Application of the *Pyemotes Tox-34* Gene to the Control of the Red Imported Fire Ant

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Motivation:

The Family Pyemotidae is represented by mite genera that are principally insect-associates, and the type genus (*Pyemotes tritici*) is a parasite of fire ants. *P. tritici* contains glands which inject a mixture of low-molecular weight proteinaceous neurotoxins into arthropod hosts. *P. tritici* debilitates by paralyzing its host and eventually causes host death. This venom has received toxocological attention and has been the focus of plant bio-engineering studies at the University of Georgia. Toxicity of the these compounds are effective at the level of 500µg/kg in test organisms (wax moths) but are not acutely toxic to mice at dosages of 50mg/kg. Thus they appear to pose little threat to humans, vertebrate wildlife, or game animals. The *Tox-34* gene codes for a major neurotoxin of *Pyemotes.*

Final Report on Bioengineering of Tox-34 gene:

The research group at Texas Tech University has had some success with the biological control of fire ants using the fungus *Beauveria bassiana*. It was my intent to contribute to this success by inserting the *Tox-34* gene into *B. bassiana* as an attempt to impose two selective forces on the fire ant, with the expectation that the ants would not be able to adapt to the two strong forces simultaneous.

I successfully completed the extraction and cloning of the *Tox-34* gene from *Pyemotes tritici.*. The insertion of the gene into the *Beauveria bassiana* genome will be accomplished in Dr. San Fransisco's lab, to facilitate a multiple-impact biological control plan. Dr. San Francisco is continuing to work on the *Beauveria* initiative with Dr. Harlan Thorvilson of Texas Tech University.

Accomplishments:

1) Completed a literature review of the mite *Pyemotes tritici* and its neurotoxin genes.
2) Initiated and expanded a laboratory culture of *Pyemotes tritici* on *Sitatroga cereallella* in preparation for the extraction of the *Tox-34* gene.
3) Completed patent search for patent restrictions concerning the characterized low-molecular weight proteinaceous toxins. Approximately 60 patent claims are held by Dr. Lois Miller (University of Georgia), none will conflict with the success of this project.
4) Hired and trained a technician to carry out steps necessary for the cloning *Tox-34*
5) Built primers (22-mers) (TOX 21A-R) for the *Tox-34* gene.
6) Constructed first strand cDNA library, and from the cDNA expression library the gene was subcloned into a plasmid.

7) The plasmid was given to Dr. M. San Franscisco for completion of the insertion stage.