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

Title of Project:
Use of genetically modified bacteria to deliver compounds that reduce fire ant fitmess.

Principal investigator:
Craig J. Coates
S. B. Vinson

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

A critical methodology and component of this project is the ability to introduce test compounds and antibiotic treatments into the fire ant diet. This is required for both of the first two objectives. We believe we have optimized the delivery method such that we can now rapidly test the efficacy of multiple test compounds as well as utilize single and multiple antibiotic treatments to determine which bacterial species are critical for fire ant survival.

The identification of target compounds will enable the selection of recombinant gene products to be expressed by the bacterial symbionts in the fire ant midgut in the second stage of objective 1. Similarly, the ability to utilize single and multiple antibiotic treatments will enable a more rapid determination of the bacterial species that are critical for fire ant survival. These species are optimal targets for genetic manipulations aimed at detrimentally affecting fire ant growth, development and expansion.

Multiple, diverse collection sites have been identified and utilized to provide additional fire ant colony samples. The analysis of the bacterial species diversity in these colonies will be critical for extrapolating our current and future results to additional geographic areas.

Goals Achieved:
Objective 1:

Test compounds associated with nucleotide synthesis and incorporation into growing DNA strands were utilized as a proof of principle for this methodology. Much of the initial effort has been directed towards the incorporation and delivery of these compounds through the fire ant diet. Preliminary results indicate that the enzymes that can produce these modified, toxic nucleotide analogs may be suitable targets for genetic modification and delivery. There are a number of classes of compounds that remain to be fully tested, including digestive inhibitors, antibacterial peptides, and pro-toxins.

Objective 2:

Significant progress has been made through the use of transmission electron microscopy studies of the location of the midgut associated bacteria. Unique structures that might be harboring bacteria were not identified, making it more likely that any important bacterial species involved in fire ant biology will be able to be propagated

using standard culturing procedures and media. However, we did find that the gut lining consists of a number of peritrophic membrane layers and bacteria can be seen both between the peritrophic membranes, as well as, in the midgut lumen.

In addition we have developed a method to reduce and eliminate most of the gut bacteria as a first step in elucidating the physiological role of the organisms in the fire ant. The effects of bacterial defaunation on the biology of the colony is currently being assessed.

Additionally, the methods and procedures developed for treating fire ants with antibiotic compounds will allow the testing of single and multiple antibiotic treatments and bacterial re-introduction to determine the importance of specific bacterial species to fire ant survival. This will assess the importance of both the currently characterized 10 species, as well as other as yet uncharacterized species. Surviving bacteria will be identified by the previously described methods, as well as an adaptation of the procedure to enable the identification of as yet un-cultured bacterial species through the use of universal PCR primer combinations.

Objective 3:

Multiple collections of fire ant colony samples have been made from several states for the purposes of characterizing the bacterial species diversity present across a broad geographic range. Ultimately this may also result in the culturing and genetic transformation of additional bacterial species not currently characterized from the local Texas populations. The completion of this portion of the study will be critical for the identification of target bacterial species that are likely to have an impact over a broad geographic range.

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

The next stage in this research project is the use of genetically modified bacterial species to produce molecules that are either directly toxic to the fire ant, or have a detrimental affect on fire ant reproduction, development, or behavior, such that they can be used for laboratory tests to determine their ability to reduce fire ant populations. Successful demonstration at this level would allow the initiation of procedures to enable field-testing of these bacterial strains.

The study of the relationships of bacteria with their host organisms is a burgeoning field of scientific interest, particularly in the social insect domain. Our ability to genetically modify these bacterial strains with a visible, non-destructive marker provides us with an opportunity to follow the distribution and function of these bacteria through and between fire ant colonies, potentially allowing for the successful application for federal funding through the National Science Foundation (NSF) to study insect associated bacteria and symbionts. It is also possible that a private industry sponsor would be interested in the mass-production and distribution of a genetically modified bacterial strain if efficacy against fire ants can be demonstrated through the completion of the proposed research objectives.

Another important aspect of this research proposal is the support of graduate student training as a significant proportion of this research will continue to be performed by Mr. Freder Medina, a Ph.D. candidate in the Department of Entomology. Freder was initially enrolled in a M.S. program, however his progress in the generation of the

preliminary data described in the proposal was such that he was approved for a change of degree plan into the Ph.D. program. There are also numerous opportunities for undergraduate research projects as a component of the proposed research, importantly, combining both field and laboratory, as well as molecular and organismal aspects.

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

1. Publications:

The submission of additional manuscripts has been delayed by the movement of two key personnel associated with this project, Dr. Haisheng Tian moved to take a position interstate and Ms. Haiwen Li relocated to a position in the Department of Biochemistry and Biophysics. It is anticipated that the remaining manuscripts detailing the work to date will be submitted in the next few months.

2. Presentations:

International Invited Symposia:

Vinson, S. B..A new appreciation for symbiotic microorganisms and the wide variety of roles they play in insects may open doors to insect management. In Symposia "Simbionts e seu papel no controle biologico". 9th Simpósio de Controle biológico. 15-19 May Recife, Brazil, 2005.

National: None

Local:.

Medina, F. "FISH (Fluorescence In Situ Hybridization) and its use in ecological studies". OCGN 689. Oral presentation.

Medina, F. Ultrastructure of the red imported fire ant (*Solenopsis invicta* Buren) midgut and associated microorganisms. BIOL 602. Poster.

Medina, F. Ninth Annual Graduate Student Forum 2006, Department of Entomology, Texas A&M University. Oral presentation.

4. Special presentations:

Medina, F. and personel from Dr Coates laboratory conducted a laboratory tour of the IFire Ant Research Laboratory Tour for the Chinese visitors. 4/4/05. Presented an overview of current research.

Signature:

Craig Coates 9-13-06

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