Texas Imported Fire Ant Research and Management Project

Final Progress Report - October 2001

Biological Control Program: Augmentation biological control of the IFA utilizing native species

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Relevance/Implication of project:
Biological control of the imported fire ant will require the mass production, dissemination, and inoculation of IFA colonies with a number of different parasitic and predatory organisms. These may be imported exotic species or they may be native species. This program was designed to focus on identifying and increasing the effectiveness of the native Biological Control species. Such information should also provide methods that can be applied to some of the exotic species to increase their effectiveness. Two extremely important groups of native organisms we believe are capable of reducing the IFA includes several protozoan diseases, particularly species of microsporidia, and other ant species that we have recently shown to predate on and destroy small IFA. Colonies.

Our research on spore shape and preliminary DNA amplification suggests that there are possibly several microsporidia or other simuliar organisms that attack the IFA, but whether these are different diseases or varieties, and whether one is more effective than another is unknown. Further, previous research indicated that this disease does not readily spread. This limitation could be circumvented through the development of the augmentative approach to biological control (mass production, storage and release). The augmentation approach is also applicable to predatory ants that could be reared and released.

Background Concerning Research Proposed.
The mass production of diseases, parasites and predators depends on knowledge regarding the nutrition of the organisms, acceptability of the prepared diet to the organisms, and the proper conditions for growth and development. Although the basic nutritional needs of most organisms are the same, each has its special preferences. Once the needs and preferences are defined, the mass production of most organisms is possible. Once the biological control organism can be mass-produced, they can be distributed wherever needed, overcoming any problems that the organisms may have had in dispersal or invasion of new host populations. Further, there is the opportunity to make additional improvements in our methods of handling and exposing the IFA to the control agents. To accomplish this goal, we proposed several steps that will provide information that can lead to the biological management of the IFA.

A. Disease organisms, are there more than one? : Microsporidia are small protozoa that are considered good biological agents because they are highly pathogenic and can kill IFA colonies, but little is known about this species. Is there more than one, if more species occour are some species more infective? We have discovered microsporidia in four species of fire ant, (*Solenopsis invicta, S. geminata, S. aurea,* and in a new fire ant species or hybrid). The morphology of the microsporidia differ in a couple of these different ant species, but their identity based on spore morphology is complicated because microsporidia often have alternate hosts and their morphology differs in different hosts. This makes the identification of microsporidia difficult, but because of their specificity, important.

I. Development of techniques to identify the various species on microsporidia.
We proposed to use molecular techniques, such as ribosomal RNA and DNA sequencing, to develop a rapid and precise method to identify microsporidia attacking fire ants. This is essential to species identification, and when perfected will allow us to confirm the presence of infection, allow us to compare the virulence of the different strains, provide a means to identify the presence of any alternate host, and allow us to unravel the biology of the disease. This will also provide a method to evaluate the impact that releases of a particular strain of microsporidia have on the IFA population.

Results
We have developed several the protocols to amplify *Thelohanea solenopsae* DNA sequences and have used this technique to confirm that *T. solenopsae* is infecting some of our ant colonies. We are also developing some additional more viable sequence regions that we hope will allow us to determine if different strains exist. We also have developed a mechanism to begin a state-wide survey to determine the occurrence and distribution of *T. solenopsae*.

II. Determine the effectiveness of native populations of the disease and to provide an insight as to how the disease is spread and the effectiveness of the different strains. (this objective was in cooperation with independently funded T. Cook).
We proposed to begin surveys to determine if *T. solenopsae* was restricted to a few locations or was more wide spread. We also wanted to know if the infection was light, a few spores in a few ants, or did an infection impact most of the individual ants, and was the infection high in the individual ants. Through surveys we initially determined that the infection appeared to be restricted to small pockets that were geographically wide spread. Infection within these pockets can range from 10 to 97% and is primarily found in polygyne colonies.

Results
Our results suggest that the disease is locally spread through the inter-colony activities of the

polygyne ant form. It is likely that the disease occurs in monogyne colonies, but rarely isolated. These data also suggest that dissemination of the disease over long distances involves either a few infected virgin queens or through an intermediate host. We continued to survey and found that the *Thelohanea* appears to occur in pockets and that these are somewhat stable, at least over the period of a few years. However, we also found more pockets of infection. These results suggest that the disease is spreading, but how quickly and to what effect is unknown. We have several projects ongoing that we hope will provide some incite into the movement of the disease. One of these was proposed earlier and is discussed next.

III. We proposed to determine whether virgin alate females become infected, determine if they fly, mate, and lay eggs as a source of infection. If the data does not support these possibilities, we should suspect that an alternate host is involved. To examine this possibility we will examine for the presence of microsporidia in various infected colony associates. This will provide information on how the infection is spread.

Results
This research is ongoing. We have collected alates before and alates that remain in the mound after a flight, as well as flight queens. Some of the flight queens are infected and we are presently examining the queens collected from mounds. These results suggest that flight queens are, at least, one source of disease spread.

IV. Determine the biology of the disease as an important step to mass production. We proposed to develop *in vitro* cell culture techniques that could be used to characterize the developmental stages of the organism and to better understand its essential needs. One aspect was to develop a IFA cell line and this depended on understanding the media in which the cells of the IFA live, ie. the hemolymph. . If successful, we could also use this technique, along with the application of molecular techniques to infected IFA cells in culture to determine the various stages of infection and to elucidate the early development of the organism.

Results
The key to this project was first characterizing the hemolymph, then the development of a cell line, followed by infecting the cell line. We characterized the hemolymph and published the results. This information was used to develop a series of cell lines. While progress was made (see next objective), we could not develop the long term cell lines required. We have been able to generate temporary IFA cell lines that we can pass through several generations, but they are not perpetual. Based on our hemolymph studies we have improved the media and this media is effective in maintaining IFA organs. That has been used in other research studies in other programs in the IFA project to meet objectives in these programs. One of these was to determine the developmental stages of the Phorid fly in collaboration with Dr. L. Gilbert, UT Austin. We have also used the improved media to grow a fungus that is present in some ants. This may be useful intransformation studies that are planned.

V. Develop *in vitro* IFA cell cultures as another important step to mass production.
Using an artificial media we proposed to develop culture techniques that would maintain IFA cells. We next planned to infect these cells with *Thelohanea* or place *Thelohanea* infected cells into our culture media. Through media adjustments we planned to develop mass production methods. The methods are outlined further under the Physiology Program Initiative.

Results
We have continued to develop fire ant all cell lines as a possible way to grow the disease. As

stated above we have cell lines that survive for several months, but as yet we have not been successful in developing a long-term viable cell line. We have also obtained several other cell lines, one from a non-ant hymenopteran that we are evaluating to see if *Thelohanea* will infect these cells. This project is also ongoing.

B. IFA predatory native ants. We have identified six species of ants prevalent in and around older homes, not including the native Fire Ants, that are capable of predating on the IFA and can even eliminate small IFA colonies. These ants, with the exception of *Monomorium pharaonsis*, also occur in relatively undisturbed areas. We proposed to evaluate these ants as potential predators or effective competitor species**.**

I. Determine which common ants are good competitors of the IFA.
The first step was to identify 5 or 6 good competitors of the IFA.

Results
We have demonstrated that a number of ant species are good competitors and several are predators. Each ant species appears to have its own method of attacking and defeating the IFA or in competing with the IFA. We have characterized many of these behaviors. Understanding the mechanisms involved in two of the species has already led to the isolation of a repellent venom that is providing a lead to the development of possible repellent. The venom from a second species of ant appears to be toxic and we are currently isolating the substance and hope to identify this agent as well. Now that we have determined which ants are effective predators of the IFA, we can focus on developing information that can be used to manage the IFA through the mass production, release encouragement, and conservation of these predacious native ant species. This is particularly important to prevent re-invasion.

II. Determine the effect of current and projected IFA pesticides on competitor-predator native ants.
We proposed to utilize laboratory colonies of the six ant species identified as good predators and competitors to determine if any are susceptible to some of the current control products. We will expose these colonies to management products that represent different toxicants and different formulations. As part of this study we will compare the acceptance of oil bait verses a protein bait, both without the toxicant. This will provide information that can be used to reduce any impact these products have on the important native ants.

Results
We have examined the effects of two different bait types, (Each with 2 formulations) on the growth and development of the IFA, as a control, and the introduced pest species, known as Pharaoh ant, as a comparison. We have examined the response of two native ants, *Monomorium* and *Diplorhoptrum*. All baits were acceptable to all 4 species. We found differences in the response of both the IFA and Pharaoh ants to the two different types of bait, however, in the end of the test all colonies treated had died. The final data on both *Monomorium* and *Diplorhoptrum* is still being collected and will be complete in Oct. 2001.

III. Determine and compare the predatory strategies of these six ant species directed towards picking the potentially most important and to document the different strategies employed by each species.
We proposed to determine if different ant species had different stratigies on dealing with the IFA.

Results

We have examined the behavioral interactions of six species of common ants in Central Texas and documented their effects in interacting with small colonies of the IFA. This group includes four native species and two exotic species. Five of the six are predators and readily invade small fire ant colonies. The other is less likely to invade but does appear to restrict the foraging of the fire ant.

We found that each ant species so far investigated have their own way of competing with and/ or killing small colonies of the IFA. For example, *Forelius* does not readily invade the colony, but will attack and kill foraging IFA workers. More importantly the presence of *Forelius* appears to prevent the IFA from foraging when they are near. As a result the IFA is unable to forage effectively and starves. In contrast, *Monomorium* readily invades a nest. They employ a venom which is repellent and appears to stimulate IFA workers to attack other IFA workers from the same nest. This has led to the development of possible IFA repellent that may have other effects. We have demonstrated that two other species are also very effective in eliminating fire ant colonies containing several hundred workers. Additional studies of the other species may yield yet other new ideas that may prove useful to IFA management.

IV. Determine the major factors that stimulate the IFA predatory activities of the predator native ants.
Although some ants are capable of predating on the IFA, the question is how can we manipulate this activity in the field. Although we have evidence that IFA predation occurs, there are a number of factors that must be understood before predatory ants can be effectively managed. Is distance between colonies a factor?, is predation influenced by or dependent on starvation?, is species packing important? The answer to these questions may differ between the IFA and the different predatory ants. Further, whether the two species will interact may depend on the strategies each species employs under the pressure of competition. We plan to examine these parameters in a simulated field in the greenhouse where we can control many of the conditions.

Results
These studies are ongoing. We have found that the interactions are a function of distance and food competition. Within several meter distances, depending on the size of colonies and species involved, we have found that *Monomorium*, for example will eliminate small IFA colonies, but the time increased as a function of distance as well as the difference in colony size between the two species.

V. Develop methods to mass-produce viable competitive predatory native ant colonies.
Some ants can be easily maintained in the laboratory but do not reproduce, while others readily reproduce. Success depends on providing the right conditions for gyne production and to stimulate mating. Mating success may depend on the strategies employed by the ant in question. Since many of the native species have a period of inactivity, we also plan to subject colonies to various conditions and determine environmental effects on reproduction and gyne production as a first step in ant production.

Results
We have initiated rearing of two of the more effective predatory species in the laboratory in an effort to begin to understand the biology directed to rearing colonies of these ants. We want to be able to re-introduce predatory ants back into an area after the IFA has been removed to induce re-infestation by the imported fire ant.