FY 2006-2007 Report on Progress

(September 1, 2006 – August 31, 2007)

Texas Imported Fire Ant Research And Management Project

Title of project: Development of *Thelohania solenopsae* as an effective biological control agent for the red imported fire ant, *Solenopsis invicta*

Principal investigator(s) and contact information:

Lay Summary of Major Accomplishments:

Technical Description of Progress on Individual Objectives:

Relevance to Achieving the Overarching Goals of the Texas Imported Fire Ant Research and Management Project (see RFP guidelines)**:**

Manuscripts Published/In Press/Submitted:

Invited and Submitted Presentations/Posters Presented at Scientific/Technical Meetings/Conferences:

PI Signatures:

Karen Snowden	1 August 2007
Signature	**Date**

S. Bradleigh Vinson	August 1, 007
Signature	**Date**

(paper copy of report contains original signatures)

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Deadline: August 1, 2007

Final Report
Texas Imported Fire Ant Research and Management Project
Year 2, August 2007

Title of Project: Development of *Thelohania solenopsae* as an effective biological control agent for the red imported fire ant, *Solenopsis invicta*

Principal Investigators:
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Lay Summary of Major Accomplishments:

The microscopic intracellular microsporidian parasite, *Thelohania solenopsae*, is an ant pathogen that has potential as a biological control agent against *Solenopsis invicta*, the Red Imported Fire Ant (RIFA). Major progress was made in the development of a semi-solid food that could be used in a bait formulation containing infective parasite spores. Experiments using tiny fluorescent beads in the bait food demonstrate the successful distribution of the beads throughout the colony simulating the transmission of the parasite through a food bait. Additional studies show native fire ants (*S. geminata*) as well as the RIFA are naturally infected with the *Thelohania* parasite. Molecular characterization of many parasite isolates suggests that different strains of the parasite may exist. Experimental data continue to support our believe that *T. solenopsae* is a promising biological control agent against the RIFA.

Technical Description of Progress on Individual Objectives:

Objective 1) Determine the horizontal transmission of *T. solenopsae* spores through RIFA colonies.

a) Simulate transmission of spores through RIFA colonies using microspheres administered in various nutrient substrates.

Early efforts at bait development using *Thelohania* spores utilized sugar water, egg yolk or peanut oil as substrates representing carbohydrate, protein and lipid nutrients. It rapidly became clear that a semi-solid substrate matrix was needed to uniformly disburse parasite spores for a more appropriate bait formulation. Therefore, a number of recipes and variations of semi-solid substrates were developed and evaluated as a bait matrix. Three semi-solid substrates composed either of a) carbohydrate, b) lipid or c) protein were optimized and tested in bait feeding simulations using microspheres. Four sizes of fluorescent microspheres (1, 2.2, 3.7 and 6 um spheres) were selected to represent the various sizes of *Thelohania* spores. Uniform distribution of microspheres in the three baits was determined by repeated sampling of various areas within the semi-solid substrates.

RIFA colonies were collected in the field (Brazos County) using standard methodology. Each colony was characterized as monogyne or polygyne by observation of one or multiple queens and by molecular evaluation of the *GP-9* gene using previously described methodology. Each colony was characterized as *Thelohania* positive or negative through microscopic evaluation of workers using calcofluor M2R staining as previously described. Uniform sized subset colonies were established from the field-collected colonies. Colonies were starved for 24 hours and then provided the microsphere substrate baits for 3 days. Subsequently, adults of all castes and 4th instar larvae were collected and evaluated microscopically for the presence of microspheres. Ingestion of microsphere substrate baits was detected through the addition of appropriate dyes in the substrate which could be visualized in individual ants.

Regardless of monogyne/polygyne status and regardless of parasite infection status, the lipid substrate bait was most success in distributing microspheres to colony members (n = 6 colonies) even though the average consumption rate of 35.5% of the bait was the lowest of the 3 substrates. Both reproductive and non-reproductive 4th stage larvae contained the high number of microspheres (average > 100 spheres/ant) of all sizes. Female alates and dealates/queens sporadically contained lower numbers of microspheres of all sizes. Workers contained no microspheres.

In colonies (n = 6) fed the protein substrate bait, the average bait consumption rate was much higher at 80%. Both reproductive and non-reproductive 4th stage larvae contained microspheres of all sizes at lower levels and on a less consistent basis. No adult stages contained microspheres.

The average bait consumption for colonies (n = 8) fed the carbohydrate substrate baits was also high at 80%, however microspheres of all sizes were found only sporadically at low levels (1-10 spheres/ant) in reproductive and non-reproductive larvae.

Although more repetitions are needed for statistical analysis, the microsphere distribution and nutrient substrate consumption appear to be similar in monogyne and polygyne colonies. Similarly, the presence or absence of *Thelohania* parasites does not appear to influence microsphere distribution or nutrient substrate consumption. Clearly, the semi-solid lipid substrate containing microspheres as a model indicates that a lipid substrate matrix is the optimum choice for continued development of a *Thelohania*-containing bait.

b) Determine the fate of spores originating from the meconia by behavioral observations and staining processes.

Fourth instar larvae near pupation were removed from colonies fed the microsphere substrate baits and allowed to pupate. As a part of the pupation process, digestive wastes are shed from each larva in the meconium. Meconia from pupating larvae were evaluated for the presence of fluorescent microspheres. Microspheres of all sizes were present in meconia in varying numbers. Since the meconial mass is reprocessed by workers, we believe that this is a possible route of horizontal transmission of *Thelohania* through the ant colony.

c) Determine if non-infected RIFA colonies can become infected through access to infected dead ant midden piles.

The midden piles of RIFA colonies fed microsphere substrates were sampled on a weekly basis for 3 weeks following bait feeding. Regardless of the substrate, variable numbers of microspheres of all sizes were found in midden piles at all time points sampled, with the midden piles of colonies fed the lipid substrate bait showing the most consistent presence of spheres in high numbers.

Objective 2) Formulate a bait with various nutrient substrates containing purified *T. solenopsae* spores and attempt experimental transmission of the infection to naive RIFA colonies.

In designing a bait containing *Thelohania* spores, the viability of spores was evaluated in several experimental conditions. In one set of experiments, the viability was determined for spores that were maintained for a week or more in suspensions at different pH values ranging from 3 to 11. A previously published fluorescent dual staining method employing Sytox Green and Calcofluor M2R staining (Green et al, J. Clin. Microbiol, 2000) was used to distinguish viable and dead spores. Preliminary results indicated that spores remained viable for several days in at a wide range of pH conditions. Experiments will be repeated using the newly developed semi-solid nutrient substrates.

Thelohania spores were also incorporated into carbohydrate (sugar water) and protein (egg yolk) substrates and evaluated for spore viability over 10 weeks using the fluorescent dual staining method. In preliminary studies, spores showed a slight decrease in viability starting at 7 weeks. These experiments will be repeated using the newly developed semi-solid nutrient substrates. These results are encouraging, suggesting that the parasites should be stable in a bait formulation suggesting that this approach should be commercially viable.

Objective 3) Use molecular and biological methods to identify and characterize variant isolates of *T. solenopsae*.

a/b) Evaluate more *S. invicta* colonies from south Texas using molecular methods to identify variant isolates of *T. solenopsae*. Establish and maintain *S. invicta* colonies infected with variant strains of *T. solenopsae* and evaluate the biological impact of parasites on the colonies.

A total of 64 fire ant colonies were collected primarily in Cameron Co., TX, in the summer of 2006. Molecular sequencing of a portion of the ribosomal RNA gene and morphologic characteristics were used to identify colonies as *S. invicta* or *S. geminata* (tropical fire ant, native to south Texas), and calcofluor M2R staining and microscopy was used to determine *Thelohania* infection status. The overall parasite infection rate in *S. invicta* colonies was 40%. This rate is similar to that found throughout the state of Texas, based on our previous survey work (Mitchell et al., 2006; Ashbaugh et al., manuscript submitted). Table 1 characterizes these colonies.

Table 1. Parasite infection rates in native and imported fire ant colonies from south Texas.

	T. solenopsae +	*T. solenopsae* -	TOTAL
S. invicta	14 (40%)	21	35
S. geminata	16 (55%)	13	29
TOTAL	30	34	64

Molecular sequencing of the ITS region of the ribosomal RNA gene of *T. solenopsae* indicated some variation among 12 *S. invicta* isolates with sequence homologies ranging from 95 to 100%. These data differ from sequences generated from 13 previously sequenced parasite isolates from various parts of the state, where sequences of this region showed 100% homology to each other. These results agree with previous data that identified variation in 2 Cameron Co. isolates collected in 2004 (Snowden et al., manuscript in preparation).

To date, *S. invicta* colonies with variant *T. solenopsae* parasite infections have not been established and maintained under experimental conditions.

c) Evaluate native *Solenopsis* species for the presence of *Thelohania*-type parasites using molecular and microscopic methods.

A total of 29 colonies of *S. geminata* were collected in south Texas in geographic areas that were co-inhabited by *S. invicta*. Microscopic evaluation of the *S. geminata* colonies indicated a higher infection rate of 55% with *Thelohania* than the nearby RIFA colonies (Table 1).

d) Characterize isolates of *Thelohania* spp. that are identified in *Solenopsis* spp. other than *S. invicta*, if present, using molecular methods.

Molecular analysis of the ITS ribosomal RNA gene region of *Thelohania* from *S. geminata* colonies showed 95 to 100% homology among 12 isolates from that ant host species. Gene homologies were similar (often 100%) when the comparing sequences of parasites from *S. invicta* and *S. geminata* hosts. Therefore, data indicate that the same parasite species infects both ant species. (Snowden et al., manuscript in preparation)

Additional Research Data Generated in this Project:

1) The objectives of a similarly funded, concurrent project authored by S. B. Vinson and T. Azizi (see their report) were to look at flight muscle degeneration, reproduction control, muscle cell apoptosis, colony foundation. As those studies were being initiated, investigators became aware that the research on these issues was compromised by the high incidence of *Thelohania* in the local fire ant population. Resources were not available to repeatedly collect ants from distant areas where the infection was low; therefore, those investigators (Vinson and Azizi) worked with Snowden (PI of this project) to initiate a project investigating the presence of *Thelohania* in archived ant samples (pre-1998) to establish to how long *Thelohania* had been in Brazos Co. and to look at some of the effects of the disease on the fire ant colonies. (See Vinson and Azizi Report).

2) While evaluating ant colonies collected in south Texas, an additional unexpected finding was the microscopic identification of the myxosporidian parasite, *Mattesia* sp. in multiple ant colonies. A total of 9 *S. invicta* colonies (26%) were positive for this new parasite, and 3 of those colonies had dual infections with *Thelohania* (Table 2A). A total of 3 *S. geminata* colonies (10%) were positive for *Mattesia*, including one dual infection (Table 2B). Molecular sequencing of a portion of the ribosomal RNA gene for 10 Mattesia isolates showed 93 to 100% homology among the isolates and matching a sequence for this parasite in Genbank. Data suggested that the same parasite species is found in both fire ant species. The pathogenicity or relative importance of this new parasite as a fire ant pathogen is totally unexplored and warrants further investigation.

Table 2A. Parasite infections in *S. invicta* colonies

	Mattesia +	*Mattesia* -	TOTAL
Thelohania +	3	11	14
Thelohania -	6	15	21
TOTAL	9	26	35

Table 2B. Parasite infections in *S. geminata* colonies

	Mattesia +	*Mattesia* -	TOTAL
Thelohania +	1	15	16
Thelohania -	2	11	13
TOTAL	3	26	29

Relevance to achieving the overarching goals of the TIFARMP:

The program goals of the Texas Imported Fire Ant Research and Management Project are "The management of imported fire ant to below economic levels on agricultural lands and to eliminate the imported fire ant as a nuisance or health threat in urban environments." Towards that goal, this research project specifically addresses the priority area of the "Elucidation of the biology of fire ant or its natural enemies (at levels of integration spanning molecular to ecological landscapes) leading to the discovery of new management methodologies or technologies with sufficiently high probabilities for near-term assessment in the field."

Specifically, the long-term goal of this research effort is to develop the microsporidian parasite, *Thelohania solenopsae*, as a biological control agent in a bait formulation that can be used to treat and/or augment other forms of control against the red imported fire ant. Specific accomplishments of this 2-year funding period are listed below:

- Major steps were accomplished in the development of a *Thelohania* spore-containing bait. Feeding trials with semi-solid microsphere substrate baits clearly indicate that a lipid based substrate was most successful in distributing fluorescent microspheres (simulating parasite spores) through the RIFA colonies. Additionally, all sizes of microspheres are distributed within the colony, suggesting that any of the *Thelohania* spore forms of various sizes could be distributed in the lipid substrate and transmitted through the ant colony.

- The confirmation of genetic variants of the *Thelohania* parasite indicate that different strains of the parasite exist. The relative virulence of these molecular variants requires further investigation. If strains with varying pathogenicity exist, it would be critical to select an appropriate strain for further development of a commercial bait using this parasite as a biological control agent.

- A major goal of this research project was to evaluate native fire ant species for the presence of *Thelohania* parasites. Data indicate that the native fire ant species, *S. geminata,* is naturally infected with *Thelohania* in areas where native and imported fire ant species both occur. Molecular data suggest that the same parasite species is found in both ant species. The pathogenicity or relevance of *Thelohania* in native fire ant species deserves further investigation. When developing a biological control agent, the impact of the pathogen (*T. solenopsae*) on native host species (native fire ant species) must be evaluated.

- An additional unexpected finding was the identification of another protozoan parasite, the myxosporidian organism, *Mattesia* sp., in both native and RIFA colonies in south Texas. The pathogenicity and relative importance of this parasite should be investigated in fire ants.

Manuscripts Published/ In Press/ Submitted:

Ashbaugh EA, KS Logan, FL Mitchell, SB Vinson, CZ Dickerson and KF Snowden. (Submitted). Correlation of colony social behavior and infection with *Thelohania solenopsae* (Microsporida: Thelohaniidae) in Red Imported Fire Ants. Environ. Entomol.

Hale MW, J Bernal and SB Vinson. (Submitted). Effects of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) infection on *Solenopsis invicta* (Hymenoptera: Formicidae) colony fitness components and queen adoption rate. J. Invert. Pathol.

Mitchell FL, K Snowden, JR Fuxa and SB Vinson. 2006. Distribution of *Thelohania solenopsae* (Microsporida: Thelohaniidae) Infecting Red Imported Fire Ants (Hymenoptera: Formicidae) in Texas. SW Entomol. 31 (4): 297-306.

Overton Katherine, A Rao, SB Vinson and RE Gold. 2006. Mating flight initiation and nutritional status (protein and lipid) of *Solenopsis invicta* (Hymenoptera: Formicidae) alates infected with *Thelohania solenopsae* (Microsporidia: Thelohaniidae). Ann. Entomol. Soc. Am. 99:524-529.

Vinson SB, T Azizi and K Snowden (ready for submission). The occurrence of *Thelohania solenopsae* (Microsporida: Thelohaniidae) in archived Imported Fire Ant *Solenopsis invicta* (Hymenoptera: Formicidae) samples in Central Texas 14 years before it discovery in the USA. J. Invert. Pathol.

Invited/Submitted Presentations/Posters at Meetings/Conferences:

Invited Symposia: "Mitchell, F.; K. Snowden, J. Fuxa, and S. B. Vinson. Statewide survey for *Thelohania solenopsae* infecting red imported fire ant, 54th Annual Meeting, Southwest. Br. Entomol. Soc. Am. Feb.27-Mar.2, Austin, TX. 2006.

Invited Symposia: Hale, Walker, A. Rao, K. Overton, M. Keck and S. B. Vinson. Effects of *Thelohania* on fire ant colonies, 54th Annual Meeting, Southwest. Br. Entomol. Soc. Am. Feb.27-Mar.2, Austin, TX. 2006.

Invited Conference presentation: Vinson, S. B., Walker Hale, Toghara Azizi and Karen Snowden. Is there potential for the use of *Thelohania solenopsae* for the management of the Imported Fire ant, *Solenopsis invicta*? International Pacific Invasive Ant Conference. Kailua-Kona, HI. May 23-25, 2007

Vinson, S. B., T. Azizi and K. Snowden. 2006. The historical occurrence of *Thelohania solenopsae* in red imported fire ant (*Solenopsis invicta*) colonies in the Brazos valley region of Texas. Annual Red Imported Fire Ant Conference, March 28-30, Mobile AL.

FY 2006-2007 Budget Audit

(September 1, 2006 – August 31, 2007)

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Title of project: Development of *Thelohania solenopsae* as an effective biological control agent for the Red Imported Fire Ant, *Solenopsis invicta*

Principal investigator(s) and contact information:

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Bradleigh Vinson (Professor, Dept. of Entomology, COALS)
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Expenditures to Date (Sept 1, 2006 – June 30, 2007):

Major items: Salary and wages, supplies and expenses for molecular analysis & DNA sequencing, fluorescent microscope supplies and travel.

Projected Expenditures (July 1 – August, 31, 2007):

No major items purchased in final 2 months of project. All funds are encumbered. I do not anticipate any unused funds.

Total: $54,000 (Snowden)
Unused Funds: $0

PI Signatures:

Karen Snowden 1 August 2007

Signature **Date**

(printed copy has original signature)

Send two copies of progress report (one paper and one electronic) to:

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Professor and Head
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Deadline: August 1, 2007

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S Bradleigh Vinson (Professor, Dept. of Entomology, COALS)
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Phone: 979 845-9754
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Expenditures to Date (Sept 1, 2006 – June 30, 2007): For Dr Vinson
(Itemize Major Items)
Spent for supplies, collecting, ant maintenance, publications, travel and salaries (51,643,50)

Projected Expenditures (August 1 – August, 31, 2007): If you anticipate having funds remaining that you will be incapable of expending before the end of the project date, please provide an estimate of the amount. Unused funds may not be carried over to the next fiscal year and will be reallocated at the discretion of TAES Administration.
(Itemize Major Items).
I have $800 encumbered that I may release to use through August.
No major items and I Do not anticipate any unused funds.

Total: 54,000 Vinson
Unused Funds: 2356.50

PI Signatures: S. Bradleigh Vinson 8-1-07

Signature **Date**

If this report is prepared by someone other than the Principal Investigator, please provide name and contact information:

Send two copies of progress report (one paper and one electronic) to:

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