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

Project Investigator's Name: Richard Deslippe

Project Title: Isolation, identification and synthesis of imported fire ant pheromones with management potential

---

Significant Accomplishment	Impact on Imported Fire Ant Management
1. We realized our major objective of obtaining the full length cDNA sequences of the two main proteins in the venom sac of queens of *Solenopsis invicta*. The sequences were similar to but different from the same sized proteins found in the venom sac of workers. In workers, these proteins are the allergens *Sol i* II and *Sol i* IV, respectively. Differences between the homologous proteins of queens and workers were greater for *Sol i* II than for the *Sol i* IV proteins.	Thus far all of our evidence indicates that at least one of the two proteins for which we have obtained the cDNA sequences is the pheromone that induces workers to kill sexual larvae. The homologue to *Sol i* II is most promising of the two proteins. We are now in the process of expressing both proteins through a vector. Once expressed, we will have sufficient material for examination of structural, chemical and biological properties of the proteins. Because these compounds appear to function in regulation of colony development, they offer great potential to be developed into a new weapon to aid in the management of *S. invicta*.
2. We determined that population growth of transformed bacteria containing the worker cDNA of either *Sol i* II or *Sol i* IV differed from the population growth of transformed bacteria containing the related queen protein cDNAs. The results indicate that the worker allergens are more bactericidal than the related queen proteins. We also determined that immunoblots using antiserum developed against the worker allergens react with the proteins found in the venom sac of workers but not with the proteins found in the venom sac of queens.	The results provide further evidence that the worker and queen proteins serve different functions. If one of the functions of the queen proteins includes regulation of colony development, as suspected, then there are direct implications for fire ant management. The implications include development of a new class of compounds that target only *S. invicta*.
3. As with *S. invicta*, we obtained the full length cDNA sequences for the two major proteins in the venom sac of the related tropical fire ant, *S. geminata*. The sequences were compared to workers of *S. geminata* and both workers and queens of *S. invicta*. The patterns of intraspecific differences were similar to those	The comparison of cDNA sequences with other related species is of interest both from an evolutionary point of view and to aid in determining cross-specificity of the proteins. The more different the proteins, the more likely that development of the proteins for management would specifically

found with *S. invicta*. As with *S. invicta*, differences were substantially greater in comparisons involving *Sol i* II than with *Sol i* IV. For completeness, we intend to obtain the cDNA sequences of the homologous proteins in the poison sacs of both the black imported fire ant, *S. richteri* and the *S. richteri/S. invicta* hybrid with some leveraged funds.	target *S. invicta*, an ideal scenario.
4. We extracted, identified and quantified the cuticular lipids from both larvae and pupae of *S. invicta*. Discriminant function analysis involving the five most abundant hydrocarbons showed separate grouping of different brood components within and among colonies. The separations were highly correlated with *n*-heptacosane and 13-methylheptacosane. However, the hydrocarbon profiles of pupal stages of queens and males were not statistically significant, providing evidence that cuticular hydrocarbons are independent of gender, at least in the pupal stages.	The principal hydrocarbons identified in all developmental stages were previously found in adult workers and in the postpharyngeal gland of mated queen, suggesting that these hydrocarbons are reliable species-specific characters regardless of developmental stage. Further, the results indicate that the hydrocarbon profiles among brood components within colonies are sufficiently different to provide a rich source of chemical information for workers to distinguish among brood. Because workers discriminate among brood components based on chemical profiles, the signals can be manipulated to deceive or be developed into attractants.
5. Bioassays were conducted to determine whether cuticular hydrocarbons play a role in brood recognition. Worker retrieval of pupae immersed in hexane for 5 min was significantly lower than retrieval of control pupae. Retrieval of pupae immersed in boiling water for 3 sec was also significantly lower relative to control pupae. The addition of *n*-docosane, the major cuticular hydrocarbon of pupae, did not increase the proportion of pupae retrieved by workers. Finally, several concentrations of cuticular extracts of sexual prepupae applied to paraffin dummies failed to induce any retrieval by workers.	Although lipid profiles differ among brood components, the results of our bioassays imply that a chemical signal other than cuticular lipids is involved in the brood recognition mechanism. The implication for management of imported fire ants is that the lipids, by themselves, are not sufficient to serve as attractants.

Conference Presentations:

HaghiPour, J., R. Renthal and R.J. Deslippe. 2005. Isolation and characterization of venom proteins of RIFA queens. 2005 Annual Red Imported Fire Ant Meeting. Gulfport, Mississippi. (Presenter: Deslippe).

HaghiPour, J. and R.J. Deslippe. 2004. Proteins in the venom sac of *Solenopsis invicta* queens. Southwestern Branch Meeting of the Entomological Society of America. Lubbock, TX.

Melvin, D. and R.J. Deslippe. 2004. Bacterial induced mortality in the red imported fire ant. Southwestern Branch Meeting of the Entomological Society of America. Lubbock, TX.

Al Diri, I. and R.J. Deslippe. 2004. Compounds extracted from the larvae and pupae of the red imported fire ant. Southwestern Branch Meeting of the Entomological Society of America. Lubbock, TX.

Publications:

a. cDNA sequences:

HaghiPour-Peasley, J.D., R.J. Deslippe, M.J. San Francisco, M. Fokar and R.D. Renthal. 2005. Cloning and sequencing of the cDNA of red imported fire ant queen venom protein, *Sol i II.* GenBank.

Deslippe, R.J., J.D. HaghiPour-Peasley, M.J. San Francisco, M. Fokar and R.D. Renthal. Cloning and sequencing of the cDNA of red imported fire ant queen venom protein, *Sol i* IV. GenBank.

HaghiPour-Peasley, J.D. and R.J. Deslippe. 2005. Cloning and sequencing of the cDNA of tropical fire ant queen venom protein, *Sol g* II. GenBank.

Deslippe, R.J. and J.D. HaghiPour-Peasley. 2005. Cloning and sequencing of the cDNA of tropical fire ant queen venom protein, *Sol g* IV. GenBank

b. Conference Proceedings:

HaghiPour, J.D., R. Renthal and R.J. Deslippe. 2005. Isolation and characterization of venom proteins of RIFA queens. Proceedings of the 2005 Annual Red Imported Fire Ant Conference. (6 pages).

c. Manuscripts Submitted:

Al Diri, I. and R.J. Deslippe. *In review*. Changes in cuticular hydrocarbon composition during development of the fire ant *Solenopsis invicta*. Submitted.

Al Diri, I. and R.J. Deslippe. *In review*. Cuticular hydrocarbons of brood entities within and among field colonies of the fire ant *Solenopsis invicta.* Submitted.

d. Theses and Dissertations:

HaghiPour, J. 2005. Isolation and characterization of venom sac proteins from fire ant queens, *Solenopsis invicta*. M.Sc. Thesis. Texas Tech University. 78 pp. (Deslippe, advisor)

Al Diri, I. 2005. Characterization of the brood cuticular hydrocarbons of the fire ant (*Solenopsis invicta*) and their possible role in brood recognition by workers. 87 pp. (Deslippe, advisor)

e. Manuscripts in Preparation:

R.J. Deslippe, J. HaghiPour and R. Renthal. In preparation. cDNA sequences of queen venom sac proteins of the red imported fire ant, *Solenopsis invicta.*

Source and Amount of Funds Leveraging Current Fire Ant Project:

Proposal Funded:

Deslippe, R.J. 2005. Characterization and Expression of Venom Proteins of Fire Ant Queens. Texas Tech University. Total grant = $2,500

Proposal Not Funded:

Sridhara, S. and R.J. Deslippe. 2004-2006. Juvenile hormone regulation of fire ant behavior and reproduction. United States Department of Agriculture.

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

Project Investigator's Name: Robert Renthal
Project Title: Isolation, identification and synthesis of imported fire ant pheromones with management potential

---

Significant Accomplishment	Impact on Imported Fire Ant Management
1. Tests of Apolipophorin-III (ApoLp-III) as a pheromone-binding protein. ApoLp-III binds to a sub-fraction of lipophorin. It does not bind cuticular hydrocarbons.	Lipophorin is known to carry pheromones in other insects. ApoLp-III will be useful for characterizing fire ant lipophorin, which may result in obtaining pheromones carried in the hemolymph prior to storage and secretion. ApoLp-III is not by itself a good candidate for obtaining fire ant pheromones.
2. Antibody to fire ant apolipophorin-III. We developed an antibody to fire ant ApoLp-III. Immunocytochemistry showed that ApoLp-III is present in the epithelium underlying antennal and head cuticle. ApoLp-III is present in 3rd & 4th instar larvae to varying extents. The highest amounts were observed in larvae found to be carrying high levels of gram positive bacteria.	Levels of ApoLp-III are known to correlate with levels of bacterial infection in some insect larvae. Anti-ApoLp-III may be useful for screening fire ant larvae for pathogenic gram positive bacteria.
3. Expression of a fire ant pheromone-binding protein. We have expressed a His-tagged varient of GP-9, a fire ant pheromone-binding protein, in *E. coli*, and we have purified it using a Ni-NTA column.	Experiments are underway to identify the pheromone carried by GP-9. This pheromone is thought to be involved in determining whether a nest has one queen or multiple queens.
4. Fire ant motion analysis. We have developed a new method of ant motion analysis using the number-average autocorrelation function, which is proportional to the average velocity of a group of ants.	Autocorrelation analysis may permit new bioassays to be devised which can detect subtle pheromone effects on interacting groups of ants.

Source and Amount of Funds Leveraging Current Fire Ant Project

Funded proposal, NIH G12 RR013646: "Genes controlling social behavior in ants." 8/1/2004 to 7/31/2007: $365,573 total direct costs.

Papers and presentations:

Published:

1. R. Renthal, "Discovering pheromones of the red imported fire ant (*Solenopsis invicta* Buren): A review of and proposed new target for pheromone disruption"; Journal of Agricultural and Urban Entomology 20, 113-121 (2004)

2. K.V.P. Guntur, D. Velasquez, L. Chadwell, C.A. Carroll, S.T. Weintraub, J.A. Cassill & R. Renthal, "Apolipophorin-III-like protein expressed in the antenna of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae)"; Archives of Insect Biochemistry and Physiology, 57, 101-110 (2004)

In preparation:

1. R. Renthal, D. Velasquez, D. Olmos, & S.B. Vinson, "Occurrence of antennal glands in ants."

2. S. Younger, A. Haiduk, D. Velasquez, J.A. Cassill & R. Renthal, "Properties of fire ant apolipophorin-III."

3. R. Renthal. "Number autocorrelation analysis of ant motion."

Presentations:

Renthal, R., Velasquez, D., Younger, S. & Cassill, J.A., "Expression and properties of ant apolipophorin-III." 52nd Annual Meeting of the Entomological Society of America, Salt Lake City, UT, Nov. 14-17, 2004

Renthal, R., Younger, S., Velasquez, D. & Cassill, J.A., "Fire ant odor and pheromone reception: a role for lipoproteins?" 2005 Annual Red Imported Fire Ant Conference, Gulfport, MS, Mar. 22-24, 2005.

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

Project Investigator's Name: S. Bradleigh Vinson and Howard Williams

Project Title: Isolation, identification and synthesis of imported fire ant pheromones with management potential.

Significant Accomplishment	Impact on Imported Fire Ant Management
A). We demonstrated that the meconium stimulates egg production by the queen (see reproduction report). We are not trying to isolate and identify the responsible components. We first designed methods to collect both seperatly and then designed an experiment to determine if the factor(s) was due to the liquid or solid part of the excreta (meconium), or both. Feeding experiments suggest that both the liquid and solid portion of the excreta are vital in stimulating egg production and removal of either results in significant steep decline in the number of eggs produced by the queen. Chemical analysis of the liquid is underway. So far the salts have been removed as responsible. We are now working on the proteins.	A). To be able to control reproduction in the IFA would be a powerful tool in the management of the IFA. This is just part of that control, but the identification of reproductive stimulants may lead the way to realizing the goal of shutting down IFA reproduction.
B). Isolation and identification of mating pheromones in the IFA. We also decided to work with both males and females, because it is not clear which sex maybe releasing a long-range attractant. To accomplish this task we have developed several techniques for our laboratory. One is the use of the solid phase absorption of volatiles that can be directly placed in the GC. The second is the development of electro-antennogram equipment (AEG) for ants. Third is the development of a mating flight stimulator in the greenhouse. Forth is the refurbishing and modification of a large flight tunnel. Initially we collected flight queens from at least 10 mating flights at the time of take-off. Volatiles were collected and chemical analysis was performed on the volatiles released. We found 1 unique volatile in queens only at this time period. We have determined the glandular source of this volatile as the venom gland of the queen. Next the isolated volatile was evaluated using the AEG equipment with	B). **A mating pheromone could provide major IFA management opportunities.** One is the development of an attractive lure that could be used to detect new IFA infestations and to monitor IFA populations. They might also be used to disrupt a mating flight, however, the use as a IFA detection tool would be a break-through. But to pheromone has been detected. Pheromones must be involved in the mating flights of the IFA, as the males fly an hour or so before females and the females must find the males and the males must recognize the females. Further, pheromones are involved in mating by other Hymenoptera including some ants. However, no bioassay has been developed (due to the nature of the mating flight with mating occurring at several hundred feet in the air and due to the infrequent occurrence of mating flights). We are taking a different approach, identifying unique volatiles released only

males to determine if this volatile had any effect on males prior to performing detailed chemical analysis and isolation of compound. To our surprise, there was no effect on males of this compound and this prompted the change in the course of pheromone research. We then concentrated on the volatiles released from the males (as described for females). We found 2 unique volatiles in males released during a mating flight. Females do respond to one of them when set up in the EAG (the other is going to be tested soon). The results thus far suggest that males are probably the ones releasing pheromone and females can respond. We are now involved in collecting enough material to identify using males from flights stimulated in the greenhouse.	during a mating flight and confirming their detection by the opposite sex by EAG.
C). The Grass project has identified that sugars are essential to IFA colony growth and the research by J, Martin (being written for publication) also provided evidence that sugar is almost exclusively used by workers as fuel for their activities. Thus, there was interest to know what sugars are most important and if some were attractive. We have isolated and identified the sugars produced by the mealy bug and demonstrated that they make up an important component of the ants diet. We are presently analyzing the sugars of the grasses that are attractive to the IFA and repellent to the IFA. We are also working in identifying the repellents from WW-B Dahl for the Texas Tec project (They can not do bioassays as they f do not have the colonies needed to do the research).	C). In order to use grass in IFA management we need to know why they prefer to nest in some grasses varieties over others. Identifying the role that resources, repellants or attractants play in the ants' choice is the first step in developing a grass management plan to suppress the IFA.
D). Some competitor ants are able to defend their food source from being taken over by the IFA (See Asha publications [Rao, Asha and S. B. Vinson 2004,Environ. Entomol. 33: 587-598]). In the case of *Monomorium minimum*, we found that the venom was very repellant to the IFA. We isolated and identified the venom components. They are repellant, but also an irritant to people. We have trued to alter the molecule to reduce the irritant effect, but not successful. We have another ant that has a repellant from a gland in the thorax, were now trying to isolate and u identify	D). Repellants could be very useful in IFA management, but at present there are no effective ant repellants available, yet other ants use them. We are trying to identify these natural ant repellants with the hope that some may prove useful.

this compound.	
E). We discovered that the glands occur only in 2 segments next to the last segment of the antennae of queen IFAs. If these 2 segments are removed the queen is killed. This suggests that the gland serves some very important function. We analyzed the glands contents but could not find any volatiles. Dr. Renthal tried to isolate possible proteins from the gland and did isolate several antennal proteins, but they do not appear to be specific to the gland. So we are revisiting the gland. We have isolated two small peptides that appear to be different from the ones isolated by Renthal and we plan to work with him in carrying forward with this project as shutting this gland down could led to queen execution	E). If we can identify the antennal gland contents we may have a new approach to IFA management, as gland removal leads to queen execution.
F). We are synthesizing a number of ∃-neuropeptide analogues and evaluating them on IFA as well as some other insects	

Source and
Amount of Funds Leveraging Current Fire Ant Project

Vinson, S. B. and Eusebio Juaristi. 2004. "Insecticidal Agents Based on Neuropeptide Analogs Containing beta-Amino Acids " Funded by Conacyt in 2004. Deals with the synthesis and evaluation of neuropeptide analogues for ant control..

PUBLICATIONS:

Vinson, S. B. and A. Rao. 2004. Inability of *Solenopsis invicta* (Hymenoptera: Formicidae) colonies to establish in a plot with a high density of *Solenopsis (Diplorhoptrum)* colonies. *Environmental Entomology*, 33: 1626-1631.

Rao, A. and S. B. Vinson. 2004. The ability of resident ants to destroy small colonies of red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), *Environmenta Entomology*, 33: 587-598.

In review:

Rao, A. and S. B. Vinson. Behavioral comparison and an analysis of several ant species in Texas. *J. Insect Behavior*.

Submitted:

Rao, A., P. Mokkarala, S. Ellison and S. B. Vinson. Effect of fire ant, *Solenopsis invicta,* baits on non-target ant species. *J. Economic Entomology*.

Katherine Overton, S. B. Vinson, R. E. Gold and A. Rao. Mating flight and nutritional

status of *Solenopsis invicta* (Hymenoptera: Formicidae) alates infected with *Thelohania solenopsae* (Microsporidia: Thelohaniidae).

PRESENTATIONS:

Rao, A Imported fire ant research and management project. As part of the Insect Chemical Ecology Workshop (ICE05), SLU, Alnarp, Sweden. *March 200.:*

Vinson, S. B. ; Rao, A., Mokkarala, P. Effect of baits commonly used in Red imported fire ant control on some resident and non-target ants. Fire Ant Symposium, Annual RIFA conference, Gulfport, Mississippi. March 2005

Vinson, S. B., A. Rao and P. Mokkarala. 2005. Effect of red imported fire ant baits on some of the non-target ants. Annual Red Imported Fire Ant Conference, March 22-24, Gulfport MS.