



Texas Imported Fire Ant Research and Management Project

Progress Report - June 2002

The Biological Control of the RIFA through Augmentation Utilizing Native Diseases

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Funding Amount/2 Years: \$332,000

Summary of Work to be Done:

- ◆ Development of molecular and immunological diagnostic tools
- ◆ Study the transmission, infectivity, and pathogenicity of each spore type of *Thelohania solenopsae* and analyze the relationships between the complex life cycle of the parasite and the organization of ant social life
- ◆ Determine the population level effects of *T. solenopsae*: prevalence, geographic distribution and spread of *T. solenopsae* in Texas, and the success rate of claustral colony founding by infected queens.
- ◆ Develop methods to isolate and purify spores and develop cell lines capable of producing *T. solenopsae* that can be scaled up to produce material for release.
- ◆ Determine the effects of environmental factors on epizootics of the pathogenic fungus *Beauveria bassiana* in RIFA

Major Accomplishments to Date:

- ◆ Discovered a third major spore type of *T. solenopsae*, the megaspore, infecting the ovaries of red imported fire ant (RIFA) adult females (Fuxa).
- ◆ Implemented a combination of trichrome staining and PCR using a newly developed specific primer set for improved detection of *T. solenopsae* in field samples of RIFA. Ant and protozoan DNA samples are archived by membrane technology (see below) and protozoan spore samples archived on permanent stained slides. (Fuxa & Mitchell).
- ◆ Developed several new primers that are being evaluated to see if they are able to detect the presence of different *T. solenopsae* strains (Vinson + Snowden).
- ◆ Developed a membrane technology that allows infected samples to be archived for future PCR (Snowden and Vinson)
- ◆ Conducted an area survey of *T. solenopsae* in the vicinity of Stephenville, TX as a trial run for the state wide survey (Mitchell)
- ◆ Determined that the presence of *T. solenopsae* infected RIFA has a positive impact on ground dwelling arthropod diversity and abundance in east Texas (Cook) and began comparing ant diversity in areas where *T. solenopsae* is common to a site where it is not in central Texas (Mitchell)
- ◆ Determined that newly mated RIFA queens infected with *T. solenopsae* weigh significantly less than uninfected queens, but that infection status does not appear to affect an alate's ability to mate (Cook)
- ◆ Determined that hemolymph vitellogenin levels, egg proteins, are reduced in infected queens (Vinson)
- ◆ Determined that newly mated queens infected with *T. solenopsae* produce significantly fewer eggs than uninfected queens, but that infection does not appear to affect the trophic: embryonated

- ◆ egg ratio (Cook)
- ◆ Began experiments to determine nanitic worker production, and colony size and caste structure of 15 week old fire ant colonies of infected vs. uninfected queens (Cook).
- ◆ Began experiments to determine resource acquisition by mature colonies infected with *Thelohania solenopsae* vs uninfected (Vinson).
- ◆ We are examining all possible ant secretions as possible sources of *Thelohania solenopsae* infection in the IFA. We have evidence of spores in some ant secretions via staining, but we are in the process of confirmation by PCR. (Vinson)
- ◆ We have examined the movement of select secretions in a fire ant colony and have data showing the some larval secretions are passed directly to queens, as well as to other larvae and workers. (Vinson).
- ◆ We have been successful in getting a *Thelohania solenopsae* infection of a non-ant insect cell line. We hope this opens the door to the development of mass spore production (Vinson and Snowden)
- ◆ Determined that the fungus *Beauveria bassiana* controlled ants significantly better in silt or sandy soil than in clay and killed significantly more ants at medium moisture levels than in wet or dry soil (Fuxa).

Goals Achieved:

- ◆ Developed improved method for screening, identification, and archival of samples to detect *Thelohania solenopsae* infection.
- ◆ Found a third major spore type of *Thelohania solenopsae* that may be involved in transmission.
- ◆ Found spores in some ant secretions that may be a source of infection and lateral disease transmission.
- ◆ Have been able to get sporulation in an insect cell culture.
- ◆ Determined that this parasite has a negative impact on weight and production of egg proteins resulting in a reduction of reproductive output of mature and newly mated queens suggesting a reduction in a newly mated queen to found new colonies and mature colonies compete.
- ◆ Determined that the presence of this parasite has a positive impact on arthropod diversity
- ◆ Determined the environmental conditions that may enhance the ability of the fungus *Beauveria bassiana* to control RIFA populations

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

- ◆ Discovery of third spore type and possible spores in ant secretions may relate to different routes of transmission, a key factor for improving biological control by this microsporidium.
- ◆ Development of the spores in a cell culture provides the opportunity to consider the development techniques for the mass production of spores for release.
- ◆ Improved detection of *T. solenopsae* in field samples will greatly aid the statewide survey to determine geographic distribution and prevalence of *T. solenopsae* and will allow us to assess the success and impact of inoculations
- ◆ Determination that infection with *T. solenopsae* negatively impacts colony founding ability of queens and impacts the competitiveness of more mature colonies allows us to consider targeting a range of colony developmental stages with inoculations to not only impact mature colonies, but prevent new colonies from forming

Products; publications submitted/published; presentations/posters presented at state and national technical conferences:

A. Refereed publications:

Sokolova, Y. and J. R. Fuxa. 2001. Development of *Thelohania solenopsae* in red imported fire ants *Solenopsis invicta* from polygynous colonies results in formation of three spore types. J. Eukaryot. Microbiol. 2001: 85S.

B. Manuscripts (in Press):

C. Manuscripts (Submitted):

Cook, T. J., M. B. Lowery, T. N. Frey, K. E. Rowe and L. R. Lynch. Effect of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) on weight and reproductive status of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), alates. *In Review*. J. of Invertebrate Pathology.

Snowden, K. F., K. S. Logan and S. B. Vinson. 2002. Simple filter-based PCR detection of *Thelohania solenopsae* (Microspora) in fire ants (*Solenopsis invicta*). *In Review* J. Eukaryotic Microbiology.

D. Manuscripts (In Preparation):

E. Manuscripts (Non-refereed publications):

F. Presentations:

Brain, M. S. and T. J. Cook. 2002. Relationship between the presence of *Thelohania solenopsae* infected imported fire ants and ground dwelling arthropod diversity and abundance. Oral presentation at 2002 annual conference of the Southwestern Association of Parasitologists, Oklahoma Biological Field Station, Lake Texoma.

Cook, T. J. 2002. Effect of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) on weight and reproductive status of female alates of the red imported fire ant, *Solenopsis invicta*. Oral presentation at 2002 annual conference of the Southwestern Association of Naturalists, Curnavaca, Mexico.

Sokolova, Y. Y., I. A. Isakova, and J. R. Fuxa. 2002. Comparative diagnosis of microsporidian infections in fire ant colonies by light microscopy techniques (Giemsa, calcofluor, and trichrome stains) and by PCR. Poster at 2002 Imported Fire Ant Conf., Athens, GA.

Vinson, S. B. and A. Rao. 2002. Survival of small colonies of *Solenopsis invicta* transplanted to *Solenopsis (Diplorhoptrum) sp.* Infested or low ant infested areas. Oral presentation. Imported Fire Ant Conf., Athens, GA.s