



FY 2006-2007 Report on Progress

(September 1, 2006 – August 31, 2007)

Texas Imported Fire Ant Research And Management Project

Title of project: Development of novel control methods using the vitellogenin and short neuropeptide F receptors that regulate reproduction in the Red Imported Fire Ant.

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Lay Summary of Major Accomplishments:

We report the expression and purification of the red imported fire ant vitellogenin receptor (*SiVgR*) fragment that was used as an antigen to produce an antibody specific against the fire ant VgR. The tissue specific expression and localization of *SiVgR* protein in virgin and mated queens were investigated with this antibody. Through immunoblot analysis, we detected an ovary-specific VgR protein of 210 kDa that was present in both alate and dealate queen ovaries. Immunofluorescence analysis of mated queen ovaries at the early stage oocyte showed that *SiVgR* protein was evenly distributed in the oocyte cytoplasm, and at the late stage oocyte showed that *SiVgR* protein was accumulated in the oocyte membrane. During this period we have begun to express the fire ant short neuropeptide receptor (sNPF) in CHO-K1 mammalian cells. The identification of a potent peptide that may activate the fire ant short NPF receptor function will be tested by using a bioluminescence assay for intracellular Ca^{2+} measurement with stably transformed short NPF receptor-expressing cells.

Technical Description of Progress on Individual Objectives:

Objective 1. Determine the Vitellogenin Receptor (VgR) localization in mated and virgin alate queens to understand Vg Receptor temporal/ spatial regulation

In order to achieve this aim we have performed the following:

1. Clustal W alignment of the LDL receptor amino acid sequences for *SiVgR* recombinant protein expression for antibody production
2. Construction of the *SiVgR* recombinant protein expression plasmid, pET28a-VgR.
3. Expression of the *SiVgR* recombinant protein and purification of recombinant protein with metal affinity resin.
4. Western blot and immunohistochemistry application for detection of the VgR expression.

Objective 2. Determine if the meconium stimulates Vg Receptor transcript abundance or Vg Receptor protein abundance

A publication by Cassill and Vinson (2007), see below, documented the organismal effects of larval secretions. We have also isolated and have a tentative identification of the factor in the meconium that is responsible for the stimulation.

We have now developed the antibody in order to further address this objective.

Objective 3. Determine the ligand of sNPF receptor by *in vitro* functional studies:

Transfection and expression of the fire ant short NPF receptor at mammalian cells

Confirmation of the ligand selectivity for sNPF of the putative sNPF receptor by a Ca²⁺ bioluminescence plate assay

In order to achieve this aim we performed the following:

1. Construction of the expression plasmid, pcDNA3.1(-)-*Sis*NPFR.
2. Transfection the plasmid, pcDNA3.1(-)-*Sis*NPFR, into CHO-K1 mammalian cells and selection of the sNPF receptor expression cells (in progress).
3. Determine the ligand of sNPF receptor by Ca²⁺ bioluminescence plate assay (pending 2).

Objective 4. Determine whether short neuropeptide F (neurohormone) stimulates ovarian growth in virgin alate females in the presence or absence of mated queen

The short neuropeptide F will likely be degraded by endogenous proteases in the fire ant. Therefore we have begun collaborating with Ron Nachman (USDA/ARS) to synthesize more stable short neuropeptide F analogs that could have a longer half-life in the insect hemolymph in order to address this experiment.

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:

1. Cassill, D. L. and **S. B. Vinson** 2007. Effects of larval secretions on queen fecundity in the fire ant. *Annals Entomol. Soc. Amer.* 100: 327-332..

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

2. **Pietrantonio, P.V.** and Chen, M.-E. 2006. The short neuropeptide F-like receptor from the imported fire ant (*Solenopsis invicta*, Buren). XV International Congress of the International Union for the Study of Social Insects. Washington DC, USA. July 30- August 4, 2006. Poster presented by P.V. Pietrantonio. Abstract published in Proceedings. Pp. 199. Poster # 470.
3. Chen, M.-E. and **P.V. Pietrantonio**. 2006. The short neuropeptide F-like receptor from the imported fire ant (*Solenopsis invicta*, Buren). Fifth International Symposium in Insect Molecular Science. Tucson, Arizona. May 20-24, 2006. Poster presented by P.V. Pietrantonio. Abstract published online in the Journal of Insect Science. <http://www.insectscience.org/>. 2006. Vol. 6:10.
4. Hsiao Ling Lu. 2006. The Tenth Annual Graduate Student Forum at Texas A&M University. August 2006. Towards developing an antibody against the vitellogenin receptor from the red imported fire ant, *Solenopsis invicta*, Buren.
5. Chen, M.-E., **Pietrantonio, P. V.** 2006. The Short Neuropeptide F Receptor from the Red Imported Fire Ant, *Solenopsis invicta* Buren (Hymenoptera, Formicidae). *Arch. Insect Biochem. Physiol.* 61: 195-208.
6. Chen, M.-E., Holmes, S. P., **Pietrantonio, P. V.** 2005. Glucose transporter 8 (GLUT8) from the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Arch. Insect Biochem. Physiol.* 62:55-72.
7. **Vinson, S. B.**; C. J. Coates, T. Haisheng, S. Rao and T. Azizi. Sorting out some of the factors that influence egg production and regulate vitellogenins in the Imported Fire Ant, *Solenopsis invicta* (Hymenoptera: Formicidae). In: Invited symposia: Current topics in reproductive physiology. "XV Congress IUSSI", Washington, DC. 2006.
8. Rao, Asha and **S. B. Vinson**. A look at the reproductive control measures of the red imported fire ant. *Ento. Soc. Am. Meeting*, Indianapolis, IN. Dec. 10-13, 2006.

PI Signatures:

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Deadline: August 1, 2007