

Progress Report
Texas Imported Fire Ant Research and Management Project
Year 1, September, 2006

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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Major accomplishments to date:

OBJECTIVE 1

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

a) Simulate the transmission of spores through RIFA colonies using appropriately sized micro spheres administered in various nutrient substrates to small colonies.

We located several small spore sized florescent micro-beads that simulate spores. The project was initiated first using a sugar substrate with microspores fed to small colonies with larvae and workers, but were queen less. The results were that no microspores were found in the larvae, but were found in the buccal pellets of workers. The study was repeated with the same design, but with a queen. This one replication showed that micro spheres were transmitted to larvae. We started to run a second replication but had difficulty digging up colonies and rarely could we locate non infected colonies (See cover letter).

b) Determine the fate of spores originating from the meconia by behavioral observations and staining processes. This is on hold until we can get good colonies.

c) Determine if non-infected RIFA colonies can become infected through access to infected dead ant midden piles. This is on hold until we can get good colonies.

OBJECTIVE 2

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

a) Administer the bait as a food source to nutrient-deprived RIFA colonies. This was initiated. The foods used consisted of a 10% and a 20% sucrose solution, an oil (Soybean) and a protein (egg yoke), each containing spores separated from infected ants. These spore containing foods were held for 10 weeks and sampled weekly to determine the viability of the spores using Calcofluor white M2R followed by Sytox Green. This technique provides a method to identify spores and determine if they are viable. The data showed that, in general, all the foods maintained at least 50% viable spores over the ten week period. A similar study was conducted by exposing spores to pH's ranging from 1 to 12 with around 40% surviving from 3 to 11.

b) Hand feed the bait to various castes and developmental stages of RIFA ants. We are now preparing this study.

OBJECTIVE 3

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

a) Evaluate more *S. invicta* colonies from south Texas using molecular methods to identify variant isolates of *T. solenopsae*.

DNA sequencing of the ITS region of the ribosomal RNA gene from *T. solenopsae* was previously completed for 17 parasite isolates from multiple locations in Texas as well as Louisiana and Florida. Two isolates from Cameron County, the southernmost county in Texas, showed significant variation from the other 15 isolates which had identical sequences (Snowden et al, manuscript ready for submission). Therefore a major focus of this project is to further evaluate parasite infection in *S. invicta* colonies from that region.

Despite the severe heat and drought that inhibited ant colony collection, a number of colonies were sampled in Cameron County during 4 collecting trips. A total of 69 colonies of several ant genera were collected during this fiscal year and are currently under analysis. To date, 19 colonies of *S. invicta* have been evaluated. Eight colonies were *Thelohania* positive using PCR, and 5 of those colonies are microscopically positive using Calcofluor M2R staining. This 42% rate of infection is similar to the overall infection rates, which ranged from less than 10% to almost 50% in a recent statewide survey depending on geographic location (Mitchell et al, manuscript submitted). DNA sequencing of the targeted ribosomal RNA gene is underway, and preliminary data suggests that at least 2 variant colonies have been identified.

b) Establish and maintain *S. invicta* colonies infected with variant strains of *T. solenopsae* and evaluate the biological impact of parasites on the colonies.

The specific locations of *S. invicta* colonies with variant *T. solenopsae* infections were noted using GPS coordinate identification. Those colonies will be collected in the near future in an attempt to establish a viable colony including queens for further evaluation of the pathogenicity of the variant *Thelohania* parasites.

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

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

Fourteen of the colonies collected from Cameron County have been identified morphologically as the native fire ant species, *S. geminata*. Eight of 14 colonies (57%) were *Thelohania* positive using PCR and microscopic methods. To our knowledge, this is the first time that this number of native fire ant species collected in the southern USA have been evaluated for the presence of the microsporidian parasite. Although the number of colonies analyzed thusfar is limited, the rate of parasite infection is unexpectedly high. DNA sequencing of the targeted ribosomal RNA gene is underway. Preliminary data suggest that variant *Thelohania* parasites are found in this species of fire ant also.

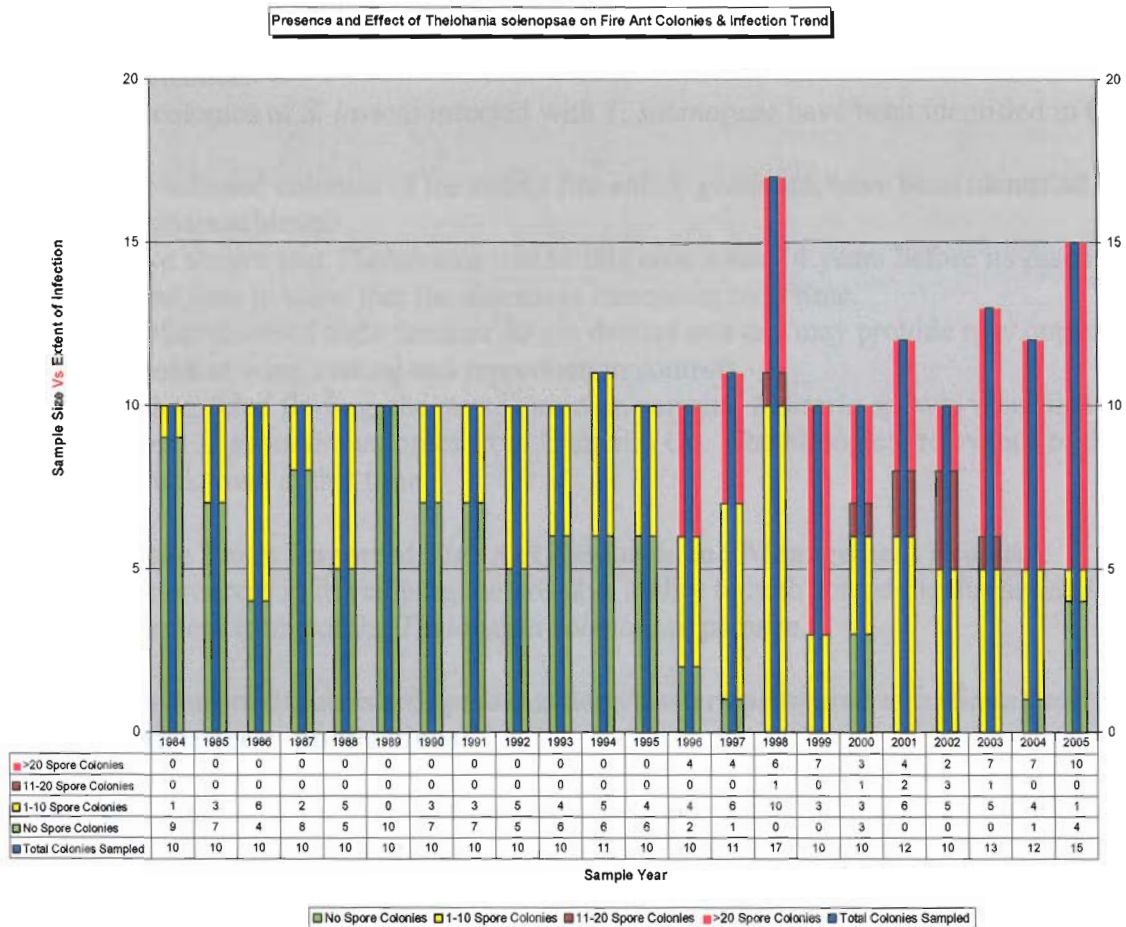
Analysis of a small number of non-*Solenopsis* ant colonies from Cameron County (n=5) showed that no *Thelohania* were present. Thirty-one additional colonies are still waiting analysis, and data should be available within the next 3 months.

Additional Research opportunities:

(Due to the environmental conditions and what appeared to be the increased incidence of *Thelohania* infection, we initiated several studies complimentary to this project).

A) Determine when *Thelohania* arrived in central Texas and to attempt to get some idea of the incidence of the disease over time.

We had been archiving samples of ants collected locally since 1984. Analysis of our archived RIFA samples (based on Calcofluor M2R staining and microscopy) demonstrate occurrence of *Thelohania solenopsae* as far back as 1984. Molecular methods (currently in progress) have confirmed the occurrence of *Thelohania solenopsae* dating back to 1990. This predates current reports by 12 and 8 years respectively, that suggest *Thelohania solenopsae* expansion into US started in 1998. In addition our data (still being analyzed) suggests that the intensity of the disease has become more intense beginning in the late 1990's (Fig.).



B) The effects of *Thelohania* on colony establishment and reproduction. We recently found that infected alate queens, when separated from the colony, do not dealate and reproduction appears to be, at least, delayed when compared to non infected alates that dealate within days and lay eggs. This may provide more insight into the effects of the disease on the colony and the control of reproduction in the IFA.

C) While evaluating ant colonies collected this year in Cameron Co., TX, one additional unexpected finding was the microscopic identification of the myxosporidian parasite, *Mattesia*, in some samples. Thus far, 6 *S. invicta* colonies and 2 *S. geminata* colonies have been infected with parasite. PCR methods were developed to confirm the identity of these parasites. Sequence analysis of the ssu rRNA gene of these 8 infections shows 98 to 100% homology with *M. geminata*. The biological significance of this finding is yet unknown.

Goals achieved:

- 1) The movement of spores in the colony to determine where the infection begins is on hold until we can get the proper colonies. We are working hard to get these.
- 2) Analysis of spore viability appears good in most types of baits that we could consider. The next question is can we get transmission directly to larvae, which is underway. Thus we are making good progress in gaining the necessary information to allow us to develop novel approaches to spread the disease (use of a bait) to new locations and IFA populations with a low disease incidence.
- 3) Additional colonies of *S. invicta* infected with *T. solenopsae* have been identified in Cameron County, TX.
- 4) *Thelohania*-infected colonies of the native fire ant, *S. geminata*, have been identified.

Opportunistic goals achieved

- A) We have shown that *Thelohania* was in this area some 14 years before its discovery. We have also data to show that the disease is increasing over time.
- B) Found that diseased alate females do not dealate and this may provide new opportunities to see look at wing casting and reproduction controls.
- C) In an unexpected finding, the myxosporidian parasite, *Mattesia* sp. was identified in both *S. invicta* and *S. geminata* colonies from Cameron Co. The biological relevance of this parasite is unknown at this time.

Relevance to the Texas Imported Fire Ant Research and Management Project:

We have made progress in developing the possible ability to both spread the disease and possibly locate more virulent forms of the *Thelohania solenopsae* parasite.

Publications submitted/published; presentations/posters presented at national/ technical meetings/ conferences: publications:

Overton, Katherine; A. Rao, S. B. Vinson and R. E. 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.

Mitchell FL, KF Snowden, JR Fuxa and SB Vinson. 2006 submitted. Distribution of *Thelohania solenopsae* (Microsporida: Thelohaniidae) infecting red imported fire ant (Hymenoptera: Formicidae) in Texas. Submitted to *SW Entomol*.

Ashbaugh EA, KS Logan, FL Mitchell, SB Vinson, K Zindler and KF Snowden. Correlation of colony social behavior and infection with *Thelohania solenopsae* (Phylum Microspora) in Red Imported Fire Ants (*Solenopsis invicta*). ready for submission to *Econo Entomol* when Mitchell et al, (listed above) is accepted for publication. (Appendix I attachment)

Snowden KF, KS Logan, J Gillespie and SB Vinson. Molecular Comparisons of Ribosomal RNA Genes from Geographically Diverse Isolates of *Thelohania solenopsae* (Phylum Microspora) from Red Imported Fire Ants (*Solenopsis invicta*). Ready for submission with approval of all authors. (Appendix II attachment)

presentations:

Ashbaugh EA, KS Logan, FL Mitchell, BS Vinson & KF Snowden "Correlation of colony social behavior and infection with *Thelohania solenopsae* (Phylum Microspora) in Red Imported Fire Ants (*Solenopsis invicta*)" oral presentation at Am Soc Parasitologists annual meeting, Mobile AL., Summer, 2005

Azizi, T and S. B. Vinson 2006. Identification of gene(s) involved in the early events concerning flight muscle degeneration in the red imported fire ant (*Solenopsis invicta*). (**Poster**) Annual Red Imported Fire Ant Conference, March 28-30, Mobile AL.

Hale, W. and S. B. Vinson. 2005. Utility of calcoflur M2R and Sytox dual staining for assessment of Microsporida *Thelohania solenopsae* invading *Solenopsis invicta*. (**Poster**). Ento. Soc. Am. Meeting, Ft. Lauderdale, FL. Nov., 6-9.

Hale, Walker, A. Rao, K. Overton, M. Keck and S. B. Vinson. Effects of *Thelohania* on fire ant colonies, Oral presentation: Southwest Branch of Entomological Society of America conference, Feb.27-Mar.2, 2006. Austin, TX.

Keck, M., S. B. Vinson and R. Gold. 2005. Invasive interactions of *Monomorium minimum* and *Solenopsis invicta* infected with *Thelohania solenopsae*. Oral presentation: Annual Red Imported Fire Ant Conference, March 22-24, Gulfport MS.

Mitchell FL, KF Snowden, EA Ashbaugh, J Fuxa and SB Vinson. " Statewide Survey for *Thelohania solenopsae* infecting red imported fire ants (*Solenopsis invicta*)" oral presentation at Southwest Branch of Entomological Society of America conference, Feb.27-Mar.2, 2006. Austin, TX.

Vinson SB, T Aziz and KF Snowden. "The Historical Occurrence of *Thelohania solenopsae* in the red imported fire ant (*Solenopsis invicta*) colonies in the Brazos Valley Region of Texas" oral presentation at the Regional Fire Ant Annual Meeting, Mobile, AL, Mar 27-

30, 2006.

Thesis:

Hale, Walker. 2006. Host parasite interactions between *Solenopsis invicta* (Hymenoptera: Formicidae) and *Thelohania solenopsae* (Microsporidia: Thelohaniidae). MS thesis Texas A&M. 50 pages.

Waits, Amy Catheranne. 2005. Intracolony transmission of *Thelohania solenopsae* (microsporidia: thelohaniidae) in red imported fire ants (Hymenoptera: Formicidae) via trophylaxis of the meconium. MS Thesis. Tarleton State University. 62 pages

Signature:

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