



# FY 2006-2007 Report on Progress

(September 1, 2006 – August 31, 2007)

## Texas Imported Fire Ant Research And Management Project

**Title of project:** Identification and behavioral testing of fire ant pheromones involved in recognition.

**Principal investigator(s) and contact information:**

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**Lay Summary of Major Accomplishments:**

We have developed methods to use pheromone binding proteins to isolate and purify pheromones and we have used the method and Dr Renthal has patented the technique. We have also isolated several fire ant binding proteins and are now in a position to use these to isolate pheromones of the fire ant. We have also isolated some venom peptides and based on work done a few years back at Texas Tec, we plan to look and ant behavioral changes when exposed to these proteins.

**Technical Description of Progress on Individual Objectives:**

1) Biochemical analysis of an antennal secretion unique to female fire ants.

We have attempted to isolate the antennal gland-specific protein using a number of techniques beginning with cutting off the gland segments and comparing to non-gland segment proteins. Although some differences have been seen using silver stain we have been unable to obtain a sufficient quantity for further analysis. This included laser capture microdissection. We are trying to determine when the secretion is being produced as this may provide an optimum time to extract.

2) Identification of a pheromone involved in fire ant colony organization.

a) We used the known sequences of the B and b alleles of GP-9 to design PCR primers, and we constructed an expression vector insert containing the complete coding sequences of the B allele gene.

b) We expressed GP-9B from *E. coli*, both with and without a His-tag.

c) We tested our affinity chromatography method for pheromone isolation with model proteins (bovine serum albumin and *Bombyx mori* pheromone-binding protein, generously provided by W. Leal, U. California, Davis).

3) Identification of a fire ant antennal protein involved in chemoreception.

a) We successfully cloned chemosensory protein 1 (CSP1), and we also found ten additional new CSP sequences in the Lausanne Fire Ant cDNA library.

b) We expressed CSP1 in *E. coli*, and purified it by ion exchange and hydrophobic interaction chromatography.

c) We found that N-phenyl-naphthylamine (NPN) binds to CSP1 with a dissociation constant of 10  $\mu$ M, providing a fluorescence binding assay for pheromones and other ligands. An extract containing fire ant cuticular hydrocarbons releases NPN from CSP1, indicating that components of the cuticle bind to CSP1.

4) Behavioral testing of worker response to a unique queen venom protein.

- a) We designed synthetic genes to clone the fire ant worker and queen venom allergens.
- b) We have expressed large quantities of fire ant worker venom protein 2 in *E. coli* and purified it by chromatography. We are now ready for tests in bioassays.

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

Identifying hormones, peptides or other biochemical factors that could be delivered to control the fire ant field populations or identifying the precise timing when known control methods could cause more impact leads this proposal under the category of elucidation of the biology of fire ant leading to new management methodologies.

**Manuscripts Published/In Press/Submitted:**

Renthal, R., Velasquez, D., Olmos, D. & Vinson, S.B., "Occurrence of Antennal Glands in Ants." submitted (2006)

Younger, S., Haidek, A., Velasquez, D., Cassill, J.A. & Renthal, R. "Properties of Ant Apolipoprotein-III." in preparation (2007)

González, D., McMahan, C., Cassill, J.A. & Renthal, R. "Fire ant chemosensory proteins." in preparation (2007)

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

Renthal, R., Velasquez, D., Olmos, D. & Vinson, S.B., "Occurrence of Antennal Glands in Ants." 53rd Annual Meeting of the Entomological Society of America, Ft. Lauderdale, FL, Dec. 15-18 (2005)

Renthal, R., Velasquez, D., González, D., Cassill, A., Baliji, S., Sunter, G., "Chemical control of fire ant behavior." Annual Imported Fire Ant Conference, Mobile, AL, Mar. 28-30 (2006)

Renthal, R., Velasquez, D., Cassill, A., Baliji, S & Sunter, G., "Task selection and protein kinase G expression in fire ants." Proc. 15th Congress of the International Union for the Study of Social Insects, pp. 108-109; Washington D.C., July 30-Aug. 5 (2006)

Renthal, R., Blanchard, O. & González, D. "Proteomics as a tool for developing IFA control strategies." Annual Imported Fire Ant Conference, Gainesville, FL, April 23-25 (2007)

**Patent awarded:**

Renthal, R. "Method for pheromone discovery in insects." United States Patent 7074572 (2006)

**New Grant funded:**

San Antonio Comparative Biology of Aging Pilot Grant Program, "Extended life span of queen ants." Funded 7-31-06, \$10,000 for 2 years. Dr Renthal

**PI Signatures:**

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**Signature**

**Date**



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**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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**Deadline: August 1, 2007**