* behavioral and functional responses that were until now unknown. The density-dependent aggregations of *P. tricuspis* observed in the laboratory are consistent with theory and field observations. Mutual interference of conspecific male and females at low densities does not appear to be significant, but may reveal itself at higher densities. The Type I functional response found was unexpected on the grounds that most parasitoids appear to have a Type II functional response. It is expected that the results obtained in this study will stimulate further research into *Pseudacteon* population ecology and test host-parasitoid theory.

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CHAPTER 4

**SPATIO-TEMPORAL DYNAMICS OF THE DECAPITATING FLY,
PSEUDACTEON TRICUSPIS BORGMEIER (DIPTERA: PHORIDAE) AT THREE
LOCATIONS IN LOUISIANA**

INTRODUCTION

Understanding the processes that affect the spatial distribution of plant and animal populations is a key subject in ecology (Tuda 2007). However, the difficulty of directly studying movement of individual animals, particularly small and numerous species such as insects, presents special problems for ecologists (Perry 1998a). For mobile animal species, spatial information is often restricted to trap counts at specific locations (Perry 1995). For any species, these count locations may be spatially arranged in regular, random, or clustered patterns without regard to the properties of the count frequency distribution (Ferguson et al. 2000). Given that population sampling relies on spatial dispersion patterns, determining the spatial population structure of organisms is important to understanding population dynamics.

Traditional methods of analyzing counts of organisms that are based on sample variance-mean relationships and derivatives thereof (see Taylor 1984) only provide information on the numeric properties of the underlying frequency distribution, and, therefore, have limited capability to describe the essential spatial information of counts (Perry and Hewitt 1991, Perry et al. 1999). For example, a highly skewed series of counts (e.g. negative binomial) with one or more very large values relative to the others may still be completely random in space (Perry and Dixon 2002). Current analyses (Spatial Analysis of Distance IndicEs [SADIE]) of spatial and temporal distribution of insects utilize location information of sample units to detect and measure degree of nonrandomness in the spatial pattern in two-dimensional space (Perry 1998a), and spatial association between data sets collected on different occasions (Winder et al. 2001). This methodology has been applied to the study of several host-parasitoid systems (e.g.

Ferguson et al. 2000, Weaver et al. 2005, and Ferguson et al. 2006). As a result, key insights into spatial dynamics of host-parasitoid systems, such as spatial aggregations of parasitoids, may be achieved by incorporating spatial information of count data into analyses.

The red imported fire ant, *Solenopsis invicta* Buren, is a ubiquitous exotic invasive insect in the United States, and is regarded as a significant economic pest (Lofgren 1986, Porter et al. 1992). Hence, recent efforts have focused on biological control of *S. invicta* by importing several species of natural enemies from the indigenous range of *S. invicta* in South America and have included the introduction of parasitic flies of the genus *Pseudacteon* Coquillet (Diptera: Phoridae). *Pseudacteon* spp. are solitary endoparasitoids of *Solenopsis* fire ants (Morrison and Porter 2005). Adults of one *Pseudacteon* species, *P. tricuspis* Borgmeier, are attracted to alarm pheromones emitted by *S. invicta* (Morrison and King 2004). *Pseudacteon* spp. females insert a single egg into the host ants' thorax, and the maggot eventually migrates to the hosts' head capsule, consumes the contents of the head over a two-week period, and eventually decapitates the host (Porter 1998).

Our understanding of phorid fly population dynamics is not very well developed (Morrison 2000). In particular, very little information is available concerning the spatial and temporal dynamics of various Phoridae (Disney 1994), particularly in the United States. Populations of *Pseudacteon* parasitoids of *S. geminata* (Fabricius) in central Texas were characterized as having significant variations in abundance, both spatially and temporally (Morrison et al. 1999). Morrison and King (2004) evaluated phorid abundances at disturbed *S. invicta* mounds at several sites in north-central Florida over

time and determined that abundances deviated significantly from a uniform distribution. However, neither of these studies evaluated phorid abundances at the same individual mounds (i.e. spatial coordinates) over time nor modeled these abundances in a spatial-temporal context.

The microsporidian parasite, *Thelohania solenopsae* Knell, Allen and Hazard, has been isolated from polygynous *S. invicta* colonies and can cause significant mortality in infected colonies (Oi and Williams 2002). However, to the extent that *S. invicta* social form or colonies infected with *T. solenopsae* influence *P. tricuspis* spatial distributions are unknown. Therefore, the objectives of this study were to: 1) characterize the spatial and temporal abundances of *P. tricuspis* populations at three study sites, and 2) attempt to relate the abundances of *P. tricuspis* to host social form and presence/absence of *T. solenopsae*.

MATERIALS AND METHODS

Study Locations

The *P. tricuspis* populations sampled in this study originated from a release conducted 17 km northeast of Covington, St. Tammany Parish, Louisiana (30° 36′ 35″ N; 90° 01′ 19″ W) during 8-13 September 1999 (2,165 flies released) (Henne et al. 1997). Populations of *P. tricuspis* were sampled weekly at three widely separated locations in Washington Parish, Louisiana between 21 September and 19 October 2005. These locations were part of a larger study conducted in the area. The GPS coordinates for each study location were recorded with a Magellan ™ GPS 315/320 (accurate to 25 m or better). The first study location was 8 km south of Bogalusa (30° 41′ 49″ N; 89° 53′ 30″ W; hereafter called Farm 2), and was approximately 70 m x 70 m unmaintained cattle pasture. The second study location was 14 km southwest of Franklinton (30° 48′ 40″ N;

90° 18′ 35" W; hereafter called Farm 4), and was approximately 40 m x 60 m mowed cattle pasture. The third study location was 16 km west of Bogalusa (30° 46′ 04" N; 90° 01′ 52" W; hereafter called Farm 7), and was approximately 170 m x 100 m unmaintained cattle pasture.

At each study site, at least 15-25 active *S. invicta* colonies were located in an arbitrarily defined area and permanently marked with a bar code to enable future location. The bar code consisted of a high durability weather-resistant polyester barcode label adhered to a 10 cm x 5 cm piece of rigid plastic sheeting that was anchored flush with the ground with 10 cm long wire staple. The barcode label assigned a unique number to each *S. invicta* mound that was retained throughout the study phase. A Symbol® MC 3000 batch mobile computer (Motorola Inc., Holtsville, NY) was used to scan and record each barcode's unique value into a database.

Decimal-degree locations of *S. invicta* mounds were taken with a GPS to obtain x, y – coordinates of sampling locations. At each location, weekly mean soil moisture levels were obtained using a Lincoln soil moisture meter (Forestry Suppliers Part No. 3052), driven into the soil to a depth of 10 cm. Ten measurements at 5 m intervals were made along a transect through each study area.

<u>Sampling *P. tricuspis*</u>

Sampling *Pseudacteon* parasitoids of *Solenopsis* involved simply disturbing host nests and awaiting arrival of flies. Two observers disturbed individual *S. invicta* mounds in the following manner: mounds were vigorously disturbed with spades (5-10 sec) and crushed ants to release large amounts of semiochemicals to attract *P. tricuspis* (Morrison and King 2004). The number of *P. tricuspis* adults that arrived at disturbed mounds

during the ensuing 5 minutes was counted. Fly surveys were conducted between 1100 h and 1700 h (CDT) and when ambient temperatures were warm enough for *Pseudacteon* spp. fly activity ($>20^0$ C) (Morrison et al. 1999).

Determination of Social Form and Presence of *Thelohania*

Two social forms of *S. invicta*, monogyne (single-queen) and polygyne (multiple-queens) occur in the United States (Glancey et al. (1973). The two forms are regulated by a single gene that is homozygous (Gp-9^B) in monogyne queens and heterozygous (Gp-9^B and Gp-9^b) in polygyne queens (Ross and Keller 1998, Krieger and Ross 2002). A sample of approximately 1 g of *S. invicta* workers was obtained from each mound on 21 September 2005 by plunging a 20 ml glass scintillation vial into the mound. The vials were coated with Fluon® to keep *S. invicta* inside the vials. After collection, the vials were filled with 95% ethanol. Social form was determined by the multiplex polymerase chain reaction (PCR) methods as described in Valles and Porter (2003). Presence of *Thelohania* in the samples was determined by multiplex PCR methods as described in Valles et al. (2002).

Analysis of *P. tricuspis* Spatial Distributions

The spatial patterns of *P. tricuspis* counts were analyzed using SADIE (SADIEShell version 1.22 [Rothamsted Experimental Station, Harpenden, Herts, UK] http://www.rothamsted.bbsrc.ac.uk/pie/sadie/SADIE_home_page_1.htm) (Perry 1995, 1998 a, b). SADIE is a spatial analysis software program designed for use in situations where species are patchily distributed into discrete aggregations (Winder et al. 2001), and is appropriate to situations such as counting numbers of adult *P. tricuspis* appearing at *S. invicta* mounds. SADIE compares the degree of spatial pattern in the observed count arrangement to the minimum effort that individuals in that sample would need to expend to

move to a regular arrangement. This is also called the distance to regularity (D) where abundances would be equivalent in each sample unit (Perry et al. 1999). Thus, spatially aggregated counts will have higher values of D than counts that are uniform (Perry and Dixon 2002). The spatial pattern is quantified by randomly permuting the observed set of counts among the sample unit locations, thereby generating expected distances to regularity (E_a) to test the null hypothesis that counts are randomly arranged with respect to one another (Perry et al. 1999). An overall aggregation index is computed as $I_a=D/E_a$ for each sample data set to establish the dispersion pattern (Perry 1995, 1998b). Values of $I_a=1$ indicate randomly arranged counts, $I_a<1$ indicates a regular pattern of counts, while $I_a>1$ indicates aggregation of observed counts into clusters. The probability, P_a, that the observed data is more aggregated than expected from a random permutation of the counts is significant at $P<0.05$.

The *P. tricuspis* spatial count data were also analyzed with SADIE to determine the degree of clustering. SADIE calculates an overall mean of the sampled population and then assigns an index of clustering (v) to each sample location. Sample locations that have counts greater than the sample mean are classified as positive (v_i) 'donor' units, while sample locations that have counts less than the sample mean are classified as negative (v_j) 'receiver' units. Clusters of count data can occur as either patches (v_i), areas of relatively high counts that are close together, or gaps (v_j), areas of relatively low counts that are close together (Perry 1995). The contribution of each sample unit to a patch or a gap is quantified with indices that measure the amount that each sample unit contributes to a patch or a gap (Perry 1998b, Perry et al. 1999). Expected clustering indices approach 1 (v_i or v_j) for random counts, $v_i>1$ for counts that belong in a patch and $v_j <-1$ for counts that belong

in a gap. To test for nonrandomness the mean value of the clustering index over the patch

units ($\overline{V}_i$) is compared with its expected value of 1. Similarly the mean value of the

clustering index over the gap units ($\overline{V}_j$) is compared with its expected value of -1.

Significance levels for v_i and v_j are established through a 2-tailed test by the 95th percentiles

of the randomized distributions (permutations), where $v_i > 1.5$ and $v_j < -1.5$ are considered

significant at the 0.025 and 0.975 levels, respectively.

Finally, spatially-referenced data that share the same coordinates can be analyzed

for spatial association (i.e. similarity in the spatial patterns of two data sets) (Perry et al.

1999, Perry and Dixon 2002). SADIE can be used to measure the evolution of temporal

change in population structure when the same species is sampled at the same spatial

coordinates over time, and can detect spatial association between two species (Winder et al.

2001, Perry and Dixon 2002). To compare consecutive weekly spatial patterns of *P.

tricuspis*, an overall spatial association index, *X* (upper case chi), was calculated by SADIE

based on the similarity of local clustering indices (v_i and v_j above) from the consecutive

weekly distributions (Perry 1998b). Similar association indices were computed for *P.

tricuspis* vs. *Thelohania* and *P. tricuspis* vs. *S. invicta* social form (except for farm 7, which

had only two *T. solenopsae*-infected mounds and was almost entirely polygyne). Spatially

associated distributions will have values of *X>0* for patch coinciding with a patch, a value

of *X=0* for random and values of *X<0* or dissociated distributions for patch coinciding with

a gap. Significance of *X* is tested against a random *X* derived from a randomization

procedure of the clustering indices, and incorporates an adjustment for small-scale

autocorrelation in the data sets (Dutilleul 1993, Perry and Dixon 2002). The null

hypothesis is that there is no spatial association between the two sets of spatially-referenced data.

A t-test was also performed on social form vs. *P. tricuspis* abundances for each sample date for farms 2 and 4 only (*S. invicta* at Farm 7 was 95% polygyne). Spatial maps of *P. tricuspis* counts at individual *S. invicta* mounds, *S. invicta* social form and presence of *T. solenopsae* were generated using S-Plus™ 7.0 (Insightful Corporation, Seattle, Washington). Contour maps showing significant gap and patch indices were generated using 3DField 2.9 (http://3dfmaps.com [©] Vladimir Galouchko).

RESULTS

T. solenopsae Infection and *P. tricuspis*

Presence of *T. solenopsae* at Farms 2, 4 and 7 are mapped in Figures 4.1-4.3 (A), respectively. Few *S. invicta* mounds were found to be infected with *T. solenopsae* (Farm 2, 25%; Farm 4, 13%; and Farm 7, 10%). No significant spatial associations were found between *P. tricuspis* abundances and mounds infected with *T. solenopsae* ($P>0.025$ for positive, and $P<0.975$ for negative spatial associations [2-tailed test]).

Social Form and *P. tricuspis*

Social form of *S. invicta* at Farms 2, 4 and 7 are mapped in Figures 4.1-4.3 (B), respectively. The majority of *S. invicta* mounds sampled at each location were polygyne (Farm 2, 67%; Farm 4, 63%; and Farm 7, 95%). A significant ($P<0.05$) positive spatial association between *P. tricuspis* abundances and the *S. invicta* social form, polygyne, was found at Farm 2 on 12 October, and was marginally significant on 5 October (Table 4.1). Significantly ($P<0.05$) more flies were often associated with disturbed polygyne than monogyne mounds (Table 4.2).

Spatio-temporal Distribution of *P. tricuspis*

Weekly counts of *P. tricuspis* at individual disturbed *S. invicta* mounds at Farms 2, 4 and 7 are shown in Figures 4.1-4.3 (C-G), respectively. Extremely variable counts of *P. tricuspis* were typical of sample locations. In general, fly abundances at Farms 2 and 7 tended to increase during the first 2-3 weeks of this study, and declined thereafter (Table 4.3). The percentage of disturbed *S. invicta* mounds that attracted *P. tricuspis* also varied at each study location from week to week, and was generally highest during maximum soil moisture levels (Table 4.3). Dynamic patterns of soil moisture levels at all three locations were identical. Soil moisture readings increased during the first two weeks of the survey, were highest during the 28 September survey, and declined during each successive survey.

Overall, *P. tricuspis* abundances showed a random spatial distribution pattern, with I_a values close to 1 (Table 4.3). However, significant ($P<0.05$) aggregated spatial distribution patterns were detected at Farm 2 on 21 September, 5 October and 19 October and at Farm 7 on 5 October. No significant temporal associations of *P. tricuspis* spatial patterns were detected for all weekly comparisons ($P>0.025$ for positive, $P<0.975$ for negative spatial associations [2-tailed test]). Clustering of *P. tricuspis* into significant gaps ($v_j<-1.5$) were identified at Farm 2 on 5, 12 and 19 October, and patches ($v_i>1.5$) at Farm 2 on 5 and 19 October and Farm 7 on 5 October. The cluster indices for these locations and dates are mapped in Figure 4.4 (A-D), (Table 4.3).

Table 4.1: Spatial associations between *P. tricuspis* abundances vs. *S. invicta* social form and presence of *T. solenopsae*

Location	Dates	Social form association index value $(X)^{1}$ and P_a	*Thelohania* association index value (X) and P_a
Farm 2	9/21	0.2726 (0.17)	0.5245 (0.04)
	9/28	0.1206 (0.39)	-0.0064 (0.38)
	10/05	0.4544 (0.03)	0.1418 (0.29)
	10/12	**0.4776 (0.004)**	0.5128 (0.04)
	10/19	0.2757 (0.18)	0.2970 (0.14)
Farm 4	9/21	0.1777 (0.35)	0.1126 (0.27)
	9/28	0.4088 (0.09)	-0.1921 (0.56)
	10/05	0.1305 (0.37)	0.3402 (0.11)
	10/12	0.4449 (0.07)	0.3051 (0.14)
	10/19	0.2694 (0.23)	0.0767 (0.36)

[1] Values in bold indicate significance ($P_I < 0.025$ for positive spatial association, or $P_I > 0.975$ for negative spatial association)

Table 4.2: Numbers of *P. tricuspis* (Mean ± SE) associated with *S. invicta* social form

Location	Date	n-Monogyne (Mean ± SE)	n-Polygyne (Mean ± SE)
Farm 2	21 September	7 (2.7 ± 0.8) a	13 (4.5 ± 1.0) a
	28 September	7 (2.7 ± 0.7) a	13 (3.9 ± 1.5) a
	5 October	7 (3.4 ± 3.1) a	14 (11.6 ± 2.1) b
	12 October	7 (0.0 ± 0.0) a	13 (2.5 ± 0.8) b
	19 October	5 (0.0 ± 0.0) a	12 (1.2 ± 0.6) b
Farm 4	21 September	5 (1.0 ± 0.5) a	10 (1.7 ± 0.7) a
	28 September	2 (0.5 ± 0.5) -	9 (4.7 ± 1.6) -
	5 October	5 (1.6 ± 0.7) a	10 (2.1 ± 0.7) a
	12 October	6 (1.3 ± 0.9) a	10 (7.0 ± 1.8) b
	19 October	4 (3.5 ± 1.3) a	10 (6.9 ± 2.1) a

Values followed by the same letter are not significantly different at P=0.05 (t-test)

Table 4.3: Summary of *P. tricuspis* spatial and temporal abundances at three locations (Farms 2, 4 and 7), aggregation indices (I_a), and gap and patch indices (v_i) and (v_j), respectively.

Location	Sample Date	Number of mounds sampled	Percent mounds with flies	Average number of flies at mounds[1]	Soil moisture probe reading	SADIE I_a and P-value (in parentheses) [2]	SADIE V_j[3] and P-value (in parentheses)	SADIE V_i[4] and P-value (in parentheses)
Farm 2	9/21	21	81	5.1 ± 0.7	0.7	**1.21** (0.05)	-1.305 (0.08)	1.132 (0.31)
	9/28	21	81	4.3 ± 1.1	2	0.79 (0.85)	-0.723 (0.85)	0.889 0.82)
	10/05	22	73	12.9 ± 1.9	0.8	**1.93** (0.03)	**-1.992** (<0.00001)	**2.073** (<0.00001)
	10/12	21	43	3.7 ± 0.9	0.3	1.05 (0.41)	**-1.184** (0.03)	0.996 (0.41)
	10/19	18	22	3.5 ± 1.2	0.1	**1.44** (0.03)	**-1.480** (0.03)	**1.433** (0.03)
Farm 4	9/21	17	65	2.6 ± 0.6	1.6	0.86 (0.59)	-0.994 (0.31)	0.613 (0.85)
	9/28	13	85	7.7 ± 2.4	5	0.81 (0.69)	-0.786 (0.67)	0.878 (0.62)
	10/05	17	65	3.5 ± 0.7	3	0.65 (0.89)	-0.658 (0.90)	0.705 (0.87)
	10/12	18	72	8.0 ± 1.6	1.8	1.30 (0.18)	-1.346 (0.33)	1.478 (0.26)
	10/19	15	87	6.5 ± 1.6	0.3	0.92 (0.49)	-0.974 (0.49)	0.822 (0.56)
Farm 7	9/21	21	43	2.0 ± 0.3	0.5	1.48 (0.10)	-1.435 (0.15)	1.460 (0.10)
	9/28	21	90	5.2 ± 0.9	3.3	0.92 (0.56)	-0.952 (0.46)	1.073 (0.33)
	10/05	21	76	7.1 ± 1.7	1.7	**1.81** (0.03)	-1.691 (0.08)	**1.853** (0.03)
	10/12	21	81	6.5 ± 1.4	0.6	0.67 (0.92)	-0.684 (0.85)	0.567 (0.97)
	10/19	21	43	5.0 ± 1.3	0.3	1.00 (0.44)	-0.960 (0.36)	1.275 (0.08)

[1] Only mounds at which flies were observed
[2] I_a values in bold indicate aggregated spatial pattern ($P<0.05$)
[3] V_j values in bold indicate significant presence of gaps ($P<0.05$)
[4] V_i values in bold indicate significant presence of patchiness ($P<0.05$)
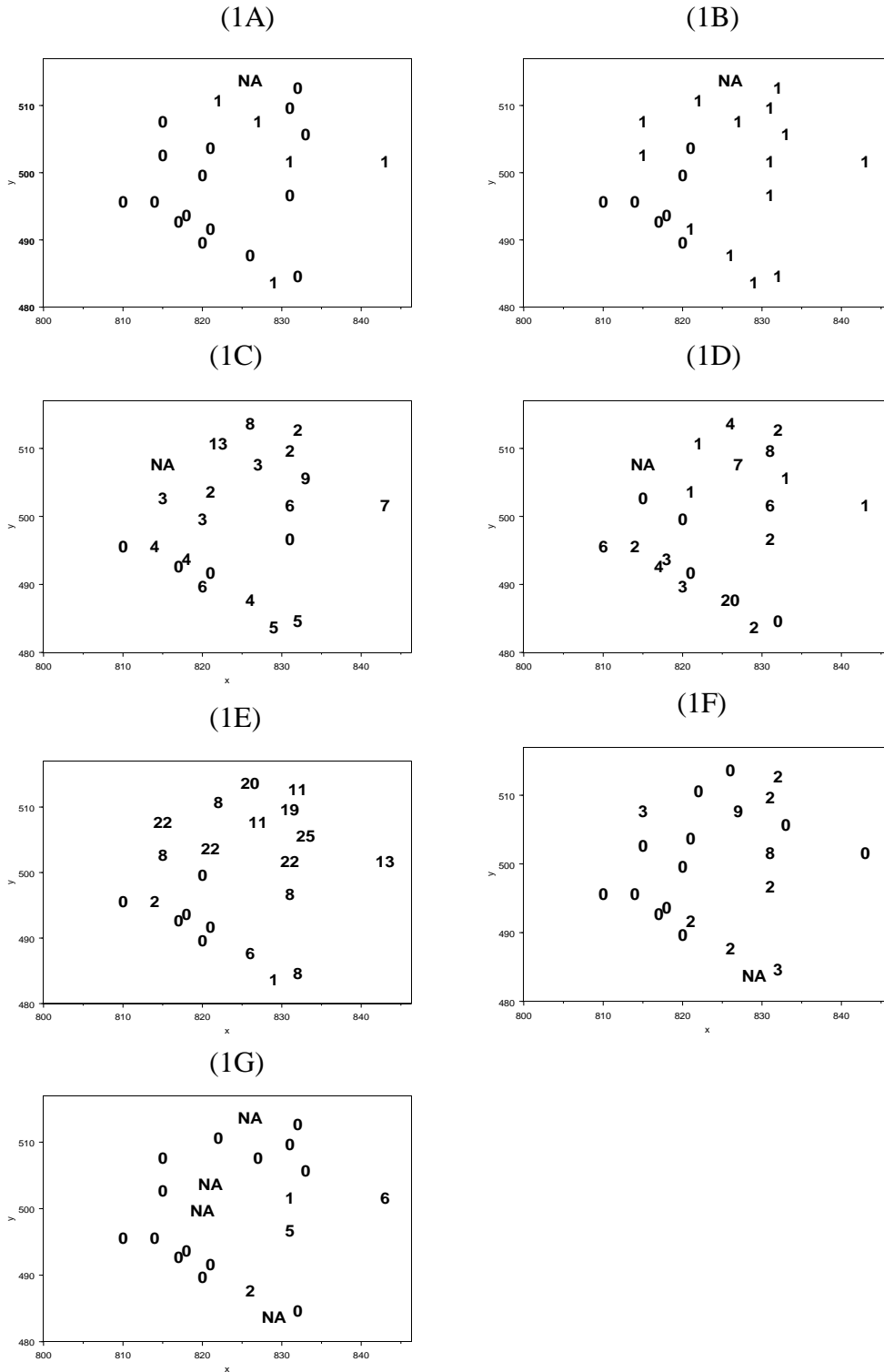
Figure 4.1: (A) Locations of *S. invicta* mounds infected with *T. solenopsae* (1=presence, 0=absence); (B) *S. invicta* social form (1=polygyne, 0=monogyne); (C (21 September), D (28 September), E (5 October), F (12 October), G (19 October)) time series of *P. tricuspis* abundances at disturbed *S. invicta* mounds at Farm 2, September-October 2005.
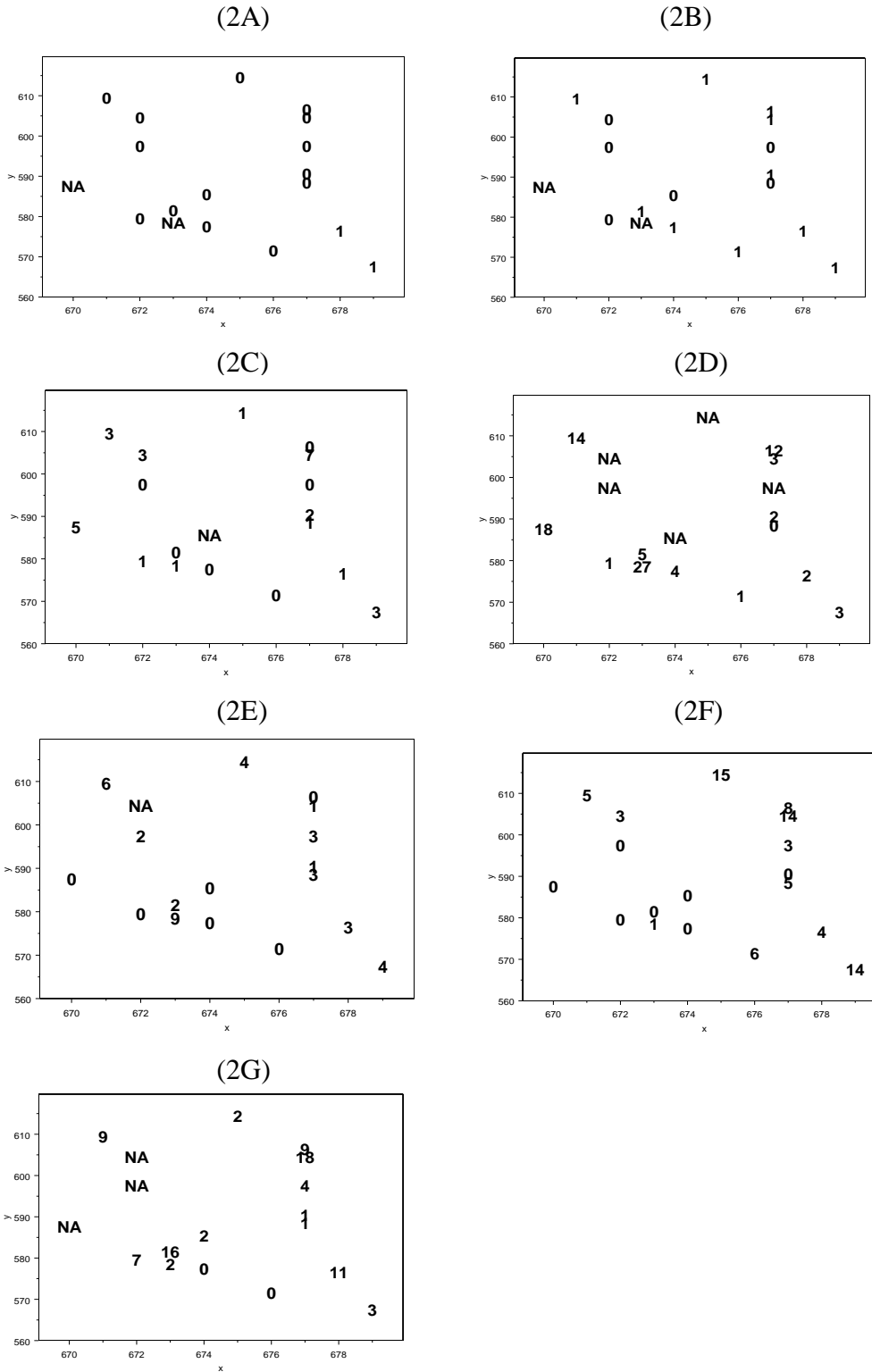
Figure 4.2: (A) Locations of *S. invicta* mounds infected with *T. solenopsae* (1=presence, 0=absence); (B) *S. invicta* social form (1=polygyne, 0=monogyne); (C (21 September), D (28 September), E (5 October), F (12 October), G (19 October)) time series of *P. tricuspis* abundances at disturbed *S. invicta* mounds at Farm 4, September-October 2005.
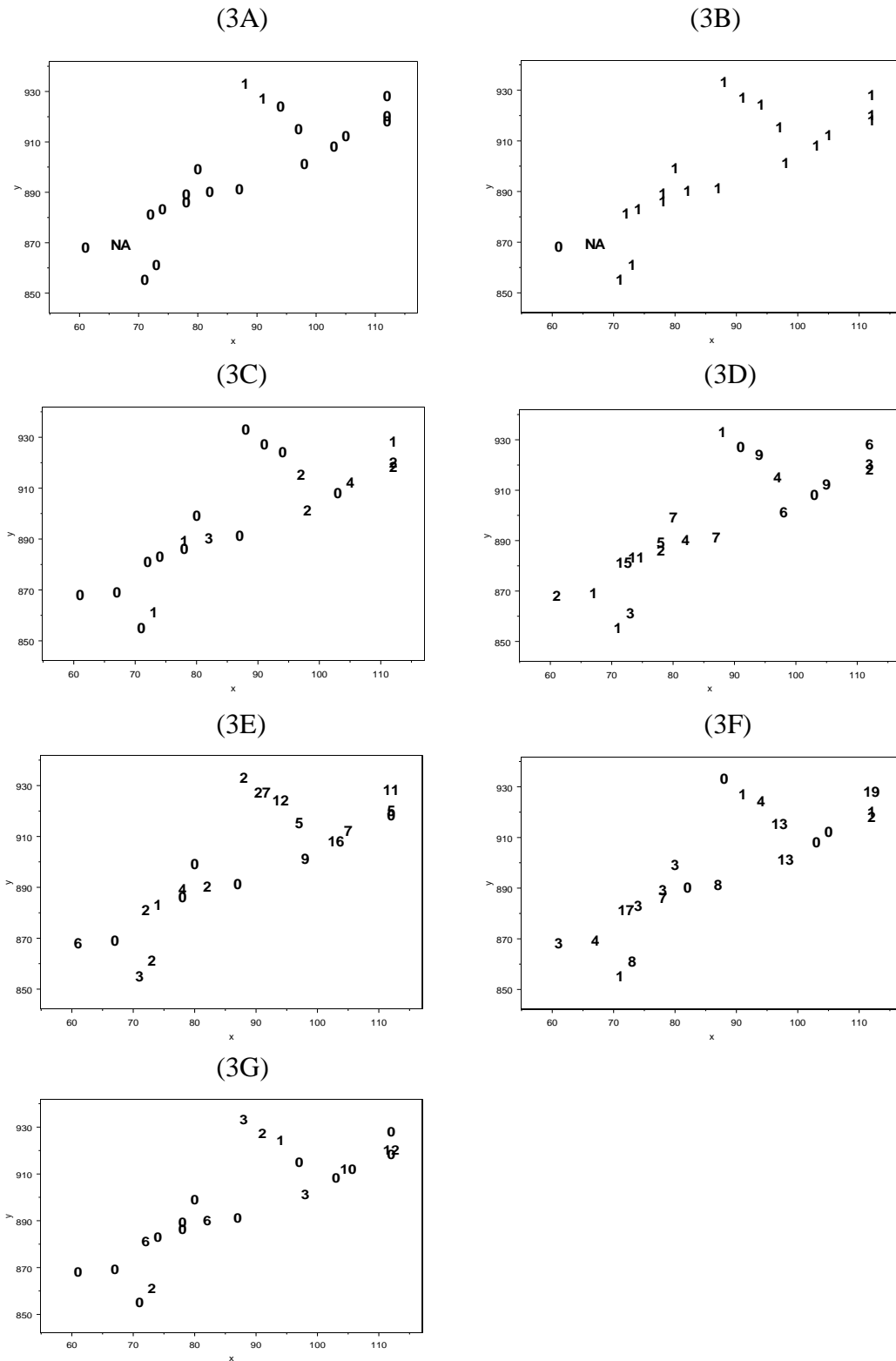
Figure 4.3: (A) Locations of *S. invicta* mounds infected with *T. solenopsae* (1=presence, 0=absence); (B) *S. invicta* social form (1=polygyne, 0=monogyne); (C (21 September), D (28 September), E (5 October), F (12 October), G (19 October)) time series of *P. tricuspis* abundances at disturbed *S. invicta* mounds at Farm 7, September-October 2005.

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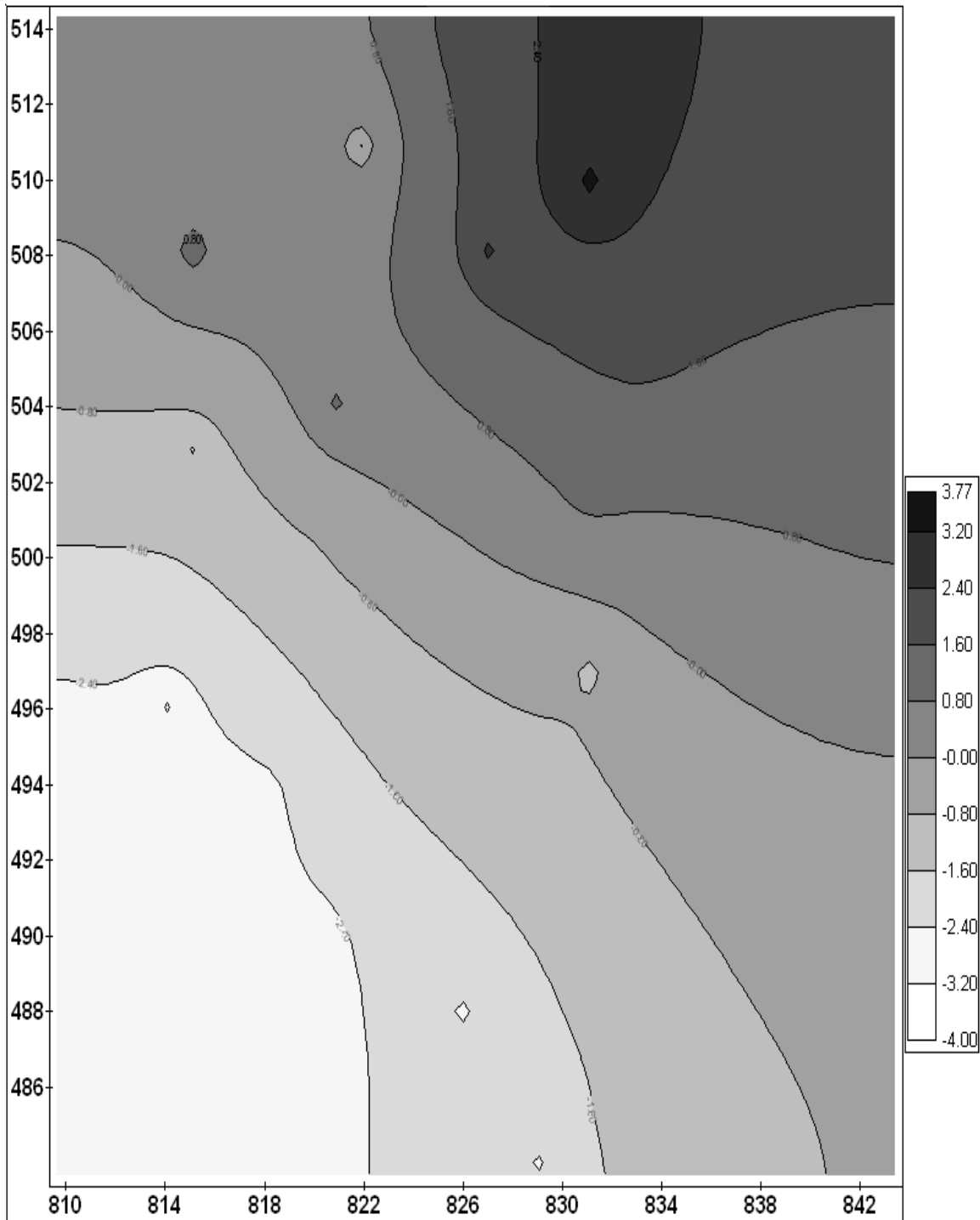
Figure 4.4: Contour maps showing areas of significant patches (darker grey) and gaps (lighter grey). A) Farm 2 (5 October 2005), B) Farm 2 (12 October 2005), C) Farm 2 (19 October 2005), D) Farm 7 (5 October 2005).

Figure 4.4 (con't)

(B)
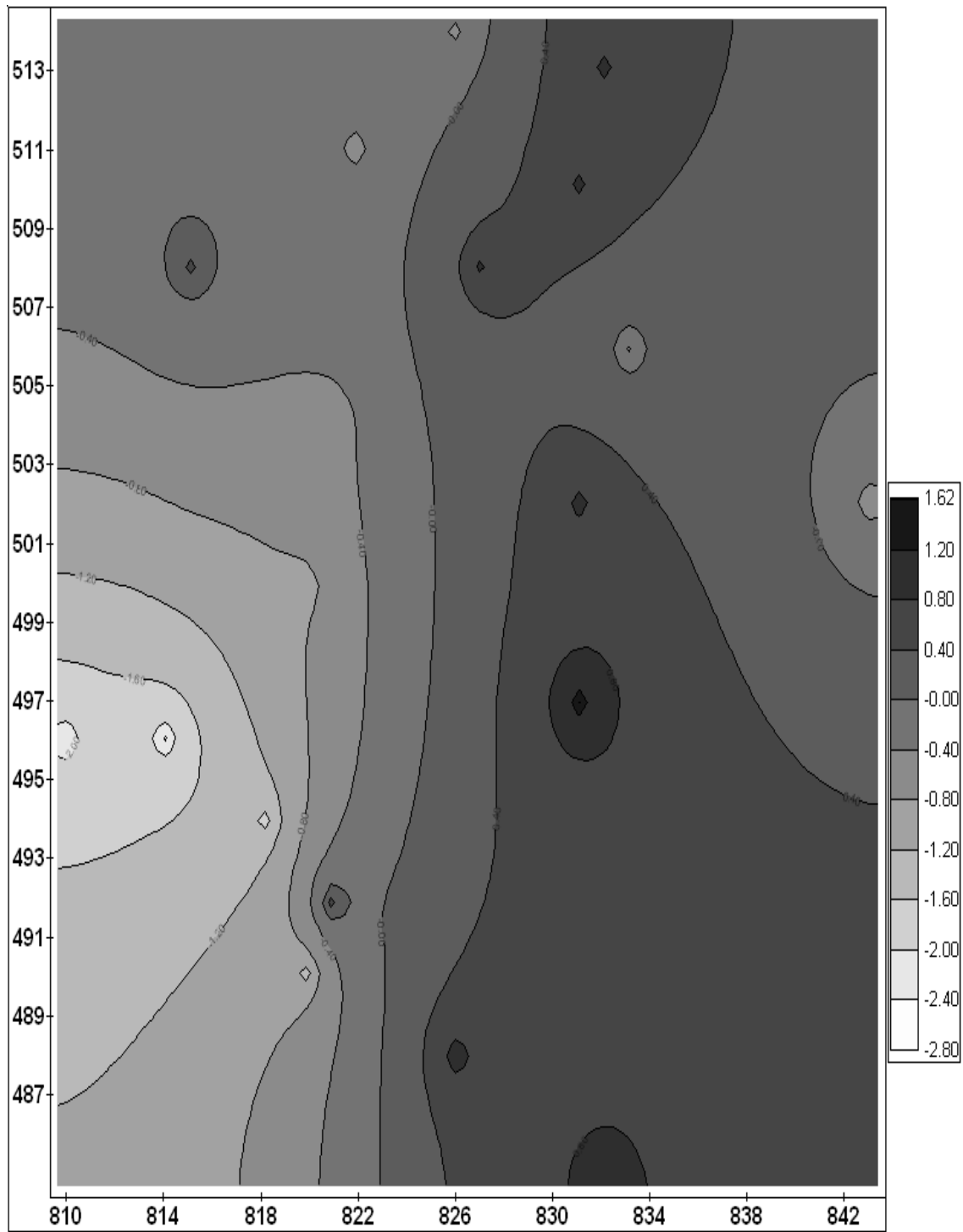
Figure 4.4 (con't)

(C)
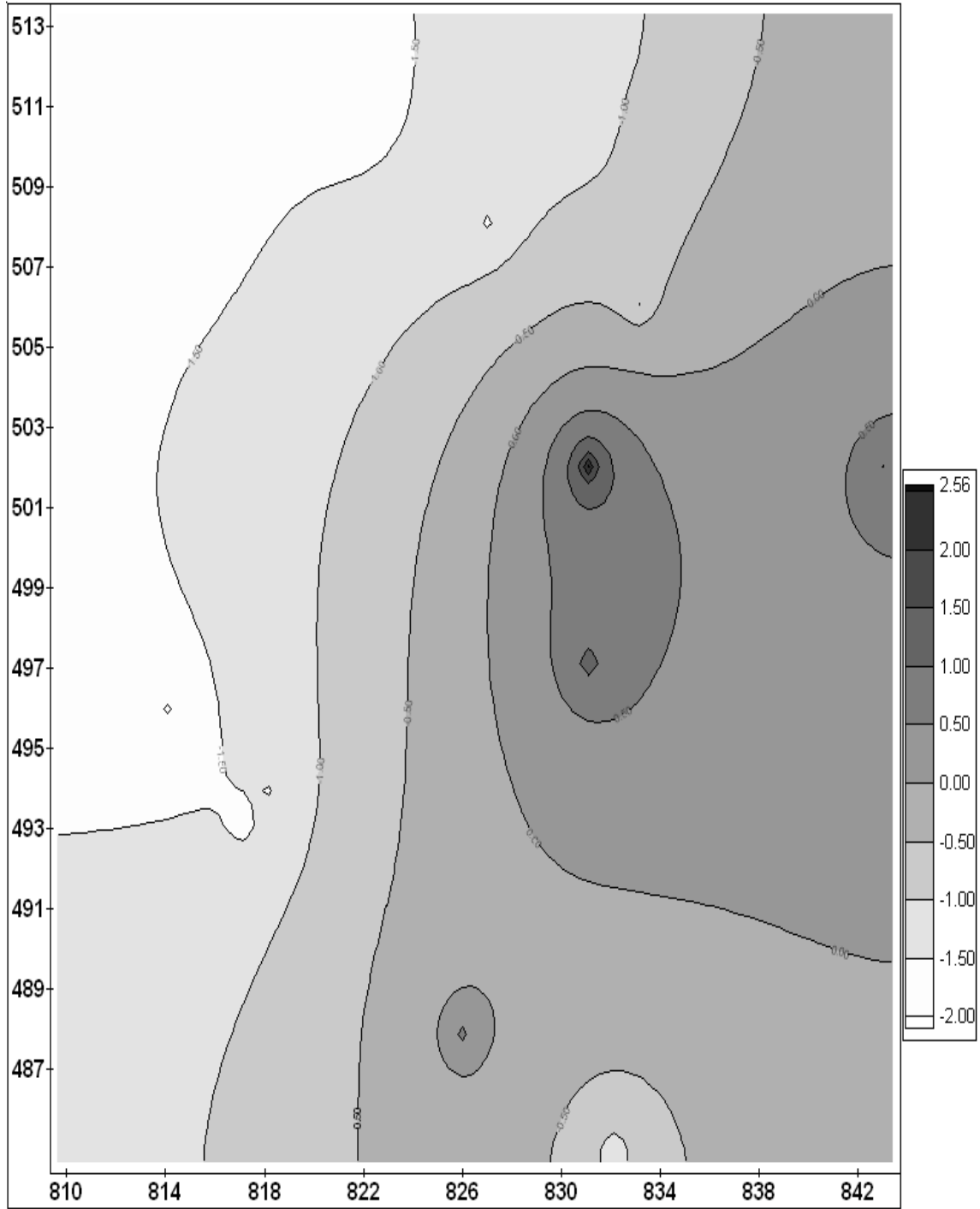
Figure 4.4 (con't)

(D)
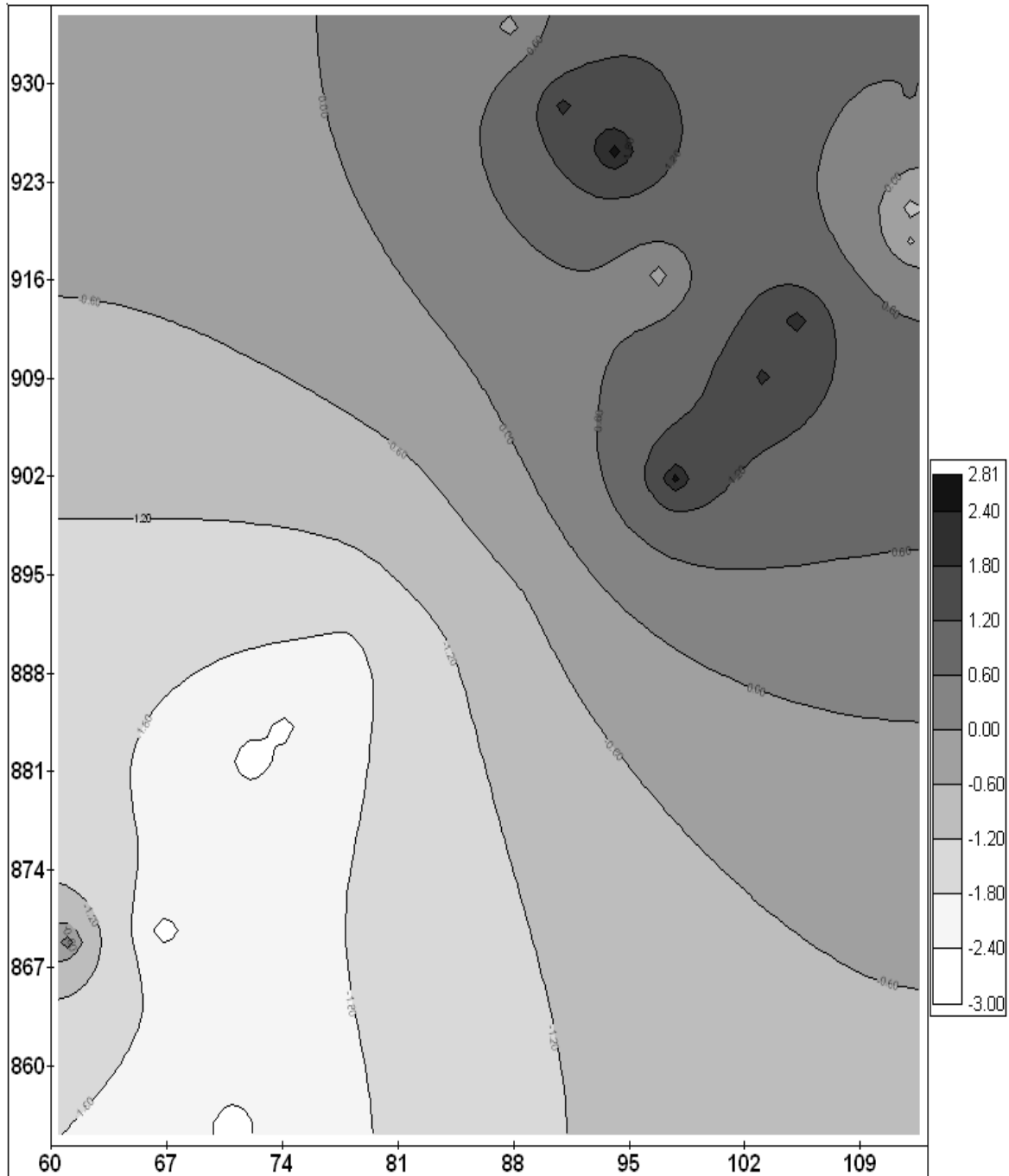
DISCUSSION

This study addressed several questions regarding spatial patterns of abundances of adult *P. tricuspis* on a spatio-temporal scale, and provides a detailed and informative perspective of *P. tricuspis* population structure on a local scale. The overall results suggest that *P. tricuspis* spatial patterns are generally random, but aggregations of high abundances occasionally occur in space. In this study, the significant positive spatial association found between *P. tricuspis* and polygyne social form at Farm 2 is difficult to interpret in biological terms. It may simply be a function of the dense population structure of polygyne *S. invicta* (Macom and Porter 1996). The number of host workers in an individual mound is not correlated with *P. tricuspis* abundances (Morrison and King 2004). However, Morrison and Porter (2005) found a positive correlation between mound area (m^2/ha) and *P. tricuspis* abundances in north-central Florida. Consequently, high host population density may translate into higher abundances of parasitoids in a direct density-dependent way.

Spatial theory predicts that patchy population distributions can arise even in continuous habitats through limited dispersal combined with host-parasitoid interactions (Maron and Harrison 1997). Certainly, *S. invicta* nest sites have limited dispersal, generally moving only a few meters (Pers. Obs.). Conversely, *P. tricuspis* can disperse several-hundred meters (see chapter 5) and can, therefore, respond to host semiochemicals at considerable distances. Our study sites were relatively homogeneous pastures with no evident patchiness in vegetation or other potential heterogeneities that could account for the significant gaps and patches of *P. tricuspis* that were identified at Farms 2 and 7. Other than the aggregated densities of polygyne *S. invicta*, there were no apparent landscape

features or environmental heterogeneity that would have potentially influenced abundances of *P. tricuspis*. Another study (Henne, unpublished data) found no correlation between *P. tricuspis* abundances and grass heights or soil moisture levels that were measured adjacent to disturbed *S. invicta* mounds. If landscape features were important, then we would expect to find some consistent temporal spatial structure in *P. tricuspis* populations. Instead, there is no consistent spatial structure of *P. tricuspis* local populations over time in homogeneous pastures. This is probably a consequence of the likelihood that *P. tricuspis* adults rarely live longer than a few days in nature (Porter et al 1995).

Morrison et al. (1999, 2000) studied the phenology of *Pseudacteon* parasitoids of *S. geminata* in central Texas and found that phorid abundances varied seasonally, with rainfall patterns possibly linked to these abundances. The last significant rainfall at the study sites prior to initiation of these surveys occurred on 29 August 2005, when Hurricane Katrina passed through southeastern Louisiana. No measurable rain fell near these study sites after 29 August 2005. The exception is Farm 4, where significant (>0.12 mm) rain fell nearby on 23-25 September 2005 (source: http://www.ncdc.noaa.gov/oa/ncdc.html). Morrison et al. (2000) also determined that soil moisture levels were often a good predictor of phorid abundance. Soil moisture levels at 10 cm depth did not rise after the Katrina rainfall until late September, a lag of approximately one-month. Abundances of *P. tricuspis* also appeared to positively respond to this rain event with a lag of approximately one month, corresponding to the development time required for *P. tricuspis*, approximately 38 days at 27° C (Folgarait et al. 2002).

We hypothesize that the rainfall associated with Hurricane Katrina (approximately 250 mm (http://www.ncdc.noaa.gov/oa/ncdc.html)) triggered widespread alate flight events after it passed through the area. Alate flight events by *S. invicta* are triggered by rain > 5mm following a period of dry weather (Markin et al. 1971, Morrill 1974). During alate flights, *S. invicta* workers swarm over the surface of the mound and adjacent vegetation in a heightened state of alarm (Markin et al. 1971), presumably to attack potential predators of alate reproductives as they leave the nest. In South America *Pseudacteon* phorids, including *P. tricuspis*, have been observed attacking fire ants swarming over mound surfaces during alate flight events (Pesquero et al. 1993). Consequently, high numbers of *S. invicta* workers may be vulnerable to attack by searching *P. tricuspis* females during alate flight events. Thus, the dynamics of *P. tricuspis* would be driven in a density-dependent manner in response to a greater availability of *S. invicta* workers during area wide alate flight events. This factor would account for the apparent synchrony in *P. tricuspis* population dynamics, particularly between Farms 2 and 7 (15 km apart). Farm 4 was located nearly 30 km away from the nearest study site (Farm 7). Large-scale spatial synchrony in animal population dynamics appears to be a general phenomenon among animal populations (Ranta et al. 1995, Heino et al. 1997). Additionally, local patchiness in alate flight events may also lead to aggregations of *P. tricuspis* in space.

The fact that *P. tricuspis* are attracted to disturbed mounds containing *T. solenopsae*-infected workers suggests that flies may not be able to differentiate between infected vs. non-infected ants from long distance cues. However, because only a few *S. invicta* colonies were infected with *T. solenopsae*, a more detailed spatial representation

was not possible. Studies to compare attack rates and/or survival of *P. tricuspis* on *T. solenopsae*-infected hosts have not been done, but would be interesting.

The results found in this study have provided several insights into the spatial structure and dynamics of *P. tricuspis* populations in a homogeneous habitat. First, it indicates that *P. tricuspis* counts have a random spatial distribution, but spatial aggregations occur when populations are high. Second, sampling *P. tricuspis* populations should be done with the distribution of *S. invicta* populations in mind, particularly where polygyne populations occur. Thus, any survey of *P. tricuspis* should attempt to sample a representative portion of the area. In a related study (Chapter 7) it was determined that a minimum of 15 mounds should be sampled to achieve an estimate of the phorid population mean that is reasonably close to the true population mean. At peak populations, significant patches and gaps in *P. tricuspis* abundances at *S. invicta* mounds can occur. Therefore, phorid sampling should be conducted in several widely separated locations to ensure that spatial heterogeneity in phorid populations can be accounted for.

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CHAPTER 5

QUANTIFYING LOCAL MOVEMENT OF THE FIRE ANT DECAPITATING FLY, *PSEUDACTEON TRICUSPIS* BORGMEIER (DIPTERA: PHORIDAE), FROM POINT RELEASE EXPERIMENTS

INTRODUCTION

As a basic process, it is well-known that organisms move at some point in their lifetimes, also known as dispersal. The term dispersal can have different meanings, but commonly refers to a form of population redistribution that describes any movement of organisms away from a source aggregation or population (Freeman 1977, Southwood 1978, Turchin 1998, Nathan et al. 2003). Entomologists and ecologists have been interested in quantifying dispersal of insects and other organisms ever since Skellam's (1951) classic theoretical treatment of dispersal. Quantifying dispersal of insects is fundamental to an understanding of insect population dynamics (Osborne et al. 2002), since local insect population abundances, their spatial structure, genetic structure and long-term persistence rely on aspects of movement (Turchin 1998).

Methods of studying dispersal in insects include the recapture or observation of members of a population released from a single point at a single time, also known as an instantaneous point release (Plant and Cunningham 1991, Turchin 1998, Cronin et al. 2001). This method is often used in mass-release-recapture or mark-release-recapture studies, and enables the researcher to identify apparent directional components of the dispersal pattern, such as would be caused by prevailing winds or spatial anisotropy (Plant and Cunningham 1991). It also enables the researcher to determine the rate at which individuals are moving to predict future spread.

Conceptual approaches to modeling dispersal depend on whether data are collected with the goal of describing movements of individual organisms (Lagrangian), quantifying population redistribution from a point in space (Eulerian) and/or long-distance dispersal (Turchin 1998, Nathan et al. 2003). The simplest model of movement

of organisms assumes a homogeneous environment and that individual movement is random (Kareiva 1983), and can, therefore, be modeled by a simple diffusion equation. Many forms of the diffusion equation exist, often in the form of partial differential equations (Turchin 1998), depending on the degree of heterogeneity of the environment, rates of loss of individuals from the population, and degree of departure of movement from random (see Turchin and Thoeny 1993, Turchin 1998). It is often assumed that dispersal from the release point will be radially symmetrical according to an exponential or normal curve (i.e. Gaussian) (Plant and Cunningham 1991).

Important opportunities for studying dispersal over geographic scales are presented when parasitoids are released during biological control programs (Godfray 1994). In most classical biological control programs, natural enemies are released at a few locations in their new environment, and then are expected to disperse on their own to locate and colonize suitable habitats (Hastings 2000, Sallam et al. 2001). However, because of their small size, limited information is available on flight behavior and mobility of biological control agents, including parasitoids (Godfray 1994, Corbett and Rosenheim 1996, Bellamy and Byrne 2001). This information could be useful when determining the number and proximity of multiple releases of biological control agents. For instance, Allee effects and natural enemy movement have been shown to be important for the successful establishment of introduced biological control agents (Hopper and Rousch 1993). Natural enemy dispersal distances from a release point should be far enough to discover hosts near the release area, but not so far that they disperse into areas that lack suitable hosts (Hougardy and Mills 2006).

Parasitic flies of the genus *Pseudacteon* have been introduced to the United States for biological control of the exotic red imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Numerous releases of *P. tricuspis* Borgmeier have been conducted in the United States (Henne et al. 2007a and references therein). However, no detailed studies of phorid fly dispersal have been attempted (Disney 1994), and no methodology for quantifying and modeling dispersal of *Pseudacteon* spp have been developed, despite many opportunities associated with the introduction of *S. invicta*-specific parasitoids. At least two studies have only given us limited insight into *Pseudacteon* dispersal. Using trays baited with *S. geminata* workers, Morrison et al. (1999) found that *Pseudacteon* parasitoids in Texas dispersed up to 650 m from the nearest *S. geminata* population. Henne et al (2007b) found that *P. tricuspis* established populations across the Mississippi River in Louisiana from populations more than 1 km away on the other side. However, no information regarding dispersal rates and population redistribution patterns or whether *Pseudacteon* dispersal fits the theoretical expectations of random diffusion is available, necessitating exploratory research into this area. In the case of *P. tricuspis* dispersal the assumption that individual movement is random and undirected may be violated because these flies orient toward alarm pheromones emitted by their hosts. This problem may be mitigated, however, provided that pheromone sources are sufficiently far apart and overlap between respective areas of attraction is minimal (Turchin 1998).

The study reported here is the first to address the above cited deficiencies in our knowledge regarding phorid fly dispersal. The objective of this study was to determine the following features of *P. tricuspis* dispersal within a generation by performing mass-

release-resighting experiments: 1) Numbers of *P. tricuspis* at various distances from a central release point at 30 minute time intervals, up to two hours after release, and 2) determine the redistribution patterns of *P. tricuspis* dispersers and fit a diffusion curve to the dispersal data. Data of this sort are useful in understanding animal movement behavior, and is necessary to develop predictive models of species spread (Turchin 1998).

MATERIALS AND METHODS

Study Organism

Pseudacteon spp. are thought to be an important factor in lower population densities of *S. invicta* in South America compared to the United States (Porter et al. 1992), and consequently may similarly suppress *S. invicta* populations in the United States. However, North American species of *Pseudacteon* that attack native North American fire ants, *S. geminata* (F.) and *S. xyloni* McCook, do not attack *S. invicta* (Porter et al. 1995). Hypothetically, native ant communities in the United States that have been displaced by *S. invicta* may rebound by reuniting *S. invicta* with several of its native *Pseudacteon* parasitoids (Porter 1998).

Phorid parasitoids find hosts by sensing volatilized ant semiochemicals (Porter 1998, Morrison and King 2004). For instance, *P. tricuspis* responds to *S. invicta* alarm pheromones that are emitted during mound disturbances, alate flights, and intra- and interspecific fighting (Williams et al. 1973, Pesquero et al. 1993, Morrison and King 2004). Female *Pseudacteon* insert an egg into the host ants' thorax, the maggot consumes the head contents over several weeks and eventually pupariates inside the decapitated head capsule (Porter et al. 1995).

There is strong evidence that parasitic phorid flies mediate competitive interactions between various ant species (e.g. Feener 1981; Feener and Brown 1992; Folgarait and Gilbert 1999; Morrison 1999, 2000; Orr et al. 1995, 2003). *Solenopsis* workers often reduce or terminate foraging activity whenever *Pseudacteon* flies are present (Feener and Brown 1992, Orr et al. 1995, Morrison 1999) with significant impacts on colony growth. For instance, a single attacking *P. tricuspis* female per 200 foraging *S. invicta* workers was shown to decrease colony protein consumption almost two-fold, and significantly reduced numbers of large-sized workers 50 days later (Mehdiabadi and Gilbert 2002). These studies demonstrate the potential for *Pseudacteon* parasitoids to reduce North American *S. invicta* populations (but see Tschinkel 2006 for a discussion of the limited potential for biological control of *S. invicta* in the United States).

<u>Pre-trial Dispersal Surveys</u>

During 2-16 June 2005, a *P. tricuspis* release was performed in a cattle pasture 14 km south of Natchitoches in Natchitoches Parish, Louisiana (31° 37′ 57″ N, 93° 4′ 7″ W) in an attempt to re-establish this species at this location (see Henne et al. 2007a). Measurement of *P. tricuspis* dispersal from release areas were conducted on three occasions during the release period by vigorously disturbing *S. invicta* mounds variable distances up to 200 m away from the release areas for two hours after flies were released. Dispersal measurements were conducted at 2-3 day intervals and in areas that were widely separated (>500m) from previous releases to allow for mortality and natural dispersal of previously released flies. Longevity of adult *P. tricuspis* under natural

conditions is unknown, but adults live only a few days in the laboratory (Henne and Johnson, Unpublished data).

<u>Dispersal Trials: Experimental Design</u>

An important requirement of mass-release-recapture studies is that a sufficient number of insects be released to enable adequate resighting frequencies for statistical analyses (Cronin et al. 2001). However, when high densities of insects are released at a single point, biased estimates of movement rates or patterns can result if movement is density-dependent (Turchin 1998). At high release densities, agitation dispersal may cause insects to disperse more widely, or movement paths to become more directed in order to minimize intraspecific encounters (Cronin et al. 2001). Whatever the biological reasons for dispersal, the result is that the population density decreases with increasing distance from the central release point in a manner similar to Brownian motion, and the data set consists of measurements of density at several points in space and time (Freeman 1977, Turchin 1998).

Four dispersal experiment trials were conducted during September and October 2005 at Montpelier in St. Helena Parish, Louisiana (30⁰ 40′ 22″ N, 90⁰ 38′ 18″ W), in an unmaintained cattle pasture that was relatively homogeneous (Table 5.1). An attempt to establish *P. tricuspis* at this location during the fall of 2000 failed as repeated post-release surveys failed to detect the presence of this species (see Henne et al. 2007a). The experimental design for evaluating *P. tricuspis* dispersal was constructed according to the following configuration: Clear plastic trays [Pioneer plastics, Inc. P.O. Box 6, 1584 Hwy 41A, North Dixon, KY 42409 #395 C (31.25 x 25.4 x 9.5 cm)] were dusted with talc to prevent escape of *S. invicta*, and were shaded with a 30 cm Styrofoam plate that was

pierced with a wire survey flag. A sheet of 21.25 cm x 27.5 cm white paper was placed underneath each plastic tray to provide a contrasting background to observe flies. Four trained observers placed these trays in a radial-pattern [similar to a design in Turchin and Thoeny (1993) and Turchin (1998) (p. 31) for quantifying southern pine beetle dispersal] at incremental distances from the release point (Figure 5.1).

Note in Figure 5.1 that only two trays are placed in the first annulus and four in each of the five succeeding annuli. Using this arrangement minimizes the potential for trays closer to the release point to compete for all of the flies that are released. The trapping grid should extend far enough to sample a substantial proportion of disperser end points, which should ideally enclose 90–95% of dispersers; however, some extrapolation beyond the recapture grid may be necessary (Turchin 1998). Attempting to maximize recapture of released organisms should not be the goal of a dispersal study, but, instead, the aim is to obtain a reasonable estimate of the spatial density of organisms (Turchin 1998). The goal of recapturing flies is to obtain an estimate of the spatial density of flies to compute density-distance curves, and the estimates serve as basic data for fitting various spatial movement models (Southwood 1978, Sutherland 1996).

There is the possibility that trays nearest the release point may attract a disproportionate number of flies, thereby depleting numbers that would otherwise reach more distant trays, and the resulting density-distance curve would be deformed from its true shape. Trays nearest the release point should attract no more than a few percent of released flies, especially when pheromones are being used to study dispersal. By placing trays that contain ants emitting pheromones too close to the release point, then it is

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ensured that most flies will be primed to respond to the pheromone and end up being

attracted to those trays, and, thus, one cannot reach any conclusions about dispersal.

Table 5.1: Dispersal trial dates at Montpelier, Louisiana, numbers of *P. tricuspis* released and resighting distances.

Trial	Date	# *P. tricuspis* released	Resighting annuli (m)
1	26 September 2005	705	25, 50, 100,150, 200, 250
2	4 October 2005	408	5, 10, 15, 20, 25, 30
3	13 October 2005	310	5, 10, 15, 20, 25, 30
4	17 October 2005	750	10, 20, 30, 40, 50, 60
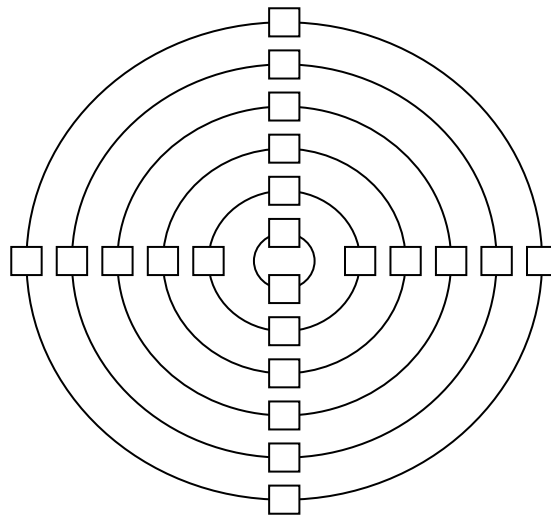
Figure 5.1: *Pseudacteon tricuspis* experimental design layout. □=trays containing nonnestmate *S. invicta*.

At the start of the field trials, preweighed ants (~0.5g each in 20-dram vials) from

two unrelated monogyne *S. invicta* laboratory colonies obtained from the Louisiana State

University Agricultural Experiment Station in St. Gabriel, Iberville Parish, Louisiana (30⁰

16′ N, 91⁰ 05′ W) were poured into each tray. In this approach, interspecific aggressive

interactions involving the release of alarm pheromones that are attractive to *P. tricuspis*

(Morrison and King 2004) were exploited. At 30, 60 and 90 minutes post-release, an additional 0.25g of *S. invicta* workers from each colony were added to each tray to maintain alarm pheromone production. Also, every time ants were poured into the trays, a portion of the ants within all trays were crushed to release additional alarm pheromones. Potential alarm pheromone contributions from the resident *S. invicta* population to dispersal of *P. tricuspis* in this study are unknown but were assumed to be negligible, provided nests were not disturbed during the experiments.

Flies were transported inside a Plexiglas cage (40 x 30 x 35cm) from the laboratory to the dispersal area. Ten plaster blocks saturated with water were placed inside the cage to maintain humidity near 80%. The cage was also placed inside a black plastic bag to limit flight activity. The cage was large enough to accommodate the flies so that confinement and agitation was minimized. As flies that are held in cages may exhibit unusually high levels of activity and movement, termed agitation dispersal (Turchin 1998), flies to be released in each experimental trial were also held in darkness or shade, for at least 30 minutes before release during which time the experimental layout was measured and trays set out.

At the start of the dispersal field trials, the cage was placed on the ground at the center of the experimental arena. The plastic bag was removed, the container lid was opened, and flies allowed to exit and disperse on their own to locate hosts. Flies were released in the center of the experimental area at approximately 1030-1100 h (CDT). All trials were conducted at temperatures >21° C, as this is considered to be the lower flight threshold for *Pseudacteon* spp. (Morrison et al. 1999).

Four release trials were conducted during September and October 2005. Numbers of flies/release ranged between 310-750 individuals (Table 5.1), because the density of flies that are released may affect movement patterns (Cronin et al. 2001). Additionally, these experimental release densities are similar to daily release densities when attempting to establish *P. tricuspis* in Louisiana (see Henne et al. 2007a). In view of the fact that sources of all previous releases of *P. tricuspis* in Louisiana have been laboratory-bred individuals, lab-reared *P. tricuspis* were used for all field experiments.

Flies were counted at 30, 60, 90 and 120 minutes post-release. Each observer would count flies at each tray, starting from the center to the edge of one transect direction. Individual observers counted flies along each transect direction only once during each trial, and would switch to a different transect during each successive count. No attempt was made in the field to determine sex ratios of flies. However, flies were collected on two occasions and sex identifications were later made in the laboratory (see below). The following meteorological variables were recorded at the release point at the time of release and just prior to each data collection period: air temperature, relative humidity, and dew point were recorded at 30 cm above ground, and wind speed and direction averaged over a 1-min period at 1.5 m above ground (Morrison et al. 2000) with a handheld digital weather instrument (Speedtech Instruments® Skymate Plus Wind Meter SM-19, Forestry Suppliers Inc. Part No. 2320).

Dispersal Distances of Fly Sexes

To determine if there are differences in average distance dispersed by fly sex, a single release trial was performed in a cattle pasture approximately 9 kilometers east of Norwood in East Feliciana Parish, Louisiana (30⁰ 59′ 8" N, 91⁰ 00′ 55" W) on 6 October

2005. A resident population of *P. tricuspis* was present in the area, having been established at this location during 2000 (Henne et al. 2007a). In order to distinguish experimental flies from resident *P. tricuspis*, experimental flies were marked with a light dusting of pink fluorescent pigment (DayGlo Color Corporation, Cleveland, Ohio). Previous laboratory experiments showed no differences in behavior or longevity between marked and unmarked flies (Henne and Johnson, Unpublished data).

Approximately 425 flies were released at 1100 h (CDT) in the same manner as described for the release trials at Montpelier. After one hour had elapsed, resident *S. invicta* colonies surrounding the release point were disturbed to attract *P. tricuspis*. A total of eight mounds were sampled, ranging from approximately 5 to 50 m from the release point. A light breeze (10km/hr) was blowing from the north and the temperature was 30° C. All flies that appeared at disturbed mounds were aspirated into individual vials and transported to the laboratory where they were frozen for later examination under a stereomicroscope to determine sex and marked vs. unmarked. Additionally, at the termination of dispersal trial #4 at Montpelier on 17 October 2005, all resighted flies at the trays were aspirated into individual vials and also brought to the laboratory as above to determine fly sex. In both trials, the average distances (in m) traveled by individuals of each sex were computed and differences were assessed using a paired t-test.

<div align="center">Statistical Analysis</div>

One intention of the mass-release-recapture experiments was to obtain information on the density distribution of flies during each census period. Figures were generated to show the numbers of resighted *P. tricuspis* in each annulus away from the

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release point during each census and the average of the censuses. The average resightings were calculated for each trial by simply taking the number of resighted flies divided by the number of trays in each annulus.

Description of Density-Distribution Curve and Fit of Dispersal to a Null Diffusion Model

Statistical analyses of insect dispersal typically avoid assumptions about any explicit model describing the dispersal process, but instead focus on estimating statistical parameters that explain the pattern of dispersal when the data are viewed as a frequency distribution (Plant and Cunningham 1991). Prior to fitting the density-distribution data to a model, methods that test for drift or non-randomness in the direction of dispersal are usually employed (Turchin 1998). Detecting drift is done by calculating the mean and variance of the spatial points where each individual is observed. By using a symmetric arrangement of spatial points to resight organisms, and assuming that drift is not significant, the expected mean displacement is then zero. Thus, the null hypothesis that drift is not significant can be tested with a t-test by determining if the mean x- and y-coordinates of resighted flies are significantly different from zero (x,y=0 at release point) (Turchin and Thoeny 1993, Turchin 1998). Also, drift is significant if the 95% confidence intervals of the mean x and y coordinates do not overlap the origin (Cronin et al. 2001). If drift is significant, the origin is reset as the mean x- and mean y-coordinates (Turchin 1998, Cronin et al. 2001). The following formula was used to compute the x-component of the average displacement of resighted flies during the census period of maximal resighting (Turchin and Thoeny 1993):

$$X_j = \frac{\sum_{i=1}^{n} x_i r_{ij}}{\sum_{i=1}^{n} r_{ij}} \qquad (1)$$

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Where r_{ij} is the number of resighted flies in tray i during replicate j, x_i is the x coordinate of the location of tray i, and n is the number of trays. The y-component was computed in the same manner. Trial 1 was excluded from analysis because it had insufficient data. Therefore tests for drift were conducted for trials 2-4. Trial four had identical numbers of maximal resightings during two consecutive census periods, and drift was computed for both periods. As x-component drift was significant for the two census periods in trial 4 (see results), the x-coordinate of the origin was computed as the average from the two census periods.

Quantitative analysis of density-distance data is normally accomplished by fitting the data to a density-distance curve (Turchin 1998). The null model is a Gaussian curve, which is one particular solution of a simple diffusion equation, and describes the instantaneous density-distribution in space based on a point-release (Turchin 1998). If a normal curve fits the data sufficiently then it is concluded that the movement pattern of the organism can be approximated by simple diffusion in a homogeneous environment (Turchin 1998). Methods for testing simple diffusion are given in Karieva (1983), Turchin (1998) and Cronin et al. (2001).

An approach similar to that described in Cronin et al. (2001) for determining diffusion of a stem galling fly, *Eurosta solidaginis* (Diptera: Tephritidae), was employed here to determine the diffusion rate of *P. tricuspis*. This experiment provided information on the density distribution of *P. tricuspis* at fixed points in time (i.e. 30, 60, 90, 120 minute post-release censuses). The null diffusion model that was tested is as follows:

$$N_r = Ae^{-r^2/B} \tag{2}$$

Where $A=\Phi N_o/4\pi Dt$, Φ is a scaling parameter that depends on observer resighting efficiency, N_o is the number of *P. tricuspis* released, D is the diffusion rate, and t is time since release. The parameter B is equivalent to $4Dt$ and was averaged over the four census periods. The diffusion rate, D, is estimated from the mean square displacement of released individuals M divided by $4t$, where t is time since release (Kareiva 1982, 1983; Turchin 1998; Cronin et al. 2001).

The diffusion model (equation 2) assumes that the diffusion rate for each census period is constant when organisms are repeatedly sampled over time (Kareiva 1982, 1983; Turchin 1998). To test this assumption, the mean square displacement of released individuals, and the diffusion rate for each 30 minute census period in trials 2-4 were calculated. Decreasing or increasing trends in diffusion rates over time were computed with Pearson's product moment correlations between diffusion rates per census period and census period (Sokal and Rohlf 1995, Cronin et al. 2001). If no significant trend was found, then the average diffusion rate D was computed. The null diffusion model (equation 2) has the linear form as follows:

$$\ln(N^r) = \ln(A) - r^2/B \qquad (3)$$

The linear form of the null diffusion model (equation 3) was fitted using a least-squares regression (Sokal and Rohlf, 1995) according to the following procedure: The sum of individual ant trays in each of the second and subsequent annuli was approximately 1 m^2 in area. However, as the innermost annulus had half the number of trays as the outer annuli, the numbers of resighted flies in the first annulus were doubled. Next, the number of flies resighted per m^2 (N_r) at each distance category was calculated by dividing the number of resighted flies by the area of the annulus upon which r is

based. Estimates of N_r used resighting data from the average of the four census periods for each trial. In cases where there were zero resightings at distances, 0.01 was added to each value of N_r and then those values were natural log-transformed. Separate least-square regression analyses were performed for trials 2-4.

Estimates of A and B for trials in which sufficient numbers of *P. tricuspis* were resighted during a census period were used to generate the expected Gaussian distribution of resighted flies (in 2-d space) (Cronin et al. 2001). From this distribution, the standard deviation (σ) and the 50% (=0.674σ) and 95% (=1.96σ) quantiles were calculated. The radius of a circle (r) containing those proportions of flies is represented by these quantiles. All statistical analyses were performed using Prism® 4.03 (GraphPad Software, Inc., San Diego, CA).

As previously mentioned, a high density of confined organisms may cause density-dependent dispersal. Many insects have initially high diffusion rates that decline as time progresses. Density-dependent dispersal can be tested by releasing flies at various initial densities. Alarm pheromones may also affect movement of flies, leading to directional attraction and flight arrestment. Trays containing alarm pheromone-releasing ants may increase by orders of magnitude the numbers of flies that are attracted. As the distance from the release point increases, the numbers of flies that reach that distance will be spread over a progressively greater area due to the area dilution effect (Turchin 1998).

RESULTS

Dispersal at Natchitoches Releases

Flies were resighted at distances up to 185 m from the release areas within two hours of release. At lower wind speeds (<10 km/h), flies were recaptured at disturbed *S.*

invicta mounds upwind and downwind from the release areas. However, on one occasion when releases were being conducted, only one fly was were resighted 25 m upwind, compared with 100 and 175 m downwind. During this release, wind speeds >20 km/h were recorded.

<div align="center">Montpelier Dispersal Trials</div>

Tests for drift indicated significant westward (x-component) displacement during the periods of maximal resighting in trial 4 only (@ 60 minutes t=2.66, df=23, p=0.01; @ 90 minutes t=2.99, df=19, p=0.008) (Figure 5.2). The prevailing wind was toward the southeast, indicating that *P. tricuspis* dispersers may have been flying upwind toward *S. invicta* pheromone sources to the west-northwest. Although not significant, there was also some displacement to the north of the release in trial 3. The prevailing wind during trial 3 was towards the south (0-5 km/h), indicating that *P. tricuspis* may have been flying upwind toward *S. invicta* pheromone sources to the north of the release point.
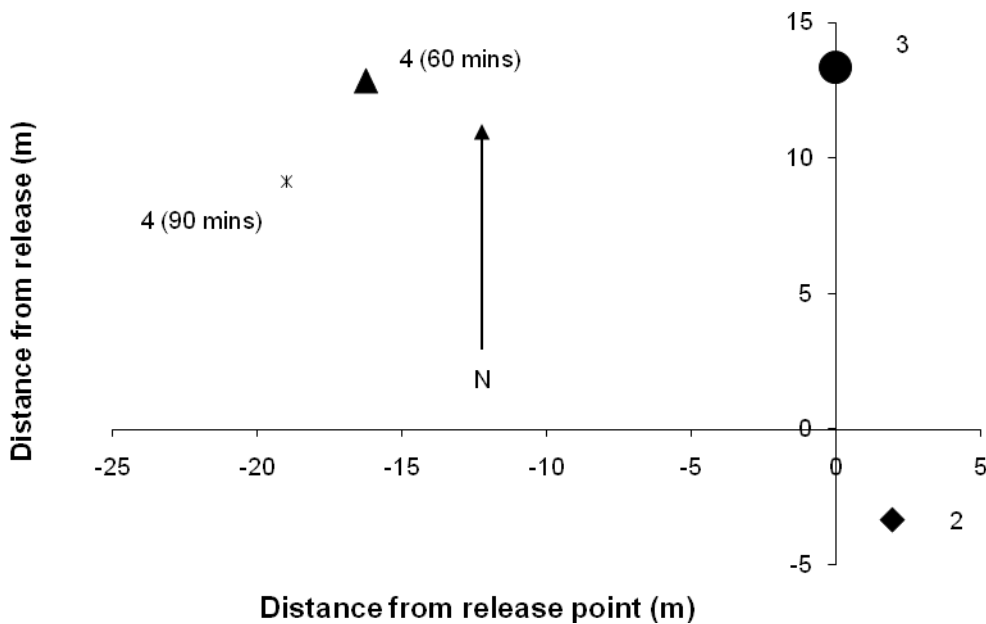
Figure 5.2: Mean displacement of *P. tricuspis* during the period of maximum resighting for trials 2-4.

<u>Recapture Rates and Fit of Movement to a Null Diffusion Model</u>

The numbers of *P. tricuspis* resighted at each time interval following release and the maximum percentage resighted are summarized in Table 5.2. In general, about 5% of flies were resighted. In trial 1, a maximum of only four flies of 705 released were resighted. Most resighted flies were observed at the 50 m annulus after 90 minutes, but a single fly was resighted at 150 m, two hours post-release (Figure 5.3A). In trials 2 and 3, the majority of flies were resighted within 15 m of the release point, but several flies were observed at 25-30 m (Figure 5.3B, C). In trial 2, most flies (70%) were resighted at trays along the east-west axis. The prevailing wind in trial 2 was approximately 10 km/hr^{-1} and blowing towards the west-northwest, as a result flies may have been orienting to pheromone sources both upwind and downwind from the release point. In trials 2 and 3, no flies were resighted at the 20 m annulus until 120 minutes post-release. In contrast, in trial 4 the highest resighting frequencies were at 20 m from the release point (Figure 5.3D). However, as indicated above, there was significant drift in this trial.

Table 5.2: Results of dispersal trials at Montpelier, Louisiana: September-October 2005.

Trial	*N* released and max. resighted per census (30, 60, 90, 120 minutes)	% max. resighted	Diffusion rate[a] ($D \pm SE$)	D_t vs t (p-value)
1	705 (0, 1, 4, 4)	0.5	*	*
2	408 (24, 19, 20, 18)	6	58.1 ± 10.3	-0.95 (0.05)
3	310 (11, 14, 13, 21)	7	58.0 ± 7.6	-0.74 (0.26)
4	750 (9, 31, 31, 22)	4	283.7 ± 99.8	-0.91 (0.09)

*=insufficient data
[a] Diffusion rate in m^2/h

The diffusion rates estimated for each 30 minute census period tended to decline over time in trials 2-4. However, Pearson product moment correlations were marginally

significant in trials 2 and 4 (Table 5.2). Diffusion rates at the 30 minute censuses were nearly twice as high as the 60 minute census, but stabilized thereafter.

(A)

(B)
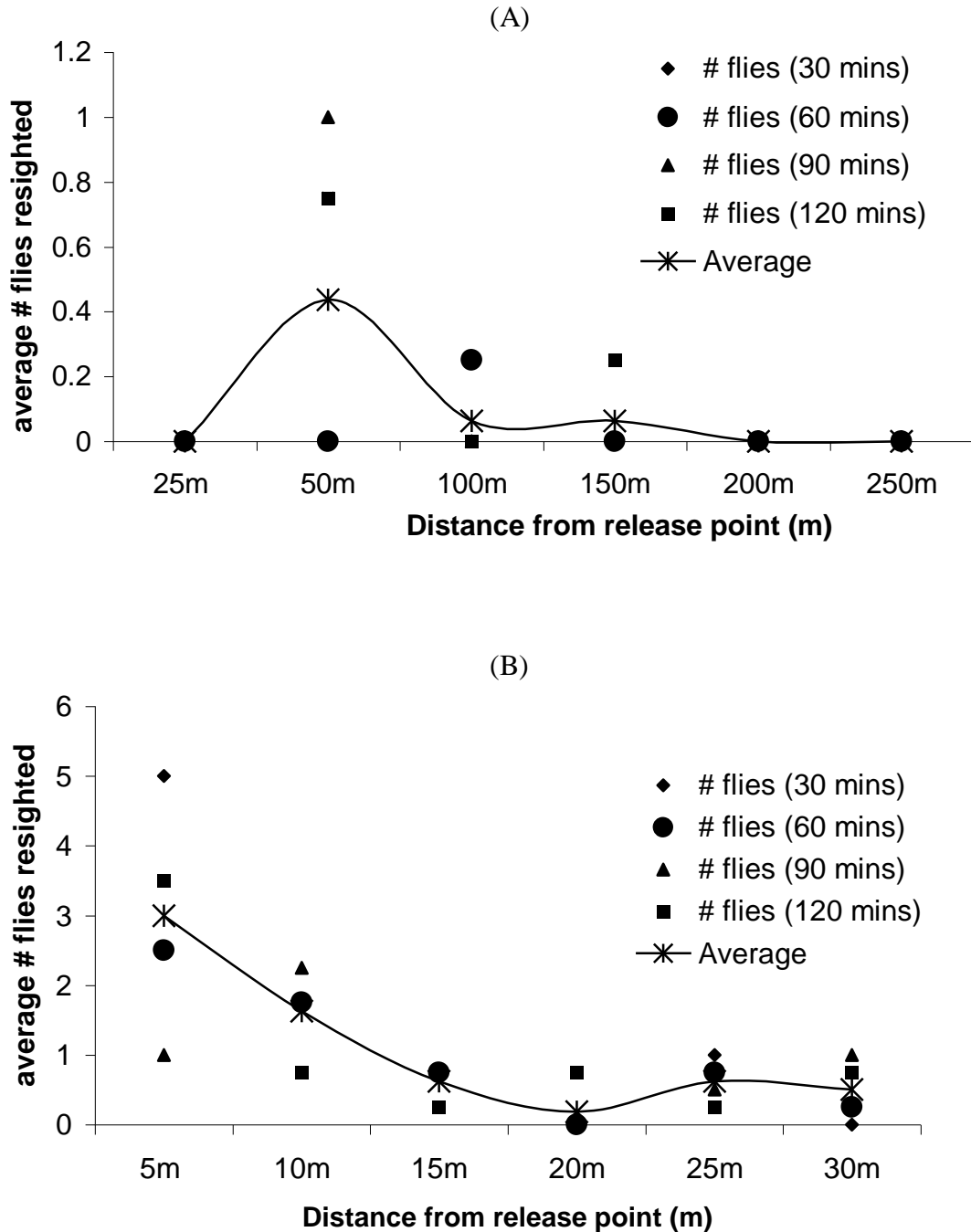
Figure 5.3: Average resightings with distances from release point: (A) Trial 1 (26 September 2005), (B) Trial 2 (4 October 2005), (C) Trial 3 (13 October 2005), (D) Trial 4 (17 October 2005). Individual data points are the average resightings at each distance.

Figure 5.3 (con't)

(C)

(D)
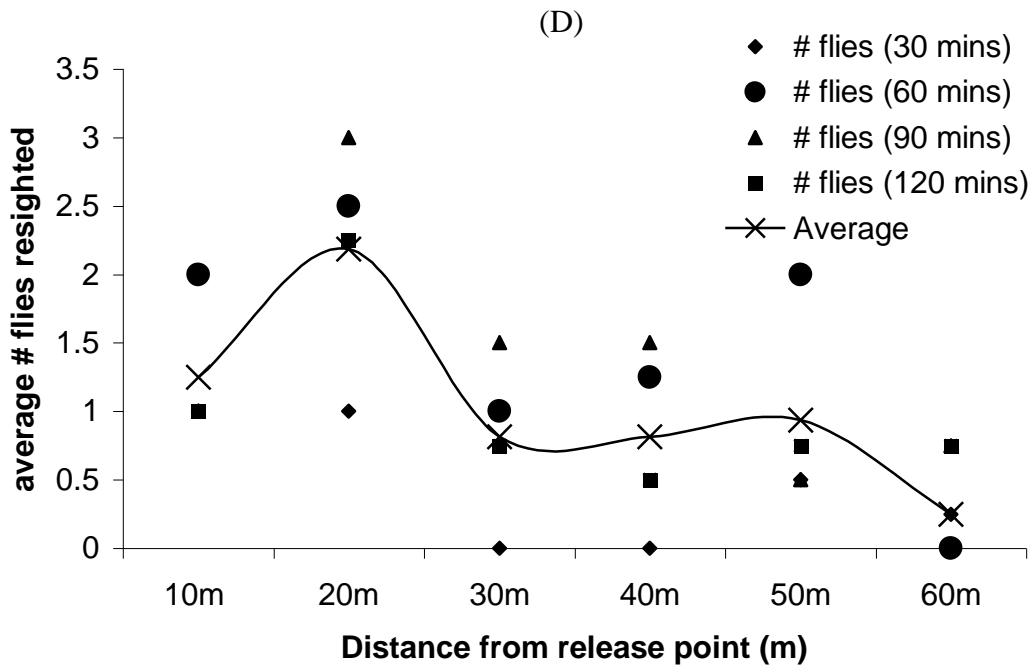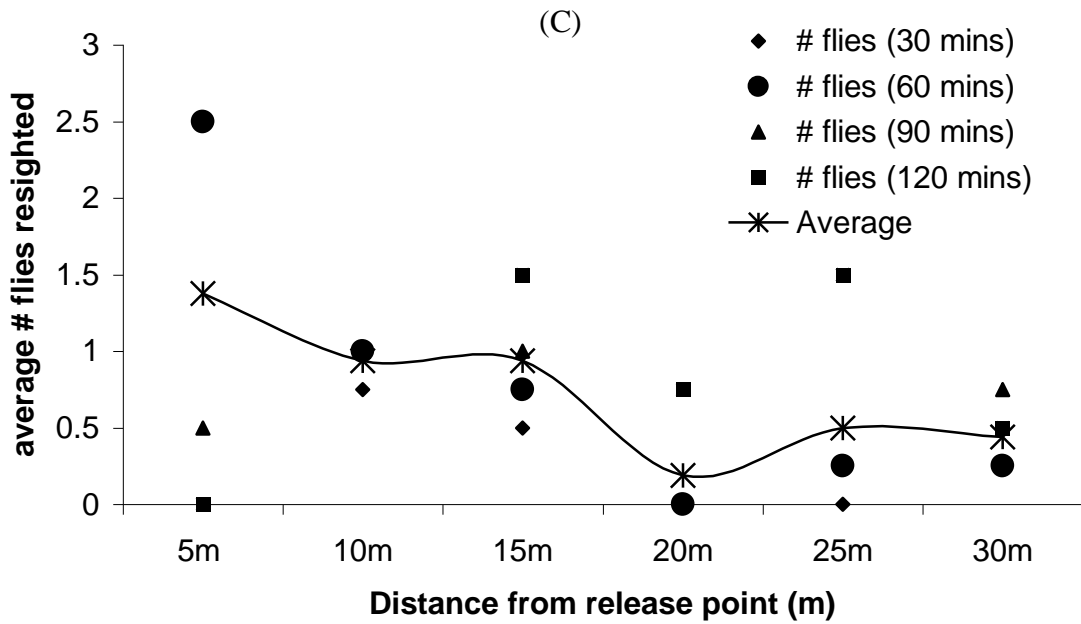
Estimated diffusion rates for trials 2 and 3 were nearly identical, but trial 4 had a much higher diffusion rate (Table 5.2). This discrepancy may have been density-

dependent, owing to the higher density of flies that were released in trial 4 (nearly 2x compared to trials 2 and 3). The redistribution pattern of *P. tricuspis* was well described by a model of random diffusion for trial 4 only, but was marginally nonsignificant for trials 2 and 3 (Table 5.3, Figure 5.4C). Dispersal quantiles, based on the predicted distribution of flies as an average of the four census periods, are presented in Table 5.3. On average, 50% of flies dispersed ≤ 10 m and 95% dispersed ≤ 29 m.
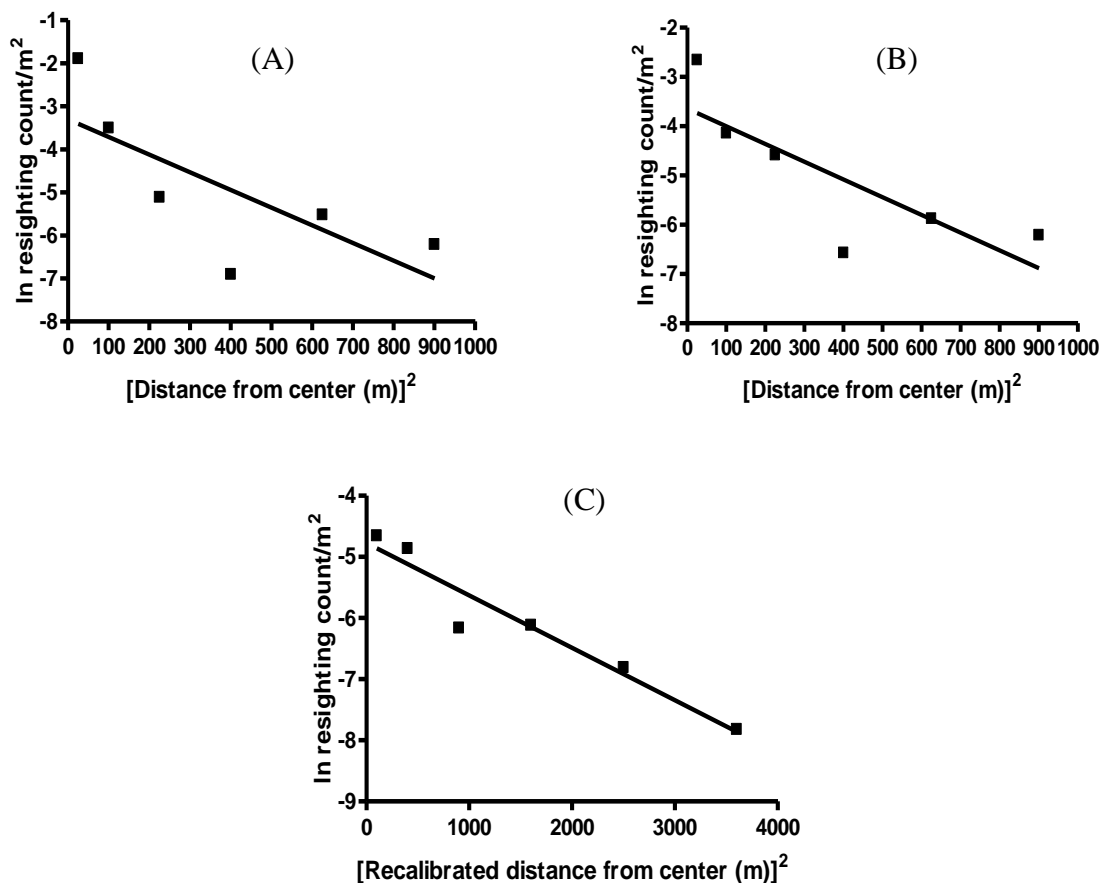
Figure 5.4: *Pseudacteon tricuspis* resightings-with-distance. A linear association between the square of the resighting distance and the logarithm of the density of resighted individuals is predicted by diffusion model (3). Lines were fitted with least squares regression. As diffusion rates were invariant with respect to time, results are the average of the four census periods: (A) trial 2 (4 October 2005), (B) trial 3 (13 October 2005), and (C) trial 4 (17 October 2005). The origin in trial 4 was recalibrated to account for significant drift.

Table 5.3: Fit of the diffusion model (3) to fly resighting data for trials 2-4, along with coefficients of determination and associated *P*-values. Dispersal quantiles are radii of a circle (m) enclosing 50% and 95% of dispersers.

			Dispersal quantiles (m)	
Trial	R^2	*P*	50%	95%
2	0.55	0.09	6.18	17.99
3	0.65	0.05	9.58	27.87
4	0.93	0.002	14.61	42.47
Mean ± SE	0.71 ± 0.11	0.05 ± 0.03	10.12 ± 2.45	29.44 ± 7.11

<u>Dispersal Distances by Fly Sex</u>

A total of 28 marked flies (7%) were recovered from the Norwood dispersal experiment (15 males, 13 females). The mean distances dispersed by sex were not different at either location (Figure 5.5) (Norwood: $t=0.5$, $df=21.4$, $p=0.6$; Montpelier trial 4: $t=0.-0.26$, $df=12.8$, $p=0.8$).

(A)
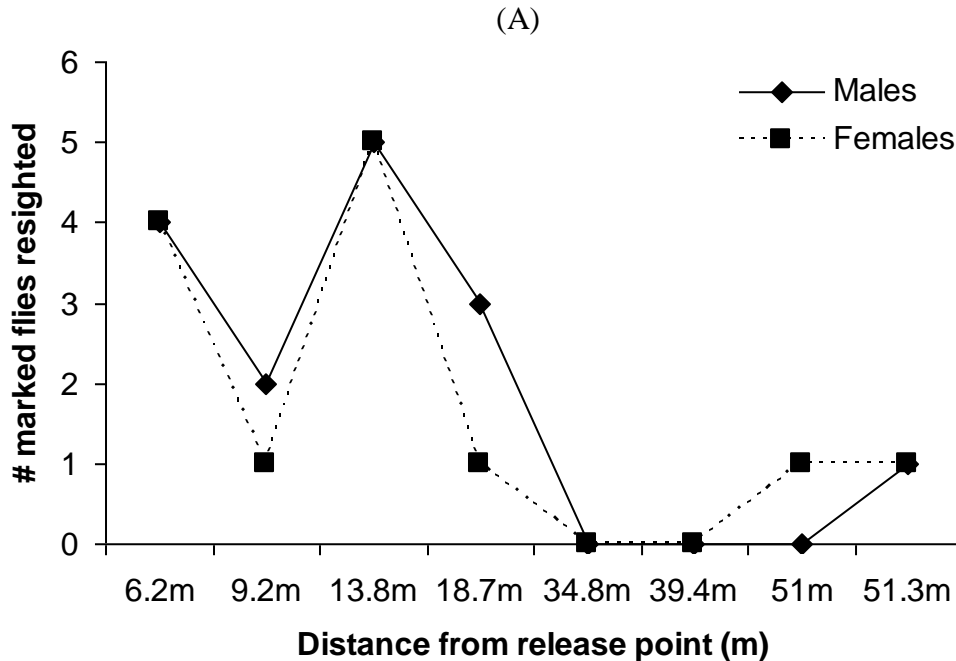
Figure 5.5: Dispersal distances of recaptured *P. tricuspis* sexes: (A) Norwood, Louisiana (6 October 2005); (B) Montpelier, Louisiana (Trial 4, 17 October 2005).

Figure 5.5 (con't)

(B)
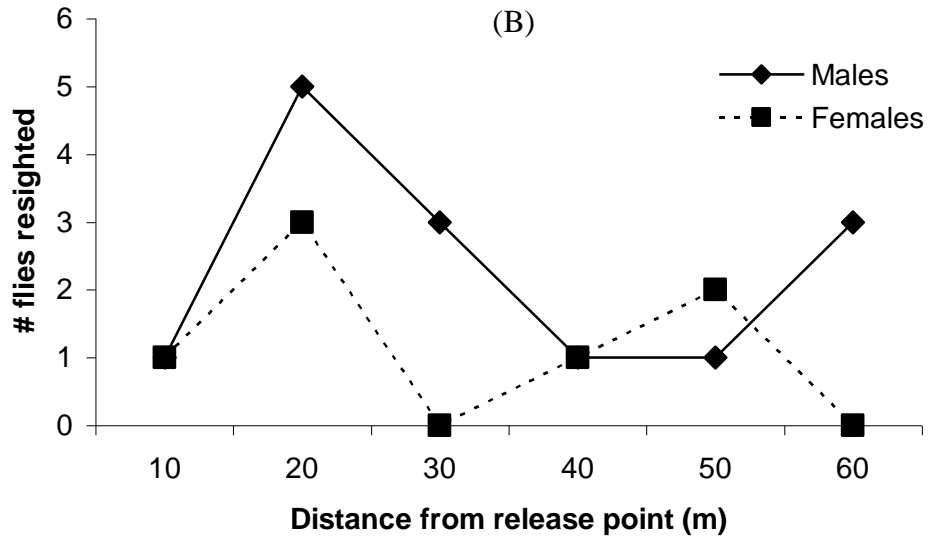
DISCUSSION

With one exception, a departure from a density distribution predicted by a simple diffusion model occurred in this study. Among insects, these departures are usually recaptures that are lower-than-expected near a point source and recaptures that are greater-than-expected farther from a point source (i.e. leptokurtic) and can occur when a population of dispersing insects is comprised of two or more subgroups that have different dispersal capabilities (Turchin 1998, Cronin et al. 2000). The lack of fit of the simple diffusion model implies that redistribution in *P. tricuspis* may be better described with a heterogeneous diffusion model (see Cronin et al. 2000). In trial 1, no flies were resighted at the innermost annulus (25 m), but were observed beyond that distance. Moreover, resighting gaps at 20 m were observed in trials 2 and 3. These resighting gaps also suggest that there are two dispersal forms of *P. tricuspis*: slow moving and fast moving dispersers. The null diffusion model failed to adequately describe redistribution patterns of *P. tricuspis* in trials 2 and 3, but fit the redistribution pattern well in trial 4.

However, trial 4 had twice the source strength of dispersers compared to trials 2 and 3, and the resighting radii were twice that of trials 2 and 3. It is possible that the random diffusion model fit the density distribution in trial 4 well because the data consisted of a majority of endpoint dispersers.

Regardless of the means of dispersal, it is apparent that both sexes of *P. tricuspis* can disperse considerable distances in only two hours. Although insufficient resighting data was obtained in the first Montpelier dispersal trial, it is nevertheless noted that at least one fly was resighted 150 m from the point of release, and flies were also resighted up to 185 m from release areas within two hours of release during preliminary trials at Natchitoches, Louisiana during June 2005. Beyond these distances it would be very difficult to observe flies, owing to a dilution effect at greater distances from the release point. However, by increasing the source strength of dispersers, it is possible to extend the limits of detection (see Nathan et al. 2003).

Rare, long-distance dispersal (LDD) events are directly linked to population spread and colonization rates (Nathan et al. 2003). Although critical to estimating the speed at which an introduced population might invade new habitats, the difficulty of quantifying tails of dispersal probability distributions (i.e. kernels) pose a challenge in ecological research because rare LDD events occur beyond observed dispersal distances, and are driven by complex and stochastic processes (Clark et al. 2001, Nathan 2006). Jump dispersal can result from intrinsic dispersal heterogeneities within a population due to differences in body size, wing morphology and movement behavior (Cronin et al. 2000, Yamamura 2002), but passive dispersal by wind can also be important (Horn et al. 2001, Compton 2002, Osborne et al. 2002).

Stratified dispersal patterns by *P. tricuspis* have been suggested as a means by which it attains annual population spread rates of 20-30 km in Louisiana (Henne et al. 2007b) or more in Florida (Pereira and Porter 2006). Like most small flying insects, *P. tricuspis* probably has a combination of long-distance undirected dispersal in upper winds and short-distance directed flights to host ants [see Compton (2002) for a recent review of wind dispersal]. High-speed atmospheric upper-level winds can transport parts of populations over considerable distances (Hengeveld 1989).

Dispersal is critical for both persistence and evolution of species, especially in changing environments where species must move or adapt to survive (Walters et al. 2006). Dispersal can be costly in the short term in terms of energy expended, particularly for small-bodied dipterans, but long-term fitness is often higher as a result (Roff 1977). In South America, *S. invicta* occupies a seasonally flooded wetland and savanna area along the Paraguay River, known as the Pantanal (Tschinkel 2006). Therefore, it is plausible that long-distance dispersal away from ephemeral or marginal habitats would have been strongly selected for by *P. tricuspis* in South America. The metapopulation dynamics of both *S. invicta* and its parasitoids in South America in relation to seasonal flooding would be an interesting study.

The pattern of displacement in trials 2 and 4 suggest that *P. tricuspis* was orienting upwind toward *S. invicta* alarm pheromones (see Figure 2). Volatile attractants are detected downwind of their source, so insects must fly upwind to locate this source. It has been shown under field conditions that parasitoids orient to host habitats via upwind (positive) anemotaxis (Compton 2002, Williams et al. 2007). In all dispersal trials at Montpelier, flies rapidly dispersed from the cage after the lid was removed, and

many were observed flying straight up in the air towards the sun. In trial 3, several flies were resighted 30 m away from the release point, and many trays at 5-10 m had 2-4 flies, less than five minutes post-release. In trial 4, a single fly was resighted 40 m from the release point at 10 minutes post-release. It does not appear likely that flies traveled these distances without the aid of wind transport, as the energy costs would be considerable (Roff 1977). Unfortunately, it is almost impossible to track individual movements of *P. tricuspis* due to their miniscule size and rapid flight.

One possible shortfall of this study is that the duration of the dispersal studies reported here may have been too short relative to the lifespan of the flies. The choice of a two hour study was decided because releases of *P. tricuspis* in Louisiana were normally done within a two hour time frame (Henne et al. 2007a). Studies to evaluate post-release loss rates and mortality of *P. tricuspis* under natural conditions have yet to be done. It is also possible that exposure to host volatiles are important to keep flies in the area. For instance, Hougardy and Mills (2006) showed that *Mastrus ridibundus* (Grevenhorst) (Hymenoptera: Ichneumondiae) females deprived of host stimuli are much more dispersive than females that were provided with hosts prior to release. The effect of presenting hosts to *P. tricuspis* females prior to release has not been evaluated.

The results obtained here should be viewed as preliminary, and more field studies are encouraged. For example, the recently described phorid fly sticky trap (Puckett et al. 2007) could be a valuable device in future *Pseudacteon* dispersal studies to ascertain LDD events and model dispersal kernels. Additionally, the putative role of wind in transporting *P. tricuspis* long distances should be tested experimentally. Nevertheless,

the study reported here provides valuable information about phorid fly dispersal and

redistribution that was previously unknown.

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CHAPTER 6

POPULATION SPREAD OF THE INTRODUCED RED IMPORTED FIRE ANT PARASITOID, *PSEUDACTEON TRICUSPIS* BORGMEIER (DIPTERA: PHORIDAE), IN LOUISIANA[1]

INTRODUCTION

Classical biological control involving the introduction of natural enemies to suppress exotic pest species has been ongoing for more than a century (see Huffaker and Messenger 1976, Coulson et al. 2000). Success of the biological control agent depends in part on its ability to establish, spread and eventually occupy the range of its host. For example, successful biological control of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), in Japan was achieved by the introduction and rapid spread (approximately 60 km/yr) of the introduced parasitoid, *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) (Moriya et al. 2002). Early models of the spread of animal and plant populations were based on the process of diffusion and predicted a simple linear rate of spread (Fisher 1937, Skellam 1951, reviewed by Hengeveld 1989, Andow et al. 1990, Okubo and Levin 2002, Hastings et al. 2005). However, empirical patterns of spread for many species are non-linear, likely attributable to appreciable rates of long-distance dispersal (e.g., Hengeveld 1989, Andow et al. 1993, Shigesada et al. 1995, Johnson et al. 2006, Muirhead et al. 2006). In these species, nascent populations appear well beyond the edge of an expanding range in what is known as stratified or "jump" dispersal (Hengeveld 1989).

For many species, human transport processes, such as the movement of the Argentine ant (*Linepithema humile* (Mayr)) by cars and trucks (Suarez et al. 2001), or the zebra mussel (*Dreissena polymorpha* (Pallas)) by boats (Buchan and Padilla 1999) are thought responsible for jump dispersal. Ignoring this component of dispersal can lead to significant underestimates of range expansion of invasive pests and natural enemies introduced for their biological control.

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The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is a ubiquitous and economically important pest in the southeastern United States (Lofgren 1986). Much has been published about the introduction, spread, biology, economic and environmental impacts, and control of *S. invicta* in the United States (see Vinson 1997, Tschinkel 2006). Early efforts to eradicate *S. invicta* with chemical control were met with limited success (Taber 2000, Tschinkel 2006). Recently, more attention has focused on the potential for biological control of *S. invicta* by importing several specialist parasitoids in the genus *Pseudacteon* Coquillet (Diptera: Phoridae) from the indigenous range of *S. invicta* in South America.

Pseudacteon tricuspis Borgmeier was the first phorid fly species introduced to the United States for biological control of *S. invicta*. It was initially released in Texas in 1995 (Gilbert 1996) and Florida in 1997 (Porter et al. 1999). In cooperation with the USDA-ARS, the first releases of *P. tricuspis* in Louisiana took place in September 1999 and May 2000. *Pseudacteon tricuspis* successfully established at each release site (Henne and Johnson, unpublished data).

Available information on dispersal and spread of *Pseudacteon* flies is limited to three studies. Using traps baited with *S. geminata* workers, Morrison et al. (1999a) found that *Pseudacteon* parasitoids in Texas dispersed up to 650 m from the nearest *S. geminata* population. Porter et al. (2004) monitored the spread of *P. tricuspis* from multiple release sites in north-central Florida and found that the average rate of spread was 10-30 km/yr. With an additional two years of data, Pereira and Porter (2006) revised these latter estimates to 26-57 km/yr. These studies did not evaluate whether *Pseudacteon* spread fit the theoretical expectations of neighborhood diffusion or that of stratified dispersal (see

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Shigesada et al. 1995, Hastings et al. 2005). Data of this sort are useful in understanding animal movement behavior and is necessary to develop predictive models of species spread (Turchin 1998). The aim of this paper is to describe and model the spread of two established *P. tricuspis* populations in Louisiana.

MATERIALS AND METHODS

Biology of *Pseudacteon* Parasitoids

Parasitic flies of the genus *Pseudacteon* contribute to maintaining lower abundances of *S. invicta* in South America (Porter et al. 1992), and thus may be useful in the suppression of *S. invicta* populations in the United States. Although there are native species of *Pseudacteon* that attack native North American fire ants (*S. geminata* (Fabricius) and *S. xyloni* McCook), they have never been observed to attack *S. invicta*. By reuniting *S. invicta* with several species of its native *Pseudacteon* parasitoids, it is hoped that the ant communities in the United States that are currently dominated by *S. invicta* may shift in favor of native ant species (Porter 1998).

Phorid parasitoids locate their hosts by detecting ant semiochemicals (Porter 1998, Morrison and King 2004). For example, *P. tricuspis* is attracted to alarm pheromones emitted by *S. invicta* during mound disturbances, alate flights, and intra- and interspecific fighting (Williams et al. 1973, Pesquero et al. 1993, Morrison and King 2004), and primarily attacks major workers (Morrison et al. 1999b). Female *Pseudacteon* inject a single egg into the host ants' thorax, the larva consumes the head contents and eventually pupariates inside the empty decapitated head capsule (Porter et al. 1995).

A considerable body of evidence suggests that parasitic phorid flies mediate competitive interactions between various ant species (e.g. Feener 1981; Feener and

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Brown 1992; Folgarait and Gilbert 1999; Morrison, 1999, 2000; Orr et al. 1995, 2003).

Solenopsis spp. workers will reduce or terminate foraging activity in response to attacks by *Pseudacteon* flies (Feener and Brown 1992, Orr et al. 1995, Morrison 1999). Mehdiabadi and Gilbert (2002) found that a single attacking *P. tricuspis* female per 200 foraging *S. invicta* workers decreased colony protein consumption almost two-fold and significantly reduced numbers of large-sized workers 50 days later. These studies demonstrate the potential for *Pseudacteon* parasitoids to reduce *S. invicta* populations (but see Tschinkel 2006).

<center>Release Sites</center>

Initial *P. tricuspis* releases in Louisiana were conducted at the following locations: 1) 17 km northeast of Covington (St. Tammany Parish) (30° 36′ 35″ N; 90° 01′ 19″ W), 8-13 September 1999 (2,165 flies released); 2) 9 km east of Norwood (East Feliciana Parish) (30° 59′ 05″ N; 91° 00′ 46″ W), 27 April-8 May 2000 (4,714 flies released). These release sites were unmaintained pastures located approximately 100 km apart and had abundant *S. invicta* populations. Adult *P. tricuspis* were released at disturbed *S. invicta* mounds over a 6-12 day period, and approximately 400 flies were released daily at ten disturbed *S. invicta* mounds. Mounds were continuously disturbed for two hours to maintain *S. invicta* activity and availability to oviposition by *P. tricuspis* (Porter et al. 2004).

<center>Evaluating Population Expansion</center>

Post-release surveys to determine the annual spread limits of *P. tricuspis* were conducted during the fall of each year (September to November) when abundances were highest (Henne and Johnson, unpublished data). Fly surveys were normally conducted

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between 1100 h and 1700 h when ambient temperatures were warm enough for fly activity (>20⁰ C) (Morrison et al. 1999a). We monitored the spread of *P. tricuspis* along transects in four cardinal directions (i.e. north, south, east, and west) from the release point. Every year, we started our survey along each transect, approximately 3 km outward from the previous year's range limit. Within a 100 m radius of that point, we located ten *S. invicta* mounds in disturbed habitat (e.g., roadsides, pastures). Two of us (D.H. and S.J.) would vigorously disturb the mounds with spades (5-10 sec) and count the number of *P. tricuspis* adults that arrived during the ensuing 30 min. Normally, flies would appear within a few minutes of mound disturbance. The sampling location was also recorded with a Magellan ™ GPS 315/320 (accurate to within 25 m) for later plotting on a computer mapping program (Maptech® Terrain Navigator Pro) or Google™ Earth.

If no flies were detected at the disturbed mounds within 30 mins., we moved approximately 1 km (the exact distance depended on the presence of suitable *S. invicta* habitat) toward the release area. If flies were present, the researchers moved 1-2 km further away from the release. The survey was continued in each direction until the limits of spread were established to within 1 km of their approximate locations. Annual surveys were conducted from 1999 (approx. 40 days post-release) to 2005 for the Covington release and from 2001-2006 for the Norwood release (approx. 1 year post-release).

<div align="center">Modeling P. tricuspis Range Expansion</div>

Average Radius of Spread

The mean radius from a point of introduction is the simplest measure of a species' range and provides an estimate of the expansion rate when it is obtained at known time

intervals (Hengeveld 1989). The change in spread radius with time is expected to take on one of three forms: linear (constant rate of spread as predicted by early diffusion-based models), accelerating (rate of spread continually increases over time), or biphasic (initially slow rate of expansion followed by an abrupt transition to an accelerating expansion rate) (Shigesada et al. 1995, Turchin 1998). The mean ± SE annual spread radius (based on four transects) for each expanding *P. tricuspis* population in Louisiana was computed. Linear (null model) and quadratic polynomials were fitted to the mean annual spread radius of both populations and compared using the extra sum-of-squares F-test in Prism® 4.03 (GraphPad Software, Inc., San Diego, CA). The linear and quadratic terms were deemed significant if the associated *P*-values were ≤ 0.05.

Annual Spread Rates

Simple models of diffusion predict the spread rate of *P. tricuspis* to be constant over time (Shigesada et al.1995, Turchin 1998). If this is not the case, we can identify time periods for which the rate of spread is low (e.g., if an Allee effect is operating during the early stages of range expansion), or accelerating. Because both populations exhibited consistent directional bias in expansion rates (see Results), separate curves were generated for each transect. To more clearly depict the latent, accelerating and plateau phases of expansion over time, we plotted the relationship between annual spread rate (spread radius in year t minus the spread radius in year t-1) and year since release. A logistic model was fit to the Covington 2000-2005 north and west annual spread rates, and to the 2000-2004 south and east spread rates using Prism® 4.03. Because the Norwood population did not exhibit any measurable spread in the first two years (zero individuals in 2001 and 2 individuals among 74 mounds in 2002), there were too few data

points to fit the logistic model to its annual spread rates. The logistic model used was a dose-response model, equivalent to a three-parameter logistic model (Motulsky and Christopoulos 2003) and had the analytic form: $y = \text{Bottom} + [(\text{Top} - \text{Bottom}) / 1 + 10^{\text{LogEC50-}x}]$. The parameters, bottom (constrained > 0) is the y-value at the bottom plateau, top is the y-value at the top plateau, and LogEC_{50} is the x-value halfway between bottom and top.

Decline of *P. tricuspis* Abundances Away From Release Points

One expectation of simple spatial spread models is that the density of the organism should decay at an approximately exponential rate with distance from the release point (Turchin 1998, Okubo and Levins 2002). When abundances are ln-transformed, the relationship is expected to be linear. Data from *P. tricuspis* transect surveys were transformed [ln $(n+1)$], where n is the total number of flies observed at ten *S. invicta* mounds. Linear regression was performed using Prism® 4.03.

RESULTS

P. tricuspis Range Expansion

Both the Covington and Norwood *P. tricuspis* releases resulted in expanding populations (Table 6.1). By the fall of 2005, the leading edges of the westward expanding Covington and eastward expanding Norwood populations were approximately 8 km apart. Based on expansion rates at that time (see below), these populations were projected to merge in 2006. Thus, the Covington survey was terminated after the 2005 survey. The Norwood population was surveyed through 2006, but the presumed merger prevented us from determining its eastern expansion limit.

For both populations, the average annual radius of spread increased nonlinearly with year since release (Figure 6.1). Adding a quadratic term to the linear models significantly improved the fit [Covington df=1,3 (F=164, P=0.001) $y = 3x^2 - 12000x + 1.2e^{0.007}$ (R^2=1.0, n=6); Norwood df=1,3(F=85, P=0.003), $y = 4.2x^2 - 17000x + 1.7e^{0.007}$ (R^2=0.99, n=6)]. In both populations, range expansion was biased to the north of each release site (Table 6.1). In Covington, northward expansion as of 2004 was 10.5 km (or 41 %) farther than the mean expansion for the other three directions. In Norwood, the difference as of 2006 was 23.5 km (or 40%).

The rate of spread of *P. tricuspis* varied tremendously among years between the Covington and Norwood releases (Figure 6.2). The annual rate of spread at the Covington site was sigmoidal over time – the spread rate was very low in the first two years following the release, then it increased rapidly during years 3-4, and finally appeared to slow down or level off at a mean maximal rate of spread of 23 km/yr (Table 6.1, Figure 6.2A). Although we do not have sufficient data from the Norwood release (Table 6.1, Figure 6.2B) to compare range expansion in the first couple of years to that from subsequent years, we do observe a steady increase in the rate of spread from years 3-5. The rates of spread during this time period are very comparable to those for the Covington release, differing only by an average of 2.6 km (or 15 %). A paired t-test (using Prism 4.3) comparing year 3-5 spread rates between both populations was nonsignificant [df=2, t=1.39, p=0.3].

Table 6.1: *Pseudacteon tricuspis* cumulative spread radius (km), annual spread distance (radius at year t minus radius at t-1; in parentheses), and estimated area occupied (km^2) for different transects and years at the Covington and Norwood releases.

Release location	Year	North	South	East	West	Mean radii ± SE	Area Occupied (km^2)‡
Covington	1999	0.1	0.1	0.1	0.1	0.1 ± 0.0	0.03
	2000	0.8 (0.8)	0.4 (0.4)	0.4 (0.4)	0.4 (0.4)	0.5 ± 0.1	0.8
	2001	1.6 (0.8)	0.8 (0.4)	1.6 (1.2)	0.8 (0.4)	1.2 ± 0.2	4.5
	2002	3.3 (1.7)	1.6 (0.8)	4.4 (2.8)	1.6 (0.8)	2.7 ± 0.7	22.9
	2003	12.4 (9.1)	11.6 (10)	14.2 (9.8)	8.6 (7)	11.7 ± 1.2	430.0
	2004	36.5 (24.1)	24.5 (13.1)	27.2 (13)	26.1 (17.5)	28.6 ± 2.7	2,569.7
	2005	59.8 (23.3)	*1	*2	47.1 (21)	50.7 ± 3.0	8,075.4
	2006	-	-	-	-	74.3 ± 5.9 *5	17,343.1
	2007	-	-	-	-	102.1 ± 9.2 *5	32,749.2
Norwood	2000* 3	0.1	0.1	0.1	0.1	0.1 ± 0.0	0.03
	2001	0.1	0.1	0.1	0.1	0.1 ± 0.0	0.03
	2002	0.1	0.1	0.1	0.1	0.1 ± 0.0	0.03
	2003	9.3	1.6	3.2	1.6	4.1 ± 1.95	52.8
	2004	19.4 (10.1)	10 (8.4)	9.1 (5.9)	8.8 (7.2)	11.8 ± 2.5	437.4
	2005	40.7 (21.3)	27.4 (17.4)	34.5 (25.4)	36 (27.2)	34.7 ± 2.8	3,782.8
	2006	82.2 (41.5)	55.2 (27.8)	*4	62.2 (26.2)	68.2 ± 9.7	14,612.3
	2007	-	-	-	-	99.3 ± 12.9 *5	30,977.6
	2008	-	-	-	-	140.3 ± 20.6 *5	61,839.4

‡ Based on the area of a circle (πr^2)

*1 Southward expansion reached Lake Pontchartrain in 2004*2 Eastward expansion merged with Mississippi *P. tricuspis* in 2005

*3 2000 - 2002 Norwood radii based on 1999 Covington radii

*4 Eastward expansion merged with Covington *P. tricuspis* in 2006

*5 Predicted radii computed from quadratic models (see text)
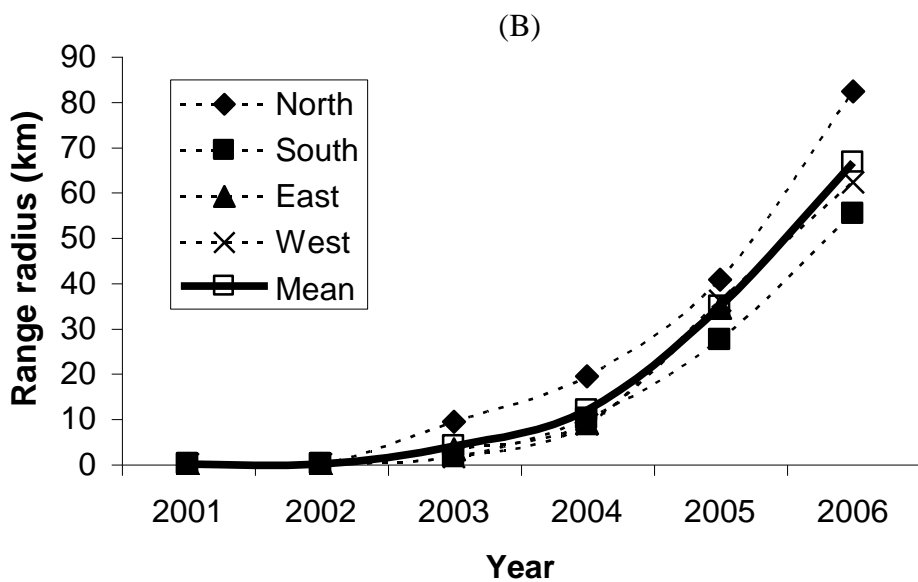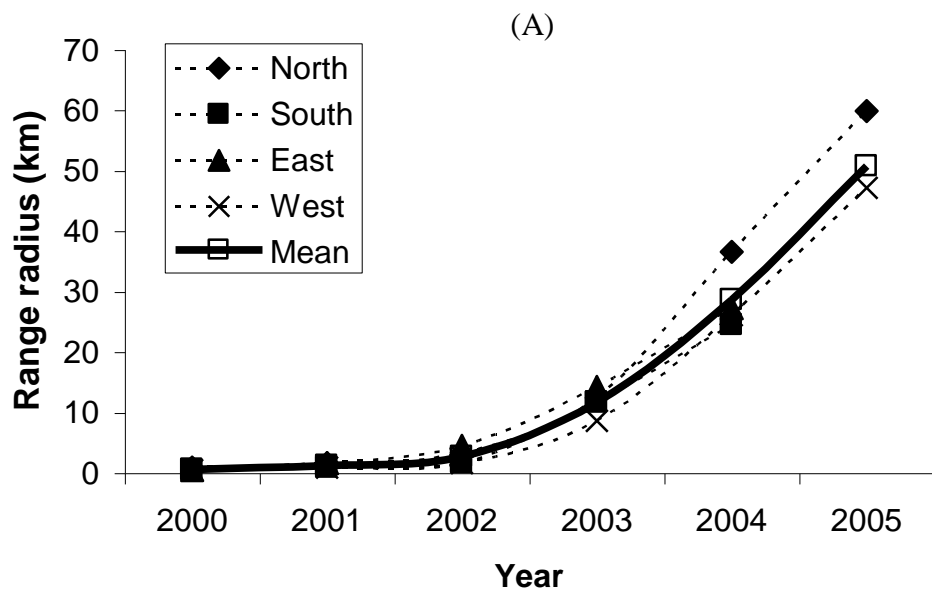
Figure 6.1: The change in *Pseudacteon tricuspis* range radius (km) over time in four cardinal directions (and mean of all directions) for two release sites: Covington (A) and Norwood (B), Louisiana. Curves are derived from polynomial least-squares regression (see Results).

The logistic models provided a very good fit to the latent, accelerating and plateau phases of the Covington spread rate data: North $y = 0.4528 + [(25.25 - 0.4528)/ 1 + 10^{\log4.199-x}]$ (R^2=0.99), South $y = 1.0\ e^{-0.007} + [(14.2 - 1.0\ e^{-0.007})/ 1 + 10^{\log3.740-x}]$ (R^2=0.98), East $y = 0.5659 + [(13.6 - 0.5659)/ 1 + 10^{\log3.636-x}]$ (R^2=0.99), West $y = 0.2096 + [(21.43 - 0.2096)/1 + 10^{\log4.336-x}]$ (R^2=0.99). Covington asymptotic spread rates are projected by the logistic models to be approximately 25 km/yr (north), 14 km/yr (south and east), and 21 km/yr year (west). The Norwood western spread distance in 2006 was similar to the 2005 spread distance (26 km vs. 27 km).

Decline of *P. tricuspis* Abundances Away From Release Points

For both point-in-time surveys of the abundances of *P. tricuspis* at the edge of the Norwood range, we found that ln fly abundances declined linearly with increasing distance from the release point, [2005 east transect df=1, 3 (F=133.5) ($R^2 = 0.98$, n=5) $p<$ 0.01; 2006 south transect df=1, 3 (F=37.59) ($R^2 = 0.93$, n=5) $p<$ 0.01; Figure 6.3].

DISCUSSION

Range expansion by *P. tricuspis* was not linear as predicted by classical models of diffusive spread. Instead, the rate of spread accelerated during the first five years post release and appeared to slow down or level off in subsequent years (at least for the Covington site). For both Covington and Norwood, populations were spreading at a rate of approximately 15-25 km/yr by the end of the study. The accelerating phase of range expansion is similar to the type 3, bi-phasic curve described by Shigesada et al. (1995). It is also suggestive of stratified dispersal in *P. tricuspis*. Rapid expansion rates, such as those observed in the 3rd through 4th years following the release of *P. tricuspis* in Covington and Norwood, can occur when a few mated female parasitoids disperse very

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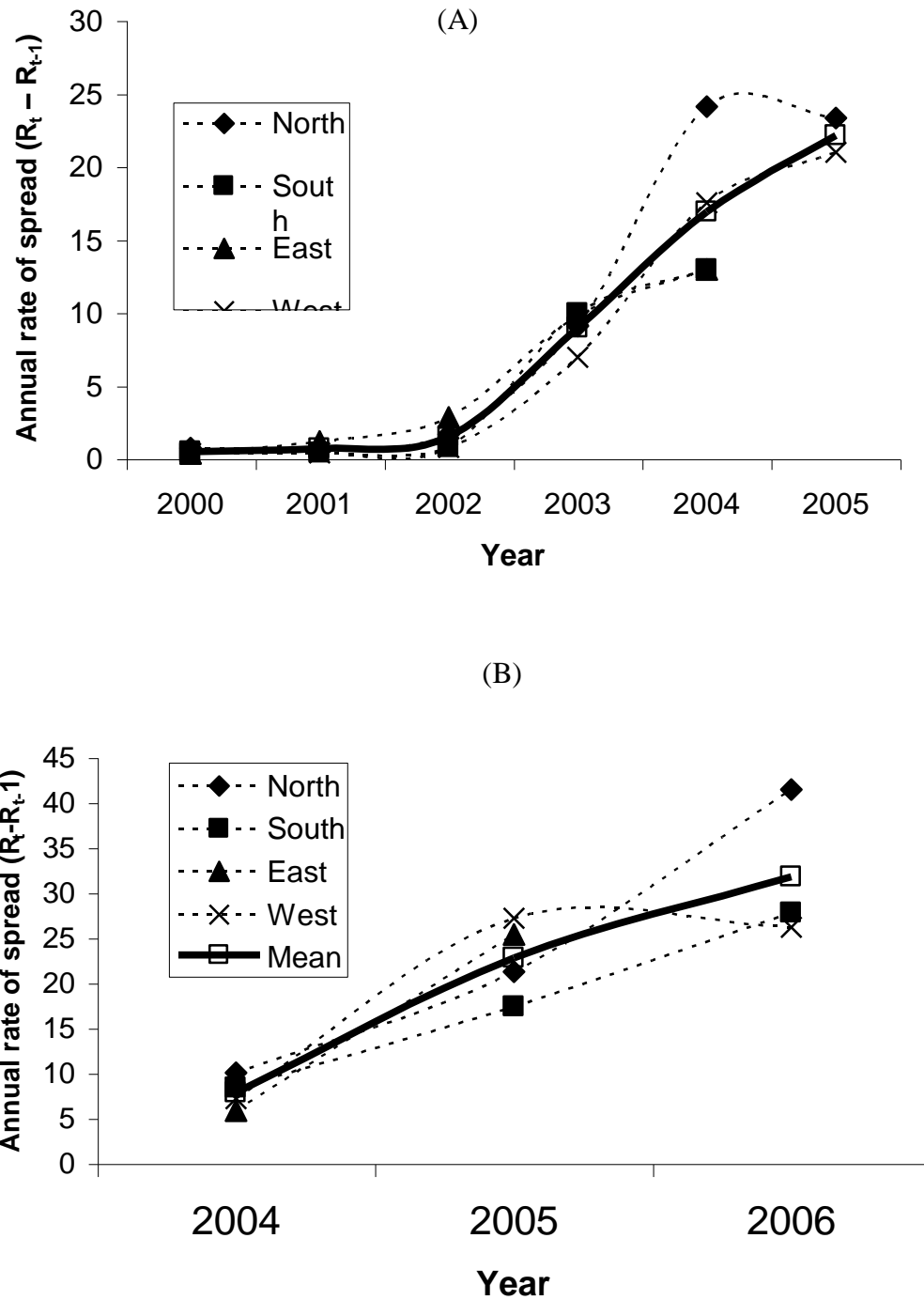
Figure 6.2: Annual directional rate of spread (km/year = radius at time$_t$- radius at time$_{t-1}$) for *P. tricuspis* at the Covington (A) and Norwood (B) release sites. A logistic growth curve (see Methods) is fit to each individual transect and the mean of all four transects.
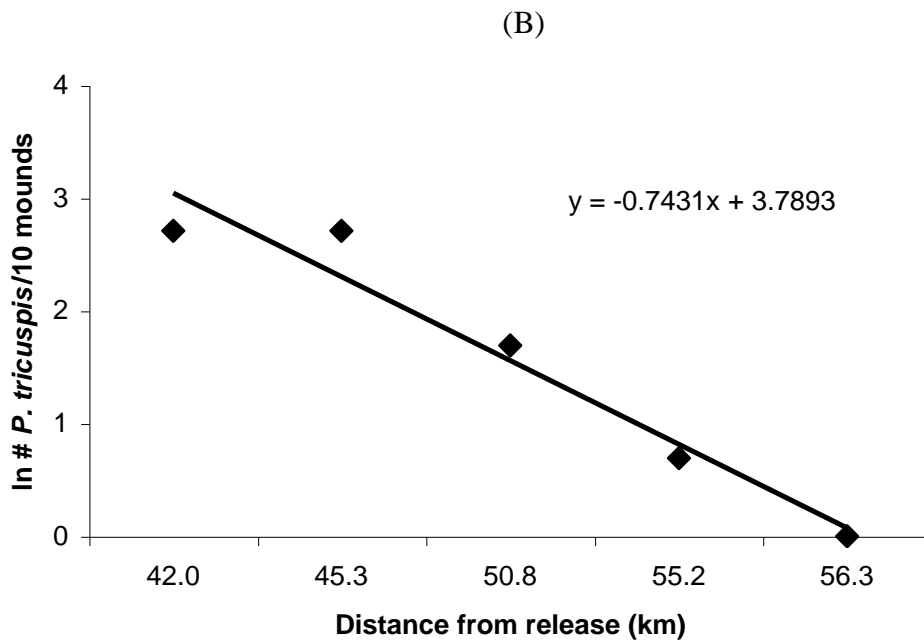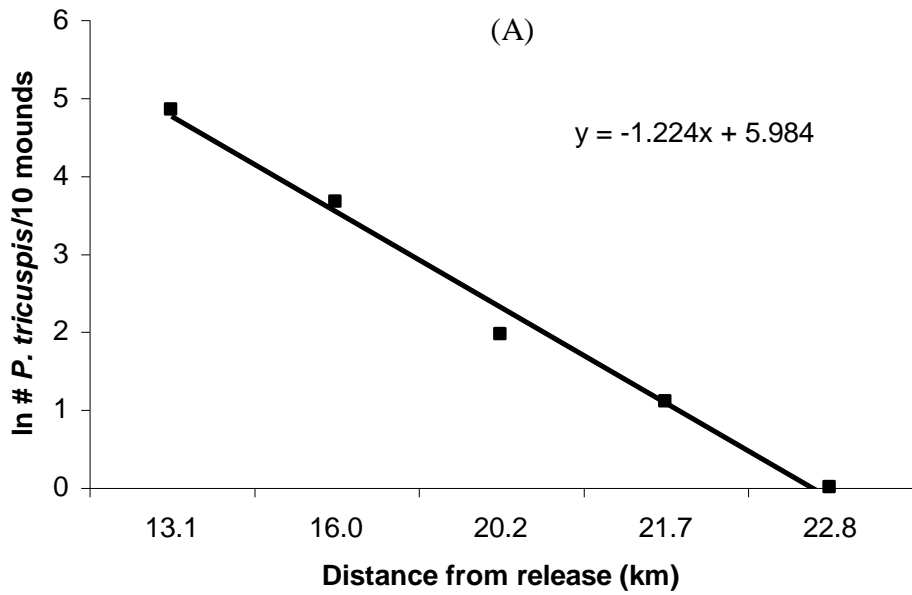
Figure 6.3: ln number of *P. tricuspis*/10 mounds at different distances from the release point at Norwood. (A) East transect, 10 October 2005, (B) South transect, 11 October 2006.

far in relation to the typical neighborhood movements of most individuals (Hastings 2000). Jump dispersal can result from intrinsic dispersal heterogeneities within a population, owing to differences in body size, wing morphology and movement behavior (Cronin et al. 2000, Yamamura 2002). It can also result from human-assisted transport of a small subset of the population (e.g., Buchan and Padilla 1999, Suarez et al. 2001). Presently, we do not have any information on whether *P. tricuspis* exhibits intrinsic differences in dispersal ability or if humans might assist in their spread.

At low initial population densities, the few long-distance dispersers would have little impact on the velocity of the advancing neighborhood diffusion wave (Hengeveld 1989). However, as densities increase, the number of long-distance dispersers will increase and may become a dominant component of the advancing wave (Hengeveld 1989). Eventually, even with stratified dispersal, spread rates should reach an asymptote as densities equilibrate; after which time the mean radius of spread (or square root of area occupied) versus time function would become linear (see Hengelveld 1989, Moriya et al. 2002, Yamamura 2002).

Many organisms introduced to a new environment undergo an initial period of little or no expansion, called a 'latent phase' (Turchin 1998). This is an important first step in the eventual establishment and spread of introduced organisms. It is a time when the population presumably adapts to local conditions and increases its numbers (Turchin 1998, Andow 1999). This latent phase may be caused by an Allee effect, whereby introduced insects dispersing into a new environment may become so rare that males and females often fail to encounter one another (Hopper and Roush 1993). In their review of the literature, Hopper and Roush (1993) found that establishment success of introduced

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parasitoids depended on the release density and number of releases. This suggests that Allee effects may represent an important constraint on the success of biological control programs using parasitoids.

Introduced organisms may also have an 'eclipse' period in which abundances shortly after the release fall below a detection threshold, (Hopper and Roush 1993). This can occur in situations when dispersal rates are high, thereby acting as a 'drain' on local populations at the release site (Kean and Barlow 2000). Despite high *P. tricuspis* abundances (>5 flies/mound) at the Norwood release site during 2000, flies were not detected at the release site one year later, and only two flies were observed the second year after introduction, despite intensive sampling. By the third year post-release, *P. tricuspis* had already spread up to 10 km away from the release area. A combination of slower-than-expected local increase at the release site (i.e., during the eclipse period), followed by rapidly increasing rates of spread beginning in the third year (Figure 6.2) , may have been responsible for the sudden appearance of *P. tricuspis* so far from the release site (Kean and Barlow 2000). Clearly, the negligible population abundances at the Norwood release site 1-2 years post-release were not a reliable indication of the true status of this population. In future releases, more intensive and wider-ranging surveys should be conducted initially when population densities are low.

Porter et al. (2004) documented *P. tricuspis* expansion rates that were comparable to the rates we found in the Louisiana releases -- 10-30 km/yr in central Florida versus 15-25 km/yr in Louisiana. The Florida releases also appeared to exhibit accelerating spread rates, although Porter et al. (2004) did not attempt to quantify this pattern. Expansion rates in Florida appeared to have accelerated more quickly than in Louisiana,

reaching approximately 23 km/yr only three years post-release. Porter et al. (2004) also found that *P. tricuspis* abundances decreased with increasing distances away from release points, and Morrison et al. (1999a) documented declining abundances of *Pseudacteon* parasitoids of *S. geminata* at increasing distances from host colonies in Texas. A species' population density tends to be highest near the center and gradually declines towards the margin of its geographical range (Guo et al. 2005). This can be attributed to an area-dilution effect (Turchin 1998). As distance from the release point increases, the numbers of organisms reaching that distance are spread over a progressively larger area. The log-linear declines of *P. tricuspis* abundances with increasing distance away from the source population suggest a probability distribution function (kernel) with a long tail of *P. tricuspis* dispersers (i.e. exponential decline in abundance). Similar declines in abundances along other transects near the range edges were observed in both Louisiana populations (Henne and Johnson, unpublished data).

Another similarity between our Louisiana releases and the Florida releases (Pereira and Porter 2006) is that there is a northward bias in *P. tricuspis* spread. Areas near coastal Louisiana are subjected to afternoon sea breezes that blow north from the Gulf of Mexico and occur almost daily during the warm season (Smith and Fuelberg 2005). Morrison et al. (1999a) suggested that most *Pseudacteon* remain close to the ground during high winds, but also that passive transport by wind may be an important factor in long-distance dispersal. In support of this claim, *P. tricuspis* have successfully dispersed across the Mississippi river (>1 km) and beyond dense forest stands (e.g. Bogue Chitto National Wildlife Refuge) that were at least five km wide and at least 20 km deep (as measured in Google[TM] Earth).

Microinsects routinely form concentrated well-defined plumes in thermal currents of rising air (Geerts and Miao 2005) suggesting that long-distance dispersal via winds may be important in the spatial spread of these species. The detection of *P. tricuspis* nearly 42 km further north of the Norwood release site in the 2006 as compared to the 2005 survey was considerably farther than any previous recorded spread distance for this species, including the Florida releases reported in Porter et al. (2004). An explanation for this may have been the influence of two hurricanes (Katrina and Rita) that made landfall in Louisiana in 2005. High winds associated with these large-scale synoptic events as they approached and moved northward through Louisiana (http://www.nhc.noaa.gov/2005atlan.shtml) would have transported dispersing *P. tricuspis* adults farther away than normal. In Florida, four hurricanes with a generally northward trajectory in 2004 (Charley, Frances, Ivan and Jeanne) (http://www.nhc.noaa.gov/2004atlan.shtml) may explain the enhanced *P. tricuspis* spread rates reported by Pereira and Porter (2006) in Florida. Several studies have shown that tropical cyclones account for long-distance transport of many insects (e.g. Larsen and Pedgley 1985, Torres 1988, Richardson and Nemeth 1991, Clarke and Zalucki 2004). Thus, model-based predictions of future expansion distances may be prone to considerable directional bias. Regardless of how directional bias occurs, *P. tricuspis* populations do spread considerable distances on an annual basis, a feature that will contribute to its ability to quickly occupy the range of *S. invicta* (see also Porter et al. 2004, Pereira and Porter 2006).

Nearly 20 species of *Pseudacteon* are known to attack *S. invicta* in South America (Porter and Pesquero 2001), and at least three species of *Pseudacteon* have already been

imported and released in the United States: *P. tricuspis* (Graham et al. 2001, Porter et al. 2004), *P. curvatus* Borgmeier (Graham et al. 2003), and *P. litoralis* Borgmeier (Porter and Alonso 1999). *Pseudacteon borgmeieri* Schmitz (Folgarait et al. 2002a) and *P. cultellatus* Borgmeier (Folgarait et al. 2002b) are currently under evaluation for possible release in the United States in the next few years. This study provides valuable information about *P. tricuspis* population spread that can be used in predicting spread rates and distances (with directional bias) for this and other *Pseudacteon* species. The fact that *P. tricuspis* spread patterns and rates are so similar in Louisiana and Florida suggests that our predictions would be robust for releases of this species throughout the southeastern United States.

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CHAPTER 7

DAILY AND SEASONAL DYNAMICS OF THE DECAPITATING FLY, *PSEUDACTEON TRICUSPIS* BORGMEIER (DIPTERA: PHORIDAE) IN LOUISIANA

INTRODUCTION

Introductions of non-native organisms for long-term biological control of exotic insects and weeds (i.e. classical biological control) have been ongoing for over 100 years (Greathead 1986, Godfray and Waage 1991). A crucial aspect of biological control programs should be the study of how populations of introduced biocontrol organisms respond to alien environments. Post-release monitoring of biological control introductions is vital, not only for assessing impacts on target pests, but also to determine population trends and develop sampling methodology. Therefore, obtaining information on daily and seasonal activity patterns of these organisms and, if possible, relating these patterns to environmental correlates should be a major thrust of biological control programs.

The red imported fire ant, *Solenopsis invicta* Buren, is an ubiquitous exotic insect in the southeastern United States, and is regarded as a significant economic pest in this region (Lofgren 1986, Porter et al. 1992). Beginning in the late 1990's, several species of parasitoids from the indigenous range of *S. invicta* in South America have been imported for the biological control of *S. invicta*. One promising attribute of this effort is based on the potentially significant role that parasitic flies of the dipteran family Phoridae play in maintaining lower abundances of *S. invicta* in South America (Porter et al. 1997). Phorid flies of the genus *Pseudacteon* Coquillet affect *Solenopsis* foraging behavior, and research has focused on how parasitic phorid flies mediate competitive interactions between various ant species (e.g. Feener 1981; Feener and Brown 1992; Folgarait and Gilbert 1999; Orr et al. 1995, 2003). Studies of *Solenopsis* foraging activity in response to attacks by *Pseudacteon* flies reveal that *Solenopsis* workers often terminate foraging

activity in the presence of phorid flies (Feener and Brown 1992, Orr et al. 1995). Reuniting *S. invicta* with several species of its native *Pseudacteon* parasitoids may ameliorate the ecological dominance currently enjoyed by *S. invicta* in the U.S. (Porter 1998).

The first species of *Pseudacteon* considered for *S. invicta* biocontrol in the United States was *P. tricuspis* Borgmeier. This species was released in Texas during 1995 (Gilbert 1996) and Florida during 1997 (Porter et al. 1999), and is now currently established in multiple states throughout the southeastern United States (Porter et al. 2004), including Louisiana (Henne et al. 2007). These parasitoids detect semiochemicals used by ants for communication, and use these cues to find their hosts (Porter 1998). For example, *P. tricuspis* are attracted to alarm pheromones emitted by *S. invicta* during mound disturbances and intra- and interspecific encounters (Morrison and King 2004), and primarily attack *S. invicta* major workers (Morrison et al. 1999a). Larvae of these flies decapitate their hosts and make use of the empty head capsule as a pupariation compartment (Porter et al. 1995).

Understanding of phorid fly population dynamics is not well developed (Disney 1994, Morrison 2000). Like most Phoridae, fundamental information about *P. tricuspis* population ecology remains unknown or is inadequate, particularly under climatic conditions unique to Louisiana. Very little information exists concerning the spatial and temporal dynamics of various *Pseudacteon spp.* (Disney 1994), particularly in the United States. Nearly 20 species of *Pseudacteon* are known to attack *S. invicta* in South America (Porter and Pesquero 2001), and three species of *Pseudacteon* have already been imported and released in the United States: *P. tricuspis* (Graham et al. 2001), *P. curvatus*

Borgmeier (Graham et al. 2003) and *P. litoralis* Borgmeier (Porter and Alonso 1999). Several other species are currently under evaluation for possible release in the United States in the next few years as well [e.g. *P. borgmeieri* Schmitz (Folgarait et al. 2002a), *P. cultellatus* Borgmeier (Folgarait et al. 2002b, *P. obtusus* Borgmeier (Folgarait et al. 2005), and *P. nocens* Borgmeier (Folgarait et al. (2006)]. Studying the population dynamics of *P. tricuspis* in Louisiana will not only provide valuable knowledge about the natural history of phorid flies, the results will also be useful in evaluating the population dynamics of other species of parasitic phorids as well.

This study addressed the following objectives to enhance our understanding of *Pseudacteon* population dynamics, particularly in Louisiana, by supplementing previous studies on *Pseudacteon* spp. in the United States by Morrison et al. (1999b) in Texas, and Morrison and Porter (2005a) in Florida: 1) determine the daily activity pattern of *P. tricuspis*, and relate these patterns to various abiotic variables; 2) determine the dynamic behavior of *P. tricuspis* populations over an extended time, determine if populations are synchronized over small and large spatial scales, and determine if populations are correlated with various abiotic variables; 3) determine the sex ratios and frequency distributions of *P. tricuspis* at disturbed *S. invicta* mounds; and 4) determine the minimum sample size and sampling methodology that will provide an estimate of the true relative population mean of *P. tricuspis* at any location.

MATERIALS AND METHODS

Daily and Seasonal Survey Sample Locations

The developmental rate of *P. tricuspis* from egg to adult is approximately 33 days at 30 °C (Morrison et al. 1997). Therefore, multivoltinism in *P. tricuspis* is likely to

occur in Louisiana and elsewhere in the southern United States. Consequently, *P. tricuspis* daily activity and relative abundances were evaluated at approximately monthly intervals from June to October 2004, and January to November 2005 at two sites in southeast Louisiana, separated by approximately 100 km. The first study site: (30° 59′ 05″ N; 91° 00′ 46″ W), located along LA 422, 9 km east of Norwood (East Feliciana Parish) was characterized as unmaintained pasture, approximately 20-30 ha in size, and surrounded by mature hardwood trees along its border. The second study site: (30° 32′ 33″ N; 90° 02′ 50″ W) located along LA 1082, 9 km northeast of Covington (St. Tammany Parish) was characterized as a horse-training facility, with approximately 6 acres of unmaintained pasture and included two large (~0.5ha) ponds. Whenever possible, both sites were sampled within a few days of one another.

2004-2005 Surveys and Sampling Methodology

Three 0.5 ha plots were permanently established at each site to obtain variance estimates of *P. tricuspis* relative abundances (i.e. # *P. tricuspis*/mound) and evaluate spatial correlations in abundances (Figure 7.1). At hourly intervals, between 0900h and 1600h Central Standard Time (CST), five *S. invicta* mounds were haphazardly selected in a subsection of each plot and marked with a wire stake flag. Plots were separated by at least 50-100 m to minimize effects of sampling *S. invicta* mounds on *P. tricuspis* populations in adjacent plots. Hourly surveys were conducted in a diagonal pattern (figure 7.1) so that consecutive surveys were conducted as far apart as possible. Morrison et al. (1999b) determined that *Pseudacteon* parasitoids of *S. geminata* near Austin, TX were attracted to *S. geminata* colonies at distances of <50 m. It is unknown if *P. tricuspis* are similarly attracted from these distances. However, the five randomly

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disturbed mounds were usually >10 m apart and were disturbed within a short period of time (1-2 mins.).
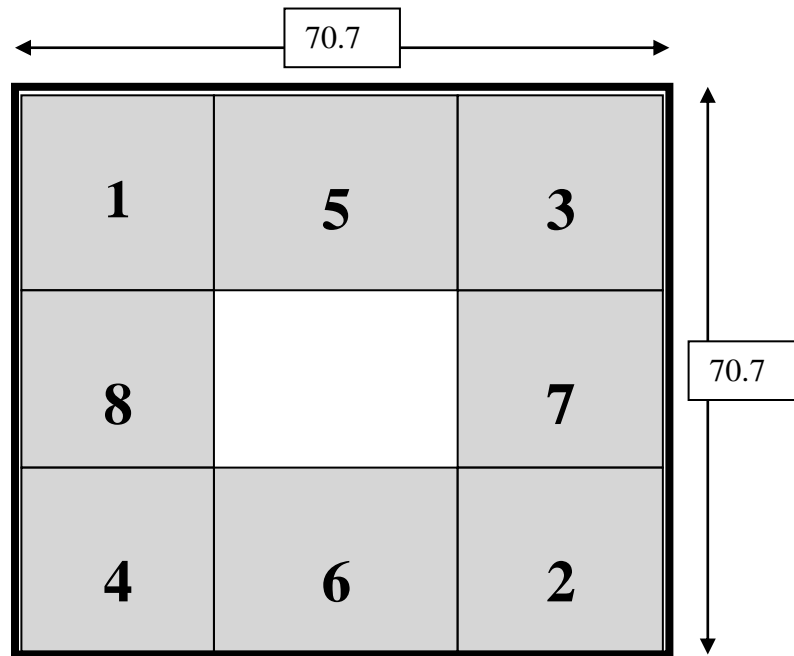
Figure 7.1: Hourly sampling pattern inside 0.5 ha survey plots. Numbers indicate order of sampling.

Williams et al. (1973) determined that a significantly greater number of phorids appear at disturbed mounds than at undisturbed mounds, and disturbing *S. invicta* mounds has been the conventional method of attracting and quantifying *P. tricuspis* populations in Louisiana since 1999. Therefore, a circular depression (approximately 15 cm diameter) was made in *S. invicta* mounds using a small spade. Wire stake-pierced Styrofoam discs (30 cm diameter) were used to shade disturbed fire ant mounds from the sun to prevent overheating and to maintain fire ant activity at the soil surface. All mounds were vigorously disturbed for at least ten seconds, and five minutes was allowed to elapse before counts of *P. tricuspis* were made. Two minutes observation time was allocated to each mound and fly counts at all mounds were made during 15-minute

periods so that all three plots could be sampled on an hourly basis. Population surveys were conducted only when air temperatures exceeded 20° C, as this temperature is considered a threshold temperature for *Pseudacteon* activity (Morrison et al. 1999b).

Before hourly surveys began, soil moisture at 10 cm depth in one plot was measured with a Lincoln soil moisture meter (Forestry Suppliers Part No. 3052), which ranks soil moisture on a scale of 1-10 (1=driest, 10=wettest). Ten measurements were made at 5 m intervals along a transect running from the corner towards the center of one plot, and the average of these values used in statistical analyses (Morrison et al. 2000). Readings were taken at approximately the same locations during each survey. To obtain an estimate of soil % moisture, three soil samples, each approximately 15 cm x 15 cm x 10 cm, were excavated with a shovel from random areas within one plot, placed in individual plastic bags and returned to the laboratory, where vegetation was carefully removed and weighed, and the soil samples dried in a desiccating oven for one week at 70º C and dry weights taken. Rainfall data was recorded with an HOBO® event recorder w/rainwise 1/100" self-emptying rain gauge (Gempler's Item No. G77651). Long-term hourly temperature and relative humidity data were recorded with HOBO® H8 Pro Series RH/Temp data loggers (Onset Computer, Pocasset, MA, Part No. H08-032-08).

At the beginning of each sample period the following variables were recorded: the air temperature, relative humidity, and dewpoint was recorded at 30 cm above ground in the shade (see Morrison et al. 2000), and wind speed and direction, averaged over a 10-second period at 1.5 m above ground with a handheld digital weather instrument (Speedtech Instruments® Skymate Plus Wind Meter SM-19, Forestry Suppliers Inc. Part No. 2320). Barometric pressure was recorded with a Brunton® ADC Summit™ weather

meter (Forestry Suppliers Inc. Part No. 89225). Maximum light intensity during a 20-second period was recorded with a light meter (Extech™ light meter, Forestry Suppliers Inc. Part No. 1393). Soil temperature at 2 cm soil depth was recorded with a temperature probe (Forestry Suppliers Part No. 89102), also at the same location over time. Temperatures recorded are correlated with *S. invicta* foraging activity (Porter and Tschinkel 1987) and were used as a proxy for ant activity due to time constraints.

2006 Surveys

The 2006 surveys were conducted on three occasions (June, July and September) at the Norwood and Covington locations described above, and on two occasions (June and October) at multiple locations (n=8) within Washington Parish, Louisiana. For each survey, at least 30-45 *S. invicta* mounds were randomly sampled and disturbed over a 3-4 hour period during the late morning and early afternoon. All *P. tricuspis* that appeared at disturbed mounds were captured into individual 2-dram glass vials with an Allen-type double chamber vial aspirator (BioQuip® #1135C), labeled and returned to the laboratory for sex determination. For each sample occasion, the percentages of mounds that attracted the numbers of the following were calculated: flies, males, females, males alone, females alone, and males and females together.

Statistical Analyses

Daily Activity Patterns

To determine if daily fly abundance patterns were correlated with measured environmental variables, total hourly fly survey counts for each of the Norwood and Covington populations during 2004 and 2005 were $\log_{10}n+1$-transformed (where n is the number of *P. tricuspis*) and regressed against the following variables: dewpoint, air, soil

surface temperature (ºC), soil temperature at 2 cm depth (ºC), relative humidity (%), light intensity (lux), air pressure (kPa), average wind speed (km/h) and time of day (CST). Adding one to fly counts was necessary to allow for $\log_{10}$-transformation of zero values. Linear and quadratic functions were fitted and compared using the extra sum-of-squares F-test in Prism[®] 4.03 (GraphPad Software, Inc., San Diego, CA). To account for changing photoperiod through the seasons, hourly sample times were also standardized according to the number hours elapsed since sunrise (see Pesquero et al. 1996) and were determined using the U.S. Naval observatory data service at http://aa.usno.navy.mil/. The linear and quadratic terms were considered significant if the associated p-values were ≤ 0.05.

Seasonal Dynamics

To determine if the three plot populations at each of the Norwood and Covington study sites fluctuated synchronously during 2004 and 2005, the total numbers of *P. tricuspis* observed in each of the three plots for each individual survey were $\log_{10}n+1$-transformed and Pearson Product-Moment Correlation (PPMC) coefficients computed. Similarly, to determine if the Norwood and Covington populations fluctuated synchronously over time, the mean $\log_{10}n+1$-transformed fly counts for each individual survey during 2004 and 2005 were also analyzed and PPMC coefficients computed. Additionally, PPMC coefficients were computed to describe how $\log_{10}n+1$-transformed individual monthly survey total fly counts at Norwood (June 2004 to October 2005) and Covington (July 2004 to October 2005) covaried with the following environmental variables: total monthly rainfall (mm), mean soil probe reading, average soil surface temperature (ºC), soil temperature at 2 cm depth (ºC), and air temperatures (ºC)

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(Morrison et al. 2000). Finally, the mean Norwood and Covington populations over the entire 2004-2005 survey were compared. The total daily fly counts for each population were $\log_{10}n+1$-transformed and compared with a 2-tailed t-test at a significance level of $\alpha=0.05$.

Time Series Analysis

Analyses of population dynamics usually employ time series methodology to analyze population abundances to determine the time lag on which negative feedback processes are acting, such as density dependence (Hunter and Price 1998, Benton et al. 2006). Data consisting of observations taken over time may be autocorrelated, where the assumption of independent error terms may not be valid (Bence 1995). Ordinary least squares procedures on autocorrelated data can lead to Type 1 errors in hypothesis testing, as well as confidence intervals that are smaller in size than they should be (Hurlbert 1984, Bence 1995, Neter et al. 1996). Here, tests for autocorrelation on *P. tricuspis* time series data were conducted using a first-order autoregressive (AR) model, followed by a Durbin-Watson test for lag-1 autocorrelation. This model assumes positive autocorrelation (i.e. population abundance at time t depends on the population abundance at time t-1), which decreases steadily with increasing time between observations (Bence 1995). The Durbin-Watson test scrutinizes the difference between consecutive errors compared to the error values themselves (Sall et al. 2005).

Time series statistical analyses were performed on the 2004-2005 Norwood and Covington survey data using the time series modeling feature in S-Plus™ 7.0 (Insightful Corporation, Seattle, Washington). Autocorrelation coefficients (ACF) and partial correlation coefficients (PACF) of $\log_{10}n+1$-transformed total daily fly counts were

computed and plotted for time lags 1-16 for both sites. The PACF has been employed as a useful tool for diagnosing the order of an AR process (Box and Jenkins 1976, Turchin 1990). Zero counts at Norwood during the February and March 2005, and Covington February 2005 surveys required the addition of one to the counts before $\log_{10}$-transformation (see Turchin 2003). Because surveys were not conducted during November and December 2004, linear interpolation was used to estimate fly counts at both locations for a single point in time between late October 2004 and early January 2005.

Analysis of Fly Count Frequency Distributions

Counts of many biological populations, including insects, are described by the negative binomial distribution (Anscombe 1949). As part of a different study, *P. tricuspis* populations were sampled during October 2006 in Washington Parish, Louisiana. Poisson and negative binomial distributions were fit to the *P. tricuspis* survey count frequency distributions using log-likelihood regression (SAS PROC GENMOD) and compared with expected frequencies using SAS PROC FREQ (SAS Institute 2002, http://www.stat.lsu.edu/faculty/moser/exst7024/distributions/discretedata-body.html).

Sample Size

Determining sample size is an important consideration for any sampling program. It should be large enough to enable suitably precise parameter estimation, but not unreasonably large (Manly 2001). The allowable precision level in ecological research is normally 10-25% (Southwood 1978), and is defined as $D = SE / \bar{x}$ where $\bar{x}$ is the sample mean abundance and SE is the standard error of the mean abundance (Zhou et al. 2004). Equations to estimate sample sizes are available, but normally apply to samples

obtained from unit areas (i.e. absolute population estimates *sensu* Southwood 1978). In this study, disturbed *S. invicta* mounds serve as 'traps' and therefore only provide an estimate of relative *P. tricuspis* abundances. To determine the minimum sample size that would provide a precise estimate of the population mean and standard error, the October 2006 Washington Parish *P. tricuspis* survey counts (n=80) were randomly subsampled using S-Plus 7.0. After three outliers were removed, random samples of 5, 10, 15, 20, 25, 30 50, 77 and 100 with replacement were taken from the truncated dataset to obtain estimates of the mean, standard error and 95% confidence intervals. Next, nonlinear curve fitting of sample sizes plotted against the standard error divided by the mean was performed using Prism® 4.03.

RESULTS

Daily Activity Patterns

The only regression variables that were significantly correlated with fly abundances over time at both locations were light intensity and time of day.   The Norwood and Covington fly activity as a function of light intensity were best fit by a straight line [Norwood light intensity (df=1, 61; F=15.61; p=0.0002), $y = 0.37 + 0.0007x$, (R^2=0.20, n=63)], Covington light intensity [(df=1, 74; F=4.52; p=0.04), $y = 0.65 + 0.0004x$, (R^2=0.06, n=76)]. Adding a quadratic term to the linear fly activity vs. time of day models significantly improved the fit: [Norwood (df=1,60; F=16.16; p=0.0002), $y = -4.64^{e-0.006}x^2 + 0.012x - 6.53$ (R^2=0.25, n=63, Figure 7.2A); Covington: (df=1,66; F=8.39; p=0.005), $y = -3.63^{e-0.006}x^2 + 0.0094x - 4.93$ (R^2=0.23, n=77, Figure 7.2B); Norwood and Covington pooled: (df=1,137; F=18.29; p<0.0001), $y = -3.85^{e-0.006}x^2 + 0.01x - 5.29$ (R^2=0.18, n=140, Figure 7.2C)].

Fly abundances at both study locations generally peaked at midday (1100-1300h CST), at approximately the time of solar maximum. The x-intercepts of the quadratic models suggest a fly activity period lasting from approximately 0700h to 1800h (CST). At Norwood, fly abundances were also positively correlated with wind speed (km/h) [(df=1, 47; F=4.46; p=0.04), $y = 0.77 + 0.04x$, (R^2=0.09, n=63)], and negatively correlated with relative humidity (%) [(df=1, 61; F=5.73; p=0.02), $y = 1.59 - 0.01x$, (R^2=0.09, n=63)] and dewpoint temperature (°C) [df=1, 61; F=6.46; p=0.01), $y = 1.83 - 0.04x$, (R^2=0.10, n=63)] At Covington, fly abundance was also negatively correlated with soil surface temperature [df=1, 30; F=13.83; p=0.0008), $y = 54.39 - 1.007\ x$, (R^2=0.32, n=32)].
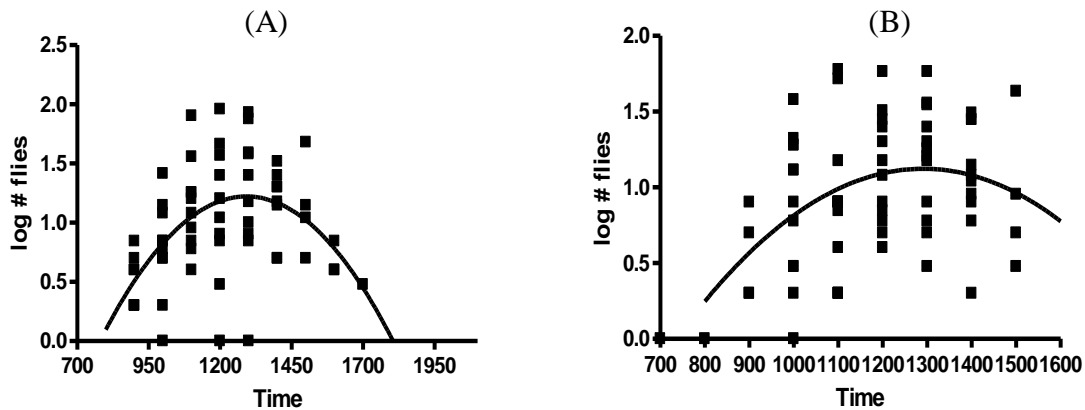
Figure 7.2: Daily activity pattern of *P. tricuspis* as a function of time of day (CST): (A) Norwood, (B) Covington, (C) Pooled.

Figure 7.2 (con't)
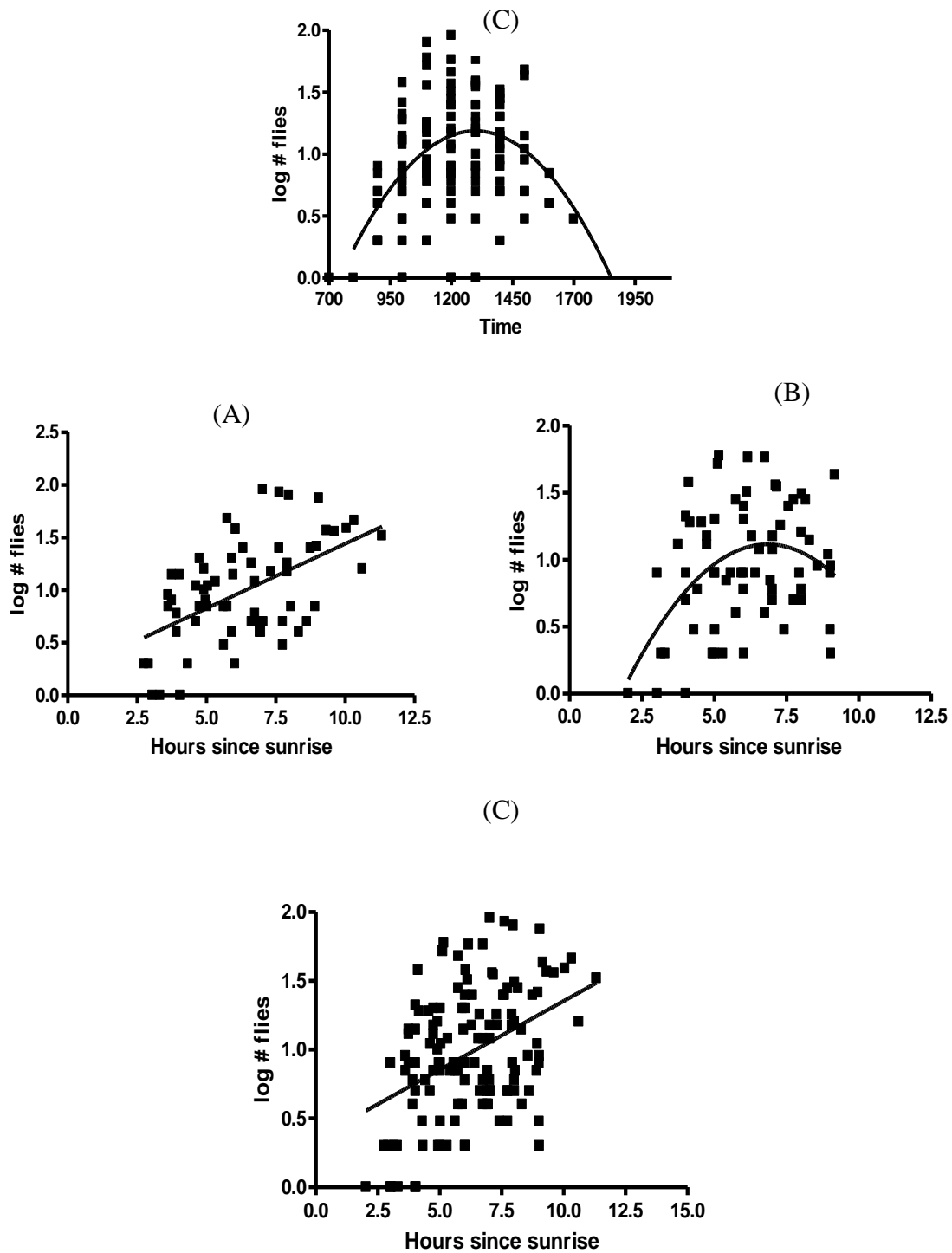
Figure 7.3: Daily activity pattern of *P. tricuspis* as a function of hours elapsed since sunrise: (A) Norwood, (B) Covington, (C) Pooled.

The hourly survey data corrected for elapsed time since sunrise gave a different pattern of results. The Norwood hourly survey data was best fit by a straight line (y=0.123x + 0.212, Figure 7.3 A). Adding a quadratic term did not significantly improve the fit (df=1, 60; F=0.58; p=0.45). However, adding a quadratic term significantly improved the fit of the Covington survey data [(df=1,66; F=8.049; p=0.006) y = -0.0436x^2 + 0.5962x - 0.9251 (R^2=0.17, n=77, Figure 7.3B)]. The combined data from Norwood and Covington was also best fit by a straight line (y=0.1x + 0.35). Adding a quadratic term did not significantly improve the fit (df=1, 129; F=3.09; p=0.08, Figure 7.3C).

<center>Seasonal Dynamics</center>

Pseudacteon tricuspis abundances at Norwood and Covington were generally highest in the late summer and early fall in Louisiana during both survey years (Figure 7.4). However, flies were still active at both locations during early January 2005, but were rare or absent during February and March 2005, despite temperatures that were warm enough for fly activity. During 2005, both populations displayed three discrete peaks in abundance: late May, late July and late September at Norwood, and late April, late June and late September at Covington. During 2004, population abundances at both locations were not significantly correlated with soil probe readings (Norwood Pearson r=-0.44, p=0.38, r^2=0.20, n=6; Covington Pearson r=-0.17, p=0.78, r^2=0.03, n=5). In 2005, population abundances at both locations were significantly correlated with soil probe readings and had nearly identical PPMC coefficients [Norwood (May to November): Pearson r=0.82, p=0.04, r^2=0.68, n=7; Covington (April to October): Pearson r=0.83, p=0.02, r^2=0.69, n=7].

<center>154</center>

(A)

(B)
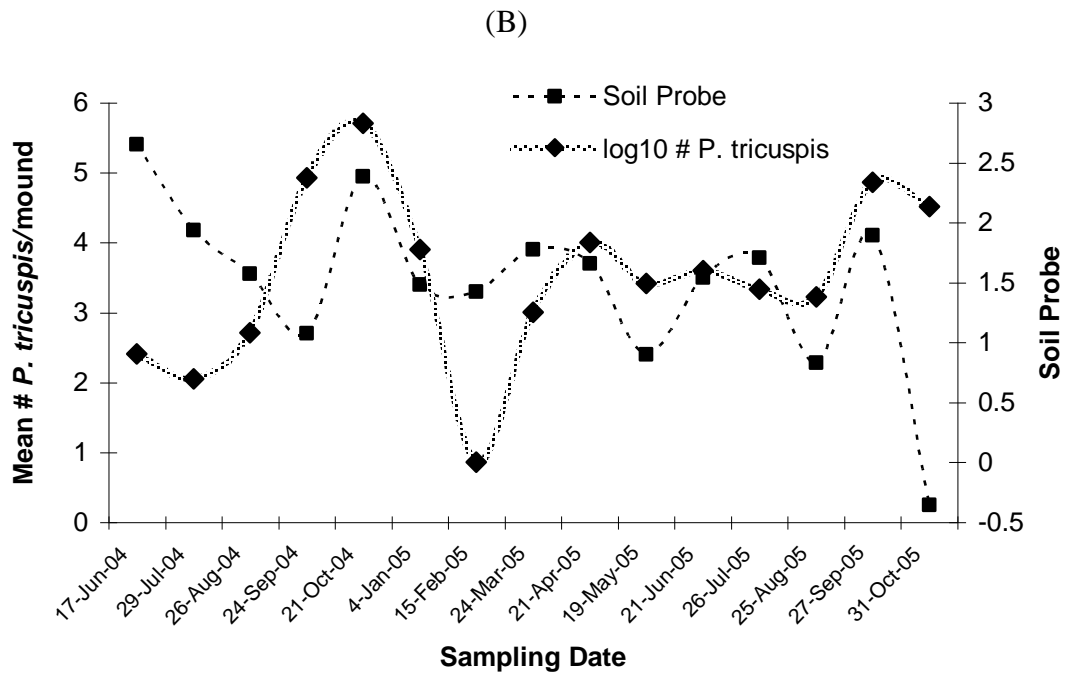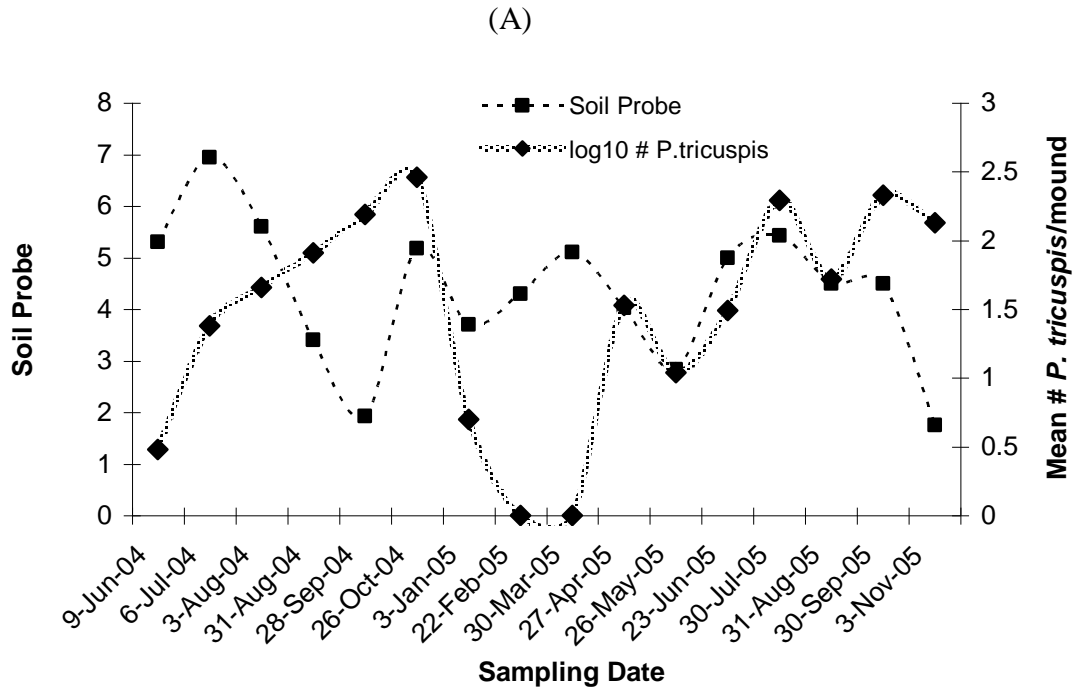
Figure 7.4: Time series graph of mean # *P. tricuspis*/mound and soil moisture probe readings: (A) Norwood, Louisiana; (B) Covington, Louisiana 2004-2005.

Pearson product-moment correlations coefficients between plot fly abundances over time were significant at Norwood (Pearson $r=0.30$, $p=0.04$, $r^2=0.09$, n=48, Figure 7.5A), but not significant at Covington (Pearson $r=0.24$, $p=0.11$, $r^2=0.06$, n=45, Figure 7.5B). However, fly abundances in plots B and C at Covington were significantly correlated (Pearson $r=0.38$, $p=0.03$, $r^2=0.15$, n=30). Overall, the time series fly abundances of the Norwood and Covington populations were significantly correlated (Pearson $r=0.69$, $p=0.004$, $r^2=0.48$, n=15).

(A)
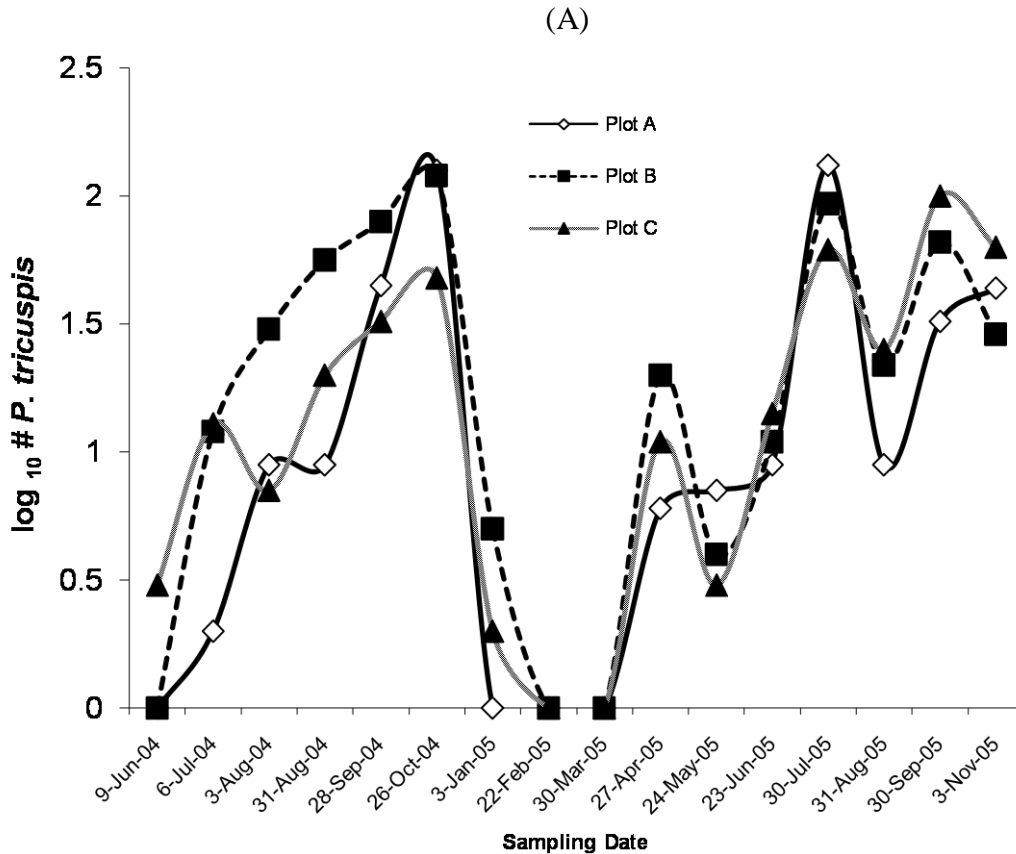
Figure 7.5: Time series graph of log-transformed *P.tricuspis* inside individual sample plots (A) Norwood, (B) Covington.

Figure 7.5 (con't)

(B)

Time Series
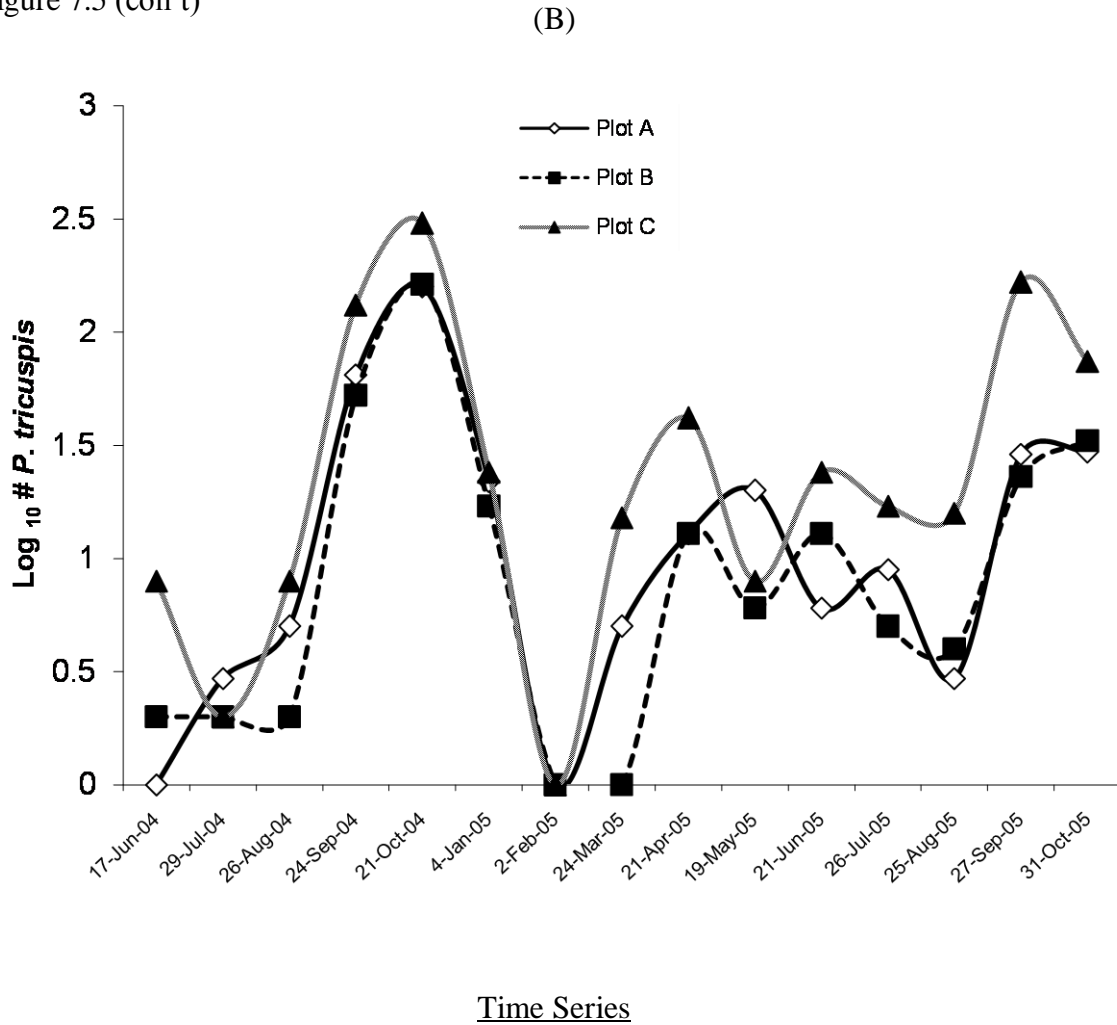
The Norwood *P. tricuspis* population autocorrelation function (ACF) plot revealed minimally significant autocorrelation at lag 1 (Figure 7.6A), indicating that the fly population at time t is dependent on the population at time t-1. In contrast, the Covington ACF plot (Figure 7.6B) showed no significant lag 1 autocorrelation. Partial autocorrelation function (PACF) graphs of both populations (Figures 7.6C, D) had single positive spikes at lag 1, but again this was only significant for the Norwood fly population (Norwood PACF 0.53, Covington PACF 0.44).

(A)

(B)
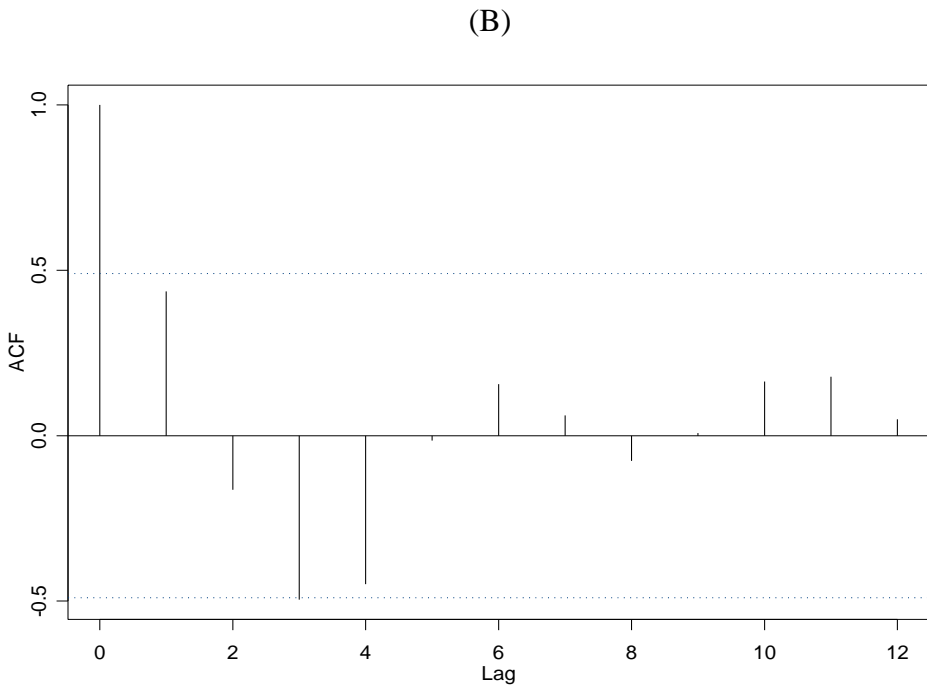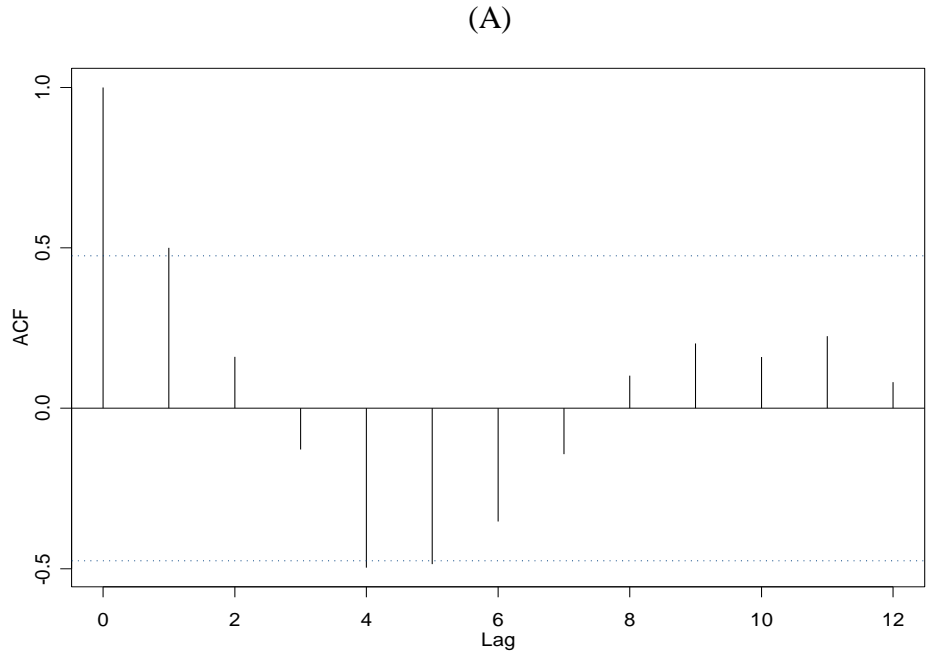
Figure 7.6: Autocorrelation (ACF) and partial autocorrelation function (PACF) plots of the *P. tricuspis* population time series: (A) Norwood ACF, (B) Covington ACF, (C) Norwood PACF, (D) Covington PACF. Dashed lines indicate 95% confidence intervals.

Figure 7.6 (con't)

(C)
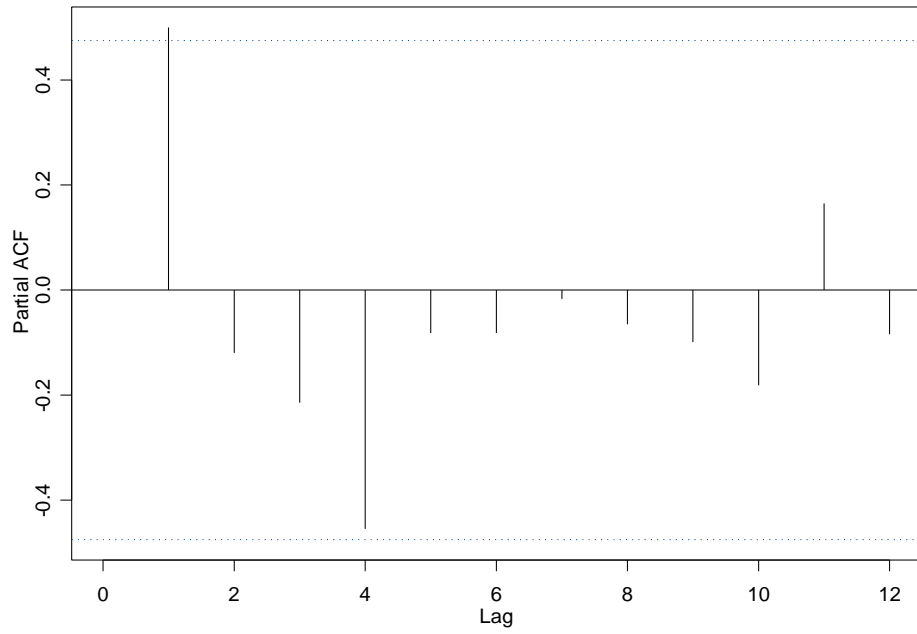
(D)
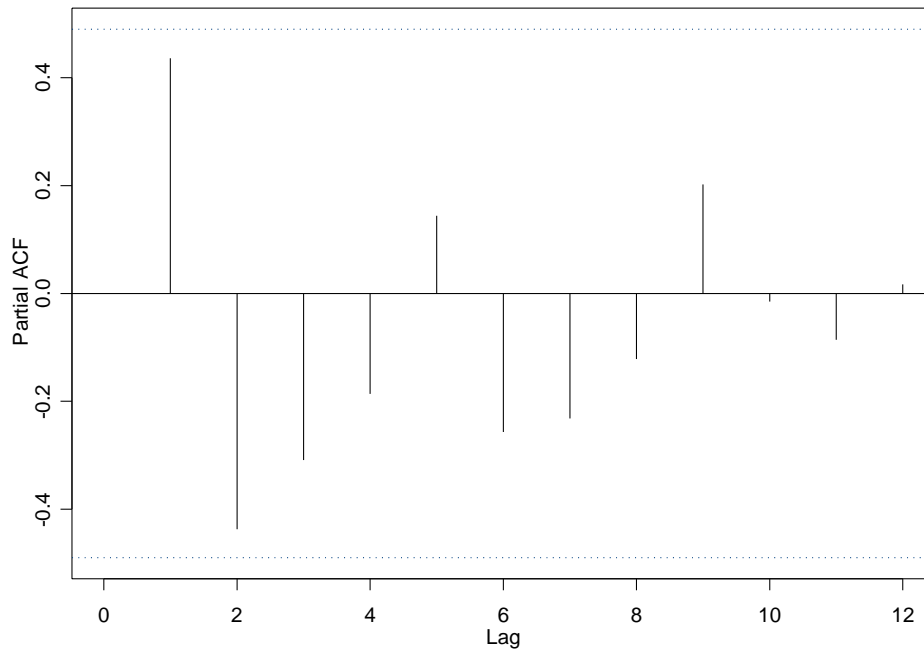
Soil probe readings were significantly correlated with lag 1-month rainfall (mm) at Norwood (Pearson $r=0.75$, $p=0.0009$, $R^2=0.56$, n=16), but not at Covington (Pearson $r=0.07$, $p=0.79$, $R^2=0.005$, n=16). The survey log-transformed mean fly populations at Norwood and Covington were not significantly different (Mean ± SE: Norwood 1.53 ± 0.18, Covington 1.59 ± 0.18, t-test $p=0.81$).

Frequency Distributions, Sex Ratios and Sample Size

Between June and November 2006, nearly 1,500 *P. tricuspis* adults were collected at 52% of disturbed *S. invicta* mounds (range: 22-96%, n=460) (Table 7.1). Of the mounds that attracted flies (i.e. positive), males appeared at an average of 88% (range: 74-100%, n=211) and females 67% (range: 40-91%, n=160). Disturbed mounds that yielded only males occurred at an average of 33% of positive mounds (range: 10-60%, n=79), and females at only 14% (range 0-26%, n=29). Males and females occurred together at an average of 55% of positive mounds (range: 4-91%, n=131). The overall male to female sex ratio at all locations was 1.75:1, at Covington 2.5:1, at Norwood 1.29:1 and at pooled Washington Parish sample locations 1.9:1.

All October 2006 Washington Parish survey frequency distributions were fit well by a negative binomial distribution, as evidenced by goodness-of-fit values close to one (Figure 7.7). Out of 80 *S. invicta* mounds that were disturbed, 75 attracted *P. tricuspis*. Males outnumbered females at 59 mounds, females outnumbered males at only eight mounds, while the other eight had equal numbers of both sexes (Table 7.1). Males appeared at 100% of mounds during the fall surveys in Washington Parish. The percentage of mounds with female appearances increased from spring to fall.

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Table 7.1: Summary of *P. tricuspis* collections made during 2006 at Norwood (N), Covington (C) and multiple locations (n=8) within Washington Parish (WP), Louisiana.

Location	Date	Mounds	Mounds w/ Flies	♂♂	♀♀	♂♂ only	♀♀ only	♂♂ + ♀♀	Total ♂♂	Total ♀♀	#/mound
C	6/1/06	45	10 (22.2%)	8 (80%)	4 (40%)	6 (60%)	2 (20%)	2 (4.4%)	12	4	1.6
N	6/5/06	75	39 (52%)	31 (79.5%)	19 (48.7%)	20 (51.3%)	8 (20.5%)	11 (14.7%)	57	28	2.2
WP	6/6/06	80	23 (28.8%)	17 (73.9%)	13 (56.5%)	10 (43.5%)	6 (26.1%)	7 (8.8%)	40	19	2.6
C	7/26/06	45	29 (64.4%)	25 (86%)	18 (62.1%)	11 (37.9%)	4 (13.8%)	14 (31.1%)	83	35	4.1
N	7/28/06	45	22 (48.9%)	19 (86.4%)	16 (72.7%)	6 (27.3%)	3 (13.6%)	13 (28.9%)	74	54	5.8
N	9/14/06	45	28(62.2%)	25(89.3%)	23(82.1%)	5(17.9%)	3(10.7%)	20(71.4%)	102	98	7.1
C	9/20/06	45	14 (31.1%)	11 (78.6%)	7 (50%)	6 (42.9%)	3 (21.4%)	4 (28.6%)	25	9	2.4
WP	10/18/06	30	27 (90%)	27(100%)	17(63%)	10(37%)	0 (0%)	17 (63%)	151	64	8.0
WP	11/1/06	50	48 (96%)	48 (100%)	43(91%)	5(10%)	0(0%)	43(91%)	386	221	12.7
		460	240 (52%)	211 (88%)	160 (67%)	79 (33%)	29 (14%)	131 (55%)			

	Total ♂♂	Total ♀♀
All sites	930	532
C	120	48
N	233	180
WP	577	304
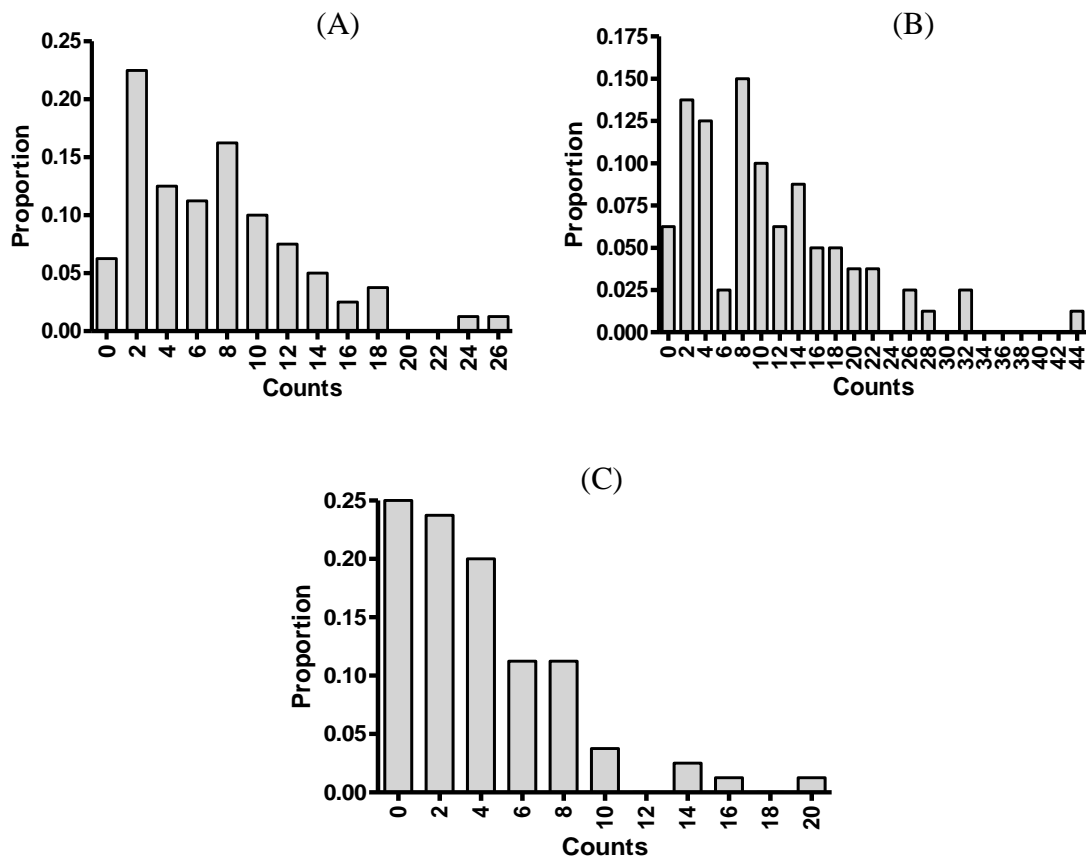
Figure 7.7: Frequency distribution of *P. tricuspis* collected from disturbed *S. invicta* mounds in Washington Parish, Louisiana October 2006: (A) males, (B) females, (C) both sexes combined.

The results of subsampling the October 2006 Washington Parish survey data set indicated no differences in mean # flies/mound when subsamples are compared (*df*=6, 220; *F*=0.938; *p*=0.47). The plot of standard error as a percentage of the mean vs. sample size was fit very well by a one phase exponential decay model (R^2=0.99) (Figure 7.8). If a standard error that is 25% of the mean is acceptable then approximately 15 samples should be taken and a 10% level would require slightly more than 50. However, small sample sizes should be viewed with caution, since confidence intervals are wider.
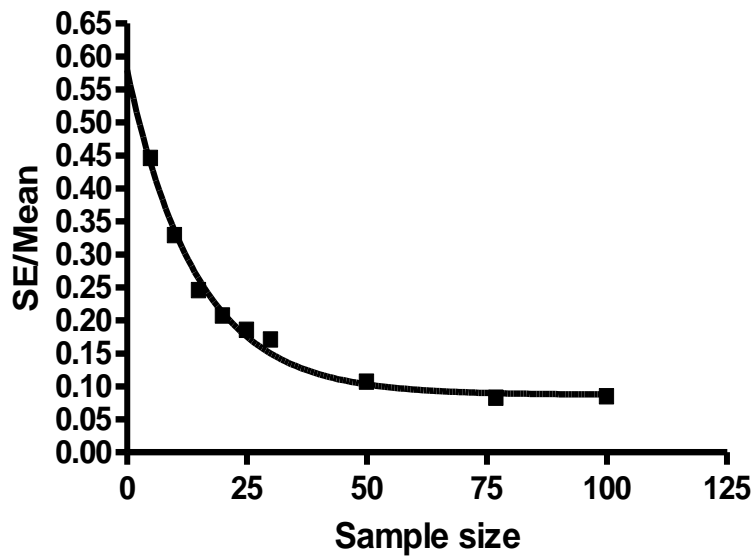
Figure 7.8: Plot of standard error percentage of mean at several sample sizes based on October 2006 Washington Parish survey data.

DISCUSSION

Daily Activity

Diurnal fly activity in Louisiana increased gradually to maximum levels at midday and early afternoon and gradually declined during the late afternoon and evening. Diurnal activity patterns of *P. tricuspis* and *P. littoralis* Borgmeier were studied in Brazil by Pesquero et al. (1996). Activity of *P. tricuspis* in Brazil peaked during midday, and abundances were significantly related to air temperature, soil temperature and humidity. Folgarait et al. (2007) found that *P. tricuspis* in western Argentina were absent for the first two hours following sunrise. In the laboratory, *P. tricuspis* emerge during the early morning hours, with many adults emerging before sunrise, and peak male emergence occurred approximately one or more hours before peak female emergence (Henne and Johnson, Unpublished data). Wuellner et al. (2002) described a similar emergence pattern for *P. curvatus* Borgmeier. The diurnal pattern of *P. tricuspis* is probably entrained by

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photoperiod during the preceding days before eclosion. Although fly abundances were also positively correlated with light intensity on certain days, this activity pattern also occured on cloudy days as well, and even when midday light intensity was very low (i.e. <200 lux). However, Wuelner and Saunders (2003) found that warmer morning temperatures resulted in *Pseudacteon* parasitoids of *S. geminata* (F.) in Texas appearing earlier in the morning. This can probably be attributed to faster physiological development after adult eclosion, allowing adults to become active sooner in the day (see also Wuellner et al. 2002, Folgarait et al. 2007). The linear pattern of fly activity based on hours elapsed since sunrise implies that fly abundance was greatest in the late afternoon and evening. This is consistent with Folgarait et al's (2007) finding that *P. tricuspis* were most abundant during the late afternoon and evening in western Argentina. However, on any given sampling day in Louisiana, *P. tricuspis* abundances peaked during the early afternoon and gradually declined into the evening in a quadratic pattern.

Even though insect diurnal activity patterns tend to be correlated with daily fluctuations in light, temperature, and other environmental variables (Disney 1994), stepwise multiple regression procedures were not utilized in this study to model *P. tricuspis* daily activity patterns with environmental correlates. Other researchers (Pesquero et al. 1996; Morrison et al. 1999a, 2000; Folgarait et al. 2003; Wuellner Saunders 2003) have found significant positive correlations between phorid abundance and air temperature, soil temperature negative correlations with humidity. However, in the studies conducted in Louisiana, relative humidity was also found to be inversely related to temperature, so correlations of phorid abundance with certain environmental variables may simply be coincidental. Using stepwise multiple regression techniques to explain patterns in nature

and to predict future trends has been severely criticized (see Whittingham et al. 2006).
Without conducting manipulative laboratory and field experiments to experimentally test
the effects of these environmental variables on phorid fly behavior, broad generalizations
about environmental correlates might be misleading and should be viewed with caution.

<u>Seasonal Dynamics</u>

Generally, the highest and lowest fly abundances were almost always found in the
same plots and plot abundances at local and regional scales fluctuated synchronously.
Morrison and Porter (2005a) also found that abundances were positively correlated among
survey sites located 8-16 km apart in north-central Florida. Additionally, fly abundances
are known to be positively correlated with *S. invicta* density (Morrison and Porter 2005b).
Although *S. invicta* mound populations were not evaluated *per se*, the plots that had higher
phorid abundances in Louisiana had more fire ant mounds. Large-scale spatial synchrony
in animal population dynamics appears to be a general phenomenon among animal
populations (Ranta et al. 1995, Heino et al. 1997), including *P. tricuspis* populations in
Louisiana that are separated by 100km.

In Louisiana, *P. tricuspis* populations fluctuated throughout the year, but were
highest during the late summer and fall and lowest during the winter and early spring. This
is consistent with findings by Morrison and Porter (2005b) in north-central Florida. Fowler
et al. (1995) evaluated seasonal activity of *Pseudacteon* spp. in Brazil and found *P.
tricuspis* to be the seasonally most abundant species. Folgarait et al. (2003) studied the
seasonal activity patterns of adult *Pseudacteon* spp. that attack *S. richteri* Forel in
Argentina and determined that *P. tricuspis* was associated with certain months, mainly
those in the fall, with greater rainfall and fewest days with frosts. *Pseudacteon tricuspis*

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is active in all months of the year in north-central Florida (Porter et al. 2004, Morrison and Porter 2005a). In Louisiana, *P. tricuspis* were rare or absent at both locations during February and March 2005. It is unknown why adults were difficult to collect during the late winter, even though ambient temperatures were warm (>25° C). However, the soil temperatures at 2 cm depth were only 18-20° C from January through March 2005 at Norwood and during January and February 2005 at Covington. Cool soil temperatures during the winter may have slowed development of phorid pupae, or they were in diapause. Folgarait et al. (2007) discuss the possibility of pupal diapause in *Pseudacteon* in Argentina.

Morrison et al. (1999b, 2000) studied the phenology of *Pseudacteon* parasitoids of *S. geminata* in central Texas and discovered that phorid abundances varied seasonally, with rainfall patterns possibly linked to these abundances. Morrison et al. (2000) also determined that soil moisture levels were often a good predictor of phorid abundance. In Louisiana, seasonal dynamics of *P. tricuspis* at both locations were significantly correlated with the soil moisture readings at 10 cm depth during 2005, but not during 2004. Frequent heavy rain occurred during much of June 2004 at both Norwood and Covington. Inclement weather would have suppressed fly activity significantly, and this was observed on several occasions during 2004 when light rain and drizzle often curtailed or stopped fly activity.

Three peaks in abundances occurred at both Louisiana locations during 2005. Morrison and Porter (2005a) also documented three seasonal peaks in *P. tricuspis* abundances in north-central Florida. These abundance peaks may be linked to *S. invicta* alate flights. Alate flight events in *S. invicta* are triggered by rainfall > 5mm following a period of dry weather (Markin et al. 1971, Morrill 1974). Populations of *P. tricuspis* in

Brazil peak during the spring, in accordance with fire ant mating flights (Fowler et al. 1995). During alate flights, *S. invicta* workers swarm over the surface of the mound and adjacent vegetation in a heightened state of alarm (Markin et al. 1971), presumably to attack potential predators of alate reproductives as they leave the nest. In South America, *Pseudacteon* phorids, including *P. tricuspis*, have been observed attacking fire ants swarming over mound surfaces during alate flight events (Pesquero et al. 1993). In this scenario, many *S. invicta* workers would be vulnerable to attack by searching *P. tricuspis* females during alate flight events. Hypothetically, the population dynamics of *P. tricuspis* may be driven in a density-dependent manner in response to a greater availability of *S. invicta* workers during area wide alate flight events that occur after a rainfall. This factor could explain the synchrony in *P. tricuspis* population dynamics in adjacent plots and in widely separated populations.

Morrison et al. (2000) discussed the importance of environmental variables on the development of *Pseudacteon* parasitoids and their population dynamics. Phorid abundances during any sampling period will be a function of environmental variables from some previous time, the effect of these environmental variables on adults of the previous generation, and the durations of larval and pupal stages in the intervening time. The environmental conditions present during sampling would not be suitable predictors of numbers of phorids attracted to ants. In other words, there would be a time lag in phorid population response to certain environmental conditions that existed between generations. The autocorrelation functions in the time series analyses behave like a damped sine wave, indicating an endogenous component in the population dynamics (Turchin 1990). However, as Berryman and Turchin (1997) warn, time series analysis

should not be employed as a test of hypotheses, but instead be used as a means of identifying potential hypotheses that can then be experimentally tested. If *P.tricuspis* population dynamics are driven by rainfall patterns and alate flight events, then this could be experimentally tested by artificially irrigating large areas of fire ant habitat after an extended dry period and leaving similar areas unirrigated as a control Additionally, local patchiness in alate flight events may also lead to aggregations of *P. tricuspis* in space. However, variance in *P. tricuspis* developmental rates may make it difficult to link *P. tricuspis* population dynamics to exogenous drivers or delayed density-dependence (see also Turchin 1990, Hunter and Price 1998).

<u>Frequency Distributions, Sex Ratios and Sample Size</u>

Male to female sex ratios in Louisiana varied by locations, but were roughly 2:1 overall. Calcaterra et al. (2005) found that *P. tricuspis* male-female sex ratios at fire ant mounds at multiple locations in three regions of southern South America were also approximately 2:1. Morrison and Porter (2005a) found male to female sex ratios of 2.65:1 in north-central Florida. Sex ratios of *Pseudacteon* parasitoids that appear at disturbed colonies and along foraging trails are often male-biased (Pesquero et al. 1993, Morrison et al. 2000, Wuellner and Saunders 2003). A discussion of sex ratio theory and *P. tricuspis* sex ratios is presented in Chapter 3.

Pesquero et al. (1993) found that many phorid males were attracted to alate swarms emanating from fire ant colonies, presumably as an assembly cue to encounter female phorids. Males of *P. tricuspis* are often present at *S. invicta* mounds and appear to feign attacks on ant workers (Porter 1998, Morrison 2000). It is thought that this behavior elicits the production of alarm pheromones by *S. invicta* workers, potentially

attracting *P. tricuspis* females to these ants, and allowing males to copulate with these females (Porter 1998).

Overall, *P. tricuspis* was collected from 52% of disturbed *S. invicta* mounds in the 2006 Louisiana surveys, with a similar pattern from surveys conducted during 2004 and 2005. In Calcaterra et al.'s (2005) study, 14 *Pseudacteon* species were collected at 51% of disturbed fire ant mounds in South America. The percentage of disturbed mounds that attracted *P. tricuspis* in Louisiana tended to increase from spring through fall.

The fly count frequency distributions were highly skewed to the left, with many counts of 1-3 flies/mound. Furthermore, the variance was much larger than the mean in all fly surveys, suggesting a negative binomial distribution. However, it is important to mention that the true spatial dispersion pattern of flies is unknown. The count frequency distributions presented here merely reflects the count distribution of flies attracted to disturbed fire ant mounds, not the distribution in the environment. Given that *P. tricuspis* aggregates at disturbed fire ant mounds, a negative binomial distribution was expected.

In contrast to findings reported by Puckett et al. (2007), mechanically disturbing *S. invicta* mounds regularly attracted many *P. tricuspis*, and is viewed as a reliable method of sampling *P. tricuspis*. During the late summer and fall in Louisiana, >100–200 flies/mound can be attracted within a few minutes of disturbance, and often appear at >90% of disturbed mounds. Extremely vigorous trauma was inflicted upon *S. invicta* mounds in the Louisiana surveys, which probably enhanced attractiveness to *P. tricuspis*. The surveys conducted in Louisiana were very labor-intensive but were necessary to determine broad spatial and temporal activity patterns.

Findings in this study indicate that the following protocols should be followed when sampling *P. tricuspis* populations in Louisiana, should disturbing fire ant mounds be the chosen method of attracting flies. Sampling should be conducted during the late morning or early afternoon, during peak fly activity, as long as temperatures are >20° C. However extremely hot temperatures (>36° C, Henne et al. 2007) or rain may curtail fly activity. In Louisiana, *P. tricuspis* population abundances can vary considerably throughout the year, but abundances consistently peak during the late summer and fall, predominantly October. Therefore, sampling should be conducted during the late summer and fall. At least 15 fire ant mounds should be sampled to obtain an estimate of the true *P. tricuspis* population mean with a precision level of 25%. As abundances can vary considerably at local spatial scales (see also Chapter 3), it is recommended that samples be taken in several locations so that a representative portion of an area is sampled. In addition to providing essential information about *P. tricuspis* population ecology in Louisiana, results of this study will be useful in conservation, augmentation, sampling and management of *P. tricuspis*.

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CHAPTER 8

CONCLUSIONS

SUMMARY

In this dissertation, laboratory and field observations and experiments on the population ecology of a red imported fire ant parasitoid, *P. tricuspis* were conducted. These studies were necessary to fill considerable gaps in our knowledge about phorid flies in general and *Pseudacteon* parasitoids in particular. The laboratory studies described in Chapter 2 revealed that parasitized *S. invicta* workers remained inside the nest during parasitoid larval development, and left the colony approximately 8-10 hours before decapitation by the parasitoid. When parasitized ants left the colony, they were highly mobile, were responsive to tactile stimuli, and showed minimal defensive behavior. Ants ultimately entered into a grass thatch layer, where they were decapitated and the fly maggots pupated. This study reveals that parasitized ants exhibit behaviors that are consistent with host manipulation to benefit survival of the parasitoid. An important outcome of this study will be for future researchers to determine the mechanisms by which the *P. tricuspis* maggot manipulates its host.

The studies conducted in Chapter 3 provided insights into *P. tricuspis* behavioral and functional responses that were unknown until now. I conducted laboratory evaluations to quantify aggregative responses of *P. tricuspis* adults to variable host densities, determine effect of direct mutual interference between pairs of ovipositing *P. tricuspis* females confined with host *S. invicta*, elucidate the effect of confining 1 or 2 additional males with already mated females on progeny sex ratios, and, lastly, determine the form of the functional response of individual ovipositing *P. tricuspis* to varying host densities. The density-dependent aggregations of *P. tricuspis* observed in the laboratory were consistent with theory and field observations. No evidence of direct mutual

interference was found when two or three female *P. tricuspis* were confined with hosts in small containers, although per capita oviposition success, measured as number of hosts killed, appeared to decline when more than two females were confined. This study did not demonstrate any reductions in estimates of searching efficiency of at least 2 or 3 simultaneously ovipositing *P. tricuspis* females. This study also did not reveal any significant effect of having additional males confined with solitary mated females. Together, mutual interference of conspecific male and females at low densities does not appear to be significant, but may become important at higher densities. However, the sex ratios trended downward toward a 1:1 ratio when the number of males confined with a single female was increased from zero to two. None of the linear parameters in the logistic models were significantly different from zero suggesting that *P. tricuspis* had constant attack rates regardless of host density under the laboratory experimental design. The Type I functional response found was unexpected on the grounds that most parasitoids appear to have a Type II functional response. It is expected that the results obtained in this study will stimulate further research into testing host-parasitoid theory with *Pseudacteon* flies.

Chapter 4 was an attempt to model the population structure of *P. tricuspis* on a local spatial scale, and relate *P. tricuspis* spatial abundances to host social form and colonies infected with the fire ant pathogen, *Thelohania solenopsae*. No significant spatial associations were found between *P. tricuspis* counts and host *S. invicta* colonies infected with *T. solenopsae*. However, significant clustering of counts occurred when *P. tricuspis* populations peaked, and were associated with polygyne host colonies. Overall, *P. tricuspis* count patterns were largely random spatially and temporally.

In Chapter 5, I was interested in quantifying local movement of *P. tricuspis* by conducting multiple mass-release-recapture studies to determine the redistribution patterns of *P. tricuspis* dispersers and fit a diffusion curve to the dispersal data. Drift of dispersing flies was found on several occasions, and was probably wind-induced. Diffusion rates ranged between 58 m^2/h and 280 m^2/h, and tended to decline over time after release. A departure from a density-distribution predicted by a simple diffusion model occurred in this study. The lack of fit of the simple diffusion model implies that redistribution in *P. tricuspis* may be better described with a heterogeneous diffusion model (see Cronin et al. 2000). The recently described phorid fly sticky trap (Puckett et al. 2007) could be a useful tool in other *Pseudacteon* dispersal studies to ascertain long-distance dispersal events and model dispersal kernels. Additionally, the putative role of wind in transporting *P. tricuspis* long distances should be tested experimentally. Nevertheless, the study reported in Chapter 5 provides valuable information about phorid fly dispersal and redistribution that was previously unknown.

In Chapter 6, the long-term pattern of spread of *P. tricuspis* was monitored in four directions at two widely separated release sites in Louisiana from 1999-2006. At both sites, *P. tricuspis* range expansion, measured as the mean radius of the range from four cardinal directions, was accelerating during the first four years post release. This pattern also contrasted with a linear pattern expected with simple diffusion, suggesting that population spread involved both a neighborhood diffusion and long-distance dispersal component. This is known as stratified or jump dispersal. This is consistent with findings in Chapter 5, where diffusion was not well described by a simple model of random diffusion. Annual rates of spread were low in the first two years post release, possibly

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owing to an Allee effect, increased rapidly in years 3-4, and slowed down or leveled off by years 5-6. Annual spread rates reached a peak of 15-25 km/yr, with the northward spread being about 40% greater than the spread in the other cardinal directions. High rates of spread in the latter years and directional bias in the spread of *P. tricuspis* may have been driven by prevailing winds and two northward-moving hurricanes. Together, the results in Chapters 5 and 6 are important contributions toward understanding animal movement.

Finally, in Chapter 7 the daily and seasonal dynamics of *P. tricuspis* were studied. I was interested in relating these dynamics to various abiotic variables, determine if populations were synchronized over small and large spatial scales, determine the sex ratios and frequency distributions of *P. tricuspis* that appear at disturbed *S. invicta* mounds, and determine the minimum sample size and sampling methodology that would provide an estimate of the true relative population mean of *P. tricuspis* at any location. Daily patterns of relative abundance followed a quadratic pattern, with peak fly activity during the afternoon. Seasonally, *P. tricuspis* relative abundances were variable and appear positively correlated with soil moisture levels. Peak seasonal abundances occurred during the late summer and fall in Louisiana, while abundances were lowest during the late winter and early spring. The following protocols were derived from the results in Chapter 7, and are recommended when sampling *P. tricuspis* populations in Louisiana, should disturbing fire ant mounds be the chosen method of attracting flies. Sampling should be conducted during the late morning or early afternoon, during peak fly activity, as long as temperatures are >20° C; however, extremely hot temperatures (>36° C, Henne et al. 2007) or rain may curtail fly activity. In Louisiana, *P. tricuspis* population

abundances can vary considerably throughout the year, but abundances consistently peak during the late summer and fall, predominantly October. Therefore, sampling should be conducted during the late summer and fall. At least 15 fire ant mounds should be sampled to obtain an estimate of the true *P. tricuspis* population mean with a precision level of 25%. As abundances can vary considerably at local spatial scales (see also Chapter 3), it is recommended that samples be taken in several locations so that a representative portion of an area is sampled.

In conclusion, a very broad range of studies were conducted to evaluate aspects of *P. tricuspis* behavior and population ecology that were either unknown or poorly known. In addition to providing essential information about *P. tricuspis* population ecology in Louisiana, results of this study will be useful in conservation, augmentation, sampling and management of *P. tricuspis*. It is expected that the findings here will applicable to other species of *Pseudacteon* that have been released in the United States for the biological control of the red imported fire ant.

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APPENDIX A

FUNCTIONAL RESPONSE SAS CODES

```
title 'functional response A';
data functional_response;
      input NO REP FATE NE;/*N0 = initial number of prey, REP =
replicate number,
      FATE: 0 = prey eaten 1 = prey alive, NE = count of prey in each
FATE */
      NO2=NO**2; /*initial number of prey squared*/
      NO3=NO**3; /*initial number of prey cubed*/
      cards;
      135 1 0 1
      135 1 1 134
      135 2 0 2
      135 2 1 133
      270 1 0 3
      270 1 1 267
      270 2 0 5
      270 2 1 265
      270 3 0 2
      270 3 1 268
      540 1 0 1
      540 1 1 539
      540 2 0 10
      540 2 1 530
      540 3 0 1
      540 3 1 539
      540 4 0 5
      540 4 1 535
      810 1 0 6
      810 1 1 804
      810 2 0 7
      810 2 1 803
      810 3 0 7
      810 3 1 803
      810 4 0 6
      810 4 1 804
      1080 1 0 2
      1080 1 1 1078
      1080 2 0 8
      1080 2 1 1072
      1080 3 0 1
      1080 3 1 1079
      1080 4 0 6
      1080 4 1 1074
      ;
PROC CATMOD DATA=functional_response;
DIRECT NO NO2 NO3;
MODEL FATE = NO NO2 NO3/ML NOPROFILE;
POPULATION NO REP;
WEIGHT NE;
DATA functional_response2;
SET functional_response;
IF FATE=0; PROPEAT= NE/NO;
PROC MEANS DATA=functional_response2;
BY NO NOTSORTED;
VAR PROPEAT;
OUTPUT OUT=funcMEAN MEAN=MEANPROP;
```

```
run;
title 'functional response B';
data functional_response;
      input NO REP FATE NE;/*N0 = initial number of prey, REP =
replicate number,
      FATE: 0 = prey eaten 1 = prey alive, NE = count of prey in each
FATE */
      NO2=NO**2; /*initial number of prey squared*/
      NO3=NO**3; /*initial number of prey cubed*/
      cards;
      135 1 0 2
      135 1 1 133
      135 2 0 5
      135 2 1 135
      135 3 0 2
      135 3 1 133
      270 1 0 3
      270 1 1 267
      270 2 0 4
      270 2 1 266
      540 1 0 2
      540 1 1 538
      540 2 0 1
      540 2 1 539
      540 3 0 4
      540 3 1 536
      810 1 0 3
      810 1 1 807
      810 2 0 6
      810 2 1 804
      810 3 0 3
      810 3 1 807
      1080 1 0 6
      1080 1 1 1074
      1080 2 0 3
      1080 2 1 1077
      1080 3 0 3
      1080 3 1 1077
      1080 4 0 4
      1080 4 1 1076
      ;
PROC CATMOD DATA=functional_response;
DIRECT NO NO2 NO3;
MODEL FATE = NO NO2 NO3/ML MAXITER=100 NOPROFILE;
POPULATION NO REP;
WEIGHT NE;
DATA functional_response2; /* obtaining means and SE's for observed
proportions eaten */
SET functional response;
IF FATE=0; PROPEAT= NE/NO;
PROC MEANS DATA=functional_response2;
BY NO NOTSORTED;
VAR PROPEAT;
OUTPUT OUT=funcMEAN MEAN=MEANPROP;
run;
```

```
title 'functional response C';
data functional_response;
      input NO REP FATE NE;/*N0 = initial number of prey, REP =
replicate number,
      FATE: 0 = prey eaten 1 = prey alive, NE = count of prey in each
FATE */
      NO2=NO**2; /*initial number of prey squared*/
      NO3=NO**3; /*initial number of prey cubed*/
      cards;
      135 1 0 1
      135 1 1 134
      135 2 0 1
      135 2 1 134
      135 3 0 1
      135 3 1 134
      270 1 0 3
      270 1 1 267
      270 2 0 4
      270 2 1 266
      270 3 0 7
      270 3 1 263
      270 3 0 5
      270 3 1 265
      540 1 0 2
      540 1 1 538
      540 2 0 5
      540 2 1 535
      540 3 0 2
      540 3 1 538
      540 4 0 7
      540 4 1 533
      810 1 0 2
      810 1 1 808
      810 2 0 6
      810 2 1 804
      810 3 0 6
      810 3 1 804
      1080 1 0 6
      1080 1 1 1074
      1080 2 0 4
      1080 2 1 1076
      1080 3 0 5
      1080 3 1 1075
      1080 4 0 1
      1080 4 1 1079
      ;
PROC CATMOD DATA=functional_response;
DIRECT NO NO2 NO3;
MODEL FATE = NO NO2 NO3/ML NOPROFILE;
POPULATION NO REP;
WEIGHT NE;
DATA functional response2; SET functional_response;
IF FATE=0; PROPEAT= NE/NO;
PROC MEANS DATA=functional_response2;
BY NO NOTSORTED;
VAR PROPEAT;
OUTPUT OUT=funcMEAN MEAN=MEANPROP;run;
```

```
title 'functional response ABC';
data functional_response;
      input NO REP FATE NE;/*N0 = initial number of prey, REP =
replicate number,
      FATE: 0 = prey eaten 1 = prey alive, NE = count of prey in each
FATE */
      NO2=NO**2; /*initial number of prey squared*/
      NO3=NO**3; /*initial number of prey cubed*/
      cards;
      135 1 0 1
      135 1 1 134
      135 2 0 2
      135 2 1 133
      135 3 0 2
      135 3 1 133
      135 4 0 5
      135 4 1 135
      135 5 0 2
      135 5 1 133
      135 6 0 1
      135 6 1 134
      135 7 0 1
      135 7 1 134
      135 8 0 1
      135 8 1 134
      270 1 0 3
      270 1 1 267
      270 2 0 5
      270 2 1 265
      270 3 0 2
      270 3 1 268
      270 4 0 3
      270 4 1 267
      270 5 0 4
      270 5 1 266
      270 6 0 3
      270 6 1 267
      270 7 0 4
      270 7 1 266
      270 8 0 7
      270 8 1 263
      270 9 0 5
      270 9 1 265
      540 1 0 1
      540 1 1 539
      540 2 0 10
      540 2 1 530
      540 3 0 1
      540 3 1 539
      540 4 0 5
      540 4 1 535
      540 5 0 2
      540 5 1 538
      540 6 0 1
      540 6 1 539
      540 7 0 4
      540 7 1 536
```

```
      540 8 0 2
      540 8 1 538
      540 9 0 5
      540 9 1 535
      540 10 0 2
      540 10 1 538
      540 11 0 7
      540 11 1 533
      810 1 0 6
      810 1 1 804
      810 2 0 7
      810 2 1 803
      810 3 0 7
      810 3 1 803
      810 4 0 6
      810 4 1 804
      810 5 0 3
      810 5 1 807
      810 6 0 6
      810 6 1 804
      810 7 0 3
      810 7 1 807
      810 8 0 2
      810 8 1 808
      810 9 0 6
      810 9 1 804
      810 10 0 6
      810 10 1 804
      1080 1 0 2
      1080 1 1 1078
      1080 2 0 8
      1080 2 1 1072
      1080 3 0 1
      1080 3 1 1079
      1080 4 0 6
      1080 4 1 1074
      1080 5 0 6
      1080 5 1 1074
      1080 6 0 3
      1080 6 1 1077
      1080 7 0 3
      1080 7 1 1077
      1080 8 0 4
      1080 8 1 1076
      1080 9 0 6
      1080 9 1 1074
      1080 10 0 4
      1080 10 1 1076
      1080 11 0 5
      1080 11 1 1075
      1080 12 0 1
      1080 12 1 1079
      ;
PROC CATMOD DATA=functional_response;
DIRECT NO NO2 NO3;
MODEL FATE = NO NO2 NO3/ML MAXITER=50 NOPROFILE;
POPULATION NO REP;
```

```
WEIGHT NE;
DATA functional_response2; /* obtaining means and SE's for observed
proportions eaten */
SET functional response;
IF FATE=0; PROPEAT= NE/NO;
PROC MEANS DATA=functional_response2;
BY NO NOTSORTED;
VAR PROPEAT;
OUTPUT OUT=funcMEAN MEAN=MEANPROP;
run;
title 'functional response 25_200';
data functional_response;
      input NO REP FATE NE;/*N0 = initial number of prey, REP =
replicate number,
      FATE: 0 = prey eaten 1 = prey alive, NE = count of prey in each
FATE */
      NO2=NO**2; /*initial number of prey squared*/
      NO3=NO**3; /*initial number of prey cubed*/
      cards;
      25 1 0 4
      25 1 1 21
      25 2 0 1
      25 2 1 24
      25 3 0 13
      25 3 1 12
      25 4 0 8
      25 4 1 17
      25 5 0 5
      25 5 1 20
      25 6 0 6
      25 6 1 19
      25 7 0 5
      25 7 1 20
      50 1 0 14
      50 1 1 36
      50 2 0 3
      50 2 1 47
      50 3 0 21
      50 3 1 29
      50 4 0 3
      50 4 1 47
      50 5 0 2
      50 5 1 48
      50 6 0 8
      50 6 1 42
      50 7 0 2
      50 7 1 48
      100 1 0 14
      100 1 1 86
      100 2 0 14
      100 2 1 86
      100 3 0 7
      100 3 1 93
      100 4 0 13
      100 4 1 87
      100 5 0 24
      100 5 1 76
```

```
      100 6 0 6
      100 6 1 94
      100 7 0 2
      100 7 1 98
      200 1 0 7
      200 1 1 193
      200 2 0 31
      200 2 1 169
      200 3 0 9
      200 3 1 191
      200 4 0 29
      200 4 1 171
      200 5 0 22
      200 5 1 178
      200 6 0 18
      200 6 1 182
      200 7 0 9
      200 7 1 191
      ;
PROC CATMOD DATA=functional_response;
DIRECT NO NO2 NO3;
MODEL FATE = NO NO2 NO3/ML NOPROFILE;
POPULATION NO REP;
WEIGHT NE;
DATA functional_response2; /* obtaining means and SE's for observed
proportions eaten */
SET functional response;
IF FATE= 0; PROPEAT= NE/NO;
PROC MEANS DATA=functional_response2;
BY NO NOTSORTED;
VAR PROPEAT;
OUTPUT OUT=FUNCMEAN MEAN=MEANPROP;
run;
```

APPENDIX B

DISCRETE PROBABILITY DISTRIBUTION SAS CODES

```
Title "Male Pseudacteon tricuspis at fire ant mounds";
Data maleflies;
 Input Y Frequency;
 Do i=1 To Frequency;
  Output;
 End;
 Keep Y;
Datalines;
 0   5
 1  10
 2   8
 3   4
 4   6
 5   6
 6   3
 7   5
 8   8
 9   4
10   4
;

/*
 * Show original frequency table
 */
Proc Freq Data=maleflies;
 Table Y;
Run;

/*
 * Examine a histogram of the data
 */
Proc GChart Data=maleflies;
 VBar Y / Discrete;
Run;

Proc Univariate Data=maleflies;
 Var Y;
Run;

/*
 * Fit a Poisson distribution to the data
 */
Title3 "Poisson Model";
Proc Genmod Data=maleflies;
 Model Y = / Dist=Poisson Link=Log LRCI;
 Estimate "Population Mean" Intercept 1 / Exp;
 ODS Output ParameterEstimates=Parms;
Run;

/*
 * Compute Expected Probabilities. These
 * will be used in a GOF test to follow.
 */
Data Expected;
 If _N_=1 Then
  Do;
   Set Parms;
```

```
   Lambda=Exp(Estimate); /* First obs is ln(lambda) */
   ELambda=Exp(-Lambda);
    Retain Lambda ELambda;
  End;
 Do Y=0 To 10;
  Prob=(Lambda**Y)*ELambda/Gamma(Y+1); /* Poisson Probability */
  Expected=63*Prob;
  Cummulative+Prob;
  InvCum=1-Cummulative+Prob;
  Output;
 End;
 Stop;
 Keep Y Prob Expected Lambda Cummulative InvCum;
Run;
Title4 "Expected Probabilities";
Proc Print Data=Expected;
Run;

/*
 * Can use PROC FREQ to do GOF test, though
 * d.f. are not correct. Since some expected
 * values will be less than 1, we will group
 * the data for Y>=4 into a common group.
 */
Proc Format;
 Value YGroup 4-High="4+";
Run;

/*
 * Since there will be 5 cells in this table,
 * PROC FREQ will compute the d.f. to be 5-1=4.
 * However, the probabilities were predicted
 * by estimating the parameter Lambda using the
 * same data. Thus we need to lose 1 more d.f.
 * Thus, d.f.=5-1-1=3.
 */
Title4 "Pearson Chi-square Goodness-of-fit Test";
Title5 "Note: Degrees of Freedom Should Be 3";
Proc Freq Data=maleflies;
 Table Y / Chisq NoCum TestP=(1.085 4.907 11.100 16.738 65.479);
 Format Y YGroup.;
Run;

/*
 * Repeat analysis using the Negative Binomial Model.
 */
Title3 "Negative Binomial Model";
Proc Genmod Data=maleflies;
 Model Y = / Dist=NegBin Link=Log LRCI MaxIter=500;
 Estimate "Population Mean" Intercept 1 / Exp;
 ODS Output ParameterEstimates=Parms;
Run;

Data Expected;
 If _N_=1 Then
  Do;
   i=1;
```

```
   Set Parms Point=i Nobs=Nobs;
   Mu=Exp(Estimate); /* First obs is ln(Mu) */
   i=2;
   Set Parms Point=i Nobs=Nobs;
   k=Estimate;     /* Second obs is dispersion parameter */
   kinv=1/k;
   VarY=Mu+k*Mu**2;
   Retain Mu k VarY kinv;
  End;
 Do Y=0 To 10;

Prob=Gamma(Y+kinv)/(Gamma(Y+1)*Gamma(kinv))*(k*mu)**Y/((1+k*mu)**(Y+kin
v)); /* Neg binomial Probability */
  Expected=63*Prob;
  Cummulative+Prob;
  InvCum=1-Cummulative+Prob;
  Output;
 End;
 Stop;
 Keep Y Prob Expected Mu k kinv VarY Cummulative InvCum;
Run;
Title4 "Expected Probabilities";
Proc Print Data=Expected;
Run;


Proc Format;
 Value YGroup 5-High="5+";
Run;


Title4 "Pearson Chi-square Goodness-of-fit Test";
Title5 "Note: Degrees of Freedom Should Be 3";
Proc Freq Data=maleflies;
 Table Y / Chisq NoCum TestP=(6.964 11.830 13.743 13.503 12.059
35.568);
 Format Y YGroup.;
Run;


Title "Female Pseudacteon tricuspis at fire ant mounds";
Data femaleflies;
 Input Y Frequency;
 Do i=1 To Frequency;
  Output;
 End;
 Keep Y;
Datalines;
 0  20
 1  10
 2   9
 3  10
 4   6
 5   6
 6   3
 7   4
 8   5
 9   3
10   0
;
```

```
/*
 * Show original frequency table
 */
Proc Freq Data=femaleflies;
 Table Y;
Run;

/*
 * Examine a histogram of the data
 */
Proc GChart Data=femaleflies;
 VBar Y / Discrete;
Run;

Proc Univariate Data=femaleflies;
 Var Y;
Run;

/*
 * Fit a Poisson distribution to the data
 */
Title3 "Poisson Model";
Proc Genmod Data=femaleflies;
 Model Y = / Dist=Poisson Link=Log LRCI;
 Estimate "Population Mean" Intercept 1 / Exp;
 ODS Output ParameterEstimates=Parms;
Run;

/*
 * Compute Expected Probabilities. These
 * will be used in a GOF test to follow.
 */
Data Expected;
 If _N_=1 Then
  Do;
   Set Parms;
   Lambda=Exp(Estimate); /* First obs is ln(lambda) */
   ELambda=Exp(-Lambda);
   Retain Lambda ELambda;
  End;
 Do Y=0 To 10;
  Prob=(Lambda**Y)*ELambda/Gamma(Y+1); /* Poisson Probability */
  Expected=76*Prob;
  Cummulative+Prob;
  InvCum=1-Cummulative+Prob;
  Output;
 End;
 Stop;
 Keep Y Prob Expected Lambda Cummulative InvCum;
Run;
Title4 "Expected Probabilities";
Proc Print Data=Expected;
Run;

/*
 * Can use PROC FREQ to do GOF test, though
```

```
    * d.f. are not correct. Since some expected
    * values will be less than 1, we will group
    * the data for Y>=4 into a common group.
    */
Proc Format;
 Value YGroup 4-High="4+";
Run;


/*
 * Since there will be 5 cells in this table,
 * PROC FREQ will compute the d.f. to be 5-1=4.
 * However, the probabilities were predicted
 * by estimating the parameter Lambda using the
 * same data. Thus we need to lose 1 more d.f.
 * Thus, d.f.=5-1-1=3.
 */
Title4 "Pearson Chi-square Goodness-of-fit Test";
Title5 "Note: Degrees of Freedom Should Be 3";
Proc Freq Data=femaleflies;
 Table Y / Chisq NoCum TestP=(5.179 15.333 22.697 22.398 34.366);
 Format Y YGroup.;
Run;


/*
 * Repeat analysis using the Negative Binomial Model.
 */
Title3 "Negative Binomial Model";
Proc Genmod Data=femaleflies;
 Model Y = / Dist=NegBin Link=Log LRCI MaxIter=500;
 Estimate "Population Mean" Intercept 1 / Exp;
 ODS Output ParameterEstimates=Parms;
Run;


Data Expected;
 If _N_=1 Then
  Do;
   i=1;
   Set Parms Point=i Nobs=Nobs;
   Mu=Exp(Estimate); /* First obs is ln(Mu) */
   i=2;
   Set Parms Point=i Nobs=Nobs;
   k=Estimate;    /* Second obs is dispersion parameter */
   kinv=1/k;
   VarY=Mu+k*Mu**2;
   Retain Mu k VarY kinv;
  End;
 Do Y=0 To 10;

Prob=Gamma(Y+kinv)/(Gamma(Y+1)*Gamma(kinv))*(k*mu)**Y/((1+k*mu)**(Y+kin
v)); /* Neg binomial Probability */
  Expected=76*Prob;
  Cummulative+Prob;
  InvCum=1-Cummulative+Prob;
  Output;
 End;
 Stop;
 Keep Y Prob Expected Mu k kinv VarY Cummulative InvCum;
```

```
Run;
Title4 "Expected Probabilities";
Proc Print Data=Expected;
Run;


Proc Format;
 Value YGroup 5-High="5+";
Run;


Title4 "Pearson Chi-square Goodness-of-fit Test";
Title5 "Note: Degrees of Freedom Should Be 3";
Proc Freq Data=femaleflies;
 Table Y / Chisq NoCum TestP=(21.871 19.264 15.250 11.620 8.681
20.066);
 Format Y YGroup.;
Run;


Title "Pseudacteon tricuspis at fire ant mounds";
Data flies;
 Input Y Frequency;
 Do i=1 To Frequency;
  Output;
 End;
 Keep Y;
Datalines;
 0    5
 1    4
 2    7
 3    6
 4    6
 5    2
 6    0
 7   11
 8    1
 9    3
10    5
;

/*
 * Show original frequency table
 */
Proc Freq Data=flies;
 Table Y;
Run;

/*
 * Examine a histogram of the data
 */
Proc GChart Data=flies;
 VBar Y / Discrete;
Run;


Proc Univariate Data=flies;
 Var Y;
Run;

/*
```

```
 * Fit a Poisson distribution to the data
 */
Title3 "Poisson Model";
Proc Genmod Data=flies;
 Model Y = / Dist=Poisson Link=Log LRCI;
 Estimate "Population Mean" Intercept 1 / Exp;
 ODS Output ParameterEstimates=Parms;
Run;

/*
 * Compute Expected Probabilities. These
 * will be used in a GOF test to follow.
 */
Data Expected;
 If _N_=1 Then
  Do;
   Set Parms;
   Lambda=Exp(Estimate); /* First obs is ln(lambda) */
   ELambda=Exp(-Lambda);
   Retain Lambda ELambda;
  End;
 Do Y=0 To 10;
  Prob=(Lambda**Y)*ELambda/Gamma(Y+1); /* Poisson Probability */
  Expected=76*Prob;
  Cummulative+Prob;
  InvCum=1-Cummulative+Prob;
  Output;
 End;
 Stop;
 Keep Y Prob Expected Lambda Cummulative InvCum;
Run;
Title4 "Expected Probabilities";
Proc Print Data=Expected;
Run;

/*
 * Can use PROC FREQ to do GOF test, though
 * d.f. are not correct. Since some expected
 * values will be less than 1, we will group
 * the data for Y>=4 into a common group.
 */
Proc Format;
 Value YGroup 4-High="4+";
Run;

/*
 * Since there will be 5 cells in this table,
 * PROC FREQ will compute the d.f. to be 5-1=4.
 * However, the probabilities were predicted
 * by estimating the parameter Lambda using the
 * same data. Thus we need to lose 1 more d.f.
 * Thus, d.f.=5-1-1=3.
 */
Title4 "Pearson Chi-square Goodness-of-fit Test";
Title5 "Note: Degrees of Freedom Should Be 3";
Proc Freq Data=flies;
 Table Y / Chisq NoCum TestP=(0.966 4.481 10.396 16.080 67.250);
```

```
  Format Y YGroup.;
Run;


/*
 * Repeat analysis using the Negative Binomial Model.
 */
Title3 "Negative Binomial Model";
Proc Genmod Data=flies;
 Model Y = / Dist=NegBin Link=Log LRCI MaxIter=500;
 Estimate "Population Mean" Intercept 1 / Exp;
 ODS Output ParameterEstimates=Parms;
Run;


Data Expected;
 If _N_=1 Then
  Do;
   i=1;
   Set Parms Point=i Nobs=Nobs;
   Mu=Exp(Estimate); /* First obs is ln(Mu) */
   i=2;
   Set Parms Point=i Nobs=Nobs;
   k=Estimate;     /* Second obs is dispersion parameter */
   kinv=1/k;
   VarY=Mu+k*Mu**2;
   Retain Mu k VarY kinv;
  End;
 Do Y=0 To 10;

Prob=Gamma(Y+kinv)/(Gamma(Y+1)*Gamma(kinv))*(k*mu)**Y/((1+k*mu)**(Y+kin
v)); /* Neg binomial Probability */
  Expected=76*Prob;
  Cummulative+Prob;
  InvCum=1-Cummulative+Prob;
  Output;
 End;
 Stop;
 Keep Y Prob Expected Mu k kinv VarY Cummulative InvCum;
Run;
Title4 "Expected Probabilities";
Proc Print Data=Expected;
Run;


Proc Format;
 Value YGroup 5-High="5+";
Run;

Title4 "Pearson Chi-square Goodness-of-fit Test";
Title5 "Note: Degrees of Freedom Should Be 3";
Proc Freq Data=flies;
 Table Y / Chisq NoCum TestP=(6.333 11.220 13.408 13.444 12.108
36.416);
 Format Y YGroup.;
Run;
```

APPENDIX C

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VITA

Donald Charles Henne was born March 3, 1968 in Winnipeg, Manitoba, Canada, to Doug and Pat Henne. As a child, he developed an intense interest in nature and how things, both living and nonliving, function. He spent much of his childhood collecting insects and exploring the seemingly endless diversity of nearby woods at the family cottage during the short northern summers. Always captivated with insects, he pursued a Bachelor of Science in Agriculture with a minor in entomology at the University of Manitoba, and later completed a Master of Science degree at the same institution. He came to Louisiana State University in 1999 to work as a research associate with Dr. Seth Johnson. In 2002 he began doctoral studies with Dr. Johnson, and will receive the degree of Doctor of Philosophy at the 2007 Commencement.