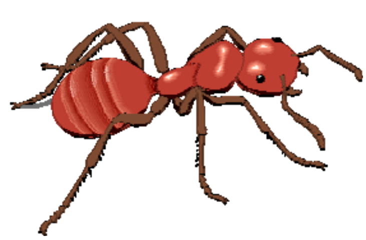
2004 ACCOMPLISHMENT REPORT

SOIL INHABITING PESTS SECTION

U.S. DEPARTMENT OF AGRICULTURE

ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE
CENTER FOR PLANT HEALTH SCIENCE AND TECHNOLOGY
ANALYTICAL AND NATURAL PRODUCTS CHEMISTRY LABORATORY



3505 25TH Ave.
Gulfport, MS 39501

2004 ACCOMPLISHMENT REPORT

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ANALYTICAL AND NATURAL PRODUCTS CHEMISTRY LABORATORY

CENTER FOR PLANT HEALTH SCIENCE AND TECHNOLOGY

PLANT PROTECTION AND QUARANTINE

U.S. DEPARTMENT OF AGRICULTURE

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These reports were prepared for the information of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service personnel, and others interested in imported fire ant control programs. Statements and observations may be based on preliminary or uncompleted experiments; therefore, the data are not ready for publication or public distribution.

Compiled and Edited by:

Anne-Marie A. Callcott

November 2005

Due to many conflicts in 2005, the 2004 IFA/SIPS Accomplishment Report was not completed as normally scheduled. This is an abbreviated version of the work that was completed in calendar year 2004. A complete update of all work that rolled-over into 2005 will be available in the 2005 Accomplishment Report. We expect that report to be available in March-April 2006.

2004 IMPORTED FIRE ANT OBJECTIVES

SOIL INHABITING PESTS LABORATORY
GULFPORT, MS

OBJECTIVE 1: Development and refinement of quarantine treatments for certification of traditional regulated articles.

- Emphasize development of quarantine treatments for field-grown/balled-and-burlapped nursery stock.
- Evaluate candidate toxicants, formulation, and dose rates for various use patterns.
- Test and evaluate candidate pesticides for use on grass sod and containerized nursery stock.
- Assist in registration of all treatments shown to be effective.

OBJECTIVE 2: Development and refinement of quarantine treatments for certification of non-traditional or non-specified articles.

- Emphasis development of treatments for baled hay, straw, pinestraw, and bee equipment.
- Evaluate candidate toxicants, formulation, and dose rates for various use patterns.
- Assist in registration of all treatments shown to be effective.

OBJECTIVE 3: Advancement of technology for population suppression and control.

- New product/formulation testing and evaluation.
- Conduct label expansion studies.
- Evaluation of non-chemical biocontrol agents, including microbial, nematodes, and predaceous arthropods.

OBJECTIVE 4: Development of survey and detection tools and technologies.

- Evaluate efficacy of survey traps
- Evaluate attractants for use in traps determining differences in seasonal preference and efficacy across species/hybrids
- Standardize trapping and survey techniques for regulatory use

OBJECTIVE 5: Technology transfer of all methods developed by laboratory.

- Provide training in quarantine treatments to stakeholders as requested
- Transfer all methods and technologies developed in lab to stakeholders through training, user's guides, web pages, etc.

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MISCELLANEOUS

CPHST PIC NO: A9M01

PROJECT TITLE: Residual Activity of TopPro Specialties/BASF Formulation of Bifenthrin, 2002

REPORT TYPE: Interim

PROJECT LEADER/PARTICIPANTS: Lee McAnally and Shannon James

INTRODUCTION:

The Federal Imported Fire Ant Quarantine Program (7CFR §301.81) states that all regulated products (nursery stock) leaving the quarantined area must be treated in a prescribed manner. Currently, treatments for containerized nursery stock include the use of granular insecticides incorporated into potting media or liquid drenches applied prior to shipping. Nursery stock treated with incorporated insecticides may be certified for 6 months to 2 years, depending on the rate incorporated into the media (10-25 ppm based on bulk density of media). This allows the grower to use less insecticide on nursery stock that will be held on site for a short period of time, and more on those that need a longer growing period prior to selling. Drench treatments are generally used just prior to shipping, and those currently approved for use in the quarantine have certification periods of 10 days to 6 months. Since drench treatments are used just prior to shipping, long residual activity is not a requirement.

Original efficacy trials evaluating bifenthrin for inclusion in the IFA quarantine as both an incorporation and a drench container treatment utilized FMC formulations of bifenthrin. TopPro Specialties, in conjunction with Micro Flo Company began the manufacture of bifenthrin in both granular (0.2%) and liquid flowable (7.9%) formulations around 2002. The granular formulation was produced on two different carriers, sand and DG lite. In August 2002 a study was initiated to determine the efficacy of TopPro bifenthrin. Each formulation was set up in treatment rates equivalent to those specified in the quarantine treatment manual for durations corresponding to the certification periods for each treatment rate.

In 2003, TopPro Specialties returned production of these bifenthrin formulations to BASF.

MATERIALS AND METHODS:

Granular Incorporation Treatment:

On July 31 and August 1, 2002 both formulations (carriers) of TopPro granular bifenthrin were blended into the MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 850 lb/cu yd) at rates of 10, 12, 15, and 25 ppm. A portable cement mixer (2 cu ft capacity) was use to blend the toxicant into the potting media, and was operated for 15 minutes per batch to insure thorough blending. Treated media was then poured into one-gallon capacity plastic nursery pots and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week. At monthly intervals, sub samples were taken from 2 pots of each treatment and composited and subjected to standard alate queen bioassay (Appendix I).

On December 1 and 2, 2003 further testing of the sand carrier granular formulation was initiated in media obtained from Windmill Nursery, Folsom, LA (bulk density 310 pounds per cubic yard) and Flowerwood Nursery, Mobile, AL (bulk density 500 pounds per cubic yard). Methods and materials for mixing, aging, and testing were the same as described above.

Drench Treatment:
Untreated MAFES media was placed in 1-gallon nursery pots and drenched with 400ml finished solution at a rate of 25 ppm. The pots were then placed under the same conditions and tested in the manner described above.

On December 2, 2003 Windmill and Flowerwood media were drench treated in the manner described above.

RESULTS:

MAFES Media:
The drench and the 10 ppm incorporation rates provided 100% mortality in 3 days or less through 6 months (Table 1). This was the planned duration for these treatment rates based on the IFA quarantine certification period, and thus the drench and 10 ppm incorporation rates were terminated after the 6 month evaluation. The incorporated 12 ppm rates with both carrier types were effective through 12 months per the quarantine certification period and were terminated at 12 months. The 15 ppm rates were also 100% effective through the 24 month certification period and were terminated at 24 months. The 25 ppm rate, used in conjunction with the Fire Ant Free Nursery Program, for continuous certification has remained 100% effective through 28 months.

The TopPro Specialties/BASF flowable and granular bifenthrin formulations in the MAFES media are as effective as the FMC formulation indicating acceptability as a product to be used in the IFA quarantine.

Flowerwood and Windmill Media:
Through 6 months post-treatment the 10 ppm incorporation rate in both media types maintained 100% efficacy and were terminated at that time (Table 2). All other incorporation rates have maintained 100% efficacy through 12 months. The Flowerwood drench provided 100% efficacy through 6 months, but the Windmill drench maintained 100% mortality through 4 months and dropped to 95% and 85% in months 5 & 6 respectively.

Slight occasional decreases in efficacy in the Windmill media has been noted in the many efficacy trials; however it does not appear that bifenthrin flowable of any formulation has been tested in this media type before. A second trial will be initiated to verify data collected in the trial above.

Table 1. Residual activity of TopPro bifenthrin in MAFES media.

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)											
		1	2	3	4	5	6	7	8	9	10	11	12
DG lite Carrier	10	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	***	***	***	***	***	***
	12	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)
	15	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)
	25	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)
Sand Carrier	10	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	***	***	***	***	***	***
	12	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)
	15	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)
	25	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)
Drench	25	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	***	***	***	***	***	***
	Check	0	0	0	0	0	0	0	0	0	5	10	5

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)											
		13	14	15	16	17	18	19	20	21	22	23	24
DG lite Carrier	10	***	***	***	***	***	***	***	***	***	***	***	***
	12	***	***	***	***	***	***	***	***	***	***	***	***
	15	100(3)	100(3)	100(1)	100(3)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(2)	100(1)
	25	100(3)	100(3)	100(1)	100(3)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)
Sand Carrier	10	***	***	***	***	***	***	***	***	***	***	***	***
	12	***	***	***	***	***	***	***	***	***	***	***	***
	15	100(3)	100(3)	100(1)	100(3)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)
	25	100(3)	100(3)	100(1)	100(3)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)
Drench	25	***	***	***	***	***	***	***	***	***	***	***	***
	Check	0	0	5	5	0	0	0	0	0	0	0	5

*** terminated based on IFA quarantine certification period

Table 1. cont.

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)							
		25	26	27	28	29	30	31	32
DG Lite	25	100(1)	100(1)	100(1)	100(1)				
Sand	25	100(1)	100(1)	100(1)	100(1)				
	Check	0	0	5	5				

Table 2. Residual activity of TopPro bifenthrin in Flowerwood and Windmill media.

Media Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)											
		1	2	3	4	5	6	7	8	9	10	11	12
Flowerwood	10	100(1)	100(1)	100(1)	100(1)	100(3)	100(1)	***	***	***	***	***	***
	12	100(1)	100(1)	100(1)	100(1)	100(3)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)
	15	100(1)	100(1)	100(1)	100(1)	100(3)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)
	25	100(1)	100(1)	100(1)	100(1)	100(3)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)
	Drench	100(1)	100(1)	100(1)	100(1)	100(3)	100(1)	***	***	***	***	***	***
	Check	0	0	0	0	0	20	0	0	5	0	10	0
Windmill	10	100(1)	100(1)	100(14)	100(7)	100(4)	100(1)	***	***	***	***	***	***
	12	100(1)	100(1)	100(7)	100(8)	100(3)	100(2)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)
	15	100(1)	100(1)	100(1)	100(1)	100(3)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)
	25	100(1)	100(1)	100(1)	100(1)	100(3)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)
	Drench	100(1)	100(1)	100(1)	100(14)	95	85	***	***	***	***	***	***
	Check	0	0	0	5	0	10	0	0	0	0	0	0

CPHST PIC NO: A9M01

PROJECT TITLE: Effectiveness of Permethrin Treated Nursery Pots in Preventing Imported Fire Ant Invasion of Containerized Nursery Stock, 2004

TYPE REPORT: Interim

PROJECT LEADERS: Shannon Wade and Lee McAnally

INTRODUCTION:

Nursery stock and other regulated articles cannot be shipped outside the imported fire ant (IFA) quarantined area unless treated with an approved insecticide (7CFR §301.81) to prevent inadvertent spread of IFA. Several treatment options are approved and registered for this use pattern. Both liquid drenches and granular insecticides (preplant incorporation treatments) are approved for use. The most frequently used treatment is incorporation of granular bifenthrin into the potting media prior to "potting up". The residual activity of the insecticide prevents IFA invasion of containerized nursery stock for up to 24 months, depending upon dose rate employed.

New technologies utilizing insecticides applied to the nursery pot or insecticides impregnated into the plastic of the nursery pot to prevent IFA invasion have been investigated by our laboratory over the past several years. Preliminary work with permethrin impregnated nursery pots showed potential for preventing IFA infestation of small nursery containers (report FA01G038 – 2000 Accomplishment Report), and a large scale trial confirmed the potential of this type of treatment for containerized nursery stock (A9M01/FA01G069 – 2002 Accomplishment Report).

In 2004, Premium Compounded Products changed the way in which it produced the insecticide impregnated containers. Instead of the insecticide being distributed throughout the plastic, there is now a 3-layer system in place for the plastic whereby 3 layers of plastic (inside, middle and outside layers) are molded into the container. Therefore the insecticide can be placed in any or all of the layers. Testing to insure the efficacy of the permethrin impregnated into the plastic of the container in this manner was initiated in 2004. The company is pursuing EPA registration of the impregnated containers.

MATERIALS AND METHODS:

One gallon nursery containers were provided by Premium Compounded Products. These containers were impregnated with 1% permethrin within the three layer plastic system. Three different types of sample pots were provided. One with the outer layer only treated, one with the inner layer only treated, and one with the inner and outer layers treated. Untreated check pots in the same size were also provided by the company. Containers were potted up at the Gulfport, MS lab with the MAFES media (3:1:1 pine bark: sphagnum peat moss: sand) on August 2, 3 and 6, 2004. A portable cement mixer was used to blend the media, and operated for 15 minutes per batch to insure thorough blending. The MAFES media was then poured into the one gallon pots provided and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1 ½ inches of water per week. Samples were taken at 2 week, 1 month, 2 month and every other month after that.

Bioassays were conducted in the laboratory in 2' x 8' test arenas (Figure 1 to right). Sides of the test arena were talced to prevent ants from climbing out and escaping. An permethrin impregnated pot was placed at one end of the arena, and an untreated check container filled with potting media was placed at the distal end of the arena. A field collected IFA colony complete with associated soil and nest tumulus was then placed in the center of the arena. Overhead incandescent light bulbs (60 watts, placed 14" above the test arena) slowly desiccated the nest tumulus so that the ants were encouraged to migrate to the more moist containers. Therefore, the IFA colony had an equal opportunity to move into either a permethrin pot or the untreated check pot. Pots were observed at 24 hour intervals for 7 days after introduction, and the estimated number of worker ants successfully invading each pot was recorded. A pot was considered infested if there were +25 workers inside the pot. There were 3 replicates per sampling interval.

<u>RESULTS</u>:

Through 4 months after potting up the inside and outside treated pots seem to be the only ones keeping fire ants out exclusively. Even at 2 weeks the inside treated only pots had >500 ants living in the pots at the end of the 7 days. A 1 month bioassay was not conducted due to Hurricane Ivan. At 2 months both the inside treated only and the outside treated only had >500 ants living in the pots at the end of the 7 days. By 4 months the inside only and the outside only pots had ants living in them, but had moved out of the treated pots by the end of the 7 days. The inside/outside treated pots are the only ones that have consistently kept fire ants out through the 7 days testing period.

Inside-outside treated   Inside treated only   Untreated

CPHST PIC NO: A9M01 (GPPS00-01)

PROJECT TITLE: Further Testing of Chlorfenapyr as an Imported Fire Ant Quarantine Treatment (2000)

REPORT TYPE: Final

PROJECT LEADER: Lee McAnally

INTRODUCTION:

The Federal Imported Fire Ant Quarantine Program (7CFR §301.81) states that all regulated products (nursery stock) leaving the quarantined area must be treated in a prescribed manner. Currently, treatments for containerized nursery include the use of granular insecticides incorporated into potting media or liquid drenches applied prior to shipping. Nursery stock treated with incorporated insecticides may be certified for 6 months to 2 years, depending on the rate incorporated into the media (10-25 ppm based on bulk density of media). This allows the grower to use less insecticide on nursery stock that will be held on site for a short period of time, and more on those that need a longer growing period prior to selling. Drench treatments are generally used just prior to shipping, and those currently approved for use in the quarantine have certification periods of 10 days to 6 months. Since drench treatments are used just prior to shipping, long residual activity is not a requirement.

Chlorfenapyr is an experimental insecticide-miticide under development by American Cyanamid (Princeton, NJ). BASF acquired American Cyanamid soon after this trial was initiated. The product is active against many pests, and works as a broad spectrum contact and stomach poison. Previously we tested a liquid formulation to determine whether the product showed significant activity against IFA in containerized nursery stock. In August 1997, we began testing a 0.5G granular formulation as an incorporated treatment (FA01G097 – 1999 Accomplishment Report).

In August 1999, we initiated an expanded test of chlorfenapyr using a 2SC liquid formulation as a drench treatment, as well as 1G, 1.5G and 2G formulations each on two different carriers (clay and corn cob grit) as incorporated treatments. All of these treatments were applied to three different potting media (FA01G019 – 2001 Accomplishment Report).

In August 2000, another trial was initiated using the 1G and 1.5G formulations on the grit carrier.

MATERIALS AND METHODS:

Granular treatments included 1% and 1.5% products formulated on a corn cob grit carrier. Each of the granular formulations was blended into the MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 785 lb/cu yd) at rates of 50, 75, 100 and 200 ppm. A portable cement mixer (2 cu ft capacity) was use to blend the toxicant into the potting media, and was operated for 15 minutes per batch to insure thorough blending. Treated media was then poured into one-gallon capacity plastic nursery pots and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week. At monthly intervals, sub samples were taken from 3 pots of each treatment and composited and subjected to standard alate queen bioassay (Appendix I). The 1.0G formulation was mixed on August 28 and the 1.5G formulation was mixed on August 29, 2000.

RESULTS:

All rates are produced 100% mortality in 12 days exposure or less through 30 months post-treatment (Table 1). While these rates are higher than traditional rates of application (25ppm) for long term incorporated quarantine treatments, economics will be the determining factor of whether this will be an acceptable quarantine treatment. Additional testing in other media types will need to be conducted to insure efficacy across media types.

Table 1. Residual activity of chlorfenapyr 1.0G and 1.5G.

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)							
		1	2	3	5	6	7	8	9
1.0G	50	100(6)	100(8)	100(11)	100(10)	100(10)	100(8)	100(13)	100(8)
	75	100(5)	100(7)	100(7)	100(9)	100(7)	100(7)	100(6)	100(7)
	100	100(4)	100(7)	100(8)	100(9)	100(6)	100(7)	100(7)	100(7)
	200	100(3)	100(7)	100(5)	100(6)	100(4)	100(3)	100(8)	100(6)
1.5G	50	100(6)	100(8)	100(11)	100(11)	100(10)	100(9)	100(7)	100(9)
	75	100(6)	100(8)	100(8)	100(11)	100(7)	100(7)	100(7)	100(7)
	100	100(6)	100(4)	100(7)	100(9)	100(7)	100(7)	100(6)	100(7)
	200	100(4)	100(4)	100(5)	100(6)	100(6)	100(7)	100(6)	100(7)
	Check*	15	10	5	5	5	5	30	5

Table 1. cont.

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)											
		10	11	12	13	14	15	17	18	19	20	21	22
1.0G	50	100(10)	100(6)	100(9)	100(11)	100(12)	100(7)	100(9)	100(10)	100(11)	100(11	100(10)	100(10)
	75	100(10)	100(6)	100(8)	100(11)	100(11)	100(7)	100(6)	100(6)	100(10)	100(10)	100(7)	100(7)
	100	100(7)	100(6)	100(7)	100(8)	100(7)	100(7)	100(6)	100(6)	100(6)	100(6)	100(5)	100(5)
	200	100(7)	100(4)	100(7)	100(6)	100(5)	100(4)	100(6)	100(4)	100(6)	100(6)	100(5)	100(5)
1.5G	50	100(10)	100(6)	100(8)	100(8)	100(11)	100(8)	100(8)	100(7)	100(11)	100(10)	100(10)	100(7)
	75	100(7)	100(4)	100(7)	100(7)	100(7)	100(7)	100(6)	100(7)	100(10)	100(10)	100(7)	100(6)
	100	100(6)	100(6)	100(7)	100(7)	100(6)	100(7)	100(6)	100(5)	100(6)	100(6)	100(7)	100(5)
	200	100(10)	100(3)	100(6)	100(5)	100(5)	100(4)	100(6)	100(4)	100(5)	100(5)	100(5)	100(4)
	Check*	10	10	5	10	5	0	0	0	5	0	10	5

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)							
		23	24	25	26	27	28	29	30
1.0G	50	100(7)	100(7)	100(11)	100(10)	100(11)	100(12)	100(12)	100(7)
	75	100(7)	100(6)	100(8)	100(7)	100(10)	100(7)	100(7)	100(6)
	100	100(6)	100(6)	100(8)	100(6)	100(6)	100(6)	100(6)	100(6)
	200	100(5)	100(6)	100(4)	100(5)	100(5)	100(5)	100(5)	100(6)
1.5G	50	100(7)	100(6)	100(9)	100(7)	100(10)	100(12)	100(12)	100(8)
	75	100(6)	100(6)	100(8)	100(7)	100(11)	100(7)	100(7)	100(6)
	100	100(6)	100(6)	100(8)	100(5)	100(11)	100(6)	100(6)	100(6)
	200	100(4)	100(2)	100(4)	100(5)	100(11)	100(5)	100(5)	100(3)
	Check*	10	10	5	10	5	10	0	0

*Check mortality is shown at longest exposure time

CPHST PIC NO: A9M01

PROJECT TITLE: Chemical Degradation of IFA Quarantine Program Insecticides Used for Incorporation into Containerized Nursery Stock Potting Media, 2004

REPORT TYPE: Interim

PROJECT LEADER/PARTICIPANTS: SIPS – Anne-Marie Callcott, Lee McAnally, Jennifer Lamont; Chemist – Joyce James

INTRODUCTION:

For certification in the Federal Imported Fire Ant Quarantine (7CFR 301.81), containerized nursery stock can be treated by incorporating granular insecticide into the potting media prior to potting. Various initial treatment dose rates result in various certification periods (e.g., 12 ppm dose rate of bifenthrin provides 12 months certification). For quality assurance, i.e. to determine whether the nursery properly applied the insecticide to the potting media, PPQ and state inspectors routinely collect media samples which are submitted to laboratories for chemical analysis to determine amount of insecticide present in the media (usually reported in parts per million – ppm). These media samples can be collected from nurseries using this quarantine treatment, as well as from nursery container shipments with suspect or confirmed IFA infestations.

Original trials to determine effective dose rates and certification periods of incorporated insecticides focused on the efficacy of the insecticide on the target insect, and no studies were conducted to determine the chemical degradation of the insecticide in potting media. In late 2004, a series of trials were initiated to determine levels of program chemicals detected by chemical analysis over the certification/aging period of the treated media. The first chemical evaluated was granular bifenthrin incorporated into different potting media. This testing is being done in cooperation with the ANPCL Chemical Analysis section who will conduct the chemical residue analyses. Data collected from these trials will allow the quarantine program to better evaluate results from chemical analyses of samples collected by inspectors.

MATERIALS AND METHODS:

Potting media used in this test were: MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 875 lb/cu yd); Windmill media (Windmill Nursery, Folsom, LA - bulk density = 310 pounds per cubic yard); and Flowerwood media (Flowerwood Nursery, Mobile, AL – bulk density = 500 pounds per cubic yard).

On August 9, 2004, untreated media of each type was submitted to ANPCL-Chemical Analysis section to establish a baseline. On November 9, 2004, treated media from another study (A9P01: Residual Activity of TopPro Specialties/BASF Formulation of Bifenthrin, 2002) was submitted for chemical analysis. Three pots of each media type were composited and four sub samples were submitted for analysis. The initial treatment rate for these samples was 25 ppm. Untreated media for each type was also submitted. The MAFES media had been aged for 27 months prior to first chemical analysis, while the Windmill and Flowerwood media had been aged for 11 months. Further samples will be submitted at 30 & 36 months post-treatment for the MAFES media and 18 & 24 months post-treatment for the Windmill and Flowerwood media. Standard IFA alate female bioassays (Appendix I) are being conducted on these samples as outlined in the project report mentioned above.

On November 9 & 10, 2004 Windmill and MAFES media was treated at the 10 and 25 ppm rate with granular bifenthrin. A portable cement mixer (2 cu ft capacity) was used to blend the toxicant into the potting media, and was operated for 15 minutes per batch to insure thorough blending. Treated media was then placed into one-gallon capacity plastic nursery pots and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week. Samples were taken as described above immediately after mixing and submitted for chemical analysis. Further samples will be submitted at 3 & 6 months post-treatment for the 10 ppm rate and 6, 12, 18 & 24 months post-treatment for the 25 ppm rate. Standard IFA alate female bioassays to determine insect mortality will be conducted on all samples, except the initial samples collected immediately after mixing.

RESULTS:

Results are summarized in the table below. To date, only the first set of samples have been submitted. The aged samples returned readings of about what was expected or higher with 100% mortality. The new MAFES samples were consistent with the theoretical dose rates; however the new Windmill samples were greatly out of line with the theoretical dose rates. This may have been caused by mixing the chemical in extremely dry media thereby causing the chemical to filter out. Further testing will be undertaken to determine the cause of this anomaly. Once these causes are sorted out and corrective procedures developed, further testing of this type will be done with other chemicals in the Federal Imported Fire Ant Quarantine Program.

Soil Type	Treatment Rate	Months Post-treatment	Mean Dry Wt. ppm (n=4)	Standard Deviation	Mean % mortality in queen bioassay
MAFES	25 ppm	27	18.21	1.75	100
Flowerwood	25 ppm	11	9.24	0.65	100
Windmill	25 ppm	11	22.25	1.81	100
MAFES	25 ppm	0	24.47	6.90	--
MAFES	10 ppm	0	9.99	2.66	--
MAFES	Check	0	<0.9*	--	--
Windmill	25 ppm	0	6.72	2.06	--
Windmill	10 ppm	0	5.87	5.13	--
Windmill	Check	0	<0.9*	--	--

* results below detectable limit of 0.9 ppm

CPHST PIC NO: A1M04

PROJECT TITLE: Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – in-field individual tree treatment, 2004 (Winter/Spring)

TYPE REPORT: Final

PROJECT LEADER/PARTICIPANTS: Shannon James, Anne-Marie Callcott, Lee McAnally, and Ron Weeks

COOPERATORS: Jason Oliver[1], Pat Parkman[2], Tahir Rashid[2], Karen Vail[2], and Nadeer Youssef[1].

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INTRODUCTION:

APHIS is responsible for developing treatment methodologies for certification of regulated commodities, such as field grown balled-and-burlapped nursery stock, for compliance with the Federal Imported Fire Ant Quarantine (7CFR 301.81). Current treatments for field grown stock, as described below, are inefficient and limited to a single insecticidal choice. Furthermore, restrictions on this insecticide, chlorpyrifos, within recent years have lead to reduced production consequently limiting its availability to growers and making compliance difficult. Thus additional treatment methods, as well as additional approved insecticides, are needed to insure IFA-free movement of this commodity.

Approximately 84% of Tennessee-grown nursery stock ships outside the Federal IFA Quarantine zone (Brooker et al. 2000). Expansion of the IFA quarantine zone in TN into areas closely associated with many producers of field grown nursery stock has prompted a critical need within this region for development of new treatments for this commodity. The Tennessee Fire Ant Research and Education Team (TFARET), comprised of faculty, students and staff of the Tennessee State University Cooperative Agricultural Research Program and the University of Tennessee Agricultural Experiment Station, are cooperating with the USDA APHIS Soil Inhabiting Pests Section (SIPS) in conducting experiments aimed at expanding treatment options for this commodity.

The currently available pre-harvest (in-field) treatment requires a broadcast application of approved bait followed in 3-5 days by a broadcast application of granular chlorpyrifos. This treatment must extend 10 feet beyond the base of all plants to be certified. After a 30-day exposure period, plants are certified IFA free for 12 weeks. A second application of granular chlorpyrifos extends the certification period for an additional 12 weeks. This method of treatment requires growers to determine which plants to certify more than a month prior to harvest, preventing any later substitutions from untreated blocks. The bait application must be conducted in weather warm enough for fire ants to actively forage on it, while harvest occurs at temperatures cold enough to ensure dormancy of plants, consequently preventing the treatment of new blocks during harvest. The ten-foot radius requirement, due to row spacing, frequently includes plants and soil that otherwise need not be treated. Thus, trials of band-style treatments for large blocks of in-field B&B (reported elsewhere) and individual plant-style treatments for select in-field plants were initiated to focus on examining efficacy of products other than chlorpyrifos, reduction of treated area, and reduction of the exposure time required prior to plant movement.

Individual tree treatment experiments conducted both in Mississippi by the SIPS group and in Tennessee by TFARET focus on treating the immediate surrounding area of nursery stock prior to digging and shipping. Unlike both the current in-field treatment and band treatments, this type of application does not require IFA to forage on bait. Therefore, this would provide an immediate short-term certification (2-6 weeks of quarantine level efficacy) during the harvest season. It would also provide the needed flexibility for growers who ship small numbers of nursery stock outside the quarantined area.

MATERIALS AND METHODS:

Mississippi: Test plots in this trial consisted of individual IFA mounds and the surrounding ground that fit within a 36"diameter circle. This size plot represents the smallest commonly harvested root ball size, 12" diameter, plus a 12" treated buffer zone surrounding the area to be harvested. Mound activity was determined by poking a wire flag in the mound and observing IFA response. Mounds with ten or more ants appearing within ten seconds of mound disturbance were considered active. Wooden stakes labeled with the plot identification number were planted in close proximity to each plot to aid in visually locating the plot and attributing results to the appropriate treatment. Hula-hoops with a 36" diameter were utilized in conjunction with orange spray paint to uniformly mark the treatment areas around each plot. Two trials were conducted in Harrison Co., MS in 2004, one January 22 – May 14 (winter/spring) and the other March 17 - August 3 (spring/summer). For each trial, one hundred twenty active IFA mounds were marked and divided into groups of fifteen replicates for each treatment. The spring applied trial also included an additional four plots per treatment without IFA mounds for use in bioassays and repellency tests.

Liquid treatments were applied using a roller pump-powered 55-gallon spray tank with a garden nozzle set on shower-pattern attached to the tank by a garden hose. Two gallons of the liquid treatments including the water control were applied on each of their respective plots. Preweighed amounts of the granular treatment were packed in individual zip-close bags and sprinkled over their designated plots by hand and then watered in with two gallons of water through the pump system previously described. The treatments in both trials are as follow:

Product	Active Ingredient	Rate of Application
Talstar GC - granular	bifenthrin	0.20 lb a.i./acre
Talstar GC - flowable	bifenthrin	0.20 lb a.i./acre
Scimitar	lambda-cyhalothrin	0.0688 lb a.i./acre
DeltaGard- granular	deltamethrin	0.13 lb a.i./acre
DeltaGard 5SC	deltamethrin	0.13 lb a.i./acre
GardStar	permethrin	27.43 lb a.i./acre
Wet Control	water	Water only
Dry Control	----	No application

Observations of mound activity after application were conducted at two days and then weekly until three months passed. Treatments showing no IFA activity at the three month reading were monitored at four week intervals thereafter until failure of treatment or field conditions prevented locating the plots. Weather conditions prevented observations in the winter/spring trial at five weeks after treatment. Temperature data throughout the duration of the trials was collected using a StowAway® data logger and accessed using BoxCar® 3.6 (Onset Computer Corp., Bourne, MA) and manual readings of air and soil temperatures were taken at the times of observation. A rain gauge located in the pasture was also checked manually.

IFA activity within the test plots, as in previously reported trials, was the primary means to determine treatment effectiveness. However, in an initial effort to verify length of treatment activity, bioassays and infestation preference tests were also set up in the lab. In the winter/spring trial four plots for each of the insecticidal treatments and the wet control were randomly selected for soil collection at two and six weeks. Alate queen bioassays (Appendix I) were set up with a single test cup for each soil sample. Survival of the five alates in each cup was monitored periodically over fourteen days. Wide variation among results within treatments seen in the two week sample bioassays lead to increased attention to maintaining the soil profile in the six week soil sample bioassays. The bioassays for the week six winter/spring trial soil samples and the bioassays for the spring/summer trial had the top 1.5" of the soil samples placed top up in the test cups and the next 1.5" placed top up in a second set of cups. The soil was then pressed firmly to eliminate any gaps between the soil and the test cup. While the bioassays were checked frequently during the fourteen-day test period by examining alate activity on the soil surface, in order to minimize destruction of the soil profile only the final check on day fourteen allowed a thorough search for all alates.

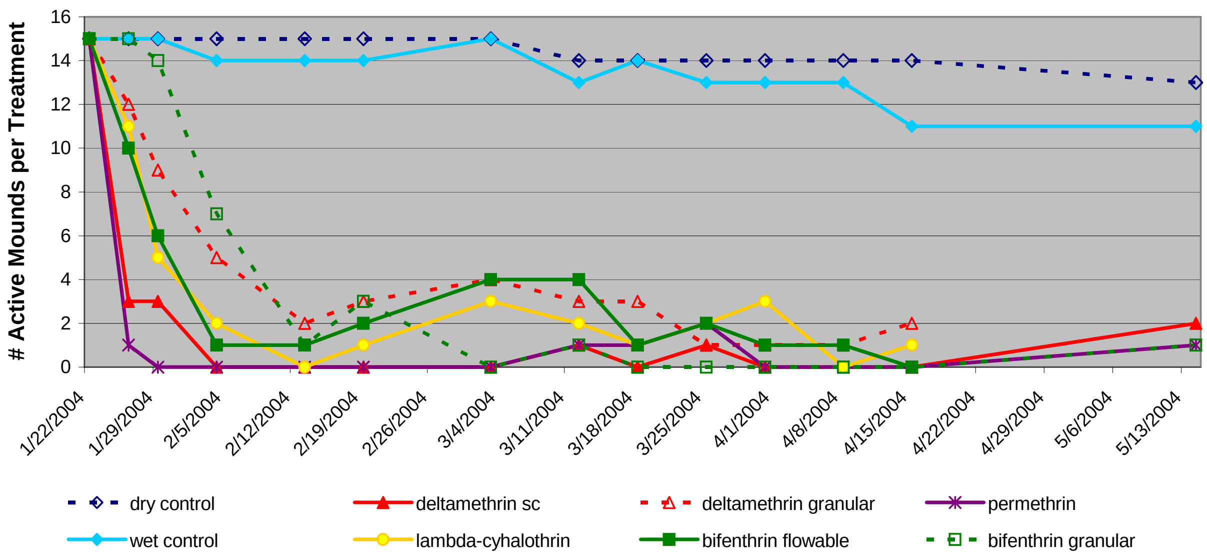
Repellency was examined in the spring/summer trial through colony exposure in a choice test. Field-collected IFA colonies were placed in the center of 2 x 7 ft arenas that had a 6 inch-diameter pot of treated soil and a similar control pot at opposite ends. The top couple inches of soil or sod was harvested from each of the extra treated plots at two days and two and six weeks after treatment for a total of four replicates of each treatment on each sample date. Drain holes in the bottom of the pots required one layer of sod to be placed top down in the bottom and a second placed top up and the soil firmly pressed to the sides to close any gaps, thereby promoting ant contact with treated surfaces. Over a seven day period ant activity was monitored through tapping on the sides of the pots. On the final observation day a thorough investigation of pot colonization was conducted by dumping the pot contents and looking for live ants. Ant activity on the pots was recorded at each observation using the following values: 0 = no activity, 0.5 = some ants, 1 = majority of the colony.

Tennessee: Members of the TFARET used three sites in Franklin Co., TN to replicate field testing on February 20, 2004. Two sites had three plots per treatment and the third site had eleven plots per treatment. Plot selection, set up, and treatments were as described in the Mississippi trials. Applications were conducted similar to Mississippi, with the exception that liquids were applied using 2-gallon watering cans. Sites were observed weekly through May 14, 2004 with the exception of weeks 1, 5, 7, and 12 for one of the three-plots-per-treatment sites. Weather data was collected by data logger through the trial's end.

<u>RESULTS</u>:

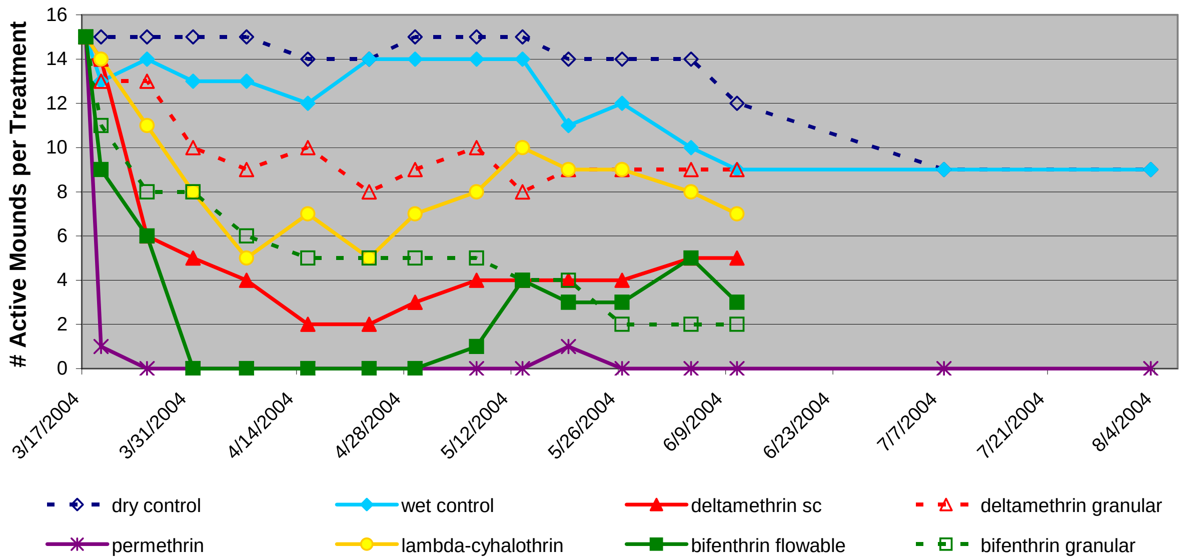
Mississippi (Winter/Spring) – Field Plots: All chemical treatments reached two or less active mounds by three weeks (Figure 1). Permethrin and liquid deltamethrin reached zero active infestations at weeks one and two, respectively, and sustained apparent control through six weeks after treatment. While all other treatments compared well against the controls, none of these can be considered to have sustained quarantine level control prior to a month post treatment.

Figure 1. Cumulative active imported fire ant mounds by treatment for field plots treated in Harrison Co., MS on January 22, 2004.



Mississippi (Spring/Summer) – Field Plots: Permethrin and liquid bifenthrin reached zero active infestations at weeks one and two, respectively, and bifenthrin sustained apparent control through six weeks after treatment while reinfestation apparently did not occur through the duration of the trial with permethrin (Figure 2). All other treatments did not reach quarantine level control.

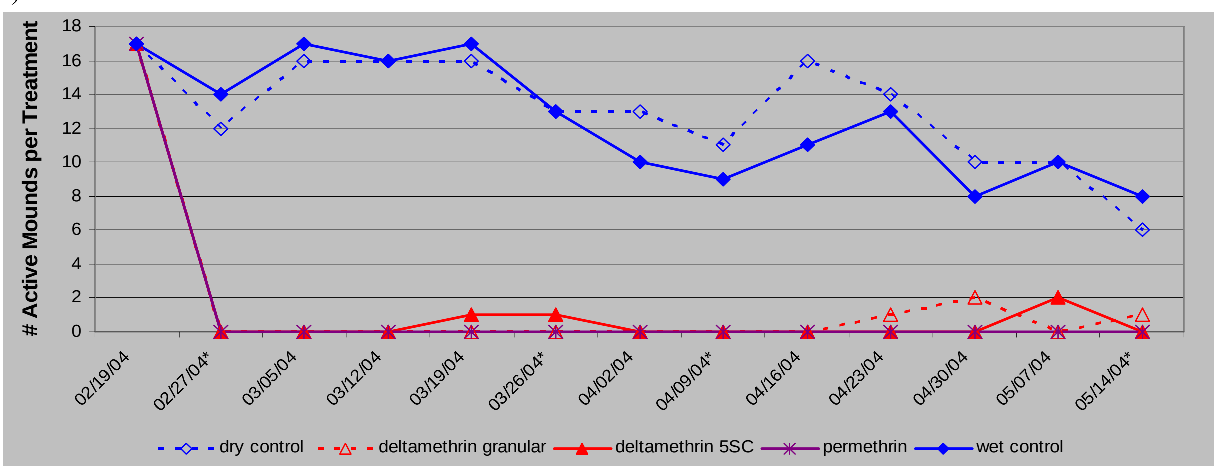
Figure 2. Cumulative active imported fire ant mounds by treatment for field plots treated in Harrison Co., MS on March 17, 2004.



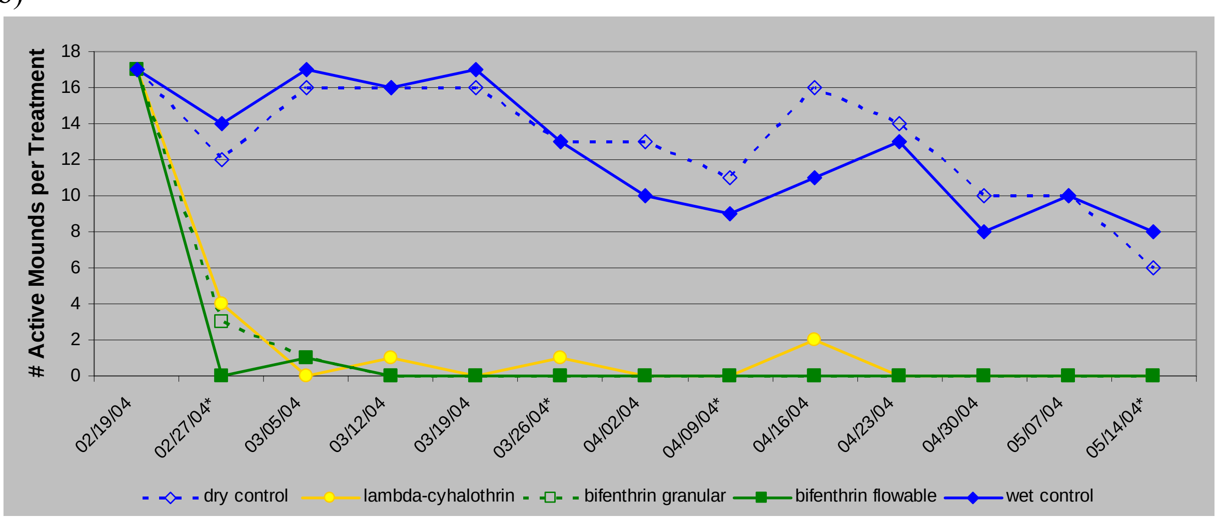
Tennessee – Field Plots: Two weeks after application all chemical treatments were down to levels of one or no active mounds, while both control treatments maintained sixteen and seventeen active mounds (Figures 3a & 3b). Permethrin (Dragnet) reached the point of zero IFA activity at one week post-treatment and maintained apparent control through the remainder of the trial. Similarly both bifenthrin treatments exhibited apparent control of IFA through the duration of the trial after reaching a sustained zero-active-mound point at three weeks. Both deltamethrin treatments reached sustained zero activity by one week. Deltamethrin granular controlled IFA in its plots through the eighth week, while there was intermittent activity in weeks 4, 5, and 11 among plots treated with the liquid formulation. Positive readings for plots treated with liquid deltamethrin were isolated and did not repeat at any point through out the remainder of the trial. The majority of the lambda-cyhalothrin plots exhibited control of IFA from week two through the end of the trial, but sustained quarantine level control for the treatment as a whole was not achieved within a month.

Figure 3a & 3b. Cumulative active imported fire ant mounds by treatment for field plots treated in Franklin Co., TN on February 20, 2004. Observation dates with an asterisk did not have data from the Pelham site giving these dates only fourteen plots per treatment instead of seventeen.

a)



b)



Mississippi – Bioassays: The results from soil samples split into top and bottom halves indicate significantly more chemical activity is available in the first 1.5 inches of treated soil (Figures 4 and 5), validating the importance of depth of soil profile in these tests. All top soil samples for lambda-cyhalothrin, deltamethrin granular, and permethrin and three of the four bifenthrin flowable soil samples taken at two days post treatment provided 100% mortality, while none of the second layer samples reached this point. Several of the top layer winter samples at six weeks produced higher mortality than the corresponding spring samples. Different weathering or some other factor between the two trials may have produced this effect, but the winter samples at two weeks can neither support nor refute this due to their composited soil layers.

Figure 4. Female alate bioassay mortality for top layer soil samples collected from Harrison Co., MS. Soil from the winter test collected on day 14 was composited, but its results are displayed for reference.

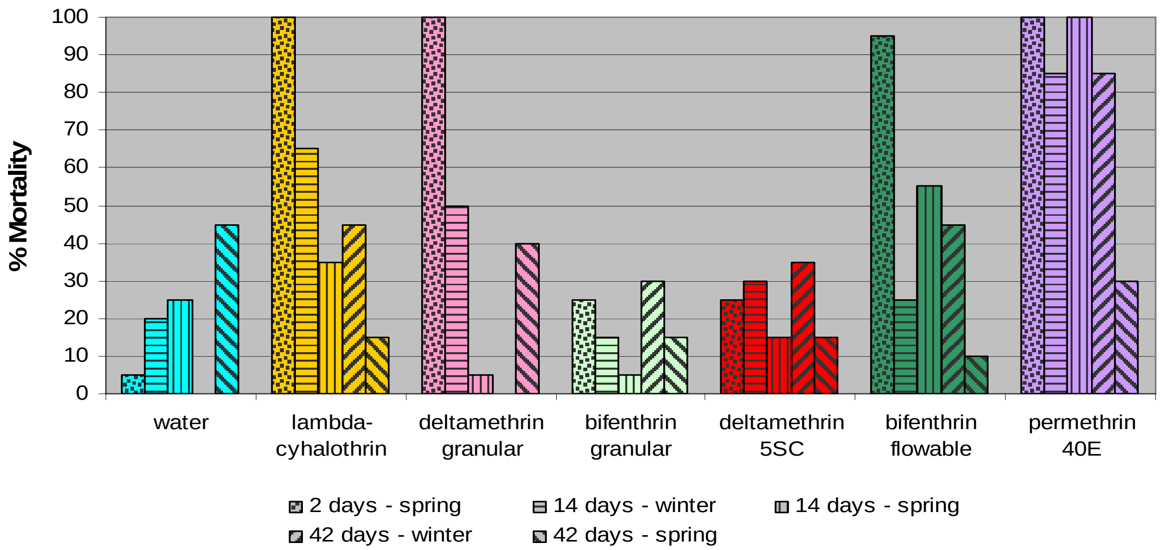
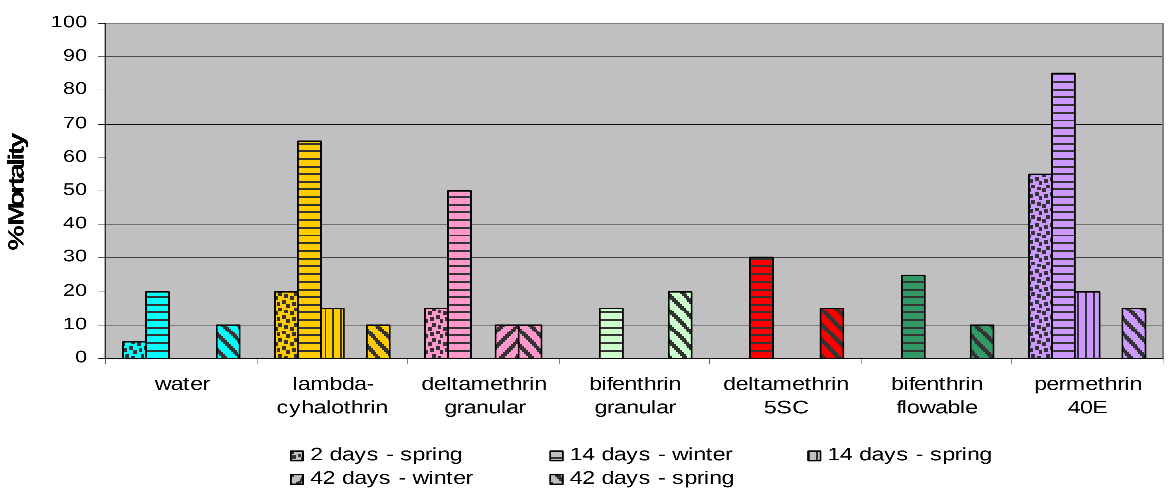
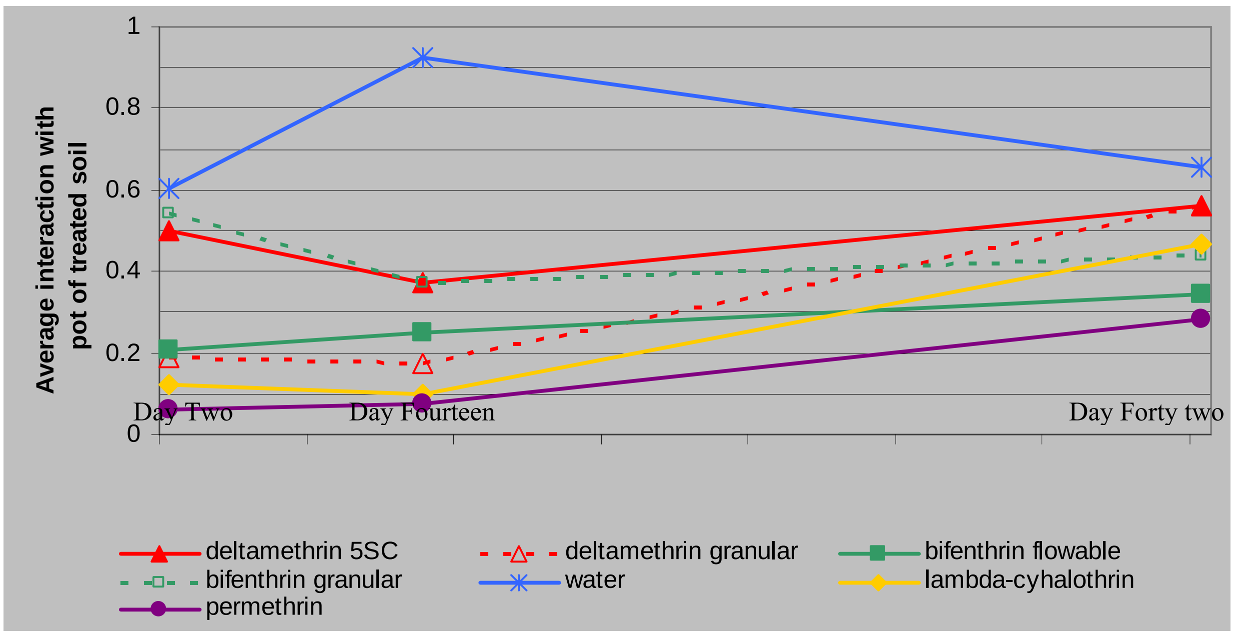


Figure 5. Female alate bioassay mortality for bottom layer soil samples collected from Harrison Co., MS. Soil from the winter test collected on day 14 was composited, but its results are displayed for reference.



Mississippi – Choice tests: All treatments at each date had at least one observation during the 7-day observation period with IFA activity (Figure 6). Permethrin, lambda-cyhalothrin, bifenthrin flowable, and deltamethrin granular each had one or more replicates from both day two and day fourteen samples with no ant activity. Ant activity on permethrin samples collected on day two and fourteen did not extend to the last observation day for either. Similarly on all three sample dates three out of the four bifenthrin flowable treated pots did not exhibit IFA activity on the final observation day. Low interaction scores and lack of ant activity on the final observation day indicate possible repellency of some treatments, but several of the samples in these treatments are noted as having dead ants under or scattered around the treated pots. Furthermore, the treatments with low interaction scores match those with high or 100% mortality in the bioassays.

Figure 6. Average scaled interaction for IFA colony fragments confined with soil samples collected from treatments applied on March 17, 2004 in Harrison Co., MS.



DISCUSSION:

Data from these single mound plot trials will be used in conjunction with those from band trials to determine new treatment regimes that meet quarantine standards and at least some of the goals mentioned earlier. Over the two years of tests by both groups, several insecticides, most notably deltamethrin and bifenthrin, are showing promise in this use pattern. Liquid formulations of both these insecticides in at least three trials have demonstrated ability to remove IFA infestations within four weeks or less after treatment and had no recurring infestation for at least a month. However, further field testing is required to determine if consistent results can be achieved at these rates or if further adjustment is warranted. Permethrin consistently rendered all its plots fire ant free by one week after treatment and maintained this status for more than a month. The rate used however was quite high and the maximum amount of active ingredient per acre per year would severely restrict ability to apply this treatment. Further testing with this insecticide will examine efficacy at lower rates. Granular formulations have worked better in TN than in MS trials. Based on differences in rainfall and speed of action in the two trial sites last year, granular treatments were watered in this year. This change did not improve the results for granular treatments in MS. Applications conducted in January generally had a quicker and more drastic impact than those in March. This is not unexpected as increased resources and temperatures in spring should increase colony vigor and decrease length of chemical exposure.

Numerous formulations of common insecticides are labeled for spot treatment of imported fire ant colonies. Imported fire ant colonies readily respond to insecticide applications made directly to the nest by relocating the colony (Collins & Callcott 1995). This insecticide-induced movement is usually over a relatively short distance (1.5 to 3.0 meters), but can be greater (AMC, personal observation). The primary objective of a quarantine treatment for field grown nursery stock is to render the plants fire ant free. Therefore, it does not matter if colonies are killed outright by the treatment or simply induced to move away from the area around each individual plant intended for harvest; however, the treatment must be proven to perform in at least one of these capacities.

Between the field plots and the laboratory tests only the repellency test for bifenthrin flowable appears to match its apparent duration in the field. Minimal interaction was observed with no interaction on the final observation day for three of the four replicates through week six, and week six was the final field reading for this treatment with no ant activity. This supports repellency results obtained by Oi and Williams (1996) in a study using pots containing bifenthrin incorporated soil. Permethrin was uniformly fatal in both lab and field tests through the second week, but the six week lab tests indicated the treatment no longer killed nor repelled while the field plots maintained lack of IFA activity. Deltamethrin granular and lambda-cyhalothrin had 100% mortality in their day-two-sample bioassays, but in the field eight and five mounds, respectively, remained active. Conversely, the field performance for both bifenthrin granular and deltamethrin 5SC indicated that the lab tests should have produced more favorable results. Handling and transit caused some lose of integrity of the soil profile for some samples prior to use in bioassays and the larger samples for the repellency tests were of uneven thickness. Switching to treated sod kept at the laboratory should remedy these problems in subsequent tests.

<u>References Cited</u>:

Brooker, J., R. Hinson, and S. Turner. 2000. Trade flows and marketing practices in the United States nursery industry: 1998. Southern cooperative series bulletin 397. University of Tennessee Agricultural Experiment Station. Knoxville.

Collins, H.L. and A-M. Callcott. 1995. Effectiveness of spot insecticide treatments on red imported fire ant control. J. Entomol. Sci. 30: 489-496.

Oi, D. H. and D. F. Collins. 1996. Toxicity and repellency of potting soil treated with bifenthrin and tefluthrin to red imported fire ants (Hymenoptera: Formicidae). J. Econ. Entomol. 89(6): 1526-1530.

CPHST PIC NO: A1M04

PROJECT TITLE: Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, fall 2003/spring 2004

TYPE REPORT: Final

PROJECT LEADER/PARTICIPANTS: Shannon James, Lee McAnally, Bob Jones, Shannon Wade, Ron Weeks, Anne-Marie Callcott

INTRODUCTION:

APHIS is responsible for developing treatment methodologies for certification of regulated commodities, such as field grown balled-and-burlapped nursery stock, for compliance with the Federal Imported Fire Ant Quarantine (7CFR 301.81). Current treatments for field grown stock, as described below, are inefficient and limited to a single insecticidal choice. Furthermore, restrictions on this insecticide, chlorpyrifos, within recent years have lead to reduced production consequently limiting its availability to growers and making compliance difficult. Thus additional treatment methods, as well as additional approved insecticides, are needed to insure IFA-free movement of this commodity.

The currently available pre-harvest (in-field) treatment requires a broadcast application of approved bait followed in 3-5 days by a broadcast application of granular chlorpyrifos. This treatment must extend 10 feet beyond the base of all plants to be certified. After a 30-day exposure period, plants are certified IFA free for 12 weeks. A second application of granular chlorpyrifos extends the certification period for an additional 12 weeks. The ten-foot radius requirement, due to row spacing, frequently includes plants and soil that otherwise need not be treated. Numerous common insecticides such as diazinon, chlorpyrifos, acephate, and others are labeled for spot treatment of imported fire ant colonies. Imported fire ant colonies readily respond to insecticide applications made directly to the nest by relocating the colony (Collins & Callcott 1995, Hays et al. 1982, Franke 1983, Williams & Lofgren 1983). The primary objective of a quarantine treatment for field grown nursery stock is to render the plants fire ant free. Therefore, it does not matter if colonies are killed outright by the treatment or simply induced to move away from the area around plants intended for harvest. Thus, trials of band-style treatments for large blocks of in-field B&B and individual plant-style treatments for select in-field plants were initiated to focus on examining efficacy of products other than chlorpyrifos, reduction of treated diameter, and reduction of the exposure time required prior to plant movement.

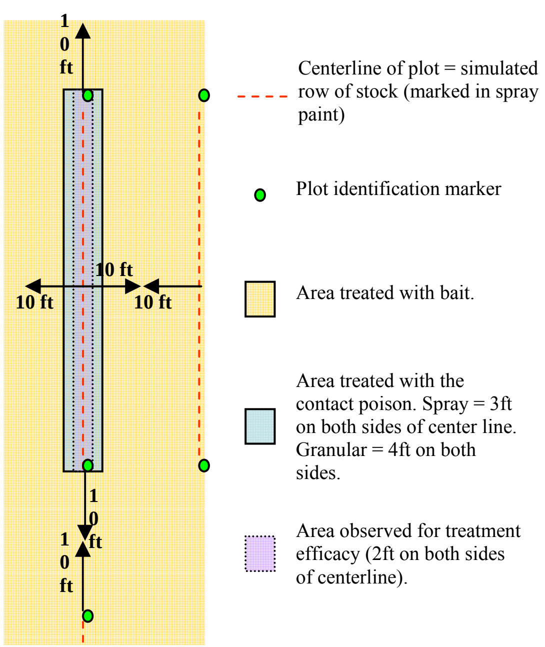
Preliminary testing initiated in Sept. 2001 assessed several liquid and granular insecticides against individual IFA mounds in the field. Results of this trial indicated promising results with acephate, bifenthrin, and deltamethrin (see GPPS01-02). Tests against individual mounds, detailed elsewhere in the 2004 Accomplishment Report, continue to provide direction for treatments utilized in the larger scale band treatments. The first two band trials applied in the fall of 2001 and spring of 2002 tested five to six-foot wide bands of bifenthrin and deltamethrin.

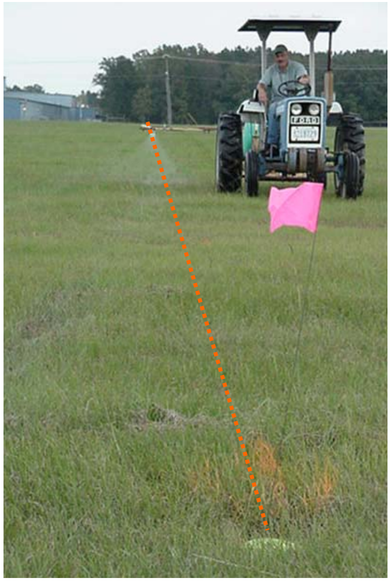
Both liquid and granular formulations showed promising results (see GPPS02-01; GPPS02-02), but showed that in band treatments contact insecticide alone was not effective enough for use in the IFA quarantine. Subsequent band trials, including the two detailed in this report, include broadcast application of bait 3-5 days prior to the contact insecticide application. The inclusion of bait in the treatment procedure has facilitated quarantine level control for several contact insecticides in these trials (Project No: A1P04; Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, Fall 2002 and Spring 2003). The trials in this report continue to explore alternative insecticides and provide supporting data for those performing well previously.

<u>MATERIALS AND METHODS</u>:

Fall 2003 Band Trial:
Bryan airport in Starkville, MS (Oktibbeha Co.) was selected as the test location for the fall trial. B&B is harvested in cold weather when trees are dormant, so it was important to initiate testing in colder weather than that found in south Mississippi, the location of the previous trials. Plots were 800-foot long strips of land containing at least five active fire ant mounds within a 4-foot wide (two feet on both sides of a center line) observation strip that ran the length of the band (Figure 1). To provide a buffer zone between plots, plot center lines, which simulated rows of plant stock, were set a minimum of twenty feet apart side to side and end to end. Pramitol®, used as an herbicide, was sprinkled at the end points of each plot, and a short wooden stake with a marker plate indicating the plot number tacked to its top was driven into the ground at each end to serve as somewhat permanent plot markers. Fluorescent orange spray paint marked the center line and was repainted as needed.

Figure 1. Plot arrangement diagram Figure 2. Application of contact insecticide to an 800 foot-long plot of simulated stock





A shop built spreader calibrated to apply 1.5 lbs/acre and mounted to a farm tractor was utilized to broadcast hydramethylnon bait across the plots designated for chemical treatment on November 4, 2003. Control plots were not treated with bait. Contact insecticide application occurred the following week on the 12th. Granular treatments were applied using a Gandy 48" granular drop spreader attached to a farm tractor. Liquid treatments were applied using a roller pump boom sprayer equipped with two standard flat spray tips (8015-SS; TeeJet Corp.) to provide a 36" band spray and a total spray volume equivalent to ca. 76 gal/acre (Figure 2). Treatments were applied on both sides of the centerline producing a band size, depending on formulation used, either 800'x 8' or 800'x 6' in each plot. The more northern location proved difficult in locating sufficient numbers of mounds per plot, thus the test consisted of the following four treatments and a control.

Chemical	Formulation	Rate of Application
bifenthrin	granular 0.2%	200 lb/acre (0.4 lb ai/acre)
bifenthrin	flowable 7.9%	40 oz/acre (0.2 lb ai/acre)
chlorpyrifos	granular 2.32%	260 lb/acre (6 lb ai/acre)
chlorpyrifos	emulsifiable 44.8%	32 oz/acre (1 lb ai/acre)

Active IFA colonies in each plot's observation area were recorded prior to bait application and after contact insecticide application at 2, 3, 4, 6, and 8 weeks and every four weeks thereafter until reinfestation occurred or six months passed. Mounds were evaluated using as little disturbance as possible, usually insertion of a wire flag into the mound. Mounds were considered active if any workers appeared. Rain data were collected using simple rain gauges located at the site, and temperature was recorded during observations by air and soil thermometers.

Spring 2004 Band Trial:
Stennis International Airport in Bay St. Louis, MS (Hancock Co.) had a high enough density of active fire ant mounds to allow establishment of three plots each of seven treatments and a control. Treatments were applied as previously described with bait application occurring June 8, 2004 and contact insecticide application two weeks later on the 22nd and the 24th. Contact insecticide treatments were as follow:

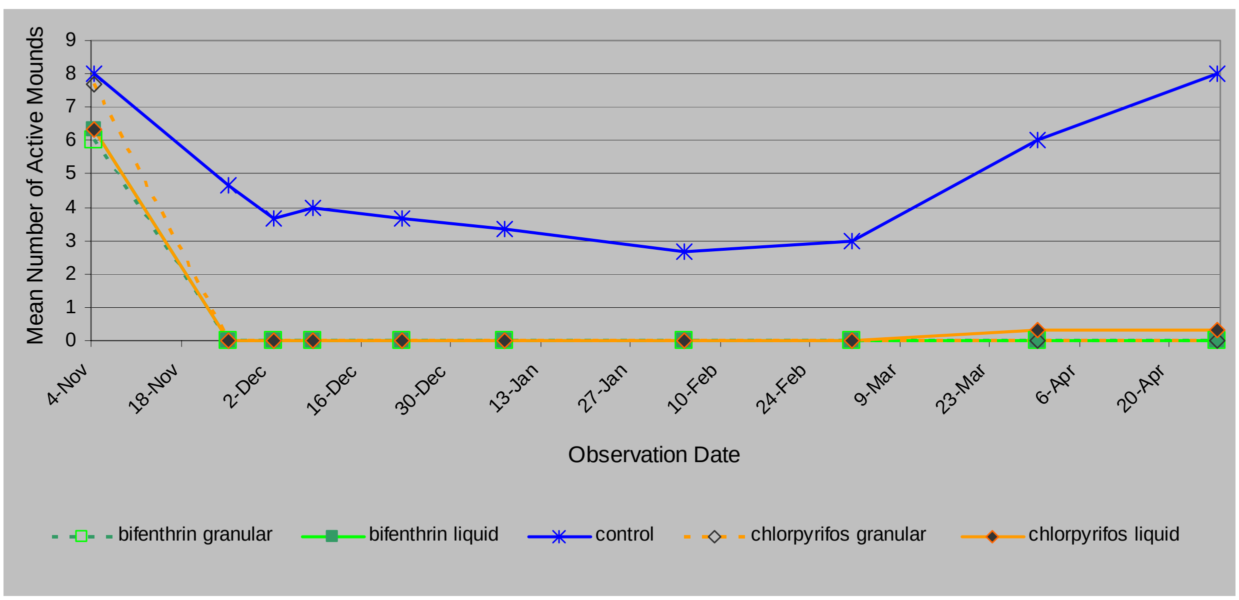
Chemical	Formulation	Rate of Application
bifenthrin	granular 0.2%	200 lb/acre (0.4 lb ai/acre)
bifenthrin	flowable 7.9%	40 oz/acre (0.2 lb ai/acre)
chlorpyrifos	granular 2.32%	130 lb/acre (3 lb ai/acre)
chlorpyrifos	emulsifiable 44.8%	32 oz/acre (1 lb ai/acre)
permethrin	emulsifiable conc. 0.1%	33 oz/acre (0.8712 lb ai/acre)
fipronil	granular 0.0143%	87 lb/acre (0.0125 lb ai/acre)
lambda-cyhalothrin	flowable 9.7%	10 oz/acre (0.06875 lb ai/acre)

Observations were conducted as previously described prior to bait application and at 1, 2, 4, 6, 8, 13, 16, and 20 after final treatment until control plots were buried during site improvements. Precipitation data was provided daily by local reports to NOAA when available as well as by manual rain gauge check at the time of observation.

RESULTS:

Fall 2003 Band Trial:
All four chemical treatments displayed 100% mortality by the first observation at two weeks after treatment (Figure 3). No active mounds appeared on any of the treated plots, until examination at and after week 20 found active mounds in two of the chlorpyrifos emulsifiable plots. The other three treatments remained without activity at the 6 month close of the trial. The two bifenthrin formulations performed identically in this trial to one conducted in south Mississippi in the fall of 2002. Chlorpyrifos was not previously tested in a fall/winter trial. The number of active mounds on the control plots declined over the winter, but resurgence in activity was seen at the same time the liquid chlorpyrifos treated plots failed. The control plots in this trial were located in close proximity to the chemically treated plots and some foraging on bait may have occurred. Weather at this site was persistently wet with frequent reports of standing water in some of the plots. The granular treatments were most likely watered in by 1.8 inches of rain between application and the first observation two weeks later.

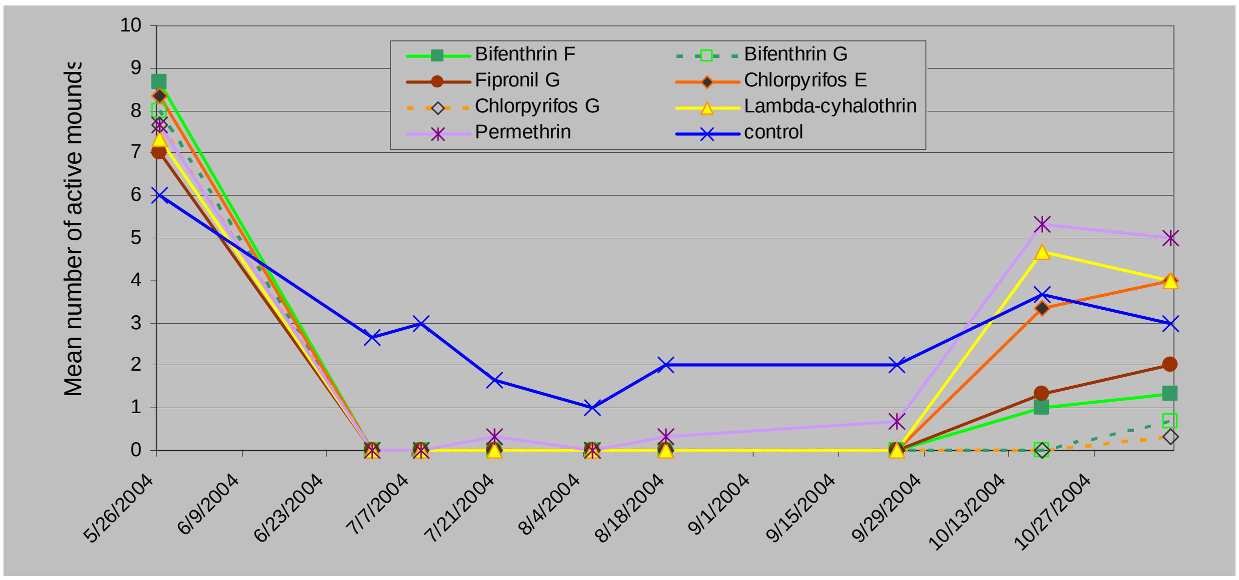
Figure 3. Fall 2003 trial – Colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide.



Spring 2004 Band Trial:
Precipitation from June 24 through July 2 was heavy at the test site, totaling 6.8 inches. Despite this significant rainfall over the last day of chemical application and the following eight days, all chemical treatments removed 100% of the IFA from the plots by the first week's examination (Figure 4). Most of the treatments appeared to be affective for three months or more with bifenthrin and chlorpyrifos granular formulations only showing reinfestation at five months after treatment. This is especially note worthy considering the chlorpyrifos granular was applied at half label rate due to lack of supply. Bifenthrin granular, chlorpyrifos emulsifiable, fipronil granular, and lambda-cyhalothrin flowable all performed almost identically in the trial conducted in the spring of 2003. Bifenthrin flowable and chlorpyrifos granular actually lasted at least five

and four weeks longer, respectively, in this trial than in the 2003 spring trial. Permethrin, which performed well for a much longer period of time in both the 2004 single tree trials, was applied at a much lower rate (0.8712 lb ai/acre) in this trial. Unlike the recommended rate for spot treatments this one falls within the 2 lb ai/acre/year maximum and was the highest labeled rate for a broadcast application against ants. It is unknown if the rate, the weather, or a combination thereof caused this treatment to fail at the one month observation. Despite the deluge at the beginning of the trial most of July through September only had minimal precipitation, thus contributing to the decline in infestation seen in the control plots. However, weather alone cannot account for lack of activity in the chemically treated plots as active mounds were regularly observed between the plots and it was during this span that the permethrin plots became infested.

Figure 4. Spring 2004 trial – Colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide.



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Williams, D.F. and C.S. Lofgren. 1983. Imported fire ant control: evaluation of several chemicals for individual mound treatments. J. Econ.

CPHST PIC NO: A1M04

PROJECT TITLE: Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, fall 2004

TYPE REPORT: Interim

PROJECT LEADER/PARTICIPANTS: Shannon James, Lee McAnally, Bob Jones, Anne-Marie Callcott, and Bruce Radsick (PPQ AEO pilot)

INTRODUCTION:

APHIS is responsible for developing treatment methodologies for certification of regulated commodities, such as field grown balled-and-burlapped nursery stock, for compliance with the Federal Imported Fire Ant Quarantine (7CFR 301.81). Current treatments for field grown stock, as described below, are inefficient and limited to a single insecticidal choice. Furthermore, restrictions on this insecticide, chlorpyrifos, within recent years have lead to reduced production consequently limiting its availability to growers and making compliance difficult. Thus additional treatment methods, as well as additional approved insecticides, are needed to insure IFA-free movement of this commodity.

The currently available pre-harvest (in-field) treatment requires a broadcast application of approved bait followed in 3-5 days by a broadcast application of granular chlorpyrifos. This treatment must extend 10 feet beyond the base of all plants to be certified. After a 30-day exposure period, plants are certified IFA free for 12 weeks. A second application of granular chlorpyrifos extends the certification period for an additional 12 weeks. The ten-foot radius requirement, due to row spacing, frequently includes plants and soil that otherwise need not be treated. Numerous common insecticides such as diazinon, chlorpyrifos, acephate, and others are labeled for spot treatment of imported fire ant colonies. Imported fire ant colonies readily respond to insecticide applications made directly to the nest by relocating the colony (Collins & Callcott 1995, Hays et al. 1982, Franke 1983, Williams & Lofgren 1983). The primary objective of a quarantine treatment for field grown nursery stock is to render the plants fire ant free. Therefore, it does not matter if colonies are killed outright by the treatment or simply induced to move away from the area around plants intended for harvest. Thus, trials of band-style treatments for large blocks of in-field B&B and individual plant-style treatments for select in-field plants were initiated to focus on examining efficacy of products other than chlorpyrifos, reduction of treated diameter, and reduction of the exposure time required prior to plant movement.

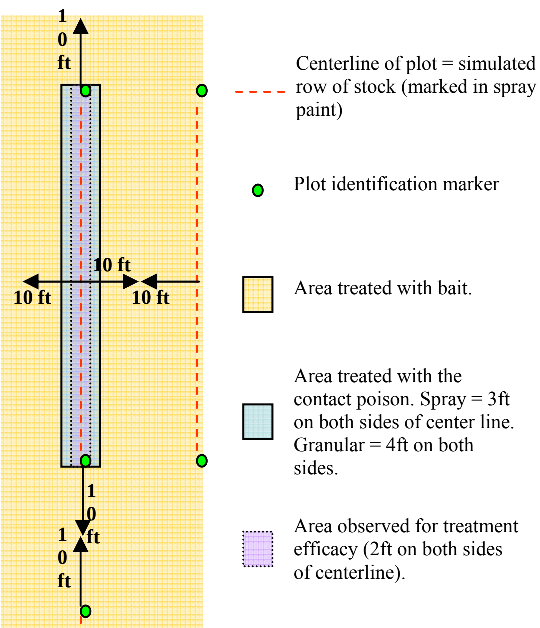
Preliminary testing initiated in Sept. 2001 assessed several liquid and granular insecticides against individual IFA mounds in the field. Results of this trial indicated promising results with acephate, bifenthrin, and deltamethrin (see GPPS01-02). Tests against individual mounds, detailed elsewhere in the 2004 Accomplishment Report, continue to provide direction for treatments utilized in the larger scale band treatments. The first two band trials applied in the fall of 2001 and spring of 2002 tested five to six-foot wide bands of bifenthrin and deltamethrin.

Both liquid and granular formulations showed promising results (see GPPS02-01; GPPS02-02), but demonstrated that in band treatments contact insecticide alone was not effective enough for use in the IFA quarantine. Subsequent band trials include a broadcast application of a bait 3-5 days prior to the contact insecticide application. The inclusion of bait in the treatment procedure has facilitated quarantine level control for several contact insecticides in these trials (Project No: A1P04; Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, Fall 2002 and Spring 2003 and Project No: A1P04; Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, Fall 2003 and Spring 2004). The trial in this report continues to explore alternative insecticides and provide supporting data for those previously performing well.

MATERIALS AND METHODS:

Fall 2004 Band Trial:
Bryan airport in Starkville, MS (Oktibbeha Co.) was selected as the test location for this fall trial. B&B is harvested in cold weather when trees are dormant, so it is important to test in colder weather than that found in south Mississippi, the location of most previous trials. Plots are 800-foot long strips of land containing at least five active fire ant mounds within a 4-foot wide (two feet on both sides of a center line) observation strip that runs the length of the band (Figure 1). To provide a buffer zone between plots, plot center lines, which simulated rows of plant stock, were set a minimum of twenty feet apart side to side and end to end. Pramitol®, used as an herbicide, was sprinkled at the end points of each plot, and a short wooden stake with a marker plate indicating the plot number tacked to its top was driven into the ground at each end to serve as somewhat permanent plot markers. Fluorescent orange spray paint marked the center line and is repainted as needed.

Figure 1. Plot arrangement diagram Figure 2. Application of bait (a) and contact insecticide (b) to the plots of simulated stock



a

b

Hydramethylnon bait was applied aerially across the plots designated for chemical treatment on October 25, 2004 (Figure 2). Control plots were not treated with bait. Contact insecticide application occurred about 28 hours later on the 26th. Normal procedure is to wait at least 3 days between bait and contact insecticide applications, but weather was prohibitive. Granular treatments were applied using a Gandy 48" granular drop spreader attached to a farm tractor. Liquid treatments were applied using a roller pump boom sprayer equipped with two standard flat spray tips (8015-SS; TeeJet Corp.) to provide a 36" band spray and a total spray volume equivalent to ca. 76 gal/acre. Treatments were applied on both sides of the centerline producing a band size, depending on formulation used, either 800'x 8' or 800'x 6' in each plot. The more northern location proved difficult in locating sufficient numbers of mounds per plot, thus the test consists of the following four treatments and a control.

Chemical	Formulation	Rate of Application
bifenthrin	granular 0.2%	200 lb/acre (0.4 lb ai/acre)
bifenthrin	flowable 7.9%	40 oz/acre (0.2 lb ai/acre)
chlorpyrifos	granular 2.5%	241 lb/acre (6 lb ai/acre)
chlorpyrifos	emulsifiable 44.8%	32 oz/acre (1 lb ai/acre)

Active IFA colonies in each plot's observation area were recorded prior to bait application and after contact insecticide application at 1, 2, 4, 6, 8, and 12 weeks thus far and will be recorded every four weeks thereafter until reinfestation occurs or six months passes. Mounds are evaluated using as little disturbance as possible, usually insertion of a wire flag into the mound. Mounds are considered active if any workers appear. Rain data are collected through the Mississippi State University Extension Service Starkville station observations http://ext.msstate.edu/anr/drec/stations.cgi?defstation=starkville and simple rain gauges located at the site. Temperature is recorded during observation by air and soil thermometers.

RESULTS:

Fall 2004 Band Trial:
All treated plots displayed a sharp decrease in active mounds at one week after treatment (Figure 3), but bifenthrin granular was the only treatment determined free of active mounds before the four week examination. Previous bait plus band trials with these insecticides produced a maximum of one to two weeks time before bands were IFA free. The longer survival of colonies in this trial is most likely due to the shorter wait between bait and contact insecticide applications preventing an effective baiting. Several days of light rain from the final day of treatment through the first observation at one week (Figure 4) served to water in granular treatments. Several observation dates thus far have recorded standing water in portions of the plots, but plot orientation within the field is such that all treated plots experience similar high and low spots. Observations are conducted when the warmth of the day encourages increased fire ant activity, but night temperatures have dropped low enough to freeze water in the rain gauge. Despite the prolonged survival of several colonies through the second week examination, all treatments are performing well at this point and are to be tested in a Tennessee location in 2005.

Figure 3. Fall 2004 trial – Colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide.

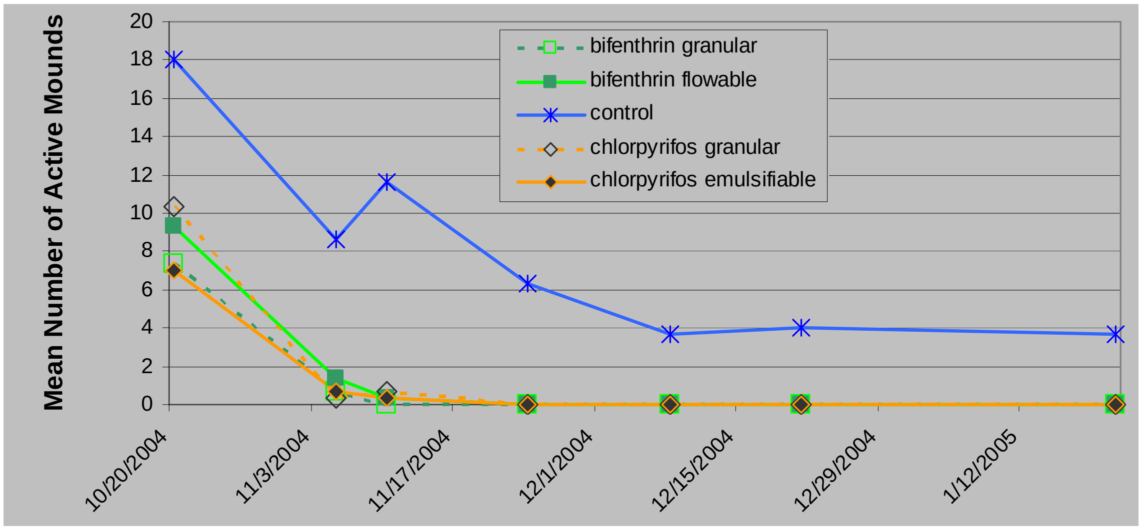
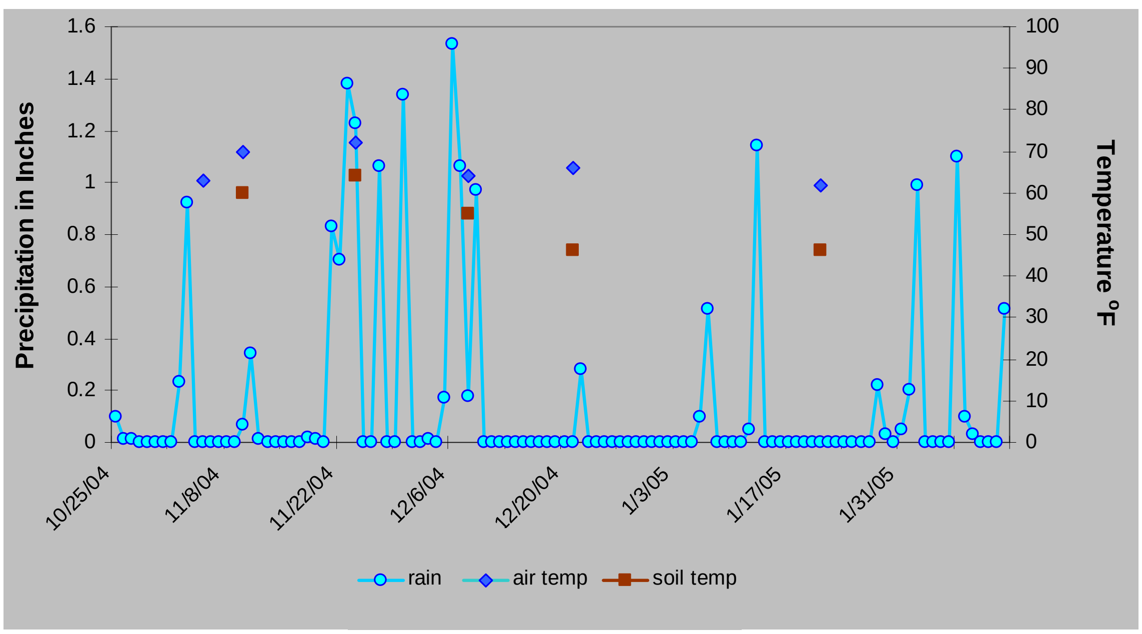


Figure 4. Weather data for the 2004 Fall band trial.



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Hays, S.B., P.M. Horton, J.A. Bass and D. Stanley. 1982. Colony movement of imported fire ants. J. Georgia Entomol. Soc. 17: 266-272.

Williams, D.F. and C.S. Lofgren. 1983. Imported fire ant control: evaluation of several chemicals for individual mound treatments. J. Econ.

CPHST PIC NO: A3M03

PROJECT TITLE: Evaluation of Methods to Prevent Imported Fire Ants From Infesting Commercial Honey Bee Pollination Operations

TYPE REPORT: Interim

PROJECT LEADER/PARTICIPANTS: Ronald D. Weeks and Dr. Gloria DeGrandi-Hoffman, Research Leader, Carl Hayden Bee Research Center, USDA-ARS,

INTRODUCTION:

Commercial pollination with honey bees is a highly mobile business, and bee colonies are frequently moved, on pallets, among holding yards and over-wintering and pollination sites. These activities increase the probability that imported fire ants (IFA) *Solenopsis spp.* will be inadvertently transported from fire ant-infested areas to non-infested areas with beehives or in soil adhering to apiary equipment. Currently, bees and bee equipment are not listed as regulated items within the Federal IFA Quarantine, however many states vigorously inspect and regulate these items coming from IFA infested states. Beehives and associated equipment are under consideration for addition to the Federal Imported Fire Ant Quarantine (Federal Code of Regulations, Title 7, Part 301.81). No quarantine treatments have been approved for assuring that transported hives are IFA free. The objectives of this research were: 1) quantify the efficacy and rates of application of permethrin and chlorpyrifos in preventing imported fire ant (IFA) foraging on commercial honey bee equipment and 2) determine if effective rates and application methods of permethrin would pose significant impacts on honey bee colony mortality or foraging rates.

IFA RESEARCH AND METHODS:

In 2004, IFA evaluations were conducted in fields infested with monogyne red imported fire ants, *Solenopsis invicta*. In 2003, pre-treatment surveys of the entire area by visual transect count showed the population level of active imported fire ant mounds to be ca. 45/acre. Routine ant sampling and visual examinations of support pallets and soil areas for IFA activity were made 3-4 days post chemical applications and at weekly intervals. A 1 x 1 inch index-card soaked in corn oil was placed on support pallets or the soil area and used as an attractant to determine the presence of foraging workers. Sampling was conducted for 45-60 minutes in each treatment.

In 2004, field trials were completed using two application rates of both permethrin (0.0042 lb A.I./gallon of water and 0.0084 lb A.I./gallon water), and chlorpyrifos (0.0087 lb A.I./gallon of water and 0.017 lb A.I./gallon water) on 5 x 5 ft soil areas. Soil area applications of both chemicals consisted of 3.0 gal finished solutions for each application rate. Five replicates of each chemical treatment and controls were evaluated. In all studies, treatments and controls were placed in a randomized block design in the field. Two bait-cards, soaked in corn oil, were placed in the center of the 5 x 5 ft treated areas to detect IFA foraging at weekly intervals.

IFA RESULTS:

In 2004, results indicate that the two application rates of both permethrin and chlorpyrifos evaluated as soil treatments (5 x 5 ft) provide excellent protection from ant foraging. The permethrin application rate of 0.0042 lb A.I./gallon of water provided protection for 3 weeks, while protection at the 0.0084 lb A.I./gallon water rate lasted 5 weeks (Table 1). Both application rates of chlorpyrifos (0.0087 lb A.I./gallon of water and 0.017 lb A.I./gallon water) were protecting bee equipment 8 weeks post-chemical treatment.

Table 1, 2004. Total number of IFA infestations in each set of replicates (n = 5) for each treatment. Soil applications of permethrin and chlorpyrifos covered a 5 × 5 ft area with 3 gal of finished solution.

Treatment	Weeks after chemical application						
	1	2	3	4	5	6	7 -8
Permethrin							
0.0042 lb A.I./gallon of water	0	0	0	1	3	3	5
0.0084 lb A.I./gallon water	0	0	0	0	0	2	4
Control	4	3	5	5	4	5	5
Chlorpyrifos							
0.0087 lb A.I./gallon of water	0	0	0	0	0	0	0
0.017 lb A.I./gallon water	0	0	0	0	0	0	0
Control	4	3	5	5	5	4	4

HONEY BEE RESEARCH AND METHODS (Results presented for permethrin 2003 – 2004, only):

Beekeepers are familiar with the properties and labeled usage of permethrin chemicals in their bee operations. Beekeepers use permethrin to control hive beetle around apiary equipment.

In the spring and fall 2003, and spring 2004, the impact of permethrin applications (0.0084 lb A.I./gallon water) on honey bee foraging activity and colony mortality were evaluated in Tucson, AZ. Permethrin was applied to support pallets and the soil area (10 x 10 ft) 24 h prior to placing honey bee colonies on treated surfaces. Support pallets were treated with permethrin using a 2.5 gal hand-pump sprayer until saturation and run-off (ca. 0.5 – 0.7 gal). Soil area applications of permethrin consisted of 6.0 gal finished solution. Control colonies received no permethrin applications. Sampling of bee colony mortality and foraging activity was conducted before and after chemical applications.

HONEY BEE RESULTS:

In 2003, in all but one instance there were no significant differences in mortality or foraging activity between treatment and control bee colonies (Tables 2 -5). In one case, pallet post-treatment mortality was higher than pre-treatment mortality, but the number of dead bees was not so great as to jeopardize colony health or survival. Results indicate that application of permethrin

(0.0084 lb A.I./gallon water) to support pallets or the soil area surrounding bee equipment (10 x 10 ft area) prior to colony placement is a relatively safe and efficacious IFA management option.

In 2004, there were no significant differences in foraging activity between treatment and control bee colonies, and in mortality before and after permethrin applications (Tables 6 -9). In one case, foraging activity of colonies after treating the pallet was significantly greater than a set of Control colonies and the other set of colonies on treated pallets. Overall results were consistent with 2003 results, indicating that application of permethrin (0.0084 lb A.I./gallon water) to support pallets or the soil area surrounding bee equipment (10 x 10 ft area) prior to colony placement is a relatively safe and efficacious IFA management option.

Table 2, Spring 2003. Average number of dead bees as determined by dead bee traps before and after treatment with permethrin (0.0084 lb A.I./gallon water) to the pallet or ground. Two colonies were used for each treatment. Five pre-treatment and five post-treatment counts were made per colony.

Treatment	Colony ID	Average number of dead bees		T	P
		Pre-treatment	Post treatment		
Control	2	1.00	0.40	1.0	0.35
Control	3	0.55	1.67	-1.52	0.20
Pallet	14	1.00	2.60	-1.55	0.16
Pallet	15	2.60	13.20	-2.69	0.05
Ground	26	0.40	0.20	0.45	0.67
Ground	27	9.00	1.80	1.83	0.14

Table 3, Spring 2003. Average number of honey bees flying from colonies during a 1.0 minute interval before and after treatment with permethrin (0.0084 lb A.I./gallon water).

Treatment	Ave. number of bees leaving /minute		Pre-treatment / Post-treatment
	Pre-treatment	Post-treatment	
Control	61.46	55.71	1.173
Pallet	61.82	61.64	1.178
Ground	74.93	69.61	1.123

No significant difference among control and treatment pre-treatment / post treatment counts ($F = 0.06$; d.f. = 2, 81; $p = 0.944$)

Table 4, Fall 2003. Average number of dead bees as determined by dead bee traps before and after treatment with permethrin (0.0084 lb A.I./gallon water) to the pallet or ground. Two colonies were used for each treatment. Five pre-treatment and five post-treatment counts were made per colony.					
Treatment	Colony ID	Average number of dead bees		T	P
		Pre-treatment	Post treatment		
Control	1	14.0	20.0	-0.82	0.44
Control	2	8.2	7.83	0.09	0.93
Pallet	3	19.4	57.0	-1.49	0.20
Pallet	4	39.6	63.7	-1.17	0.28
Ground	5	19.6	13.5	0.90	0.40
Ground	6	18.8	9.67	2.17	0.067

Table 5, Fall 2003. Average number of honey bees flying from colonies during a 1.0 minute interval before and after treatment with permethrin (0.0084 lb A.I./gallon water).			
Treatment	Ave. number of bees leaving /minute		Pre-treatment / Post-treatment
	Pre-treatment	Post-treatment	
Control	109.69	62.20	1.582
Control	151.56	76.35	1.744
Pallet	105.87	57.80	1.755
Pallet	164.69	117.70	1.265
Ground	133.25	86.05	1.444
Ground	141.31	74.05	1.696
No significant difference among control and pre-treatment / post-treatment counts (F= 1.78; d.f. = 5, 90; P = 0.125)			

Table 6, 2004. Average number of honey bees flying from colonies during a 1.0 minute interval before and after treatment with permethrin (0.0084 lb A.I./gallon water).			
Treatment	Ave. number of bees leaving /minute		Pre-treatment / Post-treatment
	Pre-treatment	Post-treatment	
Control	81.12	127.15	0.73ab
Control	31.56	60.35	0.66a
Pallet	44.25	73.45	0.68a
Pallet	44.19	50.65	1.03b
Ground	71.31	87.70	0.94ab
Ground	53.37	67.65	0.95ab
Means followed by the same level are not significantly different at the 0.05 alpha level as determined by a Tukey's W procedure. F = 3.35, p = 0.008, d.f. = 5, 90; Critical Value (W) = 0.343.			

Table 7, Summer 2004. Average number of dead bees as determined by dead bee traps before and after treatment with permethrin (0.0084 lb A.I./gallon water). Two colonies were used for each treatment. Five pre-treatment and five post-treatment counts were made per colony.					
Treatment	Sample Size	Average number of dead bees		T	P
		Pre-treatment	Post treatment		
Control	10	14.8	15.2	-0.10	0.92
Pallet	10	25.8	31.1	-0.50	0.63
Ground	10	19.9	28.7	-1.15	0.26

Table 8, 2004. Average number of honey bees flying from colonies during a 1.0 minute interval before and after treatment with permethrin (0.0084 lb A.I./gallon water). Means are based upon 12 flight counts per colony.			
Treatment	Ave. number of bees leaving /minute		Pre-treatment / Post-treatment
	Pre-treatment	Post-treatment	
Control-1	39.2	41.4	0.975
Control-2	42.0	32.0	1.58
Permethrin -1	47.7	35.4	1.70
Permethrin -2	34.1	27.1	1.37
Average pre-and post-treatment flight counts are not significantly different at the 0.05 alpha level as determined by an F-test; F=0.49, p = 0.693, d.f.=3,44.			

Table 9, 2004. Average number of dead bees as determined by dead bee traps before and after treatment with permethrin (0.0084 lb A.I./gallon water). Four colonies were used for each treatment.					
Treatment	Sample size	Average number of dead bees		T	P
		Pre-treatment	Post treatment		
Control	16	32.1	148.9	-1.30	0.23
Permethrin	16	41.0	52.7	-1.21	0.25

CPHST PIC NO: A1M03

PROJECT TITLE: Evaluation of Methods to Exclude Imported Fire Ants (IFA) From Infesting Baled Hay

TYPE REPORT: Interim

PROJECT LEADER: Ron Weeks

INTRODUCTION:

Commercial hay operations are highly mobile and imported fire ant (IFA, *Solenopsis* spp.) colonies can be moved in the commodity. As a federally regulated item, under the Federal Imported Fire Ant Quarantine (7CFR 301.81), hay stored in direct contact with the ground cannot be moved outside the quarantine. No quarantine treatments have been approved for assuring that transported hay bales are IFA-free. The objectives of this project were 1) to determine the rate of infestation of hay bales without chemical protection, and 2) to evaluate the efficacy and longevity of various permethrin application rates and application methods applied to 10 x 10 ft soil areas under either a support pallet or plastic sheet as barrier treatments for hay bales in preventing IFA foraging or nesting. Two application rates in combination with two treatment/storage methods and a broadcast bait application were evaluated.

MATERIALS AND METHODS:

Hay experiments were conducted at the White Sands Mississippi Agriculture and Forestry Experiment Station in Pearl River County, MS. In 2004, only two hay cuttings were made at White Sands; July and September. Experiments were conducted on hay bales after the first (July) cutting in a 3.5 acre field, infested with monogyne red imported fire ants *Solenopsis invicta* Buren. Routine ant sampling and visual examinations of each bale were made 4 days post hay placement and then at approximately bi-weekly intervals. A 1 x 1 in. index card soaked in corn oil and placed on the side of each bale was used as an attractant to determine the presence of foraging workers. Cards were attached to the bale with survey flags for at least 30 minutes.

Infestation rate:
On May 7, 2004, the hay field was divided into two halves and hydramethylnon bait was applied at the labeled rate to one side (ca. 2.0 acres). Hay was cut and dried and baled the week of July 12, 2004. All hay bales were removed and stacked on a fifth-wheel trailer except for 12 bales that were left "in-situ" on the ground to determine rates of infestation in unprotected hay bales. Six bales were left on the ground in the treated side of the field and 6 left on the ground in the untreated side. Sampling was conducted 4 days and 10 days after baling.

Chemical barriers:
This study evaluated the efficacy of chemical barrier applications on the soil for square baled hay stacked (4 bales per stack) on either support pallets or plastic sheeting to prevent hay contact with the chemical. In addition, for this evaluation the field was divided into two treatment sides; 1) broadcast bait application, and 2) no-bait treatment. Two application rates of permethrin (0.0042 lb A.I./gallon of water and 0.0084 lb A.I./gallon water) in a 6.0 gal finish solution on the soil treatment area (10 x 10 ft)] were compared for both pallet and plastic supports in each side of the field. Control hay stacks were evaluated in the untreated side of the field without the permethrin applications using the pallet and plastic supports. Five replicates of each treatment were set up (n = 50). Hay for this evaluation was cut and baled in an adjacent field and stored off the ground on a fifth-wheel trailer on July 19, 2004. Experiment set-up began on July 22 and sampling on July 26. Treatments were arranged approximately 7m apart in a randomized block in the field. Sampling for this experiment began on July, 26 2004 and ended on September, 22 2004.

<u>RESULTS</u>:

Infestation rate:
After 4 days in the field, bait-card samples detected only 1 of 6 hay bales that had foraging ants on the bait treated half of the field. While hay bales sampled on the untreated side showed that 4 of 6 had foraging ants and 2 of these had active colonies living under the bale. Ten days after baling, 3 of the 6 bales in the bait treated plot had foraging but no active colonies compared to 6 of 6 bales with foraging ants and active colonies in the untreated side of the field. These results support other hay trails (see annual report 2003) that broadcast bait treatments can reduce ant pressure in hay production systems, but that if left unprotected ants will forage on hay stacks.

Chemical barriers:
Broadcast application of hydramethylnon bait did not eliminate all IFA colonies or foraging frequency in the treated area. However it did significantly reduce IFA foraging frequency on stacked hay bales compared to stacked bales in the untreated side of the field (Fig. 1, only hay on support material shown).

Permethrin treatment was effective at preventing infestation of hay bales for up to 25 days, except for in several instances (Table 1). IFA infestation was detected repeatedly on a stack of hay on a support pallet (0.0042 lb A.I./gallon of water application rate) and one time on plastic (0.0084 lb A.I./gallon of water application rate) after the first and second sampling periods respectively. In the pallet supported instance a relatively large (10,000 to 50,000) IFA colony was present in the soil treatment area, under the support pallet, in the untreated side of the field. This result indicates that the permethrin application rate and method used in this study was not effective at killing an established IFA colony or eliminating foraging from the colony. Empirical observation of the colony in this instance indicated that during normal colony excavation behavior (IFA is a mound building species) the colony was able to cover-up a small portion of the treated soil area with untreated soil excavated from the interior of the colony. This result is viewed as fortuitous in that it indicates a natural limitation in the ability of the proposed chemical treatments to retard or eliminate foraging from an establish IFA colony. However, provided that hay stacks are not placed directly over an established RIFA colony the data in this report generally support bait and spot chemical approaches at protecting stacked hay bales (Table 1). In all permethrin treatments regardless of support device provided 40 days protection in the bait treated storage area.

In the bait treated areas there was no measurable difference between chemical rates of application in this evaluation, both rates protected hay for approximately 25 days post chemical application in the field (Table 1). However, comparisons of the time for hay to become infested between hay supported by pallets and hay placed on plastic show that the pallet supported hay in both application rate evaluations were still protected from IFA foraging at the conclusion of the evaluation (Table 1, 62 days). It is unclear why this difference exists, however observations in the field indicate the choice of plastic (non-permeable) was not desirable in terms of hay storage. The plastic sheets tended to collect water along creases and near the hay. This caused the hay to deteriorate and rot quickly making this an undesirable storage method. Perhaps concentrated water run-off from the plastic sheet created areas of increased chemical breakdown or dilution were ants could safely cross. Future evaluations will use permeable ground cover to allow for better water movement thus avoiding pooling around the base of the hay stack.

DISCUSSION:

Broadcast bait applications in hay fields can significantly reduce, although not eliminate, ant pressure within a field. These data suggest that the use of broadcast bait applications to storage areas (approximately 1 acre treatment areas) should improve the efficacy of contact chemical barrier treatments to reduce overall ant pressure on stored hay products. Permethrin is a relatively safe chemical that can be used in combination with a support material (pallets or plastic) to protect stored hay products for a limited period of time (up to three weeks) in non-bait treated storage areas, and up to 5 and half weeks in bait treated storage areas.

In summary, these experiments indicate that a best management approach to managing IFA infestation risk in hay production systems should include broadcast bait applications to storage areas, routine investigation and single mound treatment of residual mounds not impacted by the initial broadcast treatment, and judicious well-placed chemical application of permethrin products along with support material to protect hay products from direct contact with the chemical. Future research is planned to evaluate permeable ground covers and perimeter area chemical treatments on round hay bales.

Figure 1. Total number of IFA infestations on permethrin protected hay bales in broadcast bait treated and untreated sides of the experimental field (n = 50 hay stacks, 25/side). Sampling was conducted 5 times over a span of 62 days.

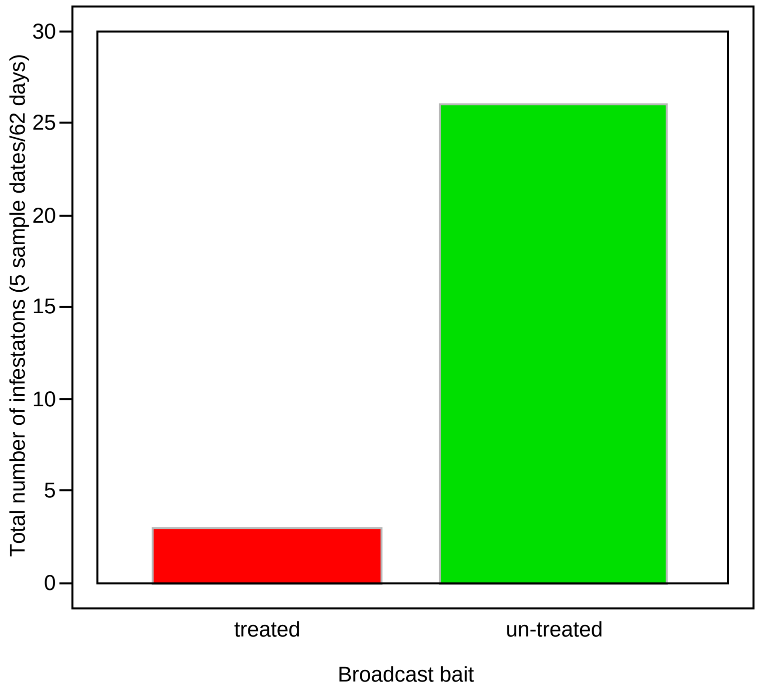

Table 1. Number of infested hay stacks in each treatment by days post-treatment. Five hay stacks in each treatment (n = 50). Three gallons finish solution of chemical in each treatment.

Treatments			Days post-treatment				
Permethrin application	Support media	Broadcast treatment	4	12	25	40	62
0.0042 lb A.I./gal water	Pallet	Treated	0	0	0	0	0
0.0042 lb A.I./gal water	Pallet	Untreated	1*	1*	1*	5	1
0.0042 lb A.I./gal water	Plastic	Treated	0	0	0	0	2
0.0042 lb A.I./gal water	Plastic	Untreated	0	0	0	3	3
0.0084 lb A.I./gal water	Pallet	Treated	0	0	0	0	0
0.0084 lb A.I./gal water	Pallet	Untreated	0	0	0	2	1
0.0084 lb A.I./gal water	Plastic	Treated	0	0	0	0	1
0.0084 lb A.I./gal water	Plastic	Untreated	0	1	0	4	4
Control	Pallet	Treated	5	5	2	5	0
Control	Plastic	Treated	5	4	3	5	3

* = active IFA colony present during hay placement and throughout sampling

CPHST PIC NO: A2M06

PROJECT TITLE: Development and Evaluation an Imported Fire Ant Survey Trap

TYPE REPORT: Interim

PROJECT LEADER/PARTICIPANTS: Robert G. Jones, Ronald D. Weeks

INTRODUCTION:

Currently, the Federal Imported Fire Ant Quarantine (7CFR301.81) has no recommended survey trap. States and researchers have used a variety of traps with no known consistency of trapping or survey results. Some traps are handmade increasing survey planning and logistic problems. Others are difficult to handle under field conditions. This means increased time needed to set out and collect traps. The collecting, preserving and recording of specimens must be done in the field when it can be done better and faster in a laboratory situation.

The traps being used or have been used include vials and jars of various sizes. The most convenient and economical is a plastic centrifuge tube which is a vial with an attached lid but it is extremely small. Index cards have been used as a substrate or holder for baits, such as peanut butter and vegetable oils. The state of California uses a plastic cage or basket to hold the bait (1/4 inch cube of SPAM®). In essence these "traps" are containers or substrates for bait attractants. The reason for this is that a foraging ant will carry some of the bait back to the nest leaving a pheromone trail for others to follow. The survey needs to demonstrate that there are established nests present. Catching IFA major and minor workers or just numerous workers provides proof of an established colony. Thus a trap that catches or entraps the foraging ant will not work for this quarantine survey.

This is a report of an effort to develop an Imported Fire Ant (IFA) survey trap that is effective, simple and easy to use. Methods used included literature surveys, questioning experienced workers and looking at a variety of containers. Both purchasing and altering a variety of containers was done. These products were then tested and observed in both the laboratory and in the field with IFA. In differing ways, a good survey trap is user friendly to both the subject insect and the surveyor. There has never been a reported test to compare the efficiency of different types of potential IFA survey traps. This work is to compare examples of these containers and holders for capturing the three genetic forms of IFA. It is also to compare a candidate trap or bait container developed to improve this survey need and improve on survey efficiency and cost.

METHODS:

Candidate Traps: Five candidate "traps" (containers and holders) are compared. This includes: 1. clear, polystyrene, 11 ml, snap lid vial; 2. opaque, polyethylene, 10 ml, hinged cap vial; 3. plastic, open mesh basket or California IFA bait station; 4. clear, polystyrene, 9X50mm petri dish bottom and 5. clear polystyrene, 9X50mm petri dish (Pall Gelman Brand), modified with

12 (1/8th inch) holes evenly spaced around sides and the top (or the Petri dishes' wide bottom) covered with aluminum foil. All containers except the basket were selected for being close to the same volume size.

Trap Bait Attractant: The same bait or food attractant will be used throughout this series of tests. The attractant is this lab's formulated survey bait that has performed well in both laboratory and field bait acceptance tests (A2M01). It will be formulated for this study in equal size pieces so that cutting and weighing baits for tests is not necessary. The bait will be prepared prior to use then packaged in small lots and refrigerated until use.

Collection of Traps: Four of the trap containers can be easily sealed to prevent loss of ants or data. To make collecting efficient and painless the basket trap is dropped into a 110 ml hinged capped, polyethylene container. All traps are then placed into a single 16 ounce plastic jar with a screw type lid. This allows for a complete test replication of all treatments to be kept together and data is recorded by replication and treatments.

Bioassay: The traps are set out individually in a line with 6 foot (± 2 feet allowing for setting on bare ground) spacing between each treatment. The next replication of the five treatments will continue on this line at the same spacing between replications and treatments. The first treatment placing of each replication is marked by a surveyor's flag and the 16 oz jar is placed there for use in collection. Traps or treatments are placed at random within each replication. In the case of poor collecting sites along the line such as wet areas, heavy grass covering or driveways, the spacing between replications will vary to maintain similarity and close proximity among treatments in the same replications. No trap location should be within 3 feet of a mound. If more than one line or replication is possible in the same pasture or location the lines should be established no closer than 10 feet apart. In the hybrid area or northern half of Mississippi the trap lines are setup along fence lines in order to place traps on bare ground. Lines or replications can be parallel, perpendicular or as a continuation of another line, but can not cross. The number of replications will be determined by setup time since collecting begins at 60 minutes after the first is set out. Soil and air temperature readings are recorded after setup and after collection. These test replications were done several times during each month dependent on favorable foraging weather.

Analyses: The numbers of IFA caught by each treatment and the numbers of traps in each treatment catching IFA will be analyzed and compared using ANOVA and LSD. There is possibility of seasonal occurrence and location providing further data. This analyses used will be determined if the need occurs.

RESULTS AND DISCUSSION:

Preliminary: The need to consider all available candidate or potential traps was important. In searching the literature and catalogues for candidates it became apparent that they all fit into three categories. These are (1) substrates or open bait holders such as index cards and the California bait station, (2) vials and jars of various sizes, and (3) lids of small containers. This simplifies the need to limit treatment numbers for field testing and still consider all the basic possibilities. Being able to see ants in the containers or traps simplifies handling. There are two types of plastic containers that make this possible. These are the clear and opaque or "milky" plastics. These types are common in the vial and jar category. It is in this category that this physical difference could be a factor by the effect of light reflection or heat accumulation in the vials. A series of observational studies was initiated on numerous containers in the laboratory with some carry over into the field. Category 1: the California Basket or IFA bait station is an accepted trap making it the one to test. Category 2: numerous candidates have potential but observations showed that the ants had no difficulty entering the straight sided vials. The opening was flat on the ground and had no obstacles to entry. Category 3: the petri dish bottom is similar to many of the container lids used and has also been used in numerous bait studies on IFA. While it may not be the most economical it can be cleaned and reused numerous times. It is easy for the ants to enter and exit. The tight fitting dish lid can easily be placed over the bottom and these petri dishes are stackable for ease in transportation. Many of the other lids examined which locked onto their container presented some obstacles for entry. The lids were also flimsy and needed care in setting out. For this test the petri dish was determined to be an excellent representative for this category. The IFA Survey Trap developed by this laboratory as a candidate was altered slightly from that reported on in 2002. The number of entrance holes or openings was increased from eight to twelve.

Field Study: This study began on June 4, 2003 with 20 replications of the five treatments. On subsequent test days this was increased to 30 replications. There have been a total of 918 replications of each treatment tested on the Hybrid IFA through August 2004. Tests involving the Red and Black were started in 2004 with too few replications to consider here. A summary of the Hybrid IFA data is found in Table 1. There were numerous variables encountered during these tests. These are too numerous to discuss and since they can not be eliminated in field testing only increasing numbers of samples will diminish their effect. These relate to microclimatic, micro ecological and micro geologic variables as well as competition from other ant species. The Little Black Ant (LBA, *Monomorium minimum*) is the most common of these in the Hybrid IFA study area. One of the species of Big Headed ants (*Pheidole* spp.) appears to be the most common in the selected Black IFA area.

The data in Table 1 shows that the laboratory produced survey trap is a very viable candidate. It is the only trap that has a reflective cover on it which may be a factor in its higher capture rates for IFA. The California Basket is at a disadvantage in capture numbers since many ants fall off or out through the large mesh openings during collection. The numbers of replicates with IFA or the % capture is the more important number in Table 1. This shows the presence of IFA which is the purpose of the IFA Survey. The survey trap had a 67.6% rate of IFA captures versus the next highest, California IFA Bait Station, with 64.3%. From this preliminary data it can be concluded that the California IFA Eradication Program made a good choice in their selection of a survey tool but maybe we can improve on it (Fig. 1).

TABLE 1. Summary of Hybrid Imported Fire Ants (IFA) Trap Captures for the Months of June 2003 through August 2004.

	California Basket	Opaque Plastic Vial	Clear Plastic Vial	Plastic Petri Dish	*Survey Trap Candidate*
% Replications with IFA Captures	64.3	60.3	56.6	59.7	*67.6*
Total IFA Captures For All Replications	25470	19947	18457	29155	*54730*
Average IFA Capture Per Replication	27.7	21.7	20.1	31.8	*59.6*

Figure 1. Laboratory produced survey trap (left open and closed) and the California trap with collection container.

CPHST PIC NO: A2M01

PROJECT TITLE: Development and Evaluation of Universally Acceptable Fire Ant Baits or Attractants for Survey Traps.

TYPE REPORT: Interim

PROJECT LEADER/PARTICIPANTS: Robert G. Jones, Ronald D. Weeks

INTRODUCTION:

Numerous baits are used to attract the imported fire ant (IFA) for both survey and research studies. There is no consistency in what is being used among the states. The use of many are laborious, messy or both. While attractants have been evaluated over the years, most researchers have not determined that one bait attractant is superior to others for use in survey traps (Lofgren et al. 1961, Brinkman et al. 2001). There are some bait attractants currently in use that have not been compared for effectiveness. None have been tested for attractiveness to all three genetic types of imported fire ants; the red, black and hybrid. The Laboratory testing was done to screen out the most promising products from the less promising. The more promising "off the shelf" products were then tested in the field.
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Developing a bait or attractant for IFA (imported fire ant) survey needs to consider both the IFA and the surveyor. The IFA like all biological organisms have specific nutritional needs. While IFA appears to eat almost anything that grows, crawls, walks or flies, it has certain basic requirements. These are oils, carbohydrates and proteins (Vinson 1968). These needs vary with season. Stein et al. (1990) found that carbohydrate baits are more attractive in lower temperatures (mean = 17°C) while proteinaceous bait is best at higher temperatures (mean = 25°C). Oils are important in any attractant (Lofgren et al.1964, Vinson 1968). With oils a carrier is necessary. Lofgren, et al (1963) found that pregelled corn worked well. Size of the particle was critical as discussed by Lofgren et al (1963) and Hooper-Bùi et al (2002). A good artificial diet media would give the IFA all its nutritional requirements throughout the year and thus be attractive to foraging IFA. This is the approach that was taken based on Jones and Brindley (1969).

The surveyor needs a bait attractant that is easy to use or handle. This means not greasy, sticky or crumbly. It also means no cutting to size or weighing. Thus, it should be a prepared bait or attractant made specific for this survey. Stability under the handling stress of most field surveys and resistance to spoilage in storage or after exposure to the elements are critical. For economic reasons the emphasis was placed on using ingredients that could be purchased in grocery stores. Pregelled corn is the exception since it is a byproduct of processing other corn products. Cost is not a factor with it and it is a proven IFA bait carrier of the correct particle size. Of the other ingredients, corn oil was chosen because of its availability and being labeled as corn oil. Peanut oil may not be available every where and soybean oil is available only when the market price of soybeans is lower than some of the other vegetable oils. Carbohydrates were chosen for both furnishing essential elements of an insect diet, their ability to hold other materials together and their properties of preservation. Both vegetable and animal protein sources were used in the final bait. The final bait is mixed and formed in equal sized units. It is stored in plastic bags under refrigeration until used (Fig. 1).

Figure 1. Laboratory produced bait attractant

1. LABORATORY TESTING:

INTRODUCTION:

Five commercial products were selected from the 2002 laboratory testing. These were Original Lay's Potato Chips®, Mini Ritz Crackers®, Fritos® (original corn chips), Kebbler's Pecan Sandies® and SPAM® (classic). In 2003 and 2004 there had been some changes in the commercial marketplace. Mini Ritz Crackers were not available so the original Ritz Crackers® were used. Original Lay's Potato Chips® apparently got a packaging facelift and are now Lay's Classic Potato Chips®. There is enough confusion in the numerous varieties of favors and dietary types of these brand name products. Now, further confusion in these commercial products is beginning to develop (www.bantransfats.com). Most of these products have trans or hydrogenated fats which maybe eliminated because of human health concerns in the near future. This could change the products' ingredients, flavor and possibly their attraction for IFA. The 2002 laboratory tests showed that corn oil was better than both peanut and soybean oils for IFA response. This created some questions so retesting was done since these results differed from those of Lofgren et al (1964). The attraction of other individual ingredients also needed testing for developing a universally acceptable IFA bait attractant.

METHODS AND MATERIALS:

The laboratory test method was a standardized Gulfport IFA Laboratory protocol based on Lofgren et al. (1961). Field collected IFA worker ants (adult and immature) with mound soil were collected with a small bladed shovel and placed in a plastic 11.4 quart dishpan or 12 qt. sweater box. The Red IFAs were collected southwest of Meridian, MS for tests at Mississippi State or in southern Mississippi for tests done in Gulfport. The Hybrid IFAs were collected on the Mississippi State University campus or up to 10 miles south in Oktibbeha Co. The Black IFAs were collected 7 miles southwest of the Boone, MS. Each treatment was composed of 5 replications of collected ants in plastic boxes. The insides of the plastic boxes where dusted with talcum powder to prevent escape. The ants were held for 3 to 5 days before testing. On the day before the test, the soil was watered and a board (1”x2”x12”) was placed in the box on the soil. The tests started with the same type of petri dish (100mm x 15mm square or round) placed on each end of the board. One dish bottom contained 4 grams of the test product and the second dish contained the 4 gram comparison standard of peanut oil and pregelled corn. After a period of 24 hours the petri dish bottoms were collected and weighed. The dishes had been numbered, weighed and recorded per replication per treatment before the tests. The finished weights were subtracted from the beginning weights. These weights were then recorded and an acceptance ratio (grams candidate bait removed/grams standard bait removed) was calculated for each replication with a mean acceptance ratio calculated per treatment (Table 1).

The baits or ingredients (Table 1) reported on were purchased and mixed within a week of the test dates. The vegetable oils, lard and mayonnaise test ingredients were mixed at 30% by weight in pregelled corn (70% by weight) to make baits. The lard and mayonnaise were heated to liquefy before mixing. All mixed and commercial baits were sealed in containers and refrigerated until used. With the exception of the pregelled corn, all ingredients were for human consumption and purchased at grocery stores. Peanut oil and pregelled corn was the standard to compare most of the other bait attractants with.

RESULTS AND DISCUSSION:

The results of the laboratory tests are presented in Table 1. Lofgren et al (1961) listed all test materials with an acceptance ratio of 75 or higher with no distinction. Since some but not all of the replications in each test either brought down or raised the acceptance ratio and none were under 83, it is our conclusion that all materials listed in Table 1. are equal to all others and the peanut oil standard. The corn oil had a high acceptance ratio in the 2002 test which brought up the average when included with the 2003 and 2004 tests. The soybean and the peanut oils were near equal as were the soybean and corn oils. In the study of Logic a syllogism is used in comparison or deductive reasoning. In this case the syllogism would be if corn equals soybean and soybean equal peanut, therefore peanut equals corn. For a final report a statistical analysis will be used but remember Logic was part of the origin of the scientific method and statistics.

In the case of the commercial products not having an acceptance ratio of 1, we were not able to grind them in a consistent particle size like the pregelled corn. Some of the replicates had some larger pieces remaining which were apparently too big for the foraging ants. The conclusion of these tests is that all materials listed in Table 1. are acceptable attractants or baits for survey use with all three genetic forms of Imported Fire Ants. This is the laboratory conclusion and any final conclusions as to use in field surveys require field testing.

Lofgren et al (1961) did some comprehensive tests with hydrogenated cottonseed oil and acceptance ratios. In 2002 we tested raw and once refined cottonseed oil and raw peanut oil. Their acceptance ratios were equal to peanut cooking oil from the grocery store. Since we have worked with vegetable oils for insecticide application we have some experience. The process of extracting the oils used to be done by simple crushing of the seeds. Many years ago the larger mills went to an extraction process using the solvent, hexane to increase the volume of oil extracted. It has been possible to find smaller mills using the old method but whether that is still true we don't know. But past work brought out the fact that the once refined oils varied from mill to mill with no industry standard. The point is that in producing a survey attractant or bait consistency is needed. The vegetable cooking oils are produced under industry and government standards and the quantity needed for survey baits doesn't justify buying in bulk at an oil mill. Safflower oil could not be located in four major grocery stores in 2004. Cottonseed oil is available through wholesale grocery dealers but not retail. The oils tested worked well and are readily available especially corn.

Table 1. Laboratory Tested Products Including Cooking Oils(Vegetable Oil, 30% on Pregelled Corn Carrier, 70%) and Commercial Products with a Soil Inhabiting Pests Laboratory (SIPS) Prototype with Acceptance Ratios (1 = no difference) for Comparison Tests on the Three Genetic Types of Imported Fire Ants.

	RED	**HYBRID**	**BLACK**
OILS			
Canola vs. Peanut	0.83	0.98	1.00
Corn vs. Peanut	1.79	1.51	1.00
Corn vs. Soybean	1.06	1.00	1.00
Olive vs. Peanut	1.01	1.15	1.00
Safflower vs. Peanut	1.00	1.00	N/A
Soybean vs. Peanut	1.00	1.14	1.00
Sunflower vs. Peanut	1.00	1.06	1.00
Lard vs. Peanut	1.00	1.00	1.32
Mayonnaise vs. Peanut	1.00	1.44	1.14
Commercial Products[1]			
Frito's Corn Chips	0.99	1.00	0.92
Lay's Potato Chips	1.32	0.91	0.85
Pecan Sandies	1.19	0.86	1.17
Ritz Crackers	1.00	0.97	0.88
SPAM	1.00	0.84	1.49
SIPS Bait	1.28	1.02	1.00

1. Compared to Peanut Oil/Pregelled Corn Standard

3. FIELD STUDIES:

INTRODUCTION: Comparing these baits in the field is the true test. The 2002 field tests worked fine in southern Mississippi on the red IFA. In northeastern Mississippi on the hybrid IFA the opposite was the result. The side by side comparisons would have IFA with one bait and native ants with the comparison. This nullified the tests and attempts to modify the test protocol and retain the simple comparisons failed. A totally new protocol was developed in 2003 for the hybrid IFA. Since IFA foraging can change with the season or temperature it is necessary to test any new bait or attractant for year round use. Due to the nature of field research, testing for 1 year is usually not enough. Two years of testing would be more appropriate and then only if the same trends or results occurred both years.

METHODS AND MATERIALS

Hybrid IFA Test Site: The South Farm at Mississippi State University was selected as the test site. This farm is composed of numerous fenced pastures totaling several hundred acres. The pastures are well managed for livestock grazing and other research experiments. All pastures are accessible on at least one side by gravel roads. In the past the vegetation had been removed by herbicide treatment under the pasture fencing. This has left long narrow strips of bare ground. Bare ground is preferred for the placement of bait holders or traps. Placement on grass or other vegetation creates too many variables to consider including increasing the foraging ant's search time for a bait. The IFA population or numbers of mounds varies from moderate (ca. 15/acre) to none observed in the different pastures. The tests were set out on the peripheral fence lines.

Preparation of the Standard Bait: The control or standard bait known to be attractive to ants was prepared by mixing peanut oil (cooking quality) and pregelled, defatted corn grits 30%:70% w/w. The standard bait was prepared mixed for each monthly series of tests and stored in sealed containers and refrigeration during this period.

Candidate Baits: The candidate baits are attractive commercial products, the standard bait and the survey bait formulation. These are 1.Fritos® original corn chips, 2. Lays® classic potato chips, 3. Kebbler's® pecan shortbread sandiest, 4. Ritz Crackers®, 5. SPAM® Classic, 6. the peanut oil standard and the SIPS formulation of animal and plant proteins, carbohydrates and oils made in the form of a cookie. The commercial products are known to have extended shelf lives but after their packages are opened each was resealed, stored in an additional sealed plastic bag and refrigerated until used. The SIPS survey bait formulation was prepared before each monthly series of tests and also sealed and refrigerated until used.

Hybrid IFA Bioassay: A new procedure was developed for the hybrid IFA. The 7 baits and the control compose the 8 treatments in each replication. Each bait is in a 9x50mm petri dish bottom on bare soil as a bait station. The stations were spaced at 6 foot (±2 feet) intervals along a line or in this case a fence row. The first station of each replication was marked with a surveyor flag and the baits are placed at random among the replication's stations. The spacing between replications was the same as between treatments except for skips where changes in ground type occurred such as driveways, low wet areas etc. The number of replications was determined by time taken to place them and return to start collecting 60 minutes after placement. This developed to 25 replications for each test date. Soil and air temperatures were recorded both after placing the bait stations and before collecting them. Collecting was done by putting the petri dish lids on the bottoms and placing the 7 treatments of a replication together in a plastic jar. Prior to going to the field all baits are weighed (1.5 ±.25 grams) and placed in petri dishes. These 7 dishes each with a different bait or treatment were stacked in a plastic jar and were randomly placed in the field.

5. Analyses of data will be done in the future using ANOVA AND LSD.

RESULTS AND DISCUSSION: The results of the 2003 and 2004 tests are summarized in Table 2. No attempt was made to statistically treat this data since testing has not been completed. The first test of the seven treatments was on June 4, 2003 and the last was in August 2004. The first 2 tests were composed of 20 replications of the 7 treatments. The remaining tests were replicated 25 times. This has made a total of 37 tests or dates totaling 915 replications of each bait type tested on the Hybrid IFA. Testing on the Red and Black Imported Fire Ants has just been started using the same methods and will be completed and reported on in 2005.

The percentage of replications with captures (Table 2) is probably the best indication of attraction of the baits. All of the baits tested were better than the standard and are all relatively close. In total numbers of IFA captures the Fritos® and SPAM® are the best. The quantity collected is not as important as the probability of demonstrating the presence or non presence of IFA. The average IFA capture per replicate is more than adequate to demonstrate this in all treatments. Some baits have the ability to attract or collect more specimens than others. . Kidd et al (1985) discusses the variations of this phenomenon on the recruitment of IFA to various baits. More ants will be on the baits with larger surface areas. Fritos and SPAM had the largest surface areas of the treatments. The ants did not carry off the bait particles as with the Standard and others. The ants sucked the oils or juice from these bait and thus had to remain in the petri dishes longer so their numbers accumulated during this feeding activity.

The percentage of replications with no ant specimens or species (Table2) is also a good indication in these more northern areas that there is competition for food from numerous native species of ants. These percentages are close demonstrating that all baits tested are good attractants for a number of ant species. The total Little Black Ant (*Monomorium minimum*) capture in all replications further demonstrates this competition for the same food as IFA.

TABLE 2. Summary of Hybrid Imported Fire Ant (IFA) and Little Black Ant (LBA, *Monomorium minimum*) Bait Captures for the Months of June, 2003 through August, 2004.

	Ritz	**Fritos**	**Standard**	**SPAM**	**PECAN SANDIES**	**Chips**	**Survey Bait**
% Reps with IFA Captures	52	58	48	60	52	54	56
Total IFA Captures: All Reps	24248	54049	29087	53683	22941	43231	25000
Avg. IFA Capture per Rep	50.9	102.4	66.7	97.3	47.9	86.8	48.9
% Reps with no species of ants	16	16.3	21	12.5	15.2	18	17.4
Total LBA Capture: All Reps	34950	24323	30587	39380	33086	23291	22502

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CPHST PIC NO: A2M02

PROJECT TITLE: Cooperative Project with ARS – Area-Wide Suppression of Fire Ant Populations in Pastures

TYPE REPORT: Interim

PROJECT LEADER/PARTICIPANTS: Anne-Marie Callcott, Lee McAnally, Ron Weeks

INTRODUCTION:

The USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE – Gainesville, FL) received a grant for a 5-year area-wide pest management demonstration project for control of imported fire ants (IFA). The CPHST Soil Inhabiting Pests Laboratory (aka Imported Fire Ant Lab) was asked to participate in the program as a Core member and Co-Principal Investigator. The Core team is responsible for oversight and review of the project and includes all external collaborators. Not only will the CPHST lab be participating in the project, but PPQ, AEO has agreed to aerially treat as many of the sites as possible (see note at end of template). In APHIS's role of safeguarding American agriculture, expanding our fire ant work from its traditional focus on quarantine methods development to including work on controlling fire ants and their impact on the environment through an integrated pest management approach, is a logical step. The Gulfport lab routinely cooperates with ARS on projects, and the expertise we bring to the program will contribute to the success of the project. For detailed information on the ARS project see http://fireant.ifas.ufl.edu.

This ARS project includes USDA-ARS, USDA-APHIS, and university and state personnel. The project is investigating the effectiveness of utilizing bait treatments combined with biological control agents to control IFA with demonstration projects in five states; FL, SC, MS, TX and OK. PPQ, AEO is providing a pilot and plane to apply bait treatments approximately twice a year, and CPHST, ANPCL, SIPS is providing coordination of pilot and plane, expertise and ground support for the aerial treatments. Due to complicated state regulations we are not aerially treating in FL.

MATERIALS AND METHODS:

There will be 2 sites per state. One will receive aerial bait applications only, and the other (referred to as IPM plot) will receive an initial bait application as well as inoculations of mounds with phorid flies and the microsporidia, *Thelohania solenopsae*. IFA mound counts within the bait treatment area of the IPM plot will trigger future bait applications. Numerous assessments will occur within each set of paired plots, including IFA populations, insect biodiversity (bait and pitfall traps), biological control agents population assessments, etc.

ARS is the lead agency on this project. State cooperators will select sites, and conduct pre-treatment site evaluations. CPHST Gulfport obtained assistance of PPQ, AEO personnel to provide aerial application of bait treatments over the course of the study.

RESULTS:

Initial aerial applications by AEO occurred in 2002 in 3 states, with a second application done in one of the original states. SIPS is providing coordination of pilot and airplane, technical expertise, and ground support for the aerial treatments. Due to complicated state regulations APHIS is not aerially treating in Florida and in 2003 SC decided to use a local applicator for logistical reasons. SIPS personnel assisted with initial aerial applications at all sites treated by AEO, and assisted with follow up applications when needed. Aerial treatments were completed in spring 2002 in MS and TX; and during the fall in TX and SC. In 2003, spring aerial applications were completed in MS and OK; and repeated in the fall in OK and MS. Weather precluded a fall 2003 treatment in TX. 2004 treatments were greatly impeded by weather during the spring. Bait applications were made during 2004 in TX and OK in the spring and in MS in the fall. SIPS anticipates continued treatments during 2005. Data for the project are being collected by state cooperators and compiled by ARS. No detailed results have been released by ARS at this time.

CPHST PIC NO: A1M01/A1F01

PROJECT TITLE: Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing Project

TYPE REPORT: Interim

PROJECT LEADER/PARTICIPANTS: Anne-Marie Callcott, Debbie Roberts, Shannon James, FL DPI, ARS-CMAVE, State Departments of Agriculture and their designees

INTRODUCTION:

In a recent USDA-APHIS survey, seven southern states ranked IFA as a top priority target organism for biological control. Most research on phorid flies has been under the direction of ARS in Gainesville, FL. Phorid flies (*Pseudacteon spp*.) from South America are promising biological control agents of IFA because they are relatively specific to IFA, are active throughout most of the year, and through suppression of fire ant activity, may allow native ants to compete with IFA for food and territory (Porter 1998). Potentially, there may be as many as 15 species or biotypes of the fly that will have an impact on IFA, and thus are candidates for rearing and release in the U.S. Phorid flies will not be a stand-alone biological control agent for IFA. A homeowner will not be able to release a few flies in their back yard and see a significant decrease in IFA mounds in the yard. However, the flies will be an important tool in IFA management programs. It is anticipated that if several species of flies are established in the IFA infested area of the U.S. over the next 10 or more years, the added stress caused by these flies on the IFA colonies will allow native ants to compete better for food and territory. This fly-native ant-IFA interaction will hopefully allow homeowners, municipalities, and others, to make fewer chemical control product applications annually to suppress the IFA to acceptable tolerance levels, lessening the impact of the IFA on humans, livestock, wildlife and the environment. USDA, APHIS, PPQ began funding a cooperative project in 2001 to rear and release this potential biological control agent for imported fire ants.

MATERIALS AND METHODS:

Preliminary research and rearing techniques have been developed by USDA, ARS for two species, with other types under development. ARS will continue to evaluate other phorid fly species for potential use in the U.S., and transfer rearing techniques to the rearing facility as the new species are ready for mass rearing. Mass rearing of flies is being conducted by the Florida Department of Agriculture, Dept. of Plant Industries (DPI), in Gainesville, FL. The CPHST biological technician assigned to the rearing facility will continue to conduct small methods development projects aimed at improving efficiency of fly production and shipping (see CPHST PIC NO: A1F01/A1M01: Progress Report of IFA Lab, Gainesville, FL 2004). In 2003, a second species of fly was transferred to the FL-DPI rearing facility, but the rearing of the first species will continue for another few years for complete distribution. Currently (winter 2004) ca. 9 attack (rearing) boxes are online producing one species of fly, *P. tricuspis*, and 5 boxes are online producing the second species, *P. curvatus*. Funding supplied in FY04 through all sources enabled an increase from 12 boxes to 14 boxes. A total of 16 boxes are available for rearing, however 1-2 boxes are maintained for research purposes to improve rearing techniques such as those described in the report mentioned above.

Rearing of these flies is extremely labor intensive, requiring 1-1.5 person(s) to maintain every 2 attack boxes. These flies cannot be reared on a special diet or medium but require live fire ants to complete their life cycle. Excellent pictorial and text descriptions of the rearing technique is available online from the FL DPI at: http://www.doacs.state.fl.us/pi/methods/fire-phorid.htm and http://www.doacs.state.fl.us/pi/methods/images/biocontrolfacilitybooklet.pdf.

Very simply, imported fire ant workers and brood are placed in a pan (from which they cannot escape) within a large attack box where adult flies are allowed to emerge, mate and lay eggs within the worker ant. The parasitized worker ants are then maintained for ca. 40 days with food and water. As the immature fly develops, the larval stage migrates to the ant's head capsule. The head capsule of the ant falls off and the larva then pupates within the head capsule. Head capsules are collected by hand and either prepared for shipping to the field for release or are used to maintain and/or increase production. Adult flies live only a few days and are very fragile, therefore it is impractical to ship and release adult flies.

Release techniques for the first fly species, *P. tricuspis*, are also labor intensive. Originally, approximately 5000-6000 parasitized worker ant head capsules were shipped to the cooperator for each release. In 2004, numbers of head capsules shipped per release were increased to ca. 10,000. The cooperator must then place the head capsules in an enclosed emergence box and allow the adult flies to emerge daily over 10-14 days. Adult flies are then aspirated into vials, carried to the field and released over IFA mounds. The mounds are disturbed frequently for 2 hours to insure worker ants are available on the soil surface for the flies to attack. One "release" encompasses 10-14 days of daily fly collection and release over mounds. Detailed instructions are available on: http://www.cphst.org/projects/Phorid_rearing/ or http://www.cphst.org/projects/Phorid_monitoring/.

Release techniques for the second fly species, *P. curvatus*, are somewhat less labor intensive. Worker ants are field collected from marked mounds and sent to the Gainesville rearing facility. The worker ants are subjected to flies to become parasitized, then returned to the collector to be re-introduced to their "home" mound to complete the fly's lifecycle.

Monitoring the success of the fly releases is conducted at a minimum annually. The best case scenario would be to monitor 2-3 times a years under optimum environmental conditions of temperature, wind, soil moisture, etc. Basically, monitoring involves returning to the original release site, disturbing several IFA mounds and visually looking for attacking phorid flies over a set period of time. If flies are found at the original release site, the cooperator moves a set distance away from the release site along the four cardinal positions and monitors for flies. Continue moving away from the original release site until no flies are found. Flies can be aspirated and submitted to this office for identification. Explicit instructions for fly monitoring can be found at http://www.cphst.org/projects/Phorid_monitoring/ or http://www.cphst.org/projects/Phorid_rearing/

RESULTS:

Rearing data: Rearing was initiated in 2001 for *P. tricuspis*, seeded by flies from the ARS-CMAVE facility. The number of rearing boxes has increased from the initial 1-2 boxes in 2001 to a high of ca. 10-12 boxes in 2003 and a decrease to 9 boxes in 2004 to make room for an increase in *P. curvatus* production. Annual rearing of *P. tricuspis* in 2003 and 2004 was ca. 1.6 million flies. *P. curvatus* rearing was initiated in late 2002, with the initial 1-2 boxes again seeded by flies from the ARS-CMAVE facility. By late 2004, 5 rearing boxes were in production. Rearing numbers are shown in Table 1.

Release data: While flies have been and will continue to be released by various research agencies, including ARS, in many states for research purposes, the goal of this project is to release flies in all federally quarantined states, and ultimately in all infested states. Releases are being coordinated through state plant regulatory officials, with a variety of state groups cooperating with the release and monitoring of the flies.

Releases began in spring 2002. From 2002 through 2004 there have been 1-5 releases in each of 12 states and Puerto Rico, with a total of 39 field releases (Figure 1) and more than 268,000 potential flies released. Of these 39 releases, 36 were *P. tricuspis* and 3 were *P. curvatus*. Additionally, the equivalent of 3 *P. tricuspis* shipments have gone to Louisiana to seed their own rearing facility, the equivalent of 2 releases have gone to New Mexico for research purposes, one *P. curvatus* release was abandoned due to site issues, and numerous small numbers of flies have been supplied to cooperators for educational purposes, such as state fair exhibits and field days. Over 57,000 potential flies have been shipped for these varied uses. California is the only state within the federal IFA quarantine that has not received flies. While California was under an eradication program, they had elected not to participate. However, they will begin receiving flies in 2005.

In the fall 2004, there were numerous hurricanes that impacted Florida, two of which impacted the phorid fly rearing facility. Electricity was off at the facility twice for 3 days each time during the 2004 hurricane season. This impacted the number of releases that occurred that fall. We anticipate 15-20 releases/shipments per year, and in 2004 only 12 releases were conducted (not including one that was terminated by the cooperator due to site problems).

Success of the program is currently being measured by successful overwintering of fly populations. Of the 27 releases conducted in 2002-2003, flies have been found after a winter at 8 of these sites (Figure 1). Those sites at which flies have not been found have not been abandoned. Cooperators and others studying the flies are finding that it may take 2-4 years for flies to build populations that are easily detected in the field. Unfortunately, this was not known early in this program and many states have conducted multiple releases at the same site when they believed no flies were present a year after a release. As resources allow, all release sites will be monitored a minimum of yearly to determine fly presence. Once flies are found at a site, cooperators move out from the site and monitor to determine spread of the flies. Collection of fly data from cooperators is fairly good and new options on collecting and transmitting that data is becoming available. A new online data entry form will be available in 2005 for all cooperators. We have also asked that IFA populations at the original release site be monitored.

This data is much slower coming in. Specific spatial data collected from releases and the subsequent monitoring of the ant and fly populations will be discussed in a future report.

Multiple releases of each fly species in each state are anticipated, depending on total acreage quarantined or generally infested within each state. Another CPHST project initiated in FY2003, utilizing spatial technology to assist in monitoring and evaluating the success of these fly releases (A3M02), will hopefully allow us to more efficiently target sites and states where each fly species would be most successful in establishment.

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Table 1. Rearing and release data for APHIS phorid fly rearing project.

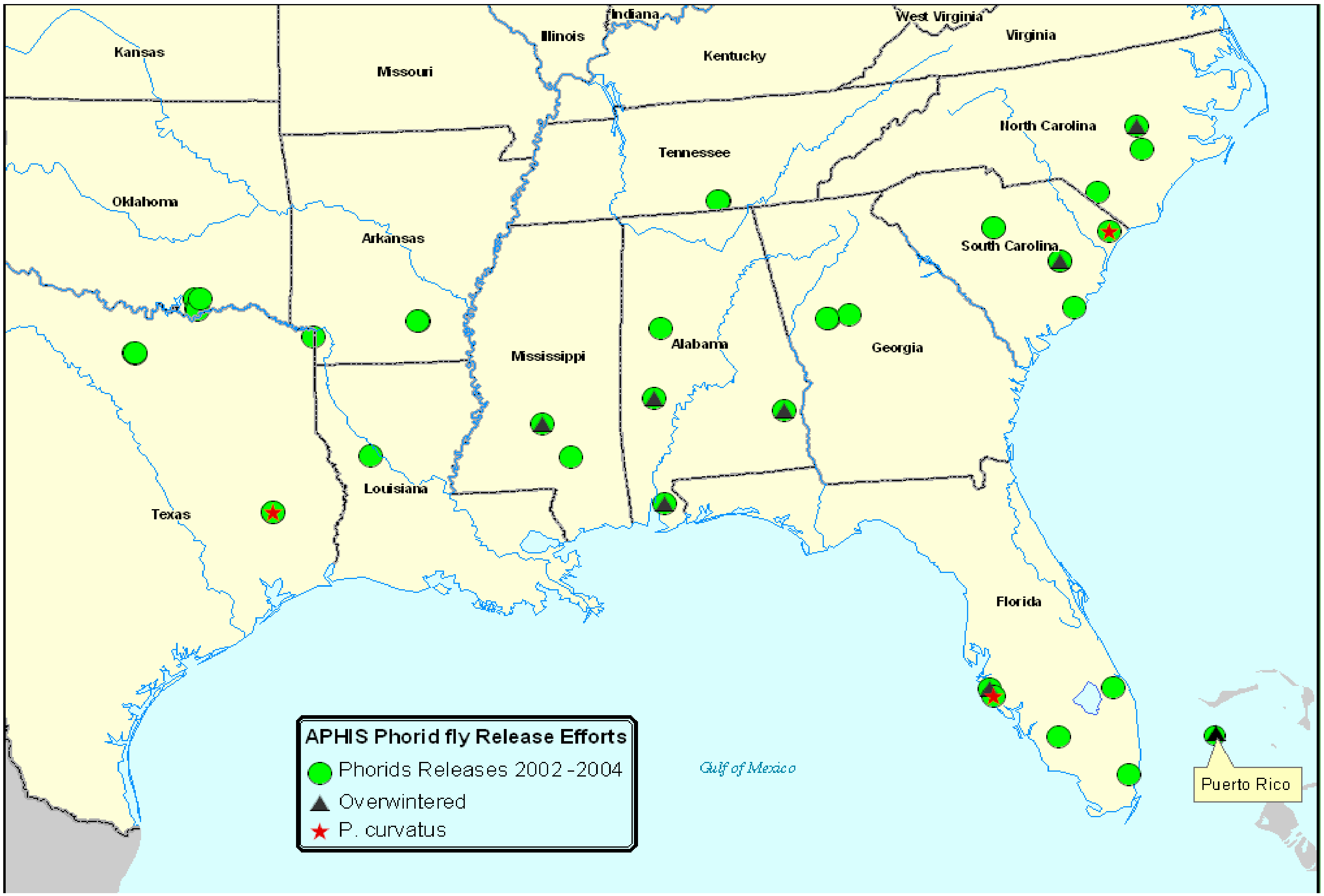
Species	Year	No. flies produced	No. pupae shipped*	No. field releases**	Mean flies/ release	Percent flies field released	Total flies shipped***	Percent flies shipped
P. tricuspis	2002	942,659	58,750	12	4,895.83	6.23	59,385	6.30
	2003	1,625,067	81,450	15	5,430.00	5.01	111,000	6.83
	2004	1,698,942	89,050	9	9,894.44	5.24	115,100	6.77

* approx. no. parasitized pupae shipped; potential flies for release
** does not include multiple shipments to LA for initiating their own rearing facility and NM for research purposes, nor multiple shipments to cooperators for educational purposes or small research projects as flies were available
*** shipped for all purposes, field release, initiate rearing, education, etc.

Species	Year	No. flies produced	Approx. no. shipped*	No. field releases	Mean flies/ release	Percent flies field released
P. curvatus	2002	7,404	0	0	0.00	0.00
	2003	121,316	0	0	0.00	0.00
	2004**	581,097	39,552	3	13,184.00	6.81

* approx. no. parasitized worker ants shipped; potential flies
** does not include one attempted release that was abandoned

Figure 1. 2002-2004 phorid fly releases in APHIS program (multiple releases at some sites; ex. Texas has done 5 releases, 3 at 2 sites in one county and 2 at one site in another county).



CPHST PIC N0: A3M02

PROJECT TITLE: Geographic Information Systems (GIS) Decision Support and Management Program for Monitoring and Evaluation of Beneficial Exotic Arthropod (i.e. phorid fly - *Pseudacteon* spp.) Releases, Establishment, and Spread in Imported Fire Ant *Solenopsis* spp. Populations

TYPE REPORT: Interim

PROJECT LEADER/PARTICIPANTS: Ronald D. Weeks, Ian Winborne, AIMS Project Manager, Center for Integrated Pest Management, North Carolina State University

INTRODUCTION:

APHIS is allocating significant funding to the rearing and distribution of phorid flies to State collaborators for releases in numerous imported fire ant (IFA) infested states and varying habitats (see Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing Project A1M01/A1F01). Spatially explicit factors that affect successful phorid fly releases and establishment include; habitat type, eco-region, land use, previous treatment strategies (poison bait applications), and IFA population densities and social form (mongyne vs. polygyne). GIS (geographic information systems) is a dynamic tool that CPHST can use to organize and compile these factors into an integrated program. This approach can be of immense value in targeting areas for efficient and effective phorid fly releases.

This CPHST program is being developed as a web-based application for delivery to State collaborators as a decision and management system. Currently, two phorid fly species are being released in the APHIS release program, *Pseudacteon tricuspus* and *P. curvatus*. As more phorid species are released, this program will provide regulatory officials a tool to monitor multiple phorid species releases, establishments, and spread. As a clearly defined and well focused project, this GIS program has served as a model for other CPHST survey and monitoring programs (i.e. *Cactoblastis cactorum*). This GIS-Phorid program can be linked with other IFA control strategies or biological control agents, which would allow for estimation of their impact on IFA populations under different management scenarios. There are two components to the GIS project; 1) development of integrated phorid fly tracking/data systems, and 2) a predictive decision and management support program. Each component of the project is structured as a stand alone program with supporting data for the predictive model relying on data from the tracking component. The tracking component of the project has been initiated in the first two years of the multi-year project. The tracking program uses GIS technology to display and organize information on phorid fly releases within states.

Spatial data are collected in two ways; 1) via web-based data entry forms maintained at NCSU's Center for Integrated Pest Management, 2) via handheld data collection units using a GPS (Teletype WorldNavigator® Global Positioning System) attached to an iPAQ® Pocket PC H3955. Additional iPAQ® hardware includes an iPAQ® Expansion Pack Plus® and 256MB SecureDigital® Card. Data are entered into GPS/Pocket PC units via customized application forms running in ARCPAD 6 (ESRI®). Application forms were designed using ARCPAD Application Builder 6 (ESRI®). Data are maintained using ARCGIS 8.3 (ESRI®) software on a Dell® Precision 650 Workstation computer in Gulfport, MS at the Soil Inhabiting Pests Section **http://www.cphst.org/sections/sips/** .

Currently, all data related to APHIS phorid fly releases and surveys are being collected by state cooperators; state agricultural inspectors, university personnel, extension personnel, etc.

Project Highlights:
There are two components to this GIS project; 1) development of integrated phorid fly tracking/data systems, and 2) a predictive decision and management support program. This program will provide regulatory officials a tool to monitor multiple phorid species releases, establishments, and spread

Completed development and posting of web page describing project, http://www.cphst.org/projects/Phorid_monitoring
Completed development and delivery of electronic data entry forms (ArcPad) and PDA/GPS handheld units
Completed 2004 surveys in Mississippi and Alabama using PDA/GPS hardware and ArcPad data collection forms (Figure 1.)
Near-completion of web-based data entry forms – collaborative effort between CPHST and NCSU's Center for Integrated Pest Management
Completed data dictionary and user manual for handheld devices and software
Completed general map of phorid fly releases in southeastern United States (Fig. 1) and detailed map of releases and establishments in Mississippi (Biological Control of IFA, A9M03) and Alabama

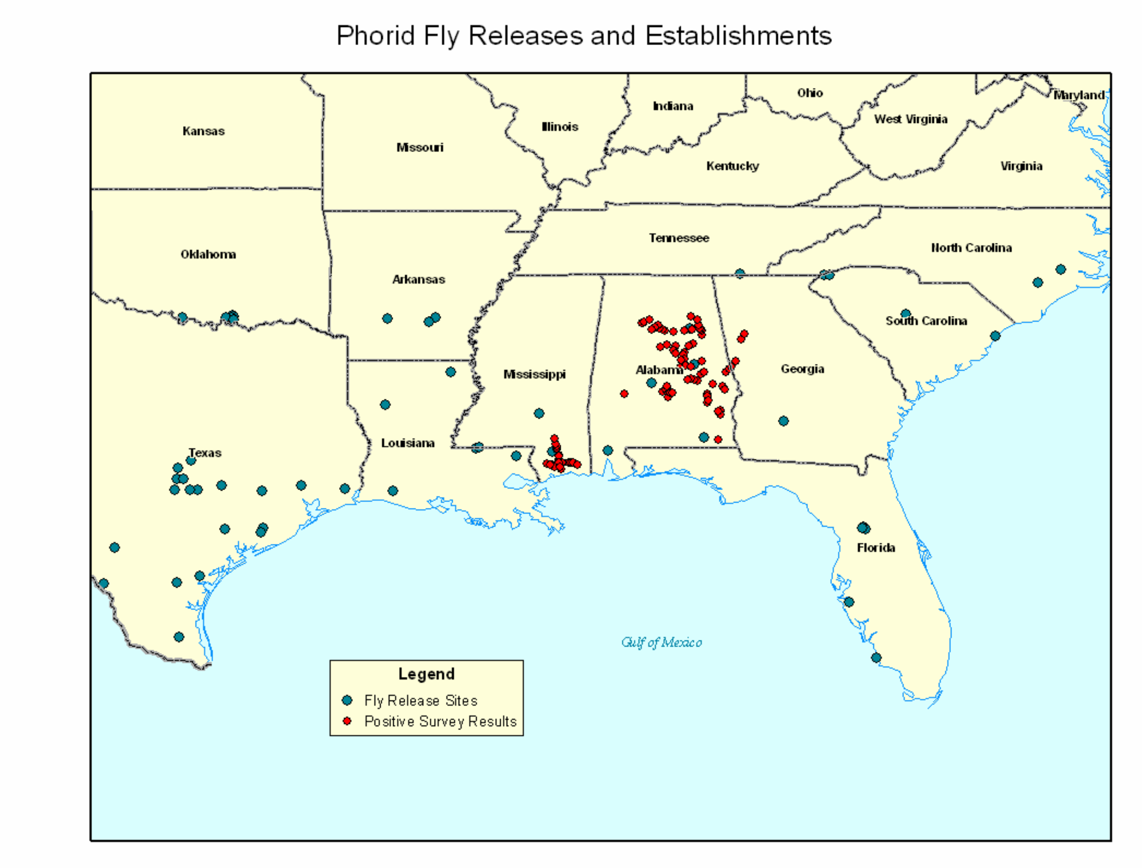


Fig. 1. Map of phorid fly releases and positive survey results in southeastern United States. Includes most APHIS releases from 2002-2004, and additional releases made by Sanford Porter (USDA, ARS, CMAVE, Florida) and Larry Gilbert (University of Texas). Survey data is complete, to date, for Mississippi and Alabama. Surveys were completed in Mississippi and Alabama using handheld data collection devices and GPS technology.

CPHST PIC NO: A9M03

PROJECT TITLE: Mississippi Phorid Fly Release Project

TYPE REPORT: Interim

PROJECT LEADER/PARTICIPANTS: Shannon James, Tim Lockley, Jennifer Lamont, and Sanford Porter (USDA, ARS)

INTRODUCTION:

Imported fire ants are pest of agricultural, environmental, urban and medical import throughout the southern half of the United States. Within their native range in South America fire ants are encountered less frequently, with fewer nest mounds per acre, and with fewer individuals per nest (Porter et al. 1992, 1997). It is speculated that lack of natural controls in the U.S., namely parasites and disease, have been responsible for this difference of abundance between the native and introduced populations of imported fire ants (Buren et al. 1978; Porter et al. 1997; Stimac and Alves 1994). The use of a complex of biological control agents through an integrated pest management program may be a successful long-term management tool for imported fire ant. Dozens of potential biological control agents have been identified in South America, and a few have been imported into United States to determine potential for release.

Species of *Pseudacteon* (phorid flies) are dipteran endoparasites of the *Solenopsis* genera of ants and are widely distributed throughout the *S. invicta* and *S. richteri* native range. Phorids impact fire ants both through parasitic destruction of individual ants and cessation of ant activities when the flies are present (Morrison 1999). Testing conducted by ARS-CMAVE determined *Pseudacteon tricuspis* safe for release in the United States. To assess their ability to establish in the wild, phorids were released by APHIS SIPS in the spring of 2000 in Harrison County, MS. Initial success of this release and others conducted in several IFA-infested states supports the rearing and distribution of these parasites, which is now conducted through the AHIS phorid fly rearing and release project, the activities of which are detailed elsewhere in this annual accomplishment report. Results from the initiation of new releases and continued observation of established releases, as described in this report, will be used to develop and improve methods for large scale release programs.

MATERIALS & METHODS:

A release site near Saucier, MS (Harrison Co.) and a paired control at the Harrison County Work Farm were selected for the study. The sites were ca. 20 km apart. Each site was similar in habitat at the time of the release, consisting of grasslands with deciduous woods and a large pond adjacent. Both the release site and the control site had ca. 100 active mounds per hectare. Conversion of the release site into a pine tree farm in 2001 moved all subsequent checks of the release site to the adjacent roadside, consequently negating the relevance of the control site.

Emerged adult flies of *P. tricuspis*, supplied by S. Porter, were released daily, per the protocol supplied by S. Porter, at the Saucier site on 11 April, 2000 with the final release occurring on 20 April. A total of 2612 phorids were released on 45 separate imported fire ant colonies. The successful establishment of phorids in the first MS release and the development of the AHIS phorid fly rearing and release project have permitted subsequent releases at other locations in southern MS. In August 2002, over 2000 phorids were released on 42 IFA colonies at the Hattiesburg Airport (Bobby Chain Air Field – Forrest Co.). Another 3000 *P. tricuspis* were released in a pasture on October 2003 near Mendenhall, MS (Simpson Co.) in collaboration with the Mississippi Department of Agriculture. These two later releases both followed the open-mound release protocol available online at http://www.cphst.org/projects/Phorid_rearing/ wherein each morning flies are aspirated into vials of 20-30 and enough mounds are opened at the site to accommodate releasing two vials each.

Post-release monitoring is conducted by opening ten IFA mounds at the release site. Mounds are "opened" by removing half a shovel full of nest soil crating an open depression in the nest. Ants are intentionally mashed in this process to increase alarm pheromone release which attracts the flies. Flies at open mounds are counted over a 30-minute period. If flies are present then the process is repeated at sites 200 m along the cardinal points from the original release. If flies are present at these locations, then the distance from the initial site is doubled and checked again. Lack of flies at a remote check site requires traveling half the distance back to the last positive site and checking again. This process is repeated until range is established. A year after release, monitoring for flies is initiated at 2 km away from the release site or at the furthest positive sites from the previous check date. Initial post-release observations are conducted three months later; this is enough time that any flies observed should be second generation or later. Subsequent checks are conducted in spring and fall.

RESULTS:

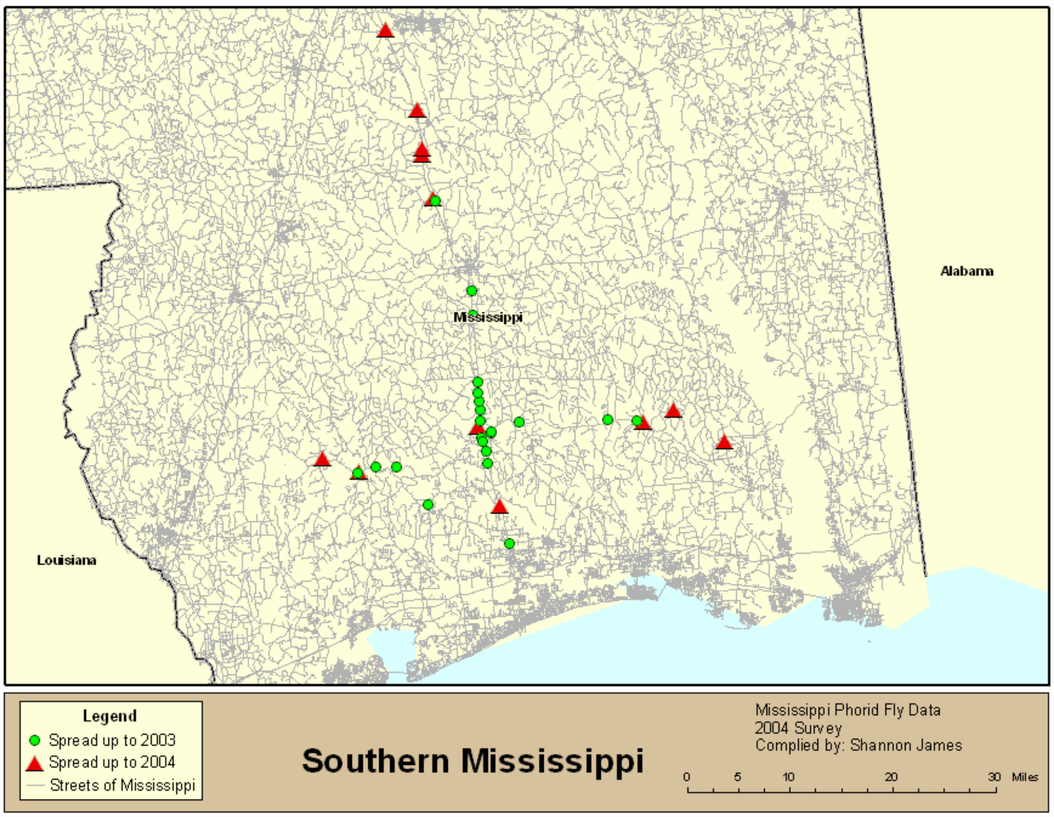
Three months after release all three sites were confirmed to have flies present. Over the past four years the Saucier release maintained fly presence and the area of fly coverage has increased. The most recent observations taken fall 2004 gave visual confirmation of fly spread 62 km north, 31 km east, 12 km south, and 26 km west of the original release site (Figure 1). A radial estimate of area from the rough center of the furthest points yields an area of approximately 4071 km^2. This is more than twice the area estimated based on the furthest points a year prior (1963 km^2)*. Even with the overall increase in territory apparently occupied by the flies, the southern most point of fly activity this year lost 7 km compared to 2003.

After the first monitoring date, no subsequent observations at the Hattiesburg site produced confirmation of fly presence. However, the furthest fly active point north of the Saucier site is at a greater distance than the other points for that site and is only about 9 km from the Hattiesburg release site. It is possible that the Hattiesburg release has been successful in establishing phorids in the area but due to unfavorable elements, such as an almost constant breeze, not at the release

* Distance and area data reported for the Saucier release in the 2003 annual accomplishment report were incorrect. The correct numbers for furthest confirmed fly spread in 2003 are 34 km north, 26 km east, 19 km south, and 21 km west.

site itself. Conversely, more than one hundred phorids were counted during the fall 2004 survey of the roadside at the original Saucier release site. One notable difference between the two locations is the Saucier roadside has a thick row of trees along it which provides a wind break. Similarly four out of five mounds abutting a wooded area at one remote site had phorid activity while the remaining mounds next to open field had none. More detailed data on these environmental factors will be collected in coming observation dates.

Figure 1. Observation sites both original and remote through 2004 for the 2000 Saucier release



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Stimac, J.L. and S.B. Alves. 1994. Ecology and biological control of fire ants. Pp. 353-380. *In* D. Rosen, F.D. Bennett, and J.L. Capinera [eds.], Pest management in the subtropics biological control: a Florida perspective. Intercept Ltd. Andover, Hampshire, UK.

CPHST PIC NO: A1F01/A1M01

PROJECT TITLE: Progress Report of IFA Lab, Gainesville, FL 2004

TYPE REPORT: Interim

PROJECT LEADER/PARTICIPANTS: Debbie Roberts and FL-DPI rearing personnel

It has been a busy year for the IFA lab here in Gainesville, with a few stumbling blocks along the way like hurricanes and surgery. Despite these setbacks we have managed to produce to date 2,012,950 phorids (*Pseudacteon triscupus* - 1,495,318 phorids in nine attack boxes; and *P. curvatus* - 517,632 in five attack boxes). We have pulled and shipped out 114,800 parasitized ant heads this year. This number is only up 2,090 from last year; it encompasses fewer releases, but we have provided greater amounts to the individual cooperators per release (see also CPHST PIC NO: A1F01/A1M01 – Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing and Release Project).

	2004	2003	2002
Field Releases	9	15	12
Fairs/Demos	4	6	3
Research Projects	3	2	-
Box Start-up/Restock	1	1	-
Total Phorids	114,800	111,810	59,385

P. curvatus has proven to be somewhat more difficult to produce. We have increased our boxes to five and plan on converting some of the *tricuspus* boxes in 2005. The *curvatus* seem to require more humidity and fair better in a little warmer temperature. Earlier in the year, Dr. Porter had recommended that we put halogen lights on the box and staple damp towels on the inside of the windows. These towels would have to be watered at least several times a week, if not daily. He assigned one of his technicians to come over every other day and maintain one of the boxes. This lasted for a couple of months, while we "came up to speed" on the rearing.

Shortly after adding the halogen lights to the boxes we saw that it kept the temperature dangerously close to the upper end of the range where we were told could be detrimental. After the lights were turned off, temperatures were monitored and found that under normal circumstances, the rooms would remain within the proper range, and the additional light/heat was too much.

The increased humidity caused by the damp towels created a proliferation of mold in the boxes, on the strings, wood, even in the white pans. The wooden doors where the towels were stapled were particularly susceptible to this mold growth. To counteract this, Debbie Roberts configured a moisture barrier cut out of black plastic sheeting and placed between the wood and the towels. This did inhibit the mold from growing on the doors, but not in the box. Our next effort involved providing additional airspace, by separating the towels. Some improvement was seen, but the strings continually grew a black mold (or mildew) that gave us concern. We were constantly having to remove the towels and washing them with bleach, a very laborious task. With the addition of floor humidifiers, the room's overall humidity was more stable. We decided to remove the towels and monitor both the humidity and temperature closely. It rarely falls below the 80% required to keep the flies "happy".

The two initial boxes were set-up in the main attack room. As we increased, we had to utilize the second attack room, now known as the *curvatus* room. These boxes have had a varying history. While our first *curvatus* box floundered for a long time, it flourished prior to the first shipment of release ants, as did the other two boxes. There is some feeling that the environmental requirements are more easily met in the second room, and that all the *curvatus* should be housed in this area. We have used these three boxes exclusively for our field release phorids, and yet their numbers equal or better those of the other room, but in general it has put a lot of pressure on production.

As for the release of *curvatus*, it has been a period of trial and error in order to fine tune the method of getting these flies into the field. Equipment was supplied by the Gulfport office. Initially the field cooperators were provided with screened vials to capture the ants that would be shipped back to the lab. Moistened cotton balls were to be added to the containers, to prevent dehydration during shipping. Unfortunately, some of the field personnel did not understand the importance of this step and neglected to do it. Several vials of ants came to us dead, or died shortly after arriving.

After a discussion with Dr. Pereira, we decided to utilize the GladLok containers that were for shipping back the ants with a dampened folded paper towel. John Cooper, one of the Texas cooperators came up with the method of poking a stick into the mound, letting the ants crawl up part way and then brushing them off into a shipping container. This "poking" method would continue until a sufficient amount of ants were obtained from each mound. This information was passed onto the remaining field cooperators, and each has adopted this in their own way. Fred Santana of the Sarasota Extension Agency utilizes a pointed piece of bamboo with a fluon collar. He puts the stick into the mound, lets the ants crawl up then he places the stick into a plastic jar with fluon around the top, and raps the stick causing the ants to fall off and become contained.

After the first group of three we received from Texas, we decided rather than putting them right into the attack box, they should be held with water and sugar tubes for a day or two (depending on when we receive them) and then introduced into the attack box. I feel that the stress of shipping and then immediately being faced with the attack of the phorids causes them excessive stress, thereby increasing the mortality rate. Texas mortality rates, although there could be many reasons, ran from 8.5% to 19%. South Carolina experienced a fluctuating mortality rate, which

I'm sure could be attributed to problems during shipping. The final shipment from SC resulted in only 5% mortality, telling me we were "getting it right". Thus far Florida has had rates of 4 and 7% consecutively.

Problem Solving

Much of Debbie Robert's time is spent coordinating, prepping and shipping for the releases, but a good percentage of her efforts go into problem solving. Some of these "problems" are extremely minor, i.e. strings too short in an attack box so either adjust or replace. Other problems are more like challenges that once solved will improve production or lighten the work load.

<u>Trash Flies</u>

We are plagued by the ever-present "trash" or casket flies. They do seem to be seasonal, but never quite die out. At the height of summer, one could look at a plaster tray of spread ant debris and it literally would be moving—from the maggots of the casket fly. Much effort goes into picking these off the trays. We firmly believe that these maggots while they are constantly eating the decaying substrate are also a cause for our precious phorid to be ejected from their protective capsule. So in our war against this enemy we have incorporated the Sticky Aphid Whitefly Trap. I screwed hooks into all the fronts of the holding boxes and we hang these traps, changing them weekly to bi-monthly based upon the abundance of the flies. For awhile we incorporated small wine traps; these seemed to work quite well, except for the mold that would grow rampantly in the cups holding the wine. The wine traps with a little sugar water added, caught the most flies. We gave this up due to our busy schedule, and forgetfulness that lead dried up or moldy containers. If and when the flies get bad again, then we may reinstate this method.

The pulling of maggots and their encapsulated pupae is a very time consuming job. From a suggestion made by of the techs, Debbie Roberts came up with a vacuum system to be used exclusively for this purpose. It has cut the time down tremendously, which is very helpful, as we now have to clear off 42 trays per week.

<u>Nesting Tubes</u>

These 16 X 150mm tubes are first filled with water, then cotton balls, then castone is poured into and lastly must be cleaned prior to usage. These tubes vary in the amount of time that they are usable depending on the habits of the ants. Many ants will burrow into the castone, chewing it up and removing it from the tube. Some eventually work their way into the cotton and can cause the tube to leak, others just find themselves a convenient place to hide and often become irretrievable. As an alternative, Debbie Roberts started working on a castone housing, unlike the one Dr. Porter was attempting to have constructed.

Dr. Porter's design utilized either an oak or Plexiglas base with two castone blocks inset from the top and an eyehook to pick it up. These were going to very cost prohibitive to construct and difficult to use. Ms. Roberts initially came up with a design that utilizes a 12 oz plastic container, like a take-out bowl at a Chinese restaurant. Cut the rim off, and shorten the height of the bowl to about 1.5 inches. Poke two holes in the top and insert a piece of brass wire to form a loop (for picking it up). Cut a couple of larger holes in the top, where water may be poured on to keep the castone damp, providing the ants with much needed humidity in their holding boxes. Trim the edge with metallic duct tape to reduce the sharpness of this exposure, and provide some added firmness. Covering the holes, fill the container with castone to within 0.5 inch of the lip. This provides a high humidity substrate upon which they hang or hide under during the thirty plus days they are shedding dead parasitized ants.

This project is still in the testing phase, and shall be resumed some time next year.

Number 6 Holding Pans

In typical fashion, now that we have found the "perfect" container for housing the ants in during the incubation period, Rubbermaid has discontinued this style of box. The replacement that they are marketing is neither affordable nor workable for out project. Between Amy Croft, Ron Rojas and Debbie Roberts, we scoured catalogs and shopped all over town for a suitable replacement. We finally compromised on a smaller less desirable 1.3 qt container that would be used exclusively for the *curvatus.*

Ms. Roberts had to experiment with different configurations for the screen air holes, but finally arrived at one that would allow the boxes to be stacked five high without offsetting them. These boxes are not square, and setting them any other way would be courting disaster.

They have been in use for several months now for attack boxes A and H. Lately we have noticed a decline in production for these two boxes, and are wondering if there is a link to the smaller size causing a stress to the ants. The materials are exactly the same as the old on, so this can be ruled out as a cause. Comparative experiments are underway on this project.

Insufficient Fluon and Escaping Ant

Many times we have put ants into the pans in the attack boxes, walked away and hours later came back to find the ants crawling all over the inside of the attack box. This can only be attributed to the fact that the fluon failed, either by the initial coverage or the humidity being too high. The latter has only occurred on a couple of occasions, when the humidifiers were stuck and ran constantly overnight filling the whole room a fog.

The coating problem has been looked at several times. Up until now we tried to solve this by better brushing techniques and more or different food coloring in the fluon. While both have improved, nothing has prevented the constant escape problem.

Based upon another experiment to be explained later, just the sides of the white pan were painted with a Krylon Fusion for Plastic paint. Once dried and the aerosols have dissipated, fluon is applied over the darker area. Any flaws in the fluon can now be seen and addressed. The fluon can easily be washed off, and the paint is not affected by scrubbing. There appears to be no adverse affect on the ants, flies or attacking. There will be a little further observation with all pans in one box being treated this way, and once we feel confident that there are no downsides to this method, it shall be incorporated throughout the attack boxes.

Contrasting Color in Attack Pan

After receiving an article on contrasting colors in attack pans from Anne-Marie Callcott (Thead & Streett 2004), a series of experiments were initiated to see if we could incorporate this into our regime. First we wanted to determine what would be the best substrate. In the article, the investigator experimented with three camouflage colors made by Krylon. The paint that he used was a flat. We tried Fusion paint by Krylon (dark green) and the Oak Panel contact paper. Whatever product was picked it needed to be washable, able to stick to the pan through repeated washing and not something that the ants would be able to cling so tightly they would be difficult to remove from the pans. Both of these treatments worked with one exception. The dark green paint was too dark; it proved to be uninteresting or made it too difficult for the flies to see the ants. The contact paper on the other hand proved to be exactly what we were looking for.

Eleven different trials were set up with *P. tricuspus* and twelve with *P. curvatus.* The experiments were set up such that seven pans were left white and seven pans were lined (on the bottom only) with the brown contact paper. These were placed in the box (Attack Box E) alternating white, brown, white, brown. Every time the ants were removed and prior to putting the new ones in, the pans were shifted, so the pan closest to the emergence door and furthest away would change. We did not want the flies to be attracted to only the closest pan over the long run.

At the end of each period of exposure, normally two or three days, the ants were collected, combining all those in the white pans together, and all in the brown into a separate group. These groups would remain separate throughout the length of the experiment. Per the protocol, the dead were disposed of after the first ten days, and then from this point the dead from each group were placed in lined plaster trays and kept in a holding box. Each tray was divided in half, and a physical barrier was placed between the white and the brown groups. This was done to prevent any heads from washing from one side of the tray to the other when watered. Two days after the dead ants were spread, they were counted. Results can be seen in Figure 1 and 2.

Figure 1. Comparative Study of Brown vs. White Pans with *P. tricuspus* attacks.

Date	Brown Pans	White Pans
5/5/04	91	7
5/7/04	364	74
5/10/04	1125	406
5/12/04	308	241
5/14/04	473	222
5/17/04	1058	613
5/19/04	438	233
5/21/04	591	384
5/24/04	1121	742
5/26/04	536	249
5/28/04	707	483

Figure 2. Comparative Study of Brown vs. White Pans with *P. curvatus* attacks.

Date	Brown Pans	White Pans
6/28/04	169	62
6/30/04	691	297
7/2/04	511	163
7/5/04	1124	628
7/7/04	1012	641
7/9/04	255	214
7/12/04	1094	416
7/14/04	775	259
7/16/04	720	428
7/19/04	1184	1072
7/21/04	896	260
7/23/04	421	109

The findings seemed to show a 2:1 difference, with the brown pans more likely to produce parasitized ants. Running statistics on the numbers, using the Tukey Comparison of Means of Parameters by Treatment, the brown pans had a mean of 679.25 while the white pans were 354.86; a significant difference. Further statistical analysis can be seen in Figure 3.

Figure 3. Analysis of Variance Table for Parameters

Source	DF	SS	MS	F	P
Ant (A)	1	81967.8	81967.8	0.90	0.3469
Trt (B)	1	1262757	126757	13.93	0.0006
Residual	43	3897628	90642.5		
Total	45	5242353			

Cases Included 46 Missing Cases 2
Caution: The sums of squares, mean squares, and F-tests are approximate for analysis with missing values. See the manual for details and instructions for constructing exact F-tests.

The next step in this test was to do box to box comparison. We put all brown pans in Attack Box E and all white pans in Attack Box F, and tracked the number of parasitized heads per tray. Results appear in Figure 4. The numbers did not prove that the brown pans, when no other options were available, increased productivity. This study deems repeating.

Figure 4. Comparative numbers between Box E and Box F.

Date	Attack Box E	Attack Box F
24-26 July	1128	1160
27-28 July	640	1064
29-30 July	1780	1172
31 Jul – 2 Aug	1200	1556
3-4 Aug	1512	1343
5-6 Aug	836	972
7-9 Aug	1188	2018
10-11 Aug	680	1152
12-13 Aug	744	1328
14-16 Aug	1464	1512
17-18 Aug	716	776
19-20 Aug	576	1092

References Cited:

Thead, L.G. & D.A. Streett. 2004. Confinement-tray color affects parasitism rates of attacking Pseudacteon curvatus (Diptera: Phoridae) in a laboratory rearing system. Proc. of Annual Red Imported Fire Ant Conf. pp. 142-147.

CPHST PIC NO: A3M01

PROJECT TITLE: Boll Weevil Identification and Forensic Examinations in Support of the Southeast Boll Weevil Eradication Program

TYPE REPORT: Interim

PROJECT LEADER: Robert G. Jones, Ph.D

INTRODUCTION:

The Boll Weevil Eradication Programs were operated for many years by USDA APHIS PPQ. The boll weevil is an introduced pest of cotton from southern Mexico. The present eradication effort started with the 1978 Trial Program in North Carolina and Virginia. This was then expanded into a North and South Carolina Program in 1983.

With the expansion into parts of Alabama, Georgia and Florida this became the Southeastern Boll Weevil Eradication Program (SEBWEP) in 1987. The Southeastern Boll Weevil Eradication Foundation was created during this expansion and was composed of representatives from each of the individual state foundations. By 1997 the Foundation had taken over the management of program operations with technical advice and funding from USDA APHIS PPQ. The SEBWEP now includes all of the formerly mentioned states with Mississippi, Tennessee and Missouri. The other boll weevil eradication programs are run by their individual state foundations in Arkansas, Louisiana, Texas, Oklahoma, Kansas, New Mexico, Arizona and California.

Identification of boll weevils is critical to eradication programs. All chemical control decisions are based on adult boll weevils being caught in survey traps. These traps are baited with an aggregation pheromone lure. While this lure is species distinct it has components found in pheromones of other weevil species. This odor as well as the trap's color and reflective attraction cause numerous species of weevils and other insects to be captured. These trapped insects must be sorted with the boll weevils identified and counted. These boll weevil counts become the data used for program operational decision making. Insecticide treatments resulting from misidentified non boll weevils are environmentally disruptive, expensive and can lead to legal problems. If boll weevils go unrecognized as such the program can have numerous costly problems. This can mean crop damage and area wide insecticide applications instead of a single field being treated.

Forensic examinations of trap caught boll weevils have four areas where it can help in program operational decision making or justification for decisions. (1) Sexing the boll weevils. Normally the male boll weevil comes to the trap in a seasonal pattern. This is early and late in the cotton plant growth cycle. To catch a male in traps during flower bud formation means one of two things, the presence of a large population or the only boll weevil present. The male boll weevil produces pheromone as he feeds to attract others to him. If others do not come he moves to pheromone sources searching for others. (2) The aging of adult boll weevils indicates different occurrences dependent on season or human interferences. For example the presence of teneral adults in traps means there has been reproduction in the immediate area or field. Of course teneral adults in traps before cotton fruiting has started means someone is tampering with the traps. This has been done by both individuals who lost their jobs with the program and growers who needed their fields sprayed for plant bugs. (3) Determining the diapause condition and (4) the reproductive condition of adult boll weevils is done by dissecting specimens. This is difficult with specimens from program traps since they are generally dead and desiccated. On occasion and when large numbers are sent in it has been possible. Boll weevils coming out of and going into the physiological diapause condition indicates problems ahead for programs. The same is true with the presence of both mated and reproductively active females.

METHODS AND MATERIALS:

Identification, sexing and aging adult boll weevils is based on Jones, Robert G. and Michael Williams. 2001. A Field Guide to Boll Weevil Identification. Mississippi Agriculture & Forestry Experiment Station. Technical Bulletin 228.10 pages.

Dissections to determine the physiological diapause and reproductive conditions are based on Brazzel, J. R. and L. D. Newsom.1959. Diapause in *Anthonomus grandis* Boh. Journal of Economic Entomology. 52:603-611 and Burke, H. R. 1959. Morphology of the Reproductive Systems of the Cotton Boll Weevil (Coleoptera, Curculionidae). Annals Entomological Society of America 52:287-294.

Materials include microscope and dissecting tools that were purchased several years ago for this project that was started in 1983. No further materials are necessary in the foreseeable future. SEBWEP pays for the overnight or two day mailing expenses of specimens.

RESULTS AND DISCUSSION:

The numbers of weevils and boll weevils submitted in 2002 and 2003 had been greatly reduced from previous years. The numbers of samples for both years was about 20. This included samples with multiple specimens and individual boll weevils and non boll weevils. The progress of the Program was evident between the two years. In 2002 samples came from several locations in northeast Mississippi, Alabama and Georgia, Tennessee. There was only one boll weevil caught in Alabama in 2003. This was the only boll weevil found in any of the cotton states east of the state of Mississippi during 2003. This was a female boll weevil that had been an adult for over three days. The trap location was at a cotton field west of Mobile, Alabama near the Mississippi state line. The field or area is near Interstate Highway 10 and on the road to a major tourist attraction. The area has a history of reinfestations since it was eradicated in the early 1990's. More information on this is found in "Robert G. Jones and James A. Wilson. 2002. Boll Weevil: Post Eradication Outbreaks in Cotton in the Southeastern United States. Proceedings of the National Cotton Council". The last boll weevil trapped in the area was in June 2002. Since this boll weevil was trapped July 22, 2003 and it was at least 3 days old, it was determined to be a "hitchhiker".

This boll weevil eradication progress has continued during 2004. This was the first year that no non boll weevil specimens were submitted. All specimens in the 10 samples were submitted for forensic examination and were correctly identified as boll weevils. All but one sample came from scattered fields well within 50 miles of the Mississippi River. The single exception came from northeast Mississippi. It was composed of many damaged specimens and parts of specimens. It was not typical of most samples coming directly from the field. Some of the parts did not match up with specimens in the sample. These parts were too small for a trapper to retrieve from traps and the whole sample looked typical of remnants of a collection picked over for the best specimens or in this case almost all specimens. The Program supervisor already had some doubts about the honesty of the trapper and questioned why so many boll weevils showed up where none had been caught for over a year. Our judgment was that some one was tampering with the detection survey.

The western Mississippi cotton fields where boll weevils were trapped in 2004 are all in areas with known historical boll weevil problems. In 1990 a study was initiated in Mississippi by USDA APHIS, ARS, the Cooperative Extension Service and the cotton growers. This was a boll weevil trapping survey using GIS mapping to show the results of a single trap in each 640 acre section of cultivated crop land in every county of Mississippi. The survey started after a record cold December 1989. The following growing season the boll weevil population was at extremely low levels so it was obvious where the best boll weevil habitat was located. This is where the last few 2004 cotton fields with boll weevils are located. This is similar to the eradication efforts of South Carolina where the USDA ARS had studied boll weevil winter survival for years. Those fields where you could historically find boll weevils after the harshest winter were also among the last fields to be eradicated. The few fields in western Tennessee are mostly in or near the suburbs of Memphis. This could mean good habitat for survival but definitely insecticide application problems. These are the last remnants of the boll weevil population in the Southeast.

The progress in Texas is advancing to the point that the sources of boll weevils for reinfestation are disappearing. Texas has two cotton growing areas that are not involved in boll weevil eradication. These are (1) the Lower Rio Grande Valley in south Texas which voted to start an eradication program in 2005 and the northern black-lands in northeast Texas which will have their ballots counted on January 25, 2005. These are the last two cotton growing areas in the United States that have not been in an eradication program. All of northern Mexico as of 2004 is either eradicated or in an active program. The Texas Department of Agriculture is developing a system of fines for quarantine violations. They requested information from Dr. Charles Allen, Texas Boll Weevil Eradication Program Manager and Entomologist, on the cost of reinfestation control efforts. He sent and recommended the previous mentioned publication by Jones and Wilson, 2002. This is the only published information on the cost and source of boll weevil infestations.

This CPHST Project A3M01 was approved for no more than 10% of the lead scientist's time. The best calculation made for 2004 was that it took 5% or less to handle the samples received and fewer samples are anticipated in the 2005 calendar year. While the work of this project may be greatly reduced, the need to do it becomes greater. To verify the eradication of the boll weevil in the Southeastern United States every questionable weevil trapped will need to be examined by someone with recognized expertise.

APPENDIX I - LABORATORY BIOASSAY PROCEDURE

PROTOCOL FOR BIOASSAY OF INSECTICIDE TREATED POTTING MEDIA/SOIL WITH ALATE IFA FEMALES

Introduction: The development of quarantine treatments to prevent artificial spread of imported fire ants (IFA) in nursery stock requires the evaluation of candidate pesticides, dose rates, formulations, etc. The use of a laboratory bioassay procedure for these evaluations provides a rapid and inexpensive means of evaluating the numerous candidates tested each year. Various bioassay procedures have been devised over the years, but the procedure currently used by the USDA, APHIS Imported Fire Ant Laboratory in Gulfport, Mississippi, is described herein. This procedure is a slight modification of the test described by Banks et al., 1964 (J. Econ. Entomol. 57: 298-299).

Collection of test insects: Field collected alate imported fire ant queens are used as the test insect. IFA colonies are opened with a spade and given a cursory examination for the presence of this life stage. Alate queens are seldom, if ever, present in all IFA colonies in a given area. Some colonies will contain only males, others may have few or no reproductive forms present, others may contain both males and queens, while some will contain only alate queens. Seasonal differences in the abundance of queens is quite evident; in the warmer months of the year 50% or more of the colonies in a given area may contain queens. However, in the cooler months, it is not uncommon to find that less that 10% of the colonies checked will contain an abundance of alate queens. Therefore, it is necessary to examine numerous colonies, selecting only those which contain large numbers of alate queens for collection. During winter, ants will often cluster near the surface of the mound facing the sun. Collection during midday on bright, sunny days is highly recommended for winter; whereas the cooler time of day is recommended for hot, dry days of summer. Once a colony (or colonies) has been selected for collection, the entire nest tumulus is shoveled into a 3-5 gallon pail. Pails should be given a liberal dusting with talcum powder on the interior sides to prevent the ants from climbing up the sides of the pail and escaping. Approximately 3-6" head room should be left to prevent escape. An effort should be made to collect as many ants as possible while minimizing the collection of adjacent soil which will contain few ants. Collected colonies are then transported to the laboratory for a 3-5 day acclimation period. The addition of food or water during this short acclimation period is not necessary. Alate queens are collected with forceps after placing a 1-2 liter aliquot of the nest tumulus in a shallow laboratory pan (Figure 1). Again, the use of talc on the sides of containers prevents escape while talced rubber gloves minimize the number of stings experienced by the collector. The forceps should be used to grasp the queens by the wings in order to prevent mechanical injury. An experienced collector can collect 200-300 queens per hour. It is generally advisable to place collected queens in a 500 cc beaker or other suitable vessel containing moist paper towels prior to being introduced into the test chamber.

Test chambers: Test chambers are 2.5" x 2.5" plastic flower pots which have been equipped with a Labstone® bottom. Labstone is generally available through dental supply firms such as Nowak Dental Supplies, 8314 Parc Place, Chalmette, LA 70043 (800-654-7623). The labstone bottom prevents the queens from escaping through the drain holes in the bottom of the pot and also serves as a wick to absorb moisture from an underlying bed of wet peat moss. Ants are susceptible to desiccation so humidity/moisture levels must be optimized. Pots should be soaked in water to moisten the labstone prior to placing potting media in the pots. The peat moss bed should be watered as needed to maintain a constant supply of moisture to the test chamber. Plastic petri dishes are inverted over the tops of the pots to prevent escape from the top of the test chambers (Figure 2). Prior to placing queens in the test chamber, 50 cc of treated potting media is placed in the bottom of each pot. Each test chamber with test media and queens is placed in a tray with a bed of wet peat moss (Figure 3). Due to possible pesticide contamination, test chambers are discarded after use.

Replicates: Traditionally, each treatment to be evaluated is subdivided into 4 replicates; with one test chamber per replicate. Five alate queens are then introduced into each replicate. This protocol is generally used for evaluation of efficacy of insecticides used to treat containerized nursery stock.

New testing of insecticides to treat balled-and-burlapped or field grown nursery stock has required the modification of the traditional replicated testing method for a variety of logistical and biological reasons. Therefore, each project/trial will define the exact queen numbers/test chamber and the number of test chambers per treatment.

Test interval: All evaluations are based on a 7-14 day continuous exposure period. i.e., introduced queens remain in the test chambers for 7-14 days. At the end of the test time the contents of each chamber are expelled into a shallow laboratory pan and closely searched for the presence of live IFA alate queens. Mortality may also be evaluated daily or at other intervals defined by the specific workplan related to each individual project/trial.

Recording of data: Results of each bioassay are entered on the appropriate data form. Conclusions regarding efficacy and residual activity of the candidate treatments are drawn from this raw data.

Time estimates: The time required to conduct a bioassay will vary greatly, dependent upon a number of factors:

1) Availability of queens; supply is primarily influenced by season. More time will be spent collecting queens in winter or during extreme droughts.

2) Number of treatments to be evaluated; e.g., if only a single treatment and an untreated check are to be evaluated only 40 queens/month are needed. Conversely, a test involving 4 insecticides at 3 rates of application (12 treatments + untreated check) will require 260 queens monthly for the duration of the test.

Duration of the trial: A successful preplant incorporated treatment for nursery potting soil must provide a minimum of 12-18 months residual activity in order to conform with normal agronomic practices of the nursery industry. Since some plants may be held for longer periods of time prior to sale, a 24-36 month certification period (residual activity) would be ideal. Therefore, most initial or preliminary trials with a given candidate treatment are scheduled for a minimum of 18 months.

Balled-and-burlapped nursery stock treatments, as well as field grown stock treatments, vary in treatment certification periods from 2 weeks to 6 months. Thus the duration of these trials is generally a maximum of 6 months.

Figure 1. Alate females being removed from nest tumulus.

Figure 2. Single test chamber with test media and alate females with lid.

Figure 3. Set up of bioassay test procedure.