

## Highlights and Impacts of FY '05 Projects Funded by the Texas Imported Fire Ant Research and Management Project

**Project Investigator's Name:** Craig J. Coates & S. Bradleigh Vinson

**Project Title:** Identification of Gene Targets and Delivery Systems for Fire Ant Control

Significant Accomplishment	Impact on Imported Fire Ant Management
Determination of the Vitellogenin Protein Profile in the IFA, demonstrating that Vg1 is ubiquitously expressed during development and in all fire ant castes, whereas Vg2 and Vg3 are specifically deposited into the developing eggs.	This finding is highly significant, as this will enable the specific targeting of Vg 2 and Vg3 as a basis for interfering with IFA reproduction and development with the ultimate goal of population reduction. This finding suggests that a control method could be developed that is specific to the IFA, rather than related non-target ant species.
Genetic Transformation of 3 bacterial species associated with the IFA and re-introduction of the transformed bacteria into fire ant colonies. The transformed bacterial could be transferred between members of the same colony and between colonies.	The 3 bacterial strains were transformed with a fluorescent marker gene, which allowed the presence of the bacteria to be detected in living organisms and to be followed throughout the colony. Importantly, this also allowed a demonstration that the transformed bacteria could be spread throughout and between colonies via normal processes, suggesting that these would be effective agents to spread deleterious gene products that could negatively impact IFA development, reproduction and longevity.
Stable Genetic Transformation using an Integrating Vector. This resulted in the incorporation of the transgenes into the bacterial chromosomes.	This is a critical advance on the previous genetic transformation of the bacterial strains. Genetic transformation via an integrating vector ensures that the transgenes are located on the chromosomes and thus will be permanently incorporated into the bacterial genome, rather than being located on transient plasmids. This will ensure that transgenes designed to negatively impact the IFA will be maintained in the bacteria and in the colonies.
Haisheng Tian, Bradleigh Vinson S, Coates CJ. (2004). Differential gene expression between alate and dealate queens in the red imported fire ant, <i>Solenopsis invicta</i> Buren (Hymenoptera: Formicidae). <i>Insect Biochem Mol Biol.</i> 34:937-49.	First Publication of cDNA library generation for the IFA

<p>Haiwen Li, Freder Medina, S. Bradleigh Vinson and Craig J. Coates (2005). Isolation, Characterization and Molecular Identification of Bacteria from the Red Imported Fire Ant (<i>Solenopsis invicta</i>) Midgut. <i>Journal of Invertebrate Pathology</i>, <i>in press</i>.</p>	<p>First description of bacteria associated with the IFA.</p>
<p>Haiwen Li, Freder Medina, S. Bradleigh Vinson and Craig J. Coates (2005). Genetic Transformation of Midgut Bacteria From The Red Imported Fire Ant (<i>Solenopsis invicta</i>). <i>Current Microbiology</i>, <i>in press</i>.</p>	<p>Genetic Transformation of bacteria associated with the IFA and demonstration of spread within and between colonies.</p>
<p>Haisheng Tian, S. Bradleigh Vinson, Craig J. Coates (2005). The Vitellogenin Genes of the Red Imported Fire Ant, <i>Solenopsis invicta</i>: cDNA cloning, protein expression, and promoter analysis. <i>Submitted to Insect Biochemistry and Molecular Biology</i>.</p>	<p>Description of unique Vg genes in the IFA.</p>
<p>Haiwen Li, Freder Medina, S. Bradleigh Vinson and Craig J. Coates (2005) Stable Transgene Integration of Midgut Bacteria from the Red Imported Fire Ant (<i>Solenopsis invicta</i>). <i>In preparation</i>.</p>	<p>Stable transformation of integrated transgenes into IFA associated bacteria.</p>
<p>Poster Presentation x 1 (Freder etc)</p>	
<p>Poster Presentation x 2 (Freder etc)</p>	
<p><b>Source and Amount of Funds Leveraging Current Fire Ant Project</b></p> <p><b>Agency:</b> NSF  <b>Title:</b> Transcriptional Regulation of Vitellogenin Synthesis in Ants  <b>Funds Requested:</b> \$596,460</p> <p>This proposal was submitted in January of 2005 and reviews were returned in June of 2005. Four of the 5 reviewers were highly supportive of the proposal and it received a ranking of “<b>High Priority</b>” but just missed out on being funded. The project officer advised that some of the broader impacts need to be addressed in more detail and this proposal will be resubmitted for the July 12<sup>th</sup>, 2005 deadline. However, it is critically important that additional preliminary data be generated over the next year (FY 2006) to ensure that this proposal is fully funded within the next 2 NSF funding cycles, ensuring that the resubmission deadlines are made and thus avoiding having to submit a completely new proposal.</p> <p>The work proposed in this NSF project has a direct impact on the study of the IFA to fully understand Vg expression so that these genes can be validated as suitable control targets and furthermore, involves the study of Vg genes in a number of closely and distantly related ant species to ensure that the basis of control would be specific to the IFA.</p>	

**Highlights and Impacts of FY '05 Projects Funded by the Texas Imported Fire Ant Research and Management Project**

**Project Investigator's Name:** S. B. Vinson and Craig Coates

**Project Title:** Identification of Gene Targets and Delivery Systems for Fire Ant Control.

<b>Significant Accomplishment</b>	<b>Impact on Imported Fire Ant Management</b>
<p>A). Determined wing muscle degeneration is an apoptotic event.</p> <p>B). Determined that wing casting following a mating flight is stimulated by JH, but that wing muscle degeneration is initiated by mating.</p> <p>C). Determined that wing muscle degeneration is initiated by mating and that the causative factor is associated with the sperm, even if dead.</p>	<p>A). Allowed us to determine if wing casting, wing muscle degeneration and reproduction initiation is triggered by the same or different events.</p> <p>B). Demonstrated for the first time that wing casting and muscle degeneration are initiated by 2 different factors.</p> <p>C). Provides a bioassay to allow for the isolation and identification of a factor(s) that could prematurely initiate wing muscle degeneration, thus preventing mating flights. <b>GOAL-</b> Inhibit the mating flight and prevent dispersal.</p>
<p>A). Developed a method to produce and separate the various types of eggs (trophic, male, or female), as well as, all male or female larvae and virgin adults for research. This method can also be used to produce colonies with only the production of sterile diploid males.</p>	<p>A). Provided samples to determine if there are different Vg's in the different types of eggs and to determine what may control the production of the different eggs (see Coates report).</p> <p>B). Provided virgin males and females for the wing casting studies above.</p> <p>C). Provide diploid males that may offer a sterile male approach. <b>GOAL-</b> interfere with reproduction and/or fertilization and female production.</p>
<p>A). Provided stages, conditions and tissues used to determine the vitellogenin protein profile (See Coates).</p> <p>B). Collecting and rearing colonies of several fire ant and competitor ant species to determine if other species also produce 3 different Vgs or if the IFA is unique.</p>	<p>A). Additional preliminary results indicate that Vg 2 and Vg3 are more abundant in the head of new founding colony queens just as the first workers are formed. Suggests that some Vg genes in specific tissues in the head are stimulated at this time by some factor related to adult worker eclosion. <b>GOAL-</b> Develop a control that is specific to the IFA.</p>

<p>A). Determined that ant larvae are the protein digesters of the colony and act as a social stomach (see publication -Cassill).</p> <p>B). found that proteins are extra orally digested, but not consumed by the larvae doing the digestion, but filtered by workers before being fed to other larvae and the queen (filtering- reported by Glancey, B. M., R. K. Vander Meer, A. Glover, C. S. Lofgren and S. B. Vinson. 1981. Filtration of microparticles by the red imported fire ant, <i>Solenopsis invicta</i>. Insect. Socio. 28: 395-401) prevents movement of infections (see Thelohania project).</p> <p>B). Determined that the larval stomach is full of bacteria.</p> <p>C). Through the collecting larvae from around the country we have determined (with Coates) that there are certain species that are commonly associated with the IFA.</p> <p>D). Currently we are determining if defaunated IFA colonies can develop without the bacteria.</p>	<p>A+B). As shown by “Sorensen, A. A., T. M. Busch and S. B. Vinson. 1983. Behavior of worker subcastes in the imported fire ant, <i>Solenopsis invicta</i>, in response to proteinaceous food. Physiological Entomol. 8:83-92” proteins go to larvae. We have shown that the larval stomach is the protein digester and suspect that bacteria play a major role in the digestion. <b>GOAL</b> –inhibit digestion (Also may explain why protein inhibitors developed by Edgar Meyer 2002 did not work and may provide a new approach to digestion inhibition.).</p> <p>C). Chagas disease has been greatly reduced as a threat by modifying symbiotic bacteria that kill the disease organism. This approach may provide a new management approach by transforming the bacteria to release physiological triggers that reduce the competitiveness of the IFA or release transgene products that impact the IFA. . <b>GOAL</b>- Reduce IFA competitiveness or trigger physiological processes at appropriate times through the release of triggers by transformed symbiotic bacteria placed in a bait.</p>
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**Source and  
Amount of Funds Leveraging Current Fire Ant Project**

**Grant proposals:**  
See C. Coates report.

**Publications :**

Haisheng Tian, Vinson SB, Coates CJ. (2004). Differential gene expression between alate and dealate queens in the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Insect Biochem Mol Biol. 34:937-49. (First Publication of cDNA library generation for the IFA.)

Haiwen Li, Freder Medina, S. Bradleigh Vinson and Craig J. Coates (2005). Isolation, Characterization and Molecular Identification of Bacteria from the Red Imported Fire Ant (*Solenopsis invicta*) Midgut. Journal of Invertebrate Pathology, *in press*. (First description of bacteria associated with the IFA.)

Haiwen Li, Freder Medina, S. Bradleigh Vinson and Craig J. Coates (2005). Genetic Transformation of Midgut Bacteria From The Red Imported Fire Ant (*Solenopsis invicta*). Current Microbiology, *in press*. (Genetic Transformation of bacteria associated with the IFA and demonstration of spread within and between colonies.)

Haisheng Tian, S. Bradleigh Vinson, Craig J. Coates (2005). The Vitellogenin Genes of

the Red Imported Fire Ant, *Solenopsis invicta*: cDNA cloning, protein expression, and promoter analysis. *Submitted to Insect Biochemistry and Molecular Biology*. (Description of unique Vg genes in the IFA.)

Haiwen Li, Freder Medina, S. Bradleigh Vinson and Craig J. Coates (2005) Stable Transgene Integration of Midgut Bacteria from the Red Imported Fire Ant (*Solenopsis invicta*). *In preparation*. (Stable transformation of integrated transgenes into IFA associated bacteria.).

Cassill, D., J. Butler, S.B. Vinson and D. Wheeler. Cooperation during prey digestion between workers and larvae in the ant, *Pheidole spadonia*. *Insectes Sociaux* (In press)

**Dissertations directed:**

Toghara Azini-Babane Aug. 2004. Initiation of flight muscle histolysis in red imported fire ant (*Solenopsis invicta*). Texas A&M University. 60p. (Directed by S. B. Vinson).

**Presentations:**

Invited Symposium: Vinson, SB. "Invasion of *Solenopsis invicta*, a discussion of some events that appear to be having an impact on local populations in Texas". In; Section 2 "Invasions of social insects" (C. Van der Woude and C. Allen, Organizers). XXII International Congress of Entomology. Brisbane, Queensland, Australia, August 15-21, 2004.

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

Li, H., F. Medina, S. B. Vinson and C. Coates. 2005. Isolation and molecular characterization of bacteria from the red imported fire ant (*Solenopsis invicta*) midgut. **(Poster)** Annual Red Imported Fire Ant Conference, March 22-24, Gulfport MS.

(370) Li, H., F. Medina, C. Coates and S. B. Vinson. 2005. Genetic engineering of midgut bacteria from red imported fire ant (*Solenopsis invicta*). **(Poster)** Annual Red Imported Fire Ant Conference, March 22-24, Gulfport MS.