# MATING FLIGHT INITIATION AND NUTRITIONAL STATUS OF *Solenopsis invicta* (HYMENOPTERA: FORMICIDAE) ALATES INFECTED WITH *Thelohania solenopsae* (MICROSPORIDA: THELOHANIIDAE)

A Thesis

by

KATHERINE JANE OVERTON

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

MASTER OF SCIENCE

December 2003

Major Subject: Entomology

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Approved as to style and content by:

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| Roger E. Gold<br>(Chair of Committee) | S. Bradleigh Vinson<br>(Member) |
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# ABSTRACT

Mating Flight Initiation and Nutritional Status of *Solenopsis invicta* (Hymenoptera: Formicidae) Alates Infected with *Thelohania solenopsae* (Microsporida: Thelohaniidae). (December 2003)

Katherine Jane Overton, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Roger E. Gold

*Thelohania solenopsae* Knell, Allen and Hazard, is a microsporidian pathogen that infects the red imported fire ant *Solenopsis invicta* Buren. This four part study examined the effects that *T. solenopsae* had on fire ant queens as they matured for their mating flights. For the first study, a total of 878 alates were collected at two timed intervals during a nuptial flight and after to determine if *T. solenopsae* affected their ability to initiate a mating flight. Infection rates in alates that left the colony early during a flight were 23.75% while alates that did not leave the colony were 66.16%. Two other studies examined whether *T. solenopsae* affected protein and lipid stores in future queens. Protein stores were not significantly different in infected and uninfected ants, while lipid stores were significantly less in alates that were infected with *T. solenopsae* (10.69% in infected versus 13.98% in uninfected). The final analysis was done with all of the combined data, which showed that alates infected with *T. solenopsae* were significantly smaller than uninfected alates.

# DEDICATION

I would like to dedicate this to my husband, Todd Overton, who was a constant source of strength and encouragement from the time we met early in my graduate career. Thank you for always listening, always being interested (or at least pretending to be), and always having faith in me. Having you believe that I am strong and capable of anything helps me to believe it too. To my parents, who always supported me, even when I insisted on doing things my way, thank you for teaching me values and ethics, and the very important lesson that one must have a good brain and a good sense of humor to survive in this world.

## ACKNOWLEDGEMENTS

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Thank you to the many outstanding professors I encountered. You made this degree an absolute joy to complete. I never thought that I would be so interested in the many facets of entomology. Thank you to Dr. Wharton and Dr. Olsen from the entomology department and Dr. Snowden from the veterinary parasitology department for classes that challenged me and opened my mind.

Thank you to Dr. Mark Wright for patiently answering my many questions about chemistry, computers, life and everything else. A big thanks to Bobb Gorena for being brave enough to try to explain statistics to me. I have to thank Laura Nelson for her help in everything administrative and her friendship.

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# CHAPTER I

## INTRODUCTION

When the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), was introduced into the United States in the 1930's, it found an almost ideal environment in which it established and spread. In its native environment in South America, *S. invicta* makes up only 23% of endemic populations, and is not necessarily the dominant ant species. In contrast, in the U.S. today, fire ants (for the purposes of this study fire ant refers to *S. invicta* unless otherwise specified) account for 97% of ant species collected along roadsides in some areas (Porter et al. 1992). Since its introduction into the U.S., fire ants have spread rapidly each year, competing with native ant species for space and resources. They have also increased their numbers exponentially, becoming a serious agricultural and urban pest. Fire ants cause major monetary losses in the livestock and agricultural industries through attacks on animals or infestation of crops and equipment. Imported fire ants infest much of the southern United States and their range is expanding every year. The presence of *S. invicta*, especially polygyne (multiple queen) colonies, is associated with substantial losses in biodiversity among arthropod communities (Porter and Savignano 1990). In urban areas, fire ants disrupt landscapes and yards and are a medical hazard because of their sting. Although insecticides can be successful in controlling populations of fire ants, the use of chemicals over large areas is

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This thesis follows the format of the Journal of Economic Entomology.

not economically feasible in most cases, and pesticides are becoming increasingly undesirable in many situations.

Biological control is a non-chemical option for fire ant control. Biological control is the use of natural enemies or pathogens to keep a pest population at a manageable or non-damaging level. The advantage of biological control is that a well-planned program can be effective, environmentally friendly, and self-sustaining. Biological control of social organisms, such as fire ants, presents some unique challenges and opportunities. Controlling social insects can be difficult because they usually live in a cryptic, sheltered environment. Fire ants live in subterranean nests where the queens are well protected and defended by thousands of workers. These workers can fight off attacks from predators and parasitoids, and they also protect the colony from parasites and pathogens by behaviors such as grooming and colony hygiene that can remove potential control agents (Schmid-Hempel 1998). Alternatively, social insects can present a good target for biological control because of the ready availability of similar individuals. A parasite or pathogen could do quite well if it exploited some of the characteristics of social insects, such as food sharing and reproductive caste partitioning. A control agent passed through the colony in food or passed from the queens to offspring could potentially have a devastating effect on a colony.

A microsporidian parasite, *Thelohania solenopsae* Knell, Allen and Hazard (Microsporida: Thelohaniidae), is of interest for study because of its potential as a biological control agent against fire ants. *Thelohania solenopsae* is a common pathogen of

fire ants in their native South America, and Briano et al. (1995a) found that the presence of microsporidia infection was associated with reduced densities of black fire ant (*Solenopsis richteri* Forel) colonies. Laboratory studies of *S. invicta* and *S. richteri* showed that infected colonies have increased mortality, decreased numbers of workers and brood, and reduced fecundity in queens (Briano and Williams 1997, Williams et al. 1999). Williams et al. (1999) and Oi and Williams (2002) have been successful in inoculating fire ant colonies in the field with microsporidia. These infections were associated with a decrease in populations of *S. invicta.*

The research objectives for this study were designed to examine the relationship between fire ants and microsporidia. Since *T. solenopsae* is a potential biological control agent, an understanding of the complex interactions between the two species is essential before any control program can begin.

The first objective of this thesis was to examine the effect *T. solenopsae* has on mating flight initiation. This experiment tested whether virgin queens infected with *T. solenopsae* preferentially flew earlier, or later, or not at all during a nuptial flight. A difference between if or when infected queens fly for a nuptial flight could have an effect on dispersal capabilities of both fire ants and the microsporidian pathogen.

The last two objectives examined what effect *T. solenopsae* had on nutritional stores of protein and lipids in virgin queens. New queens are very dependent on their nutritional stores when founding new colonies; therefore, if microsporidia affected these resources, infected queens could be at a disadvantage when starting new colonies. In addition to protein and lipid analysis, a comparison of overall body weights was also done.

# CHAPTER II

## REVIEW OF LITERATURE

There are several factors that can help explain the proliferation of *S. invicta* in the United States. A favorable climate, similar to their native environment in South America, has aided fire ants in establishing and spreading to the limits of their cold tolerance in North America. Another advantage for fire ants is their status as a "weed species", meaning that they selectively prefer habitats that have been disturbed. Forest thinning, clear cutting, road construction, and many of our urbanization processes favor the spread of *S. invicta* (Tschinkel 1986). Along with their high reproductive potential and ability to utilize many food sources, these factors all contribute to fire ant's massive pest potential. One final factor for the success of fire ants, and the only one that can be feasibly altered, is that in the United States fire ants lack efficient competitors and many of their natural enemies. Porter et al. (1997) suggested that escape from natural enemies is one of the most likely explanations for high densities of fire ants in North America. Natural enemies of fire ants in South America are varied and abundant. They consist of parasitoids (phorid flies and parasitic wasps), nematodes, microorganisms, and socially parasitic ants (Porter et al. 1997). In North America, by contrast, very few natural enemies of fire ants exist.

Fire ants became even more of a threat because *S. invicta* have both monogyne (single queen) and polygyne (multiple queen) forms, giving them added flexibility to adapt to new environments. Porter et al. (1991) found that colonies of *S. invicta* in the

United States are usually the monogyne form, except in Texas, where the polygyne form is predominant. Where polygyne forms are found, the number of fire ant mounds per hectare is two to three times as high (Porter et al. 1991, Macom and Porter 1996) and the number of workers per hectare is almost twice as high as areas with monogyne colonies (Macom and Porter 1996). This higher density creates situations with greater potential losses and damage due to fire ants (Porter et al. 1991). Controlling fire ants might or might not be more difficult when dealing with polygyne forms. Higher mound densities could require more labor and more insecticide to be used for individual mound treatments. Individual polygyne fire ant mounds could require more insecticide per mound, since the colonies contain more workers than monogyne fire ants (Macom and Porter 1996). As an alternative theory however, polygyne populations could potentially be easier to control, since they are not territorial against other polygyne colonies and even share resources (Macom and Porter 1996).

Microsporidia are a very diverse group of unicellular protozoa, all of which are obligate intracellular parasites. They are characterized by small size, thick walls, and a specialized organelle called a polar filament. Microsporidia are unusual eukaryotic organisms in that they lack mitochondria, peroxisomes, and other typical eukaryotic structures (Keeling and McFadden 1998). Debates have been ongoing as to the correct taxonomic classification of these organisms for some time (Müller 1997).

Microsporidia have a unique method of parasitizing cells. After ingestion by the appropriate host, microsporidia enter host cells by extruding the coiled polar filament, which penetrates the host cell, and microsporidial sporoplasm is injected into the cell,

initiating a new infection (Müller 1997). Microsporidia are known parasites of all major animal groups, and have been found in every tissue and organ system of their host(Weiss and Vossbrinck 1999). Almost half of the described genera of microsporidia are insect parasites, usually found infecting the fat body and the midgut epithelium. Infections in insects are mostly sub-lethal or chronic, with insects showing little or no outward signs of parasitization. In general, infected insects are less fit and do not live as long as uninfected insects (Becnel and Andreadis 1999).

Microsporidia were first discovered in fire ants in Brazil. Allen and Buren (1974) found "cyst-like" bodies in the gaster of *Solenopsis invicta*. These bodies were determined to be *Thelohania* spores enclosed in fat body cell membranes. Knell et al. described *Thelohania solenopsae* in 1977. In 1998 *T. solenopsae* was found in the United States from Florida, Mississippi, and Texas where it had infected the fat bodies of workers and sexuals, and the ovaries of queens (Williams et al. 1998). A microsporidian infection produces no outward signs of infection or behavioral changes in *S. invicta*, but it does cause hypertrophy of the fat body and leads to reduced life expectancies (Jouvenaz 1983). Spores (the transmission stage) are found in the late stage pupae, adult workers, and ovaries of queens. The vegetative stages are found in the eggs, larvae, pupae, and queen ovaries (Williams et al. 1999). Transmission of microsporidia is accomplished through both vertical and horizontal means. Vertical transmission occurs when infected females pass the parasites transovarially to offspring (Dunn and Smith 2001). Horizontal transmission occurs naturally and has been accomplished through the introduction of live infected brood into uninfected colonies, both in the laboratory and in

the field (Williams et al. 1999, Oi et al. 2001). The exact method of horizontal transmission within a colony has not yet been determined, but infections occur when live infected brood is adopted and reared by colonies. Oi et al. hypothesized in 2001 that the microsporidia spores were being transferred through feeding secretions from workers to larvae and then to queens.

In fire ants, *T. solenopsae* forms two distinct spore types in the same tissues at the same time. One of these is uninucleate and bound in groups of eight cells, called "octospores." The other type are binucleate, unbound "free spores" (Jouvenaz 1986). Both types of spores are visible with a 400X magnification of wet mount slides of smears of ant gasters (Williams et al. 1998). The infected cells are usually hypertrophied, forming cysts (Jouvenaz 1986). Williams et al. (1999) found that infected queens weigh less, have decreased oviposition rates and decreased longevity than uninfected queens.

Microsporidial infections in *Solenopsis invicta* colonies have been found to cause a reduction in numbers of brood (Oi et al. 2002), decreased volume of mounds (Cook 2002, Briano et al. 1995b), and an overall slow decline and eventual death of the colony (Oi et al. 2002).

Some of the characteristics of *T. solenopsae* make it a good potential candidate for a pathogenic biological control agent of *S. invicta*. R.M. Anderson (1982) outlined the optimal characteristics that a pathogen should have in order to be successful as biological control including:

1) An intermediate pathogenicity combined with the ability to reduce host

reproduction. *Thelohania solenopsae* produces an infection that is not especially virulent, but causes an overall weakening of the colony, increased mortality, and reduced fecundity (Williams et al. 1999);

2) High transmission efficiency, microsporidia have both vertical and horizontal transmission routes. Vertical transmission occurs when an infected queen passes her infection on to new eggs. The mechanism of the horizontal transmission is not completely understood, but a colony can be infected by placing brood from an infected colony in the uninfected colony (Williams et al. 1999, Oi et al. 2001), and;

3) The ability to produce large numbers of long-lived infective transmission stages. All microsporidia have a stable transmission stage (the spore) that can resist many environmental stresses and survive in the environment for long periods of time. Some spores can remain viable after months or even years in exposed, dry conditions (Maddox 1973).

Due to these factors, *T. solenopsae* should be the subject of further investigations to determine if it could be the subject of a biological control program for red imported fire ants.

# CHAPTER III

# MATING FLIGHT INITIATION

Mating or nuptial flights occur when fire ant reproductives leave the colony for the purpose of mating and forming new colonies. These flights occur predominantly in the spring, and fall on humid days that follow a rainfall (Morrill 1974). If conditions are acceptable (high humidity, low wind) the workers will begin opening the top of the mound for alates. Alates will leave from the mound or climb onto vegetation for a take-off point. *Solenopsis* ants can release hundreds or thousands of alates in an hour (Hölldobler and Wilson 1990).

This experiment tested the hypothesis that *Thelohania solenopsae* had no effect on fire ant's ability to leave the colony on a nuptial flight.

## Materials and Methods

**Insects.** *Solenopsis invicta* alates (winged reproductives, for this study all alates were virgin females) were collected from eight sites during nuptial flights for the years 2001-2003. Samples of workers were obtained to determine if sites were infected with *T. solenopsae*. Workers were collected by inserting a 50 ml plastic centrifuge tube (BD Falcon®, Franklin Lakes, NJ) into the center of the mound. The inside rim of the tubes were dusted with talc to prevent the ants from escaping. Ants were then killed by freezing. To determine infection status, 50-100 worker ants were homogenized in 1-2 ml of

water in a glass tissue grinder (Pyrex 7725-16). A drop of the resulting solution was examined under 400X magnification with a Nikon Alphaphot-2 light microscope for the presence of *T. solenopsae* spores. Eight sites in central Texas were selected that had microsporidia infections. Sites 1 through 7 were located in Brazos County, Site 8 was in Burleson County. The eight sites were:

1) Roadside on Hwy. 47 (N30º36.847' W096º25.135'), nuptial flight on 9 June 2001;

2) Cattle pasture on Hardy Weedon Rd. (N30º39.897'W096º13.622'), nuptial flight on 8 May 2002;

3) Edge of pond on Hardy Weedon Rd. (N30º39.972'W096º13.634'), nuptial flight on 30 October 2002;

4) Edge of pond on Hardy Weedon Rd. (same location as site 3), nuptial flight on 2 May 2003);

5) Field at corner of F & B Rd. and Hwy. 2818 (N30º36.619'W096º22.236'), nuptial flight on 22 June 2002;

6) Field on private property on Wilson Pasture Rd. (N30º54.113'W096º14.870'), nuptial flight on 6 June 2001);

7) Field at corner of F & B Rd. and Hwy. 2818 (same location as site 5), nuptial flight on 12 June 2003); and,

8) Corner of Hwy. 21 and Hwy. 50 (N30º37.354'W096º32.636'), nuptial flight on 6 April 2001).

Alates were collected from traps or from the top of the mounds using an insect aspirator. Collections were made at 10-15 min intervals for the duration of the swarming flight. The emergence traps were constructed of a cone shaped frame of plastic tubing covered with mesh window screening material, to allow free movement of workers but restrict the movement of alates, which are larger. After the nuptial flight had ended, workers were collected from each colony by inserting a plastic centrifuge tube into the colony as previously described. Alates that did not leave for the nuptial flight were collected by removing dirt from the mound and aspirating the alates. The collected ants were killed by freezing and held at –5ºC until analysis could be performed. Infection with *T. solenopsae* for a colony was determined in the manner described previously in this section. The infection status of individual alates was determined from a wet mount slide made from the fat body and ovaries. The slides were examined at 400X magnification for the presence of *T. solenopsae* spores.

**Analysis.** The data from each site where microsporidia infection was present at the time of nuptial flight were compared separately. The alates were divided into different time categories, including two successive time periods that occurred during the nuptial flight (time one and two), and those alates that were left in the colony after the flight ended (time three). To determine if there were statistical differences in the percent infected at each time period, a post-hoc Fisher's Least Significant Distance (LSD) analysis was performed. Data were analyzed using SPSS for Windows version 10.1 (2000) and the results of the Fisher's LSD for all sites are provided in Appendix A.

**Voucher Information.** Vouchers of the ants used in these studies were placed in the Texas A&M University Insect Collection. The Texas A&M University Insect Collection can be found on the second floor of the Minnie Bell Heep Building on the Texas A&M University campus in College Station, TX.

## Results

**Mating Flight. Site 1 (Roadside on Hwy. 47, 9 June 2001).** Site 1 had the largest sample size of the eight sites included in the study, with 305 virgin queens being analyzed. Figure 1 shows the percentage of infected alates at each time period. There was a significant difference ($\alpha = 0.05$) in the proportion of infected ants between each time period, with the highest percentage of infected ants found in those ants that did not leave the colony during the nuptial flight (82.83% infected).

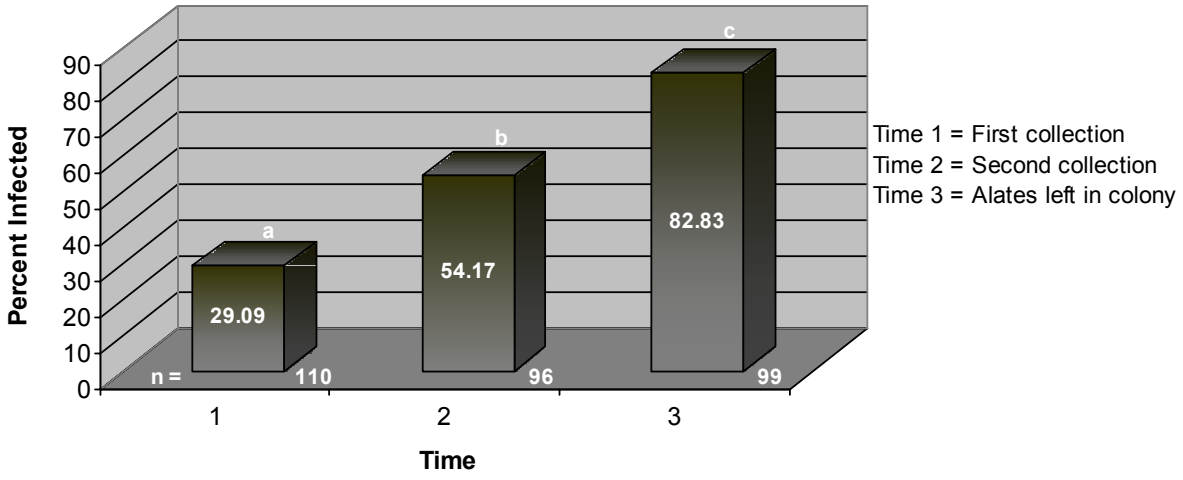


**Figure 1.** Percentage of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at each time period for Site 1.
* Bars topped by different letters were significantly different at $\alpha = 0.05$.

**Site 2 (Cattle Pasture on Hardy Weedon Rd., 8 May 2002).** Site 2 had a sample size of 163 alates. There were no significant differences in the proportion of infected alates for the two times during the nuptial flight. There was, however, a significant difference ($\alpha = 0.05$) in the percentage of infected alates that left during the flight and those that remained in the colony. The were infection rate for those alates left in the colony after the nuptial flight had ended was 60.78%, compared to 17.95% for the alates that left during the nuptial flight. The graph of infected alates at each time period is shown in Figure 2.

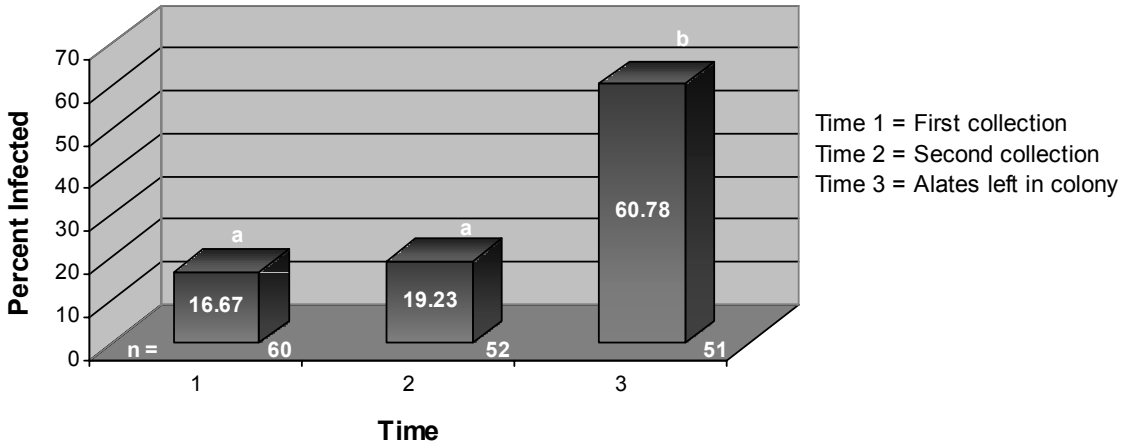


**Figure 2.** Percentage of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at each time period for Site 2.
* Bars topped by different letters were significantly different at $\alpha = 0.05$.

**Site 3 (Edge of Pond on Hardy Weedon Rd., 30 October 2002).** Site 3 had a sample size of 126 alates. The trend in this sample was similar to Site 2. There were no significant differences in the percentage of infected alates during the nuptial flight; however, there was a significant difference ($\alpha = 0.05$) in the numbers of infected alates that left during the flight and those that were left after the flight ended. Figure 3 shows the percentage of infected alates at each time period.

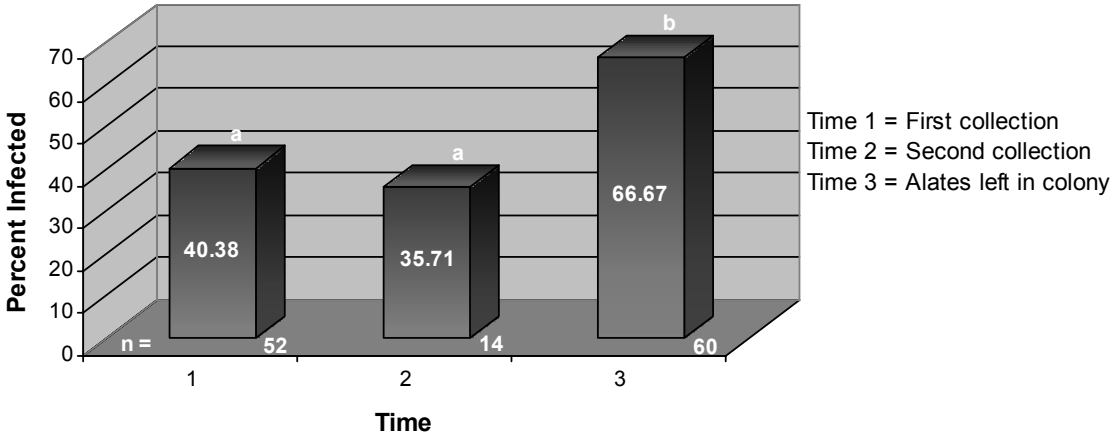


**Figure 3.** Percentage of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at each time period for Site 3.
* Bars topped by different letters were significantly different at $\alpha = 0.05$.

**Site 4 (Edge of Pond on Hardy Weedon Rd., 2 May 2003).** The sample size for Site 4 was 225 alates. The percentage of infected alates increased with time during the nuptial flight. There were significant differences ($\alpha = 0.05$) in the proportion of infected alates for the two time periods during the flight; however, the second time collection was not significantly different from those left in the colony. The data is shown graphically in Figure 4.

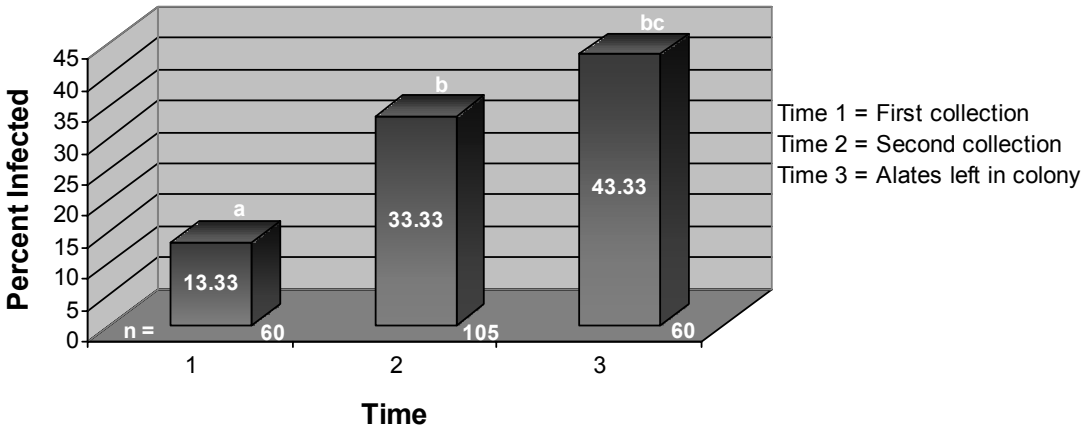


**Figure 4.** Percentage of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at each time period for Site 4.
* Bars topped by different letters were significantly different at $\alpha = 0.05$.

**Site 5 (Field at Corner of F&B Rd. and Hwy. 2818, 22 June 2002).** Site 5 had a sample size of 61 alates. The trend for this data was similar to Site 4. The two collections during the nuptial flight were significantly different ($\alpha = 0.05$), but the later collection was not different from the percentage of infected ants that were left in the colony. The proportion of infected ants at each time is shown in Figure 5.

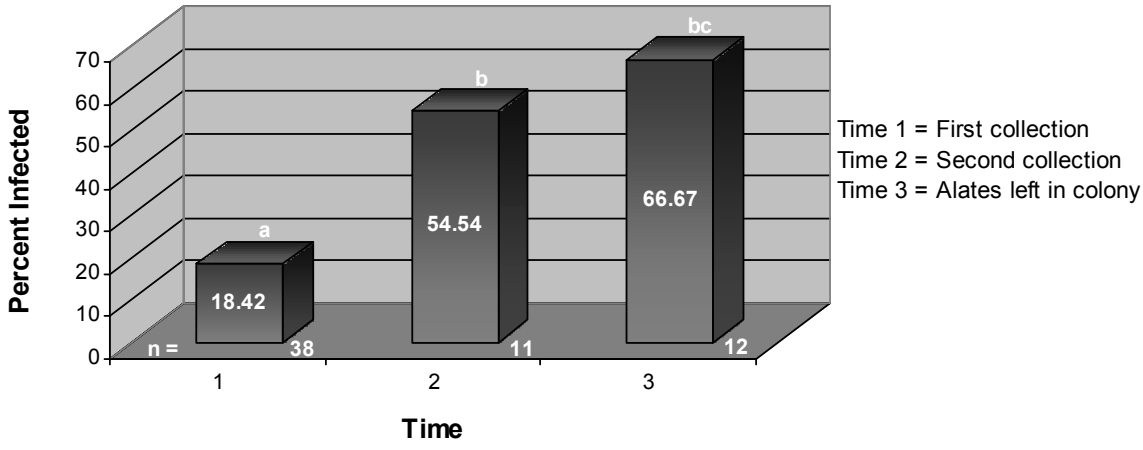


**Figure 5.** Percentage of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at each time period for Site 5.
* Bars topped by different letters were significantly different at $\alpha = 0.05$.

**Site Totals.** For the last analysis all the data from the five sites were combined to provide an overall trend. All time periods are significantly different from each other, as shown in Figure 6, with the proportion of infected alates increasing through time, and the majority of alates left in the colony (66.16%) infected with microsporidia.

**Sites 6, 7, 8.** Colonies that had nuptial flights on the days of collection were not infected with microsporidia and therefore were not used for analysis.

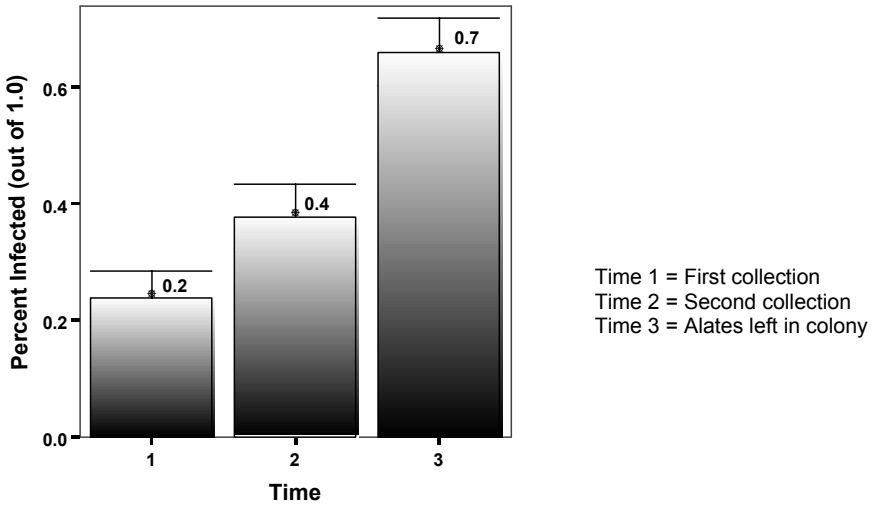


**Figure 6.** Percentage of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at each time period for all sites combined.

## Discussion

**Mating Flight.** This experiment was designed to determine if microsporidia had an effect on the dispersal capabilities of virgin *S. invicta* queens during nuptial flights. Whether infected ants leave on nuptial flights as readily as uninfected ants is an important piece of information to take into consideration for a control program.

Based on the data collected, microsporidia had an effect on if and when infected alates left during a flight. The alates that left the nest early during nuptial flights were uninfected (less than 25% infected), while those that remained in the colony were mostly infected (over 65%). This implied that there may have been some sort of selection process going on during the flight, where healthy ants were more likely to leave and infected ants were left behind in the colony because they did not or could not depart. There also was a trend during nuptial flights for infected alates to leave later in the flight, which may have resulted in lower probability of being mated by waiting males.

# CHAPTER IV

## NUTRITIONAL STATUS

New colonies of fire ants can be formed in several ways. One method is through claustral colony founding. In this event, the reproductives leave the colony during a nuptial flight, mate and then return to the ground to shed their wings. The newly mated queens then search for an acceptable colony site and seal themselves in the new colony chamber to begin laying eggs. Another type of colony founding is through a process called budding, where a mated queen leaves the colony accompanied by workers that will assist her in starting a new colony (Hölldobler and Wilson 1990).

Whether a queen founds a colony by independent claustral means or by dependent budding, she is reliant upon her nutritional reserves. In a claustral situation, the amount of resources she has stored in her body are entirely responsible for the success or failure of the colony. Since she has no brood to help, her bodily reserves must be sufficient to sustain her and the developing larvae until they become workers and can feed her. If colonies are founded by budding, nutrients still play an important role in colony success, as heavier queens are more likely to survive (Bernasconi and Keller 1996) and produce more brood (Vander Meer et al. 1992).

Colony founding is a metabolically stressful event for a new queen. During the process, queens metabolize the fat body and wing muscles for the production of eggs and the subsequent larvae (Hölldobler and Wilson 1990). Histolysis of the flight muscles begins when a queen locates an acceptable nesting site and sheds off her wings. The wing

muscles are thought to be a source of protein needed for energy during early colony founding. Another source of protein is that stored in the fat body. During the 12 hours immediately following a nuptial flight, protein reserves are depleted by 50% (Toom et al. 1976b). The importance of nutritional reserves was highlighted by further studies by Toom et al. (1976a) that show that during the time required to rear the first workers, fire ant queens lose almost 50% of their body mass.

The objectives for this part of the study were to compare protein and lipid stores in virgin fire ant queens that were leaving the nest on a nuptial flight. Both protein and lipid are stored in the fat body of insects (Chapman 1998). Since microsporidia infect the fat body, it is possible that they could cause a decrease in nutrients that are needed for new colony founding.

An additional analysis was done to determine if infection with microsporidia had an effect on overall body mass.

## Materials and Methods

**Protein Assay.** Female *Solenopsis invicta* alates were collected from sites with known *Thelohania solenopsae* infection after nuptial flights. Alates were collected with an insect aspirator from mounds that were dug up with a shovel. Ants were then killed by freezing and were held at –5ºC until analysis was performed. Each ant was weighed on an electronic balance (A&D Company, Limited ER-182A). Ants were then homogenized in a glass tissue grinder (Pyrex 7725-16) with 250 µl of distilled water for

approximately one minute, or until there were no large pieces of tissue remaining. To determine infection status, 15 µl of the homogenized material was removed from the tissue grinder and used for a wet mount slide preparation. The slides were examined under 400X magnification using a Nikon Alphaphot-2 light microscope to detect the presence of *T. solenopsae* spores. A protein assay was then performed in the manner of Wheeler and Buck (1996) using a bicinchoninic acid assay kit purchased from Pierce Chemical Co. (Rockford, IL) with modifications to account for determining the presence or absence of microsporidia infection. The procedure deviated from that recommended by Pierce Chemical Co. by the addition of distilled water.

After determining infection status, 1 ml of 1N sodium hydroxide was added to the tissue grinder and the solution was mixed well. From the tissue grinder, 1 ml of the preparation was removed and pipetted into a 16x13 mm glass test tube. A bicinchoninic acid reagent was added to the test tube in the amount of 1 ml and the test tubes were heated at 60˚C for 60 minutes in a dry bath incubator (Fisher Scientific, Pittsburgh, PA). After the solution had cooled to room temperature, the samples were read in a Beckman DU-7 spectrophotometer (Fullerton, CA) at 562 nm using a NaOH blank.

Protein concentrations were determined from a standard line (Figure 7) that was generated using a range of concentrations of bovine serum albumin in 1N NaOH. A standard line generated by following the kit instructions was not sufficient as samples gained color due to the process of adding water. The standards were treated as an ant sample would be. After raw concentrations were found, calculations were done to show protein concentration as a percentage of body weight. The raw concentrations of pro-

tein were divided into the total body weight for each ant to give the percent body weight data.

The means of percent protein for infected and uninfected alates were compared using independent samples t-tests (SPSS, 2000; $\alpha = 0.05$).

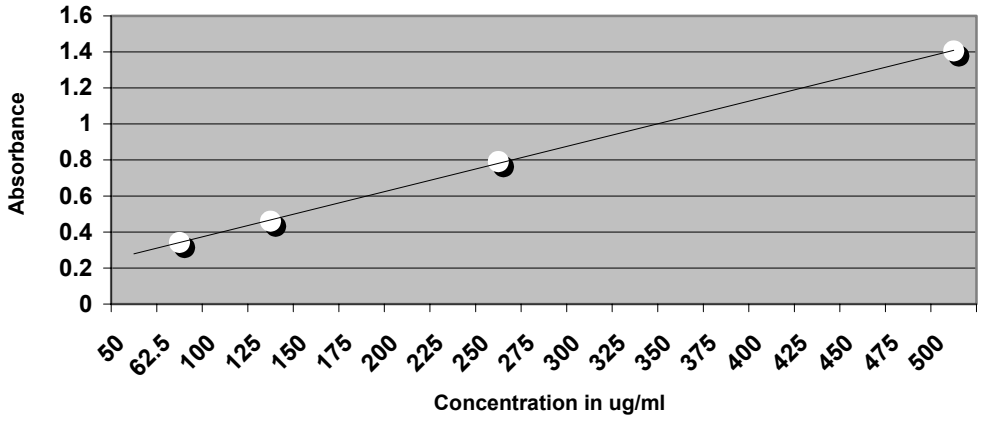


**Figure 7.** The best fit standard line for the protein assay for *Solenopsis invicta.*

**Lipid Assay.** Female *Solenopsis invicta* alates were collected from sites with known *Thelohania solenopsae* infection after swarming flights. Ants were collected with an insect aspirator from mounds that were dug up with a shovel. The ants were then killed by freezing and held at –5ºC until analysis.

The lipid assay was performed in the manner of Van Handel (1985) with modifications to account for determining the presence or absence of microsporidia infection.

The procedure differed from that of Van Handel by the addition of water. Each ant was weighed on an electronic balance. Ants were then homogenized in a glass tissue grinder with 250 µl of water for approximately 1 minute, or until there were no large pieces of tissue remaining. To determine infection status, 15 µl of homogenized material was removed from the tissue grinder and used to prepare a wet mount slide preparation. The slide was examined under 400X magnification for the presence of *T. solenopsae* spores. A 1:1 mixture of chloroform and methanol was then added to the tissue grinder in the amount of 0.5 ml, and the mixture was well agitated. The contents of the tissue grinder were then carefully poured into a 16x13 mm glass test tube. From this point on, the assay followed the procedure set forth by Van Handel (1985).

Test tubes with the ant homogenate and 0.5 ml of chloroform-methanol were heated at 100 ± 5˚C until the solvent had evaporated. Tubes were allowed to cool to room temperature, and then 0.2 ml of 85% sulfuric acid was added to each tube. Tubes were agitated to mix well and heated at 100 ± 5˚C for 10 minutes. After tubes had cooled to room temperature, 4.8 ml of a vanillin-phosphoric acid reagent was added to each test tube. The reaction was allowed to proceed for 5 minutes while the color developed. The samples were read in a spectrophotometer at 525 nm using a blank of the vanillin-phosphoric acid reagent.

Lipid concentrations were determined from a standard line (Figure 8) that was generated by using standard concentrations of a commercially available vegetable oil in chloroform. A standard line generated in the manner of the original assay was not sufficient as lipids were lost when the samples of ant homogenate were removed to

determine infection status. The lipid standards were treated as an ant sample would be. After raw concentrations were measured the data was used to calculate lipid concentration as a percentage of body weight. The raw concentrations of lipids were divided into the total body weight for each ant to give the percent body weight data.

The means of percent lipids for infected and uninfected alates were compared using independent samples t-tests (SPSS 2000).

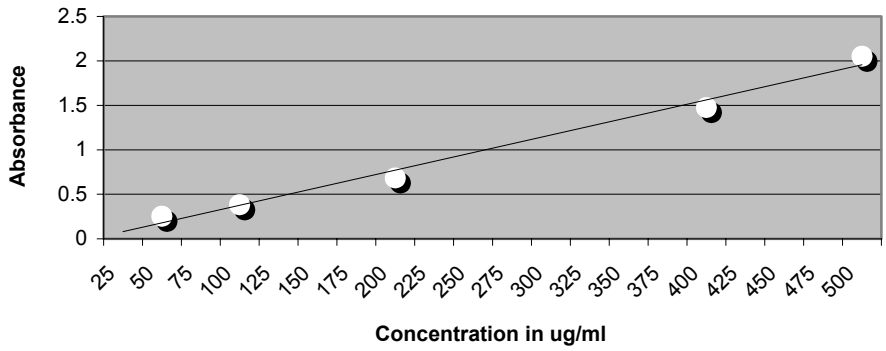


**Figure 8.** The best fit standard line for the lipid assay for *Solenopsis invicta.*

**Infection Status and Weight.** The information gathered for previous experiments provided a large sample size for an analysis of weight and infection status. The data from the mating flight experiment and the protein and lipid assays were pooled and an independent samples t-test was done to determine if there was a difference in the mean weight of infected and uninfected alates (SPSS 2000, $\alpha = 0.05$).

## Results

**Protein Assay.** The data for the protein assay are shown in Appendix B, Tables B1 and B2. Figure 9 shows the data as a comparison of the protein percent body weight of ants uninfected and infected with *T. solenopsae*. The mean protein percent body weight was 22.63 ± 0.87% and 24.21± 0.72% for uninfected and infected ants, respectively. This difference was not significant (F = 0.050; df = 58; P = 0 .169). The mean concentration of protein for uninfected ants was 2166.33 ± 76.58 µg, while for infected ants the mean was 2193.33 ± 59.48 µg. This difference was not significant (F = 3.214; df = 58; P = 0.782).

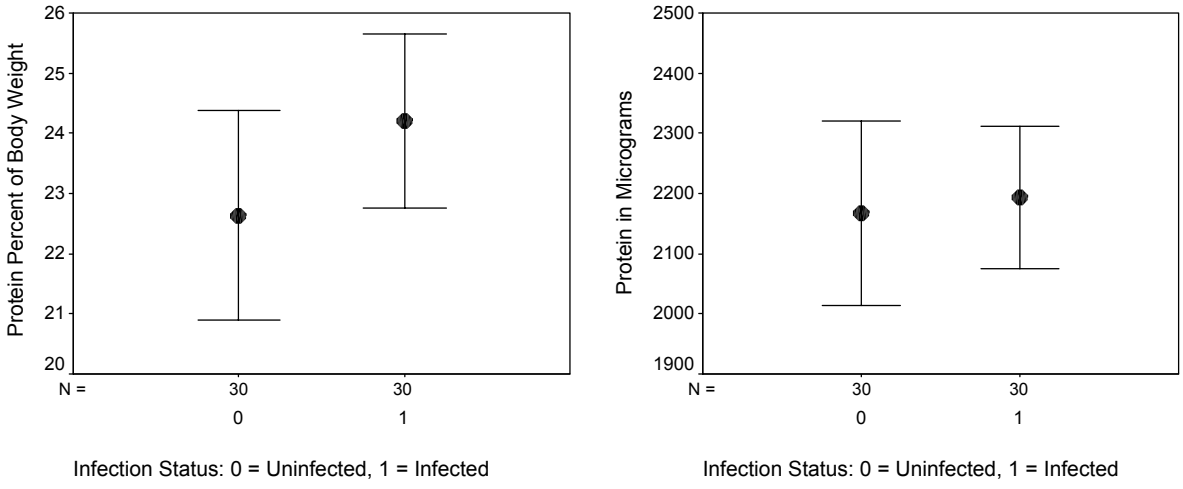


**Figure 9.** Results of the protein assay for *Solenopsis invicta*, where protein as a percent of body weight is shown on the left and protein as a raw concentration on the right. Bars represent the standard error of the mean.

**Lipid Assay.** The raw data from the lipid assay is provided in Appendix C, Tables C1 and C2. Figure 10 shows the percent lipid and raw lipid data. The mean amount of lipids as a percent of body weight for uninfected ants was 13.98 ± 0.49% and for infected ants it was 10.69 ± 0.68%. Statistical analysis showed that this was a significant difference ($F = 3.112$; $df = 58$; $P < 0.001$). For raw concentrations of lipids, the mean weights were 1353.97 ± 53.83 µg and 1072.83 ± 66.88 µg for *T. solenopsae* uninfected and infected ants, respectively. This difference was statistically significant ($F = 2.493$; $df = 58$; $P = 0.002$).

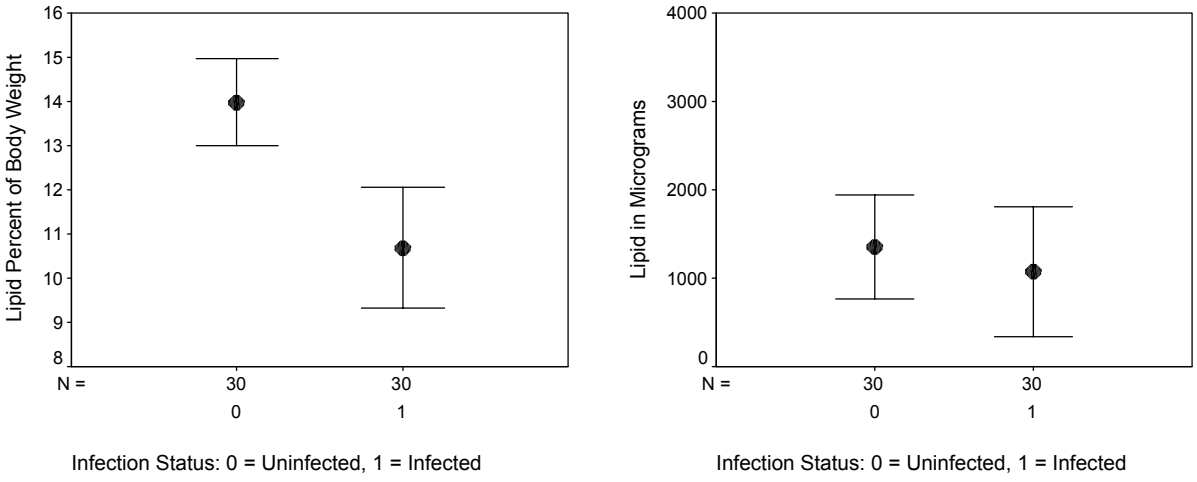


**Figure 10.** Results of the lipid assay for *Solenopsis invicta,* where lipid as a percent of body weight is shown on the left and lipid as a raw concentration on the right. Bars represent the standard error of the mean.

**Infection Status and Weight.** Based on statistical analysis of the sample, there was a significant weight difference between infected and uninfected alates. The mean weight for uninfected ants was 10307.83 ± 76.48 µg and for infected ants the mean was 9818.91 ± 86.56 µg . This difference was significant (F = 0.160; df = 996; P < 0.001). The data is shown in graphical form in Figure 11.

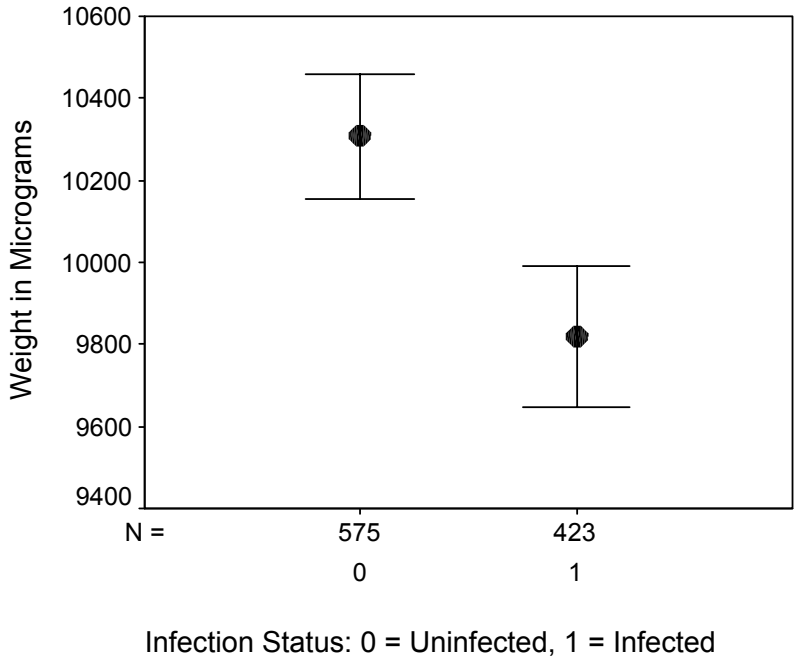


**Figure 11.** Comparison of weights for *Solenopsis invicta* alates infected with *Thelohania solenopsae* and uninfected.
Bars represent the standard error of the mean.

## Discussion

**Protein Assay.** Although *Thelohania solenopsae* infected the fat body of fire ants, there was not a significant difference in the amount of stored protein in uninfected and infected ants. When looking at the percent protein graph, it appeared that infected ants had more protein than uninfected ones, even though the difference was not statistically significant. This unexpected result could possibly be explained in several ways. One theory is that the addition of the microsporidial parasite, which has protein of its own, contributed to the outcome of the assay. Another possibility is that the ants were compensating for being parasitized by producing more protein. It also may have been that uninfected ants survived with fewer protein stores and a smaller body mass than infected ants. These smaller ants with less protein may have caused the data for the percent protein graph to become skewed. This explanation may be the most likely since the graph of the raw protein data shows that there was a broader range of protein stores in uninfected ants, suggesting that they can survive with less protein than infected ants.

Overall, it did not appear the *T. solenopsae* had an effect on the protein stores of future fire ant queens.

**Lipid Assay.** The data showed that there was a significant difference in the amount of lipid stores between ants that were infected with *Thelohania solenopsae* and those that were not infected. Infected ants contained significantly less lipid than uninfected ants. The difference was visible with both the raw data and the data expressed as

a percent of body weight. Another interesting observation was that infected ants had a much broader range of lipid concentrations than uninfected ants. The lowest and highest amounts of lipid in infected ants were less than for uninfected ants. The low end of the range for infected ants was 400 μg lower than the bottom end of the range for uninfected ants. This data strongly suggests that *T. solenopsae* has a detrimental effect on lipid stores.

**Infection Status and Weight.** It is not unusual that a parasite would have a detrimental effect on its hosts body mass since it is taking nutrients from the host. Cook et al. (2003) found that infected female alates usually weighed less. While the 488 μg difference that microsporidia made in fire ant body weights between infected and uninfected alates does not seem like very much, it could have an effect on the potential success of each individual ant and their future colonies. The weight of a queen is a major determinant of fecundity (VanderMeer et al. 1992), so smaller queens would most likely produce fewer eggs and brood. A queen that cannot produce sufficient offspring during early colony founding may be at a disadvantage for long-term survival of the colony.

# CHAPTER V

## SUMMARY AND CONCLUSIONS

The first objective of this thesis was to determine if microsporidia had any effect on the nuptial flights of *S. invicta*. The outcome of this experiment showed that the first alates to leave during a flight were largely uninfected with microsporidia, while alates that remained in the colony were largely infected with *T. solenopsae*. What this information could mean in terms of a control program is unclear. This could be a positive outcome, because fewer ants may be leaving to start new colonies. However, since very few alates actually survive the swarming process (Hölldobler and Wilson 1990), it remains to be seen whether microsporidia have much of an effect on the numbers of new colonies that are successfully started. This data also implies that there could potentially be a problem with microsporidia having self-limiting dispersal capabilities. With many infected alates remaining in the nest, the pathogen may not be spread in the environment as well as it needs to for successful control measures. The original experiment planned for this objective called for collecting mated queens that had returned to the ground after a mating flight. This proved difficult to accomplish, as very few alates that were collected after a mating flight were infected with microsporidia. There could be several reasons for this occurrence. Ants infected with *T. solenopsae* may not have survived the flight and mating processes. Since fire ant queens can fly long distances, and microsporidia infection occurs in pockets, it may be difficult to collect a large sample of mated, infected alates at one location. Another reason appeared after analyzing the data

from the revised experiment since the outcome showed that infected alates may not be as able to leave the colony during a nuptial flight.

The other objectives for this study focused on the effects microsporidia had on nutritional stores in alates, in the form of protein, lipid and overall body weight. It was shown that *T. solenopsae* did not affect the protein stores of alates, but it did have a deleterious effect by reducing lipid stores and body mass. Ants that were infected with microsporidia had significantly less lipid and weigh significantly less than uninfected ants. This could put infected ants at a serious disadvantage when starting new colonies. With fewer nutritional stores, they may not survive long enough to produce brood that will help feed and care for the new colony. New colonies that are infected with microsporidia could be smaller in size, since smaller queens produce fewer brood (Vander Meer et al. 1992), which could make them more susceptible to ecological competition from other ants and control methods. Female alates that weigh less or have less nutrient stores may not be able to fly as long on their nuptial flights (Vogt et al. 2000). This could result in infected alates having a decreased chance of mating successfully.

Based on the findings of this research, it seems that microsporidia are a possible option for biological control of the red imported fire ants. *Thelohania solenopsae* appears to interfere with a reproductive's ability to initiate a mating flight and it depletes some of the nutritional reserves and body mass of the queens.

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# APPENDIX A

## STATISTICS FROM MATING FLIGHT DATA

Table A1. Fisher's LSD results from mating flight at Site 1. Significance indicates a difference in the percentages of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at different times during a nuptial flight.

| | | Mean Difference (I – J) | Standard Error | Significance |
|---|---|---|---|---|
| (I) Time | (J) Time | | | |
| 1 | 2 | -0.26* | 0.063 | <0.001 |
| | 3 | -0.54* | 0.064 | <0.001 |
| 2 | 1 | 0.26* | 0.063 | <0.001 |
| | 3 | -0.27* | 0.063 | <0.001 |
| 3 | 1 | 0.54* | 0.063 | <0.001 |
| | 2 | 0.27* | 0.064 | <0.001 |

* Means followed by an asterisk are statistically significant at $\alpha = 0.05$.

Table A2. Fisher's LSD results from mating flight at Site 2. Significance indicates a difference in the percentages of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at different times during a nuptial flight.

| | | Mean Difference (I – J) | Standard Error | Significance |
|---|---|---|---|---|
| (I) Time | (J) Time | | | |
| 1 | 2 | -0.04* | 0.081 | 0.581 |
| | 3 | -0.43* | 0.081 | <0.001 |
| 2 | 1 | 0.04 | 0.081 | 0.581 |
| | 3 | -0.38* | 0.084 | <0.001 |
| 3 | 1 | 0.43* | 0.081 | <0.001 |
| | 2 | 0.38* | 0.084 | <0.001 |

* Means followed by an asterisk are statistically significant at $\alpha = 0.05$.

Table A3. Fisher's LSD results from mating flight at Site 3. Significance indicates a difference in the percentages of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at different times during a nuptial flight.

| | | Mean Difference (I – J) | Standard Error | Significance |
|---|---|---|---|---|
| (I) Time | (J) Time | | | |
| 1 | 2 | 0.12 | 0.145 | 0.418 |
| | 3 | -0.26* | 0.092 | 0.005 |
| 2 | 1 | -0.12 | 0.145 | 0.418 |
| | 3 | -0.38* | 0.143 | 0.009 |
| 3 | 1 | 0.26* | 0.092 | 0.005 |
| | 2 | 0.38* | 0.143 | 0.009 |

* Means followed by an asterisk are statistically significant at $\alpha = 0.05$.

Table A4. Fisher's LSD results form mating flight at Site 4. Significance indicates a difference in the percentages of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at different times during a nuptial flight.

| | | Mean Difference (I – J) | Standard Error | Significance |
|---|---|---|---|---|
| (I) Time | (J) Time | | | |
| 1 | 2 | -0.20* | 0.073 | 0.007 |
| | 3 | -0.30* | 0.082 | <0.001 |
| 2 | 1 | 0.20* | 0.073 | 0.007 |
| | 3 | -0.10 | 0.073 | 0.171 |
| 3 | 1 | 0.30* | 0.082 | <0.001 |
| | 2 | 0.10 | 0.073 | 0.171 |

* Means followed by an asterisk are statistically significant at $\alpha = 0.05$.

Table A5. Fisher's LSD results from mating flight at Site 5. Significance indicates a difference in the percentages of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at different times during a nuptial flight.

| | | Mean Difference (I – J) | Standard Error | Significance |
|---|---|---|---|---|
| (I) Time | (J) Time | | | |
| 1 | 2 | -0.36* | 0.150 | 0.019 |
| | 3 | -0.48* | 0.145 | 0.002 |
| 2 | 1 | 0.36* | 0.150 | 0.019 |
| | 3 | -0.12 | 0.183 | 0.510 |
| 3 | 1 | 0.48* | 0.145 | 0.002 |
| | 2 | 0.12 | 0.183 | 0.510 |

* Means followed by an asterisk are statistically significant at $\alpha = 0.05$.

Table A6. Fisher's LSD results form mating flight for all sites combined. Significance indicates a difference in the percentages of *Solenopsis invicta* alates infected with *Thelohania solenopsae* at different times during nuptial flights.

| | | Mean Difference (I – J) | Standard Error | Significance |
|---|---|---|---|---|
| (I) Time | (J) Time | | | |
| 1 | 2 | -0.14* | 0.037 | <0.001 |
| | 3 | -0.42* | 0.038 | <0.001 |
| 2 | 1 | 0.14* | 0.037 | <0.001 |
| | 3 | -0.28* | 0.039 | <0.001 |
| 3 | 1 | 0.42* | 0.038 | <0.001 |
| | 2 | 0.28* | 0.039 | <0.001 |

* Means followed by an asterisk are statistically significant at $\alpha = 0.05$.

# APPENDIX B

## RAW DATA FROM PROTEIN ASSAY

Table B1. Protein assay data for *Solenopsis invicta* alates uninfected with *Thelohania solenopsae*.

| Weight (µg) | Protein (µg) | Protein % of Body Weight |
|---|---|---|
| 12100 | 2370 | 19.59 |
| 11400 | 2230 | 19.56 |
| 10600 | 2350 | 22.17 |
| 12000 | 2600 | 21.67 |
| 5900 | 1200 | 20.34 |
| 7700 | 1520 | 19.74 |
| 7900 | 1630 | 20.63 |
| 10900 | 2510 | 23.03 |
| 9400 | 2560 | 27.23 |
| 8400 | 2250 | 26.78 |
| 10600 | 2570 | 24.24 |
| 11800 | 2610 | 22.12 |
| 12600 | 2370 | 18.81 |
| 9300 | 2460 | 26.45 |
| 8700 | 1750 | 20.11 |
| 8200 | 1500 | 18.29 |
| 12400 | 2180 | 17.58 |
| 6400 | 1400 | 21.87 |
| 5700 | 2150 | 37.72 |
| 13400 | 2790 | 20.82 |
| 8100 | 2440 | 30.12 |
| 11200 | 2320 | 20.71 |
| 11500 | 2270 | 19.74 |
| 7800 | 1730 | 22.18 |
| 6300 | 2030 | 32.22 |
| 14900 | 1870 | 12.55 |
| 10200 | 2450 | 24.02 |
| 11700 | 2560 | 21.88 |
| 8000 | 1850 | 23.12 |
| 10400 | 2470 | 23.75 |
| **Average** 9850 ± 43.12 µg | 2161 ± 7.66 µg | 22.63 ± 0.87% |

Appendix Table B2. Protein assay data for *Solenopsis invicta* alates infected with *Thelohania solenopsae*.

| Weight (µg) | Protein (µg) | Protein % of Body Weight |
|---|---|---|
| 10400 | 2220 | 21.35 |
| 10200 | 2730 | 26.76 |
| 8800 | 2200 | 25.00 |
| 7400 | 2180 | 29.46 |
| 9400 | 2280 | 24.25 |
| 10800 | 2360 | 21.85 |
| 5300 | 1900 | 35.85 |
| 8500 | 1870 | 22.00 |
| 10200 | 2040 | 20.00 |
| 10200 | 2440 | 23.92 |
| 7700 | 1480 | 19.22 |
| 7700 | 2100 | 27.27 |
| 10800 | 2270 | 21.02 |
| 8400 | 1740 | 20.71 |
| 9800 | 2140 | 21.84 |
| 11500 | 2300 | 20.00 |
| 11300 | 2460 | 21.77 |
| 9800 | 2400 | 24.49 |
| 10000 | 2870 | 28.70 |
| 9000 | 1650 | 18.33 |
| 7500 | 2210 | 29.46 |
| 9100 | 2230 | 24.50 |
| 8700 | 2500 | 28.73 |
| 7400 | 1980 | 26.76 |
| 8900 | 1920 | 21.57 |
| 7300 | 1670 | 22.88 |
| 8700 | 2160 | 24.83 |
| 10500 | 2690 | 25.62 |
| 7900 | 2260 | 28.61 |
| 13100 | 2550 | 19.46 |
| **Average** 9210 ± 29.14 µg | 2190 ± 5.95 µg | 24.21 ± 0.72% |

# APPENDIX C

## RAW DATA FROM LIPID ASSAY

Appendix Table C1. Lipid assay data for *Solenopsis invicta* alates uninfected with *Thelohania solenopsae*.

| Weight (µg) | Lipid (µg) | Lipid % of Body Weight |
|---|---|---|
| 10500 | 1870 | 17.81 |
| 9400 | 1140 | 12.13 |
| 7500 | 565 | 7.53 |
| 8500 | 1235 | 14.53 |
| 8000 | 1450 | 18.12 |
| 10300 | 1760 | 17.09 |
| 9800 | 1440 | 14.69 |
| 12300 | 1350 | 10.97 |
| 8200 | 1295 | 15.79 |
| 9000 | 1545 | 17.17 |
| 9700 | 1625 | 16.75 |
| 10200 | 1550 | 15.20 |
| 10700 | 1325 | 12.38 |
| 6100 | 720 | 11.80 |
| 9800 | 1490 | 15.20 |
| 11300 | 1505 | 13.32 |
| 10400 | 1555 | 14.95 |
| 9600 | 1475 | 15.36 |
| 6500 | 850 | 13.08 |
| 10100 | 1645 | 16.29 |
| 8700 | 910 | 10.46 |
| 6700 | 915 | 13.66 |
| 10100 | 1265 | 12.52 |
| 10500 | 1125 | 10.71 |
| 12600 | 2010 | 15.95 |
| 11000 | 1620 | 14.73 |
| 7800 | 825 | 10.58 |
| 12800 | 2245 | 17.54 |
| 9900 | 1360 | 13.74 |
| 8300 | 780 | 9.40 |
| **Average** 9540 ± 30.86 µg | 1353 ± 29.14 µg | 13.98 ± 0.49% |

Appendix Table C2. Lipid assay data for *Solenopsis invicta* alates infected with *Thelohania solenopsae*.

| Sample | Weight (µg) | Lipid (µg) | Lipid % of Body Weight |
|---|---|---|---|
| 1 | 8400 | 1540 | 18.33 |
| 2 | 7700 | 550 | 7.14 |
| 3 | 7800 | 600 | 7.69 |
| 4 | 6600 | 595 | 9.01 |
| 5 | 11200 | 1730 | 15.45 |
| 6 | 8100 | 1300 | 16.05 |
| 7 | 9700 | 1065 | 10.98 |
| 8 | 6300 | 375 | 5.95 |
| 9 | 7600 | 710 | 9.34 |
| 10 | 8800 | 810 | 9.20 |
| 11 | 5700 | 435 | 7.63 |
| 12 | 6700 | 385 | 5.75 |
| 13 | 8500 | 630 | 7.41 |
| 14 | 12300 | 1750 | 14.23 |
| 15 | 9600 | 1030 | 10.73 |
| 16 | 10500 | 1565 | 14.90 |
| 17 | 8400 | 450 | 5.36 |
| 18 | 7400 | 800 | 10.81 |
| 19 | 5900 | 620 | 10.51 |
| 20 | 9000 | 930 | 10.33 |
| 21 | 10200 | 1515 | 14.85 |
| 22 | 8800 | 1325 | 15.06 |
| 23 | 9000 | 1200 | 13.33 |
| 24 | 8300 | 865 | 10.42 |
| 25 | 12800 | 1930 | 15.08 |
| 26 | 8800 | 1305 | 14.83 |
| 27 | 9900 | 870 | 8.79 |
| 28 | 11300 | 610 | 5.40 |
| 29 | 6600 | 375 | 5.68 |
| 30 | 10100 | 1050 | 10.40 |
| | **Mean** 8730.00 ± 32.96 µg | 1072.83 ± 66.88 µg | 10.69 ± 0.68% |

# VITA

Katherine Jane Overton was born on August 16, 1974 in Bristol, Tennessee, to N.N. and Brenda Smith of Kentucky. She had the privilege of living overseas in Saudi Arabia before her family settled in Texas. She received a B.S. degree in zoology from Texas A&M University in 1998 and then became employed at the Center for Urban and Structural Entomology. During her time working as a research assistant and being a graduate student there, she oversaw a correspondence course in termite biology and control for Orkin® and Texas pest control operators, and had the opportunity to be a teaching assistant for Dr. Roger Gold for the class Insects and Human Society (ENTO 222). She completed her M.S. degree in entomology in 2003.

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