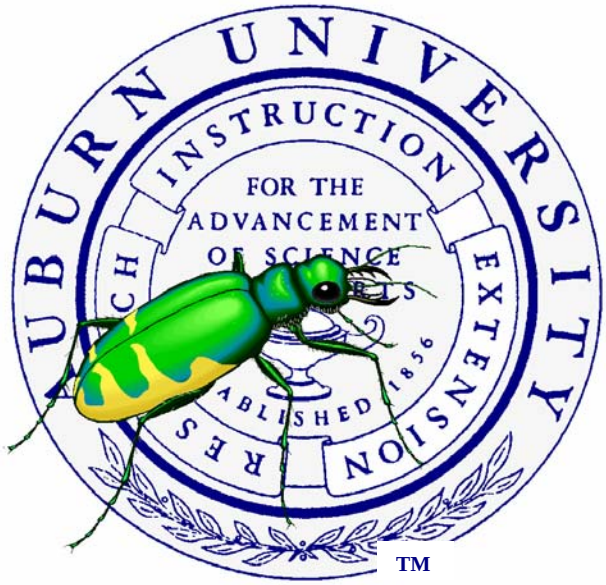
ALABAMA FIRE ANT MANAGEMENT PROJECT PROGRESS REPORT - FY 99*



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THE DEPARTMENT OF ENTOMOLOGY AND PLANT PATHOLOGY AT AUBURN UNIVERSITY

* This report was edited by Fudd Graham and David Gaylor

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Introduction

Imported fire ants now occur throughout the Southeast, and have recently invaded California. More than $12 million is spent annually for control of fire ants in Alabama. Despite this expense, there is general dissatisfaction among the public with current control strategies.

The Alabama Fire Ant Management Project, based at Auburn University, is charged with conducting both research and educational programs on imported fire ants. Beginning in FY 2000, colleagues at Alabama A & M will be joining the project. Our goal is to develop long-term management programs that will make the fire ant easier to live with and to transfer this information to Alabama residents, and others, in a timely manner.

Unfortunately, the biology of the fire ant does not allow the development of an eradication program, such as was successful with the boll weevil. Research studies are designed to develop strategies to reduce populations of fire ants and to maintain these populations at an acceptable level. Educational programs include information on the biology of the fire ant that is needed to properly design a control strategy for a local area.

Our basic biology projects provide insight into the growth, development and spread of the fire ant. Ecological studies will determine the impact of fire ant management on other insects and related organisms in pastures and crops. Four study sites, established in 1997 in cooperation with local Cooperative Extension agents, are used to demonstrate how to get maximum fire ant control with minimum input. One site will be turned over to the local school district once school personnel have been trained to use the integrated pest management tactics developed at the site. Two biological control agents have been released in Talladega and Macon counties in conjunction with the USDA. The decapitating fly has currently reduced mound size and number and has spread into an adjacent control site in a localized area in Macon County.

Our educational programs are intended to increase the general knowledge level about imported fire ants. Various media resources are used to inform the public of our program. Several Extension publications are available from county Extension offices. In October, Alabama Fire Ant Management Program personnel manned a booth at the Alabama National Fair in Montgomery. Faculty, staff and students were available to answer questions. Fire ant educational materials were available for children and adults. Data from research projects are presented each year at professional meetings results are published in professional journals.

This project is funded by the Alabama State Legislature, through the Alabama Department of Agriculture and Industries. Five subprojects were funded in 1999. A progress report is included for each subproject conducted in 1999. In addition, proposals for projects that were funded during the current fiscal year are included in Appendix C.

Introduction

Alabama Fire Ant Management Project Personnel and Cooperators

Auburn University
Department of Entomology
& Plant Pathology

Advisory Committee
M. L. Williams, Ph.D. & Dept. Chair
A. G. Appel, Ph.D.
K. L. Flanders, Ph.D.

Mickey Eubanks, Ph.D.
Nannan Liu, Ph.D.
Kathy McLean, Ph.D.
Gary Mullen, Ph.D.
Fudd Graham
Linda Carter
Zandra Delamar
David Gaylor
Marla Tanley
Xin Yue
Yuping Wei
Guy Shelton
Matt Aubuchon
Amelia Williams
Michelle Perdue
Christa Parrish
Stewart Blackwell
Carol Lawson
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Ken Ward, Ph.D.
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USDA-ARS
Sanford Porter, Ph.D.
David Oi, Ph.D.

Alabama Agricultural Experiment Station

Local Cooperators & County
Rhonda & Albert Childress - Marshall
Mike Duke – Talladega
Henry Easley - Talladega
L. E. Ensminger - Lee
Susan & Tim Gaasch - Macon
Robert Harris - Montgomery
David Hodges – Marshal
Pyron Keener - Montgomery
Greg Myrick - Talladega
George Robertson – Chambers
Sarah & David Spivey - Baldwin
Tuscaloosa County School System
Mike Williams - Macon
David & Doug Wilson - Talladega

Acknowledgements

Thanks to the following people and companies for donating fire ant bait.

Kyle Miller – American Cyanamid
Robert Cartwright – Novartis
Scott Lawson – Novartis
Harry Wisdom – Universal Cooperatives
Joe Chamberlain – Valent

Sincere apologies from the editors to anyone inadvertently omitted from this list.

Educational Materials, Presentations, Publications
See Appendices A & B

Alabama Fire Ant Management Program Publication

The Hidden Truth About Red Imported Fire Ants. Youth activity book reproduced with permission from an Arkansas Cooperative Extension Service publication created by Donna Shanklin and Brian Richardson.

Extension Publications

Flanders, K. L., S. Porter, and D. Oi. 1999. Biological control of imported fire ants. Ala. Coop. Ext. Sys. Circular ANR-1149.

Flanders, K. L., L. Weatherly, and L. Craft. 1999. Getting the most out of your fire ant bait. Ala. Coop. Ext. Sys. Circular ANR-1161.

Flanders, K. L. 1999. New fire ant bait products. Ala. Coop. Ext. Sys. Timely Info. Sheet.

Flanders, K. L. 1999. Stop chasing those fire ants around!. Ala. Coop. Ext. Sys. Timely Info. Sheet.

Television & Radio

ALFA prepared a news release on fire ants at our lab. The release was aired locally during the nightly news on WSFA and WRBL. Others may have used the release.

One radio program appearance.

Magazine & Newspaper Articles

Flanders, K. Nov. 1998. Rosalind, fire ant queen, forage bites. Alabama Cattleman's Magazine. p.47

Flanders, K. Jan. 1999. Tips for making fire ants easier to live with, forage bites. Alabama Cattleman's Magazine. pp. 43-44.

Flanders, K. Feb. 1999. Are fire ants costing you money?, forage bites. Alabama Cattleman's Magazine. pp. 43-44.

Flanders, K. L. Apr. 1999. Fire ant bait most effective control tool. Southeast Farm Press, p.2.

Flanders, K. L. Apr. 1999. Fire ant bait control. AFC Farming News, p. 9.

Six newspaper articles plus information to county agents for local articles.

Local Presentations

Four presentations to local groups.

Two poster presentations at ALFA field days.

Articles in Refereed Journals

Shelton, T. G., J. T. Vogt, A. G. Appel and F. M. Oi. 1999. Observations of *Reticulitermes* spp. in *Solenopsis invicta* mounds (Isoptera: Rhinotermitidae, Hymenoptera: Formicidae). Sociobio. 33: 265-275.

Vogt, J. T. and A. G. Appel. 1999. Discontinuous gas exchange in the fire ant *Solenopsis invicta* Buren: caste differences and temperature effects. J. Insect Physiol. [In Press]

Vogt, J. T. and A. G. Appel. 1999. Standard metabolic rate in the red imported fire ant, *Solenopsis invicta* Buren: effects of temperature, mass, and caste. J. Insect Physiol. 45: 655-666.

Vogt, J. T., A. G. Appel, and M. S. West. 1999. Flight energetics and dispersal capability of the fire ant, *Solenopsis invicta* Buren J. Insect Physiol. [Accepted]

Educational Materials, Presentations, Publications
See Appendices A & B

Published Abstracts

Shelton, T. G., J. T. *Vogt, A. G. Appel and F. M. Oi. 1998. *Solenopsis invicta* Buren mounds as *Reticulitermes* spp. habitats. Proc. Nat'l. Conf. Urban Entomol., San Diego, CA.

Vogt, J. T. and A. G. Appel. 1998. Red imported fire ant (*Solenopsis invicta* Buren) flight energetics. Proc. Nat'l. Conf. Urban Entomol., San Diego, CA.

Non-refereed Journals

Shelton, T. G., J. T. Vogt, and A. G. Appel. 1999. Dangerous liaisons? Native termites inhabiting red imported fire ant mounds. Highlights of Ag. Res. Ala. Ag. Exp. Stn. 46(2): 5-6.

Papers presented at professional meetings

Flanders, K. L., D. H. Oi, F. M. Oi, and P. P. Cobb. 1998. Evaluation of integrated pest management methods for red imported fire ants in Alabama. Poster presented at the annual meeting of the Entomological Society of America, Las Vegas. NV.

Flanders, K., A. Appel, G. Mullen, N. Liu, and L. Graham. 1999. The Alabama fire ant management project. Imported fire ant conference, Charleston, SC. (poster)

Liu, N. and X. Yue. 1999. Role of cytochrome P450 in red imported fire ants. Imported fire ant conference, Charleston, SC.

Porter, S. D., L. A. Nogueira de Sa, K. Flanders, and L.Thompson. 1999. Field releases of the decapitating fly, *Pseudacteon tricuspis.* Imported fire ant conference, Charleston, SC.

Vogt, J. T. and A. G. Appel. 1999. An examination of the flight capability of *Solenopsis invicta* Buren alates. Imported fire ant conference, Charleston, SC.

Vogt, J. T. and A. G. Appel. 1999. The energetic cost of flight in *Solenopsis invicta* Buren, the red imported fire ant. Southeastern Branch Entomological Society of America, Destin, FL.

Progress Reports – FY99

Evaluation of Integrated Pest Management Methods for Red Imported Fire Ants in Alabama

Kathy Flanders, Fudd Graham, Pat Cobb, and Henry Dorough, $55,000,
Third year of funding

Objective 1: **Demonstrate the efficacy and efficiency of targeted broadcast baiting and mound treatments in urban landscapes and rural agricultural operations/home sites.**

Four study sites have been established since 1997 to demonstrate that targeting ants in priority areas can extend suppression of fire ant populations. This strategy requires maintaining low levels of fire ants to kill invading fire ant queens attempting to establish new colonies. These sites are a school in Tuscaloosa Co., a cattle ranch in Montgomery Co., a broiler operation in Marshall Co. and a cemetery in Baldwin Co. The studies are conducted in cooperation with Extension agents Lloyd Weatherly and Larry Craft. Applications of fire ant bait has resulted in a marked reduction in fire ant mounds, but a more demanding criterion for control will be to see how long these reductions last. Maintenance will continue at the cattle ranch and cemetery sites. The poultry site will be dropped, while workers at the Tuscaloosa Co. school site will be trained to continue the program established there. According to the school superintendent, this may be expanded to other county schools.

Objective 2: **Release and evaluate the effectiveness of two fire ant biological control agents: a phorid fly and a microsporidian.**

The first population of the decapitating fly, *Pseudacteon tricuspis*, was released in 1998 in a pasture in Talladega County, in cooperation with county agent Henry Dorough and with Sanford Porter of the USDA-ARS. No captures have been made in Talladega Co. to date. A new population of *Pseudacteon* was released in a Macon Co. pasture in May 1999, with Dr. Porter's assistance. The release was performed over a period of two weeks. Flies have been captured from the second generation in Macon Co. and have been positively identified by Sanford Porter. At least four generations of the fly occurred during the summer and fall.

The microsporidian, *Thelohania solenopsae*, was released in a pasture in Macon Co. in 1998. Fire ant workers have been monitored for presence of *Thelohania* spores and infected workers were found in Aug 1999. Fire ant broods in the infected mound are now being examined for presence of the vegetative stage of *Thelohania*.

Characterization of Arthropod Communities in Fire Ant Managed Areas in Alabama

Gary R. Mullen, and Matt Aubuchon, $6,500, Second year of funding

Field evaluations were conducted to assess the ecological impact of chemical control of the red imported fire ant on the arthropod communities associated with grazed pasturelands. Extinguish7, a bait formulation of S-methoprene, was the commercial product chosen for these studies. The investigation was conducted at two privately owned sites: a 42-acre farm in Lee County and a 40-acre farm in adjacent Chambers County, with a distance of less than 2 miles between them. A randomized complete block design was used, comprised of 6 blocks of treatments with untreated controls, replicated 5 times. All treated and untreated plots were 1 acre in size. Extinguish7 was applied as (a) a perimeter treatment with a band width of 10 feet, and (b) a broadcast treatment, to compare the efficacy of these two methods for managing fire ants. A buffer zone of at least 30 meters was provided between plots to minimize inter-plot effects. Treatments were made June 19 and June 22 at the Lee Co. and Chambers Co. sites, respectively.

Pre-treatment mound counts were made, and mound locations within each plot were mapped using the Global Positioning Systems (GPS) and Arcview 3.0 Geographic Information System software. These data are being used to calculate a USDA Population Index to measure treatment efficacy, and for comparison with post-treatment measurements at 8 weeks and 16 weeks. Aerial photo images of both study sites have been obtained for georeferencing and use in conjunction with mapping data.

Pitfall traps and Berlese substrate samples were taken to monitor ecological effects of the fire-ant bait on other arthropod species within plots. The pre-treatment samples and post-treatment samples at 1, 3 and 6 weeks have been collected to date. These represent 576 pitfall samples and 576 Berlese samples, one-half of the total 2,016 samples that will be collected by the end of the study. The field investigation is at the midpoint. Specimens recovered in the pitfall and Berlese samples are currently being processed.

Because of the amount of time and effort required in laying out field plots and collecting samples at 3-week intervals, there has not been sufficient time to begin taxonomic identification of the arthropods collected thus far. The effort to date has required a graduate student on a 1/3 - time assistantship and the employment of 2 hourly-paid students working a combined 40-45 hours per week to handle the field work and routine processing of samples.

Flight Energetics and Desiccation Tolerance of Red Imported Fire Ant Alates

Arthur G. Appel, $15,000, Third year of funding

A simple energetics model indicates that flight capability of winged fire ants (alates) is limited to <3 mi in the absence of wind. This is the first biologically and physiologically-based estimate of potential dispersal distances in this species. Experiments were conducted to estimate these flight capabilities. These experiments (1) quantified energetic expenditure during fixed flight, (2) characterized metabolic substrate of male and female alates, (3) estimated flight speed of male and female alates, and (4) quantified wingbeat frequency and water loss during flight. Both male and female alates had an increase in metabolic rate during fixed flight, with the females rate being higher than that of the males. Female alates had mean respiratory quotient (RQ) of 0.999, indicating the use of carbohydrates for flight fuel. Mean RQ of males was significantly lower (0.867), indicating the use of lipids for flight fuel. Female flight speed on a circular flight mill averaged ≈2.3 ft/sec, and increased with temperature but decreased with body mass. Male flight speed was 43% greater (≈3.3ft/sec) and increased linearly with temperature and increasing body mass. Female alates lost ≈1.8 mg water/hr during flight.

The discontinuous gas exchange cycle (DGC), the cyclic release of carbon dioxide (CO_2) and uptake of oxygen (O_2), was investigated in workers and sexuals of the fire ant using real-time flow-through respirometry. The DGC type ventilation strategy is thought to be an adaptation to prevent water loss and reduce metabolic costs. CO_2 burst volume of all castes decreased as temperature increased, and increased with body mass. This effect was most pronounced at lower temperatures, a phenomenon consistent with DGC use by insects. All castes of the fire ant displayed a DGC in the temperature range .60-80°F, but only male alates and workers ventilated discontinuously at .85°F. Thus workers and male alates are capable of reducing their water loss at times of high energy use and high temperatures. Frequency of CO_2 emissions increased for all castes as temperature increased.

Technical Support and Development of Educational Materials

Kathy Flanders, Arthur Appel, Nannan Liu, Gary Mullen, and L. C. Graham, $11,500, First year of funding

A booth was rented at the Alabama National Fair in Montgomery to promote the Alabama Fire and Management Project. Over 6000 activity books, *The Hidden Truth About Fire Ants*, were distributed to children at the fair. An attractive display publicized all research, extension and educational aspects of the fire ant program. A mound cast, live fire ants, and live decapitating flies were on display. Other publications on fire ants were available by request and over 1500 were distributed to fair patrons. Alabama Fire Ant Management Project personnel manned the both and were available to answer questions.

Cytochrome P450 Monooxygenases in Red Imported Fire Ants

Dr. Nannan Liu, $11,500, Second year of funding

Our studies provide information for understanding the role of cytochrome P450 in fire ant development and important tools for the study of population genetics and diversity in the fire ant. Cytochrome P450 monooxygenases are enzymes that occur in all types of organisms from bacteria to higher plants to mammals. In insects, cytochrome P450 monooxygenases are involved in the synthesis and degradation of many insect hormones and pheromones, and play an important role in growth and development.

We have successfully cloned and sequenced a cytochrome P450 gene from the red imported fire ant. It has been named the CYP4C gene. This gene is in a new P450 subfamily and is the first P450 gene that has been identified in the fire ant. It has been suggested that cytochrome P450 genes in family 4 function primarily in endogenous metabolic pathways. We can use the cloned CYP4C gene as a genetic marker to investigate the gene flow in fire ant populations.

In order to study population genetics of fire ants and the molecular basis involved in the developmental regulation of fire ants, two specific objectives were addressed in 1999. The first objective was to clone and sequence the cytochrome P450 gene(s) in the fire ant. The second was to study the developmental expression of cytochrome P450 gene(s) in the fire ant. These studies will help us to understand the functional role of P450 in the growth and development of fire ants and provide very important information and tools for the study of population genetics of fire ants and their diversity.

Developmental expression of CYP4C gene in different life stages of fire ant has been studied by northern hybridization analysis using a ^{32}P labeled CYP4C gene fragment as a probe. Studies showed that the CYP4C gene was expressed in larvae, male and female alates, but not in workers and pupae. This suggests that the CYP4C gene is involved in the regulation of fire ant development.

Paper in preparation:

Liu, N., X. Yue, J. T. Vogt, A. Appel, and K. Flanders. In Preparation.. Isolation and characterization of a cytochrome P450 gene in the red imported fire ant, *Solenopsis invicta*. Insect Biochem. Mol. Biol.

APPENDIX A

RESEARCH HIGHLIGHTS

Site Map – Fire Ant Bait Demonstration

Site Map – Characterization of Arthropod Communities

Article – Fire Ants and Termites

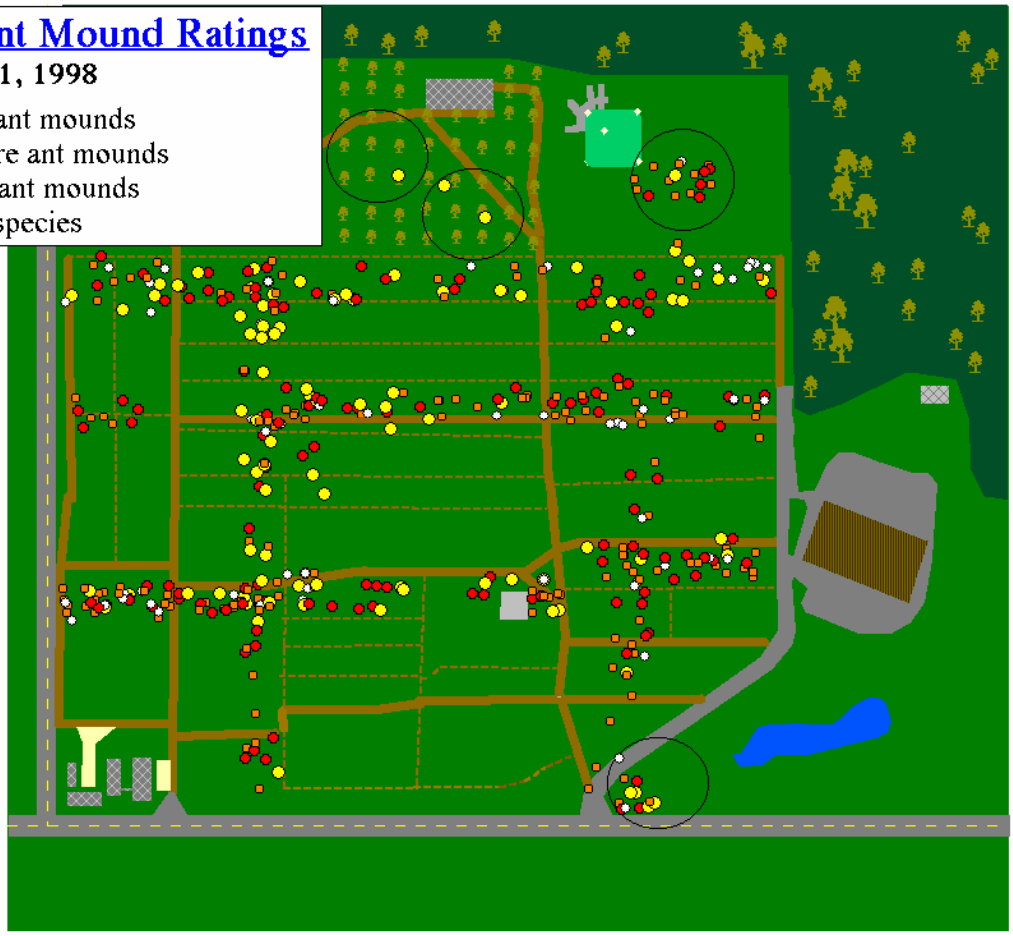
Cytochrome P450 Gene

Baldwin Co. Cemetery Site
Pine Rest Cemetery

1998 Pretreatment Mound Ratings

July 21, 1998

- Small fire ant mounds
- Medium fire ant mounds
- Large fire ant mounds
- Other ant species



1999 Posttreatment Mound Ratings

Sept. 02, 1999

- Small fire ant mounds
- Medium fire ant mounds
- Large fire ant mounds
- Other ant species

APPENDIX C

FY 2000 – CURRENT PROJECTS

Evaluation of Integrated Pest Management Methods for Red Imported Fire Ants in Alabama

Lawrence Graham, Kathy Flanders, Dept. of Entomology & Plant Pathology, Auburn University, Henry Dorough, Alabama Cooperative Extension System, Ken Ward, Alabama A&M University, $45,000, Fourth Year of Funding

Objective 1: Demonstrate the efficacy and efficiency of targeted broadcast baiting and mound treatments in urban landscapes and rural agricultural operations/home sites.

In 1997, study sites were established in areas with economic and/or emotional factors that provided a need and desire to support a long-term management program for imported fire ants. Targeted areas included public recreational areas and agricultural operations: 1) cemeteries, 2) schoolyards and athletic fields, 3) poultry operations, and 4) cattle ranches. A managed treatment plan was developed for all sites. The treatment plan was developed to consider use patterns of the properties in order to target sensitive areas for control and leave unmanaged areas to maintain low levels of natural control. Sensitive areas and unmanaged areas were based on interviews with property owners and the terrain. GPS/GIS was used to map study sites and fire ant locations before and after treatment. The mapping allows for timely spatial documentation of changes in fire ant densities. In 2000, we will turn the school site over to maintenance personnel following an IPM training session and terminate the poultry sites. At the two remaining sites, we will continue the monitoring and treatment program as in past years.

Objective 2: Release and evaluate the effectiveness of three fire ant biological control agents: two species of phorid fly and a microsporidian

Phorid flies will be purchased from the USDA-ARS lab in Gainesville, Florida, and released in Alabama. This will be the third year of this program. Phorids have been sighted, but not collected at the Talladega site established in 1998. At a newly established site in Macon Co., second generation phorids have been collected and identified by Sanford Porter. We plan to release a new phorid species in pastures in Talladega Co., maintain the newly established site in Macon Co. and continue sampling the existing Talladega site. The microsporidian *Thelohania solenopsae* was released in Macon Co. in 1998. We plan an additional site for 2000 in north Alabama in cooperation with Ken Ward, Alabama A&M University. The biological control agents and fire ants will be monitored at all release sites and corresponding control sites. Additionally, non-target impacts of the established phorid populations will be assessed.

Expected outcomes:

Targeted baiting demonstrations will be used to develop recommendations for fire ant control for homeowners, small farms, and public use areas and to demonstrate effectiveness of these recommendations.

With the successful establishment of the biological control agents, fire ant populations will be monitored for spread of the agents, effectiveness of the agents, and hardiness of the agents. This data will be entered into a data base for the southeastern United States to determine if mass rearing and release will be effective.

Proposed projects will be carried out as demonstrations with the participation of county agents in the Alabama Cooperative Extension System. Results will be reported through county meetings, field days, news releases, and fact sheets.

Desiccation Tolerance and Water Budgets of Red Imported Fire Ant Alates

Arthur G. Appel, $16,000, Fourth Year of Funding

Objective 1: Determine the tolerance of alate red imported fire ants to various rates of desiccation.

Desiccation rates of alate red imported fire ants will be determined in still and moving air. In still air, freshly collected alates will be desiccated at 30°C and 0% RH. Mass loss will be measured initially and every other hour for 12 hours and at 24 hours. Water content will be determined by drying at 50°C for 5 days. In moving air, tolerance of alates will be determined by placing preweighed alates into a 10 ml syringe and exposing them to air velocities of 10-500 ml/min regulated by a mass flow controller. Alates will be reweighed hourly and their condition and ability to fly determined. Several air temperatures and relative humidities will be used.

Objective 2: Quantify cuticular and respiratory water loss in alate red imported fire ants.

Cuticular and respiratory water loss will be measured in a flow-through respirometry system. Alates will be attached to one end of a thin wire (as above). The other end of the wire will be inserted into a small ball of modeling clay and positioned inside a 60 ml syringe barrel so that the alate ant is held in the center of the syringe. Dry, carbon dioxide-free air will be drawn through the syringe barrel and across the alate ant. A small magnetic stir bar will be positioned in the syringe and in direct contact with the alate's legs. Cuticular and respiratory water loss of resting alates will be recorded, the stir bar removed, and flight induced. Increased carbon dioxide, decreased oxygen, and increased water vapor concentrations in the air leaving the syringe will be analyzed with a computer controlled Sable Systems TR-3 respirometry system. Temperature in the respirometer chamber will be maintained with a peltier-effect temperature controller and cabinet.

Objective 3: Construct water budgets for alate red imported fire ants.

Water budgets will be based on the percentage of time spent in specific activities such as flight, walking, feeding, and resting; multiplied by the amount of water lost or gained during those behaviors. Metabolic water production will be estimated by determining RQs of male and female alates during flight, walking, and rest and converting RQ-values to μg of water produced during metabolism. Itemized water budgets (i.e., both water influx and efflux pathways) will be constructed.

Expected outcomes:

Our previous studies have indicated that alate red imported fire ants only contain enough energy to fly <5 km in the absence of wind. These studies were necessarily conducted at a single temperature and RH, but do not reflect the range in natural environmental conditions encountered by alates. The proposed studies will provide further quantification of the flight capacity of alate red imported fire ants. This research will result in at least 2 publications and several presentations. Most importantly, however, our results will allow better estimates of the ability of alate red imported ant to disperse via flight.

Characterization of Arthropod Communities in Fire Ant Managed Areas in Alabama

Gary Mullen and Matt Aubuchon, $7,500, Third Year of Funding

Objective 1: Evaluate the effectiveness of perimeter treatments of Extinguish7 (S-methoprene) for control of S. invicta in grazed pasture lands.

Objective 2: Assess the effects of treatment for S. invicta with Extinguish7 on associated arthropod communities

Procedures:

This is a continuation of the field studies initiated in 1999 at two pasture sites in Lee County and Chambers County. These studies are approximately at their midpoints, with population indices at 8 weeks and 16 weeks yet to be conducted, and post-treatment sampling at 9 weeks, 12 weeks, and 15 weeks (totaling 1,008 pitfall and Berlese samples) yet to be done. Based on experience during the first half of this extended field project, the average processing time per sample will take ca. 35 minutes (range 5-120 minutes). Once all of the sorting and labeling of samples is completed, the extremely time-consuming and arduous task of identifying the collected arthropods and tabulating species indices, assessing efficacy of treatments, plotting pre-treatment and post-treatment mound activity in Arcview, etc. begins. In addition to assisting in the field, significant student hourly help will be needed for slide mounting mites and other small arthropods to facilitate taxonomic determinations. Selected arthropod taxa will be indentified which exhibit the best potential as indicator organisms for the development of quantitative ecological indices for use in assessing the environmental impact of fire-ant management practices.

Expected Outcomes:

Results of this project will help to establish: (1) The efficacy of the fire-ant bait Extinguish7 as a perimeter treatment in grazed pastures, compared with broadcast treatment of this product; (2) The ecological effects of the insect growth regulator S-methoprene on ant species other than *S. invicta* and other groups of arthropods (including soil insects and mites) in pasture communities; (3) The effectiveness and practicality of pitfall traps and Berlese samples for monitoring ecological effects of fire-ant treatments in pastures on both beneficial and harmful components of the associated arthropod communities; (4) Identification of index species or groups of arthropods that can be effectively used to monitor ecological effects of fire-ant management programs; and (5) A basis for monitoring both favorable and unfavorable ecological changes that may take place during initiation, long-term maintenance, and/or termination of fire-ant management programs, using a standardized sampling scheme and index species found in the soil-ground layer community.

Implementation of a Fire Ant Management Program in the Tuscaloosa School System

Lloyd Weatherly, Lawrence Graham, and Kathy Flanders, $1,440, First Year of Funding

Objective: Transfer the Tuscaloosa school fire ant management demonstration over to the stakeholders.

During the past three years, we have developed a site specific fire ant management program for three Tuscaloosa schools. The strategy of mapping fire ant mounds to determine priority areas for treatment with a fire ant bait has worked well. After the second year of the program, fire ant mounds were reduced by 90%. The school principals are very pleased with the results. They have received fewer complaints about fire ants, and have not had to spend so much time or money on fire ant control.

Fire ant project personnel have been doing the mapping and the treatment. It is now time to transfer the management of these sites over to the grounds maintenance personnel. We plan to train the maintenance personnel at the three school sites, and to provide them with equipment needed to apply fire ant baits. An all day training session will include principles of fire ant biology, pesticide safety, site mapping techniques, and bait application procedure. A backpack granular bait blower will be purchased and kept at the Tuscaloosa County Office. Maintenance people will be able to borrow the blower to apply the bait as needed. Each school will be expected to provide their own fire ant bait.

Co-PI's Graham and Flanders will conduct the training session. Co-PI Weatherly will help the maintenance personnel to map fire ant mounds and apply the bait.

Expected outcomes:

Improved knowledge of fire ant biology and management by maintenance personnel. Maintenance of excellent level of fire ant control at the sites. Increase interest extending the fire ant management concepts to other school yards and public landscapes in Tuscaloosa Co.

The Ecological and Economic Significance of Fire Ants in Field Crops

Micky D. Eubanks, $16,000, First Year of Funding

Objective 1: What insects are predated by fire ants in field crops?

Use intensive visual observations of foraging workers on corn, cotton, and soybean plants to identify insects that are being attacked or carried by workers (isogenic and transgenic lines of each crop since insect densities are much higher on Bt transgenics). Compare identity of attacked insects with background densities of insects on foliage (from Objective 3) to get a rough estimate of prey choices under field conditions. Conduct choice experiments with fire ant workers and selected prey on caged corn, cotton, and soybean plants to get a better estimate of prey choice by foraging workers.

Objective 2: How many insects are predated by fire ants in field crops?

Visual observations of foraging workers will allow calculation of number of prey captured per hour of observation per acre of crop. Can then calculate total number of prey taken by foraging workers per acre of crop. Can use density of foraging workers (from Objective 3) to calculate number of prey taken per active worker per crop. This will allow relatively quick comparisons of the effect of fire ants across crops when an estimate of fire ant density is available. This estimate is easy to obtain (see Objective 3 below).

Objective 3: Are fire ants of net benefit in field crops?

Take weekly sweep net samples of untreated (control) and treated (with Extinguish) field crops (isogenic and transgenic). Treatment with Extinguish will provide a range of fire ant worker densities from relatively high (untreated) to relatively low (treated). At the same time, use baited test tubes to estimate density of foraging fire ant workers. Correlate density of foraging workers with density of pest insects (caterpillars, tarnished plant bugs, aphids, etc.) and density of beneficial insects (predators such as ladybird beetles, lacewing larvae, etc., as well as parasitoid wasps). Estimate yield of plots and damage from herbivorous insects and correlate with worker and mound densities.

Expected Outcomes:

Fire ant workers will be the most abundant natural enemy in all three field crops. Fire ants will be important predators of many pests, but are also likely to disrupt the efficacy of other important natural enemies (e.g., ladybird beetles and big-eyed bugs).

Development and Dissemination of Materials That Increase the Public's Knowledge of Imported Fire Ant Biology and Management

Kathy Flanders, Lawrence Graham, Arthur Appel and Kathy McLean, $8,650
First Year of Funding

Objective: Develop and disseminate training materials on fire ant biology and management in Alabama

State funding has been provided for research and education directed toward the control of fire ants. This year, the following projects are proposed:

Exhibit at the Alabama National Fair, October, 2000

A display highlighting on-going projects, and providing fire ant control information. Costs will include free standing display system, booth rental, and travel to and from Montgomery.

Fire ant coloring books:

Fire ant coloring books will be disseminated at the fair, and through selected school systems. Costs will include printing costs for 10,000 coloring books and the cost to distribute coloring books in schools.

Fire ant video:

An Arkansas-developed video on fire ant biology and management will be adapted for Alabama, with the assistance of the Alabama Cooperative Extension System Communications Department. Biological control information and specific information for Alabama residents will be added. Videos will be distributed to county extension agents and state legislators. Cost will be for reproduction of 300 videotapes.

Colored fact sheets

Two new fact sheets will be developed with the assistance of the Alabama Cooperative Extension System: 1) Protecting Electrical Equipment from Fire Ant Damage; and 2) How to Quickly Get Rid of a Troublesome Fire Ant Mound.

Regional Publication for Reference Purposes

Two thousand copies of the regional publication "Managing Imported Fire Ants in Urban Areas" will be purchased. This publication will be distributed to county Extension office, legislators, and other key individuals.

County agent in-service training

An in-service training session will be held in Scottsboro, AL on April 5, 2000. Funds requested will cover expenses to bring speakers to the training session.

Expected Outcomes:

Improved knowledge of fire ant biology and management strategies by the public and increased visibility of Alabama Fire Ant Management Program.

Population Genetics, Development, and Reproduction of the Red Imported Fire Ant

Nannan Liu, $12,000, Third Year of Funding

Introduction:

The red imported fire ant arrived in Mobile, Alabama from South America during the 1930's has spread into much of the southeastern United States. Approximately 270 million acres currently infested. However, the population genetics of fire ants and their diversities remains unknown. In our laboratory, cytochrome P450 CYP4C gene has been cloned and sequenced from the red imported fire. It can be used as a genetic marker to investigate the gene flow in fire ant population.

One of the major limitations in the control of fire ants is the lack of understanding the molecular basis involved in the development and reproduction of these pests. Primer pheromones are natural chemicals produced by fire ant queens that control development and reproduction of colony members. Identification and isolation the gene(s) involved in the pheromone regulation in fire ant queens will provide useful information for developing the novel strategies to control the imported fire ant.

Objective 1: Investigate the expression of CYP4C gene in fire ants throughout Alabama.

Fire ants will be sampled throughout Alabama. Total RNAs will be extracted from different samples of fire ants by the acidic guanidine thiocyanate-phenol-chloroform method (Chomczynski and Sacchi 1987). Reverse transcription-Polymerase chain reaction (RT-PCR) will be conducted using a primer based on the CYP4C gene sequence. PCR products will be cloned into PCR^{Tm} 2.1 vectors with a PCR^{Tm} 2.1 Original TA Cloning Kit (Invitrogen) as described by the manufacturer. The sequences of the clones will be verified by automated sequencing (Scott-Ritchey Research Center, Auburn University, Auburn, AL). Sequences of clones will be aligned and compared to the CYP4C gene.

Objective 2: Initiate the study of identifying genes or mRNAs related to pheromone regulation in fire ant queens by differential display.

Fire ant workers and queens will be used in this study, because both are female. Unlike queens, workers are completely sterile, lack functional reproductive organs, and do not produce pheromones.

Total RNA will be extracted from workers and queens. cDNA from samples that are to be compared will be amplified by RT-PCR using a combination of ^{35}S labeled random primer pairs. The denatured RT-PCR products will be separated by electrophoresis on polyacrylamide gel. The gel will be exposed to X-Ray film. Among thousands of amplified fragments, those that correspond to differentially expressed genes will be identified by virtue of differences in signal intensity of radioactivity. The gene(s) that are expressed in queens (including genes involved in pheromone regulation) will be identified. cDNA bands of interest will be cut out from the gel, diffused by boiling, and recovered by ethanol precipitation. cDNA will be amplified by PCR use same primer pair and cloned into the PCR^{TM} 2.1 vectors. Clones will be sequenced, aligned, and compared to the database using a Blast search of GenBank.

Fungal Antagonistics Associated with Imported Fire Ants in Alabama

Kathy Mclean, $3,000, First Year of Funding

Objective 1: The objectives of this research are to identify naturally occurring fungi associated with fire ants in Alabama and to screen potential fungal isolates for possible microbial insecticide activity.

Ants and eggs will be collected from ant hills in the Northern valley, Appalachian Mountain, Eastern Valley, Piedmont Plateau, Upper Plains, Prairie, coastal Plain and Gulf areas of Alabama. Ants and eggs will initially be scanned utilizing dissecting microscope to observe any fungal spores attached to the ants exoskeleton. All ants and eggs will be placed on nutrient mediums utilizing aseptic techniques to culture fungal isolates. All fungal isolates will be increased in liquid media, diluted to a standardized inoculum density and applied to selected fire ant mounds by drenching. Pathogenicity will be determined by number of ant deaths over a period of time.

Expected Outcomes::

The Department of Entomology and Plant Pathology will have a collection of all naturally occurring fungi associated with fire ants and their eggs. Fungal isolates which could potentially be a microbial insecticides will be screened for efficacy of fire ant control.

Coordination and Support for the Alabama Fire Ant Management Program

Michael Williams and Lawrence Graham, $25,200, Second Year of Funding

Objective 1: Provide general assistance in the coordination and implementation of fire ant projects.

Objective 2: Conduct project outreach efforts to improve the public's knowledge of fire ant control strategies, and to promote the efforts of the Alabama Fire Ant Management Program.

Objective 3: Pay for on-going expenses for the fire ant program