2005 ACCOMPLISHMENT REPORT

SOIL INHABITING PESTS SECTION

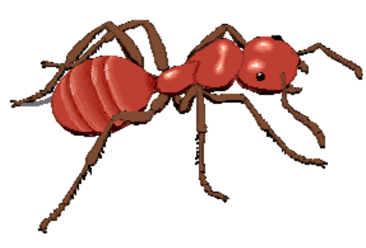
U.S. DEPARTMENT OF AGRICULTURE

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

2005 ACCOMPLISHMENT REPORT

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

Results of insecticide trials are reported herein. Mention of trade names or proprietary products does not constitute an endorsement or recommendation for use by the U.S. Department of Agriculture.

Compiled and Edited by:

Anne-Marie A. Callcott

May 2006

Available online at the CPHST-SIPS website:

http://cphst.aphis.usda.gov/sections/SIPS/

2005 IMPORTED FIRE ANT OBJECTIVES

SOIL INHABITING PESTS SECTION
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 and straw 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

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 35 months. The final 36 month result (data sheet) was lost due to hurricane Katrina.

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 19 months. Bioassays were not set up in months 20 through 23 due cleanup activities associated with hurricane Katrina. The 24 month bioassay indicated an unexpected drop in efficacy especially in the Flowerwood media. The 15 ppm rates were terminated after 24 months (scheduled), the 25 ppm rates will continue to be bioassayed through 36 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	33	34	35	36
DG lite Carrier	10	***	***	***	***	***	***	***	***	***	***	***	***
	12	***	***	***	***	***	***	***	***	***	***	***	***
	15	***	***	***	***	***	***	***	***	***	***	***	***
	25	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(3)	100(4)	100(7)	100(4)	100(4)	***
Sand Carrier	10	***	***	***	***	***	***	***	***	***	***	***	***
	12	***	***	***	***	***	***	***	***	***	***	***	***
	15	***	***	***	***	***	***	***	***	***	***	***	***
	25	100(1)	100(1)	100(1)	100(1)	100(1)	100(1)	100(3)	100(1)	100(5)	100(4)	100(3)	***
Drench	25	***	***	***	***	***	***	***	***	***	***	***	***
	Check												

*** terminated based on IFA quarantine certification period
*** Data sheet lost due to hurricane Katrina

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

Table 2. (cont.) 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)											
		13	14	15	16	17	18	19	20	21	22	23	24
Flowerwood	10	***	***	***	***	***	***	***	***	***	***	***	***
	12	***	***	***	***	***	***	***	***	***	***	***	***
	15	100(1)	100(1)	100(3)	100(4)	100(7)	100(7)	100(10)	***	***	***	***	10
	25	100(1)	100(1)	100(3)	100(1)	100(5)	100(4)	100(7)	***	***	***	***	20
	Check	0	0	0	0	0	5	15					
Windmill	10	***	***	***	***	***	***	***	***	***	***	***	***
	12	***	***	***	***	***	***	***	***	***	***	***	***
	15	100(1)	100(1)	100(4)	100(4)	100(5)	100(7)	100(7)	***	***	***	***	75
	25	100(1)	100(1)	100(3)	100(4)	100(7)	100(7)	100(6)	***	***	***	***	100
	Check	0	0	0	5	0	10	5					

*** Data sheet lost due to hurricane Katrina

*** Bioassays not run due to post-Katrina clean-up

CPHST PIC NO: A9M01

PROJECT TITLE: Effectiveness of Mustard Based Nematicide when used as a Pre-plant Incorporation Treatment for Containerized Nursery Stock, 2005

REPORT TYPE: Final

LEADER/PARTICIPANTS: Shannon Wade, 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 (bifenthrin, tefluthrin or fipronil) 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 (chlorpyrifos, diazinon or bifenthrin) 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. However, actual products with labels for use in the IFA quarantine are limited; therefore we regularly screen potential insecticides for these use patterns.

New insecticides and technologies are routinely tested for efficacy against IFA in nursery stock. Peacock Industries (Hague, Saskatchewan), supplied us with a mustard based nematicide for testing.

MATERIALS AND METHODS:

In discussions with the company, who had observations regarding potential efficacy of this product against IFA, rates of application were determined. The company suggested that 1/16-inch of material broadcast over a mound would either kill or induce the colony to move from the area. That 1/16-inch layer rate was converted into the amount to cover a trade gallon nursery container, and that amount incorporated into potting media for standard testing. A ½-rate was also tested.

Trade 1 gallon nursery containers were potted up at the Gulfport, MS lab with the MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 850 lb/cu yd) on June 1, 2005. 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 2 weeks, 4 weeks and bi-monthly there after standard alate female bioassays (Appendix I) were conducted.

RESULTS:

Bioassays were conducted at 3 weeks and 5 weeks due to scheduling conflicts. Bioassays would have continued every 2 months there after but unfortunately Hurricane Katrina destroyed all of the pots. At 3 weeks and at 5 weeks there was no significant difference in the mortality of the checks and both rates of mustard. At this time there are no plans to test this nematicide any further unless there is continued interest with the company.

CPHST PIC NO: A9M01

PROJECT TITLE: Residual Activity of DuPont DPX E2445 as a Containerized Drench Treatment

REPORT TYPE: Final

LEADER/PARTICIPANTS: 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 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.

DuPont DPX E2445 is a candidate pesticide for use as a drench treatment.

MATERIALS AND METHODS:

On June 7, 2005 untreated MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 850 lb/cu yd) was placed in 1-gallon nursery pots and drenched with 400ml finished solution at a rates of 10, 25, 50, and 100 ppm. The pots were weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week. At 2 weeks and thereafter at monthly intervals, sub samples were taken from 3 pots of each treatment and composited and subjected to standard alate queen bioassay (Appendix I).

RESULTS:

The trial was scheduled to run for six months. However, it was terminated after 2 months due to a lack of sufficient efficacy. None of the treatment rates exhibited greater than 70% mortality during the trial and decreased efficacy was noted over time. Results are summarized in table 1.

Table 1. Residual Activity of DuPont DPX E2445 as a Containerized Drench Treatment

Rate of Application (ppm)	Mean % mortality to alate females at indicated post-treatment interval		
	2wks	1 month	2 months
10	70	40	25
25	70	45	35
50	60	55	50
100	50	70	45
Check	10	5	5

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

LEADERS: Shannon Wade, Lee McAnally and Anne-Marie Callcott

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 together to make 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 each treated layer of the 3-layer plastic system. Three different types of sample pots were provided. One with the outside layer only treated, one with the inside layer only treated, and one with the inside and outside layers treated. Therefore, the inside and outside treated containers contain twice as much active ingredient than the single layer treated containers. 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. A 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 18 months after potting up, the only containers still working well are the outside only treated pots (Figures 1 and 2). At 2 months the outside only treated pots had >500 ants living in them at the end of the 7 days evaluation period. At 4 months the same pots would have ants living in them during the evaluation period, but would move out before the end of the 7 days. But, by 10 months the outside treated pots have basically excluded ants, and continue to do so through 18 months.

Even at 2 weeks the inside only treated pots had 500 or more ants living in the pots at the end of the 7 days (Figure 3). And after 6 months the efficacy of these pots significantly declined. Through 18 months these pots have continued to do poorly. By weeks end one or more of the treated pots have had half of the ants living in them and the other half living in the check pots.

Initially, at 4 months after potting up the inside/outside treated pots seemed to be the only ones keeping fire ants out through the 7 days, with only one pot at 2 months containing ca. 100 workers. But around 10 months these pots had ants move into them by the middle of the weeklong evaluation period, and then move out by weeks end. And at 15 months 2 of the 3 pots had around 100 ants living in them by the end of the 7 days.

This trial was interrupted by Hurricane Katrina. However, we did not lose any containers and we will continue to evaluate these pots every other month.

Outside only treated | Inside only treated | Untreated

DISCUSSION:

These results indicate that treating the inside layer only of containers with 1% permethrin does not exclude IFA, probably due to the ants being able to avoid or limit contact with the inside of the container. While ants did infest the outside only treated containers early in the trial, we speculate they were able to infest the containers by pulling "untreated" media from the container around the drain holes (similar to the inside-only treated picture above), allowing the ants to enter and exit the container while limiting contact with the treated plastic. However, over time the media in the drain hole area may take on some chemical through leaking, thus providing the extra barrier needed to exclude IFA from the container. Unfortunately, this extended period of time required to achieve exclusion is not acceptable under the IFA Quarantine. The inside/outside treated containers showed good results through 12 months, but the break in efficacy at 15 months is of concern.

Previous testing showed that containers treated with 1% permethrin impregnated throughout the plastic were effective in excluding IFA for 16-18 months, depending on the definition of infested (less than 25 or less than 50 workers). Data from those trials completed in 2002 was more consistent than data generated by the containers treated by the "new" 3-layer system reported on here. While this application method of treating containers is under consideration for use in the IFA Quarantine, the results from the new 3-layer containers does cause concern. A 1% permethrin impregnated container contains more active ingredient than a container with 1/3 or 2/3 of it's plastic impregnated with 1% permethrin. This may be enough to cause the prolonged time to achieve efficacy and the shorter effective time period. Thus, this technique is under review and will be actively discussed with the cooperating company.

Figure 1. Percentage of containers infested with IFA (>25 workers) at indicated time after potting up.

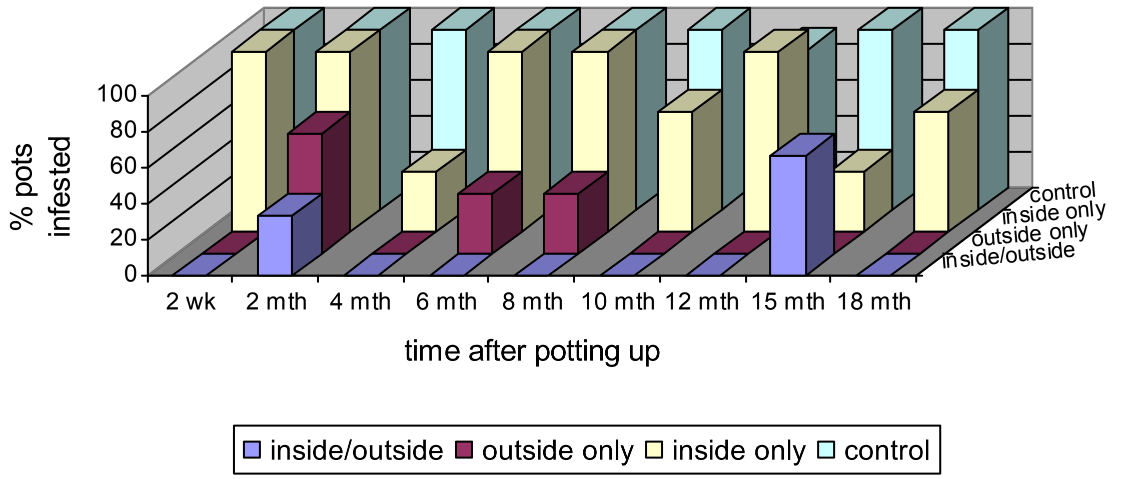


Figure 2. No. workers infested each replicate at indicated time after potting up for outside treated only containers.

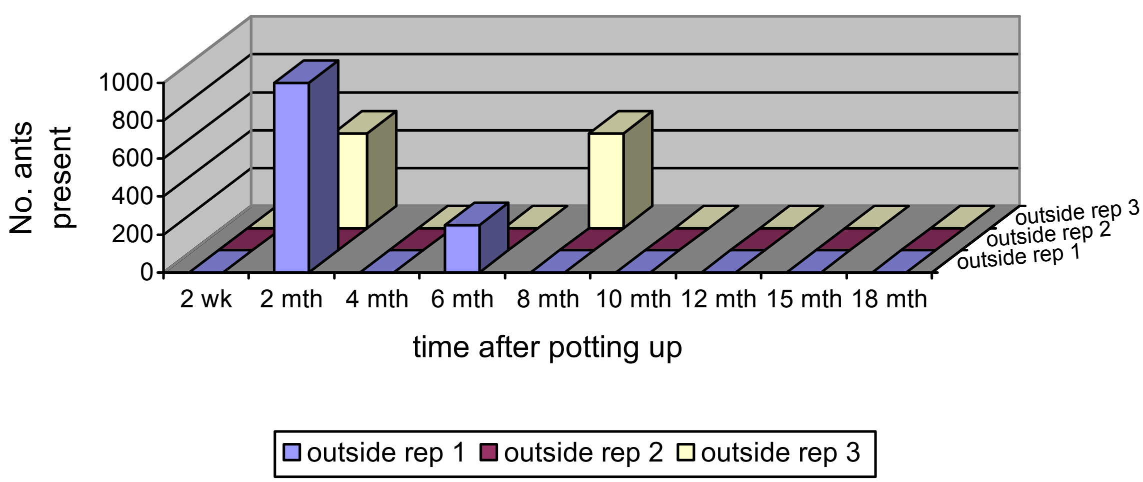


Figure 3. No. workers infested each replicate at indicated time after potting up for inside treated only containers.

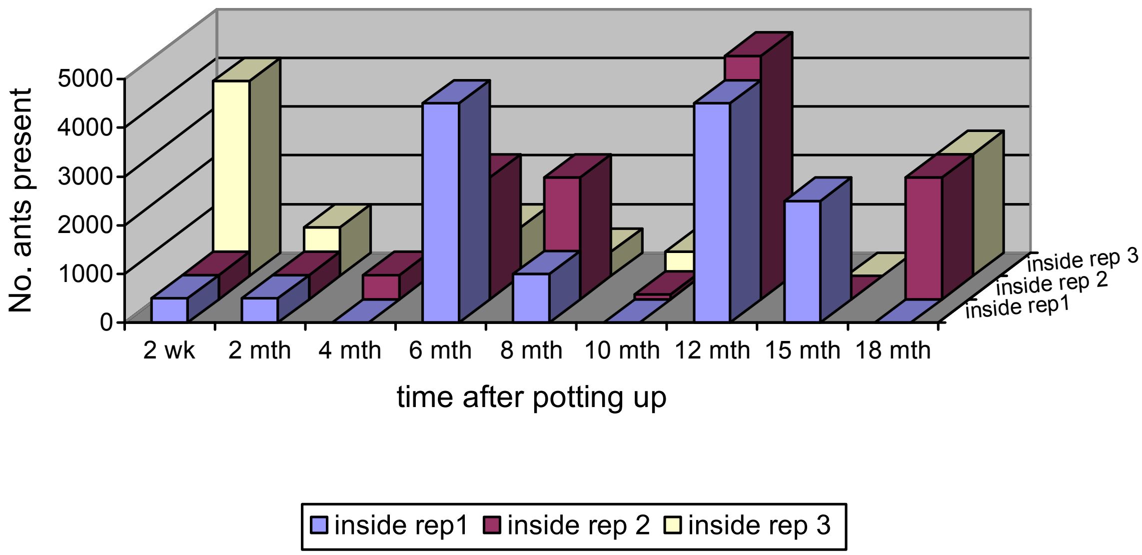
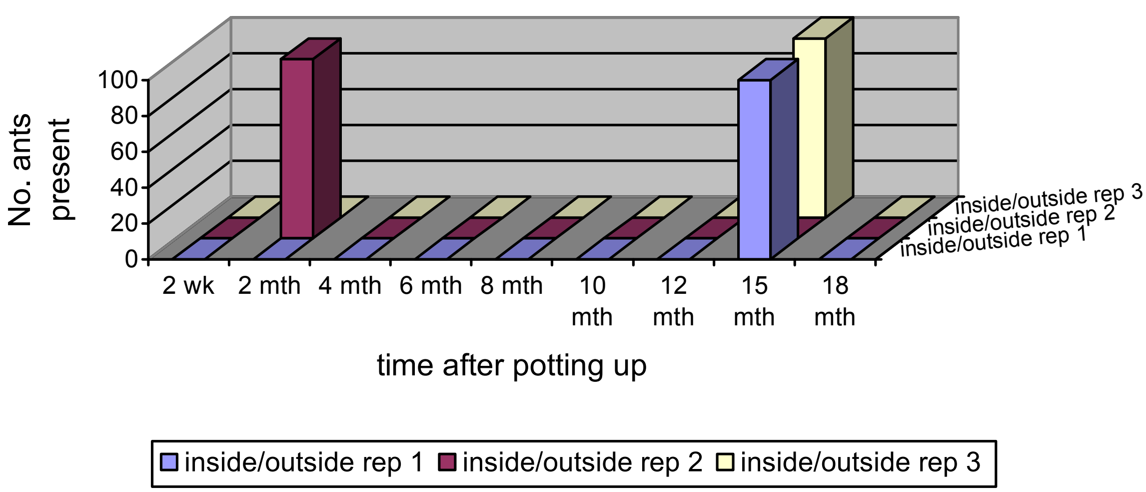


Figure 4. No. workers infested each replicate at indicated time after potting up for inside and outside treated containers.



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

LEADER/PARTICIPANT(s): Anne-Marie Callcott, Lee McAnally, Jennifer Lamont; Physical Scientist – 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.

In an effort to determine the effect of media moisture content at the time of mixing, Windmill media was treated at the 10 and 25 ppm rate with granular bifenthrin as described above. One set for each rate was mixed dry and one set for each rate had water added to the media in the mixer immediately prior to the addition of the granular bifenthrin on February 14, 2005. Samples were taken on the same schedule listed above.

RESULTS:

Results are summarized in Table 1. This test was prematurely terminated due hurricane Katrina. The data generated by the limited sampling was inconsistent and highly variable, and no significant conclusions can be formed with this data. As a result, a new trial will be initiated in 2006 incorporating lessons learned about the sampling and mixing procedures. In hindsight, the water added to the media in the mixers should have been mixed more thoroughly prior to the addition of the granular bifenthrin to allow a more uniform moisture content. In addition, testing in the laboratory to determine importance of moisture content on even distribution of the insecticide in the media and on sampling techniques will be conducted in conjunction with field conditions mixing and sampling.

Table 1. Chemical Analysis and Bioassay Mortality for Bifenthrin Incorporated into Various Potting Media and Aged

Soil Type	Treatment Rate (ppm)	Months Post-treatment	Mean Dry Wt. ppm (n=4)	Standard Deviation	Mean % mortality in queen bioassay
MAFES	25	27	19.28	1.82	100
		30	36.375	1.97	100
		36	12.025	0.40	**
Flowerwood	25	11	9.4	0.69	100
		17	3.5	0.26	100
		24	<0.9*	NA	20
Windmill	25	11	21.36	1.65	100
		17	14.325	1.52	100
		24	<0.9*	NA	100
MAFES	25	0	27.09	6.94	***
		6	25.925	1.78	100
MAFES	10	0	10.61	3.07	***
		3	21.05	1.97	100
		6	8.875	1.16	100
MAFES	Check	0			
Windmill	25	0	6.93	1.96	***
		6	4.7	1.97	95
Windmill	10	0	5.83	5.07	***
		3	7.325	1.33	100
		6	24.05	2.63	65
Windmill	Check	0			
Windmill Wet	25	0	47.975	6.85	100
		3	22.025	1.96	100
		6	19.625	0.30	**
Windmill Dry	25	0	20.83	12.77	100
		3	9.725	2.82	100
		6	22.975	5.38	**
Windmill Wet	10	0	13.825	1.80	100
		3	7.725	1.05	100
		6	7.55	0.59	**
Windmill Dry	10	0	4.50	4.19	40
		3	9.525	1.01	95
		6	5.825	0.68	**

* results below detectable limit of 0.9 ppm
** Data sheets lost due to hurricane Katrina
***Bioassay not run at 0 months post-treatment

CPHST PIC NO: A1M04

PROJECT TITLE: Alternative IFA Quarantine Drench Treatments for Balled-and-Burlapped Nursery Stock, Fall 2003 and Spring 2004

REPORT TYPE: Final

LEADER/PARTICIPANT(s): Shannon James, Lee McAnally, Anne-Marie Callcott; Jason Oliver, Sam Dennis, and Nadeer Youssef of Tennessee State University; Michael Reding and Jim Moyseenko of USDA-ARS

INTRODUCTION:

APHIS is responsible for developing treatment methodologies for certification of regulated commodities, such as field grown balled-and-burlapped nursery stock (B&B), for compliance with the Federal Imported Fire Ant Quarantine (7CFR 301.81). Current treatments for field grown stock are inefficient and limited to a single insecticidal ingredient, chlorpyrifos. Furthermore, restrictions on this insecticide 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.

Current certification options for harvested B&B stock are immersion in a chlorpyrifos solution (dipping) or watering twice daily with a chlorpyrifos solution for three consecutive days (drenching). Likewise, the only approved post-harvest treatment for Japanese beetle (*Popillia japonica* Newman) in B&B requires dipping in chlorpyrifos. Since both pests are a concern for the Tennessee field-grown nursery industry, the trials detailed in this report were conducted in cooperation with personnel at the Tennessee State University Nursery Research Center (TSU-NRC) and the USDA-ARS Horticultural Insects Research Laboratory, Wooster , OH with the goal of determining treatments useful against both pests.

Standard testing of chemical treatments for both application methods has been through female alate bioassays on soil core samples from the treated root balls (Appendix I). Erratic results from soil core bioassays for drenches conducted in 2002 and spring 2003 indicated insufficiency in either pesticide application or the mode of testing. The certification drench for B&B does not specify the volume of solution to apply per plant but instead requires application to the point of runoff. Enough pesticide solution to equal $^{1}/_{5}$ th the volume of the root ball, an adaptation of the treatment volume for containerized plants, was used in these trials in order to standardize the amount each root ball received. However, when dry, the burlap on the root balls appeared to restrict penetration by liquids and runoff was achieved very quickly. Thus this amount may not have adequately covered or penetrated the surface of the root balls. The fall 2003 and spring 2004 drench trials applied in Tennessee therefore focused on application volume to address the question of amount of solution required to cover and penetrate root balls.

MATERIALS and METHODS:

B&B plants with 25-inch diameter root balls were drenched twice daily over three consecutive days for each treatment in drench trials initiated in October 2003 and March 2004. Insecticidal solutions were prepared in 30-gal drums with polypropylene liners and pumped through a hose attached to a shower-headed nozzle using a Shur-Dri battery-powered pump (Figure 1). Pesticide treatments were applied at the same final rate in one of two solution volume treatments. The 1X volume was applied in amounts of 2.56 ℓ totaling 15.36 ℓ per plant by the end of treatment. Each application out of the six total applications in the 1X regimen was equivalent to 1/30 the total root ball volume (Appendix II; larger 25-inch root ball dimensions used) and sufficient to achieve runoff. The 2X volume was twice the amount of solution at half the rate of active ingredient giving a final available amount of chemical equal to the 1X volume but presented in a total of 30.72 ℓ of solution. All products were tested at both the 1X and 2X volumes, with the exception of imidacloprid which was tested only at the 2X volume. Products and rates used were as follow:

Product	Active Ingredient	Rate (lb a.i./ 100 gal H_2O)
Flagship 25WG ™	Thiamethoxam	0.260
Dursban TNP ™	Chlorpyrifos	2.000*
Talstar Lawn & Tree Flowable ™	Bifenthrin	0.230
Scimitar GC™	Lambda-cyhalothrin	0.034
Marathon 60WP ™	Imidacloprid	0.400
Control	----	0.000

* The rate used for chlorpyrifos treatments (2.0 lb ai/100 gal H_2O) is the rate required for the U.S. Domestic Japanese Beetle Harmonization plan. The IFA quarantine rate is much lower at 0.125 lb a.i./100 gal H_2O.

Figure 1. Drench application

Figure 2. Soil core sample collection from the "top" of the root ball.

After final treatment, the plants were maintained outside to weather naturally. Four replicate plants were selected out of the twenty root balls in each treatment group at 0.5, 1, 2, 4, and 6 months after final treatment for soil core sample collection. Three locations, top-surface, base-surface, and middle, were sampled on each plant to explore coverage and penetration. The two surface samples were collected from within the first four inches and the middle sample was collected at depths between four and eight inches. The "top" of the root ball was the upper most surface during treatment application (Figure 2) not the surface the tree trunk protruded from, and the base was the surface the root ball rested on during treatment. The top side was marked with orange spray paint after treatment completion so the correct sides could be sampled through the duration of the trial. After collection, the samples were frozen and sent to the CPHST-ANPCL Soil Inhabiting Pests Section in Gulfport, MS where they were utilized in standard alate queen bioassays (Appendix I). Each soil sample was split into four sub-samples and five alates were tested per sub-sample (Figures 3 & 4). Results were analyzed by ANOVA using JMP 5.1 (SAS Institute Inc., Cary, NC). Means were separated using Tukey's HSD at $\alpha = 0.05$.

Figure 3. A tray of alate mortality bioassay cups

Figure 4. Orange circles indicate the locations of clusters of female alates within this bioassay cup.

RESULTS and DISCUSSION:

The fall 2003 and spring 2004 trials were intended to be replications of the same experiment, so in an initial examination all data from both trials were analyzed together. Overall the 2003 trial had slightly higher mortality than the 2004 trial ($F = 8.4878$, $df = 1$, $P = 0.0037$), but, when examined in conjunction with treatment, only imidacloprid (Marathon 60 WP) and to a lesser extent thiamethoxam (Flagship 25WG) indicated a difference due to trial (trial x treatment interaction $F = 4.0244$, $df = 10$, $P = 0.0001$).

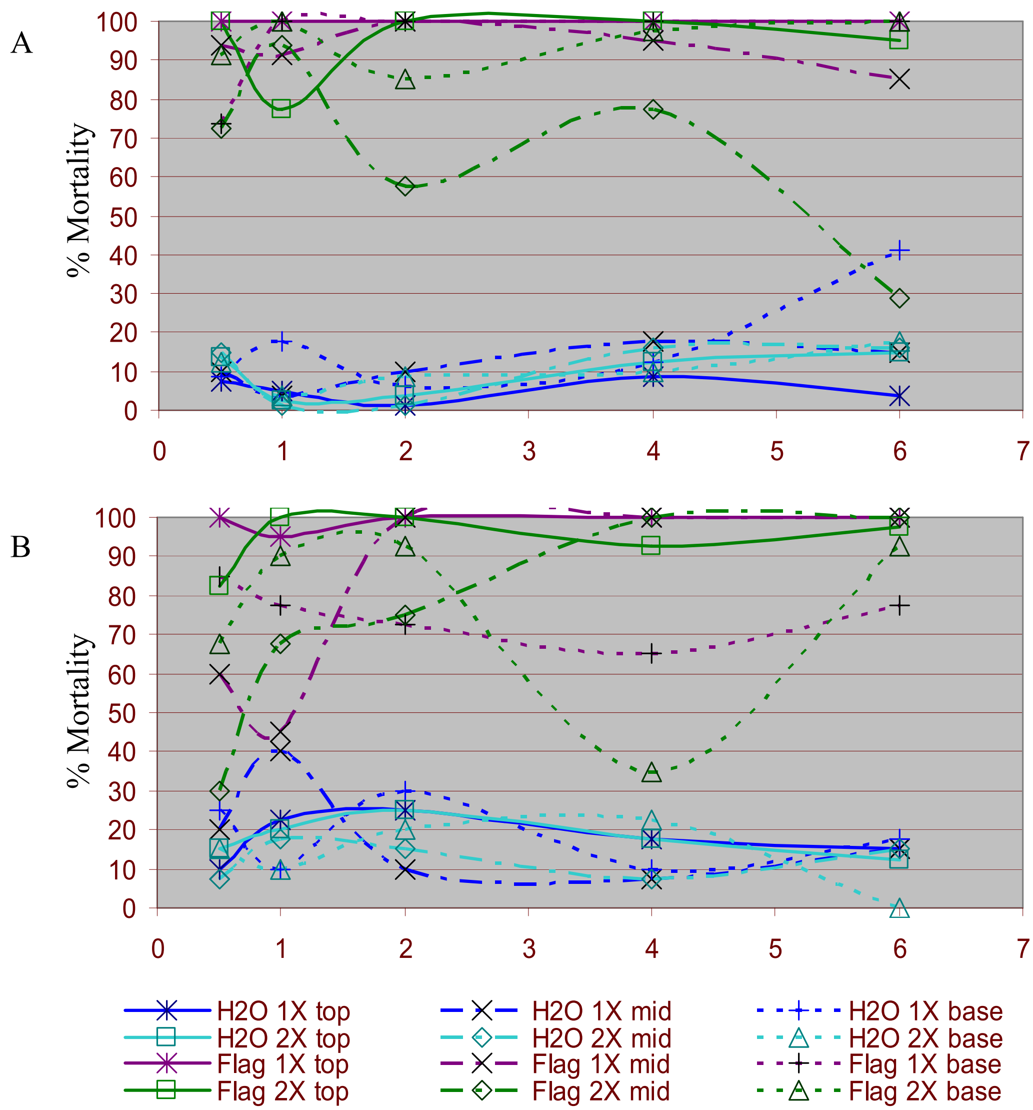
The influence of sample site in the general model indicated top samples were significantly more lethal than the middle or base samples ($F = 26.4258$, $df = 2$, $P = 0.0001$). Also, when the treatment mean of a sample site deviated from the other two sample sites within a treatment or fluctuated over time, it was usually either the middle or base sample (Figures 5 – 9). Considering top samples came from the surface of direct application, it was not surprising that in general they would exhibit higher mortality over time. However, it was interesting that the middle samples, which were collected further from the surface, frequently displayed higher mortality than base samples in the same treatment at the same date. When the combined effect of sample site x treatment was considered ($F = 3.5603$, $df = 20$, $P = 0.0001$) individual treatments had different relationships between sites, and lambda-cyhalothrin (Scimitar GC) and thiamethoxam further displayed differences in the mortality within a sample site across application volumes.

Treatment ($F = 255.9165$, $df = 10$, $P = 0.0001$), date x treatment ($F = 2.0253$, $df = 40$, $P = 0.0002$), and trial x date x treatment ($F = 2.1464$, $df = 40$, $P = 0.0001$) all were significant effects. All pesticide treatments provided significantly higher mortality than the water control applications. Different application volumes of the same chemicals performed similarly in the general model with the exception of thiamethoxam. Over the six month sampling period of the experiment both the 1X and 2X volumes of bifenthrin (Talstar Lawn & Tree Flowable), chlorpyrifos (Dursban TNP), and thiamethoxam had similar relationships among sample dates within treatment and were similar across volume within chemical at the same date. Lambda-cyhalothrin at the 1X volume was significantly more effective at two weeks than at any other sample dates. However, the effectiveness of lambda-cyhalothrin at the 2X volume at two weeks was similar to that at four months but significantly higher than at two months. Likewise, imidacloprid samples at two weeks were similar to those at six months but significantly better than the four-month sample results.

Due to the complex web of effects present in the whole experiment analysis and the importance of the effect of insecticide used, further analysis was conducted comparing all treatments within a single chemical with the water controls. Further discussion of results is divided by chemical for clarity.

Thiamethoxam (Flagship 25 WG), especially in the 2X application, displayed great disparity among sample sites and inconsistency across time (Figure 5 A & B). The lack of cohesion among sample site results was generally higher in the spring 2004 trial than the fall 2003 trial, though both displayed significant difference ($F=6.1324$, $df=2$, $P=0.0024$), and the 1X application was more lethal and more cohesive across sample sites than the 2X application (treatment: $F=662.6418$, $df=3$, $P=0.0001$; treatment x site x date: $F=1.5582$, $df=24$, $P=0.0474$). Top samples for both 1X and 2X treatments in both trials continued to exhibit complete or nearly complete control of IFA through the final sample date at six months. With the exception of the mean mortality for 2X at two weeks in spring 2004, all drops in top sample means were due to surviving ants in soil from one replicate, and samples from those replicates with survivors displayed 100% kill at later sample dates (non-repeating). Since top samples came from the surface of direct application where surface coverage should have been complete, this indicates the collected soil in these samples extended beyond the depth of treatment penetration. Middle samples for 1X and 2X in both trials, however, frequently had several replicates with surviving ants suggesting that thiamethoxam does not easily penetrate to the central depth from which the middle samples were collected, regardless of volume of solution applied. Base samples in the spring 2004 trial also showed multiple replicates with surviving ants which may indicate either a lack of penetration or incomplete coverage for the side the plants rested on during treatment.

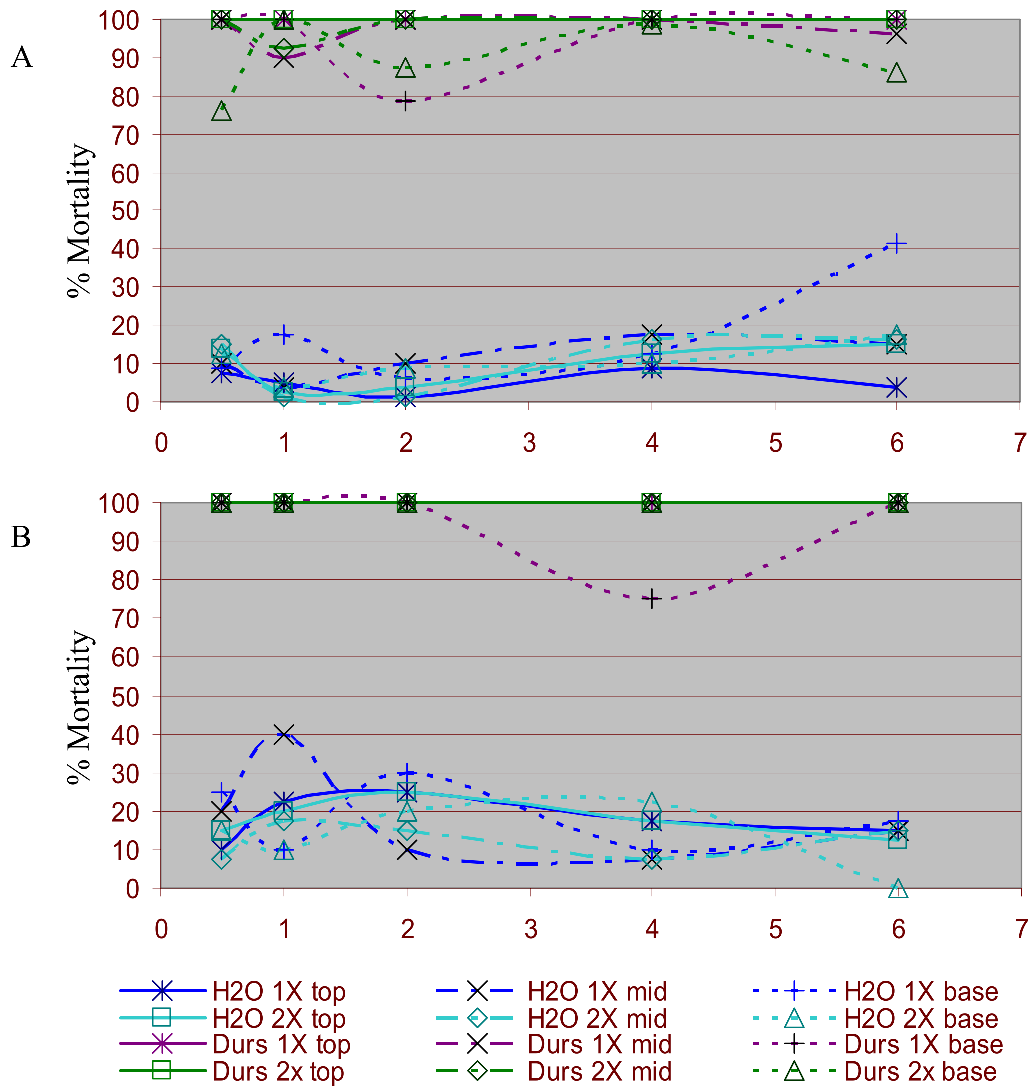
Figure 5 A & B. Comparison of efficacy over time of thiamethoxam (Flagship 25WG) treated soil core samples and water treated soil core samples in controlling female alate IFA across three sample sites and at two application volumes. Figure 5 A illustrates the fall 2003 applications and 5 B the spring 2004 applications.



Among chlorpyrifos (Dursban TNP) treatments across both trials, the top soil samples stayed at 100% kill through the six month trial duration (Figures 6 A & B). Greater frequency of deviation among samples occurred in the fall 2003 trial, with most of that due to frequent fluctuation in results for the base samples of the 2X application. Significance in the trial x site x date combined effect ($F=2.5902$, $df=8$, $P=0.0092$) indicates that the only difference occurred between the fall 2003 and spring 2004 middle samples at one month and the fall 2003 and spring 2004 base samples at two months. Otherwise, no statistical differences existed among the chlorpyrifos samples regardless of sample site or application. These results were not wholly unexpected, since all deviations in the mean for this chemical were due to single non-repeating low mortality replicates while all other replicates at the same sample date yielded 100% mortality. Furthermore, the chlorpyrifos treatments were applied at the Japanese Beetle

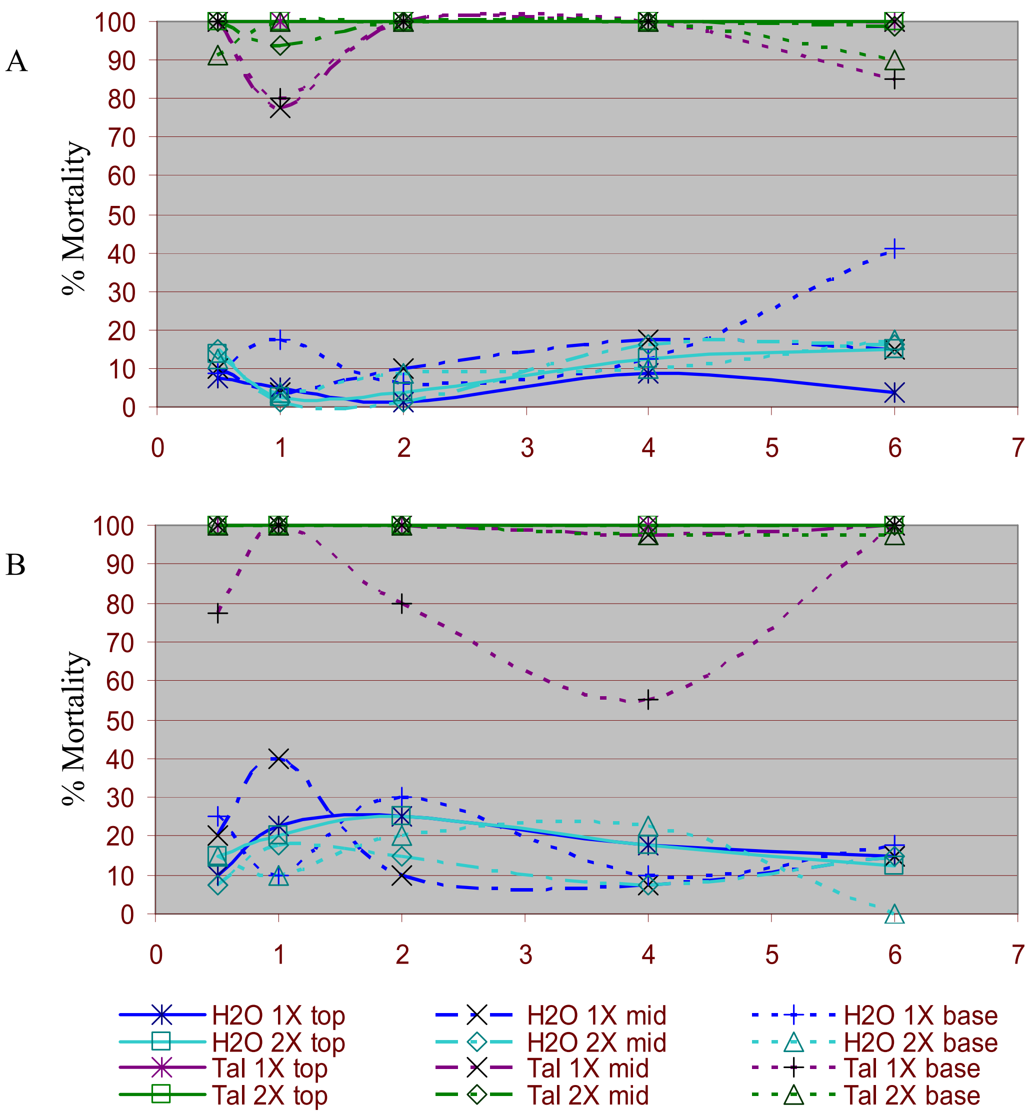
Harmonization Plan dip rate of 2 lbs a.i. per 100 gal of water which greatly exceeds the IFA rate of 0.125 lb a.i. per 100 gal water.

Figure 6 A & B. Comparison of efficacy over time of chlorpyrifos (Dursban TNP) treated soil core samples and water treated soil core samples in controlling female alate IFA across three sample sites and at two application volumes. Figure 6 A illustrates the fall 2003 applications and 6 B the spring 2004 applications.



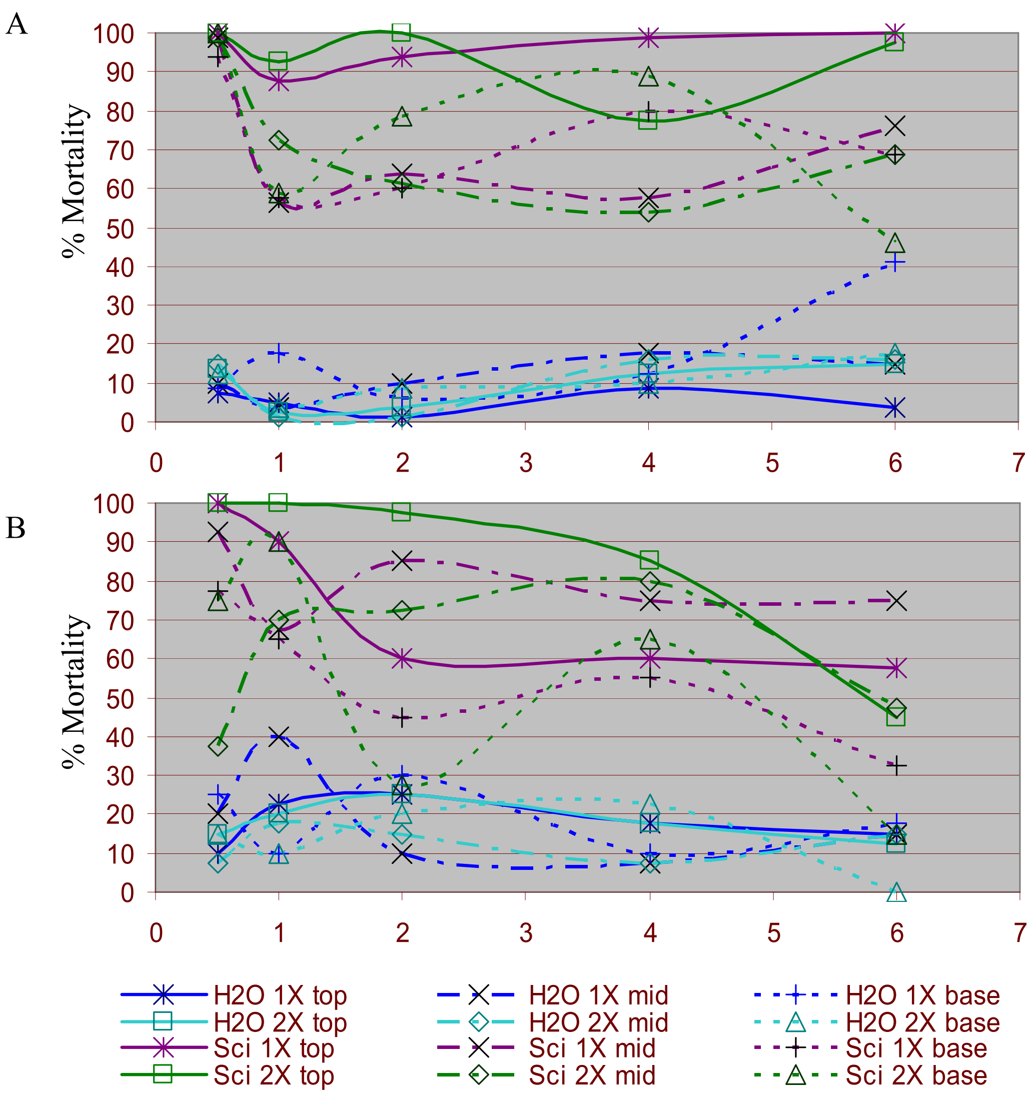
Bifenthrin (Talstar Lawn and Tree Flowable) treatments at both volumes provided complete control at the top sample location at all sample dates in both trials (Figures 7 A & B). With the exception of the one-month samples in the fall 2003 trial, middle sample results produced IFA mortality at 95 to 100%. The 2X volume treatment base samples maintained control at 90 to 100% in both trials while 1X base results were significantly worse than other site x treatment combinations (F=4.0880, df=6, P=0.0006) mostly due to performance in the spring 2004 trial (trial x date x site x treatment F=1.9006, df=24, P=0.0072). While most apparent points of low efficacy on the graphs in figure 7 were due to single replicates with survivors, the low means at the one-month sample date in fall 2003 had multiple replicates with surviving ants both in 1X and 2X as did the lowest point for the 1X base in the spring 2004 trial. The 2X volume appears slightly better than the 1X in coverage and penetration for bifenthrin; however, this improvement is not statistically significantly and under treated samples (cold spots) still occurred.

Figure 7 A & B. Comparison of efficacy over time of bifenthrin (Talstar Lawn and Tree Flowable) treated soil core samples and water treated soil core samples in controlling female alate IFA across three sample sites and at two application volumes. Figure 7 A illustrates the fall 2003 applications and 7 B the spring 2004 applications.



Lambda-cyhalothrin (Scimitar GC) lacked cohesion across sample sites at individual sample dates regardless of application volume (Figure 8 A & B). Top samples were significantly more likely to kill the test ants (F=4.6078, df=6, P=0.0002) than middle or base samples, and the lower mean mortality seen in the middle and base samples usually was due to several replicates with surviving ants at each sample date. The two-week samples for all sites in the fall 2003 trial and the top samples for both volumes at all sample dates in the fall 2003 trial had at most one replicate with surviving ants at each sample date. This suggests that the highest potency of this chemical was at the top sample site and that the other sites declined rapidly after the two-week sample. However, at least one or more replicates among the middle and base samples at each date still produced 100% mortality.

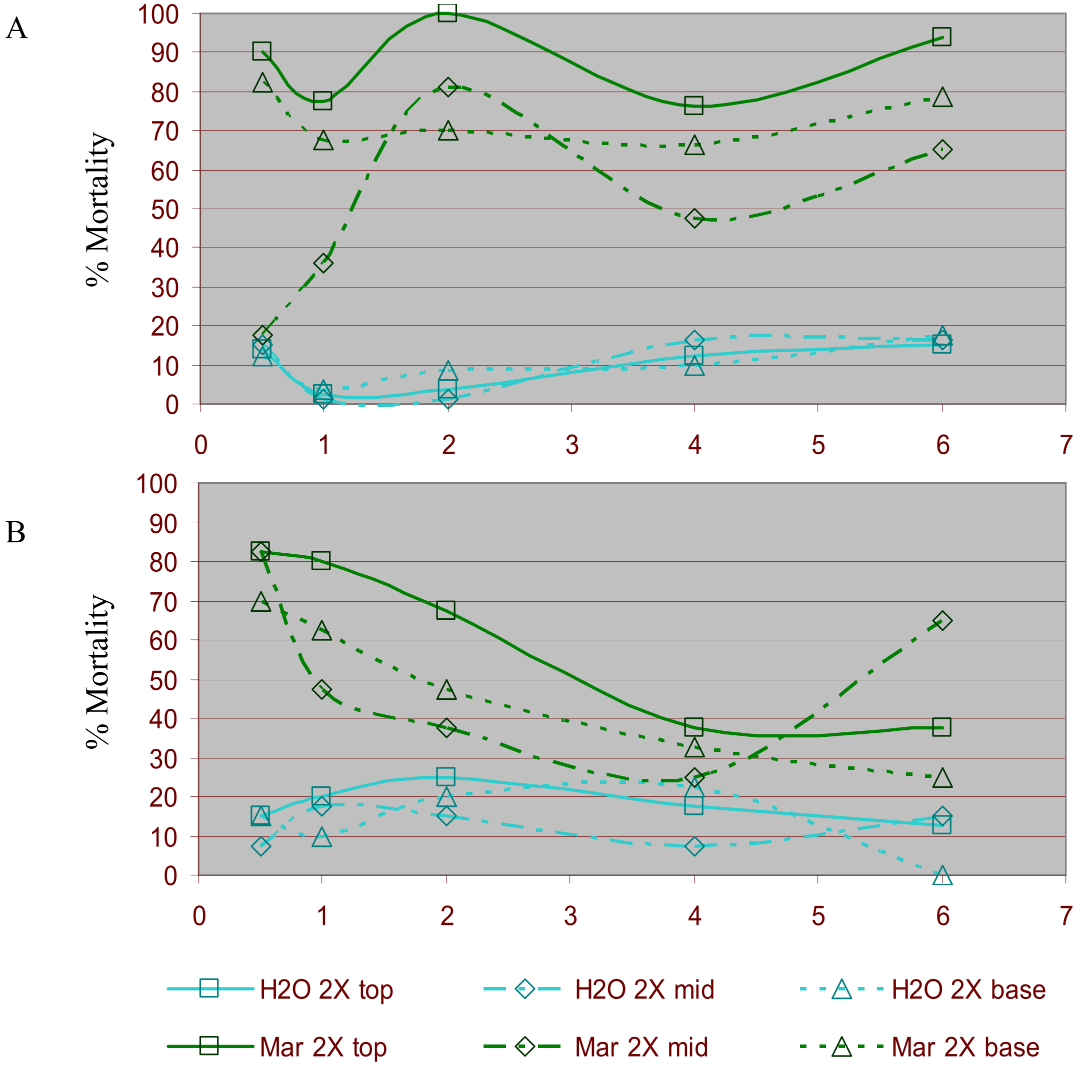
Figure 8 A & B. Comparison of efficacy over time of lambda-cyhalothrin (Scimitar GC) treated soil core samples and water treated soil core samples in controlling female alate IFA across three sample sites and at two application volumes. Figure 8 A illustrates the fall 2003 applications and 8 B the spring 2004 applications.



The lambda-cyhalothrin treatments in the fall 2003 trial provided greater overall control of IFA than those in spring 2004 ($F=5.2838$, $df=3$, $P=0.0014$). Consistency within site wavered over time for both chemical applications and in both trials, but the irregularity within the fall 2003 trial was further demonstrated through the statistical similarity between the two-week sampling and six-month sampling while the one-month samples were significantly less lethal ($F=5.1766$, $df=4$, $P=0.0005$). It is possible given the apparent rapid loss of efficacy within the top samples of the spring 2004 trial compared to those in fall 2003 that weather related degradation may have had a greater impact over the late spring to summer period in which the spring 2004 trial was conducted than the fall to winter period of the fall 2003 trial. A progressive increase in the number of replicates with surviving ants over the sample dates in the spring 2004 trial, but not in fall 2003, seems to support this possibility.

The difference among results from the three soil collection sites in imidacloprid (Marathon 60WP) remained remarkably constant through most sample dates (Figure 9 A & B). The highest IFA mortality for each sample date typically came from the top sample site while the lowest came from the middle site ($F=4.8814$, $df=2$, $P=0.0086$) suggesting generally inefficient penetration of the root ball by imidacloprid. Between the two trials the pesticide yielded a greater overall mortality in the fall 2003 trial than that in spring 2004 ($F=9.5450$, $df=1$, $P=0.0023$). Efficacy of chemical treatment appeared to waver but showed no definitive decline through the span of the fall 2003 trial. Throughout the six months of the fall 2003 trial, low mean mortalities in top, base, and middle samples respectively were usually due to non-repeating single replicates with surviving ants, two replicates with survivors, and two to four replicates with survivors. Within the fall 2003 trial the two-week, four-month, and six-month samples were similar to all dates, while the two-month samples were more lethal than those at one month ($F=2.6261$, $df=4$, $P=0.0362$). This relationship among sample dates in the fall 2003 trial is in contrast to the apparent progressive degradation over time seen in the spring 2004 trial. The first sample date in the spring 2004 trial not only demonstrated significantly higher control of the IFA than the last three sample dates but also was the only sample date with a majority of the replicates achieving 100% control. Imidachloprid thus mimics the trial by date by treatment relationship previously seen in lambda-cyhalothrin suggesting that the longevity of both chemicals was heavily influenced by seasonal factors. However, it should be noted that even in the spring 2004 trial there is at least a single replicate within each sample date that killed all ants.

Figure 9 A & B. Comparison of efficacy over time of imidacloprid (Marathon 60WP) treated soil core samples and water treated soil core samples in controlling female alate IFA across three sample sites. Figure 9 A illustrates the fall 2003 applications and 9 B the spring 2004 applications.



In summary, doubling the volume of a chemical application did not reduce the occurrence of erratic readings across time or among replicates. Nor did it appear to improve penetration into the soil. The uniformity of results across sample sites in bifenthrin and chlorpyrifos indicated these active ingredients had the greatest ability to penetrate the soil in lethal levels of concentration; however, even these treatments had occasional samples with surviving ants.

By its very nature, doubling the volume of solution ensures a more complete coverage of the exposed surfaces on the root balls. However, since erratic results continued and immersion applications using the same rates of these chemicals have provided consistent results, this implies the issue has not been due to the chemicals themselves nor the surface coverage of the application but most likely has been due to collecting samples beyond the depth of chemical penetration. This does not, however, mean that drench treatments cannot be used for quarantine.

If a drench application can render root balls free of fire ants by eliminating established colonies and providing a barrier to new infestation, then it would still be effective despite the absence of insecticide in deeper soil.

Bioassays for drench treatments utilizing infestation of intact root balls, instead of alates exposed to soil core samples, would not compromise any barrier treatment on the root ball surface and thus could verify actual effectiveness of the drench application. Therefore, future drench application testing will include the use of intact plant bioassays, as well as, a variety of drench application regimens to determine and verify effectiveness of application methods that are both more economical to apply and ensure even application over the whole root ball.

Portions of this project performed by TSU-NRC were partially funded through a research grant from USDA-CSREES Pest Management Alternatives Program Project 2003-34381-13660.

CPHST PIC NO: A1M04

PROJECT TITLE: Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Fall 2004 and Spring 2005

REPORT TYPE: Interim

LEADER/PARTICIPANT(s): Shannon James, Lee McAnally, Anne-Marie Callcott, Jennifer Lamont; Jason Oliver, Sam Dennis and Nadeer Youssef of Tennessee State University; Michael Reding and Jim Moyseenko of USDA-ARS

INTRODUCTION:

APHIS is responsible for developing treatment methodologies for certification of regulated commodities, such as field grown balled-and-burlapped nursery stock (B&B), for compliance with the Federal Imported Fire Ant Quarantine (7CFR 301.81). Current treatments for field grown stock are inefficient and limited to a single insecticidal choice, chlorpyrifos. Furthermore, restrictions on this insecticide 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.

Current certification options for harvested B&B stock are immersion in a chlorpyrifos solution (dipping) or watering twice daily with a chlorpyrifos solution for three consecutive days (drenching). Likewise, the current treatment for Japanese beetle (*Poppillia japonica* Newman) in B&B requires dipping in chlorpyrifos. Since both imported fire ants (IFA) and Japanese beetle (JB) are a concern for the Tennessee field-grown nursery industry, the trials detailed in this report were conducted in cooperation with the Tennessee State University Nursery Research Center (TSU-NRC) with the goal of determining treatments useful against both pests. The JB testing portion of this trial was planned and conducted by TSU-NRC and the USDA-ARS Horticultural Insects Research Laboratory in Wooster, OH, and they report the details and results for that portion of these trials.

Standard IFA testing of chemical treatments for both dip and drench applications has been conducted through female alate bioassays on soil core samples from the treated root balls (Appendix A). Soil core bioassays for drenches conducted in 2002 and spring 2003 yielded erratic results over time and among replicates within treatments. The same chemicals at equal or lower rates, when applied by immersion, were consistent, thus indicating insufficiency in either application or the mode of testing for the drench applied treatments. Drench trials conducted in fall 2003 and spring 2004 determined that doubling the volume of solution applied failed to eliminate inconsistent results.

During drenching, B&B normally rests on a single side of the root ball throughout the three-day drench process. This possibly restricts treatment coverage on the resting side, while giving the surface of direct application a higher concentration of chemical and deeper penetration. Trials initiated in fall 2004 and spring 2005 were designed to examine whether changes in plant handling during application improve penetration and coverage and possibly allow reduction in the number of days required to complete a drench.

MATERIALS and METHODS:

In October 2004 and March 2005 TSU-NRC and USDA-ARS personnel completed drench applications on B&B plants with 25-inch diameter root balls at the TSU-NRC in Warren Co., TN. Drench treatments consisted of one of three chemical solutions or a water control in each of four application methods. In order to focus on the effect of application variation, the variety of chemicals applied was reduced from previous trials to three of the more promising insecticides that demonstrated control with both IFA and JB. Solutions, final rates, and handling which composed the treatments are listed in the table below.

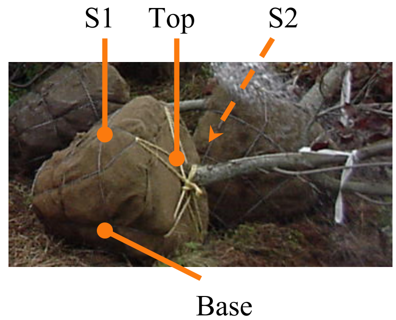
Product	Active Ingredient	Rate (lb a.i./ 100 gal H_2O)	Handling			
			F2	F4	F6	NF
Flagship 25WG	Thiamethoxam	0.260	X	X	X	X
Dursban TNP	Chlorpyrifos	2.000*	X	X	X	X
Talstar Lawn & Tree Flowable	Bifenthrin	0.230	X	X	X	X
Control	----	0.000				X

* The rate used for chlorpyrifos treatments (2.0 lb ai/100 gal H_2O) is the rate required for the U.S. Domestic Japanese Beetle Harmonization plan. The IFA quarantine rate is much lower at 0.125 lb a.i./100 gal H_2O.

Insecticidal solutions were prepared in 30-gal drums with polypropylene liners and pumped through a hose attached to a shower-headed nozzle using a Shur-Dri battery-powered pump (Figure 1). Solutions were applied twice daily (once in the morning and again in the afternoon) and between these applications in the flip-handled regimes the root balls were rotated or flipped to expose a different side to the direct application. The three regimes with flip-handling were two drench applications in one day (F2), drench applications twice daily for two consecutive days (F4), and drenches twice daily for three consecutive days (F6). Each root ball received approximately 0.67 gallons of drench solution at each drenching totaling 1.354 gallons a day. The amount used per drench application was based on the amount needed to achieve, “the point of runoff,” required in the IFA quarantine. Although the volume of solution applied increased as the number of days drenched increased, the amount of chemical in the solution was adjusted so that within a single chemical group, regardless of the number of drench days, each plant was exposed to the same total amount of pesticide by the conclusion of its final drench. Each pesticide and the water control also had a no-flips (NF) treatment group that remained stationary, while receiving two applications a day for three consecutive days like the F6 group.

Figure 1. Drench application

Figure 2. Soil core sample collection sites. The root ball is resting on an edge, so the base is visible and the lateral side two (S2) is opposite the camera.



After final treatment, the plants were maintained outside to weather naturally. Four replicate plants were selected out of the twenty root balls in each treatment group at 0.5, 1, 2, 4, and 6 months after final treatment for soil core sample collection. Four locations corresponding to the four sides of a root ball, top-as-planted (top), lateral side 1 (S1), lateral side 2 (S2), and the lateral side the plant rested on at the first drench application (base), were sampled on each plant to explore evenness of coverage (Figure 2). Samples were collected from within the first four inches of soil depth for testing against red, black, and red x black hybrid IFA as well as analytical chemical analysis. TSU-NRC is responsible for conducting and reporting testing on locally available black and hybrid IFA and chemical analysis. The samples for testing against red IFA were frozen and sent to the CPHST-ANPCL Soil Inhabiting Pests Section in Gulfport, MS where they were utilized in modified alate queen bioassays. Each soil sample was split into two sub-samples and ten alates were tested in each (Figures 3 & 4). Results were analyzed by ANOVA using JMP 5.1 (SAS Institute Inc., Cary, NC). Means were separated using Tukey's HSD at $\alpha = 0.05$.

Figure 3. A tray of alate mortality bioassay cups.

Figure 4. Orange circles indicate the locations of clusters of female alates within this bioassay cup.

Through the course of testing samples from the fall 2004 initiated trial, several control samples had rapid onset of high mortality, which is indicative of chemical contamination. The chemical analysis from the same plants revealed that indeed a portion of the control group received some accidental chlorpyrifos application. Since no other treatment groups had contamination and within each round of applications chlorpyrifos was always the final chemical in the drench apparatus and the control was always first, it is expected contamination occurred due to a failure to clean before the next round of drench application began. The drench apparatus was normally washed between chemical treatments by running the pump for 20 seconds in a soap solution followed by 20 to 30 seconds of clean water. After the incident with high mortality in the controls, a second group of control samples using Mississippi soils was added to the groups tested in the spring 2005 drench as a backup verification of the health of the alates used. At this point testing of the spring 2005 drench samples is still in progress.

RESULTS AND DISCUSSION:

Testing of the spring 2005 drench trial is ongoing, so statistical analysis comparing trials will be rendered after all soil samples have completed testing in 2006.

Fall 2004 trial:
Treatment, site, date, treatment x site, and treatment x date were significant factors affecting the overall run of the fall 2004 drench trial. Among the treatments, thiamethoxam F4 had significantly lower levels of IFA mortality than either bifenthrin or chlorpyrifos in the same application regime, and all pesticidal treatments were significantly better at controlling IFA than the water control ($F=168.0602$, $df=12$, $P=0.0001$).

Mortality across the different application regimes was not statistically significant within the same chemical, but the improvement in the flipped treatments compared to the ones that remained stationary is apparent (Figures 5-8). All of the NF groups had about half of their lower means due to multiple replicates within a date with surviving ants. Low means in the flipped treatments, with the exception of thiamethoxam F4, resulted from single replicates thus indicating more uniform treatment coverage or penetration. Incongruent lower mortality means occurred more frequently in the NF treatments and usually came from base side samples.

The lethality of some treatments varied by sample site ($F=1.9861$, $df=36$, $P=0.0006$). The base samples of bifenthrin NF yielded significantly lower mortality than all other sample sites in that same treatment but were similar to all other bifenthrin bases. Thiamethoxam NF base samples had significantly lower IFA mortality than either S1 or S2 samples in the same application regime and were also significantly lower than the bases of the other application regimes for thiamethoxam.

Both the chlorpyrifos and the bifenthrin rates were apparently high enough that any degradation over time did not reach the point where efficacy against IFA was lost. Over the course of the sample dates thiamethoxam in the F2, F4, and F6 regimes lost significant efficacy by the six-month sample date ($F=3.7147$, $df=48$, $P=0.0001$). The NF thiamethoxam as seen in figure 8 C also degraded by the final sampling date but was not statistically different from its results at other dates. While each of the thiamethoxam treatments was no longer consistent across sample sites by the final sample date, the amount of difference between highest and lowest mortality means varied with treatment from a difference of about 48% for F4 to 9% for the NF.

Figure 5 A , B & C. Insecticidal efficacy over time for treated soil cores from plants rotated once during the course of two drench applications in a single day (F2). Bifenthrin, chlorpyrifos, and thiamethoxam are represented in graphs A, B, and C respectively.

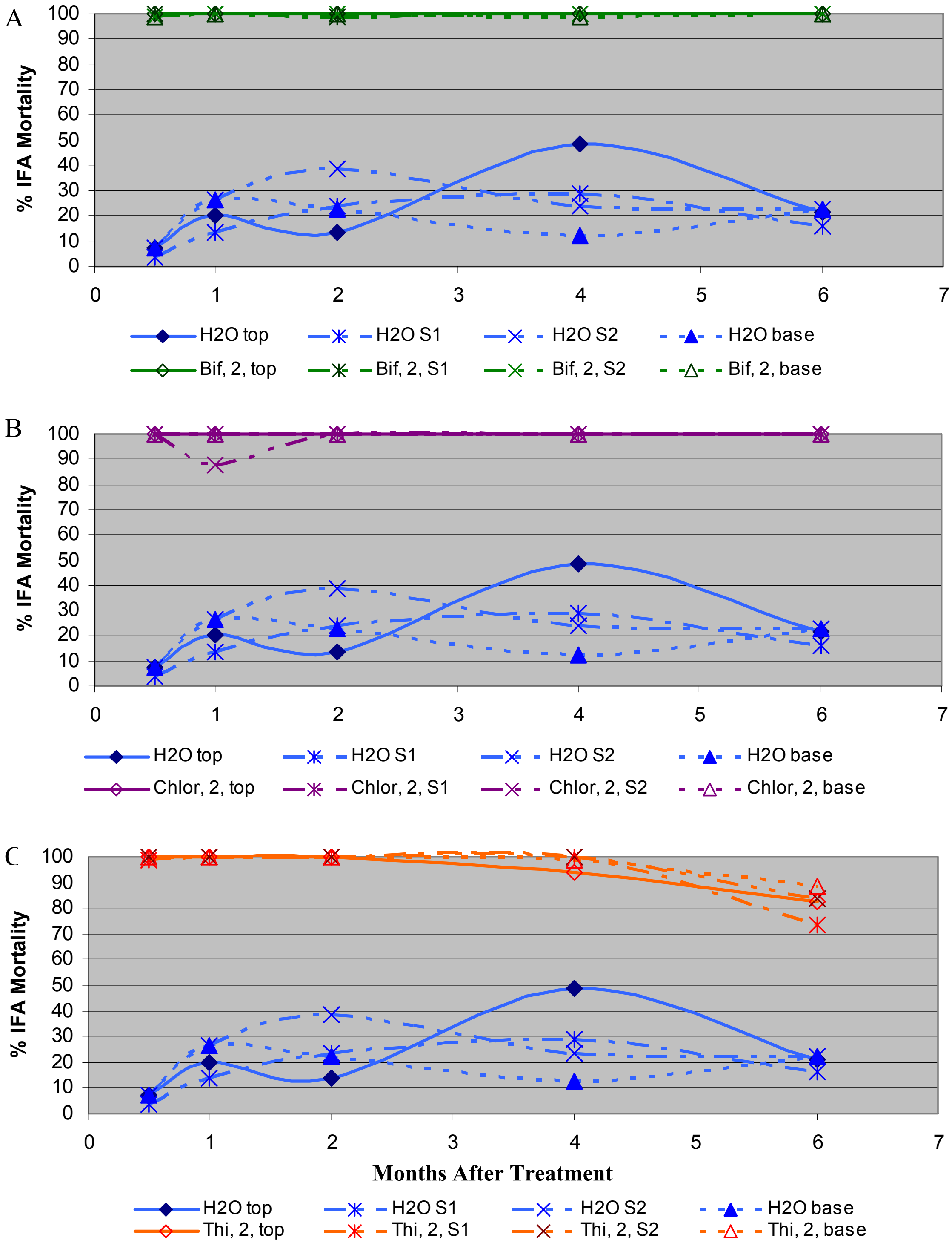


Figure 6 A , B & C. Insecticidal efficacy over time for treated soil cores from plants rotated twice during the course of four drench applications over two days (F4). Bifenthrin, chlorpyrifos, and thiamethoxam are represented in graphs A, B, and C respectively.

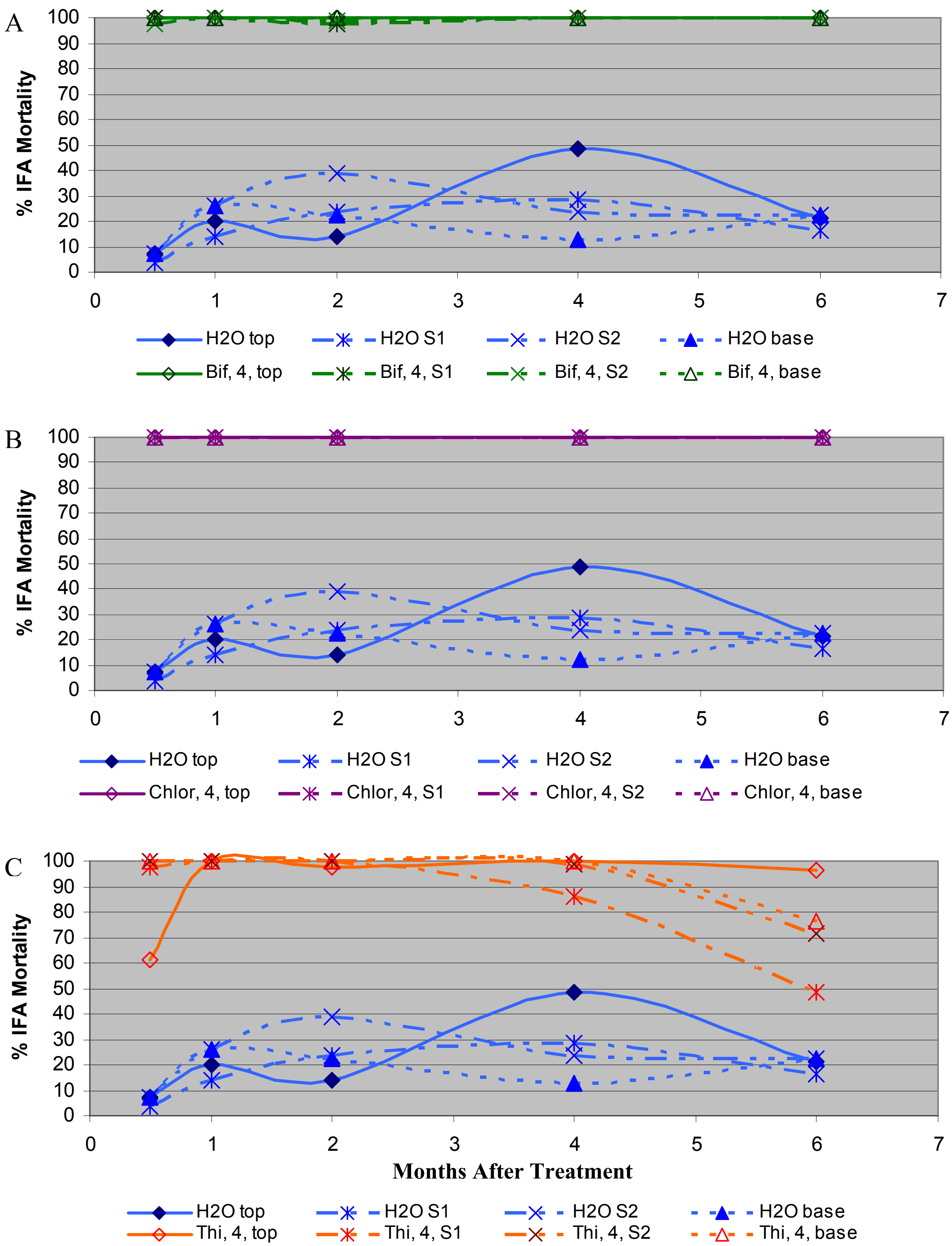
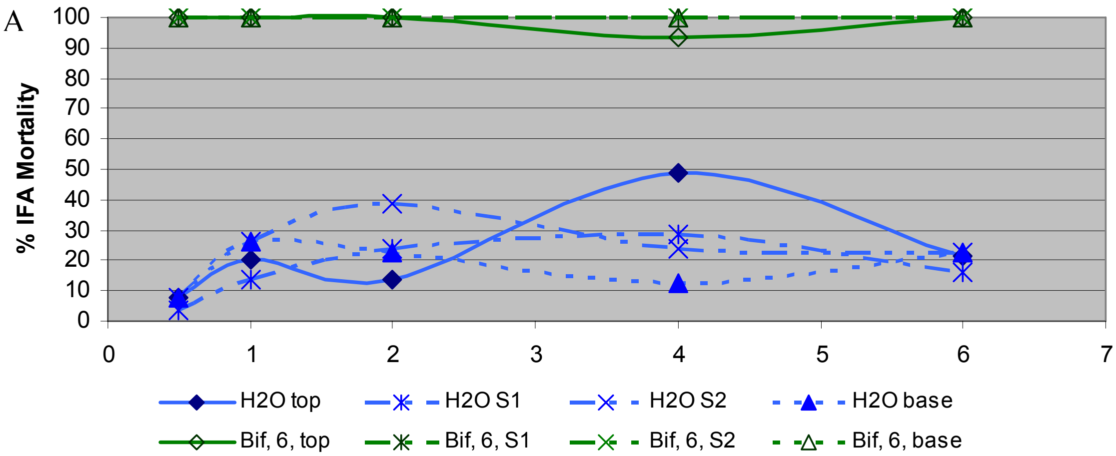
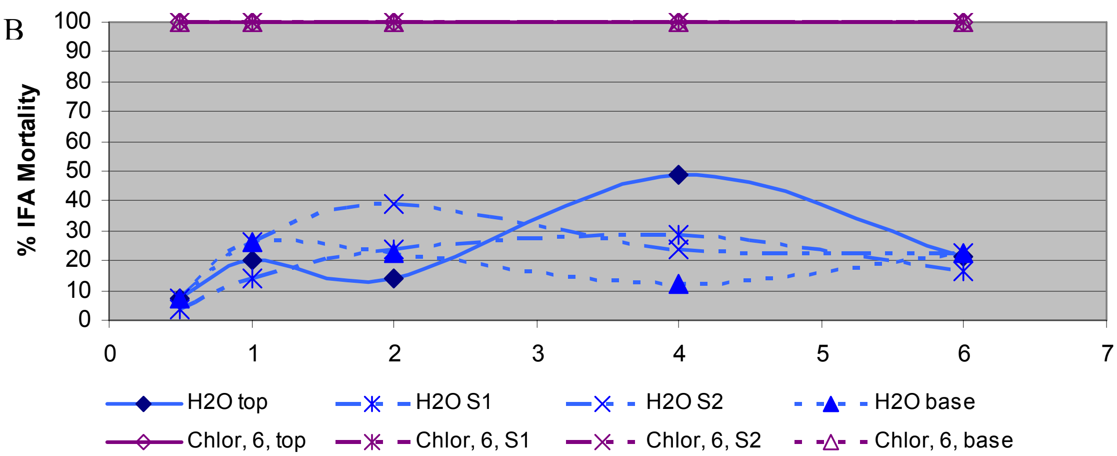


Figure 7 A , B & C. Insecticidal efficacy over time for treated soil cores from plants rotated three times during the course of six drench applications applied over three consecutive days (F6). Bifenthrin, chlorpyrifos, and thiamethoxam are represented in graphs A, B, and C respectively.

A



B



C

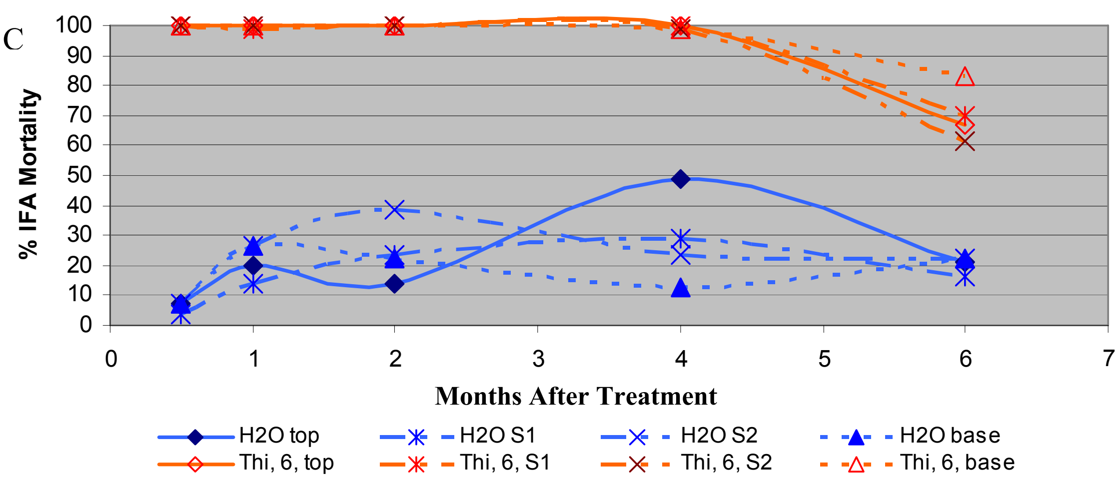
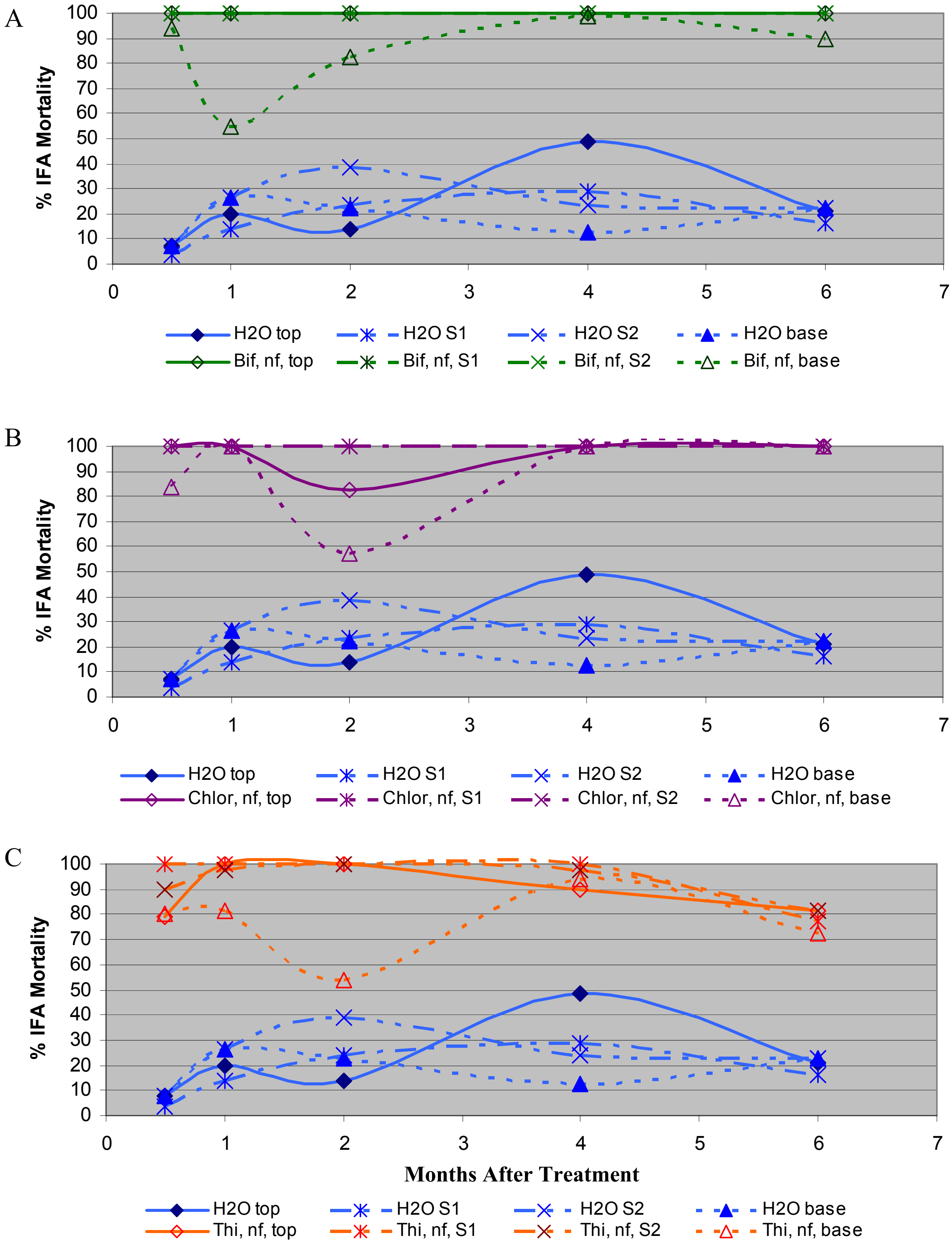


Figure 8 A , B & C. Insecticidal efficacy over time for treated soil cores from plants remaining stationary during the course of six drench applications applied over three consecutive days (NF). Bifenthrin, chlorpyrifos, and thiamethoxam are represented in graphs A, B, and C respectively.



Spring 2005 trial:
Treatments in this trial have shown more inconsistent results than the same treatments in the fall 2004 trial. While all chlorpyrifos treatments have demonstrated 100% control of IFA at this point, all bifenthrin treatments have had at least one instance of surviving IFA (Figures 9 & 10). The thiamethoxam treatments have had surviving ants in at least three sample sites each (Figure 11). Low mean mortalities have usually resulted from surviving IFA in multiple replicates in this trial. Most of the surviving ants in bifenthrin treatments were exposed to soil from base sites and were split almost evenly between the two completed sample dates. IFA surviving the thiamethoxam treatments did not seem to favor a particular sample site at this point, and while the number of samples with survivors was equally split between sample dates, there were more surviving ants from two week samples than from those at one month.

Figure 9. IFA control achieved in bifenthrin treated soil samples collected at four surface sites from four application regimes at 0.5 and 1 month after final drench application.

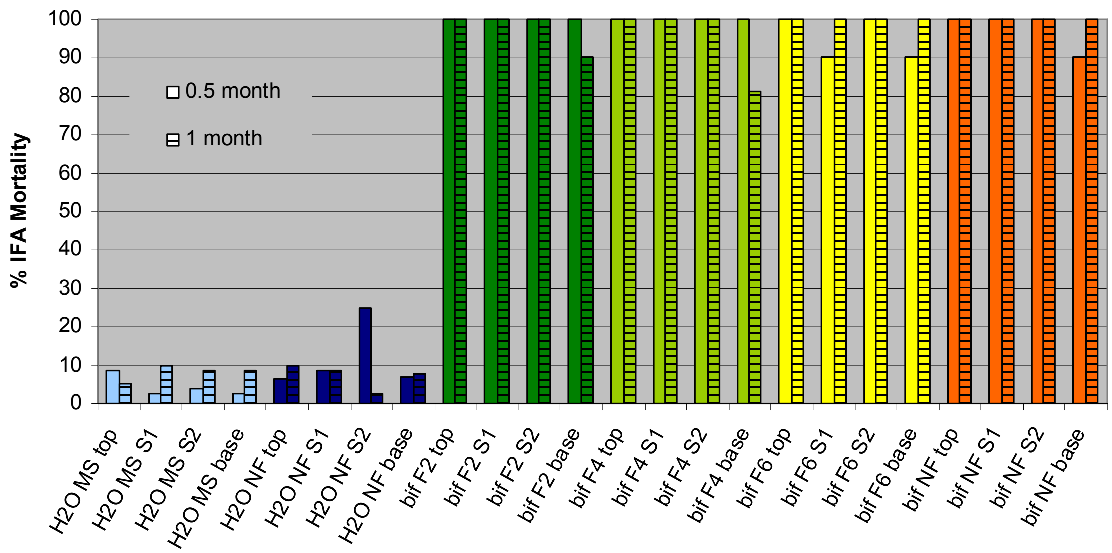


Figure 10. IFA control achieved in chlorpyrifos treated soil samples collected at four surface sites from four application regimes at 0.5 and 1 month after final drench application.

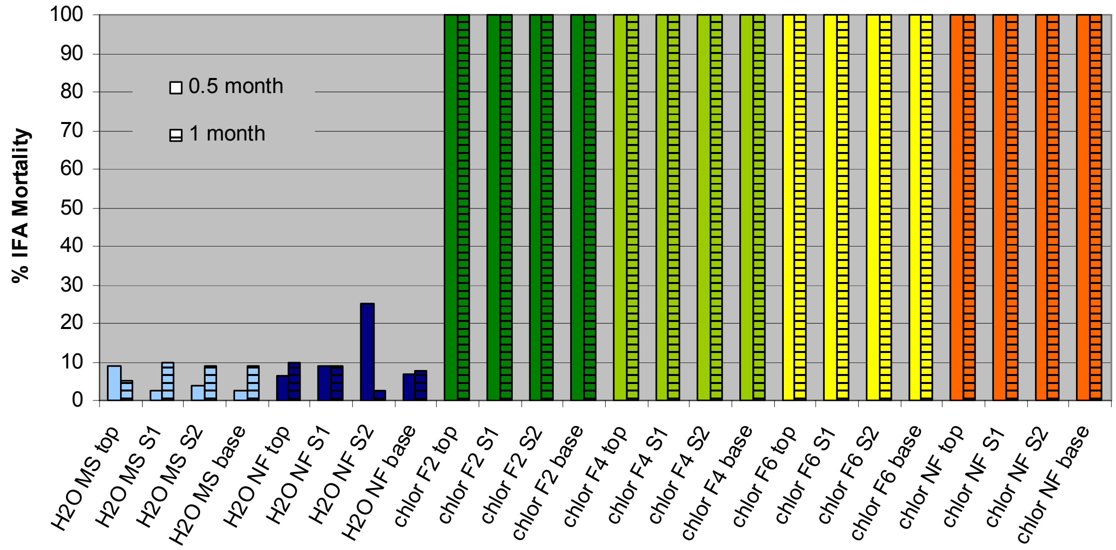
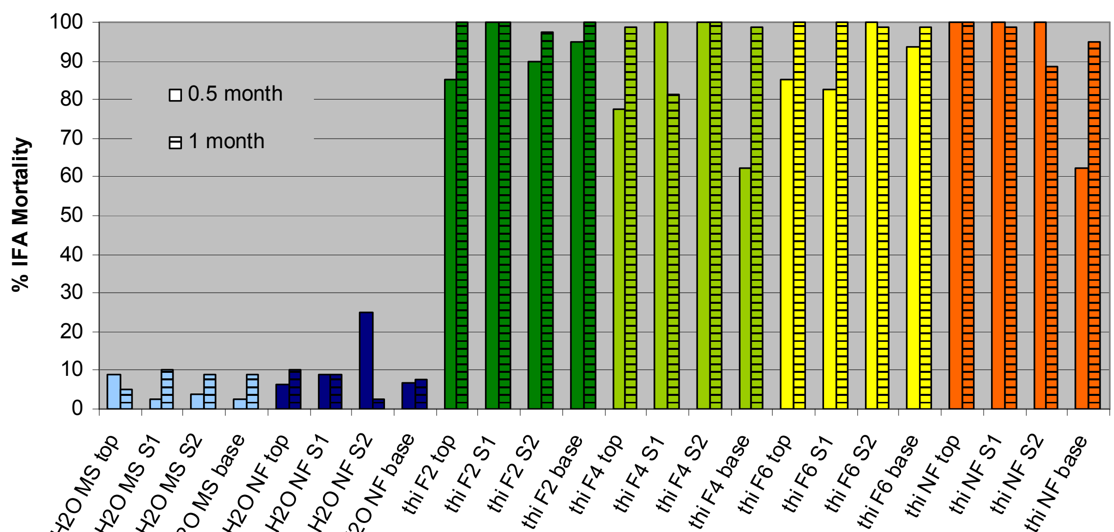


Figure 11. IFA control achieved in thiamethoxam treated soil samples collected at four surface sites from four application regimes at 0.5 and 1 month after final drench application.



In summary, the 2004 fall drench strongly suggested that rotating root balls during treatment, regardless of frequency, improved the consistency of bioassay results and could potentially cut the number of days spent applying drenches from three down to one. However, results from the first two sample dates for the spring 2005 drench have failed to match those of the previous trial, and even the improvement seen in the 2004 trial did not completely eliminate inconsistent sample results. Since erratic results continue in drench treatments, and immersion tests using the same rates of these chemicals or lower provide consistent results, this indicates the issue is related to collecting soil samples beyond the depth of chemical penetration.

Apparent lack of penetration of drench applied insecticides does not, however, mean that drench treatments are not useful for quarantine. If a drench application can render root balls free of fire ants by penetrating and eliminating established colonies and providing an outer layer of barrier to new infestation, then it is effective despite absence of the insecticide in deeper soil. Bioassays that utilize infestation of intact root balls (see "Soil Core and Intact Root Ball Bioassay Tests on Alternative Drench Treatments for use in the IFA Quarantine, 2004," found elsewhere in this annual accomplishment report), unlike soil core collection, do not compromise any barrier treatment on the surface. Through the ability of IFA to infest the treated root balls, the intact root ball bioassay can determine if treatments which appear promising by virtue of prior soil core bioassay actually prevent or eliminate infestation. Beyond use of intact plant bioassays, a variety of drench application regimes will continue to be examined in the coming years in both Mississippi and Tennessee to determine application methods that are both more economical to apply and ensure even application over the whole root ball.

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CPHST PIC NO: A1M04

PROJECT TITLE: Soil Core and Intact Root Ball Bioassay Tests on Alternative Drench Treatments for use in the IFA Quarantine, 2004

REPORT TYPE: Final

LEADER/PARTICIPANT(s): Shannon James, Lee McAnally, Anne-Marie Callcott, and Jennifer Lamont

INTRODUCTION:

APHIS is responsible for developing treatment methodologies for certification of regulated commodities, such as field grown balled-and-burlapped nursery stock (B&B), for compliance with the Federal Imported Fire Ant Quarantine (7CFR 301.81). Current treatments for field grown stock are inefficient and limited to a single insecticidal choice, chlorpyrifos. Furthermore, restrictions on this insecticide 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.

Current certification options for harvested B&B stock are immersion in a chlorpyrifos solution (dipping) or watering twice daily with a chlorpyrifos solution for three consecutive days (drenching). Standard testing of chemical treatments for either application method has been through female alate bioassays on soil core samples from the treated root balls (Appendix I). Erratic results from soil core bioassays for drenches conducted in 2002 and 2003 indicated insufficiency in either pesticide application or the mode of testing.

Drench application of pesticides in the 2002 – 2003 trials followed the pattern of the current certification procedure in being applied twice daily for three consecutive days. The amount applied to each root ball at each application in these trials appeared possibly to be too little to adequately cover the surface of the plant. When dry, the burlap used on the plants also appeared to restrict penetration by liquids. The 2004 drench trial in Mississippi therefore reduced the number of pesticides tested but added two new application styles to address the observed issues.

Drench applications do not penetrate into the root ball as deep or with the same evenness as immersion, which can lead to inconsistent results within the trials if test soil samples are collected beyond the depth the insecticide penetrated. However, if a drench application can render root balls free of fire ants by penetrating and eliminating established colonies and providing a barrier to new infestation, then it is effective despite absence of the insecticide in deeper soil. An intact root ball trial using small colonies to infest root balls before and after chemical application was thus initiated to verify efficacy of some of the drench treatments from soil-core-tested trials.

MATERIALS and METHODS:

Soil-core Tested Drench Trial:
Treatment for this trial was initiated on April 19, 2004 at the Gulfport, MS USDA-APHIS facility. Seventy-two box wood shrubs (*Buxus microphylla*) with 16" diameter root balls grown in sandy loam soil from George Co., MS were each treated with one of the following chemicals or control.

Active Ingredient	Trade Name	Rate (lb ai/100 gal H2O)
Bifenthrin	Bifenthrin Pro (flowable)	0.100
Lambda-cyhalothrin	Scimitar	0.034
Chlorpyrifos	Dursban 4E	0.125
Water	Control	--

Each root ball received a total of 1.02 gallons (3.86 ℓ) of the prescribed treatment, an amount equal to $^1/_5$ the volume of the root ball. The application volume listed for the current certification treatment is ambiguous, so the $^1/_5$ volume minimum drench treatment for containerized plants was used for our standard total volume across all treatments. Treatments were applied either in a single drench (single or S), a single drench preceded 24 hours prior by an equal volume of water (pre-wet or PW), or the regulatory twice daily for three consecutive days (2x3). Treatments applied in the 2x3 regime received 0.17 gallons (0.644 ℓ) at each of the six applications. Each solution/application style combination was tested on six replicate plants. After final treatment, the plants were maintained outside to weather naturally. Soil core samples were collected from the upper most surface (top), side (mid), and the surface the plant rested on (base) at 0.5, 1, 2, 4, and 6 months after treatment. Soil samples were frozen after collection until time of testing at which point they were thawed and used in a modified female alate bioassays (Figures 1 & 2). Each soil sample was split into two sub-samples and five alates were tested in each. Results were analyzed by mixed model ANOVA with REML fitting using JMP 5.1 (SAS Institute Inc., Cary, NC). Means were separated using Tukey HSD at $\alpha = 0.05$.

Figure 1. A tray of alate mortality bioassay cups.

Figure 2. Orange circles indicate the locations of clusters of female alates within this bioassay cup.

Intact Root Ball Tested Drench Trial:
Drench rates and application methods used in this trial mimic treatments from the drench initiated April 19 – 21, 2004 to allow comparison with standard soil core bioassays. The test plants in this trial were 90 box woods of the same size and origin as the previous test. Starting October 12, 2004, as test space allowed, each plant received treatment with the previously listed rates of chlorpyrifos, bifenthrin, or water control using the previously listed application styles. Lambda-cyhalothrin was not included in this trial due to lack of resources.

Each solution/application style combination was tested against ten root balls, half of which were infested prior to treatment and the other half infested after. Wild collected IFA were removed from their nest soil by dripping (Banks et al. 1981), and at infestation approximately a $^{1}/_{3}$ ℓ volume ball of workers with brood was placed on top of each root ball. The ants were given a minimum of 48 hours to move into the root balls prior to treatment on those plants that were infested first. Plants at the time of testing were kept in 26" diameter by 7" deep (66 x 18 cm) plastic Plantainer™ pans (Mac Court, Denver, CO) which were painted on the inside surface with Fluon (AGC Chemicals Americas Inc., Bayonne, NJ) to prevent ant escape (Figure 3).

Figure 3. An infested root ball

Figure 4. Splitting a root ball at the final observation to determine infestation

Air temperature in the glass house where trials were conducted was continuously recorded through out the trial by a StowAway® data logger and the data accessed using BoxCar® 3.6 (Onset Computer Corp., Bourne, MA). Manual readings of air and soil temperatures were taken at the times of treatment and final observation. Observations of IFA infestation of treated plants were conducted at seven and fourteen days after treatment application. At these observations the side of the root ball was firmly hit and the plant stem shaken to agitate any live IFA in the soil. If workers appeared within a minute of this disturbance, the plant was considered to have an active infestation. Plants that did not show active infestation at day 14 were split open and searched for live IFA (Figure 4). Results from the intact root ball trial were analyzed by logistic nominal models using JMP 5.1. Means were separated using Tukey HSD at $\alpha = 0.05$. Data from the 0.5 month soil core samples were reduced from counts to the binary designation of "inactive" (90% mortality or higher) or "active" (less than 90% mortality) for comparisons with the intact root ball trial.

RESULTS AND DISCUSSION:

Soil-core Tested Drench Trial

Even though the pre-wet and single applications appear more even than the 2x3 application as seen in Figures 5, 6, and 7, when comparing the three applications at each site within each insecticide, frequently there was no significant difference. All top samples within a chemical were statistically similar, mid samples within bifenthrin and within chlorpyrifos were similar, and there was no difference among the applications in the base samples of bifenthrin. However, within lambda-cyhalothrin mid and base samples of pre-wet had significantly higher mean mortality than the analogous 2x3 samples, and among chlorpyrifos base samples the single application was significantly more lethal than the 2x3 ($P = 0.0005$).

The 2x3 application was the least even of all application methods (Fig. 5) with the top sample sites yielding significantly higher mortality of IFA than both the middle and base samples within the same insecticide for all insecticides tested ($P = 0.0005$). The single application results as displayed in figure 6 are much more cohesive across sample sites than those in the 2x3 application but do not yield a significant increase in IFA mortality. This is most likely due to the fact that while the single application style had no significant differences across sample sites within chlorpyrifos and lambda-cyhalothrin, the top samples were significantly more lethal than base samples in bifenthrin. The pre-wet application, however, resulted in significantly higher overall IFA mortality than the 2x3 ($P = 0.0036$) and within each insecticide pre-wet showed no significant differences across sample sites. This indicates that both of the alternative drench application styles do improve evenness of chemical activity across the surface of the root ball with a slight but significant advantage to those already wet or moist at the time of chemical application.

Each chemical was overall significantly different from the others ($P < .0001$) such that by order of impact on test ants from highest mean mortality to lowest they are bifenthrin, lambda-cyhalothrin, chlorpyrifos, and the control. Since each of the insecticides is capable of achieving 100% mortality within the first month, this statistic is heavily influenced by the length of apparent activity of each chemical ($P < .0001$). Chlorpyrifos had its highest IFA mortality at 0.5 and 1 month sample dates, was in significant decline by the 2 month sampling, and in its 4 and 6 month samples was similar to the water control. Lambda-cyhalothrin remained at its most lethal through the 2 month sample, was in significant decline by the 4 month sample, and similar to water by the 6 month. Bifenthrin remained significantly different from the water controls even through the 6 month sample.

Figure 5. Mean mortality of female alate IFA exposed to soil samples collected from three sites (top, mid (vertical sides), and base) over the course of five sample dates (0.5, 1, 2, 4, and 6 months after chemical application) with the chemicals applied in a twice-daily-for-three-consecutive-days application style drench.

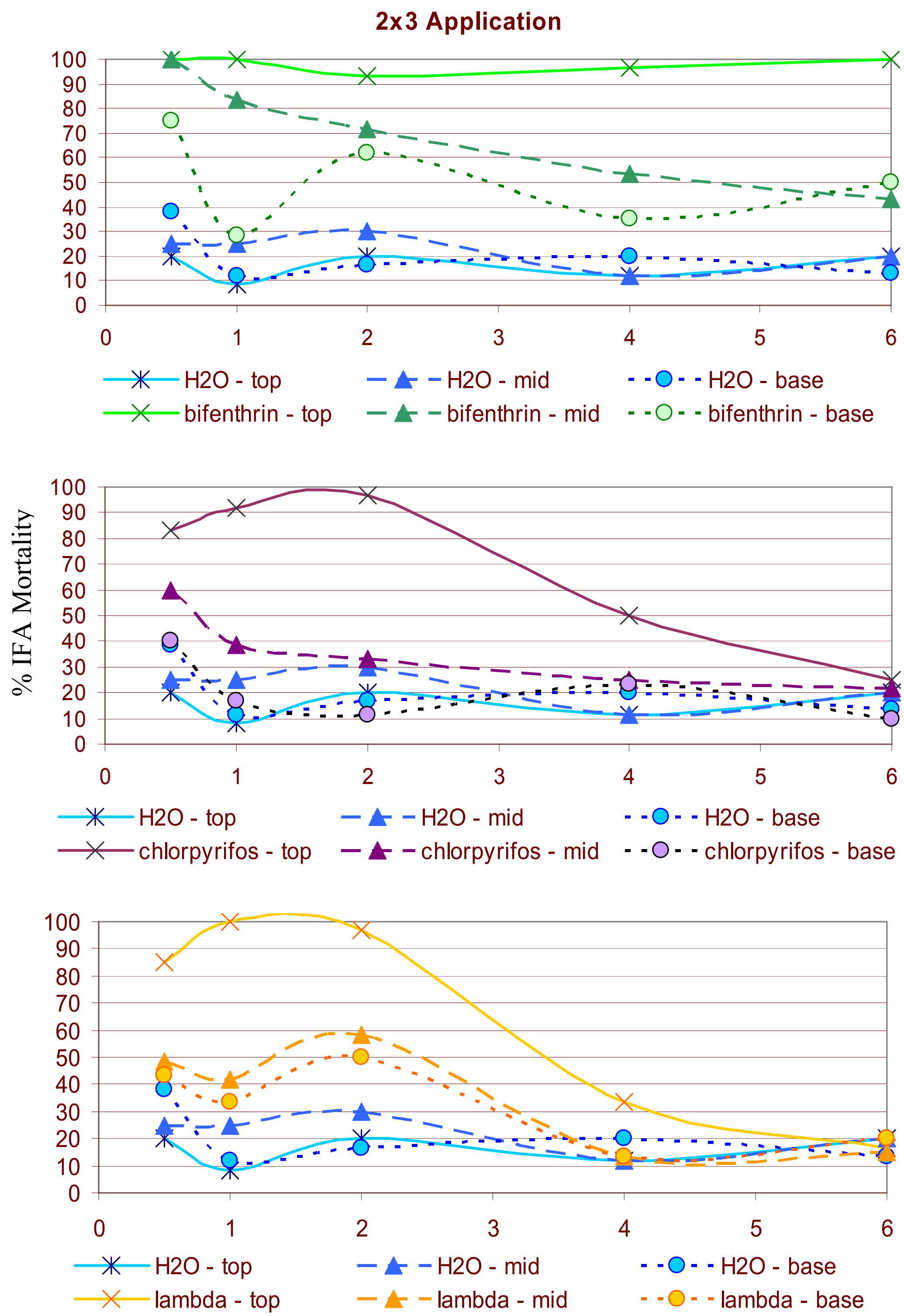


Figure 6. Mean mortality of female alate IFA exposed to soil samples collected from three sites (top, mid (vertical sides), and base) over the course of five sample dates (0.5, 1, 2, 4, and 6 months after chemical application) with the chemicals applied in a single application drench.

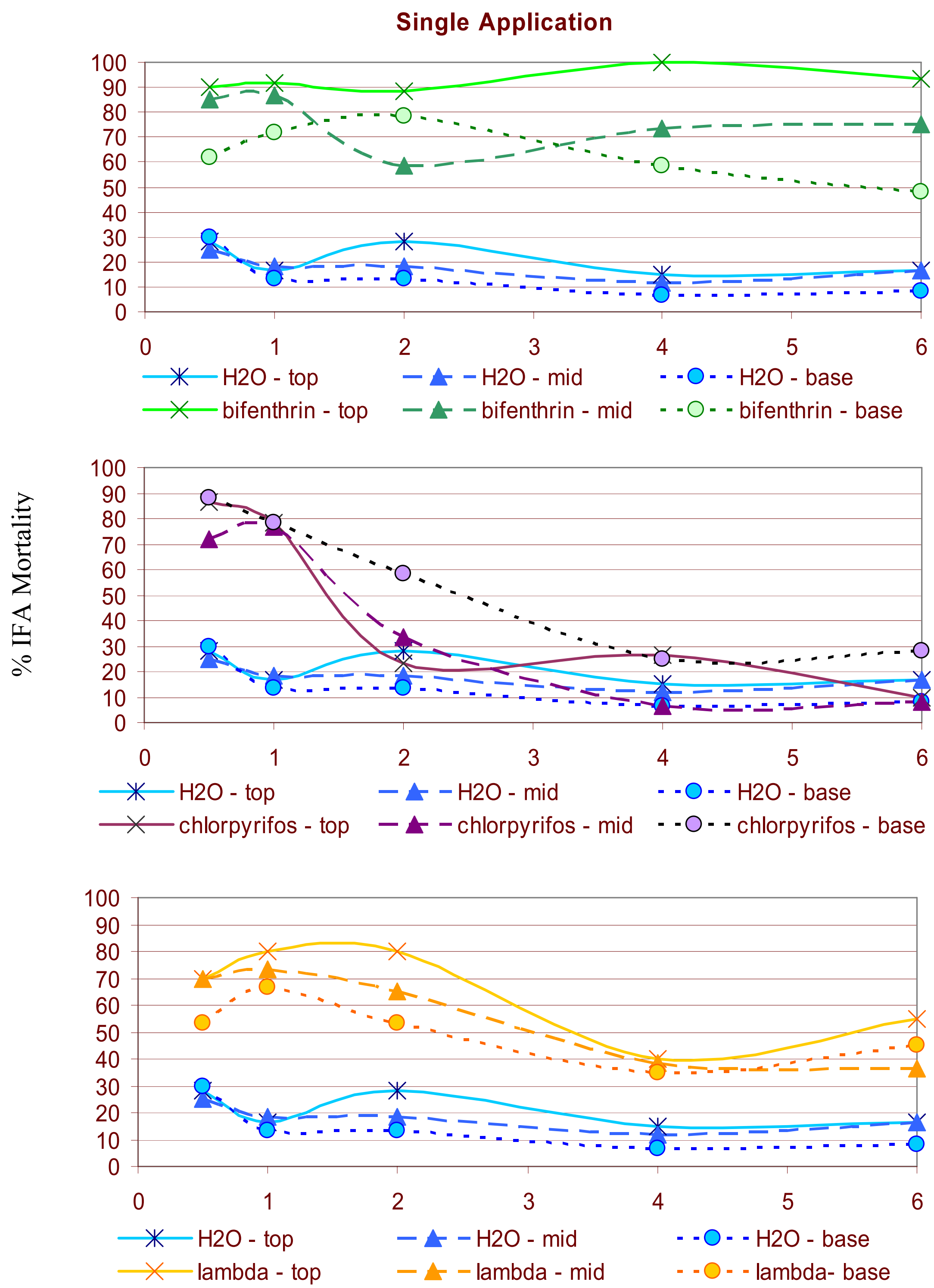
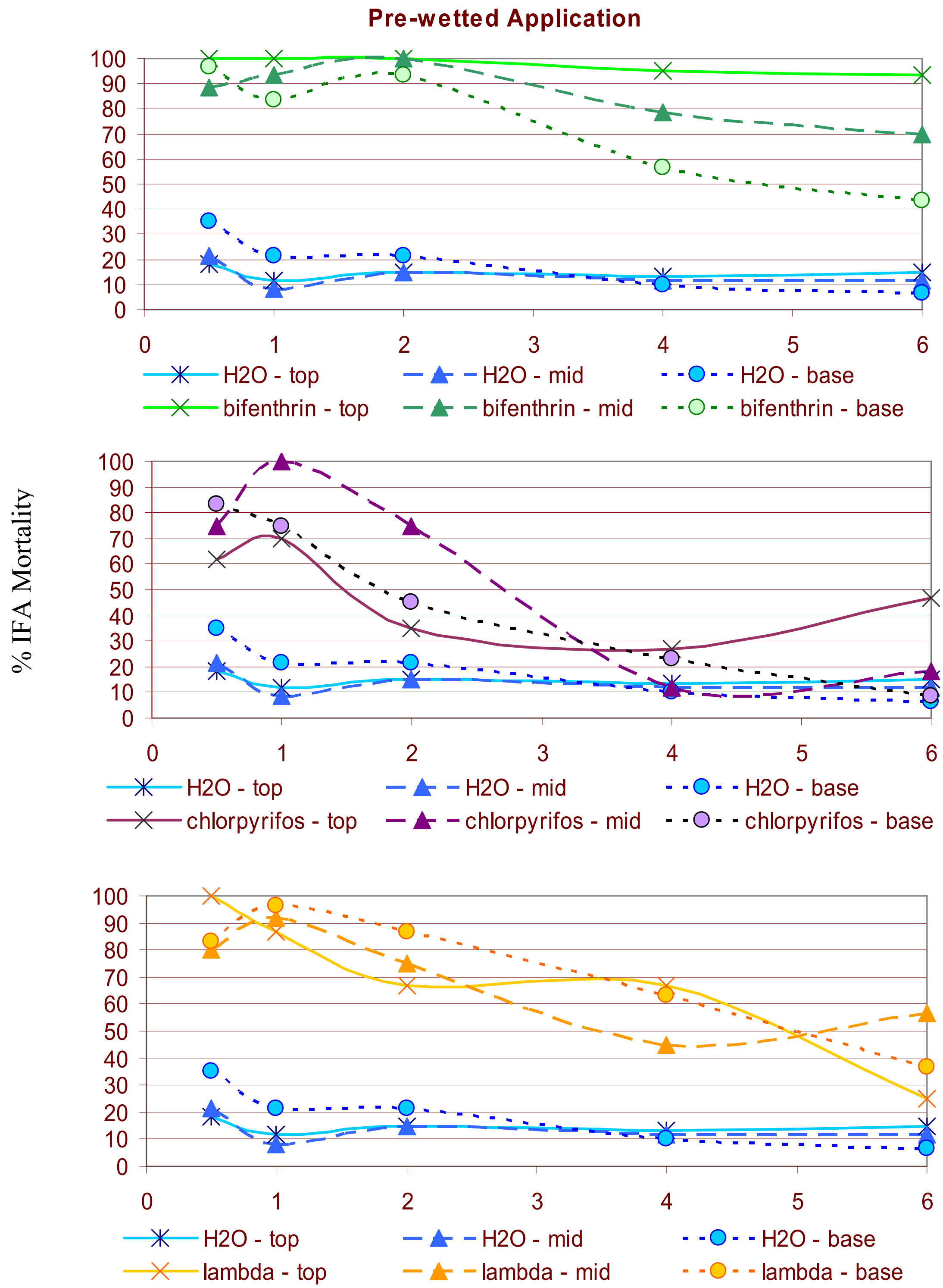


Figure 7. Mean mortality of female alate IFA exposed to soil samples collected from three sites (top, mid (vertical sides), and base) over the course of five sample dates (0.5, 1, 2, 4, and 6 months after chemical application) with the chemicals applied in a single application drench after the plants were drenched in an equal amount of water the previous day.



Over previous drench trials the main indicator of a problem either existing in application evenness or testing method has been the occurrence of inconsistent results both among replicates and over time. This trial was no exception. Frequently four or five replicate soil samples would kill all alates in less than a week, but the remaining one or two replicates would have survival equal to that found in the water controls. The influence of this situation is displayed in the sample date significance of the combined bifenthrin treatments wherein the chemical activity of the two week samples was statistically more effective than the one month samples ($P < .0001$) but statistically similar to the 2 month samples. It is not likely that the chemical had resurgence in effectiveness at 2 months. Also if the questionable results had been due to a greater susceptibility of the ants during the 2 month test then a similar pattern would have been witnessed in at least one of the other chemicals since they were tested at the same time using ants from the same colonies.

It is interesting that neither the interaction of sample date x chemical x application nor the interaction of sample date x chemical x site x application were significant, since each of the component effects had a significant influence and the means of each treatment over time indicated striking differences in longevity of chemical activity based on application style. I expect this lack of significance is due to the inconsistency of results, among replicates and across time, muddying the treatment at this smaller sample size level, $n = 24$ for each unique chemical x application x time group instead of $n = 360$ as seen in each unique chemical group. If results from soil core trials on drenches could be improved through shallower sample collection and more even application styles, I expect the interaction of chemical x sample site x application style x time would show significance.

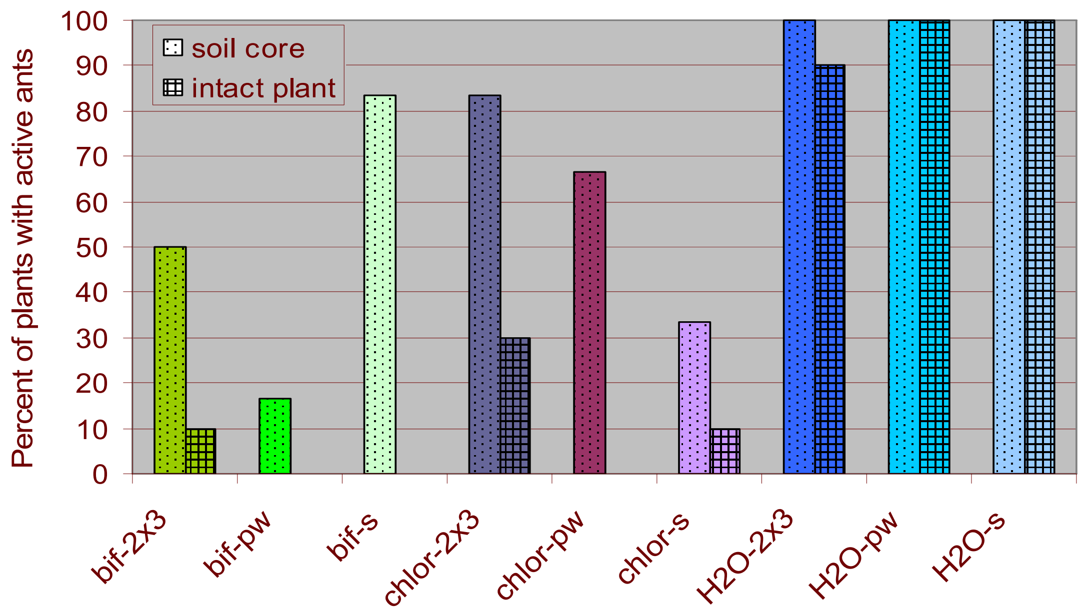
Intact Root Ball Tested Drench Trial:

Ten percent of the plants treated with an insecticide had active ants at the end of two weeks. Half of the ant colonies that remained active were introduced before treatment was applied. Statistically only the chemical used in the treatments was significant ($P < 0.0001$), and bifenthrin was not different from chlorpyrifos. However, five out of the six root balls that maintained infestation despite chemical treatment were treated with chlorpyrifos, and four out of the six had the 2x3 application regime. A higher amount of replication than seen in this initial trial may enable further statistical differences among the combinations of chemical and application style to emerge.

The higher number of failures within the 2x3 application style in the intact plant trial echoed the results from the soil core trial. Regardless of chemical used, the soil core sample tests showed the largest disparity between the mortality for top samples and base samples in the 2x3 applications with the base samples averaging half or less the mortality rate of top samples. When active ants were found on the intact test plants they usually were located in the base of the plant. Thus, it is likely that in the 2x3 application method the small amount of solution applied at each individual application soaked mostly into the immediate application site instead of continuing over the remaining surface of the plant. Observations in Tennessee of typical certification drenches applied by growers on actual stock, however, indicate that the plants are subjected to higher volumes of insecticidal solution at each application than the plants in these tests (Jason Oliver personal observation).

When examining the potential of each whole plant in the soil core trial to allow infestation, there are several points of striking difference between the soil core trial and the intact trial (Fig. 8). Generally far fewer intact plants sustained infestation than was indicated by the number of plants with one or more soil core samples that sustained infestation. Three of the intact treatments had no infestation at the end of two weeks, yet the soil core tests had some infestation in each treatment. Most notably though in the soil core test the single application of bifenthrin and the pre-wet application of chlorpyrifos both indicated more than half the plants should be infested, while the corresponding treatments in the intact test had none.

Figure 8. Apparent efficacy of treatment as measured in both the soil core and intact plant tests.



The 0.5 month soil core samples for each plant sampled were converted to an "active" (< 90% mortality) or "inactive" (≥ 90% mortality) result designation and averaged so an appropriate comparison could be made with the intact plant test results.

While the soil core testing on drenches did not yield a definitive answer to the question of quarantine level treatment lethality over time, it did provide some indication of the duration and evenness of the treatments. The frequent inconsistency among soil core samples both across time and among replicates and the disparity between the soil core and intact trials when looking at complete plant results as either infested or not, indicate that soil core collection does frequently sample beyond soil containing an otherwise effective insecticidal treatment. The intact plant tests, while truer to real field efficacy of treatments, have high resource requirements for both plants and space and allow fewer treatments to be tested simultaneously. The collection and consequent testing of soil samples from three locations on each plant in the soil core trial provided a higher amount of replication of treatment for analysis, thus allowing significance to be seen between applications in soil core testing when it was not seen in the intact testing. Further, the splitting of the plants is necessary for the final determination on not infested plants, which means in this method of assay an unrealistic number of plants would be needed for testing the efficacy of treatments over time. The soil core tests are thus useful and cost effective for initial determinations of treatment worth especially when the number of plants available for testing is a detrimentally limiting factor, but intact plants trials are preferable to make the final determinations of quarantine level efficacy of treatment.

REFERENCES CITED:

Banks, W. A., C. S. Lofgren, et al. (1981). Techniques for collecting, rearing, and handling imported fire ants, USDA, SEA, AATS-S-21, 9 p.

PROJECT NO: A1M04

PROJECT TITLE: Alternative Immersion Treatments for Balled-and-Burlapped Nursery Stock for use in the IFA Quarantine, Mississippi and Tennessee 2003-2005

REPORT TYPE: Final

LEADER/PARTICIPANT(s): Shannon James, Lee McAnally, Anne-Marie Callcott, Jennifer Lamont, and Shannon Wade of USDA-APHIS; Jason Oliver, Sam Dennis, and Nadeer Youssef of Tennessee State University; Michael Reding and Jim Moyseenko of USDA-ARS

INTRODUCTION:

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

Current certification options against imported fire ants for harvested B&B stock are immersion in a chlorpyrifos solution (dipping) or watering twice daily with a chlorpyrifos solution for three consecutive days (drenching) both at a rate of 0.125 pounds of active ingredient (a.i.) per 100 gallons of water. Likewise, the current treatment for Japanese beetle (*Popillia japonica* Newman) in B&B requires dipping in chlorpyrifos but at a rate of 2.0 lb a.i./100 gal water (Figure 1). Thus, a cooperative research effort to screen other liquid insecticides for inclusion in imported fire ant (IFA) quarantine treatments for B&B, with priority given to products effective for Japanese beetle (JB), was initiated with the Tennessee State University Nursery Research Center (TSU-NRC) and the USDA-ARS Horticultural Insects Research Laboratory, Wooster, OH. Trials conducted in 2002 - 2003 indicated several chemicals could potentially be used in addition to chlorpyrifos in treatment of B&B nursery stock.

Figure 1. Commercial dipping of B&B nursery stock. Image A depicts equipment used and plants prior to treatment and image B shows submerged plants receiving treatment.

MATERIALS AND METHODS:

Five separate trials are covered in this report, four initiated by TSU-NRC and one by USDA, APHIS, PPQ, CPHST, SIPS. Testing against JB grubs and chemical analysis were conducted only in TN and thus are not included in this accomplishment report.

Mississippi 2004 Trial:
Treatments were applied on site at the USDA APHIS facility in Gulfport, MS on April 23, 2004. B&B plants with 16 inch-diameter root balls, grown in George Co., MS sand loam soil with an average pH of 6.0, were submerged in insecticidal solutions in large plastic garbage cans until cessation of bubbling. Treated root balls were placed on pallets to prevent cross contamination by run off from other treatments and stored outside on these pallets through the duration of testing. Treatments and rates were as follow:

Product	Active Ingredient	Rate (lb ai/100 gal H_2O)
Talstar Lawn & Tree Flowable™	Bifenthrin	0.025
Dursban 4E™	Chlorpyrifos	0.125
Dursban 4E™	Chlorpyrifos	0.0625
Control	Water	------

Soil core samples were collected for each of the six replicates within the treatments at a depth between two and six inches. Collection occurred at 0.5, 1, 2, 4, and 6 months after application and the soil was then either frozen until resources for testing were available or immediately used in female alate IFA bioassays (Appendix I) (Figure 2 A). A single bioassay cup was used for each soil sample in this trial, differing from procedures used in prior trials, in which four bioassay cups were used for each soil sample. Each cup exposed five female alate IFA to treated soil for a period of up to 14 days (Figure 2B). Alate mortality was recorded several times a week during the exposure period and dead alates were removed from bioassay cups during these observations (Figure 2C). Results were analyzed by ANOVA using JMP 5.1 (SAS Institute Inc., Cary, NC). Means were separated using Tukey's HSD at $\alpha = 0.05$.

Figure 2. (A) General laboratory set up of bioassays. (B) A single bioassay cup (visible alates highlighted in circles). (C) Soil sample scattered in pan to locate alates.

Tennessee Fall 2003 and Spring 2004 Trial:
Treatments were performed October 20-22, 2003 and March 15-16, 2004 at the Nursery Research Center by personnel from TSU-NRC and USDA-ARS. A commercial grower in

Warren Co., TN provided plants with 12 to 15 inch-diameter root balls in strongly acidic (pH 5.1 to 5.5) loam to clay loam soil. The root balls in both trials were immersed for one minute in a dip tank that consisted of one of the following treatments:

Product	Active Ingredient	Rate (lb a.i./ 100 gal H_2O)
DeltaGard GC 5SC™	Deltamethrin	0.065
Dursban TNP™	Chlorpyrifos	2.000*
Dylox 80 T&O™	Dimethyl phosphonate	4.000
Flagship 25WG™	Thiamethoxam	0.065
Marathon 60WP™	Imidacloprid	0.200
Orthene T&O 75WP™	Acephate	0.375
Scimitar GC™	Lambda-cyhalothrin	0.034
Sevin SL™	Carbaryl	4.000
Talstar Lawn & Tree Flowable™	Bifenthrin	0.115
Control	----	0.000

* Only the Japanese beetle rate for chlorpyrifos was used in these two trials.

Due to limitations of resources and the dual nature of this project, treatments applied by TSU-NRC were occasionally conducted at higher rates of application than may have been needed to control IFA in attempt to control JB grubs. Past testing has indicated susceptibility of JB grubs to some insecticides is seasonally influenced. Thus, treatments conducted by TSU-NRC were generally tested in both a spring and fall season trial and, except where noted, were tested in an identical manner.

After treatment, the plants were maintained outside to weather naturally. Soil core samples were collected from the surface and center of four replicates within each treatment at 0.5, 1, 2, 4, and 6 months post-treatment. Samples for testing against red imported fire ants were shipped to the CPHST-ANPCL Soil Inhabiting Pests Section in Gulfport, MS where the samples were frozen until they could be utilized in alate female bioassays. The inherent uniformity of dip application allowed modification of the standard alate bioassay to meet resource limitations faced during the bioassay period for the spring 2004 trial. A single bioassay cup containing five female alates was utilized for each soil sample in the spring 2004 trial, while prior tests, including the fall 2003 trial, subdivided soil samples into four cups with five alates each. Female alate mortality was recorded several times a week during the 14-day exposure period, and dead alates were removed from bioassay cups during these observations. Results for the fall 2003 trial were analyzed in conjunction with results from the matching spring 2004 trial using JMP 5.1. The analysis employed was ANOVA with residual maximum likelihood (REML) using plant identification number as the random effect. Means separation was performed through Tukey's HSD at $\alpha = 0.05$.

Tennessee Fall 2004 and Spring 2005 Trials:
Treatments for the fall 2004 and spring 2005 matching trials were applied October 17, 2004 and March 15-17, 2005, respectively. Application was performed using the same methods described above for the previous TSU-NRC applied dip trials and used plants of the same size from the

same location. The following treatments were utilized in both the fall 2004 and spring 2005 dip trials:

Product	Active Ingredient	Rate (lb a.i./ 100 gal H_2O)
DeltaGard GC 5SC™	Deltamethrin	0.065
DeltaGard GC 5SC™	Deltamethrin	0.040
Dursban 4E™	Chlorpyrifos	2.000*
Dursban 4E™	Chlorpyrifos	0.125*
Flagship 25WG™	Thiamethoxam	0.130
Marathon 60WP™	Imidacloprid	0.400
Discus™	Cyfluthrin & Imidacloprid	0.12 & 0.50
Talstar Lawn & Tree Flowable™	Bifenthrin	0.025
Talstar Lawn & Tree Flowable™	Bifenthrin	0.0125
Control	----	0.000

* Both the JB rate 2.0 and the IFA rate 0.125 were tested in these two trials.

After treatment, the plants were maintained outside to weather naturally; however, root balls used in the fall 2004 trial were buried in straw on December 17, 2004 to prevent freeze damage to the plants (Figure 3). The fall 2004 trial plants remained straw insulated through the remainder of the trial while spring 2005 plants remained bare. Soil core samples were collected from the surface and center of four replicate plants within each treatment at 0.5, 1, 2, and 4 months post-treatment in the fall 2004 trial and at 1, 2, and 4 months post-treatment in the spring 2005 trial. A decrease in the number of root balls available for testing subsequently reduced the number of sample dates in which soil was collected for both the fall 2004 and spring 2005 trials.

Figure 3. Treated plants maintained outside either (A) insulated under straw or (B) bare.

After collection, samples were handled as described in the 2004 spring trial with those marked for red IFA testing sent to CPHST-ANPCL SIPS. Concern over the small number of alates tested, five per sample in the spring 2004 dip trial, lead to an increase to ten alates per test cup and an increase to two test cups from each soil sample for both the fall 2004 and spring 2005 trials. Worker testing on top soil samples from each treatment was included in these trials to support data from TSU-NRC. TSU-NRC personnel intended to test soil samples against

Tennessee black and hybrid red x black IFA groups to verify efficacy of treatment across all IFA species, but only tested workers due to insufficient numbers of female alates. Alate bioassays at the Gulfport facility were conducted as previously described, but the worker cups were checked for mortality only at seven and fourteen days of exposure to reduce the possibility of escape.

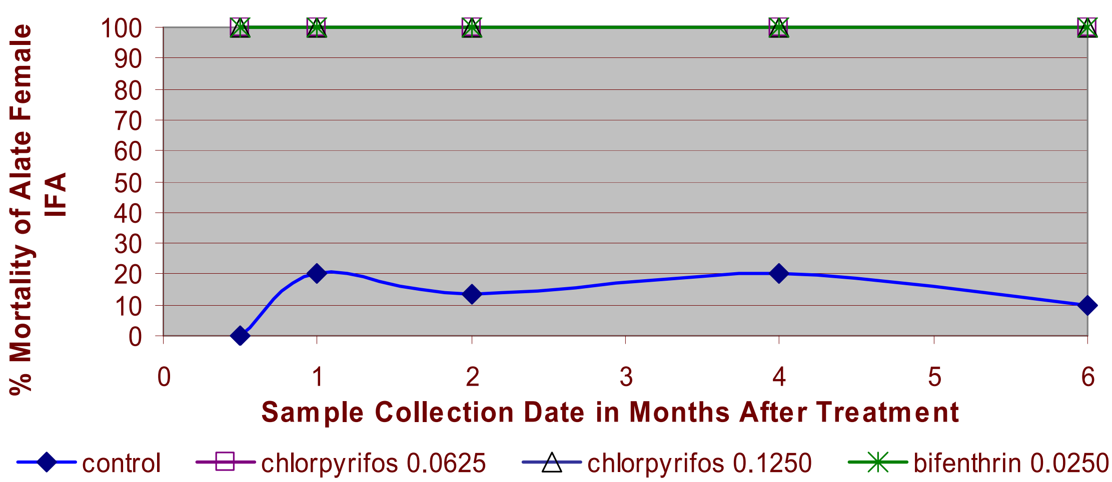
Results were analyzed by ANOVA using JMP 5.1 (SAS Institute Inc., Cary, NC). Means were separated using LSD at $\alpha = 0.05$. The fall 2004 and spring 2005 trials were set up as seasonally separate replicates of the same experiment, so it was important to analyze the data from both trials together. Since the fall 2004 trial included samples collected at 0.5 months after treatment, while spring 2005 did not, the data from 0.5 months was excluded from the analysis conducted on data sets including both trials. Separate analysis of the fall data sets reintroduced the 0.5-month results. The unbalanced use of sample site and caste required dividing data analysis so that results from workers and alates tested on top soil samples were separated from alate only comparisons between middle and top sample sites. Separate analysis of the alate data also allowed some limited comparison with past trials.

RESULTS AND DISCUSSION:

Mississippi 2004 Trial:

All three insecticide treatments provided 100% control of alate female IFA at each sample date (Figure 4). All insecticide treatments were significantly more effective than water ($F=1114.351$; $df=3$; $P=0.0001$), but similar to one another. Chlorpyrifos at 0.125 and bifenthrin at 0.025 were tested in MS soils in the 2003 Mississippi trial and performed identically in 2004. This was the first dip-applied trial examining a rate of chlorpyrifos at less than the quarantine treatment required rate. The exploration of lower rates of chlorpyrifos could potentially yield a certification treatment with this chemical that displays reduced phytotoxic responses in sensitive plants and a reduced hazard to workers and the environment.

Figure 4. Duration of efficacy of treatments for the Mississippi 2004 dip trial.



Tennessee Fall 2003 and Spring 2004 Trials:
Overall, the fall 2003 trial had a slightly higher kill of IFA female alates than the one in spring 2004 ($F=10.9381$; $df=1$; $P=0.0010$). However, when trial was examined in conjunction with treatment, only thiamethoxam and imidacloprid were significantly more lethal in the fall trial, and acephate and the control treatments actually displayed higher mortality in the spring trial (trial x treatment interaction $F=14.6873$; $df=9$; $P=0.001$). During the period of time when the spring 2004 samples from one and two months were used in bioassays, there was an outbreak of *Metarhizium anisopliae* among the bioassays. Fruiting bodies of this entomopathogenic fungal infection appeared frequently among the dead in both the control and acephate treatments. Upon sanitation improvements, visible instances of the infection decreased along with the alate death rate in the control treatment.

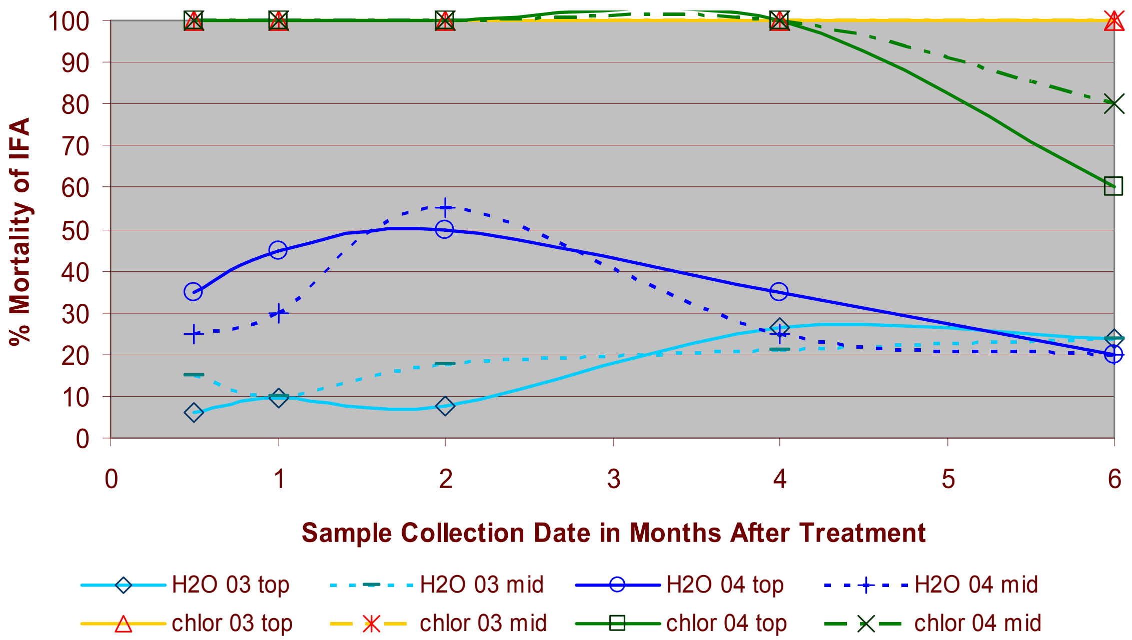
Sample date was an important effect ($F=38.8812$; $df=4$; $P=0.0001$), and the relationship among sample dates within the fall 2003 trial was different from that in the spring 2004 trial (trial x date interaction $F=19.5532$; $df=4$; $P=0.0001$). The half-month sample date was the most lethal within the fall 2003 trial, while all other dates were statistically similar to each other. The higher mortality for that first sample date was most likely due to the brief lethal activity of dimethyl phosphonate and carbaryl treatments, which decreased abruptly by the next sample date. All other treatments basically maintained their respective levels of IFA control through the rest of the trial. The spring 2004 trial, however, exhibited a steady decrease in chemical effectiveness against IFA. The half-month samples again had the highest mortality rate, but the samples from months one and two displayed a higher mortality rate than those at four months, which were in turn more lethal than samples taken at six months after treatment. Only bifenthrin and lambda-cyhalothrin treatments did not lose efficacy over time in the spring trial. This difference between fall and spring trials over time may mean either IFA were progressively less susceptible during the period when bioassays were run for the spring trial or that chemical degradation pressure was more pronounced during the spring 2004 trial.

The sample site effect for the combined results of both trials indicated samples collected from the middle of root balls were significantly more lethal than top samples ($F=7.9126$; $df=1$; $P=0.0051$). However, within the site x date x treatment interaction, only acephate, carbaryl, and thiamethoxam were significantly different between their sample sites within a sample date ($F=1.7711$; $df=36$; $P=0.0044$). Though not statistically significant, chlorpyrifos and deltamethrin also lost greater efficacy in top samples than in middle samples towards the end of the spring 2004 trial. The effect of middle samples being more lethal than top samples was most likely due to weather induced chemical breakdown in the more exposed top samples. Yet, some middle samples of bifenthrin, lambda-cyhalothrin, and deltamethrin displayed anomalous surviving ants in single replicates, while all other replicates from both sample sites at the same date provided 100% control. This may indicate that, at least for some chemicals, one minute of immersion may have been insufficient to completely penetrate the root balls.

Treatment ($F=238.4731$; $df=9$; $P=0.0001$), the interaction of date x treatment ($F=8.5380$; $df=36$; $P=0.0001$), and the interaction of trial x date x treatment ($F=3.9787$; $df=36$; $P=0.0001$) all significantly influenced results. The results for each treatment are discussed separately in the following paragraphs for clarity. The best performing treatments (chlorpyrifos, bifenthrin, lambda-cyhalothrin, and deltamethrin) are discussed first and are followed by the treatments that were progressively less reliable or less effective.

The current IFA quarantine treatment only provides a 30-day certification period after immersion in chlorpyrifos (0.125 lb ai/ 100 gal H_2O); however, the chlorpyrifos treatment used at the JB rate (2.0 lb ai/ 100 gal H_2O), as seen in the fall 2003 and spring 2004 trials, lasted six and four months after application, respectively (Figure 5). Between the two sample sites there were no significant differences, which indicates good penetration of the treatment. Over the duration of the sampling period, the six-month sample results in the spring 2004 trial were significantly lower than all previous sample results and significantly lower than the six-month sample from the fall 2003 trial (F=3.9787; df=36; P=0.0001). Even though efficacy in the spring appears shorter lived, a grower dipping B&B for JB grubs would potentially qualify for an extended certification period of four months or more for IFA.

Figure 5. Efficacy duration of chlorpyrifos 2.0 lb a.i./ 100 gal H_2O treatments against female alate IFA in fall 2003 and spring 2004.



Bifenthrin uniformly provided high levels of IFA mortality at both sample site locations throughout the six-month duration of both trials (Figure 6). Only a single sample within the six-month sample date in the 2004 spring trial failed to provide 100% mortality. Subsequent trials will explore rates lower than the 0.115 lb ai/100 gal H_2O used in these two trials, to maximize efficient chemical use for appropriate B&B shipping periods.

Lambda-cyhalothrin was another top performing treatment in these two trials (Figure 7). With the exception of a single replicate with surviving alates in both the one and two month sample dates from the spring 2004 trial, lambda-cyhalothrin yielded 100% mortality through the six-month trial end. There were no significant differences in results between the sample sites, trials, or among sample dates for this chemical. Future dip trials should include this chemical at this and lower rates to clear any inconsistency in treatment and explore shorter terms of efficacy.

Figure 6. Efficacy duration of bifenthrin 0.115 lb a.i./ 100 gal H_2O treatments against female alate IFA in fall 2003 and spring 2004.

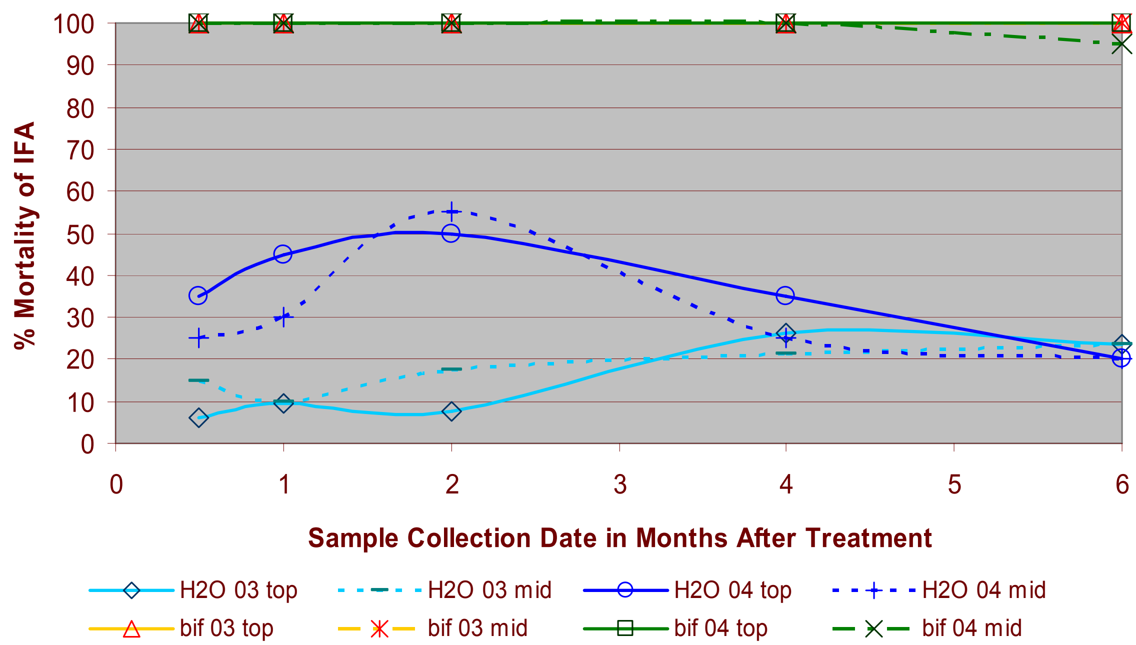
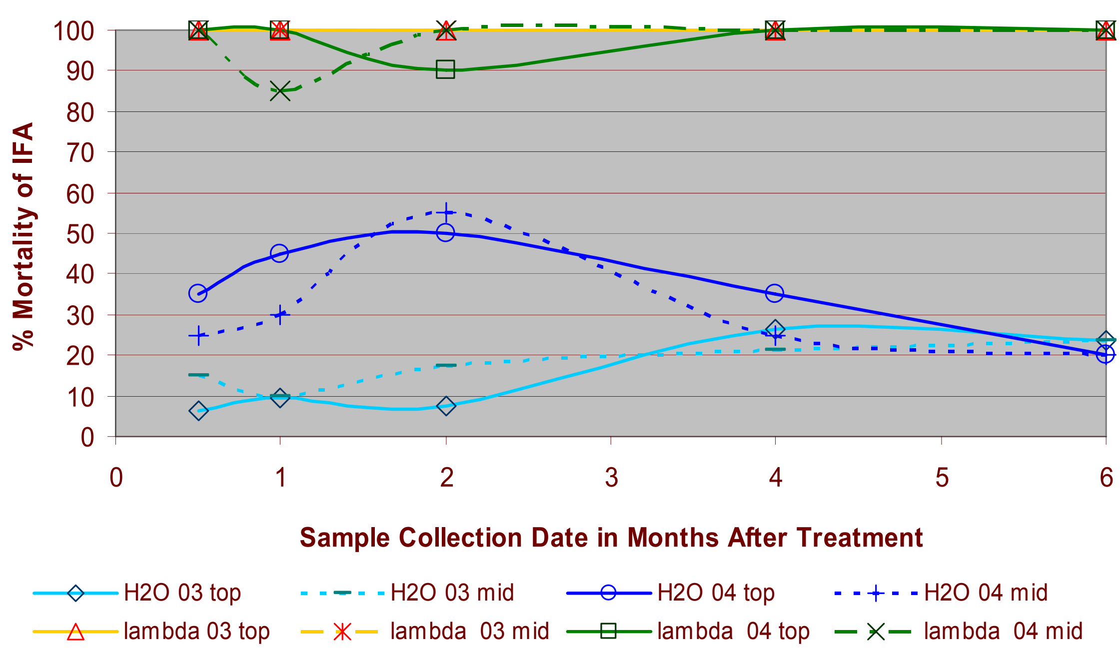
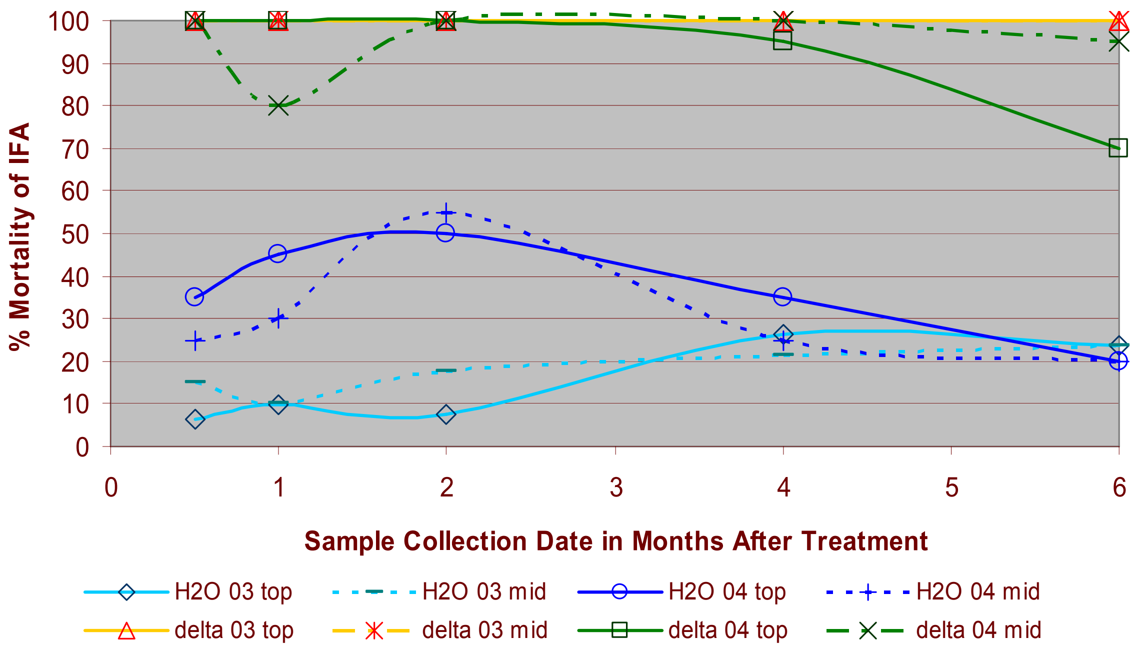


Figure 7. Efficacy duration of lambda-cyhalothrin 0.034 lb a.i./ 100 gal H_2O treatments against female alate IFA in fall 2003 and spring 2004.



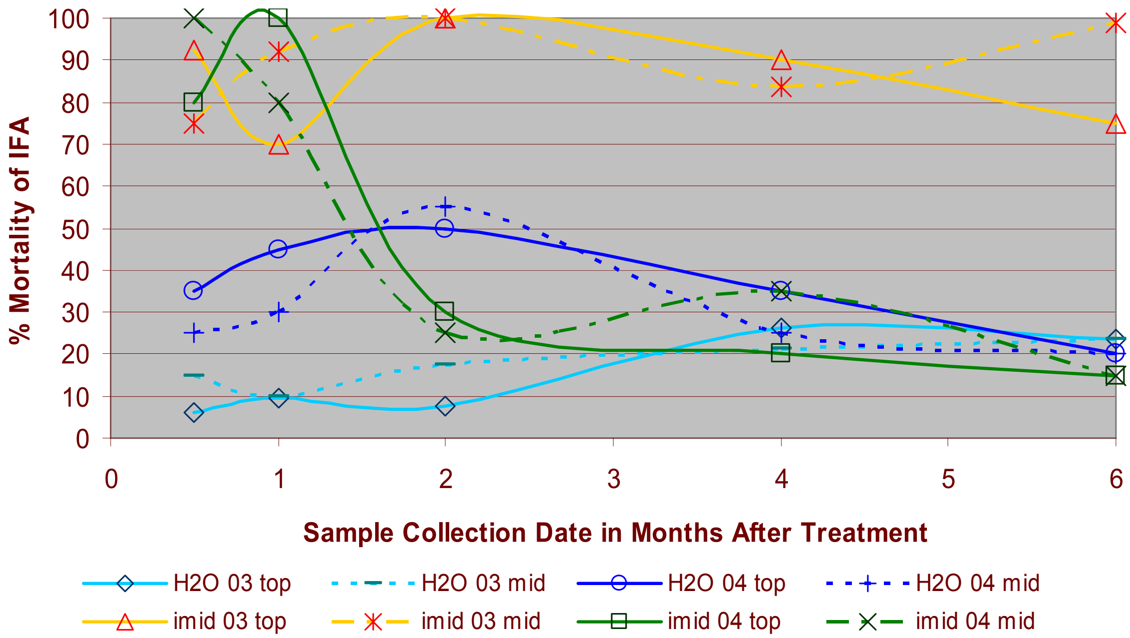
Deltamethrin provided complete control through six months in the fall 2003 trial, but an apparent decrease in efficacy began around four months in the spring 2004 trial (Figure 8). The six-month sample results for the spring 2004 trial were significantly lower than the half and two-month samples within the same trial and the six-month sample in the fall 2003 trial ($F=3.9787$; $df=36$; $P=0.0001$). There was never a statistical difference between the top and middle samples in this treatment. However, when the top samples yielded less than 100% control of IFA, it was due to survivors in multiple replicates, while in middle samples only a single replicate was involved. This may indicate some protection of the chemical from degradation in deeper soil. Future dip trials should include this chemical at this and lower rates to clear any inconsistency in treatment, explore shorter terms of efficacy, and examine longevity difference based on seasonal effects.

Figure 8. Efficacy duration of deltamethrin 0.065 lb a.i./ 100 gal H_2O treatments against female alate IFA in fall 2003 and spring 2004.



Imidacloprid at the 0.2 lb a.i./ 100 gal H_2O rate tested in these two trials was inadequate for quarantine purposes (Figure 9). While the fall 2003 application appeared to maintain some efficacy against IFA through the end of the trial, all but one of the sample dates had multiple replicates with surviving ants. The spring 2004 trial application on the other hand, which was significantly less effective than the fall 2003 application ($F=14.6873$; $df=9$; $P=0.0001$), had a strong showing in the half- and one-month results but dropped sharply by the two-month sample period. The two, four, and six-month sample means for the spring 2004 trial were statistically similar to water. The means from these dates were also significantly less effective at killing IFA than the two earlier sample dates in the same trial and less effective than the analogous dates in the fall 2003 trial ($F=3.9787$; $df=36$; $P=0.0001$). Imidacloprid should either be left out of future quarantine testing against IFA, or higher rates should be explored to improve consistency of fall/winter-only applications.

Figure 9. Efficacy duration of imidacloprid 0.2 lb a.i./ 100 gal H_2O treatments against female alate IFA in fall 2003 and spring 2004.



Thiamethoxam appears to display degradation in efficacy influenced by seasonal weathering (Figure 10). The mortality in fall samples was significantly higher than spring at two, four and six months ($F=3.9787$; $df=36$; $P=0.0001$). The middle samples in the fall 2003 trial consistently produced 100% kill, while the exposed top samples from the same trial wavered in efficacy. Furthermore, while both sample sites of the spring 2004 trial declined in efficacy over time, the top samples displayed a much sharper decline than the more protected middle samples and the sample sites were significantly different at two and four months ($F=1.7711$; $df=36$; $P=0.0044$). Unless higher rates of this chemical perform more consistently, thiamethoxam cannot be considered for quarantine use against fire ants.

Dimethyl phosphonate initially caused high mortality in the alates, but efficacy declined rapidly in both trials and both sample sites (Figure 11). Middle samples were significantly more effective at killing IFA than top samples at one and two months, suggesting some protection from chemical degradation in the deeper soil of the root balls (F 1.7711; $df=36$; $P=0.0044$). Top samples were statistically similar to control samples by one month, so at this point it is unlikely that this chemical would be useful as an IFA quarantine treatment.

Carbaryl and acephate never provided adequate IFA mortality and were generally statistically similar to the control through out both trials and regardless of sample site (Figures 12 & 13). The half-month sample for carbaryl was the only date where that treatment was significantly more effective than the control and acephate never was more effective than the control ($F=3.9787$; $df=36$; $P=0.0001$). Neither carbaryl nor acephate treatments appeared useful for the IFA quarantine.

Figure 10. Efficacy duration of thiamethoxam 0.065 lb a.i./ 100 gal H_2O treatments against female alate IFA in fall 2003 and spring 2004.

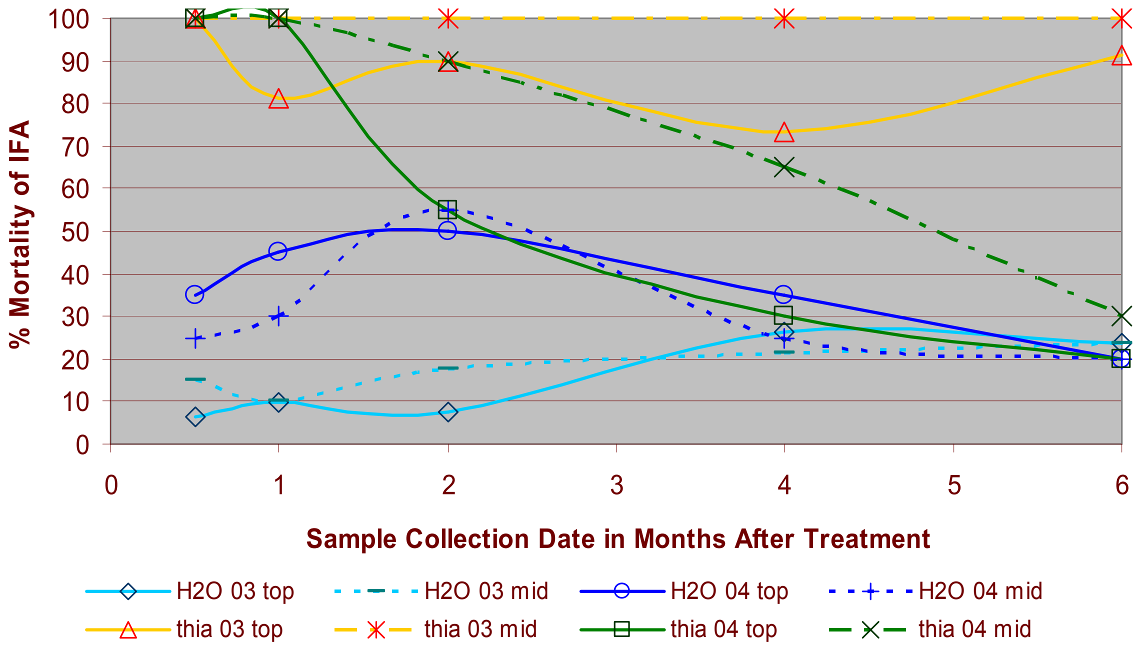


Figure 11. Efficacy duration of dimethyl phosphonate 4.0 lb a.i./ 100 gal H_2O treatments against female alate IFA in fall 2003 and spring 2004.

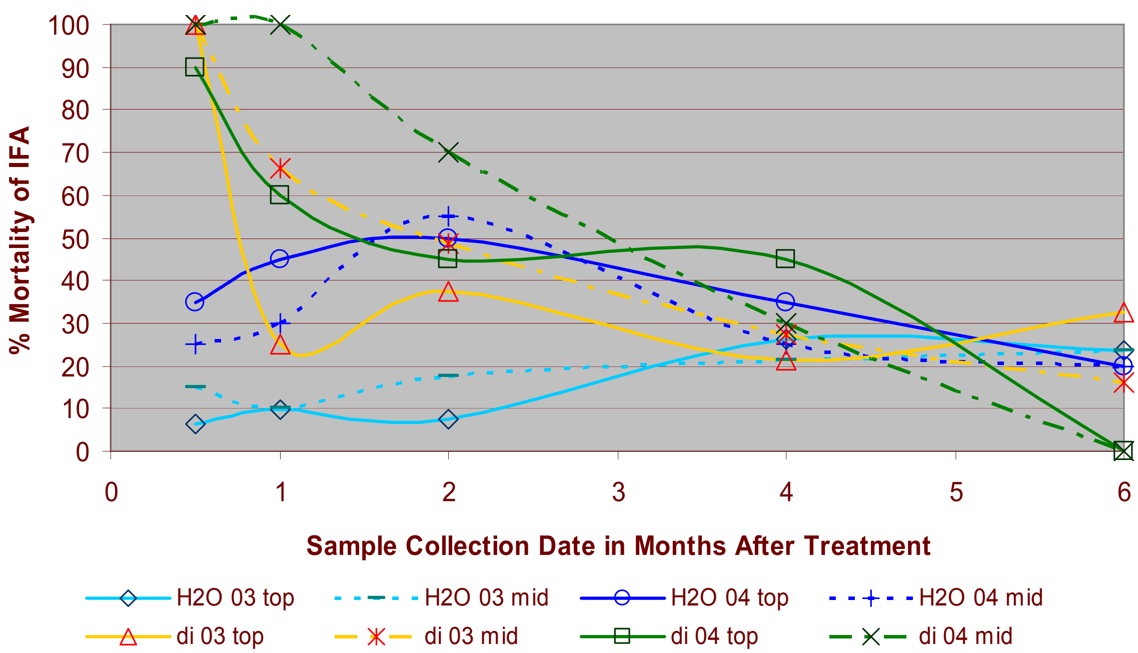


Figure 12. Efficacy duration of carbaryl 4.0 lb a.i./ 100 gal H_2O treatments against female alate IFA in fall 2003 and spring 2004.

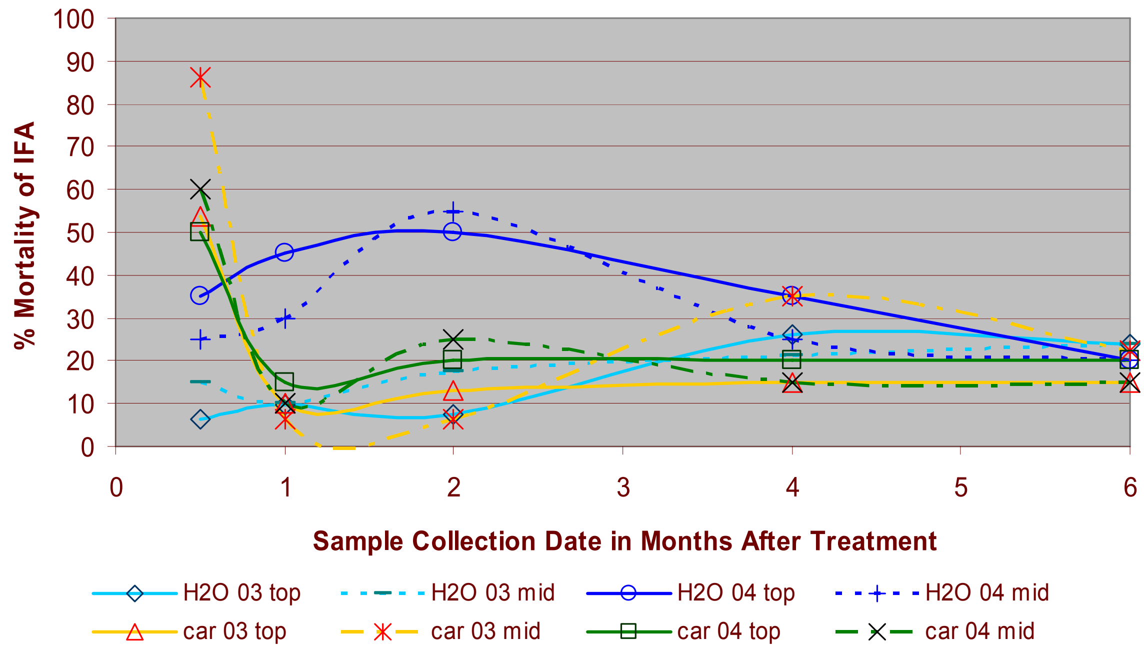
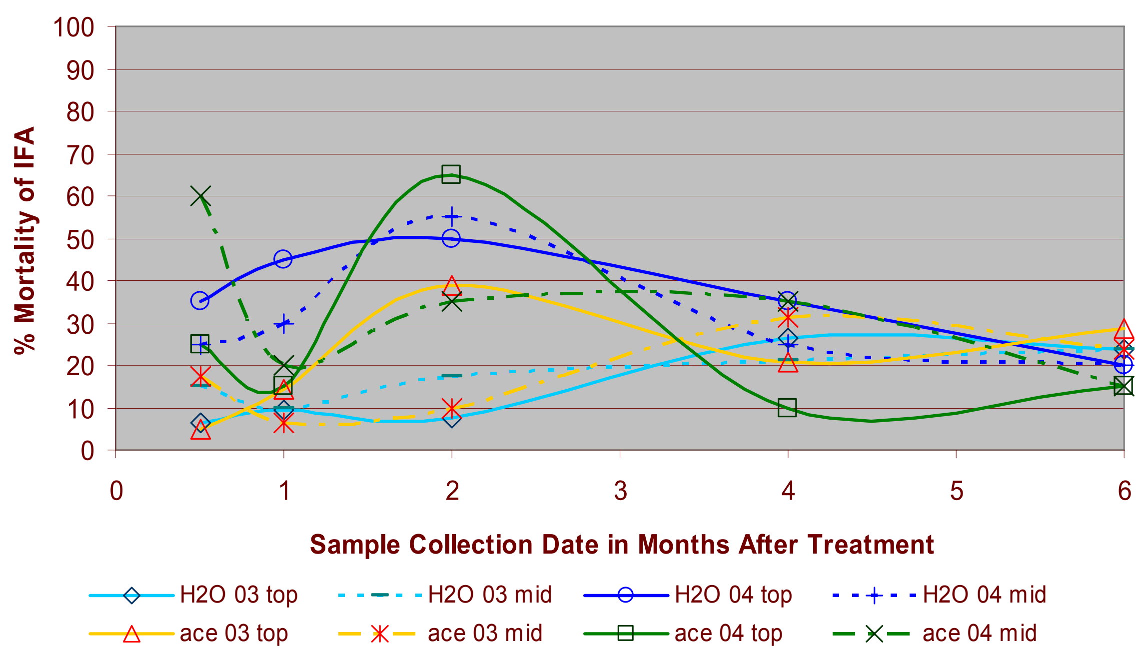


Figure 13. Efficacy duration of acephate 0.375 lb a.i./ 100 gal H_2O treatments against female alate IFA in fall 2003 and spring 2004.



Tennessee Fall 2004 and Spring 2005 Trials:
As stated in the methods section, results comparing sample site and caste were analyzed separately. The following discussion of results uses TM to refer to analysis examining the relevance of top and middle sample sites, while WA refers to analysis focused on comparisons between workers and alates.

Analysis of the combined fall 2004 and spring 2005 trials found significance in the effects of date (TM, F=29.6167; df=2; P=0.0001: WA, F=13.5124; df=2; P=0.0001), trial x date (TM, F=16.8965; df=2; P=0.0001: WA, F=4.5761; df=2; P=0.0109), treatment (TM, F=157.0949; df=9; P=0.0001: WA, F=195.6336; df=9; P=0.0001), date x treatment (TM, F=5.0658; df=18; P=0.0001: WA, F=3.1345; df=18; P=0.0001), and trial x date x treatment (TM, F=1.9486; df=18; P=0.0125: WA, F=2.5781; df=18; P=0.0005) for both the TA and WA data sets. Additionally, within their respective combined-trial analyses, the TM set found significance in the interaction of trial x treatment (F=3.2352; df=9; P=0.0009), and the WA set found significance in the caste interactions of date x caste (F=7.5130; df=2; P=0.0006), trial x date x caste (F=7.6515; df=2; P=0.0006), treatment x caste (F=4.3254; df=9; P=0.0001), trial x treatment x caste (F=2.1300; df=9; P=0.0265), and date x treatment x caste (F=2.0163; df=18; P=0.0086).

Both the trial and sample site main effects were significant in the previous fall 2003/spring 2004 set of trials but not in the fall2004/spring 2005 trials. This was probably due to a combination of difference in treatments used and the reduced number of sample dates in the fall 2004/spring 2005 trials. Between sets of trials only the chlorpyrifos at 2.0 lb ai and deltamethrin at 0.065 lb ai treatments were common to both. The results from the most recent trials for these treatments echoed their results in the fall 2003/spring 2004 trials up to the four-month sample date (Figures 5, 8, 15, and 20), it is within the six-month sample date, where significant difference in seasonal degradation occurred for these treatments. Additionally, the separate analysis of the fall 2004 alate results, which had four sample dates, indicated significant difference in treatment efficacy between the top and middle samples of imidacloprid (treatment x site: F=2.8212; dF=9; P=0.0038). Meanwhile, the spring 2005 alate results, which had only three sample dates, did not show significance even though the raw difference between middle and top sample means at four months was greater than that seen in the analogous sample date in the fall (Figure 22).

The general trend for sample date was of reduced chemical efficacy by the fourth month after treatment (date, TM and WA). When separated by caste and trial though, the fall tested alates and spring tested workers responded similarly within caste across dates, the spring tested alate results across dates mimicked the general influence of date, and the fall worker-tested samples displayed a peak efficacy at one month post-treatment with the other dates statistically similar to each other (trial x date x caste, WA). Despite the different patterns of significance seen within alate and worker groupings, there was no overall significant difference between workers and alates in the fall trial within the same sample date. However, alate mortality was higher than that of workers tested in the first two sample dates in the spring trial and then fell significantly below worker mortality by the final sample date.

At the broadest level, any chemical treatment provided higher mortality of IFA than those exposed to the water control samples, and imidacloprid was significantly less effective than all other chemical treatments (treatment, TM and WA). This relationship of treatment efficacy was constant regardless of caste. Treatment used was such an important effect that all other result-influencing effects were further defined by their interaction with treatment. Because of the complexity of the interaction relationships and the use of multiple analyses on slightly different data sets, in order to maintain clarity, continued discussion of the results for the fall 2004 and spring 2005 trials has been divided by treatment. The best performing treatments (chlorpyrifos at 2.0 lb ai, chlorpyrifos 0.125 lb ai, and Discus™ [an imidacloprid and cyfluthrin blended product]) are discussed first and are followed by the treatments that were progressively less reliable or less effective.

The Discus™ and the chlorpyrifos at 2.0 lb ai treatments produced identical results, 100% IFA mortality regardless of sample site, caste, trial season, or date (Figures 14 and 15). Chlorpyrifos at 0.125 also performed exceptionally well, but had one surviving alate from a top sample at four months (Figure 16). All three treatments were very consistent and out lasted the current IFA quarantine 30-day certification period. The 2.0 and 0.125 lb ai chlorpyrifos treatments were also examined in the fall 2003/spring 2004 and Mississippi 2004 trials, respectively. As previously mentioned, the JB rate treatment of 2.0 lb ai chlorpyrifos performed identically in both sets of Tennessee trials up to the point sample dates coincided. The IFA rate of 0.125 lb ai chlorpyrifos when tested in Mississippi soil was able to kill all alates through six months of testing. The performance of the IFA rate in Tennessee soil was comparable up to its last sampling at four months. Since the IFA rate applied to Tennessee soil only sampled through the fourth month, it is impossible to determine if the lone surviving alate in the TN soil samples was an outlier or if it was the beginning of a decline in efficacy in the top soil samples for that treatment.

Figure 14. Duration of efficacy of the Discus® (0.5 lb a.i. imidacloprid and 0.12 lb a.i. cyfluthrin per 100 gal H_2O combined product) dip treatments against female alate and worker IFA in fall 2004 and spring 2005.

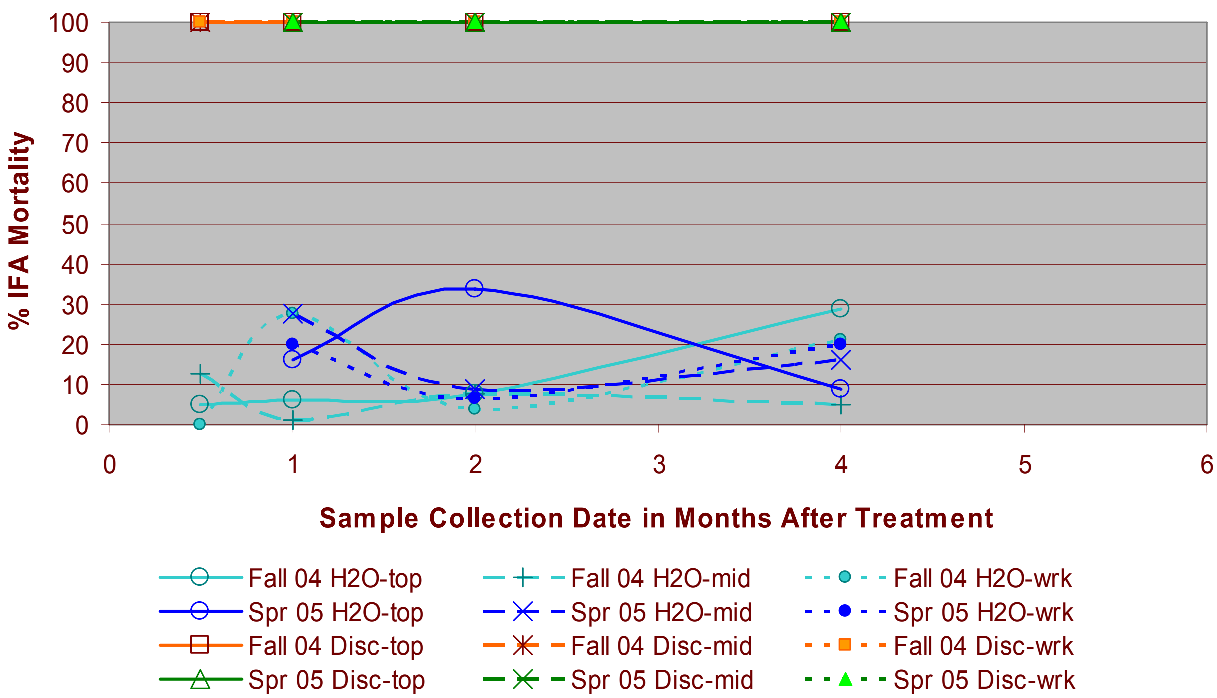


Figure 15. Duration of efficacy of the 2.0 lb a.i./ 100 gal H_2O chlorpyrifos dip treatments against female alate and worker IFA in fall 2004 and spring 2005.

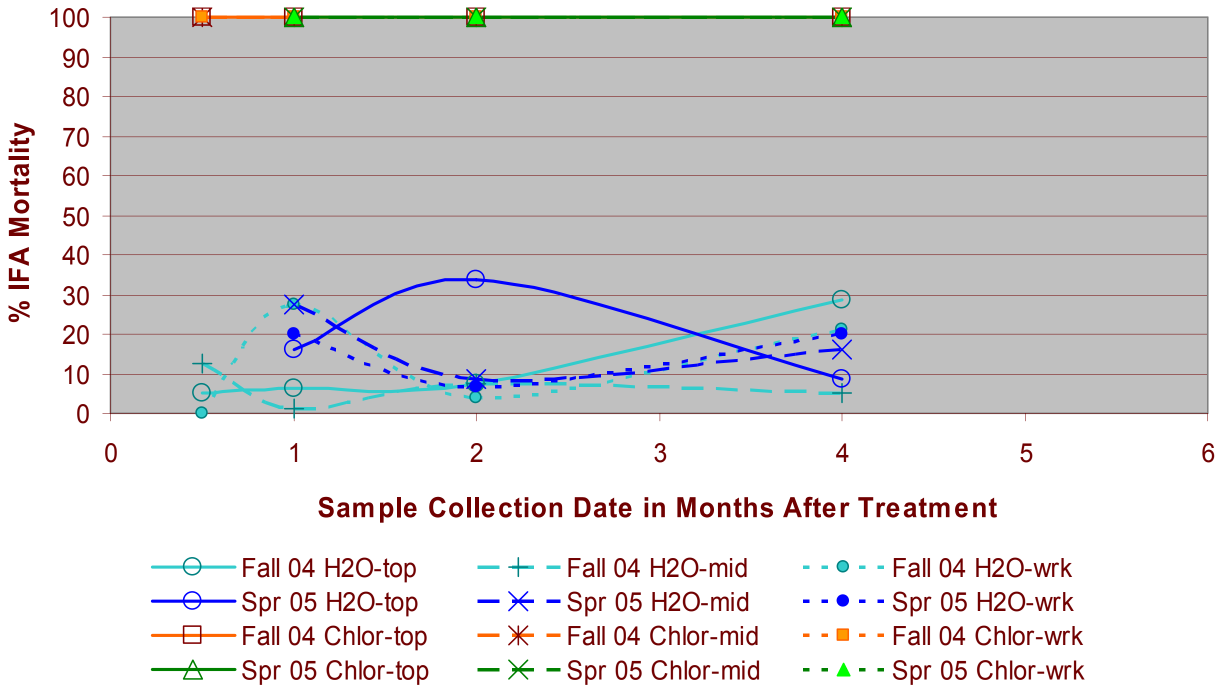
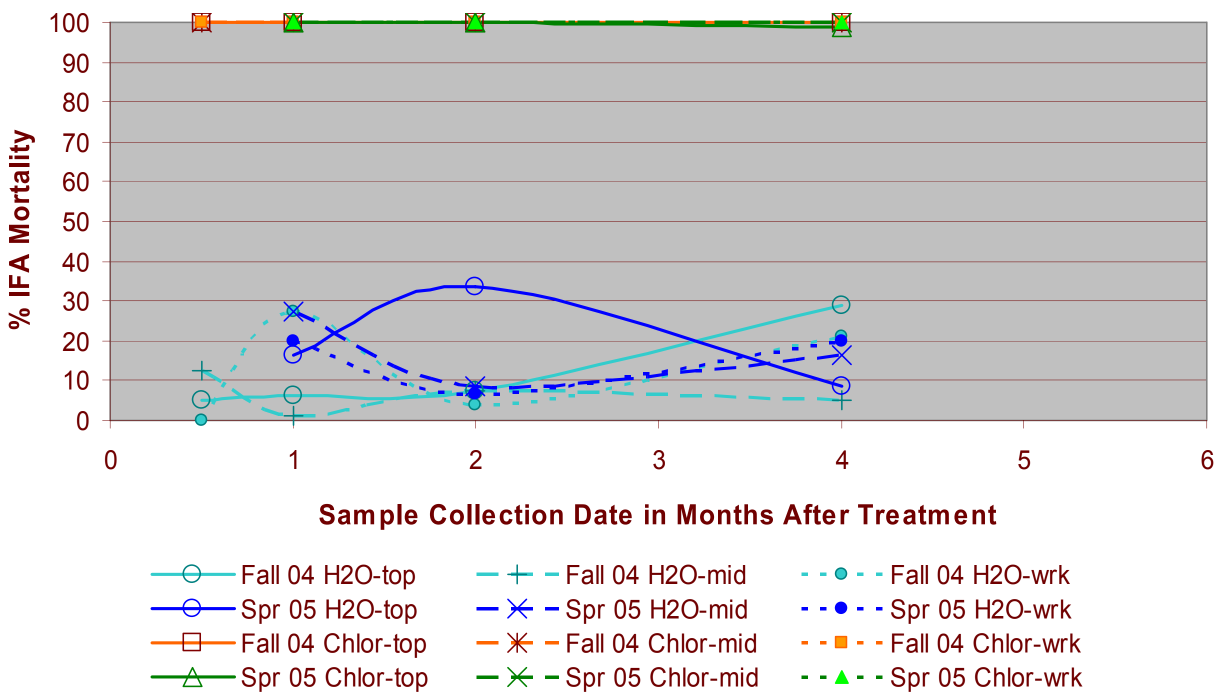


Figure 16. Duration of efficacy of the 0.125 lb a.i./ 100 gal H_2O chlorpyrifos dip treatments against female alate and worker IFA in fall 2004 and spring 2005.



Previous trials using Tennessee soils at higher rates of bifenthrin (0.23 and 0.115 lb ai) produced near to complete control of IFA alates through six months after treatment regardless of trial season or sample site location. The 0.025 lb ai rate was previously tested using Mississippi soil in 2003 and 2004 and provided six months of 100% control in each trial as well. The 0.025 lb ai rate used in the fall 2004 (Tennessee) trial similarly provided 100% control of IFA regardless of sample site, caste, or sample date (Figure 17). The lower rate (0.0125 lb ai) in the fall 2004 trial, while performing strongly through the final sampling, was not as consistent as the higher rate (0.025 lb ai) and had surviving alates on some two-month middle samples and on one of the top samples from the final date (Figure 18). Surviving alates in middle samples were seen for the first time when bifenthrin was at 0.0125 lb ai. This may indicate a lowered ability to penetrate the root balls at this low rate. The spring 2005 trial saw both rates of bifenthrin lose efficacy against alates after the two-month sample date. The higher 0.025 rate was actually significantly less effective by four months than at its earlier sample dates (trial x treatment x date, TM and WA). Due to the influence of the middle samples, this relationship was the same for the lower 0.0125 rate when examining the TM data set (trial x treatment x date, TM). However, within the WA data set there was no difference across all dates in the lower rate.

No workers survived either rate of bifenthrin at any date in either trial. Within the results for the 0.025 lb ai rate from the final sample, workers were reduced significantly more by the treated soil than alates were (treatment x date x caste, WA). Though alates and workers at the lower 0.0125 lb ai rate were never significantly different from each other, the loss of efficacy against alates displayed at this and the higher rate is important when considering certification duration for quarantine purposes. Examining the worker data alone from the bifenthrin treatments would have been misleading.

Figure 17. Duration of efficacy of the 0.025 lb a.i./ 100 gal H_2O bifenthrin dip treatments against female alate and worker IFA in fall 2004 and spring 2005.

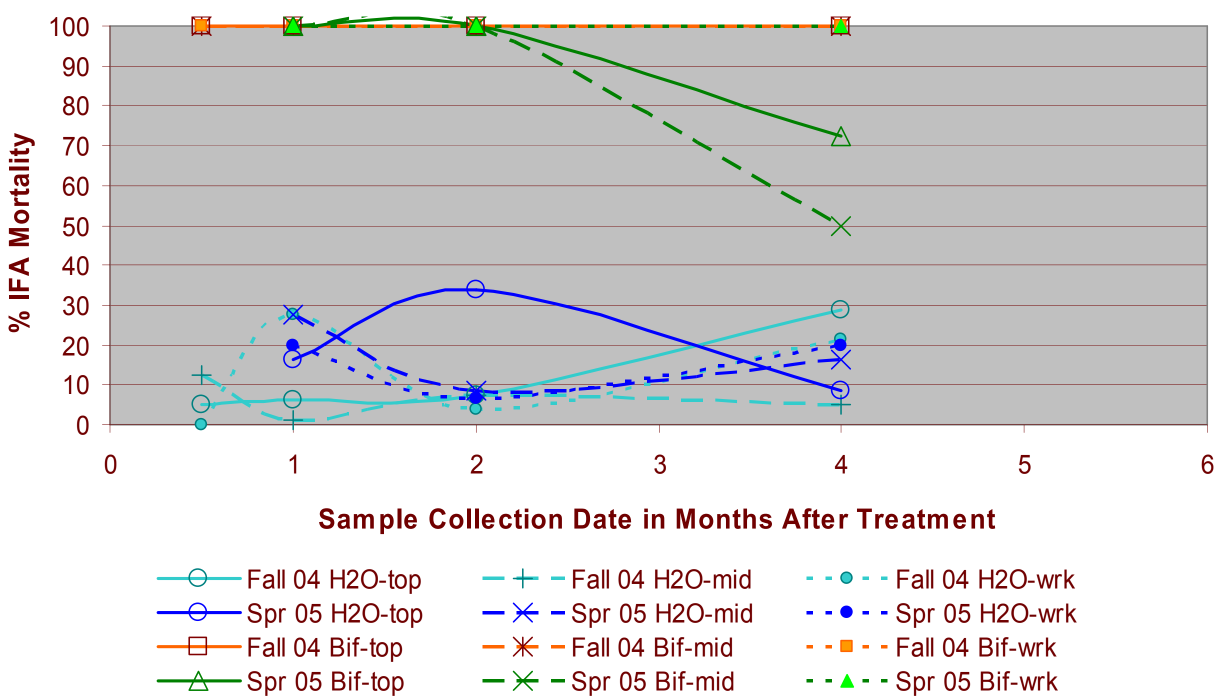
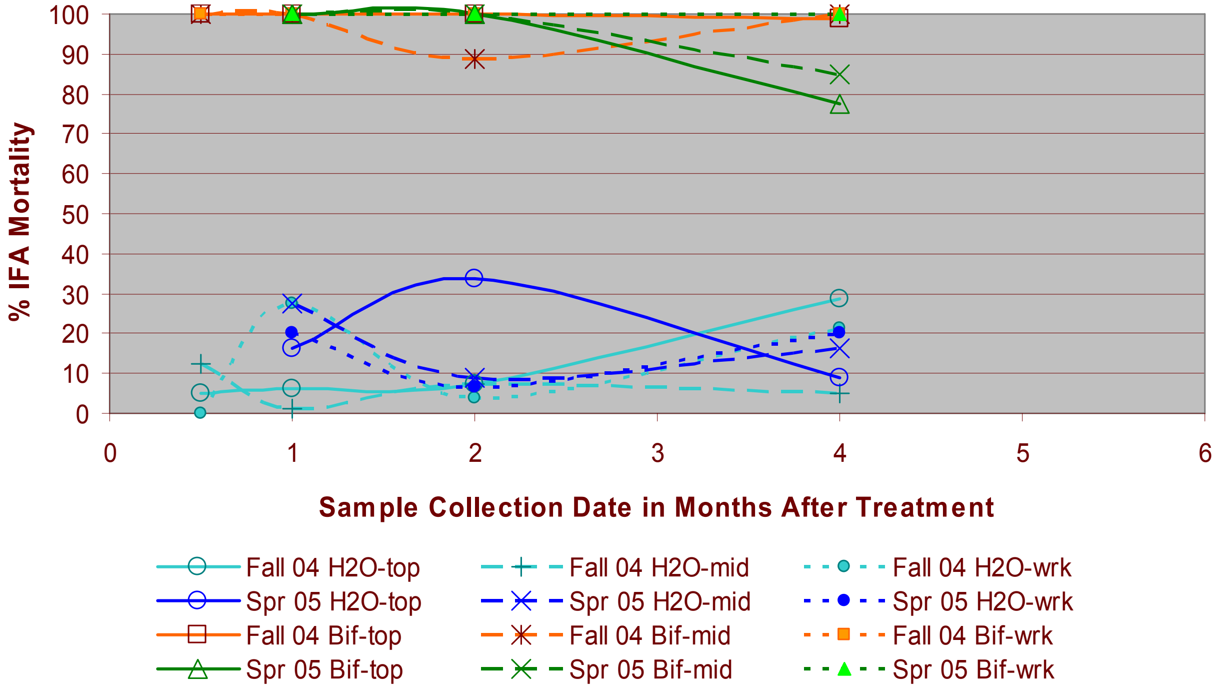


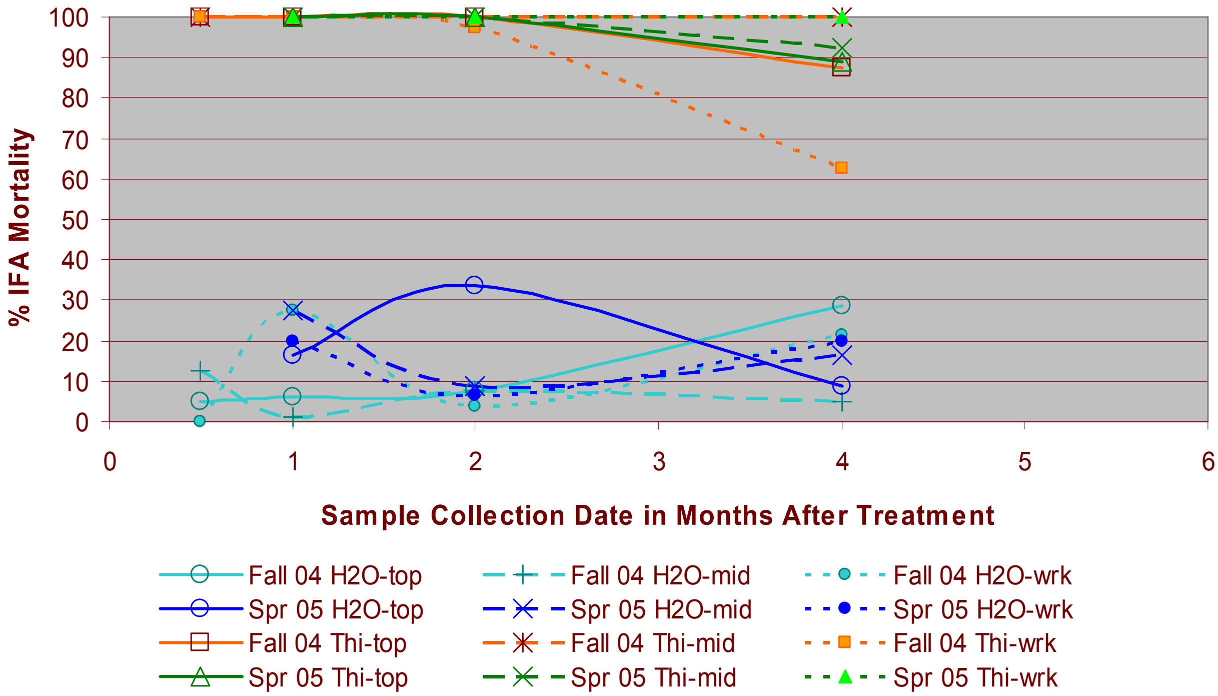
Figure 18. Duration of efficacy of the 0.0125 lb a.i./ 100 gal H_2O bifenthrin dip treatments against female alate and worker IFA in fall 2004 and spring 2005.



Thiamethoxam at the 0.13 lb ai rate was tested previously in trials conducted in spring 2002, fall 2002, and spring 2003. Though sample dates and sites were not exactly the same as the fall 2004 and spring 2005 trials, their results generally supported those of the most recent trials (Alternative B&B immersion or drench treatments for use in the IFA quarantine, 2002 and 2003 in the 2003 Annual Accomplishment Report for SIPS). Complete control of alates was produced in both the fall 2004 and spring 2005 trials through the two-month sample date and remained high through four months (Figure 19). When all alates were examined over trial and sample date, the decrease in efficacy was slight enough that results from all dates were considered similar. However, workers tested against the final samples in the fall 2004 trial appeared less affected by the treatment than the alates. Analysis of the WA data set does indeed find survival skewed significantly in favor of the fall four-month sample when workers are included (date x treatment, trial x date x treatment, trial x treatment x caste, and date x treatment x caste; WA). The disparity between workers and alates in the fall at four months in this treatment is of little importance since by that time both castes show survivors.

This higher rate of thiamethoxam appears little affected by seasonal differences compared to the lower rate used in the fall 2003 and spring 2004 trials. Both fall 2004 and spring 2005 top samples had almost identical means, while the top samples from the fall 2003/spring 2004 set were only similar at the half-month sample date. Also, the 100% kill seen through the final date in middle samples from fall 2004 was mimicked in all but one of the four-month replicates in the spring 2005 middle samples, unlike the prior trial set that saw seasonal deviation by two months post treatment. The improvement in consistency and duration of a thiamethoxam treatment applied at 0.13 lb ai mean that it could possibly be considered at this rate for use in the IFA quarantine.

Figure 19. Duration of efficacy of the 0.13 lb a.i./ 100 gal H_2O thiamethoxam dip treatments against female alate and worker IFA in fall 2004 and spring 2005.



Deltamethrin at 0.065 lb ai provided results strikingly similar to bifenthrin at 0.0125 lb ai within this same fall 2004/spring 2005 set of trials, and it further supported results previously seen for the same deltamethrin treatments in the fall 2003/spring 2004 trial set (Figures 20, 18, and 8). Fall 2004 trial results for deltamethrin at 0.065 lb ai showed complete kill of workers and alates on top soil samples, and while some alates survived in middle soil samples at two months, none survived at other dates. Again in the spring 2005 trial, the middle samples had a few surviving alates at one sample date, but this was followed by complete control at the next date. The top soil samples in the first two sample dates in spring 2005 displayed control of both alate and worker castes, but by the fourth month, workers were apparently more affected by the residual chemical than alates were.

Deltamethrin at the lower rate of 0.04 lb ai delivered results similar to the higher rate but with surviving alates in more middle soil samples and a significantly greater decrease in efficacy by the final sampling at four months post-treatment (trial x date x treatment, TM) (Figure 21). The few surviving alates in middle samples at both rates of deltamethrin did not yield statistically different results from top samples at the same date; however, the frequent occurrence of surviving alates does impact the potential use of the deltamethrin treatments for quarantine certification. When the deltamethrin 0.04 lb ai treatment was somewhat degraded at the four-month sampling in the spring trial, workers were significantly more susceptible to the treatment than alates (trial x treatment x caste and date x treatment x caste, WA). Worker response in this treatment could have been misleading if used alone when considering quarantine treatment potential. Like the bifenthrin 0.0125 lb ai treatment, both deltamethrin treatments displayed survivors in middle samples at early sample dates, raising the question of whether these rates are too low to effectively penetrate the root balls or if longer submersion is required.

Figure 20. Duration of efficacy of the 0.065 lb a.i./ 100 gal H_2O deltamethrin dip treatments against female alate and worker IFA in fall 2004 and spring 2005.

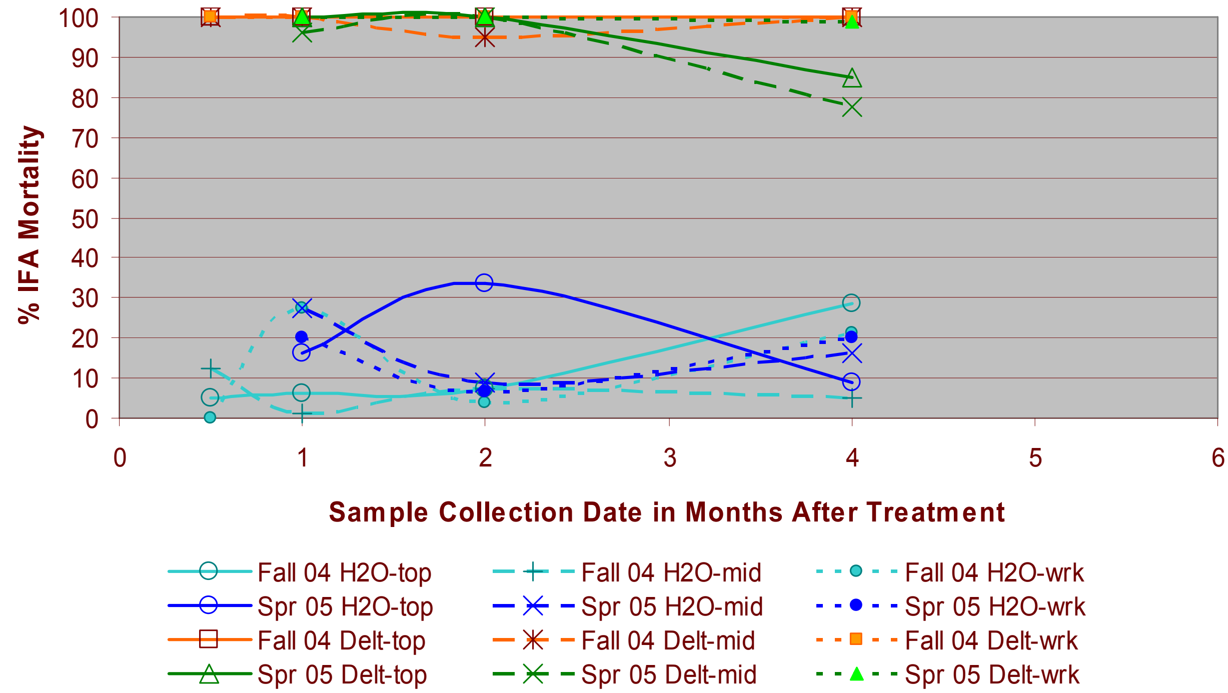
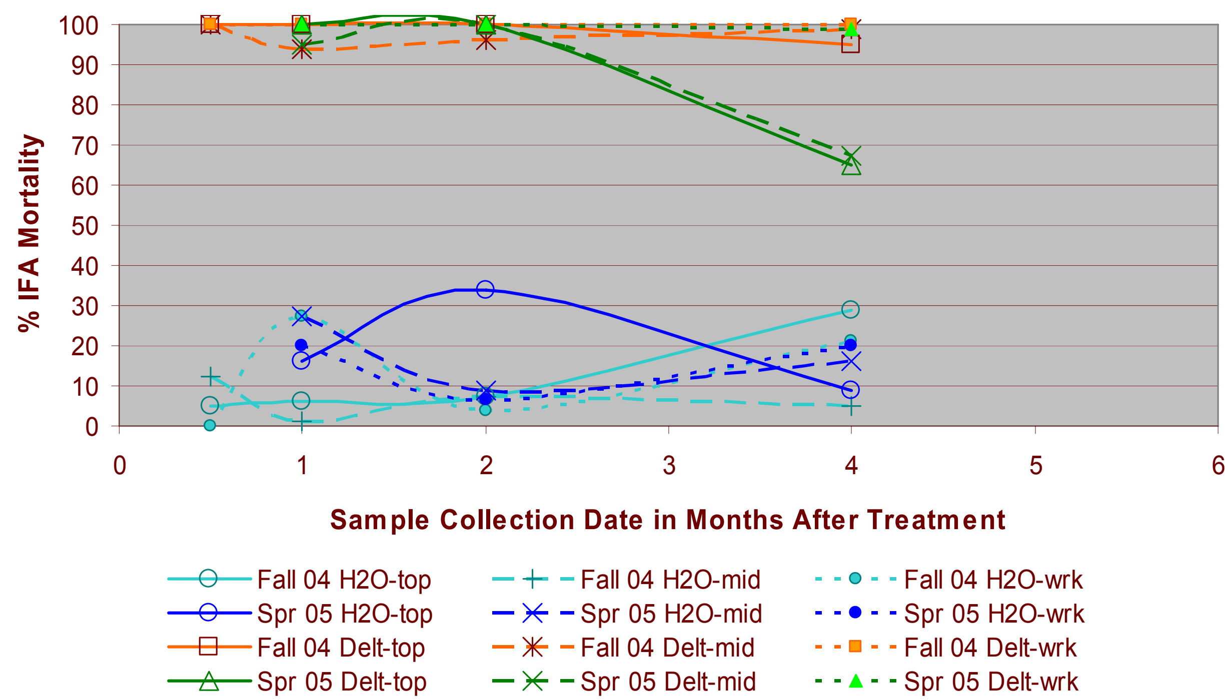


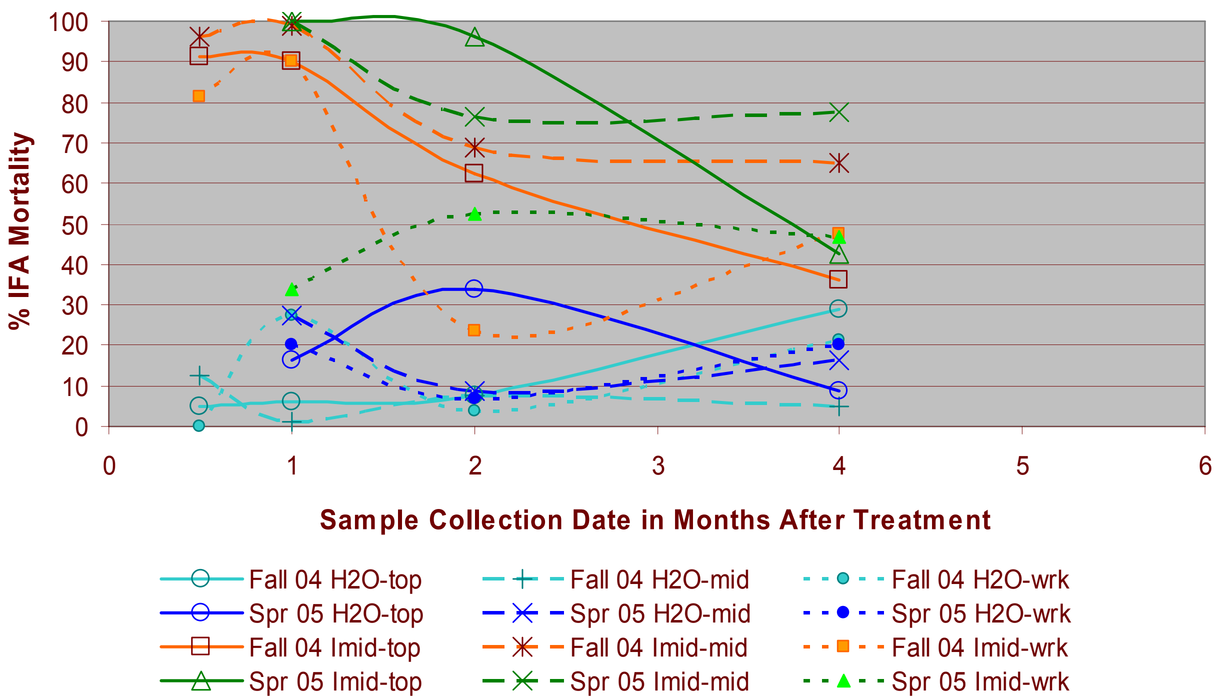
Figure 21. Duration of efficacy of the 0.04 lb a.i./ 100 gal H_2O deltamethrin dip treatments against female alate and worker IFA in fall 2004 and spring 2005.



Cumulatively across trials and dates, imidacloprid was the least effective of the chemical treatments (treatment, TM and WA); however, it did attain high, though incomplete, levels of IFA control at its earliest sample dates (Figure 22). The fall 2004 trial saw imidacloprid results drop significantly and progressively after the one-month sample date (trial x date x treatment, TM and WA). This was unexpected, since the imidacloprid treatment in the fall 2003 trial displayed residual activity through six months and was applied at half the rate used in fall 2004/spring 2005 trials. Contrary to the precedent set by the fall 2003/spring 2004 trial set, the spring 2005 results at the two-month sampling provided significantly higher IFA mortality than the fall 2004 samples from the same sample date (trial x date x treatment, TM and WA). Also, the difference between sample sites at the same date was not significant in the fall 2003/spring 2004 trial set, but the fall 2004 top samples had significantly more survivors than middle samples at the final sample date (treatment x site, TM fall 2004 only data set). Although not significant, the spring 2005 sample sites differed widely at both the two- and four-month sample dates.

Workers generally were less affected by the imidacloprid treatment than alates (treatment x caste, WA). This was significantly prominent in the spring 2005 trial, especially in the samples collected at one and two months post-treatment (trial x treatment x caste, WA; date x treatment x caste, WA). Since reproductive females, represented in these tests by female alates, are necessary for the continued survival of a colony, worker results, if considered alone, could give a false sense of loss of efficacy. It is interesting that this treatment displayed the reverse of the caste reactions seen for the high rate of bifenthrin and the low rate of deltamethrin. However, regardless of direction of discrepancy, since presence of workers currently is key in determining commodity infestation, it precludes use of these treatments, as they currently act, in quarantine certification.

Figure 22. . Duration of efficacy of the 0.4 lb a.i./ 100 gal H_2O imidacloprid dip treatments against female alate and worker IFA in fall 2004 and spring 2005.



CONCLUSION:

Across the five trials discussed in this report, Discus™, chlorpyrifos at 2.0 and 0.125, bifenthrin at 0.115 and 0.025, and thiamethoxam at 0.13 stand out due to their consistency in providing quarantine level control for a period of time greater than 30 days. The Discus™, 0.025 rate of bifenthrin, and 0.13 rate thiamethoxam need to be tested through another set of Tennessee soil trials for verification. However, the 2.0 rate of chlorpyrifos and the 0.115 rate of bifenthrin have proven in these and other trials (reported in previous annual accomplishment reports) to be capable of protecting treated root balls throughout the duration of the B&B harvest and shipping season. The current certifying rate of chlorpyrifos is certainly adequate for quarantine as currently described in the Federal Imported Fire Ant Quarantine, and given further testing, may be granted an increased shipping period. Based on all of our test data, there is no doubt that a grower treating for Japanese beetle grubs using the 2.0 chlorpyrifos dip rate has also effectively treated for imported fire ants.

Treatments with lambda-cyhalothrin at 0.034, bifenthrin at 0.0125, and deltamethrin at 0.065 and 0.04 all demonstrated extended periods of high level IFA control, but had some surviving alates at a date or dates before the apparent point of degradation-influenced lost efficacy. The anomalous lack of efficacy for a few soil samples suggests not all soil in the root ball is uniformly treated, thus leaving areas where insecticide levels are insufficient to provide 100% IFA control. It is possible at these rates that these pyrethroids, by their chemical nature, may have trouble effectively penetrating the root balls. It is also possible that an increase in time spent immersed in these solutions would allow more thorough penetration. Currently, the time of immersion for IFA quarantine dipping is, "until bubbling ceases." Trials conducted using Tennessee-grown plants were subjected to approximately one minute of immersion to reach bubbling cessation, while Mississippi trials immersed plants between 5 and 17 minutes to reach cessation of bubbling. Mississippi trials yielded longer residual efficacy at lower rates than TN trials. The larger pore space in the sand-loam Mississippi soil compared to the clay-loam Tennessee soil undoubtedly allows more rapid penetration and, due to there being more air space to fill, prolongs the dip time. Cessation of bubbling, therefore, may be an inadequate indicator of treatment completion for denser soils.

Trial season was a factor introduced because of its importance to the control of JB grubs. The inactivity of JB grubs during winter months appears to influence the success of certain treatments (Jason Oliver personal communication). Since IFA are active year round, it was not expected that there would be a seasonal effect on their survival. However, in an ANOVA run on the alate data from the common sample dates of these four TN trials, mortality in the control samples was higher during spring sample testing than during that for the immediately preceding fall trials ($F=18.124$; $df=3$; $P=0.0001$). Personal accounts by Anne-Marie Callcott and Lee McAnally indicate a seasonally linked increase in IFA mortality in untreated soil has occurred in the past. It may be that IFA from south Mississippi are either less fit, or that *Metarhizium anisopliae* is more active in soils during this time.

Since the general trend during spring testing is for decreased alate survival, it could be expected that spring trials would appear to have a longer lasting control of IFA. However, for several chemicals survivors appear much earlier and are more prevalent in spring trials compared to fall trials. So in this way, the higher background mortality rate serves to highlight the seasonal increase in chemical degradation in the spring. The trials conducted through fall and winter coincide with the typical harvest and shipping times for field-grown B&B, and thus, performance during these trials is generally viewed as being closer to what could be expected in actual practice. The Imported Fire Ant Quarantine, however, applies to all B&B within the IFA-infested zones in the United States, including areas with milder weather that may lend to faster degradation, similar to what was demonstrated in the spring trials. Detailed records of environmental factors treated plants are exposed to should be recorded in future trials to determine their role in insecticide degradation. This information could potentially be used to develop a schedule of treatment duration growers could reference based on their local weather conditions.

Worker and alate castes of IFA demonstrated different responses to pyrethroids and neonicotinoids. Workers in spring 2005 appeared more susceptible than alates to the effects of the pyrethroids bifenthrin and deltamethrin. However, the neonicotinoids imidacloprid and thiamethoxam demonstrated less of an effect on workers than on alates. Both chemical classes are nerve toxins, so it is not readily apparent why they would produce different caste based effects. A difference in caste response to pesticidal exposure raises issues with the use of those treatments for quarantine purposes, regardless of which caste is less affected and the physiological cause. A colony without a viable queen cannot survive and cannot produce new colonies to infest an area. However, since workers are the most visible indication of infestation, their presence is used to determine infestation of goods moving out of the quarantined areas. Neonicotinoid treatment may lead to unnecessary delay or rejection of shipments due to presence of workers when the treatment still has lethal effects on queens and alates. Conversely, lack of detectible workers in pyrethroid treatments could allow viable queens and alates to slip through undetected if B&B were shipped past its certification period.

CPHST PIC NO: A1M04

PROJECT TITLE: Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – in-field individual tree treatment, 2005 winter

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Shannon James, Anne-Marie Callcott, Lee McAnally, and Jennifer Lamont of USDA-APHIS; Jason Oliver and Nadeer Youssef of Tennessee State Univ.; Pat Parkman, Tahir Rashid and Karen Vail of Univ. of Tennessee

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.

<u>MATERIALS AND METHODS</u>:

Field Trial:
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 mark the treatment areas around each plot (figure 1).

Figure 1. A field trial plot

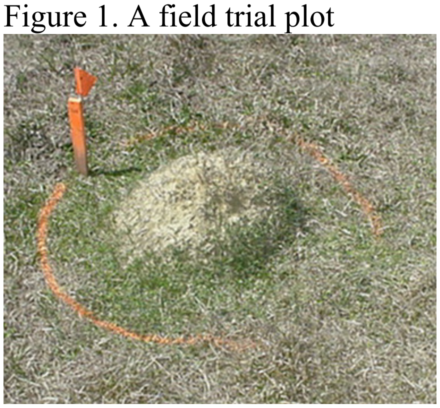


Figure 2. Application of water to a control plot

The field trial treatments were applied to fifteen replicate plots each, in a Harrison Co., MS pasture on January 26 and 27, 2005. 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 (figure 2). Each treatment was applied in two gallon amounts to each of its assigned plots. The treatments were as follow:

Product	Active Ingredient	Rate of Application
BifenthrinPro - flowable	bifenthrin	0.20 lb a.i./acre
BifenthrinPro - flowable	bifenthrin	0.40 lb a.i./acre
Scimitar	lambda-cyhalothrin	0.0688 lb a.i./acre
Scimitar	lambda-cyhalothrin	0.1375 lb a.i./acre
DeltaGard 5SC	deltamethrin	0.13 lb a.i./acre
Dursban 4E	chlorpyrifos	1.0 lb a.i./acre
Wet Control	water	Water only

A problem with an unidentified contaminate in the spray tank occurred on the 26th requiring location of fifteen new plots each and, after thorough tank cleaning, reapplication for the control and both rates of bifenthrin. The contaminated treatment plots were monitored along with the uncontaminated to determine effect if any of the impurity. Contaminated treatments when discussed in the results are labeled with an "A" following the treatment name while analogous clean treatments are indicated with a "B."

Observations of mound activity were conducted weekly until three months passed. Treatments showing no IFA activity at the three month reading which had achieved control within four weeks, were monitored every other week for an additional month until field conditions prevented locating the plots. Due to hosting the Annual Imported Fire Ant Conference, no mound readings occurred in the eighth week. Temperature data throughout the duration of the trials were collected using a StowAway® data logger and accessed using BoxCar® 3.6 (Onset Computer Corp., Bourne, MA) and supplemented by manual readings of air and soil temperatures taken at the times of observation. Precipitation was measured through a rain gauge located in the pasture.

Laboratory Tests:
IFA activity within the test plots, as in previously reported trials, was the primary means to determine treatment effectiveness. However, in an effort to verify length of treatment activity, bioassays and infestation preference tests in the laboratory were also examined. Sod was the selected material for these tests because prior trials attempting to collect directly from the field site provided soil samples primarily consisting of soil beyond the depth that the treatment penetrated (see Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – in-field individual tree treatment, 2004 Winter/Spring). The sod used in the current experiment was only between one to two inches thick and, unlike the field soil, was not compacted and thus better promoted treatment penetration.

Initially pieces of sod were placed in the pasture with the field trial and treated there at the same time and with the same equipment as the ant infested plots; however, the activities of a hungry cow required a second set of sod treatments to be set up on the grounds at the laboratory. Sod plots at the laboratory were treated identically to the field plots except the contaminated plots were not replicated and two-gallon watering cans were used to apply the treatments. These sod plots were allowed to weather naturally and were thus exposed to similar conditions through out the trial as the corresponding field plots. Soil samples were collected from the sod plots at 1, 2, 4, 8, 12, and 16 weeks after treatment application. Fifteen soil samples of each treatment were collected and divided into five replicates for use in the alate queen bioassays (Appendix I), dual-choice repellency tests, and single-choice repellency tests. The edges of the soil plugs were pressed firmly to the cup to eliminate gaps between the soil and the test cup, then the soil samples were frozen until ants and space were available to run the tests.

Alate queen bioassays were set up with a single test cup for each soil sample. Survival of the five alates in each cup was monitored and recorded periodically over fourteen days. In order to preserve the integrity of the treated surface, monitoring activities on days other than the final day were limited to examining alate activity on the soil surface. Since samples were discarded on day fourteen, any cups with unaccounted for alates were thoroughly searched by emptying the cup contents into a pan and digging through the soil samples until all alates were found.

Repellency is being examined through exposing workers with brood to soil samples in either a dual-choice test, where they are presented with both chemically treated and untreated control soil samples, or single-choice tests, where only a treated cup is available for colony nesting. In the dual-choice, control soil plugs have been collected from completely untreated sod so that even the water treated sod has an opposing control cup. Plastic pans measuring 9.5” x 11.5” at the base with 5.5” high walls are used as test arenas. Pots, identical to those used in the mortality bioassays, containing the soil sample nest material are centered two inches from the edge of the short end and approximately 1500 workers are then placed either between prospective nesting cups (dual-choice) or at the opposite end of the test pan (single-choice). Soil samples are kept moist by direct application of small amounts of water as needed. Butcher paper is also draped over the tops of the test arenas to further inhibit ant and soil sample desiccation. Over a seven day period apparent general health (live, sick, or dead) and preferred location (arena pan, inside treatment pot, exterior of treatment pot, inside of control pot, exterior of control pot) of the workers is recorded daily. The number of ants inside soil is estimated by subtracting the numbers recorded in other location categories in the test arena from 1500. On the final observation day a thorough investigation of pot colonization is conducted by dumping the pot contents and looking for live ants. Due to a number of prohibitive factors the repellency lab tests are only in the earliest stages of testing and will be reported at a later date.

RESULTS AND DISCUSSION:

Field Trial:
All six insecticidal treatments caused a decrease in active mounds, with chlorpyrifos and both rates of bifenthrin apparently reaching 100% control by two weeks after treatment (figure 3). However, deltamethrin appears to be the only treatment that reached 100% control before the week four observation and maintained an unbroken period of control for at least four weeks. Even the two uncontaminated bifenthrin treatments had an occasional active mound. The contaminated bifenthrin plots (bifenthrin 0.4-A and bifenthrin 0.2-A) had many more active readings throughout the trial (figure 4) than the analogous uncontaminated plots, even though they demonstrated the same initial drop in activity.

Figure 3. Cumulative active imported fire ant mounds (plots) within each treatment counted at weekly observations over the course of the field trial.

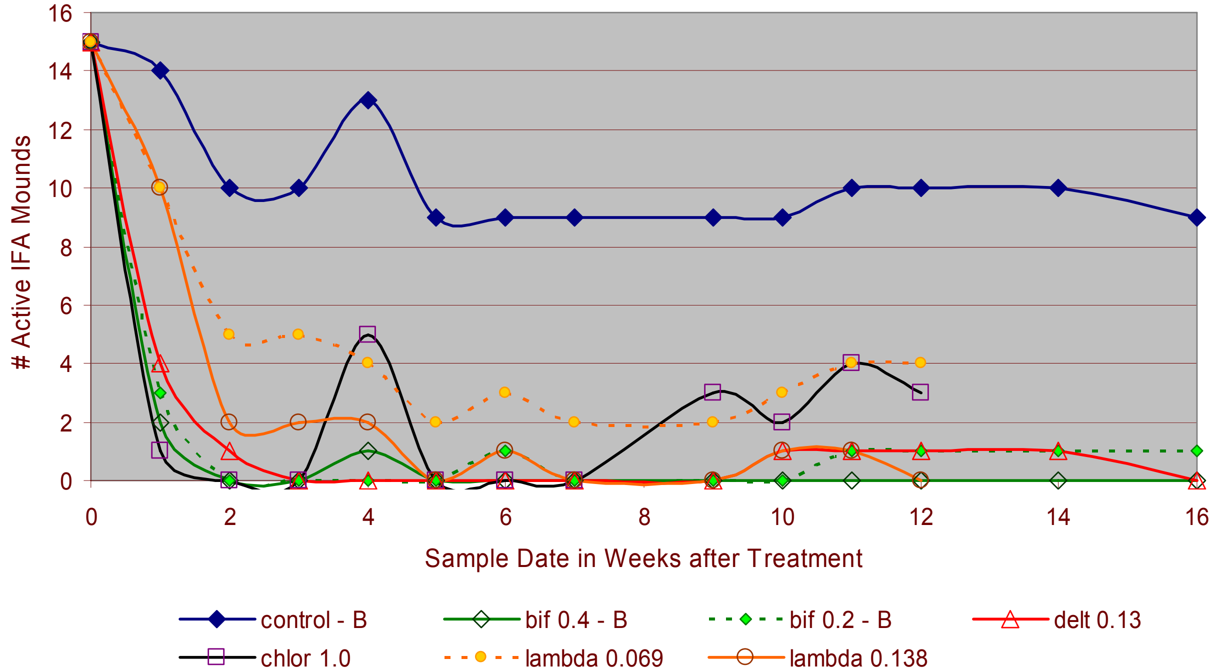
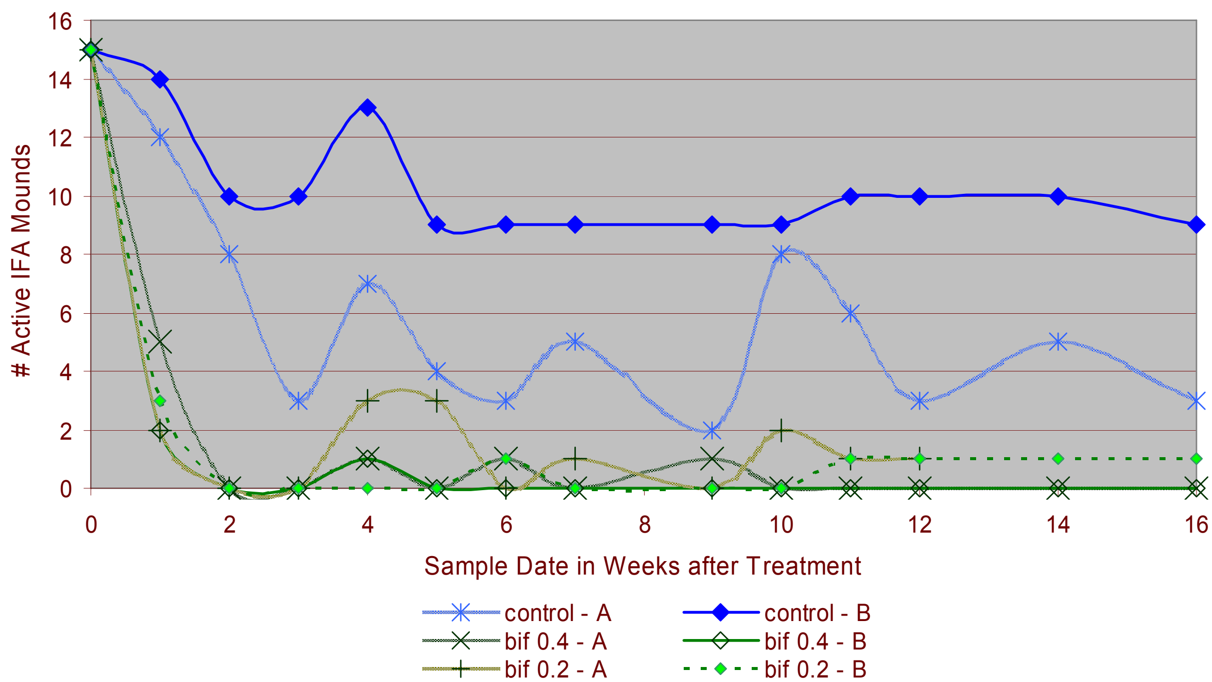
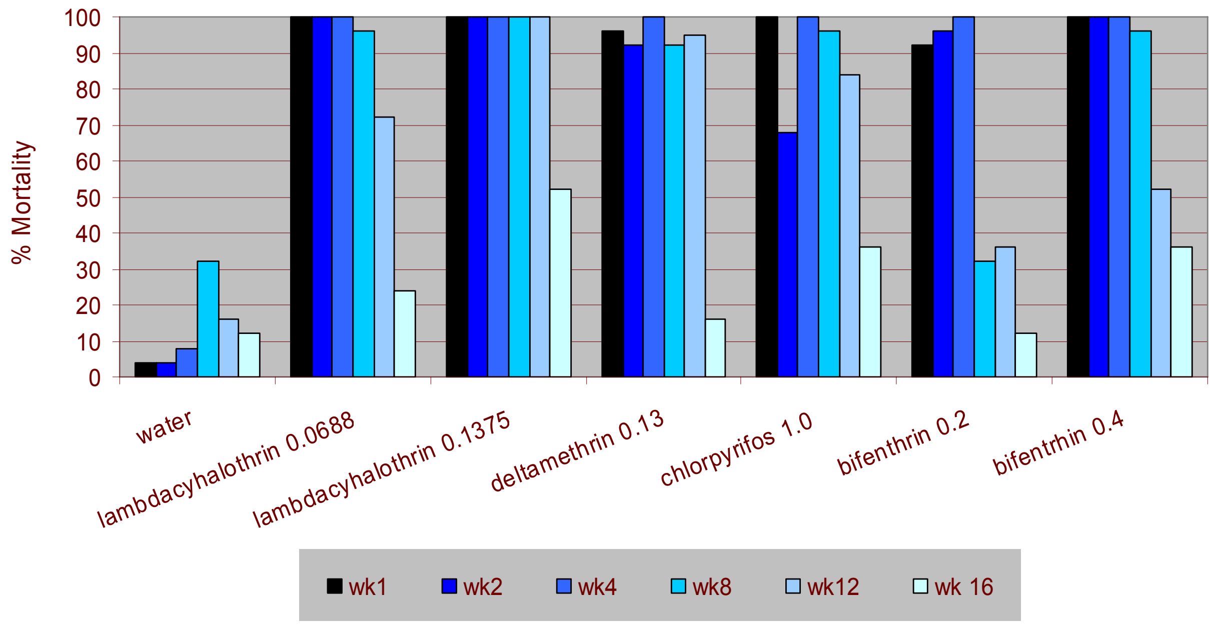


Figure 4. Cumulative active imported fire ant mounds (plots) within each treatment counted at weekly observations over the course of the field trial. Treatments marked with the letter "A" were applied with an undetermined contaminate in the spray tank. Treatments marked with the letter "B" were applied after the tank was thoroughly cleaned.



Laboratory Conducted Alate Bioassay:
Alate mortality for all insecticidal treatments was 90% or greater when tested against the one week soil samples (figure 5). The higher rate of lambda-cyhalothrin gave the longest and most consistent control providing 100% kill through the twelve week soil sample. Both the high rate of bifenthrin and the low rate of lambda-cyhalothrin were consistently lethal through week four samples and by the eighth week had only dropped to 96%. The other three treatments, deltamethrin, chlorpyrifos, and the low rate of bifenthrin, may have had uneven applications since they overall performed well, but occasionally alates survived in a replicate or two while other replicates killed them all.

Figure 5. Percent mortality of alate IFA confined with treated soil samples for fourteen days. Treated soil samples were collected at 1, 2, 4, 8, 12, and 16 weeks after application of treatment.



Among the insecticidal treatments deltamethrin was the most similar in its results between the laboratory bioassays and field test (figure 6). Chlorpyrifos looks like it might have been similar in duration across the two tests, but the active mounds in the field test at four weeks and the surviving alates in the bioassays on the two week soil samples make actual duration uncertain. Both rates of lambda-cyhalothrin appear to have been more lethal in the bioassays (figure 7) than in the field. Bioassays provide no escape from the treated soil, so perhaps in the field the surviving colonies simply were able to cover or remove treated soil. Bioassays on the two bifenthrin treatments indicated that reinfestation should have occurred by eight weeks on the lower rate and by twelve weeks post-treatment on the higher rate (figure 8). The completion of the repellency tests in 2006 will hopefully clarify whether the apparent extended activity of bifenthrin in the field trial is due to repellency or just a lack of reinfestation.

Figure 6. Comparison of results for field trial and laboratory bioassays for deltamethrin and chlorpyrifos.

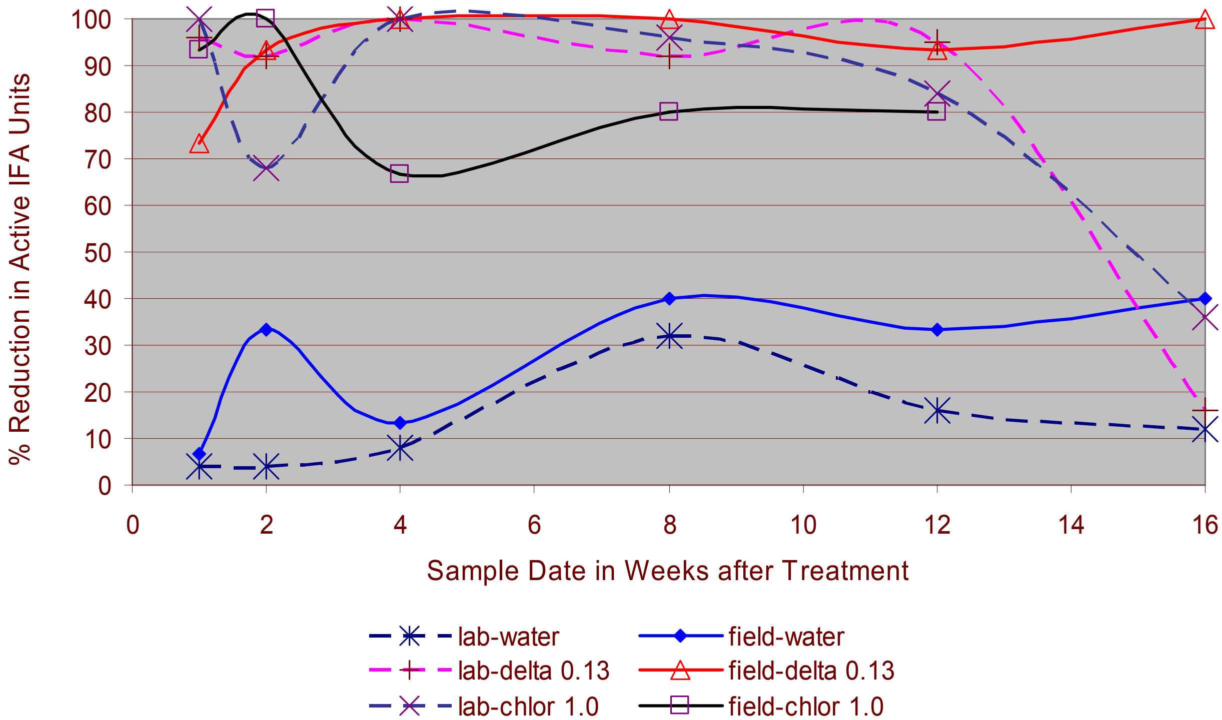


Figure 7. Comparison of results for field trial and laboratory bioassays for lambda-cyhalothrin applied at 0.1375 and 0.0688 pounds active ingredient per 100 gallons of water.

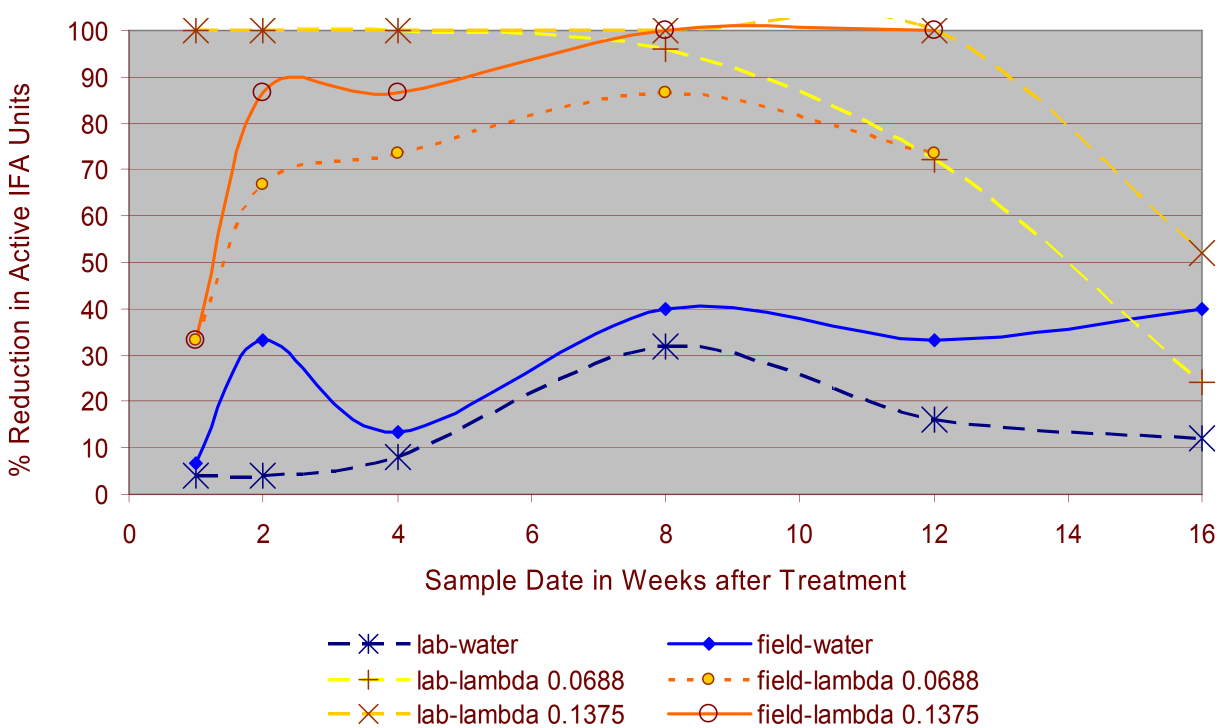
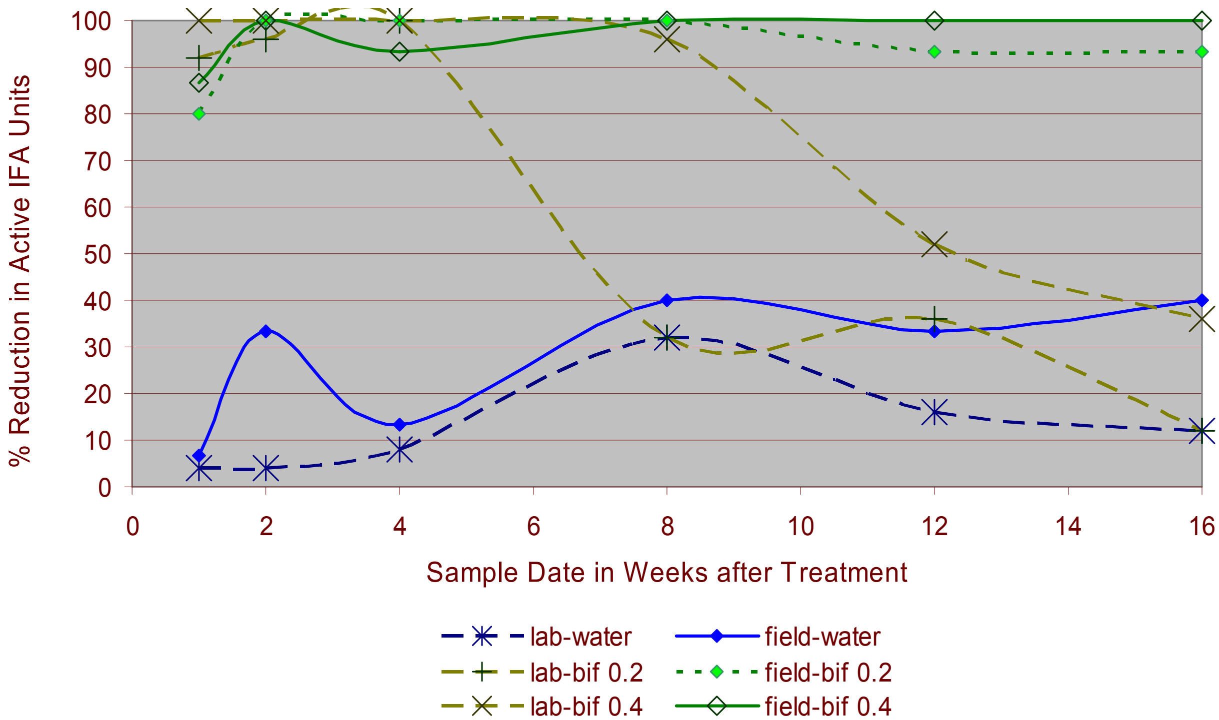


Figure 8. Comparison of results for field trial and laboratory bioassays for bifenthrin applied at 0.4 and 0.2 pounds active ingredient per 100 gallons of water.



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CPHST PIC NO: A1M04

PROJECT TITLE: Development of Alternative Quarantine Treatments for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, Fall 2004 & Spring 2005

TYPE REPORT: Final

LEADER/PARTICIPANTS: Shannon James, Lee McAnally, Bob Jones, Anne-Marie Callcott, Jennifer Lamont, Shannon Wade, 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 nursery stock, as described below, are inefficient and limited to a single insecticide. 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. The primary objective of a quarantine treatment for field grown nursery stock is to render the plants fire ant free. 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). 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 2005 Accomplishment Report, continue to provide direction for insecticides 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 have included a 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 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 trials in this report continue to explore alternative insecticides and provide supporting data for those previously seen to perform 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 was important to test insecticides in colder weather than that found in south Mississippi, the location of most previous trials. Plots consisted of 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). Plot center lines, which simulated rows of plant stock, were set a minimum of twenty feet apart side to side and end to end to provide a buffer zone between plots. Wooden stakes with plot identification numbers were planted at the plot ends and Pramitol®, an herbicide, was sprinkled around them to keep the grass from obscuring the stakes. Fluorescent orange spray paint marked the center line of each plot and was repainted as needed.

Figure 1. Plot arrangement diagram

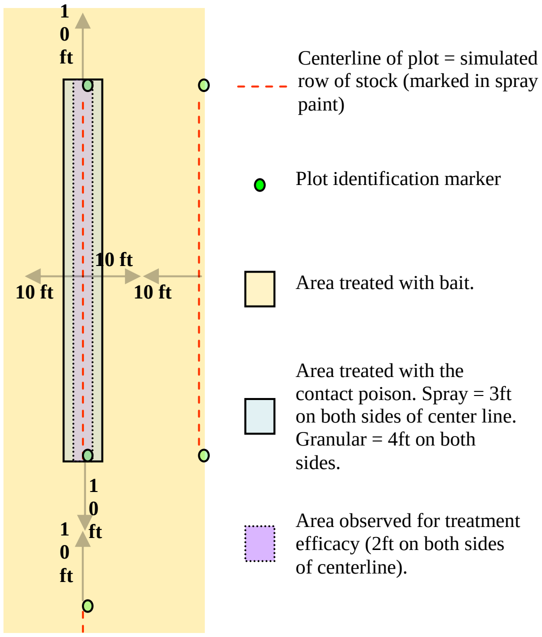
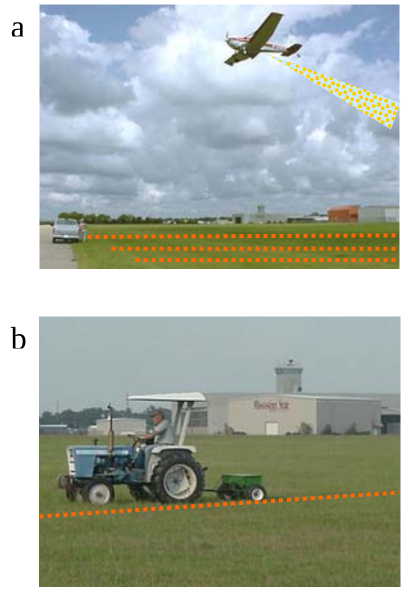


Figure 2. Application of bait (a) and contact insecticide (b) to the plots of simulated stock



On October 25, 2004 hydramethylnon bait was applied aerially at a rate of 1.5 lb/acre across the plots designated for chemical treatment (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 consisted of three replicates each of the following treatments.

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)
control	---	---

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 and every four weeks thereafter until 32 weeks passed. Mounds were evaluated using as little disturbance as possible, usually through insertion of a wire flag into the mound. Mounds were considered active if any workers appeared. Rain data were 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 was recorded during observation by use of air and soil thermometers.

Spring 2005 Band Trial:
Bobby Chain Memorial Airport in Hattiesburg, MS (Forest Co.) was selected as the test location for the spring trial due to the large amount of IFA infested land available. Plots were set up and marked as described in the fall trial. A total of thirty plots were divided into groups of three replicates for the following treatments with an additional three plots each assigned to lambda-cyhalothrin and fipronil for the examination of the potential for a second chemical application to extend treatment duration.

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)
fipronil	granular 0.0143%	87 lb/acre (0.012 lb ai/acre)
lambda-cyhalothrin	flowable 9.7%	10 oz/acre (0.06875 lb ai/acre)
deltamethrin	concentrate 4.75%	39 oz/acre (0.13 lb ai/acre)
control	---	---

Hydramethylnon bait was applied to insecticide treatment plots on April 22, 2005 at a rate of 1.5 lb/acre through the use of a shop built spreader mounted to a farm tractor. Contact insecticide application started three days later on the 25^{th} and due to weather and the number of treatments finished on the 29^{th}. The equipment utilized to apply the insecticides in the spring 2005 trial was the same as used in the fall 2004 trial and with the exception of deltamethrin was applied in the same manner. Due to statements on the label of deltamethrin, total spray volume for that treatment was doubled to ca. 152 gal/acre. The second applications for the lambda-cyhalothrin and fipronil re-treat plots were to be applied at twelve weeks after the initial application using the same rate and volume as before. However, continued ant activity in the fipronil plots meant the three lambda-cyhalothrin re-treat plots were the only ones treated on July 18.

Active IFA colonies in each plot's observation area were evaluated and recorded as previously described with observations occurring prior to bait application and approximately 1, 2, 4, 6, 8, 12, 16, 24, 28, 32, 37, and 42 weeks after contact insecticide application. Chaos in the aftermath of hurricane Katrina prevented observations between 16 and 24 weeks after treatment. Weather data were obtained through the airports weather station observations which are reported at http://www.wunderground.com. Due to damage from the hurricane, weather data were either incomplete or unavailable from August 29^{th} through September 13^{th}. Supplementary temperature readings were recorded during treatment application and most monitoring sessions by use of 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. Some observation dates recorded standing water in portions of the plots at times through out the trial, but plot orientation within the field is such that all treated plots experience similar high and low spots. Observations were conducted when the warmth of the day encouraged increased fire ant activity, but night temperatures during the coldest months still dropped low enough to freeze water in the rain gauge and subsequently break the gauge. Despite the prolonged survival of several colonies through the second week examination, all treatments performed well with the first active mounds in chlorpyrifos granular and bifenthrin flowable appearing at 24 and 32 weeks after treatment respectively. Future fall testing is expected to take place in Tennessee and include chemical analysis on soil samples to determine actual longevity of the chemical. The repeat success of the bifenthrin formulations in three fall trials completed thus far indicate that, if it performs similarly in the next fall trial, it will be considered successful and another promising pesticide will be able to move into the subsequent fall trial treatment lineup.

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

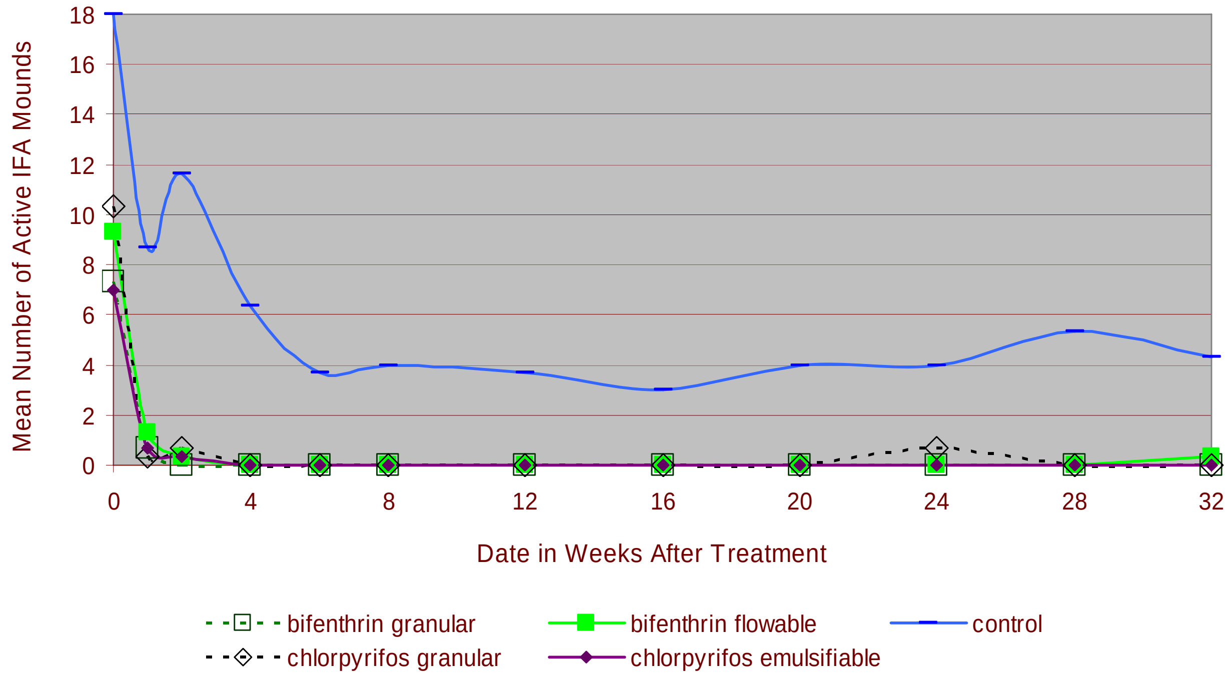
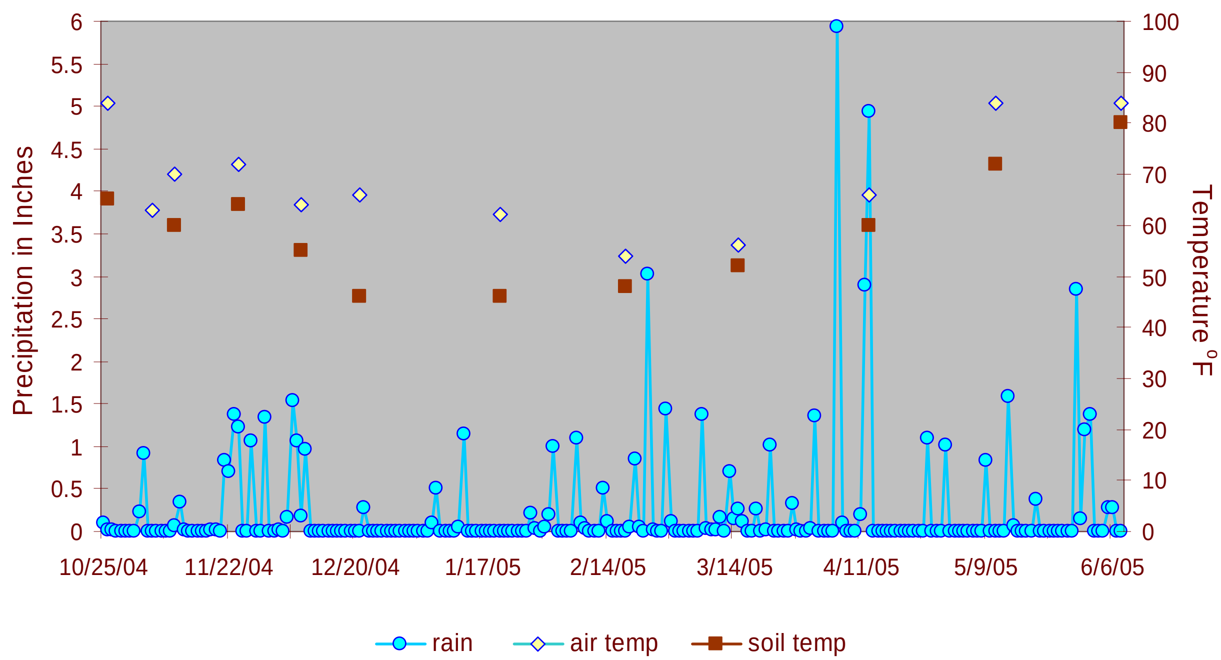


Figure 4. Weather data for the 2004 fall band trial.



Spring 2005 Band Trial:
Both of the bifenthrin treatments and the granular chlorpyrifos treatment demonstrated the most rapid onset of IFA control as each reached the point of no active mounds by two weeks after treatment (Figure 5 a). The shorter amount of time taken to reach this point of control for the bifenthrin liquid and chlorpyrifos granular treatments indicated that the bait application in the spring 2005 trial, which had a three-day wait before contact insecticide application, was more effective than the bait application in the fall 2004 trial despite the advent of light rain in the night following the spring bait application (Figure 6). Liquid chlorpyrifos, deltamethrin and lambda-cyhalothrin treatments each reached the point of no active mounds by the observation at four weeks; the fipronil however still had lingering active IFA mounds (Figure 5 b). The day after the last insecticide was applied the trial area received 0.42 inches of rain which should have aided speed of efficacy for the granular treatments, including fipronil, by watering them into the soil.

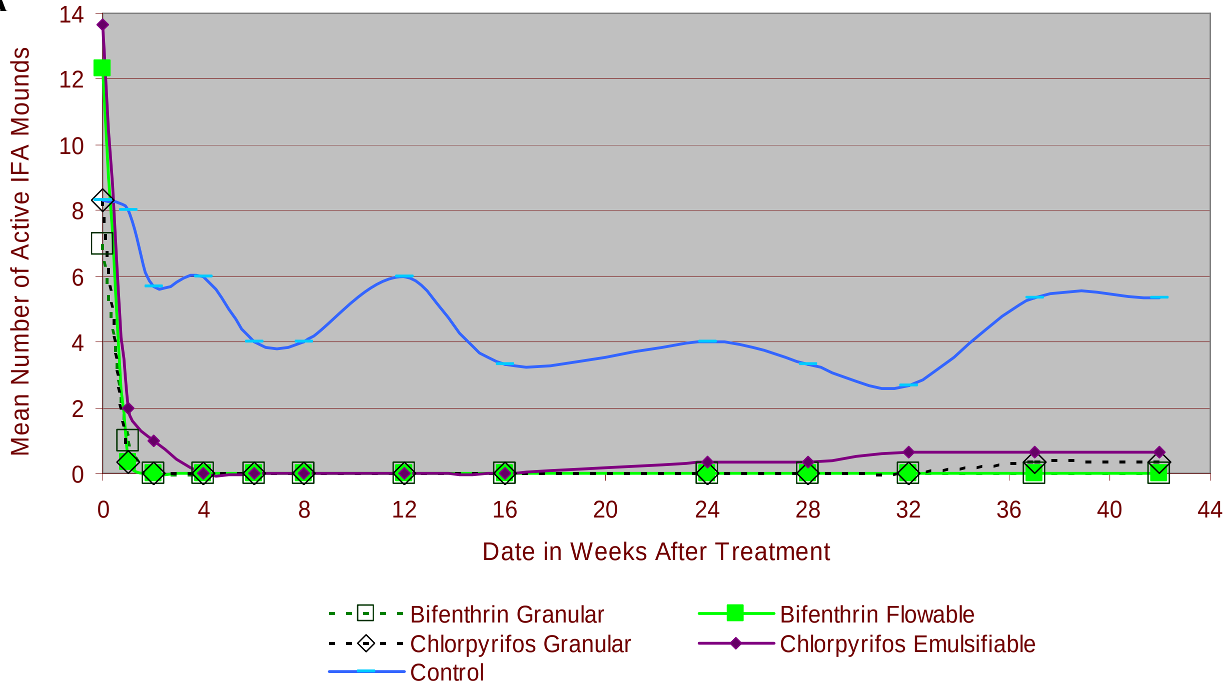
Bifenthrin granular, bifenthrin liquid, and the re-treat plots of lambda-cyhalothrin, after achieving the point where they were free of active mounds, all remained without active IFA mounds through the end of the trial. Fipronil, deltamethrin, chlorpyrifos liquid and the single-application lambda-cyhalothrin treatments each had an edge plot, a plot adjacent to a large untreated area, and the edge plot in all of these treatments appears to have been the first to succumb to reinfestation. Interestingly, the lambda-cyhalothrin re-treat group also included two edge plots, but no reinfestation occurred on these. Observations of the chlorpyrifos granular treatment yielded no active mounds for a thirty-week period, and the chlorpyrifos liquid treatment provided apparent control for fourteen weeks. Deltamethrin and the single-application plots of lambda-cyhalothrin both had a single active mound appear during a monitoring date that occurred after the plot had been IFA-free for multiple observation dates but also well before re-infestation was indicated through sustained activity over multiple dates. The deltamethrin and the single-application lambda-cyhalothrin treatments, with the exception of the previously mentioned single unsustained mounds, respectively, provided eight and twenty eight weeks without active mounds on their plots. The eight-week duration of control in the deltamethrin treatment is an apparent improvement over its performance in the 2003 spring trial wherein control was not achieved.

The fipronil did not achieve a quarantine-level of control in this trial; therefore, its plots marked for retreat at twelve weeks did not receive the second application of chemical. The poor performance of this pesticide in this trial compared to previous trials in spring of 2004 and 2003 gave rise to concerns about the age and potential degradation of the batch of product used. A laboratory bioassay (Appendix I) using this batch of fipronil product in potting media at 25 and 5 ppm was conducted after the spring 2005 band trial. Lower than expected mortality results from this bioassay indicated that the fipronil used in the bands may have been degraded prior to band application.

Most of the treatments in the spring 2005 trial appeared to last longer than they had in previous spring trials. The cause for this is unknown but the low amount of precipitation during the trial most likely influenced the behavior of the IFA, reducing reinfestation movement and mating flights and making the IFA workers reluctant to expose themselves to desiccation through defense or rebuilding of the mound when disturbed. Future band trials initiated in the spring will also include soil sampling for examination of continued pesticide presence. The resulting data from the chemical analysis should be able to support field results and guide our exploration of the use of reapplication to extend treatment longevity.

Figure 5. Spring 2005 trial – Colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide. The results are divided into two graphs for visual clarity.

A



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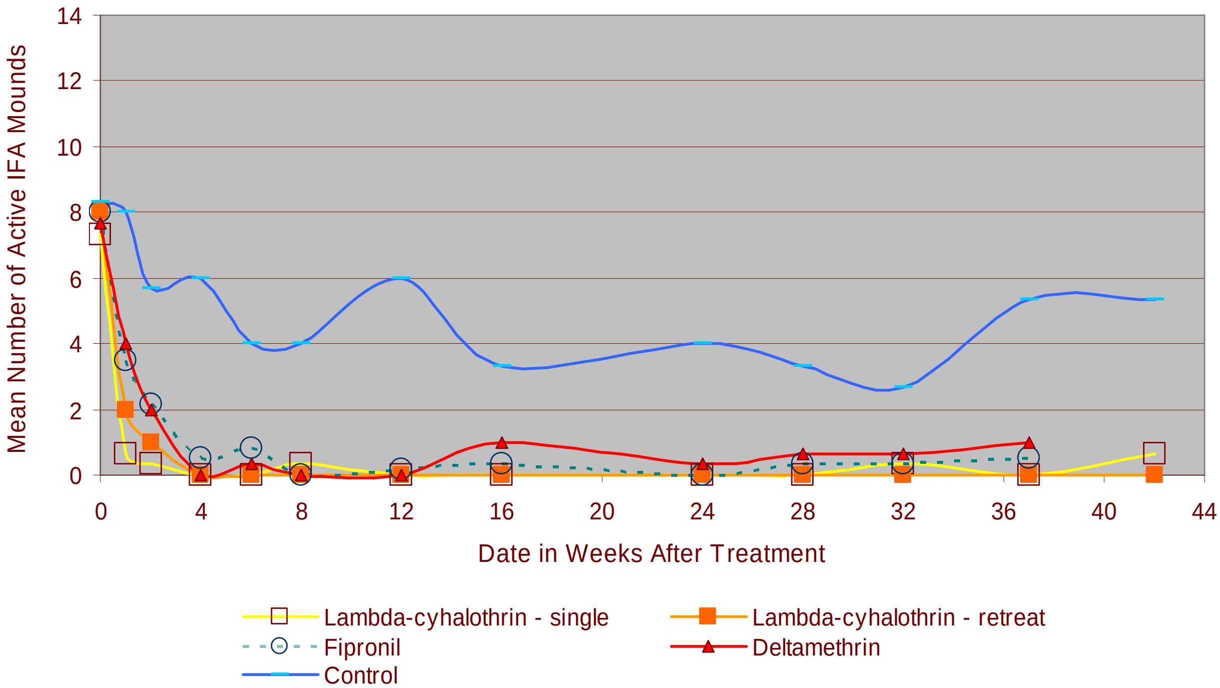
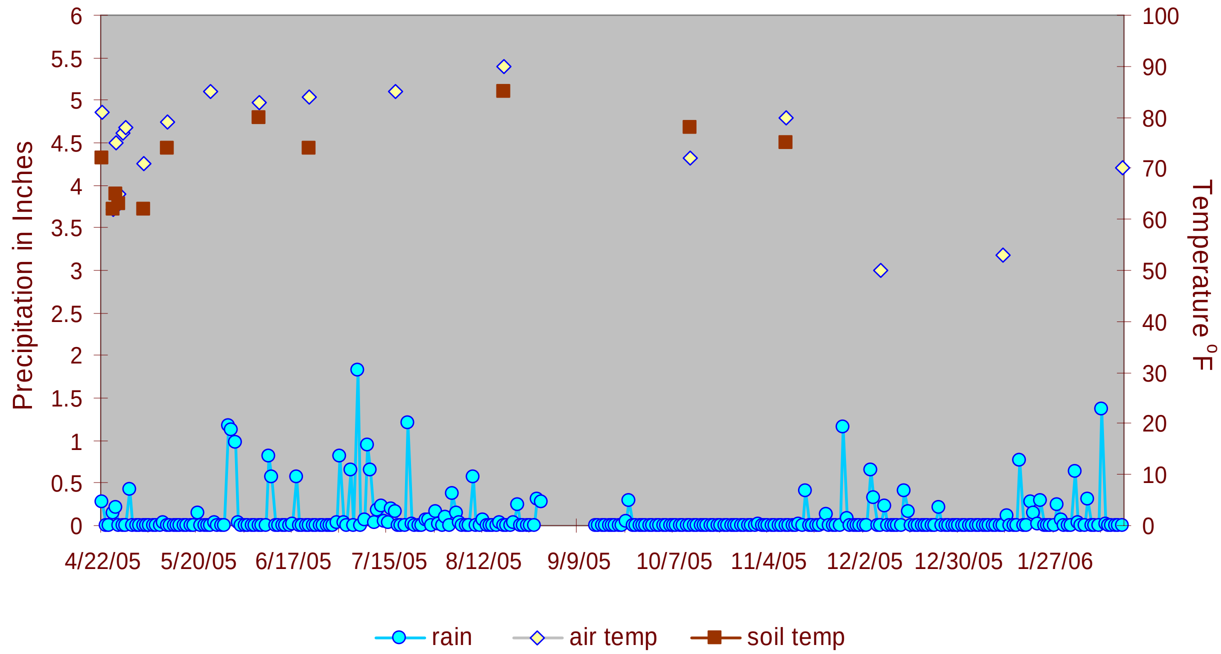


Figure 6. Weather data for the 2005 spring band trial.



<u>References Cited</u>:

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Franke, O.F. 1983. Efficacy of tests of single mound treatments for control of red imported fire ants. Southwest. Entomol. 8: 42-45.

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 2005

LEADER/PARTICIPANTS: Ronald D. Weeks and Bob Jones

INTRODUCTION:

Commercial pollination with honey bees is a highly mobile business, and bee colonies are frequently moved among holding yards, 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 Imported Fire Ant Quarantine (Federal Code of Regulations, Title 7, Part 301.81), however many states vigorously inspect and regulate these items coming from IFA infested states. No quarantine treatments have been approved for assuring that transported hives are IFA free. The objectives of this research are to develop Best Managements Practices (BMP) that focus on control at both the field and commodity level that may be practical and useful against IFA. Field level broadcast bait applications may be useful for reducing IFA populations in bee yards and holding areas (1/4 acre to larger areas). Contact insecticides may be applied strategically to support pallets or the soil area (3-5m^2) where bee hives are to be stored before shipment.

DELIVERABLES:

- Updated Program Aid 1670: Beekeepers Don't Transport Imported Fire Ants – 2006. A best management guide for beekeepers (release date February 2006).
- One-page Best Management Practices (BMP) overview to SPRO's in IFA infested states.

MATERIALS AND METHODS:

In 2005, IFA evaluations were conducted in north Mississippi by Dr. Bob Jones CPHST SIPS. Field trials were completed using permethrin soil applications (0.0084 lb A.I./gallon of water) on 5 x 5 ft soil areas. Soil area applications 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.

Routine ant sampling for IFA activity was completed 3-4 days post chemical applications and at weekly intervals. A 2.5 cm^2 index-card soaked in corn oil was placed on the soil area and used as an attractant to determine the presence of foraging workers within plots. Sampling was conducted for 30-45 minutes in each treatment.

RESULTS AND DISCUSSION:

Results from trials conducted in north Mississippi show that the labeled rate application of permethrin may be an effective barrier treatment for IFA infestation for 4-5 weeks (Fig. 1). These results support other SIPS research on permethrin barrier treatments (see Annual Reports 2003 and 2004). Results in north Mississippi indicate that several other ant species were able to cross the permethrin treated areas (Fig. 2). This result indicates resistance or tolerance of permethrin at the rate we used does occur in some ant species. It also demonstrates the need for caution when making ant identifications to avoid any false records or actions. To date, all studies conducted by SIPS have shown that the permethrin treatments are effective against RIFA (*Solenopsis invicta*).

Figure 1. Efficacy of permethrin soil applications as a barrier against IFA foraging in treated plot areas.

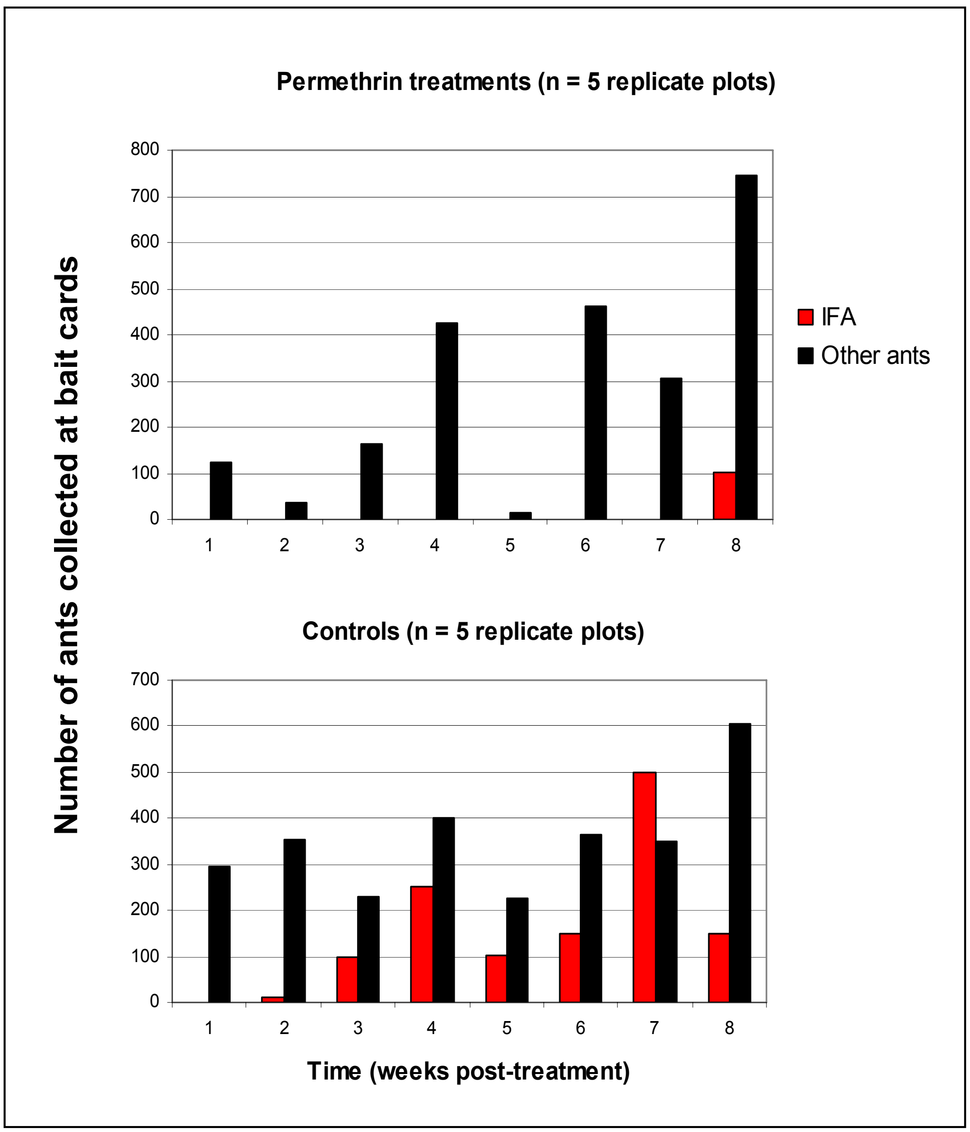
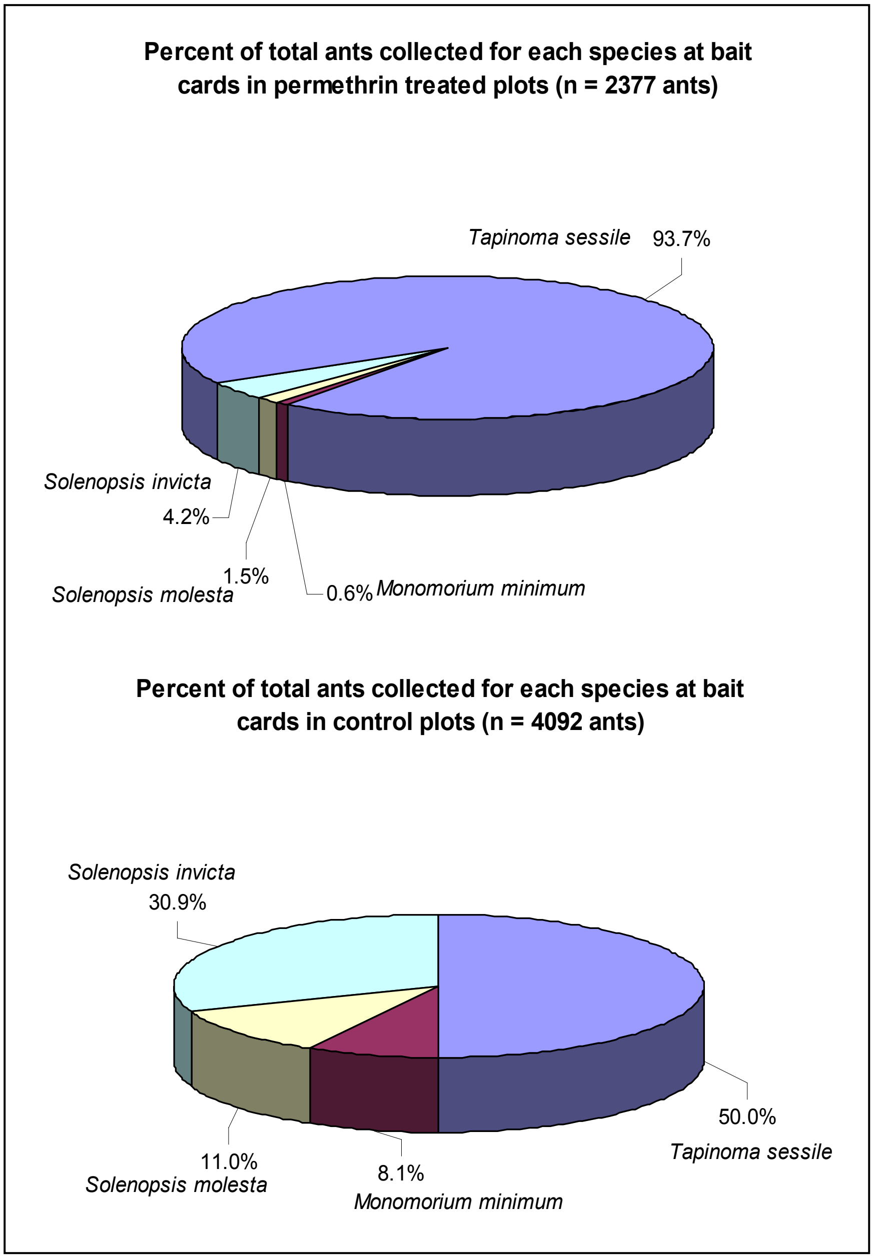


Figure 2.


Percent of total ants collected for each species at bait cards in permethrin treated plots (n = 2377 ants)
Tapinoma sessile 93.7%
Solenopsis invicta
4.2%
1.5%
Solenopsis molesta
0.6% Monomorium minimum
Percent of total ants collected for each species at bait cards in control plots (n = 4092 ants)
Solenopsis invicta
30.9%
50.0%
Tapinoma sessile
11.0%
Solenopsis molesta
8.1%
Monomorium minimum

CPHST PIC NO: A1M03

PROJECT TITLE: Exclusion Methods for Imported Fire Ants (IFA) in Hay-Transport Operations

TYPE REPORT: Interim 2005

LEADER: Ronald D. Weeks

INTRODUCTION:

As a federally regulated item, under the Federal Imported Fire Ant Quarantine (7CFR 301.81), baled hay stored in direct contact with the ground cannot be moved outside the quarantine area. This poses significant limits on commodity transportation and access. Currently, there are no quarantine treatments approved for assuring that transported hay bales are imported fire ant (IFA) free. Previous research (see Annual Report 2003 – 2004) suggests that a Best Management Practices (BMP) approach which focuses on control at both the field and commodity level is useful in developing steps and applications to use against IFA. Broadcast bait applications are useful for reducing IFA populations in half-acre to larger field areas. Contact insecticides may be applied strategically around or under the commodity with few caveats. The objectives of this project are to evaluate the efficacy, longevity, rates and formulations of contact chemicals applied as barrier treatments around hay bales in preventing IFA infestation. In 2005, two experiments were set-up, one after the first cutting (June) and another after the second cutting (August). The August trials were terminated shortly after set-up (August 29) due to disruption caused by Hurricane Katrina. This report summarizes the first cutting trials.

MATERIALS AND METHODS:

Hay trials were conducted at the White Sands Mississippi Agriculture and Forestry Experiment Station in Pearl River County. Trials were conducted in a 2.25 ha field, infested with monogyne red imported fire ants *S. invicta* Buren. Hyrdamethylnon bait was broadcast applied at the labeled rate to the field 4 weeks prior to trials. Previous SIPS studies have shown that broadcast applications of commonly applied ant baits may kill up to 90% of colonies from the treatment area. The percentage of hay bales infested by IFA was compared for two chemical barrier methods using two rates of permethrin (half-rate = 0.0042 lb A.I./gallon of water and full-rate = 0.0084 lb A.I./gallon of water) application. Barrier methods comprised; 1) applying permethrin to a 3.6 x 3.6 m area directly under a hay bale then placing a permeable landscaping ground cloth between the hay bale and chemically treated soil (Fig. 1) and 2) application of permethrin to a 0.9m wide band around each hay bale. Soil applications of chemicals consisted of 3.0 gal finish solution for each application rate. Control and treatment bales were placed in a randomized block design spaced approximately 4 m apart. Four replicates of each treatment were set up (n = 20). Routine ant sampling of each bale was completed 3-4 days post hay placement and at weekly intervals. A 2.5 x 2.5 cm. 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 30 minutes. Hay for this evaluation was cut and baled in an adjacent field and moved within 24 hours to the experimental field.

RESULTS and DISCUSSION:

Ants were detected foraging on control bales the second and all remaining weeks of the 7 week study. These ants may have come from colonies that escaped the initial broadcast bait application or they could have been moving in from outside the treated area. There were significant differences between control and contact insecticide barrier treatments (Table 1). Both application rates of permethrin and barrier methods provided protection to bales from IFA infestation for up to six weeks. No IFA foraging was detected in the half-rate area treatment. This result is likely a combination of factors such as the six week protection window provided by the barrier treatments and the chance of not being near any active ants during the 6 and 7 week samples (i.e. sampling error). Windows of opportunity for IFA-free hay transport may provide best management practices that hay operators may use to temporarily store hay bales on the ground before shipping to non-quarantined areas. This practice appears to be working on individual hay bales. However, more replicate studies are needed and future work needs to be completed on larger stacks of hay.

Table 1. Percent of hay bales infested by imported fire ants in treatments during each of the 7 weeks of the study (number of hay bales = 20, with four replicates in each treatment).

Treatment	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**
Strip application							
Half-rate	0	0	0	0	0	50	75
Full-rate	0	0	0	0	0	25	25
Area application							
Half-rate	0	0	0	0	0	0	0
Full-rate	0	0	0	0	0	25	0
Control	0	25	100	50	75	75	100

Figure 1. Round hay bale on landscape ground cloth.

CPHST PIC NO: A1M03

PROJECT TITLE: Exclusion Methods for Imported Fire Ants (IFA) in Pine Straw

TYPE REPORT: Interim 2005

LEADER: Ronald D. Weeks

INTRODUCTION:

As a federally regulated item, under the Federal Imported Fire Ant Quarantine (7CFR 301.81), pine straw stored in direct contact with the ground cannot be moved outside the quarantine area. This poses significant limits on commodity transportation and access. Currently, there are no quarantine treatments approved for assuring that transported pine straw is imported fire ant (IFA) free. The objectives of this project are to evaluate several non-chemical and chemical treatments for pine straw that prevent IFA infestation.

MATERIALS AND METHODS:

On August 24, 2005, a storage experiment was set-up at the SIPS laboratory in Gulfport, MS. The experiment evaluated whether IFA infesting pine straw could be killed as a result of storage in a steal container (Fig. 1 a and b). The hypothesis tested was that IFA infesting pine straw would be unduly stressed and die due to high temperatures experienced within a sealed storage container during summer months. The experimental design consisted of infesting 4 pine straw bales with IFA (approximately 1000 ants and some brood but no queens) and placing infested bales inside 1m plastic round swimming pools coated with Fluon® to prevent ant escape (Fig. 1a). Two infested bales were stored in a steel container (Fig. 1b) and two were placed under a tractor shed open to the front. Visual examinations of IFA were made at weekly intervals. IFA were scored as either being alive or dead depending on whether any active ants could be found in the pine straw or swimming pool. Unfortunately, experiments in the tractor shed were terminated shortly after set-up (August 29) due to disruption caused by Hurricane Katrina. This report summarizes the storage shed results.

RESULTS and DISCUSSION:

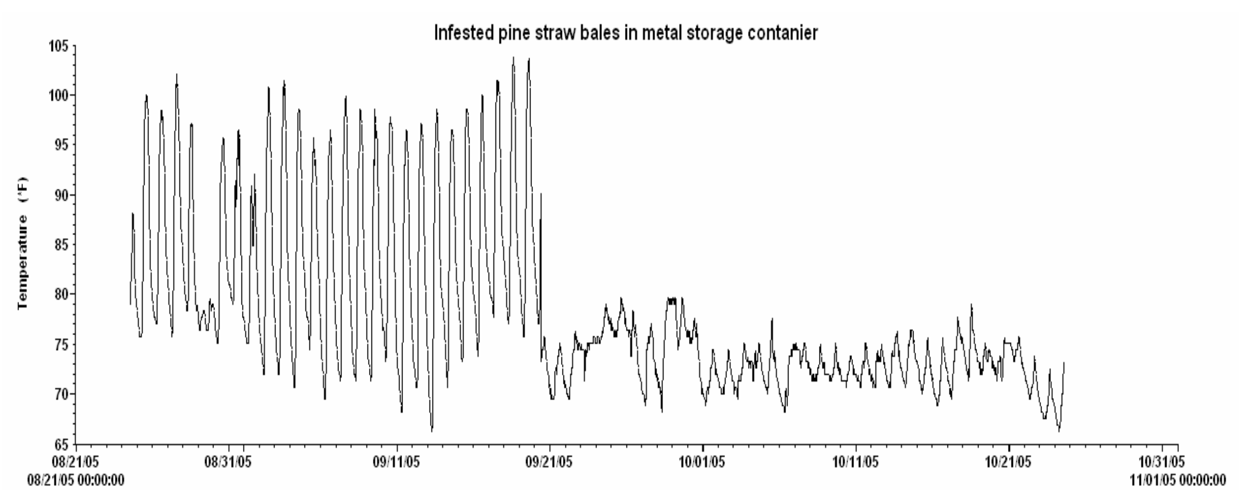
Ants survived and were active in the pine straw for the first 41 out of the 61 days following placement in the storage container. The maximum temperature experienced in the study was 103.7° F, the minimum was 66° F and average was 78.3° F. Temperatures approached the maximum value regularly during the forty-one day period of ant survival (Fig. 2). These results indicate that storage of pine straw bales infested with ants in metal storage containers is not a viable quarantine treatment option. Other areas of investigation include chemical barrier treatments to prevent infestation.

Figure 1. a) IFA on pine straw bale with food and water. b) Pine straw bale in storage container.

a)

b)

Figure 2. Temperature measured inside of metal storage container between August 24 and September 24, 2005.



CPHST PIC NO: A2M06

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

TYPE REPORT: Final

LEADER/PARTICIPANTS: Robert G. Jones, Anne-Marie A. Callcott

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. Detection surveys that require a large number of survey traps are considerably different from research studies. Traps need to be easy to place and retrieve. The surveyors are not researchers and their interests differ. The simpler the whole survey procedure is the better the end result will be. Using traps that are difficult to handle under field conditions causes some surveyors to develop simpler methods. This may cause inconsistency with the results of others and thus affect the survey results.

The traps being used or have been used include vials and jars of various sizes. Index cards have been used with peanut butter or soaked with a vegetable oil. Lids of petri dishes and other small containers have been used. 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 must carry some of the bait back to the nest. This ant as it carries the bait leaves a pheromone trail for others to follow back to the food source. Thus a trap that catches or entraps the foraging ant will not work for this quarantine survey. The detection survey needs to discover if there are established nests present. Catching major and minor workers or just numerous workers provides proof of an IFA colony. The need for trapping is that the nests may not be as observable as the mounds in older infested areas.

This is a report of the development and comparative testing of an Imported Fire Ant (IFA) survey trap that is effective, simple and easy to use. 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 representative examples of the basic containers and/or bait attractant holders for capturing the three genetic forms of IFA (Red-RIFA, Black-BIFA and hybrid-RxBIFA).

METHODS:

Preliminary Studies: Methods used included a literature survey, questioning experienced workers and looking at a variety of containers. Both purchasing and altering a variety of containers was done as preliminary to this study. These products were then tested and observed in both the laboratory and in the field with IFA. This information went into developing and building the SIPS proto-type IFA Trap used in this test. The selection of the other candidate traps was from these preliminary studies.

Candidate Traps: Five candidate "traps" (containers and holders) were selected for comparison in field tests. This included: 1) plastic, open mesh basket or California IFA bait station; 2) opaque, polyethylene, 10 ml, hinged cap vial; 3) clear, polystyrene, 11 ml, snap lid vial; 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. These holes were matched through both top and bottom sections. With a slight twist these entrance holes were closed and the ants captured. The trap top (or the Petri dishes' wide bottom) was covered with aluminum foil to combat intense heat during summer months. All containers except the basket were selected for being close to the same volume size.

Trap Bait Attractant: The same bait or food attractant was used throughout this series of tests. The attractant is this lab's formulated survey bait (SIPS IFA Bait – PIC A2M01) that has performed well in both laboratory and field bait acceptance tests. It was formulated for this study in equal size pieces (1.5 ± .25 grams) so that cutting and weighing baits for tests was not necessary. The bait was prepared no more than 3 months before use. Small bait lots were sealed in plastic bags and refrigerated until used.

Collection of Traps: Four of the selected trap containers can be easily sealed to prevent loss of ants or data. To make collecting efficient and painless the basket trap was dropped into a 110 ml hinged capped, polyethylene container. All traps for one replication were then placed into a single 16 ounce plastic jar with a screw type lid. This allowed for a complete test replication of all treatments to be kept together with data being recorded by replication for treatments. The jars were placed in a freezer for 48 hours before counts and identifications of species were made.

Bioassay: The traps were 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 continued on this line at the same spacing between replications and treatments. The first treatment placing of each replication was marked by a surveyor's flag and the 16 oz jar was placed there for use in collection. Traps or treatments were 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 attempted to maintain similarity and close proximity among treatments in the same replications. No trap location was within 3 feet of a mound or obvious foraging tunnel. If more than one line or replication was possible in the same location the lines were established no closer than 10 feet apart. Lines or replications were parallel, perpendicular or as a continuation of another line, but did not cross. Traps were collected after being in place for approximately 60 minutes. Soil and air temperature readings were recorded after setup and after collection. These test replications were run during most months of the year dependent on favorable foraging weather.

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 simplified 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 was a possibility in this category that this physical difference could be a factor by the effect on 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 most 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 (SIPS) 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.

<u>Field Study:</u> This study began on June 4, 2003 with the RxBIFA on the Mississippi State University South Farm, Oktibbeha Co. Test replications were done at least 12 different locations on the farm through August 2004. Tables 1 & 2 present the general results of this study. Tests involving the RIFA and BIFA were started in late 2004 and continued through October 2005. 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 (Tables 1 & 2). 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 both Oktibbeha and Prentiss Co. study areas. There were several other native ant species competing for the baits in both areas and had a distinct effect on the percent of traps with IFA. While there were some specimens of native ants caught in Harrison and Rankin Co., they were not a factor in this test.

The data in Tables 1 & 2 shows that the SIPS IFA survey trap is a very viable candidate. It is the only trap that has a reflective cover on it which may be a factor its higher capture rates for IFA. The California Basket is at a disadvantage in capture numbers since ants fall off or escape through the large mesh openings during collection. The numbers of replicates with IFA or the % capture is the more important number (Table 2). This shows the presence of IFA which is the purpose of the IFA Survey. If the traps are full of IFA you probably don't need a trap survey to find the nests. The escape rate from the traps could be a factor if you lost the only ones there during collection. But as long as the surveyor saw the escapees then more traps could be placed in the location to determine identity. The SIPS survey trap had a 66% rate of overall IFA captures (1227 replications) versus the next highest, the California IFA Bait Station, with 63.8%. From this data it can be concluded that the California IFA Eradication Program made a good choice in their selection of a survey tool but we feel that we have improved on it from the ease of handling by the trapper (Fig. 1). The SIPS IFA Survey Trap is a handmade proto-type. It is made to be stackable and contained in a carrying tube so that large numbers can be taken to and returned from the field with minimum effort. It was designed with blow mold production in consideration but only the need for large numbers will make this an economic reality. The handmade proto-type with care can be used numerous times.

Table 1. The Average Number of RIFA (Harris and Rankin Co., MS), RxBIFA (Oktibbeha Co.) and BIFA (Prentiss Co.) caught by each Trap Type by Number of Replications and average for all locations.

Location	Replications	Basket	Opaque Vial	Clear Vial	Petri Dish	SIPS Trap
Harris Co.	25	86.84	56.9	37	47.56	110.3
Rankin Co.	75	45.6	45.6	46.5	50.6	100.9
Oktibbeha Co.	918	28.8	21.73	20	30.67	59.6
Prentiss Co.	209	29.9	18.4	18.9	23.4	46.54
All Locations	1227	30.7	23.0	21.8	31	60.9

Table 2. The Per Cent of Treatment Replications That Caught IFA by Location and Accumulated Total.

Location	Replications	Basket	Opaque Vial	Clear Vial	Petri Dish	SIPS Trap
Harris Co.	25	96%	88%	84%	84%	88%
Rankin Co.	75	69%	76%	76%	64%	76%
Oktibbeha Co.	918	64.3%	60.3%	56.6%	59.7%	67.6%
Prentiss Co.	209	55.7%	47.1%	48.1%	51.4%	52.4%
All Locations	1227	63.8%	56.9%	56.9%	59.6%	66%

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

PROJECT NO. A2M01

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

TYPE REPORT: Final

LEADER/PARTICIPANTS: Robert G. Jones, Anne-Marie A. Callcott

INTRODUCTION:

The Federal Imported Fire Ant (IFA) Quarantine (7CFR301.81) has no recommended survey attractant for use in detection of IFA. Numerous attractants, including canned meats, potato or corn chips, peanut butter, etc. are used to attract the imported fire ant for both survey and research studies. The consistency in them is that they contain vegetable oils or animal fats. Most are messy to handle and must be cut or measured into trap size proportions. While many attractants have been evaluated over the years, most researchers have not determined that any one attractant is superior. There are some bait attractants currently in use that have not been compared for effectiveness, and none have been tested for attractiveness to all three genetic types of imported fire ants; the red (RIFA, *Solenopsis invicta*), black (BIFA, *Solenopsis richteri*) and hybrid (RxBIFA, *Solenopsis richteri x invicta*). There were 3 main parts of this study after the laboratory testing of a large variety of food products in 2002 and 2003. These parts or sections of this report are 1) the development of a SIPS IFA survey bait, 2) the laboratory testing of the SIPS bait and the commercial products selected for field studies against all 3 genotypes of IFA and 3) the field testing of the promising products as IFA attractants or baits.

SECTION 1: The Development of a SIPS IFA Survey Bait

INTRODUCTION: 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 (pregelatinized corn starch) 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 year round. This is the approach that was taken based on Jones and Brindley (1970). More thorough discussions of this subject have recently become available in a book by A.C. Cohen (2004).

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.

METHODS AND MATERIALS: The methods used were a combination of those used by cooks in preparing cookies or granola bars and those used in mixing insect diets. The ingredients listed in the recipe (Table 1) were tested in the laboratory on IFA prior to selection. They were selected for being essential to insect nutrition and attraction as well as being preservatives and glues to hold the ingredients together. These are food products found in most grocery stores. They are basic products that have not been adulterated with additives or by removing ingredients such as gluten. The pregelatinized corn starch acts as a sponge when mixed with vegetable oil and maintains it general size and shape. When mixed in water it dissolves and becomes a thickening agent. The recipe in Table 1 was developed through trial and error to get the right consistency of the end product. The mixture of ingredients are mixed and heated to a malleable form for spreading over sheets of individual molds. Each mold is in the shape of half a sphere with a diameter of 17 mm.

RESULTS AND DISCUSSION: The ingredients of the developed bait are in Table 1 and the finished product shown in Figure 1. Mixing instructions are as follow:

1) The cornmeal and pregelled corn are weighed out and mixed together in separate bowl or beaker
2) The corn oil, honey, and egg are mixed or beaten together in separate beaker
3) The oil, honey, and egg mix is added to cornmeal-pregelled corn and mix thoroughly
4) The corn syrup and sugar are mixed together in a 1 quart sauce pan; these sugars are heated to melting, careful not to burn
5) The remainder of the ingredients are mixed into the hot sugar; this is heated while mixing and pressing mix together for assured consistency
6) When hot to the touch stop heating and spoon on to the molds and with a putty knife or spatula press into individual molds
7) Once each sheet of molds is filled, go to next and when completed, set molds aside to air dry at room temperature for 24 hours
8) The molded baits are then removed from forms and placed on wax paper to dry for 24 hours more
9) When hardened the baits are counted, placed and sealed in plastic bags. The bags are stored in a refrigerator or freezer.

Figure 1. Laboratory produced bait attractant

Table 1. Recipe of Ingredients for SIPS
IFA Survey Attractant or Bait.

Ingredients	Amounts
Stone Ground Natural Yellow Cornmeal	84 grams
Pregelled Corn (pregelatinized starch)	63 grams
Corn Oil	54 grams
Clover Honey	21 grams
Egg (chicken)	1 large
Corn Syrup	63 grams
Granulated White Sugar	21 grams

<u>SECTION 2:</u> The Laboratory Testing of SIPS Bait and the Commercial Products Selected for Field Studies with All 3 Genotypes of IFA.

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 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. The commercial products used directly from their packages were ground or cut in to particles similar in size to the pregelled corn. 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 2. Lofgren et al. (1961) listed all test materials with an acceptance ratio of 0.75 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 0.83, it is our conclusion that all materials listed in Table 2 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. 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 to remove. The conclusion of these tests is that all materials listed in Table 2 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 peanut oil (water was still present). 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 was not found at our retail stores. The oils tested worked well and are readily available, especially corn.

Table 2. Laboratory tested products including cooking oils (30% vegetable oil on defatted pregelled corn carrier, 70%), commercial products and a Soil Inhabiting Pests Section (SIPS) prototype bait compared to a standard of peanut oil on defatted pregelled corn. Acceptance ratios of 0.83 or higher are acceptable in these comparisons tested 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 and SIPS Bait			
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

SECTION 3: Field Tests Of Five Commercial Products, A Standard Bait And The SIPS Developed Survey Bait in Four Spatially Different Areas of Mississippi.

INTRODUCTION: The Federal Imported Fire Ant (IFA) Quarantine (7CFR301.81) has no recommended survey attractant or bait for use in detection of IFA. This project has generated a short list of commercially available and acceptable attractants. This list was sent to the National Plant Board through Bob Gronowski (NPB IFA committee chair) in 2002. The recommendations from this work are included in the PPQ Imported Fire Ant Program Manual published on line in 2004. The following report is the completion of this work with the bioassay of the attractants on field populations.

METHODS AND MATERIALS: Field Test Sites: 1) The South Farm at Mississippi State University was selected as the hybrid IFA 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 of about 12 different pastures. This set of test was done from June 2003 through August 2004. After June 2004 the tests were alternated at only 2 pastures because of weed growth. 2) The BIFA test site was the margin of a cotton field. This location was in Prentiss County about 3.5 miles SW of Booneville, MS. It was on the NW corner of US Highway 45 and Blacklands Road. The woods edge on the field margin included an incline with Eastern Red Cedars on the east and drainage ditches on the west side. 3) The RIFA site in Rankin County was 10 miles north of Pelahatchie, MS along an old road between large corn and soybean fields. 4) The other RIFA site was in Harrison County with some of the highest populations of RIFA in the state of Mississippi.

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 no more than 3 months before field testing and stored in sealed containers and refrigerated during this period.

Test Baits: The candidate baits are attractive commercial products, the standard bait and the survey bait formulation. These are 1) Ritz Crackers®, 2) Fritos® original corn chips, 3) peanut oil standard, 4) SPAM® Classic, 5) Kebbler's® pecan shortbread sandies, 6) Lays® classic potato chips, and 7) the SIPS IFA Survey Bait. 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. New products were purchased about 3 months after their original packages were opened.

Field Bioassay: All baits were placed in 9x50mm petri dish bottoms. The baits were 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 seven treatments continued on this line at the same spacing between replications and treatments. The first treatment placing of each replication was marked by a surveyor’s flag and the 8 oz jar was placed there for use in collection. Baits or treatments were 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 attempted to maintain similarity and close proximity among treatments in the same replications. No bait location was within 3 feet of a mound or obvious foraging tunnel. If more than one line of test replications was possible in the same location then lines were established no closer than 10 feet apart. Lines or replications were parallel, perpendicular or as a continuation of another line, but did not cross. Baits were collected after being in place for approximately 60 minutes. Soil and air temperature readings were recorded after setup and after collection. These test replications were run during most months of the year dependent on favorable foraging weather.

Collection of Baits: By placing the petri dish lids on the bottoms, the baits were easily sealed to prevent loss of ants or data. All baits in their petri dishes for one replication were then placed into a single 8 ounce plastic jar with a screw type lid. This allowed for a complete test replication of all treatments to be kept together with data being recorded by replication for treatments. The jars were placed in a freezer for 48 hours before counts and identifications of species were made.

RESULTS AND DISCUSSION: The results of the field tests are presented in Tables 3 and 4. The field testing based on the average IFA capture numbers (Table 3) shows the favorite bait attractants to be the ham product and the corn chips. Unfortunately, this demonstrates as well the differences of baits of varying size. This has been discussed by Hooper-Bui et al. (2002). In the laboratory test all these food products performed close to the same (Section 2). In those tests all products were ground or broken up in to small particles. In the field attractant tests the products that could be carried off as small particles, the standard, cookies and SIPS baits, had the lowest average capture rates. The solid products could not be carried off without be torn apart or had the liquid sucked out. This meant that the IFA workers spent more time at the baits. This in part accounts for the larger capture numbers. In the trap tests (A2M06 of this annual report) the SIPS IFA Survey Bait was the only bait used in different traps. Its capture rate was 50% higher than in this test. The other difference was that it was in a trap that was slightly harder to get out of than the others.

Table 4 shows the percentage of bait treatment replications with IFA captures. In a detection survey the collection of large numbers is not as important as just collecting some. These percentages show that all baits were about the same and all were slightly better than the standard bait. The conclusions from this field study are that any of these food products are attractive enough for IFA detection surveys. The choice of one over the others should be based on consistency of availability over time, efficiency for use by the surveyors and cost of survey with its use. If these tests were to repeated it is suggested that the spacing between baits be increased to more than 10 feet apart and double or triple the number of replications. The number of uncontrollable variables encountered was tremendous. This makes experiment design difficult to impossible especially testing products that are proven in the laboratory to be attractive.

Table 3. The average number of RIFA (Harrison and Rankin Co. MS), RxBIFA (Oktibbeha Co.) and BIFA (Prentiss Co.) caught by each bait type by number of replications and average for all locations.

Location	Replications	Crackers	Corn Chips	Standard	Ham Product	Cookies	Potato Chips	SIPS Bait
Harrison County, MS	30	56.6	113.4	43.23	221.8	70.9	98.6	70.3
Rankin County, MS	100	77.4	53.6	27.4	103.8	24.2	46.5	38.1
Oktibbeha, County, MS	914	25.6	59.2	31.8	58.8	25.1	44.7	27.6
Prentiss, County, MS	225	46.6	67.0	30.8	64.7	22.2	39.5	32.1
All Locations	1269	34.1	57.6	29.8	65.9	25.1	46.2	29.5

Table 4. Percent of bait treatment replications that attracted IFA by location and for all locations.

Location	Replications	Crackers	Corn Chips	Standard	Ham Product	Cookies	Potato Chips	SIPS Bait
Harrison County,MS	30	93.3%	90%	80%	100%	96.7%	96.7%	80%
Rankin County, MS	100	62.4%	57.6%	52.8%	64.8%	60.8%	60%	60%
Oktibbeha County, MS	914	84%	83.7%	79%	87.5%	84.8%	83%	82.6%
Prentiss County, MS	225	56%	61.8%	52%	60.4%	54.7%	58.2%	60.9%
All Locations	1269	77%	77.2%	72%	81%	78%	76%	77%

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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

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

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, SIPL 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. In 2005, treatments were made in OK and TX in the spring, and in OK and MS in the fall. SIPS anticipates continued treatments during 2006. 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.

Loading airplane with IFA bait.

Bait application – calibration is conducted at the airport prior to treating test sites.

CPHST PIC NO: A1M02

PROJECT TITLE: Efficacy of Advion® Fire Ant Bait when Formulated on Tast-e-Bait® Carrier

REPORT TYPE: Final

LEADER/PARTICIPANTS: Anne-Marie Callcott, Lee McAnally, Shannon Wade, Jennifer Lamont, Ron Weeks, Shannon James

INTRODUCTION:

Baits are an important part of the Federal Imported Fire Ant (IFA) Quarantine (7CFR301.81) and are an environmentally friendly treatment method for both the quarantine affected industry and the general public. Testing of new bait formulations for inclusion in the IFA Quarantine is an ongoing process with new baits routinely added to the list of approved insecticides. Fire ant baits are utilized in the IFA Quarantine 1) in combination with a granular chlorpyrifos treatment for certification of field grown nursery stock, and 2) as an environs treatment within the Fire Ant Free Nursery Program. Traditional IFA baits are formulated on a pregelled corn grit carrier with soybean oil. Over the years, there have been some concerns in the industry regarding the availability of corn grit. Therefore, new carriers need to be tested to determine efficacy of fire ant baits on any new carrier. Tast-e-bait® is a carrier product formulated from the by-products of bakery products by Advanced Organics. This carrier has been tested by this laboratory for several years and has proven to be an acceptable carrier. Several active ingredients have been tested in the past using this carrier, with similar efficacy as using the traditional corn grit carrier. We tested the efficacy of Dupont's Advion fire ant bait formulated on the traditional carrier vs. on Tast-e-Bait carrier on field colonies of imported fire ants.

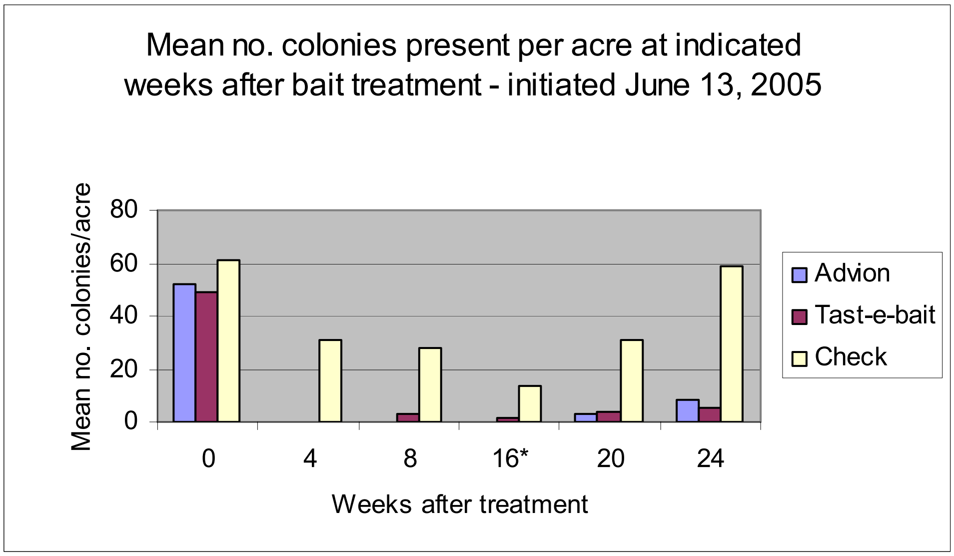
METHODS AND MATERIALS:

Dupont provided Advion fire ant bait on traditional carrier, and Advanced Organics provided a bait with the same percent active ingredient formulated on Tast-e-Bait carrier. Test plots were set up in Harrison county Mississippi. Each plot was one acre in size with a ¼-acre efficacy subplot located in the center of the test plots. There were 3 replicates per treatment. Treatments were applied on June 13, 2005 using a shop-built spreader on a farm tractor at a rate of 1.5 lbs/acre. Prior to treatment and at 4 week intervals thereafter, evaluations of IFA populations are made in each ¼-acre efficacy subplot using the procedures described by Lofgren and Williams (1982) and Collins and Callcott (1995). Evaluations continue until reinfestation is observed.

RESULTS:

At 4 weeks after treatment, both treatments provided 100% control of IFA in the treated areas (Figure 1). Traditional Advion maintained 100% control through 16 weeks and >90% control through 20 weeks. Tast-e_Bait with Advion also maintained >90% control through 20 weeks. Both treatments showed reinfestation at the 24 week evaluation. The checks were typical for a hot Mississippi summer. Significant decreases in observable colonies/mounds during the hot dry summer are normal in this area. However, populations rebounded as expected by the 24 week evaluation. No evaluations were conducted at 12 weeks due to Hurricane Katrina, and in the 5 weeks prior to the 16 week count there was no rain, and extremely high temperatures.

Figure 1. Advion/Tast-e-Bait Trial – initiated June 13, 2005; Harrison Co., MS



* no 12 week count due to Hurricane Katrina
no significant rainfall for 5 weeks prior to 16 week count

<u>DISCUSSION</u>:

Both formulations of Advion fire ant bait performed as expected and similar to other IFA baits, providing >90% control of IFA within 4 weeks of application. Fire ant baits are not formulated to provide residual activity, however both Advion formulations acted in a similar fashion to all baits, whereby rapid reinfestation is not observed in the treated area for up to 20 weeks after an application.

<u>REFERENCES CITED</u>:

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CPHST PIC NO: A9M03

PROJECT TITLE: Mississippi Phorid Fly Release Project

TYPE REPORT: Interim

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 APHIS 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://cphst.aphis.usda.gov/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 five years the Saucier release maintained fly presence and the area of fly coverage has increased. The most recent observations taken June 16-23, 2005 gave visual confirmation of fly spread 62 km north, 36 km east, 16 km south, and 34 km west of the original release site. An egg-shaped area using the furthest cardinal points with flies as its furthest reach in those directions encompasses approximately 4,118 km^2. Interestingly a similar release conducted by Louisiana State University has also produced an egg-shaped spread. This suggests prevailing winds from the Gulf of Mexico hinder southward spread in the coastal region while encouraging northward growth. The fall monitoring session was cancelled due to the impact of hurricane Katrina on the monitoring area and personnel. This is unfortunate since fall readings typically show a much greater increase than those in spring.

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 site itself. Conversely, about one hundred phorids were counted during the spring 2005 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 in the fall 2004 monitoring session had phorid activity while the remaining mounds next to an open field had none. More detailed data on these environmental factors will be collected in coming observation dates.

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CPHST PIC NO: A1M01/A1F01

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

TYPE REPORT: Interim

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 and sustained through FY05 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.html.

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, and 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 7-9 boxes in 2004-2005 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, with a decrease in 2005 (with the increase in *P. curvatus*) to 1.3 million (Table 1). *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 with additional boxes going online in 2005. Production has dramatically increased from 121,000 in 2003 to 1.3 million in 2005 (Table 2). Combined production is shown in Table 3.

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 2005 there have been 1-8 releases in each of 13 states and Puerto Rico, with a total of 56 field releases (Table 3; Figure 1) and more than 448,000 potential flies released. Of these 56 releases, 46 were *P. tricuspis* and 10 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 research or educational purposes, such as state fair exhibits and field days. Louisiana completed its first release from LA-reared flies in 2005. Over 88,000 potential flies have been shipped for these varied uses.

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). Despite hurricanes in 2005, only 2-3 potential releases in fall 2005 were impacted, with 17 releases that year, the best since the program was initiated.

Success of the program is currently being measured by successful overwintering of fly populations. Of the 39 releases conducted in 2002-2004, flies have been found after a winter at 17 (43%) of these sites; 14 *tricuspis* sites (AL, AR, FL, GA, MS, NC, PR, SC, TX) and 3 *curvatus* sites (FL, SC, TX). 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 was made 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.

REFERENCES CITED:

Porter, S.D. 1998. Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). Fla. Entomol. 81: 292-309.

Table 1. Rearing and release data for APHIS phorid fly rearing project – *Pseudacteon tricuspis*.

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
	2005	1,381,650	91,175	10	9,117.50	6.60	123,350	8.93

* approx. no. potential flies shipped 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.

Table 2. Rearing and release data for APHIS phorid fly rearing project – *Pseudacteon curvatus*.

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
	2005	1,383,641	88,638	7	12,662.57	6.41

* approx. no. potential flies shipped for release
** does not include one attempted release that was abandoned

Table 3. Rearing and release data for APHIS phorid fly rearing project – all species combined.

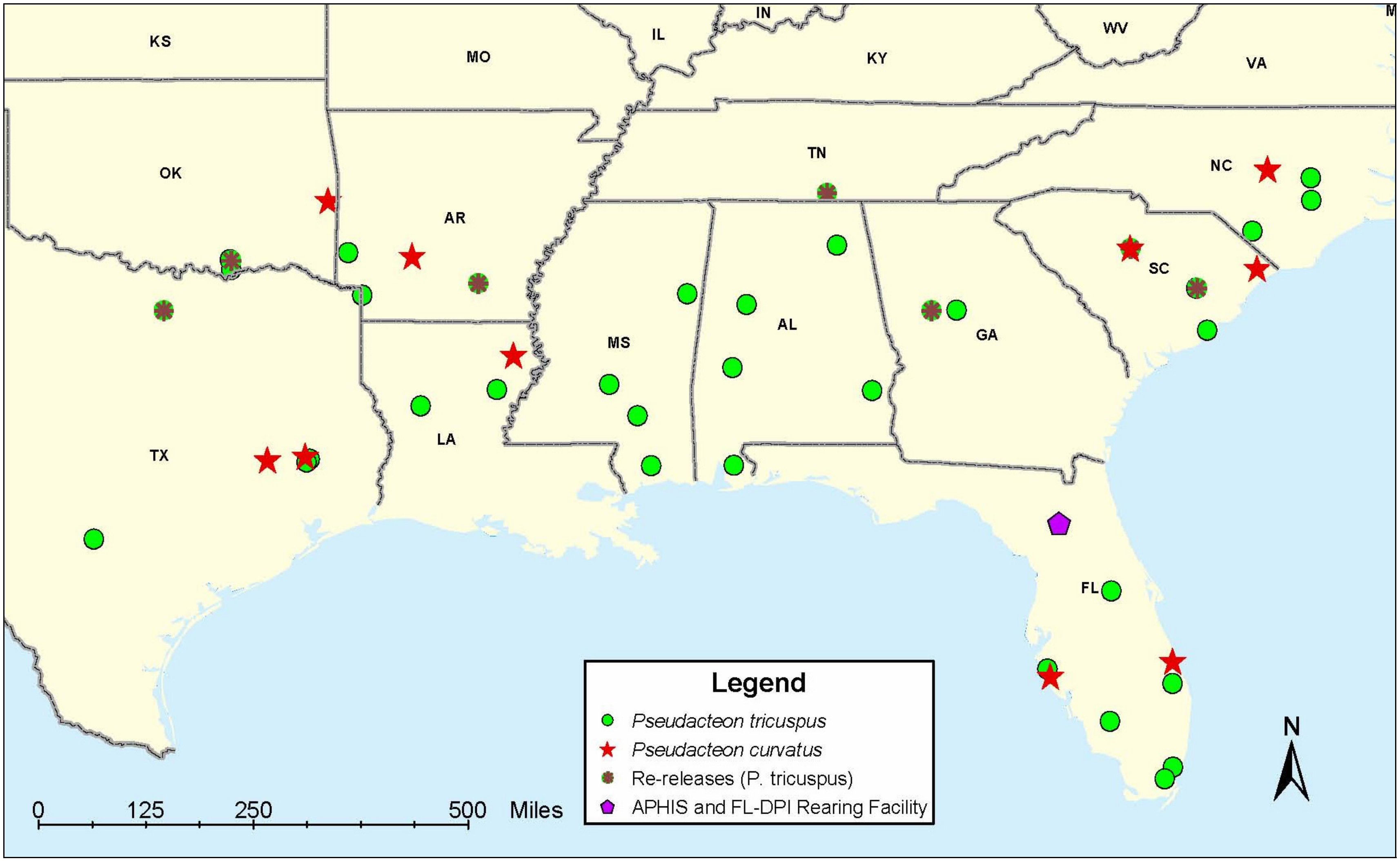
Species	Year	No. flies produced	Approx. no. shipped*	No. field releases**	Mean flies/ release	Percent flies field released	Total flies shipped***	Percent flies shipped
tri,cur	2002	950,063	58,750	12	4,895.83	6.18	59,385	6.25
tri,cur	2003	1,746,383	81,450	15	5,430.00	4.66	111,000	6.36
tri,cur	2004	2,280,039	128,602	12	10,716.83	5.64	154,652	6.78
tri,cur	2005	2,765,291	179,813	17	10,577.24	6.50	211,988	7.67

* approx. no. potential flies shipped 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.

Figure 1. 2002-2005 phorid fly releases in APHIS program; both *P. tricuspis* and *P. curvatus* (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). Releases in CA (2005) and Puerto Rico (2002, 2005) not shown on this map.



CPHST PIC NO: A1M01/A1F01

PROJECT TITLE: Progress Report from IFA Lab, Gainesville, FL 2005

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Debbie Roberts, FL-DPI personnel

This year has proven to be a better year for *Pseudacteon curvatus* while *P. tricuspis* has remained status quo. There had been talk from Dr. Porter earlier in the summer about dropping one of the *P. curvatus* boxes that has failed to excel, and bring over *P. littoralis* or *obtusus*. This has not occurred, partly because *obtusus* has not been removed from quarantine yet. The box in question is the H box, and while it has improved in production numbers, it has never improved enough to be used in the release program. Environmental differences between the two attack rooms are being attributed to it's less than stellar performance. On the other hand, the rest of the *curvatus* population has ballooned, frequently out pacing the *tricuspis* boxes.

There has been produced this year, 1,381,650 *tricuspis* and 1,383,641 *curvatus* for a total of 2,765,291 phorids.

The following is a breakdown of the shipments of *tricuspis* and *curvatus* this year in comparison to the three previous years.

		2005	2004	2003	2002
Field Releases					
	tricuspis	9	9	15	12
	curvatus	7	4		
Fairs/Demos		9	4	6	3
Research Projects		4	3	2	-
Box Start-up/Restock		-	1	1	-
Total	*tricuspis*	125,580	114,800	111,810	59,385
	curvatus	79,008	44,040	0	0

Most of the "bugs" were worked out with the *curvatus* releases by the end of last year. There were only a couple of boxes that came in that had any appreciable amount of dead due to dehydration. There was one incident where there was a hole in one of the lids on a holding pan and the ants found it and made their escape. These were not recoverable.

Hurricanes had the most deleterious effect, canceling one entire release, and cutting short another. Several states were under severe drought and delayed their releases. Cold weather also shortened or knocked out releases.

The following items are projects and/or problems that were approached, worked on, and or proven to be of no value during the year to further the IFA program. These are listed in no particular order, but all had impact on performance of the daily workings of the project.

#6 Lids

Rubbermaid #6 pans are a mainstay of the program. Last year when no "real good" substitute could be found for both sizes of ants, a replacement was bought and used for the *curvatus.* These proved to be cumbersome as far as stacking and quite difficult to get a good sized screen glued to them for air exchange. Through a continued effort, we were able to reduce the number of #6 pans by consolidation within an individual group. More of the larger pans became available for both groups so we stopped using these smaller pans except on rare occasions.

The reason we had wanted a replacement was the long term usage had caused many of the lids to crack around the rim. Tape was used to try to prevent the ants from escaping, but this had to be replaced after each washing. I was familiar with a substance known as Plasti-Dip® (Plasti Dip International); a multi-purpose rubber coating that is normally used to coat the handle of tools. A can was purchased and a trial was run on eight lids. The Plasti-Dip® was applied in thin layers, with a drying time between layers, over the cracks. These lids were marked with a date and put into the system. After several weeks, the repaired areas were holding strong. Some of the cracks were small and easily repaired. Others were quite large, almost to the point of having to throw the lid away. The Plasti-Dip® is elastic enough that it can withstand continual use (not abuse) and washing and still remain strong.

The next time a repair job was done, the entire lid collection was evaluated and all cracked lids were removed, cleaned and coated. The application process was modified slightly however. Heavier coats were applied each time, reducing the number of coats that needed to be applied, thus shortening the repair time per lid. Many of the lids were in need of repair, and it has become a task that must be undertaken every few months.

Shipping Containers for *P. curvatus*

During the 2004 shipping season there were four releases of IFA parasitized with *P. curvatus*. Each and every time upon receiving these ants from the field, there would be escapees in the pillowcases. Sometimes it was minimal while other times it would be quite extensive. In order to cut back on mortality in 2005, a new shipping container was needed. After searching many catalogs and visiting a half dozen retail stores, a new product was found; a container called Lock and Lock (Heritage Mercantile). Size and shape were the determining factor. The container had to be small enough to fit an entire collection into one of the coolers (in order to cut back on shipping cost), yet large enough to hold 3 to 5 grams of ants without causing overcrowding while in transit. A 1.7 cup container was selected and sent for.

A second problem with the shipping was trying to maintain a humidity level within the container in order not to dehydrate the ants while in transit. Previously a folded dampened paper towel was placed inside the container, but this caused a two fold problem: degradation of the fluon and extreme difficulty in extracting all the ants from the paper towel upon receipt. The answer came in the form of a miniature nesting tube. A 13 X 100 ml culture tube was prepared in the same way as the larger nesting tubes were. Eight small holes were drilled in the bottom of the Lock and Lock container and garden wire was looped through the holes. Hot glue was applied to both sides of the box at the juncture of the wire and box to seal up any possible escape holes. Two of these smaller version nesting tubes were placed in the box, and the wire was twisted (2 per tube) to hold the tube in place. Additional moisture was provided by two dampened cotton balls that were wedged under the nesting tubes.

Seven releases of *P. curvatus* of ants were accomplished in 2005 with no tubes ever breaking during shipping. The ants would migrate to the inside of the tubes and would be much easier to extract by untwisting the tubes and tapping out the ants, then having to meticulously pick off ants clinging to damp paper towels. This has cut way back on the time needed to unload and weigh the ants prior to putting them into the system.

Occasionally the tube must be replaced due to drying out, and a twist tie repaired because the wire will break after many repeated usage, but this is a small price to pay vs. the previous method.

One additional change to the *P. curvatus* shipping flow was added. Scheduling of shipments was found to be optimal if the releaser collected and shipped the ants on a Monday, so that the lab would receive them on Tuesday. Ants would be stored on the shelf until Thursday, with food and water and allowed to regain strength and "de-stress". Thursday they would be put into attack boxes and taken out on Monday, giving them a full four days of parasitization. We noted a much healthier population, with even a marked increase in weight after the rest period. Ants were always weighed upon arrival, prior to being put into the attack boxes, and prior to shipment. On the rare occasion when ants did not arrive on Tuesday, the period of time to rest was shortened or dispensed with entirely. Much higher mortality rates were seen, cutting in to the potential numbers of parasitized ants returning to the field.

<u>Feeding/Watering Tubes for Phorids</u>

After information was received from Dr. Henry Fadamiro on the longevity of phorids that have been allowed to feed and/or drink, tubes were placed in closed proximity to the entrance into the attack box as well as on one of the cross beams above the pans. Phorids generally "walk" out into the attack box area prior to flying, so it was thought that this would be a good area to offer them sustenance. Many observations were made, and only three times were any flies seen on the cotton balls of the water/sugar tubes. Two flies actually became stuck to a sugar tube cotton ball. The tubes were removed after it was determined that they were not of any benefit and may actually prove to be a detriment to the phorids.

Nylon Eyebolts/Strings

The attack boxes were constructed over a long period of time and by two different carpenters. An early version was constructed with large stainless steel eyebolts to support the string and housing cup used in the attack procedure. The remainder of the boxes were built utilizing two different sized eyebolts made of brass or galvanized material. Over time, these eyebolts had deteriorated, rusting, breaking away from their anchored position, or oxidize causing the galvanizing material to become very rough. These eyebolts have caused a deterioration of the strings, and we are constantly having to replace them—they have broken, become unraveled or are frayed down to a single strand.

Two different fixes are being applied. First, a replacement eyebolt constructed out of an isopolymer plastic was researched and finally purchased from CraftTech Industries. This company was found via an internet search and negotiations were made with the owner on size and number as well as providing a nylon nut to finish the application. The original samples were received, both with and without a collar. The one with the collar proved to be perfect with the exception of a small ridge on the inside of the "O" of the eyebolt. This was due to the manufacturing process; the eyebolts are formed in a mold and where the mold separates, this line appears. A small file was used to remove this ridge and the eyebolt was now ready to be put into operation. A quarter inch hole had to be drilled in the Plexiglas, the eyebolt placed in the box and the nut screwed down on the top of the bolt on the outside of the box. Observations were made over several weeks.

The eyebolts proved to be extremely strong, durable and unbreakable. Enough to complete all the boxes were ordered (350 pcs). Prior to being placed in the box, the ridge must be filed off. One box at a time is being refitted with this new eyebolt. Not all the strings were replaced at the time of refitting the boxes, so it is as yet undeterminable whether or not the string will not have to be replaced due to fraying. Regardless, there have been no more broken eyebolts, and it appears that this will be a permanent fix.

The second fix will be replacing the nylon twine with a more substantial material. Several options have been experimented with. A craft plastic string that became completely stretched out after only 24 hours and a plastic coated stainless steel wire that is used in jewelry making. This worked quite well for a couple of weeks, but it to eventually frayed and broke on the rough brass eyebolts. This was a rather costly wire too, $8.00 for only 100 ft. Finally an 80 lb test fishing line was tried. While it is difficult to tie, it has held up without stretching or breaking for more than four months. Different methods of attaching the string to the cup have been attempted. The safety pins first used have long since been replaced with jewelry lanyards. The pins rusted and broke. Some of the lanyards are also rusting. The humid environment within the boxes plays havoc with most materials.

Fishing paraphernalia has once again been utilized in experimentation. Double swivels and snap swivels have been placed in E box. The double swivels were used to try and help prevent the tangling that the cup string and the monofilament holding string do after repeated liftings. These do not allow for hooking and unhooking, and must be either incorporated with a lanyard or tied to the end of the sting on both ends. Not an option for replacement for the cup string. The snap swivel replaced the lanyard, but is extremely difficult to clip and unclip, and fatigues the technicians working with them. Long term, these have held up well, and do not need to be removed very often. This type or something very similar may be our next item to replace the now slowly failing lanyard population.

Fluon Replacement

Dupont informed us about three months ago, that they would no longer be manufacturing Teflon PTFE T-30 due to the constituent C8 and its links to cancer. Teflon PTFE TE-3859 has now totally replaced the old product. Experiments were done to see if it would work as well as the old product, and there appears to be no difference. The change over was seamless.

Orphan Box Dead

At times there is so much material being extracted from the ant boxes and placed on the plaster trays, that it becomes extremely difficult to read the trays. The thought occurred to me that the orphan box may not be yielding enough parasitized heads do make it worth going through all that extra detritus. A small experiment utilizing only the dead piles from seven of the attack boxes were separated out and placed on a plaster tray to see how many parasitized heads were actually there. Results were as follows: A = 10; B = 19; C = 44; D = 25; E = 20; F = 30; G = 13. While these may not be significant numbers, it was decided that we would rather not lose these potential phorids. No change was instituted in procedure.

Field Gathering of *P. curvatus*

In early November, Dr. Porter called about going out in the field with some of our own ants and exposing them to local phorids in order to boost our laboratory population numbers and "freshen" the gene pool. Amy and I accompanied Dr. Porter and Darrell along with a visiting Bio Sci Lab Tech from Stoneville, MS out into the natural area just east of our building. With eight pans of fire ants, we set them out hoping for a few attacks. What we saw was incredible. After only twenty minutes or so, there were attacks in several of the trays that would rival one of the best pans in our attacks boxes. We stayed out there for two hours. Upon returning to the lab, these ants were set up exactly as those that would have come out of the attack box. We went out to the same area twice more over the next week with approximately the same amount of ants and exposed them for the two hour time period. The location was ideal; recently disturbed area with woods nearby, and close proximity to a pond. The weather was perfect for all three days, with the slight exception of a strong breeze on the second trip.

These ants formed group X and over the next month were treated as any other group. The trays that were created from this group were placed through out the *curvatus* boxes based on counts from the trays from that same date; thus the tray with the lowest count would have the lowest number of emerging flies and would determine placement in the needy attack box. Ultimately this endeavor introduced 12,652 new phorids into the population. We were amazed at the ease with which the boxes could be replenished, at least for this species. Far fewer *tricuspis* flies were observed, but this may have been due to the size of ants we chose to expose.

Summary

It's been an eventful year with quite a few set backs, changes in personnel, weather situations and yet we managed to exceed the production and distribution of the previous years. Barring unforeseen circumstances, it will be the goal of this lab to equal or exceed these accomplishments in 2006.

PROJECT NO: A3M02

PROJECT TITLE: Geographic Information Systems (GIS) Decision Support and Management Program for Monitoring and Evaluation of Phorid flies (*Pseudacteon* spp.) in Imported Fire Ant *Solenopsis* spp. Populations

TYPE REPORT: Interim 2005

LEADER and COOPERATOR: Ronald D. Weeks, Jr. and Dr. Karl Suiter, Center for Integrated Pest Management, North Carolina State University

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

- Maintain project websites –
  Rearing - http://cphst.aphis.usda.gov/projects/Phorid_rearing
  Monitoring - http://www.cphst.org/projects/Phorid_monitoring
- Completed 2005 surveys in Mississippi and Puerto Rico using PDA/GPS hardware and ArcPad data collection forms
- Posted of web-based data entry forms – collaborative effort between CPHST and NCSU's Center for Integrated Pest Management: Web-site is currently available for collaborators: http://test.cphst.org/fireAnts/main.cfm. The site is password protected.
- Developed data dictionary and user manual for handheld devices and software
- Developed GIS layers of phorid fly releases and establishments in southeastern United States (Figures 1 and 2).

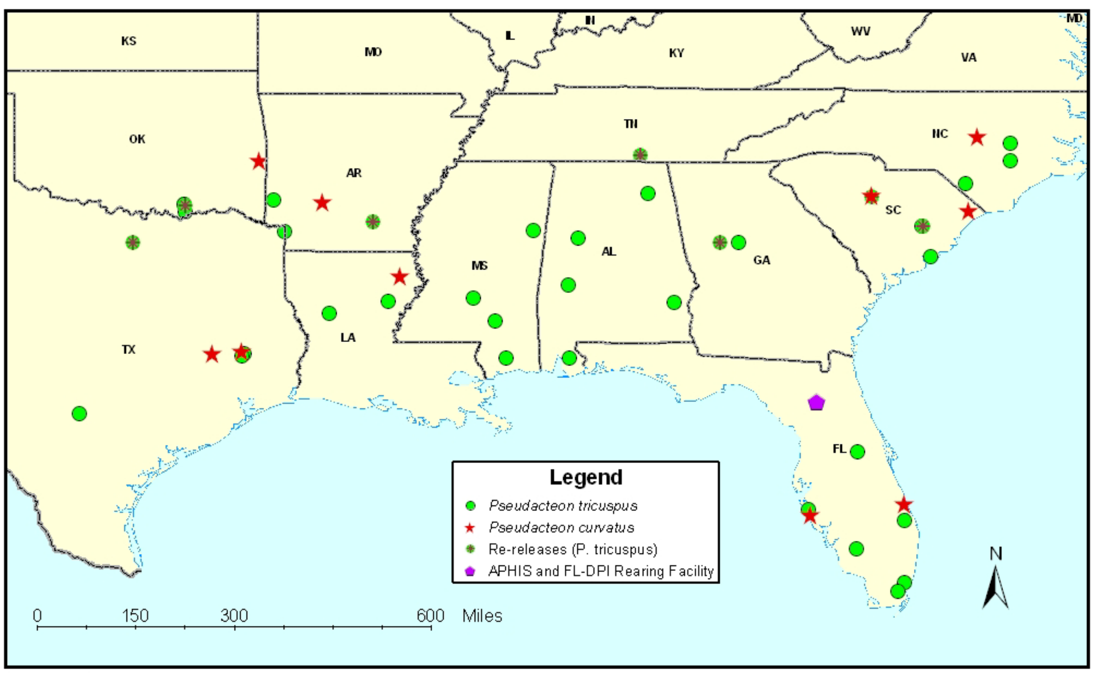
APHIS is allocating significant funding to the rearing and distribution of phorid flies to State collaborators for releases in numerous imported fire ant (IFA) infested states and varying habitats (see Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing Project A1M01/A1F01). 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 project 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. 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.

Spatial data are collected in several ways; 1) via web-based data entry forms and download PDF documents maintained at NCSU's Center for Integrated Pest Management, 2) via handheld data collection units using GPS/PDA handheld units. Data are entered into GPS/PDA 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/** .

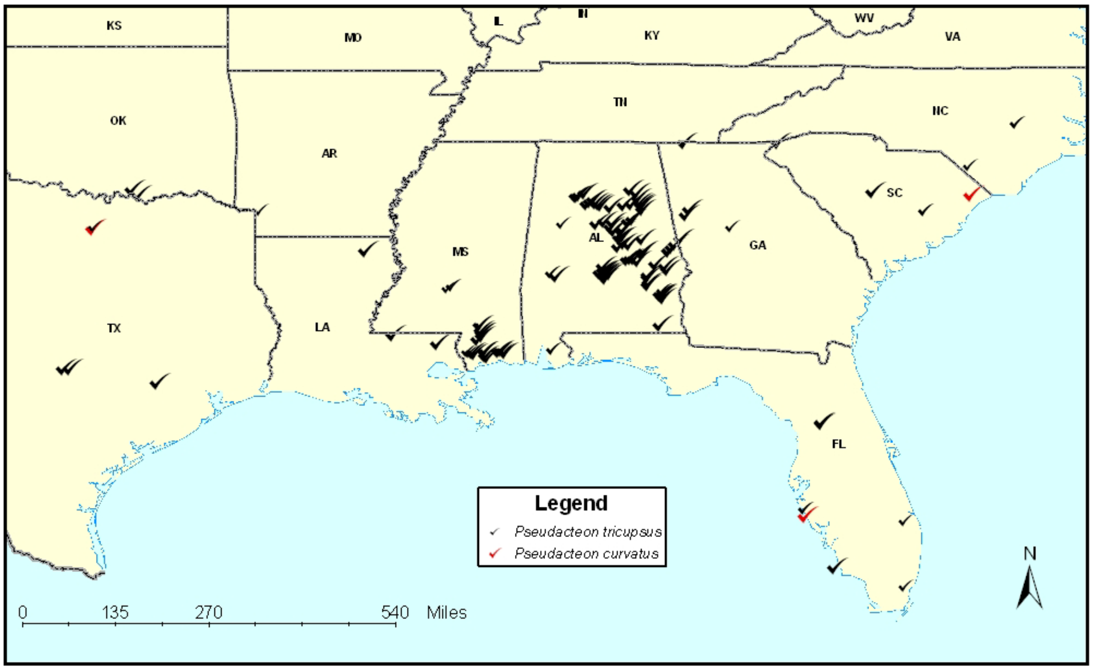
Currently, all data related to APHIS phorid fly releases and surveys are being collected by state cooperators; state agricultural inspectors, university personnel, extension personnel, etc.

Figure 1. USDA, APHIS, PPQ, CPHST Phorid fly rearing and release efforts 2002-2005. California (2005) and Puerto Rico (2002, 2005) releases not shown.



Source: SIPS
Created by: Ronald D. Weeks, Jr.,
USDA- APHIS- PPQ-CPHST-SIPS,Gulfport, MS
January 26, 2006

Figure 2. Phorid fly detections reported by cooperators and USDA-APHIS personnel. Survey efforts in Alabama and Mississippi were conducted as beta testing surveys for GPS/PDA units. More survey effort, data reporting and coordination are planned for other states. Puerto Rico detections positive but not shown. (Data from USDA and state organizations)



Source: SIPS
Status: Draft copy
Created by: Ronald D. Weeks, Jr.,
USDA- APHIS- PPQ-CPHST-SIPS,Gulfport, MS
February 3, 2006

PROJECT TITLE: Ad Hoc Project – Imported Fire Ant (IFA) Survival in Bulk Granular Urea

CPHST PIC NUMBER: Ad-Hoc Request

TYPE REPORT: Final

LEADER: Anne-Marie Callcott

INTRODUCTION:

In May/June 2002, two cargo vessels with bulk urea (fertilizer) were offloading their cargo onto barges when the cargo on some of the barges was noted to have numerous imported fire ants (IFA) crawling on the surface. After many consultations with numerous regulatory and scientific experts, it was determined that the ants were newly mated queens (NMQs) from a mating flight(s) in the U.S. Cargo on those barges infested with NMQs which were destined to areas outside the federal IFA quarantine areas had to stay within the quarantined areas. The prolific mating flight(s) during that period were the result of an extended dry period (4-6 weeks) ending in several days of rain during the ants primary mating season. The next IFA-NMQ incident with urea cargo offloading onto barges occurred again in late May 2005 after several weeks of dry weather was broken with numerous days of intermittent rainfall. This precipitated the question of the true risk of NMQs in bulk urea. An ad-hoc request was submitted with the following requests. Laboratory studies are needed to determine: 1) if IFA queens are attracted to granular urea; 2) the length of time newly-mated IFA queens can survive in granular urea; 3) if newly-mated IFA queens can establish a viable colony in granular urea. Deliverables: A report no longer than 10 pages in length that provides supporting information, results, and any conclusions that can be reached regarding the above studies.

MATERIALS AND METHODS:

IFA colony founding in urea: To determine the length of time NMQs can survive in urea and if NMQs can produce a viable colony in bulk granular urea, bulk urea (supplied by Shawn McGreevy, Koch Nitrogen, Wichita, KS) was placed in a 2" square plastic nursery pot and one NMQ and her associated brood was placed on top of the urea on June 10, 2005. Each nursery pot was placed in a 6x9x1.5(h)-inch pan (sides painted with fluon to prevent ant escape). In this trial the NMQ was confined to the area surrounding the urea filled pot, but not physically confined to the pot itself. Ten replicates were set up. Each NMQ had been collected ca. 10 days prior to the test and placed in a standard laboratory rearing tube, therefore each queen had already produced some eggs/brood. No water or food was supplied.

Alate female survival in urea: Alate females are the reproductively viable or threatening life stage of the IFA. Since newly mated queens are not available year-round or easily found in great quantities, we tested alate female survivability in granular urea vs. survivability in top soil. A standard alate female bioassay was set up, whereby 2x2-inch nursery pots are filled half full with either top soil or granular urea. Five replicates of each were initiated on July 22, 2005. Ten alate females were introduced into each replicate and confined to that pot with the media (lid placed on top of each pot). Mortality was determined twice weekly for 2 weeks.

IFA NMQ attractiveness to urea: We can not determine if NMQs are attracted to granular urea as they descend from a mating flight. Instead, in discussions with the ad-hoc requestor, it was decided to test the attractiveness of granular urea to NMQs on the ground. Several tests were initiated. In one test, granular urea was placed in an open standard square petri dish and top soil was placed in a second dish. Dishes were placed in a 6x9x1.5(h)-inch pan (sides painted with fluon to prevent ant escape). One NMQ was placed in the space between the 2 petri dishes and allow free choice of substrate in which to found a colony. Five NMQs, all collected on the day of the test (July 1, 2005), were tested in this manner. In a second test also initiated on July 1, 2005, the urea and top soil were placed in loose piles at opposite ends of the test pan (removing the possible barrier of the petri dish lip) and a NMQ placed in the pan between the piles and given free choice of a substrate in which to produce a colony. In a final test, granular urea and "clean" potting media (retains moisture better than top soil in lab conditions) were placed in 2x2-inch nursery pots. One of each media was placed in a 6x9x1.5-inch pan as above. NMQs were collected on July 22, 2005 and 2 NMQs were introduced into each pan between the pots containing the media. There were 10 replicates. Potting media was moistened as required but the urea was left as is.

<u>RESULTS</u>:

IFA colony founding in urea: Ten NMQs with associated eggs/brood were introduced individually onto urea on June 10, 2005 (NMQs collected May 31, 2005). Five days after introduction, all queens were alive and all were still on top of the urea. NMQs like to burrow/crawl underneath soil for protection, but the urea granules appear to be too heavy for the queens to move. At day 10, 3 of the NMQs had moved out of the container with the urea. By day 14, 2 NMQs were dead (outside the urea container), and only one of the remaining queens was still on the urea. By day 20, the remaining 8 NMQs were dead in the pan outside the urea container.

Alate female survival in urea: At 3 days after introduction all alates within the urea replicates were alive and one alate in the top soil replicates was dead (Table 1). However, by 7 days after introduction, an average of 80% of the alates in the urea replicates was dead. At 10 days, all alates in the urea were dead.

Table 1.

Treatment	Cumulative mean % mortality of 5 replicates at indicated days after alate female introduction to media		
	-3 days-	-7 days-	-10 days-
Urea	0	80	100
Top soil (control)	2	4	6

IFA NMQ attractiveness to urea: In the trial using petri dishes to contain the urea and soil, four days after introduction, 2 NMQs had moved into the top soil, and the remaining 3 were under the petri dishes containing the top soil. None were in the urea. In the trial with media piled at the ends of the pan, 4 of the 5 NMQs moved into the top soil within 3 hours of introduction. One NMQ moved into the urea and remained there until day 10 when she had moved into the top soil. All the NMQs died within 2 weeks of the initiation of these two trials negating any true survival testing for the NMQs in either media. Obviously our testing protocol was flawed which led to the third trial. Three days after introducing the NMQs to the test arena, 1 out of 20 NMQs was under a urea pot, 2 (from different replicates) were under potting media pots, and the remaining NMQs appeared to have moved into the potting media pots through the drain holes in the bottom of the pots. Urea pots were dumped and examined for NMQs to insure no NMQs were inhabiting these pots. On day 7, there was still one NMQ under the same urea pot, and only one NMQ under a potting media pot. By day 10, there was a dead NMQ in the replicate with the urea pot that had had a NMQ under the pot. By 5 weeks after introduction (Aug. 26, 2005), only one other NMQ had died, with the remaining 18 surviving in the potting media. Unfortunately, Hurricane Katrina arrived on Aug. 29, 2005, and the trial was terminated.

<u>DISCUSSION</u>:

While NMQs in flight may be visually attracted to bulk granular urea, once they have landed the urea appears to be detrimental to successful colony founding as well as NMQ survival. When NMQs are introduced onto granular urea and confined only to the area surrounding the urea, queen death resulted within 20 days. Alate females introduced into and confined to granular urea were dead within 10 days. Without a productive queen to produce more eggs/brood, colony development has been halted. When given a choice of media in which to start a colony, the limited work done here indicates that the majority of the NMQs will chose to inhabit top soil/potting media rather than granular urea. These trials were conducted under optimum temperature conditions (±78°F), unlike those conditions within an enclosed barge in June on the southern Mississippi River where the temperatures can probably exceed 120°F, thus speed of mortality may be enhanced by higher temperatures. We were not able to look into that aspect in the timeframe of this project.

<u>SUMMARY</u>:

Testing indicates that the risk of newly mated imported fire ant queens landing on bulk granular urea and surviving long enough to produce a viable colony is very low. However, to insure newly mated queens do not move from the inhospitable environment of the granular urea to an environment conducive to survival and colony founding, it is suggested that granular urea believed to be infested with newly mated IFA queens (through inspection or observation by marine surveyors or workers) be maintained on the barge within the IFA quarantine area for a minimum of 20 days prior to shipment to final destination.

CPHST PIC NO: A3M01

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

TYPE REPORT: Final

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. In 2005 the state foundation of Georgia left the SE Foundation and is now a separate operation.

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 on flower buds. This pheromone is an aggregation pheromone to attract both sexes to his locale. If others do not come he starts searching for pheromone sources which is when he can be trapped. (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 have been necessary. SEBWEP paid 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". Since this boll weevil was trapped July 22, 2003, the last boll weevils trapped in the area was in June 2002 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.

In 2005 based on samples received for examination, the boll weevil has issued a wake up call to the eradication effort. They can not relax until eradication has been achieved. The number of samples was 56. Many of these were teneral adults trapped in July through September in Mississippi and Missouri. This indicated boll weevil reproduction in a few fields. The older adult specimens indicated movement back into eradicated areas of Lafayette, Pontotoc, Tippah, Union and Yazoo Counties of Mississippi. These specimens demonstrate the importance of continued trapping behind the active Program. This importance was further emphasized in North Carolina with the capture of one apparent hitch-hiking boll weevil in Martin County. I supervised the eradication effort there in 1978-1980. This was the only trap capture east of the state of Mississippi in two years.

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 were not involved in boll weevil eradication. These are (1) the Lower Rio Grande Valley in south Texas and (2) the northern black-lands in northeast Texas which both started eradication in 2005. These are the last two cotton growing areas in the United States that had not been in an active 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 2005 was that it took 5% or less to handle the samples received 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.

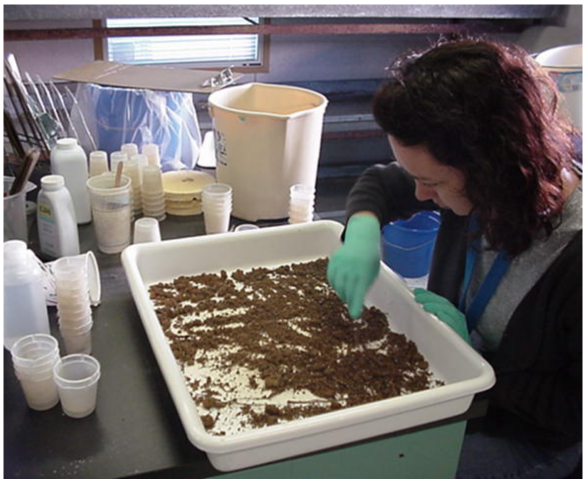


Figure 2. Single test chamber with test media and alate females with lid.

Figure 3. Set up of bioassay test procedure.

APPENDIX II. CHART USED TO DETERMINE VOLUME OF BALLED-AND-BURLAPPED ROOT BALLS.

B&B Wire basket dimensions

Volume formula for Cone = pi (R^2 + rR + r^2) h / 3

R = Radius of top of cone, r = radius of bottom of cone, h = cone height, pi = 3.1415926535

Top Diameter (in.)	Bottom Diameter (in.)	Height (in.)	R^2	r *R	r^2	Ball Volume (in^3)	L	Gal	**1/5 Volume Per Ball (gal)**	**1/6**	**1/8**	**1/10**	**1/20**	**1/30**
16	8	10	64	32	16	1172.9	19.2	5.1	**1.02**	0.85	0.63	0.51	0.25	0.17
17	10	11	72.25	42.5	25	1609.8	26.4	7.0	**1.39**	1.16	0.87	0.70	0.35	0.23
20	12	12	100	60	36	2463.0	40.4	10.7	**2.13**	1.78	1.33	1.07	0.53	0.36
22	15	13	121	82.5	56.25	3536.1	58.0	15.3	**3.06**	2.55	1.91	1.53	0.77	0.51
25	10	12	156.25	62.5	25	3063.1	50.2	13.3	**2.65**	2.21	1.66	1.33	0.66	0.44
25	13	16	156.25	81.25	42.25	4687.3	76.8	20.3	**4.06**	3.38	2.54	2.03	1.01	0.68
28	14	13	196	98	49	4669.5	76.5	20.2	**4.04**	3.37	2.53	2.02	1.01	0.67
30	17	18	225	127.5	72.25	8006.3	131.2	34.7	**6.93**	5.78	4.33	3.47	1.73	1.16
32	15	15	256	120	56.25	6789.8	111.3	29.4	**5.88**	4.90	3.68	2.94	1.47	0.98
34	21	24	289	178.5	110.25	14520.4	238.0	62.9	**12.58**	10.48	7.86	6.29	3.14	2.10
40	20	23	400	200	100	16859.9	276.3	73.0	**14.60**	12.17	9.13	7.30	3.65	2.43
60	22	26	900	330	121	36783.9	602.9	159.3	**31.86**	26.55	19.91	15.93	7.96	5.31

Lantenn Nursery presently using: 100 gal / 600 plants (16 inch B&B).

If we assume their 16 inch B&B is the same dimensions as above, then they should be using 1 gallon per plant to meet the 1/5 ball volume requirement. In actuality, they are using 0.17 gal per plant equals about 1/30 volume (1.36 pints). Wayne Milstead at Lantenn indicated that 0.17 gal per 16 inch B&B was sufficient to achieve runoff, which is what the IFA Quarantine protocol indicates is necessary on page 8 under "For Balled and Burlapped (B&B) Plants".