



# Texas Imported Fire Ant Research and Management Project

*Progress Report - June 2002*

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## **Physiology of the antennae and the isolation and identification of pheromones leading to new IFA control technology**

**Principal Investigator(s):** R. J. Deslippe, P. Pietrantonio, R. Renthal, E. L. Vargo, S. B. Vinson and H. J. Williams.

**Funding Amount/2 Years:** \$428,658

### **Summary of Work to be Done:**

Within the next few months, we will complete the sequencing of two of the proteins found in the poison sac of queens, objective 1. We have targeted these proteins because they increase in amount at about the same time after dealation that the killing of sexual larvae and pupae by workers can be stimulated by poison sac extracts. After sequencing, we will clone the relevant genes, insert them in a microbial vector and express the proteins. We anticipate that this procedure will generate sufficient quantities of the proteins for bioassays to test their roles in the killing of sexual larvae. Objective 2 concerns the isolation of sex pheromones. We have discovered some mating queen specific volatiles. We have collected material and are in the process of identification and determining their role as sex pheromones. The objective (3a) for this part of the project is to isolate and sequence the major male pheromone-binding protein, and then use this protein to obtain its pheromone ligand. Subsequently, the same methods will be used for worker and queen pheromone-binding proteins. The objective (3b) for this part of the project is to characterize odorant receptors. We have made progress and are working on the isolation of these receptors.

### **Major Accomplishments to Date:**

We have demonstrated that a protein(s) from the venom sac of queens influences reproductive larval survival. We have identified two proteins in the poison sac of queens. Both alate and dealate queens produce a 16,000 kDa protein, but only dealate queens produce a 14,000 kDa protein. The smaller protein is found in both fertilized and unfertilized wingless queens, and we have demonstrated that it is expressed within days of dealation (Renthal and Deslippe).

Partial amino acid sequences of the two proteins were determined and compared to sequences of the four proteins found in the venom of workers. The queen venom proteins are different from any of the worker venom proteins (Renthal).

We cloned a sugar transporter (possibly a glucose transporter) from whole fire ant cDNA. We synthesized cDNA from antenna of worker ants and determined that the cDNA was present by amplifying a positive control DNA corresponding to the subunit c of the V-ATPase that we previously cloned from the ant. Amplification of antenna cDNA with specific primers

for the glucose transporter gave the DNA PCR product of the expected size. This is evidence that the glucose transporter is present in the antenna of worker ants (Pietrantonio).

We found that a protein related to apolipophorin-III is one of the major proteins of the male fire ant antenna. Apolipophorin-III may have a role in antennal and pheromone metabolism, since it is known to be expressed a moth sex pheromone-producing gland (Renthal). We have developed purification methods for GP9, reported to be the pheromone-binding protein involved in genetic determination of polygyny (Renthal).

We have found several volatile compounds are released from preflight alate female reproductives. We have found that these volatiles decrease following a mating flight and dealation. We have isolated these alate reproductive caste volatiles and we are completing their identification (Consoli and Williams).

We have found differences in the protein composition of glanded and non-glanded antennal segments, but no difference in the lipids. However, we also have some indication that the secretion may be only released at specific times. Based on this we are completing a time sequence and re-evaluating the data (Vinson, Williams and Renthal).

We have demonstrated that queens lacking the antennal gland are executed, but we have also found that the antennal club that supports the gland is very important in queen feeding. Thus, the role of the gland in the queen is not yet clear (Vinson).

### **Goals Achieved:**

One of our major goals has been to identify the structure and analyze additional chemical properties of a queen pheromone that induces workers to kill sexual larvae. Several bioassays have revealed that the chemical of interest is likely a protein and located in the poison sac. As a result, we have searched for the proteins in the venom sac of queens, and have found two compounds expressed in abundance. We are well on our way to characterizing the entire amino acid sequences of both proteins, and are working on expressing the proteins in a vector system to generate an abundance of the proteins for testing in bioassays.

We have cloned and fully sequenced a putative sugar transporter from the fire ant. We have evidence that the mRNA for this protein is present in the antennae of worker ants. There are no other sugar transporters known from insects with the exception of *Drosophila* where a similar gene has been identified.

We have completed amino-terminal sequencing of the male and worker antennal proteins. RT-PCR was used to identify the male protein.

We have obtained a sufficient quantity of purified pheromone-binding protein (GP9) to test the affinity column pheromone purification method.

We have some success in the isolation of volatiles associated to reproduction and in the isolation of the components of the antennal gland.

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

We have developed a program focused on identifying the molecular controls of communication in the IFA. The strategy involves an investigation of the identification, synthesis, and release of vital pheromones and the molecular mechanisms involved in their detection. The pheromones we pursue include worker-queen attraction pheromones, sexual larvae elimination pheromones, and pheromones controlling mating, dispersal and reproduction. Because these pheromones are key to the social organization and survival of the IFA, any alteration in the production, detection or timing of the compounds will greatly reduce the IFA infestation.

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

**A. Refereed publications:**

Holmes, S. P., S. K. Frazier, and P. V. Pietrantonio. 2000. Cloning of the 16-kDa V-ATPase proteolipid subunit from the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) *Arch. Insect Biochem. Physiol.* 45:109-116.

**B. Manuscripts:**

Deslippe, R. J. 2002. The killing of nestmate queens in ant colonies. *Southwestern Entomologist*. (In press).

Klobuchar, E. and R. J. Deslippe. 2002. A queen pheromone induces workers to kill sexual larvae in colonies of the red imported fire ant (*Solenopsis invicta*). *Naturwissenschaften*. (In press).

Pietrantonio, P. V. , S. P. Holmes, C. Jagge and S. K. Frazier. 2002. Cloning of troponin C and other gene fragments from the red imported fire ant *Solenopsis invicta* Buren (Hymenoptera, Formicidae). *Southwestern Entomologist Supplement on the Red Imported Fire Ant*. (In press).

Renthal, R., Velasquez, D., Hoog, S., Carroll, C. & Weintraub, S.T. 2002. Analysis of antennal proteins of the red imported fire ant. *Southwestern Entomologist Supplement on the Red Imported Fire Ant*. (In press)

**C. Manuscripts (Submitted):**

Renthal, R., Velasquez, D., Olmos, D., Hampton, J. & Wergin, W. P. "Sensilla structures and patterns on the antenna of the red imported fire ant." submitted to *Arthropod Structure and Development* (2002)

**D. Manuscripts (In Preparation):**

Pietrantonio, P. V., and Holmes, S. P. 2002. Cloning and characterization of a sugar plasma membrane transporter from worker fire ants (*Solenopsis invicta*): evidence for expression in antennae.

**E. Manuscripts (Non-refereed publications):**

Deslippe, R. J., E. A. Klobuchar, D. Velasquez and R. Renthal. 2002. A queen pheromone induces workers to kill sexual larvae in colonies of the red imported fire ant (*Solenopsis invicta*). Proceedings of the 2002 Annual Imported Fire Ant Research Conference. Athens, Georgia. (In press).

**F. Presentations:**

Deslippe, R. J., E. A. Klobuchar, D. Velasquez and R. Renthal. 2002. A queen pheromone induces workers to kill sexual larvae in colonies of the red imported fire ant (*Solenopsis invicta*). Entomological Society of America. San Diego, CA.

Deslippe, R. J., E. A. Klobuchar, D. Velasquez and R. Renthal. 2002. A queen pheromone induces workers to kill sexual larvae in colonies of the red imported fire ant (*Solenopsis invicta*). Joint meeting of the Southwestern Branch of the Entomological Society of America and the Sociedad Mexicana de Entomologia. Guanajuato, Mexico.

Deslippe, R. J., E. A. Klobuchar, D. Velasquez and R. Renthal. 2002. A queen pheromone induces workers to kill sexual larvae in colonies of the red imported fire ant (*Solenopsis invicta*). 2002 Fire Ant Conference. Athens, GA.

Renthal, R., K. Guntur; D. Velasquez; J. A. Cassill, 2002. Fire ant pheromone-binding proteins. 2002 Imported Fire Ant Conference, Athens, GA.

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**Summary of Work to be Done:**

Our ultimate objective is to identify the primer pheromones that suppress dealation and egg production in a monogyne colony. Because the primer pheromone is relatively non-volatile we also need to produce a synthetic version of the queen attractant pheromone that will cause workers to pick up the primer pheromone and spread it around the colony. We have tentatively identified this attractant pheromone's chemical components and their relative concentrations, and are currently testing a synthetic version to verify the composition. Once we have a functional synthetic version of this attractant pheromone, we will use it to attract workers to baits containing the putative primer pheromones. This delivery system will allow us determine the chemical composition of this inhibitory compound. We will also use an electro-antennal detector to record the response of virgin queen and worker antennae to queen-produced compounds.

**Major Accomplishments to Date:**

Using a combination of behavioral assays and analytical chemical techniques, we have tentatively identified several components of a pheromone produced by the queen that elicits worker attraction. These components are invictolide, alpha pyrone, z-9-tricosene and z-9-pentacosene. One or more alkanes also appear to be involved, but these have not yet been identified. We have also isolated a 20- carbon aldehyde that has some inhibitory effect on reproduction when used alone.

**Goals Achieved:**

We had five goals for this project: 1) to isolate, purify and structurally characterize the pheromone responsible for queen recognition; 2) to synthesize the pheromonal components to confirm structure; 3) to determine if the queen recognition pheromone also has primer effects; 4) to determine if the queen recognition pheromone or other queen-produced compounds elicit antennal firing from virgin queens and workers, and if so, to identify the active compounds; 5) to isolate, purify and structurally characterize the primer pheromone. We have almost completely achieved objectives 1 and 2, which we expect to finish shortly, and we are currently working on 3, 4 and 5.

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

The results of these studies will lead to the identification of queen pheromones, setting the stage for

possible development of biorational methods for managing fire ant populations. Altering the pheromonal environment of fire ant colonies should significantly disrupt the social organization and survival of the fire ant.

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

C.S. Brent, E.L. Vargo (2002). Characterization of a putative queen tending pheromone in *Solenopsis invicta*. Imported Fire Ant Research Conference, Athens, GA.

C.S. Brent, E.L. Vargo (2001). Measures of JH biosynthesis rates and attractant pheromone composition in queens of the red imported fire ant, *Solenopsis invicta*. Entomological Society of America, San Diego, CA.