Annual Progress Reports due September 13,
Texas Imported Fire Ant Research and Management Project

Title of Project: Develop products to initiate wing muscle degeneration in alate virgin females leading to IFA management (and also initial egg laying).

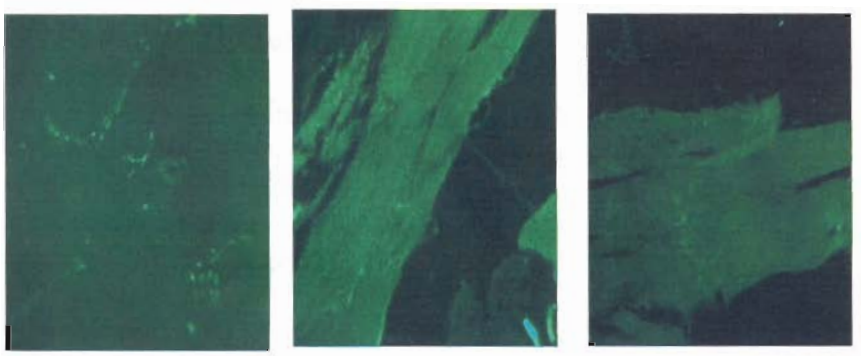
Principal investigator: S. B. Vinson
Co-investigator: T. Azizi

Major accomplishments to date (September 1, through August 31, 2006)**:**

Obj. 1.

We have used our Wing Muscle Cell Apoptosis bioassay to begin to purify the active fraction in the seminal fluid + sperm (Obj.1). Obj. 1 involves the isolation and identification of the components of the male seminal fluid that initiate wing muscle apoptosis. To accomplish this goal we used a size cut off filter followed by centrifugation to separate sperm from seminal fluid. Next sperm was extracted and assessed for its viability. Mature 14 days old female were artificially inseminated with (A) Seminal fluid + sperm, (B) the isolated sperm and (C) the seminal fluid with no sperm. Using our apoptosis bioassay for the initiation of flight muscle degeneration we evaluated each step. Our finding showed no initiation of muscle breakdown with the exception of seminal fluid + sperm which confirmed our earlier results (Fig. 1).

The results suggest the initiation is more complicated. Either two components are involved (one in the seminal fluid and another in the sperm), or the sperm are not functioning naturally. However, more progress has not been made due to drought (see below).



A B C

Fig. 1 TUNEL assay to study the effect of Sperm and Seminal Fluid on initiation of fire ants queen flight muscle histolysis.

A) Flight muscle from virgin queens, inseminated by injection of 1 µl of diluted sperm and seminal fluid into ovary.
B) Flight muscle from virgin queens, inseminated by injection of 1 µl of diluted sperm into ovary.
C) Flight muscle from virgin queens, inseminated by injection of 1 µl of diluted seminal fluid into ovary.
Fixed dissected flight muscle tissues from each group were embedded into paraffin and 5µm thick cross section was obtained from each block. TUNEL labeling of apoptotic nuclei was performed for A through C section. No TUNEL- positive nuclei were detected in B and C, TUNEL- positive nuclei can be observed in artificially inseminated section (A). Live cells stained green, dead cell have dark background, and apoptotic nuclei are light spots.

Obj. 2. This objective involved isolation of a hemolymph factor that is correlated to wing muscle apoptosis. However, progress this year has been slow because we have not had any sign of a good mating flight, due to the very dry conditions. Although we have had a few rains these have not soaked in very deep and the next day or two was followed by poor environmental conditions conducive of flights (See cover letter) .

Obj. 3. We have made no progress on this objective due the lack of newly mated queens (See cover letter).

Obj. 4. We have also had no progress on this objective due to the same problem of the lack of a mating flight (See cover letter).

However, we have found something new in trying to produce viable males so we could at least start objectives 3 + 4 by producing viable males that we could use to artificially mate females to overcome the problems in getting newly mated queens that we are experiencing. This involves separating virgin alate females that delate and lay eggs that lead to males. See (Bashir, Mir; S. Bradleigh Vinson; and Jorge A. Piedrahita. 2003. "Artificial maturation of female alates for the purpose of the production of only male *Solenopsis invicta* (Hymenoptera: Formicidae)". Southwestern Entomologist. 28:19-26.).
We set up alate females from infected and non infected colonies. The females in the non-infected colonies dealated within 3 days, as expected. The females from the infected colonies have not. This opened up a new research opportunity: to ask the question as to why these infected queens have not delated during the last month and are not reproductive. This is ongoing.

Goals Achieved:
Although a little progress has been made and none of the goals have been achieved, we have been able to do more on the *Thelohania* project.

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

Although the goals we presented have not been achieved, we have taken advantage of the high incidence of *Thelohania* infection. We have initated a study to determine why infected delates do not delate and Dr. T. Azzizi has worked on several issues concerning *Thelohania*. That information is placed in the report on the *Thelohania* project. Some of her efforts are documented there.

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

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.

Azizi, Toghara; J. Spencer Johnston and S. Bradleigh Vinson 2006. A Gene Involved in Post-Mating Flight Muscle Degeneration in Red Imported Fire Ant Queens. Submitted to the *Ann. Entomol. Soc. Am.*

Azizi, Toghara and S. Bradleigh Vinson. Initiation of Wing Casting and Flight Muscle Histolysis in the Red Imported Fire Ant *(Solenopsis invicta).* Ready for submission once we decide the best journal outlet.

Signature:

S Bradleigh Vinson 9-13-06

Toghara Azizi-Bahaee

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