2007 ACCOMPLISHMENT REPORT

IMPORTED FIRE ANT SECTION

GULFPORT LABORATORY

CENTER FOR PLANT HEALTH SCIENCE AND TECHNOLOGY

PLANT PROTECTION AND QUARANTINE

ANIMAL AND PLANT HEALTH INSPECTION STATION

U.S. DEPARTMENT OF AGRICULTURE

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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:

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May 2008

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2007 IMPORTED FIRE ANT OBJECTIVES

CPHST-Gulfport Laboratory, Imported Fire Ant Section

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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CPHST PIC NO: A9F01

PROJECT TITLE: Chemical Degradation of IFA Quarantine Program Insecticides Used for Incorporation into Containerized Nursery Stock Potting Media, 2006

REPORT TYPE: Interim

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

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, 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 was done in cooperation with the CPHST Gulfport Lab Chemical Analysis section which conducted 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.

The initial test was prematurely terminated due to hurricane Katrina. The data generated by the limited sampling was inconsistent and highly variable, and no significant conclusions could be formed with this data. As a result, a new trial was initiated in 2006 incorporating lessons learned about the sampling and mixing procedures.

MATERIALS AND METHODS:

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

The MAFES media is being tested in two ways; bulk mixing where the individual components of the untreated media was premixed in a large quantity, then measured out into 1.5 cu. ft. loads then chemical treatments applied, and batch mixing where each individual component was measured out in the correct proportions for one 1.5 cu. ft. mixer load and chemical treatment added at the same time. The same amount of chemical was added based on the single premixed bulk density of the MAFES media. Windmill media is obtained in bulk and required no difference in handling.

To insure consistency over the quarantine all incorporation applications are made based on the dry weight bulk density of the media. However, the question on efficacy of bifenthrin and/or adsorption of bifenthrin to the media when mixed in thoroughly saturated media or very dry media has been raised. Each media/mixture type was then mixed either wet or dry at 10 and 25 ppm. Dry mixing meant that no additional moisture other than what was already in the media was added. The wet mixes were done by adding approximately 1 liter of water per mixer load (1.5 cu. ft.). The wet loads were allowed to mix for approximately 5 minutes to ensure uniform moisture content before the chemical treatments were added. A portable cement mixer (2 cu ft capacity) was used to blend the chemical into the potting media, and was operated for 15 minutes per load 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.

Immediately after potting, samples were taken for chemical analysis. Each sample consisted of one full pot and three such samples (replicates) per treatment were submitted for analysis. Samples were taken at 0, 3, and 6 months post-treatment for the 10 ppm treatments and at 0, 6, 12, 18 and 24 months post-treatment for the 25 ppm treatments.

<u>RESULTS</u>:

Results to date are summarized in Table 1. The figures reported in the table are the average of the three replicates for each treatment. The analytical method used returns an initial result in ppm which is then adjusted for moisture content. The limit of detection (LOD) and the limit of quantification (LOQ) for the initial analytical results are 0.9ppm and 3.0ppm, respectively. Several replicates returned initial results that were below the LOQ (not adjusted for moisture content). For those replicates, an initial reading of 1.95 ppm (median of LOD and LOQ) was assumed, and this number adjusted based on moisture content of the sample. This allowed us to obtain an adjusted dry weight average ppm for the three replicates in each treatment.

All treatment types and rates of application showed a consistent rate of degradation (Table 1, Figures 1 & 2). All started at or near the theoretical dose rate with the exception of the MAFES 25 ppm batch mixed dry treatment which was nearly double the theoretical dose rate initially. This is unexplainable since the subsequent samples appeared to be at or near the same strength as the other 25 ppm treatments. The 10 ppm treatments showed a drop between 0 and 3 months and little change from 3 to 6 months. The 25 ppm treatments showed a similar drop from 0 to 6 months followed by a plateau at 6 and 12 months and another drop at 18 months. Overall, this trial indicates that in the first 12 months after mixing, there is an average decrease in bifenthrin detected by chemical analysis of approximately 41-43%, with a range of 19-72%. Most of this decrease occurs in the first 3 months after mixing (36% decrease). By 18 months, the decrease in bifenthrin has jumped to 66% of the initial amount with a range of 53-81%.

The 10 ppm treatments were terminated after 6 months as scheduled. The 25 ppm treatments were scheduled to be terminated after 24 months. However, since extra treated material was left over after mixing due to batch sizes, these treatments will also be analyzed at 30 months.

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

Soil Type	Mixing Method	Soil Moisture	Treatment Rate	PPM at indicated months post-treatment (Mean of 3 samples)				
				0 mth	3 mth	6 mth	12 mths	18 mths
MAFES	Bulk	Dry	10 ppm	7.4	5.6	4.8	-	-
			25 ppm	25.0	-	15.4	14.7	11.2
		Wet	10 ppm	14.4	5.3	4.0	-	-
			25 ppm	18.8	-	13.5	13.4	8.7
	Batch	Dry	10 ppm	9.8	6.5	6.7	-	-
			25 ppm	46.7	-	15.4	16.2	9.1
		Wet	10 ppm	11.6	7.2	6.9	-	-
			25 ppm	26.6	-	15.4	14.9	9.6
Windmill	Bulk only	Dry	10 ppm	15.2	11.5	12.3	-	-
			25 ppm	26.7	-	16.0	14.2	8.6
		Wet	10 ppm	13.2	9.0	6.2	-	-
			25 ppm	24.2	-	16.7	16.3	5.8

Red indicates mean based on one or more initial readings below LOQ (see text for details)

Figure 1. Results of chemical analysis of potting media incorporated with granular bifenthrin at 10 ppm.

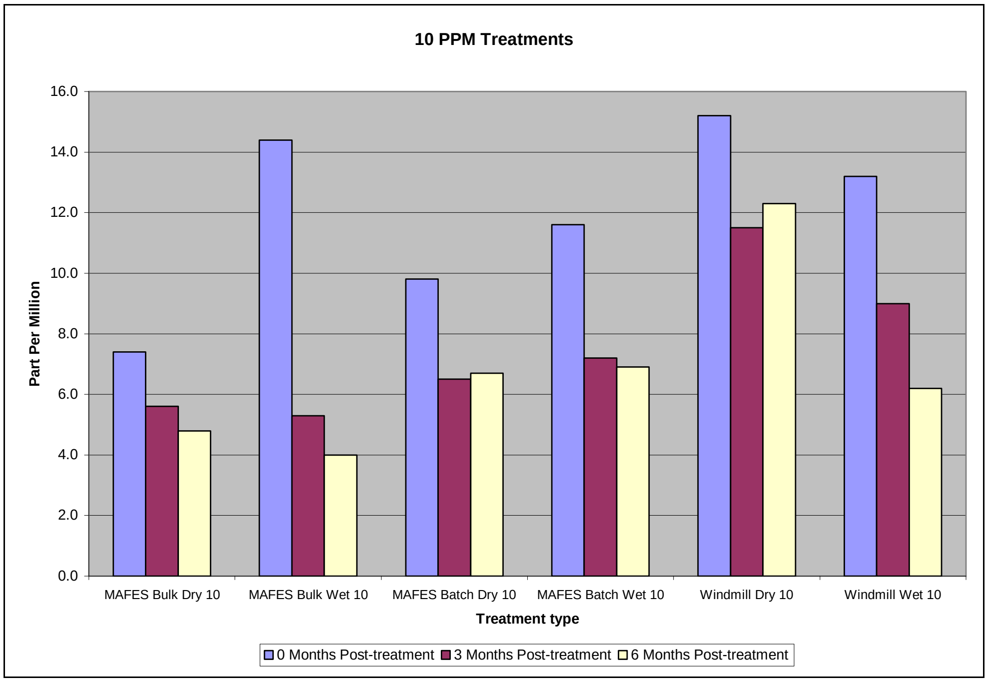
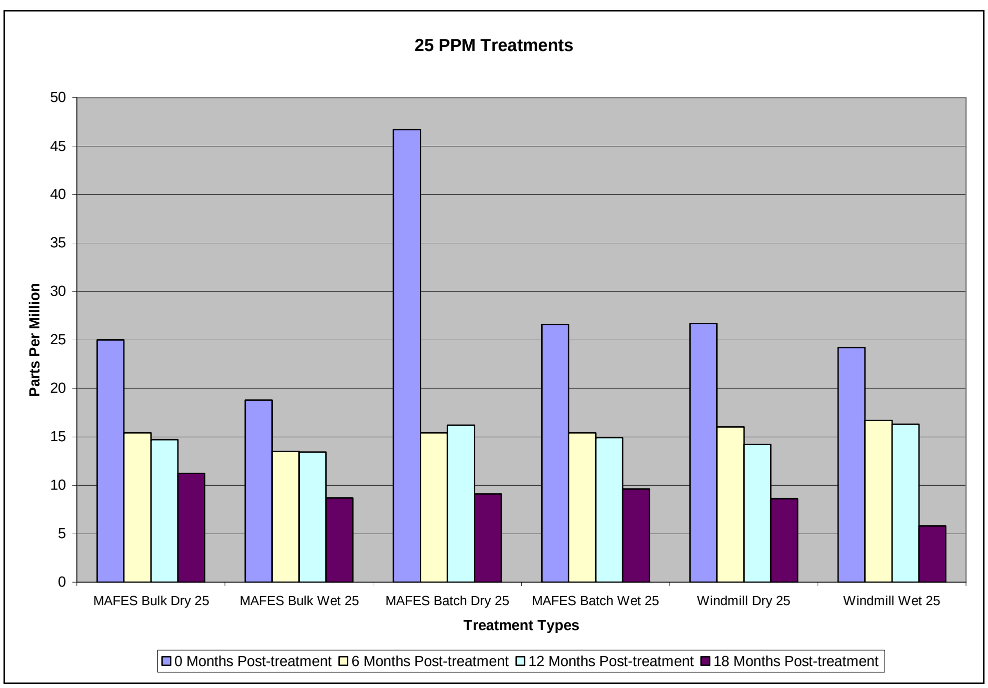


Figure 2. Results of chemical analysis of potting media incorporated with granular bifenthrin at 25 ppm.



CPHST PIC NO: A1F04

PROJECT TITLE: Summary of Numerous Dip/Immersion Trials for Balled-and-Burlapped Nursery Stock 2002-2007

REPORT TYPE: Status Report

LEADER/PARTICIPANTS: Anne-Marie Callcott

INTRODUCTION:

Since 2000, one of the primary focuses of this laboratory has been to find alternative treatments or insecticides for use as imported fire ant quarantine treatments for field grown nursery stock. Current treatments rely solely on the use of the insecticide chlorpyrifos. The infield treatment requires applying a broadcast treatment of a toxic fire ant bait followed in 3-5 days by a granular chlorpyrifos treatment. Alternatively, a post-harvest treatment of the balled-and-burlapped (B&B) stock requires a dip/immersion treatment with a chlorpyrifos solution, or a twice daily for 3 consecutive days drench/watering in treatment with a chlorpyrifos solution. Alternatives are critical to insure continued movement of field grown nursery stock to areas outside the federally regulated imported fire ant (IFA) areas.

While many people have worked on these projects, the primary leaders of all the trials summarized here are Shannon James (USDA-APHIS-PPQ-CPHST Gulfport resigned) and Jason Oliver (Tennessee State University, McMinnville, TN). Many of these trials were conducted in conjunction with testing of insecticides against Japanese beetle, another soil dwelling pests of regulatory concern to tree growers in Tennessee and other areas in the northern area of the fire ant quarantine area. Not all results or insecticides tested are presented here, but those which show promise or are of interest to the growing community are presented.

MATERIALS AND METHODS:

Specifics can be found in previous annual reports in the individual project trial reports. Root balls were obtained from various nurseries in both Mississippi and Tennessee for testing and ranged in size from 12-inches to 24-inches. Insecticides were mixed at the testing rates and placed in dip tank/container large enough to dip the largest root ball being tested (Table 1). Root balls were immersed in the liquid for approximately 1 minute or until bubbling ceased. Balls were then stored outdoors for aging. At specific intervals, soil samples were collected using a soil corer. Samples were taken either from the middle/core of the root ball, or from the surface/top of the root ball. There were generally 3-4 replicates (root balls) per treatment in each trial, and samples were generally collected at 0.5, 1, 2, 4, and 6 months after treatment, although there was some variation in sampling. Testing was initiated in both fall and spring months.

All IFA bioassays were conducted in Gulfport, MS at the APHIS-PPQ-CPHST laboratory. Field collected IFA alate females were subjected to the soil samples and mortality was accessed at 14 days after continued confinement to the soil sample. While the number of females per sample varied due to changes in the protocol and resources, a minimum of 10 alate females per replicate, and thus a minimum of 30 alate females per treatment per sampling interval were always used. A few trials also included bioassays conducted against IFA worker ants.

Table 1. Insecticides tested for use as an imported fire ant quarantine dip/immersion treatment for balled-and-burlapped nursery stock.

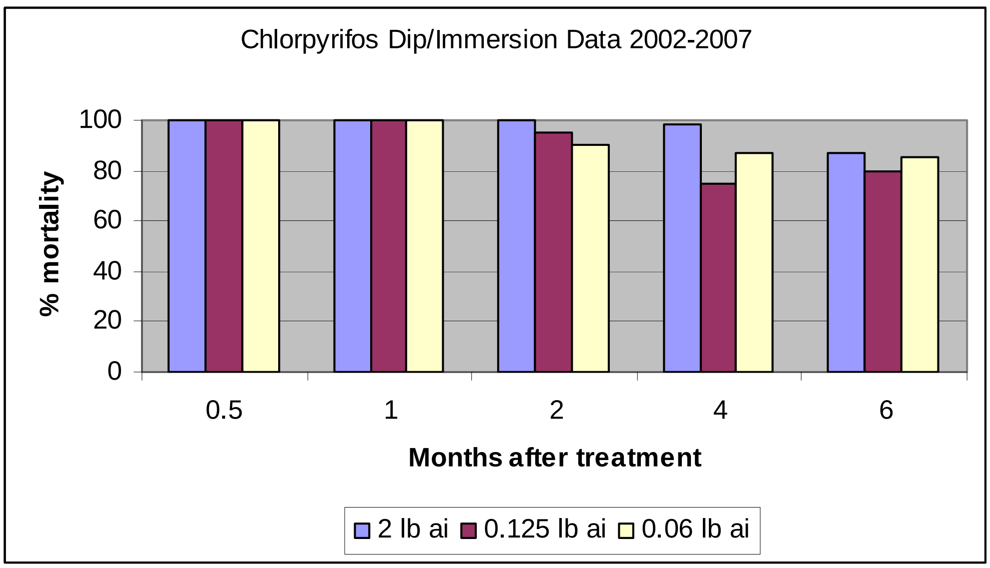
Insecticide	Rate of Application (lb ai/100 gal water)	Insecticide	Rate of Application (lb ai/100 gal water)
Acephate	0.375	Cyfluthrin+Imidacloprid	0.5+0.12
	0.75	(Discus)	
Bifenthrin	0.006	Deltamethrin	0.04
	0.0125		0.065
	0.025		0.13
	0.0575		
	0.115	Imidacloprid	0.2
	0.23		0.3
			0.4
Carbaryl	4.0		
	8.0	Lambda-cyhalothrin	0.017
			0.034
Chlorpyrifos	0.06		
	0.125	Thiamethoxam	0.065
	2.0		0.13

RESULTS:

For the purpose of this summary report, all soil sample sites (middle or top) as well as life stages tested were combined. Several of the insecticides tested did not show promising results against IFA at the rates tested in these trials. These included acephate, carbaryl, and imidachloprid. Thiamethoxam at the highest rate tested of 0.13 lb ai/100 gal water provide quarantine level efficacy through 1 month, but was losing efficacy at 2 months.

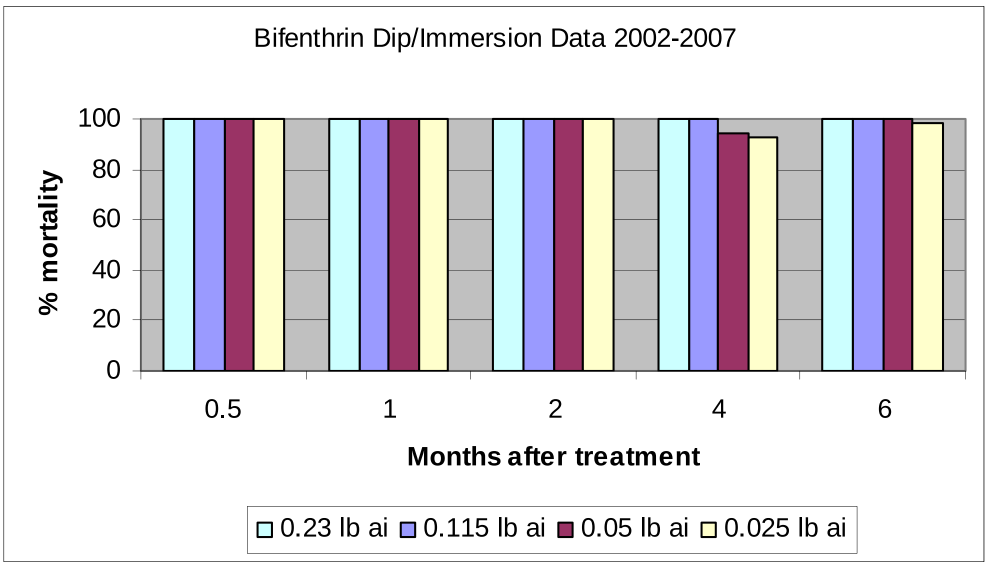
Chlorpyrifos: Currently, chlorpyrifos is the only insecticide that can be used to support the IFA regulatory dip/immersion treatment for B&B nursery stock. It is mixed at a rate of 0.125 lb ai/100 gal water and provides a certification period of 30 days. Our testing confirms this rate and certification period (Figure 1). These trials indicate that a lower application rate (0.06 lb ai) may also be effective at eliminating IFA for 30 days. This data will be examined in more detail to determine whether the current regulations could be changed to this lower application rate, saving growers money and protecting the environment by using less insecticide (half the current rate). The 2.0 lb ai rate was also tested routinely because it is the current rate of application to certify movement of B&B nursery stock for the Japanese beetle (JB) Harmonization Plan and some growers must treat for both pests. The high JB rate provided excellent control of IFA for 4 months.

Figure 1. Efficacy of chlorpyrifos as a dip treatment for balled-and-burlapped nursery stock. Compilation of data from numerous trials in MS and TN from 2002-2007.



Bifenthrin: Bifenthrin tested as a dip/immersion treatment for balled-and-burlapped nursery stock provided consistent and long-term control against imported fire ants. Rates as low as 0.115 lb ai/100 gal water provided 100% control for 6 months in numerous trials in both MS and TN during both spring and fall applications (Figure 2). Label changes to a FMC bifenthrin label to include this use pattern at the 0.115 lb ai/100 gal rate are underway by the company and USDA-APHIS fully supports the development of this label and process, and plans to officially approve this treatment once labeling has been completed. Lower rates with shorter certification periods will also be considered when labeling is completed.

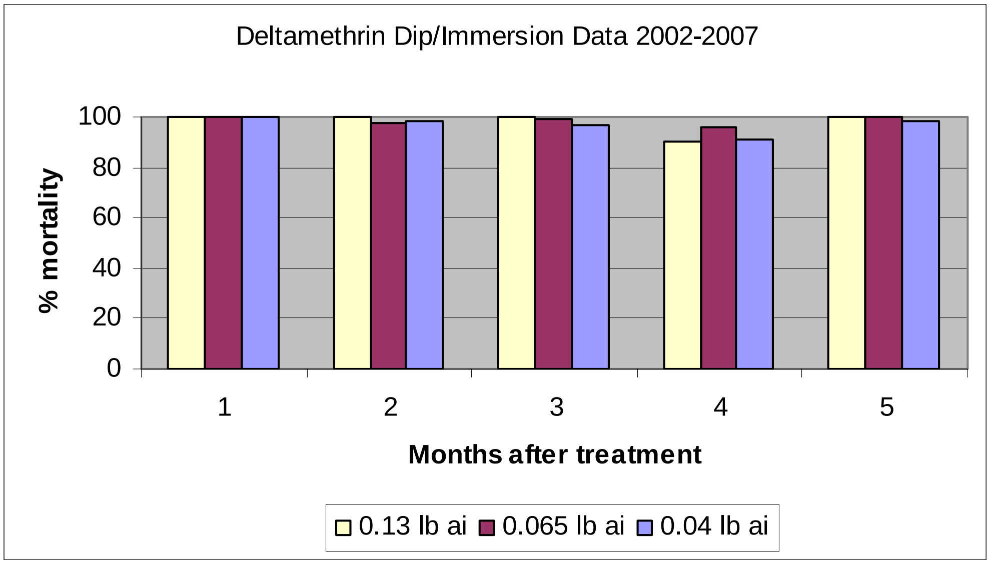
Figure 2. Efficacy of bifenthrin as a dip treatment for balled-and-burlapped nursery stock. Compilation of data from numerous trials in MS and TN from 2002-2007.



Imidachloprid + Cyfluthrin: This combination of insecticides is currently available in the labeled product Discus™ (OHP Inc.). Several tests at a single rate of application (0.5 imidachloprid+0.12 cyfluthrin) have been conducted with this combination insecticide; however, data in all trials has only been collected through 4 months. The imidacloprid + cyfluthrin combination has been 100% effective against IFA for 4 months. Further testing to provide efficacy at 6 months at this rate of application is planned and testing at lower rates of application will be planned.

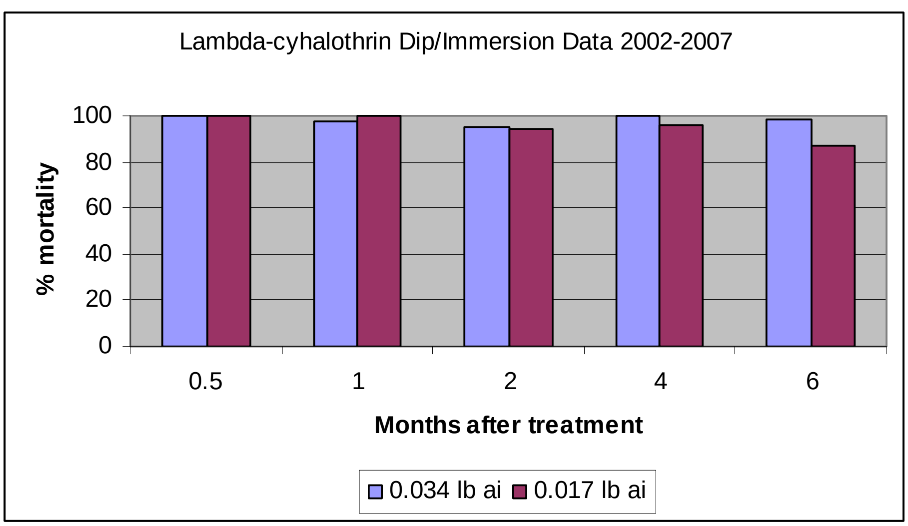
Deltamethrin: This insecticide shows promise however with rates selected for testing, it has not been as consistent as previously mentioned insecticides (Figure 3). The two higher rates do provide excellent control of IFA through 2 months.

Figure 3. Efficacy of deltamethrin as a dip treatment for balled-and-burlapped nursery stock. Compilation of data from numerous trials in MS and TN from 2002-2007.



Lambda-cyhalothrin: Lambda-cyhalothrin has not been as consistent at the rates tested as the previously mentioned insecticides (Figure 4). This product may be a good choice for a short term treatment (1 month certification). However, it continues to show great promise and therefore additional testing at other rates may be pursued.

Figure 4. Efficacy of lambda-cyhalothrin as a dip treatment for balled-and-burlapped nursery stock. Compilation of data from numerous trials in MS and TN from 2002-2007.



PROJECT NO: A1F04

PROJECT TITLE: Alternative Immersion Treatments for Balled-and-Burlapped Nursery Stock for use in the IFA Quarantine, Tennessee Fall 2007

REPORT TYPE: Interim

PROJECT LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Lee McAnally, Craig Hinton of USDA-APHIS; Jason Oliver, Nadeer Youssef of Tennessee State University; Michael Reding and Jim Moyseenko of USDA-ARS, Horticultural Insects Research Laboratory

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 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 past few years indicated several chemicals could potentially be used in addition to chlorpyrifos in treatment of B&B nursery stock.

MATERIALS AND METHODS:

Tennessee Fall 2007 Trial

Treatment applications were made October 16-17, 2007 at the Nursery Research Center by personnel from TSU-NRC and USDA-ARS. A commercial grower in Warren Co., TN provided plants with 12 and 24 inch-diameter root balls in strongly acidic (pH 5.1 to 5.5) loam to clay loam soil. The 12” root balls were immersed for one minute in a dip tank (Fig.1 A) that consisted of one of the treatments in Table 1. The 24” root balls were immersed using power lifting device (Fig.1 B) in the solution of one of the three treatments in Table 2.

Figure 1. (A) Workers dip 12" plants in chemical solution for one minute. (B) Power-lifting device was used to dip the 24" B&B nursery stock.

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.

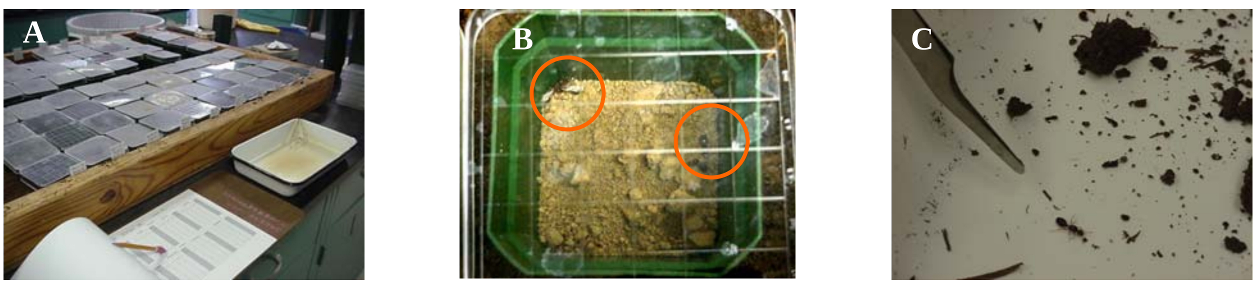


Table 1. List of treatments for 12 inch immersion trial

Material	Active Ingredient	Rate #ai/100 gal.	Amount Product/gal
Talstar N F	bifenthrin	0.115	6.53 ml
Talstar N F + Sevin SL	bifenthrin + carbaryl	0.0125+0.25	0.71 + 2.37 ml
Talstar N F+ Dylox 80TO	bifenthrin + dimethyl phosphonate	0.0125+.25	0.71ml+1.42g
Talstar NF+ Marathon II	bifenthrin + imidacloprid	0.2+ 0.253	11.36+4.79 ml
Onyx Pro 23%	bifenthrin	0.05	4.35 ml
Discus (0.72 gal)	cyfluthrin + imidacloprid	0.1875+.045	27.09 ml
Allectus	imidacloprid+ bifenthrin	0.0625+.05	5.26 ml
Arena 50 WDG	clothianidin	0.2	3.63 g
Arena 50 WDG	clothianidin	0.4	1.81 g
Safari 20 SG	dinotefuran	0.54	12.25 g
DPX-E2Y51	unknown	0.42	9.52 ml
Control	--	--	--

Table 2. List of treatments for 24 inch immersion trial

Material		Rate #ai	Amount product/gal
Discus 0.96 gal	cyfluthrin + imidacloprid	0.25 + 0.06	36.15 ml
Talstar NF+ Sevin SL	bifenthrin + carbaryl	.0125 + 0.25	0.71 + 2.37ml
Talstar NF + Dylox 80TO	bifenthrin + dimethyl phosphonate	0.0125+0.25	0.71ml+1.42g
Control	--	--	--

After treatment, the plants were maintained outside to weather naturally. Soil core samples were collected from the surface of five replicates within each treatment at 0.5, 1, 2, 4, and 6 months post-treatment. Soil core samples from the surface and the middle of five replicates (root balls) were collected at 5 months to insure penetration of the insecticide(s). Samples for testing against red imported fire ants were shipped to the CPHST Lab in Gulfport, MS where the samples were frozen until they could be utilized in alate female bioassays. A single bioassay cup containing 10 female alates was utilized for each soil sample (replicate). Female alate mortality was recorded two times a week during the 14-day exposure period, and dead alates were removed from bioassay cups during these observations.

RESULTS AND DISCUSSION:

All dip treatments in the 12-inch root balls were very effective in against IFA alate females through 4 months, except the dinotefuran (Safari) and the experimental DPX-E2Y51 product (Figure 3). The bifenthrin + dimethyl phosphonate (Dylox) had a slight decrease in efficacy at 1 month and the clothianidin 0.2 rate had a slight decrease at 4 months. The dinotefuran was 100% effective at ½ month, but showed significant decreases in efficacy thereafter, while the experimental DPX product was not different from the control at any evaluation period.

The three combination treatments in the 24-inch root balls were also 100% effective through the 4 months of evaluation to date (Figure 4).

These products will continue to be evaluated to replicate efficacy as needed.

Figure 3. Efficacy of B&B dip treatments against IFA alate females in 12-inch root balls; Tennessee Fall 2007.

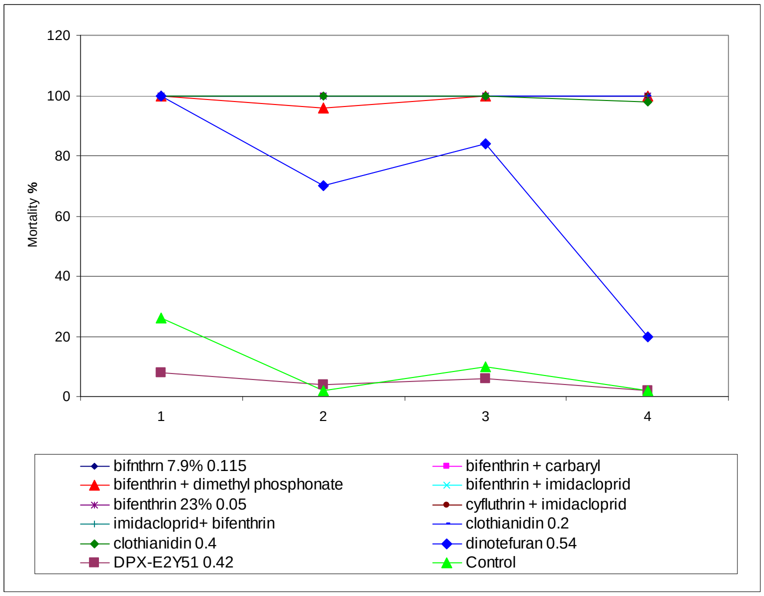
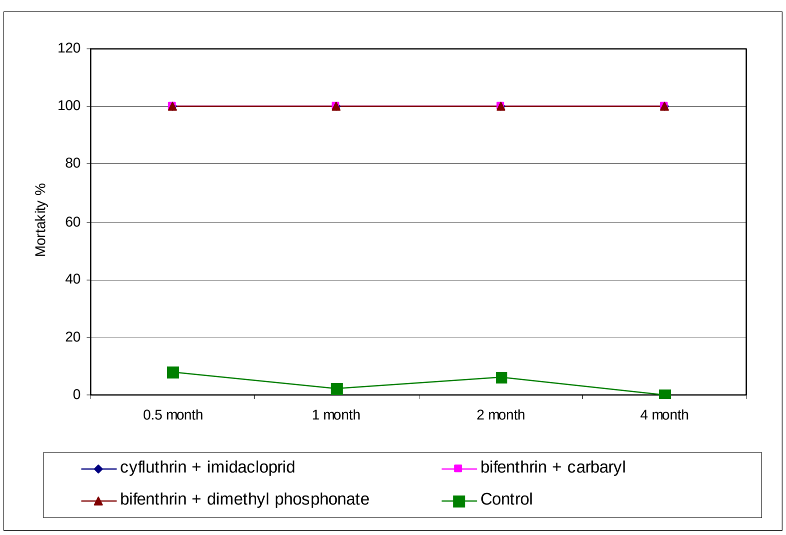


Figure 4. Efficacy of B&B dip treatments against IFA alate females in 24-inch root balls; Tennessee Fall 2007



CPHST PIC NO: A1F04

PROJECT TITLE: Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Fall 2007 in Tennessee

REPORT TYPE: Interim

LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Lee McAnally, Craig Hinton; Jason Oliver 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. Soil core bioassays for drenches conducted in 2002 and spring 2003 yielded erratic results over time and among replicates within treatments. Results from the same chemicals at equal or lower rates, when applied by immersion, were consistent, thus indicating insufficiency in application of the drench treatments. Doubling the volume of solution in drench application conducted in fall 2003 and spring 2004 failed to eliminate inconsistent results. The search for the cause of the inconsistency problem become narrower and has pointed to coverage and penetration of the drench solutions.

During drenching, B&B normally rests on one side of the root ball throughout the three-day drench process. This was true for all drench treatments done before fall 2004. This drench method possibly restricts treatment coverage on the resting side, while giving the surface of direct application a higher concentration of chemical and deeper penetration. The 2004 fall drench strongly suggested that rotating root balls during treatment, regardless of application frequency, improved the consistency of bioassay results and could potentially cut the number of days spent applying drenches from three down to one. Trials were repeated in spring 2005 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.

Fall 2007 drench trials in TN again focused on examining some promising insecticides and application /plant handling methods for drench treatment. Multiple chemicals, application frequencies, and plant handling methods (rotating vs. non-rotating) were investigated.

MATERIALS AND METHODS:

In October 2007 TSU-NRC and USDA-ARS personnel completed drench applications on B&B plants with 24-inch diameter root balls at the TSU-NRC in Warren Co., TN. Drench treatments consisted of one of four chemical solutions or a water only control. In order to focus on the effect of application variation, the variety of chemicals applied was reduced to three 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 for 6NF regimen** (lb a.i./ 100 gal H_2O)	Handling		
			1F1	2F2	6NF
Lorsban 4E 0.125	Chlorpyrifos	2.000*	X	X	X
Talstar 7.9%	Bifenthrin	0.230	X	X	
OnyxPro 23%	Bifenthrin	0.115	X	X	
OnyxPro 23%	Bifenthrin	0.230	X	X	
Control	----	----			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.
** Rate modified to insure same amount a.i. per root ball (based on 6NF regimen) regardless of drench regimen.

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 (Figure 2). The plant handling methods are described below. **1F1:** one drench in the morning; then in the afternoon, flip the tree and drench the other side of the ball. This method requires minimum chemical and days of application for drench treatments. **2F2**: one drench in the morning and in the afternoon on one side of the root ball. The next day, flip the tree and drench two more times (morning and afternoon) for the other side of the root ball. **6NF**: this is the conventional and currently approved drench method included in the trial for chlorpyrifos only as a standard comparison. This method requires applying drenches twice a day for 3 consecutive days without flipping the root balls. The water control also followed no-flips (6NF) treatment application method.

Fig.1. TN personnel applied drench treatment to B&B trees

Fig.2. Root balls were rotated (flipped) once during the entire drench treatment applications

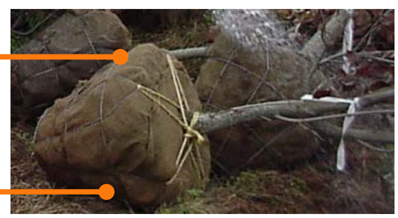


Fig.3. Soil core sample collection sites.

Each root ball received approximately 0.67 gallons of drench solution at each drenching totaling 1.35 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.; a rate based on the amount of a.i. applied to a root ball during the 6NF regimen.

After final treatment, the plants were maintained outside to weather naturally. Five replicate root balls were selected out of the 8 plants in each treatment group at 0.5, 1, 2, 4, and 6 months after final treatment for soil core sample collection. Two locations corresponding to top (up-facing side of the root ball) and bottom (the lateral side the plant rested on the ground at the first drench application), were sampled on each plant to explore evenness of coverage (Fig. 3). Soil samples were collected from within the first four inches of soil depth for testing against red IFA. The samples for testing against red IFA were frozen and sent to the CPHST Lab in Gulfport, MS where they were utilized in female alates bioassays. Each soil sample was tested with ten female alates of red IFA (Figs 4 & 5).

Figure 4. A tray of alates mortality bioassay cups.

Figure 5. Orange circles indicate the locations of clusters of female alates within this bioassay cup.

<u>RESULTS AND DISCUSSION</u>:

Soil samples have not yet been collected for 6 month sampling period at the time this report was written; therefore results are only through 4 months. The treatment application method 1F1 showed promise because for all treatments tested, regardless chemicals, rates, and most importantly top and bottom sampling sites, 100% in mortality were achieved. It was not surprising that both of the chemicals investigated in this study, chlorpyrifos and bifenthrin, at the tested concentrations performed well but the good results associated with the treatment application method 1F1 was the goal of this investigation (Figures 6, 9, & 10). However, the application method 2F2 surprisingly did not do as well as 1F1 in solution coverage. Chlorpyrifos 4E 2.0 (bottom sample) and bifenthrin 0.115 (top sample) applied using 2F2 method were both less than 100% mortality at month two (Figures 7 & 10). The reason for this is unknown and is difficult to explain because 2F2 should be at least as good as 1F1 in application coverage. Observation during treatment application in TN clearly showed that the second application penetrated better than the first drench application in the same treatment and that chemical solution should be able to reach into the root balls reasonably well. However, the concentration in drench solution used in 2F2 was only half of that used in 1F1 (even though the total dosage of the test chemical the root balls received in these two treatments was the same); this may have some effects on the efficacy although it is not clearly known how this would impact. Since the 4 month results were 100% for all treatments of the 2F2 application method, the slightly inconsistence in 2 month result may not caused only by treatment application.

Soil sample collected from bottom of root balls treated with chlorpyrifos 4E 2.0 using application method 6NF gave a mortality of only 40-60%, again showing that 6NF is not the most effective application method (Figure 8). Bioassay results from bifenthrin treated root balls were fairly consistent with only one soil sample giving less than 100% mortality at month 2 (Figure 7).

Figure 6. IFA control achieved with various chemicals in treated soil samples collected at two surface sites from the application 1F1 regime at 0.5, 1, 2, and 4 months after final drench application.

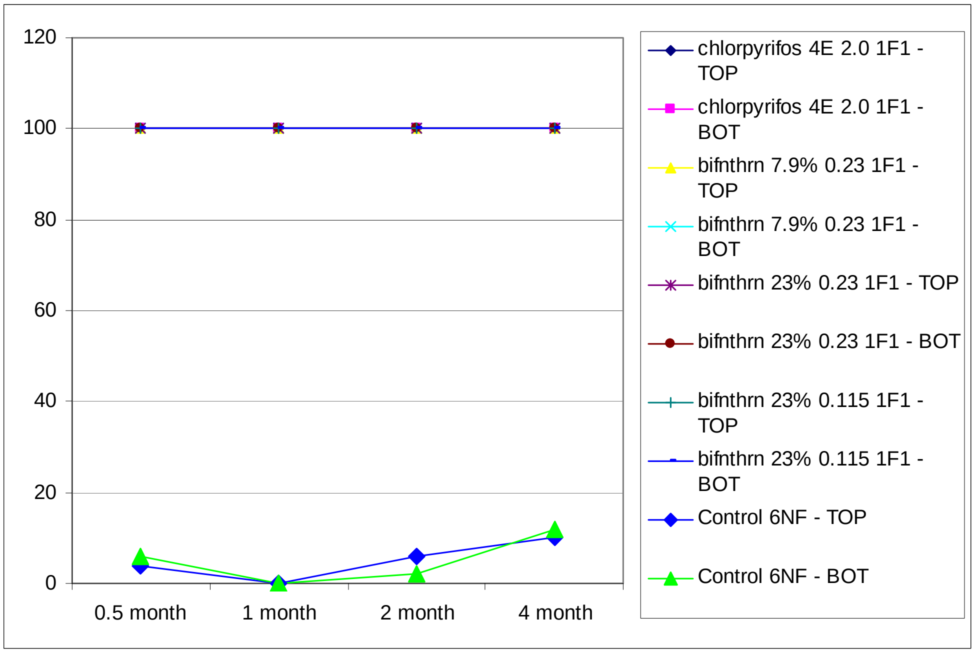


Figure 7. IFA control achieved with various chemicals in treated soil samples collected at two surface sites from the application 2F2 regime at 0.5, 1, 2, and 4 months after final drench application.

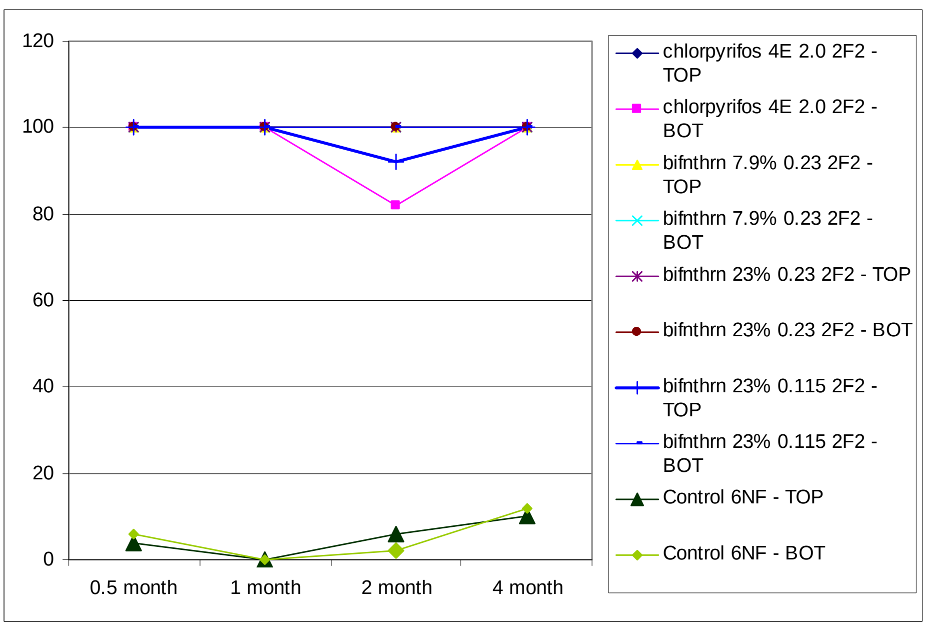


Figure 8. IFA control achieved with chlorpyrifos-treated soil samples collected at two surface sites from 1F1 and 2F2 application regimes at 0.5, 1, 2, and 4 months after final drench application.

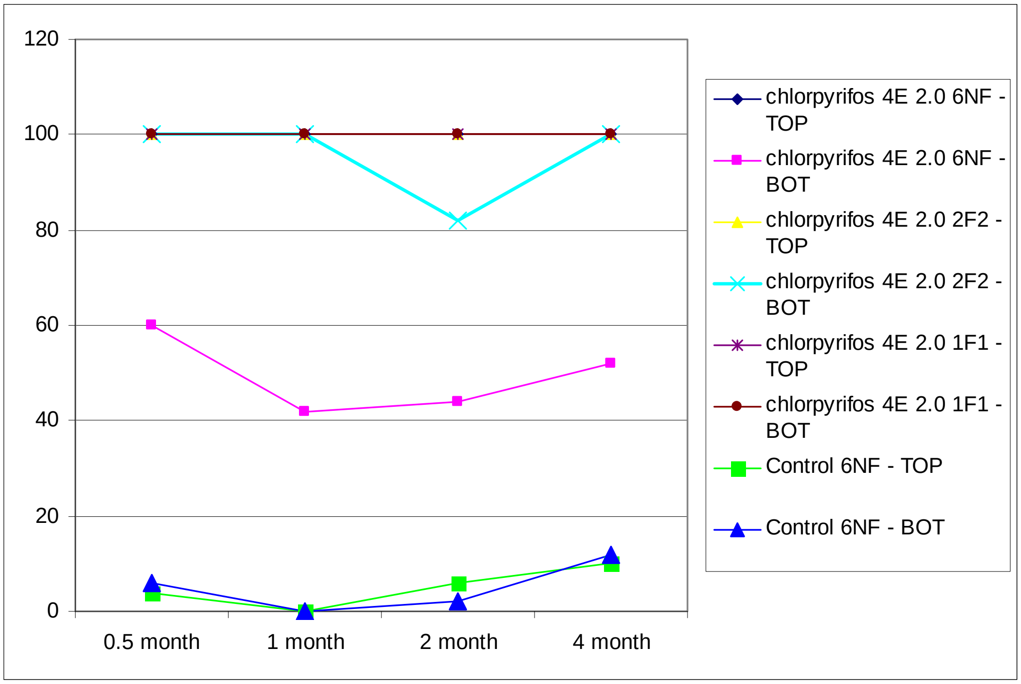


Figure 9. IFA control achieved with bifenthrin flowable (0.23 lb ai/100 gal) treated soil samples collected at two surface sites from 1F1 and 2F2 application regimes at 0.5, 1, 2, and 4 months after final drench application.

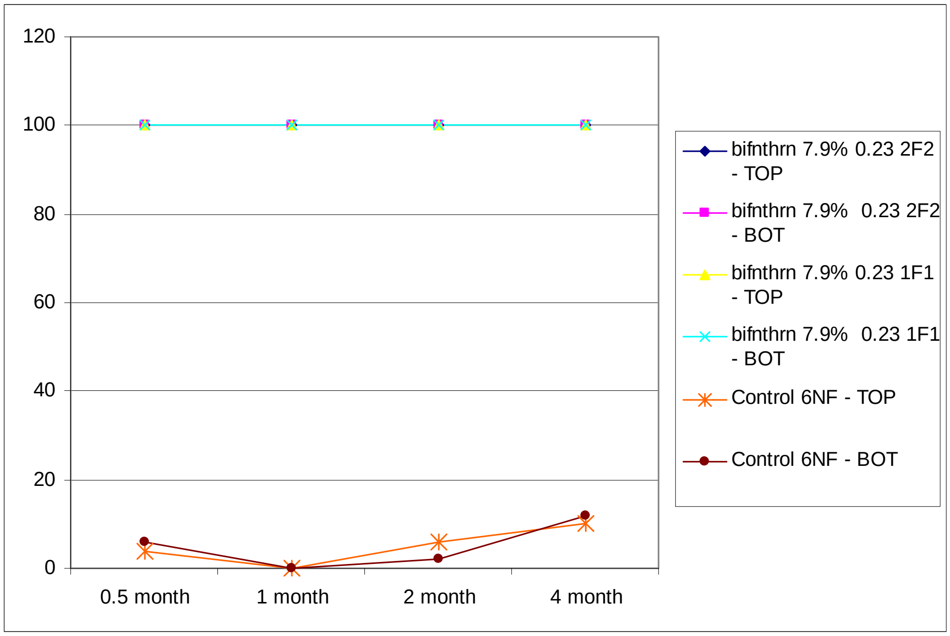
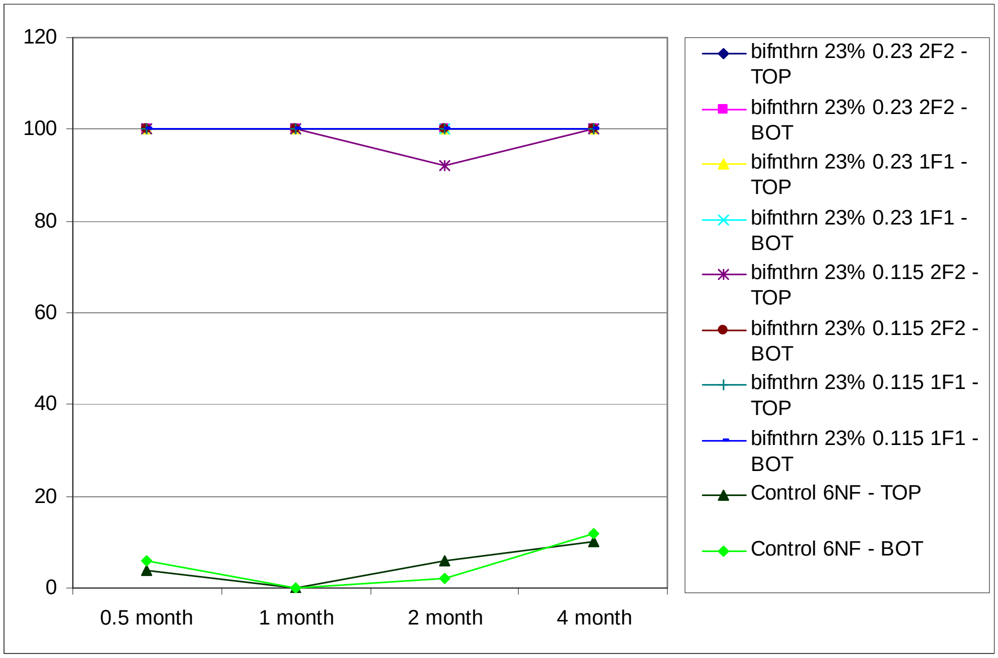


Figure10. IFA control achieved with two rates of bifenthrin EC treated soil samples collected at two surface sites from 1F1 and 2F2 application regimes at 0.5, 1, 2, and 4 months after final drench application.



Since it became obvious from TN drench trial that water-repelling burlap caused run-off problems affecting the penetration of chemical solution into the root balls, we included a procedure of pre-wetting the root balls with diluted surfactant solution to reduce run-off problem for better penetration when we did drench trial in CPHST Lab in Gulfport MS (see MS drench report 2007). We followed TN's "1F1" treatment method with a slight modification: pre-wet the root balls with surfactant (or dish-washing liquid) followed by the "1F1" application method.

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: A1F04

PROJECT TITLE: Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Gulfport MS in Fall 2007

REPORT TYPE: Interim

LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Lee McAnally and Craig Hinton

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 ensure 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 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. 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 however, gave consistent results, thus indicating insufficiency in either application or the mode of testing for the treatments applied through drench. Drench trials conducted in fall 2003 and spring 2004 determined that doubling the volume of solution applied failed to eliminate inconsistent results.

Until fall of 2004, drenching was done without rotating the root balls and B&B normally rests on one side of the root ball throughout the three-day drench process. This possibly restricts treatment coverage on the resting side of the ball, while giving the surface of direct application a higher concentration of chemical and deeper penetration. The 2004 fall drench strongly suggested that rotating root balls during treatment, regardless of application frequency, improved the consistency of bioassay results and could potentially cut the number of days spent applying drenches from three down to one. Trials were repeated in spring 2005 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. Fall 2007 trials in TN continued examining the following treatment/plant handling methods for drench application.

The drench trial conducted in Gulfport Lab was meant to complement those conducted in TN in fall 2007 where multiple chemicals, application frequencies, and plant handling methods (rotating vs. non-rotating) were investigated. The TN trial was initiated first and while applying insecticidal drenches in TN it became obvious that the water-repelling burlap caused run-off problems affecting the penetration of chemical solution into the root balls. Therefore, in the MS trial we included a procedure of pre-wetting the root balls with diluted surfactant to reduce run-off problem for better penetration of the drench solution. We followed TN's "1F1" treatment method (one drench in the morning, then in the afternoon flip the root ball and drench again) with a slight modification: pre-wet the root balls with surfactant (or dish-washing liquid) followed by the "1F1" application method. We called this "1F1 plus".

MATERIALS AND METHODS:

In October 2007, we completed drench applications on B&B plants with 25-inch diameter root balls at the facility of CPHST Lab, Gulfport MS. Drench treatments consisted of two concentrations for each of the two formulations of bifenthrin (Onyx Pro 23% EC and Bifenthrin Pro 7.9% Flowable) and a water only control. Since we considered this trial a supplemental to the similar trial conducted in TN, we used only one handling method "1F1plus": apply one drench in the morning, let dry for a couple of hours and then flip the root balls and drench one more time in the afternoon. The word "plus" here takes a meaning that we would use surfactant solution (or dish washing liquid at the rate of 1 tsp per gallon of water) to wet the entire root ball before any drench with chemical solution so that the burlap will not cause run-off while drenching. However, because of continuous rain in the days prior to the trial, the root balls in the experiment were already completely wet and extra pre-wetting would not be helpful, therefore the pre-wet process was skipped in this trial. But since the completely wet burlap surface did not cause run-off any more, the results generated from this trial are still considered no different from having root balls pre-wetted for application method discussion purposes. Solutions, final application rates, and handling which composed the treatments are listed in the table below.

Table 1. Treatment list for 1F1 drench trial in Gulfport, Mississippi Fall 2007

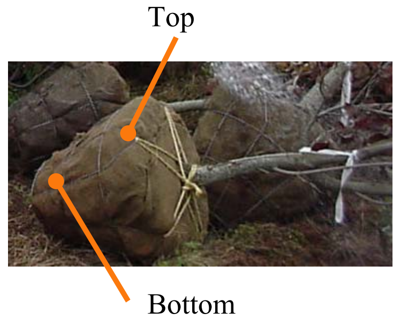
Material	#	Rate #ai/ 100 gal	Rate ml prod./gal	Water vol./drench	Amount of Insecticide per drench	Total Amount Applied to 4 root balls
Bifenthrin Pro 7.9%	1	0.115	6.53	4 gal	26.12 ml	52.24 ml
	2	0.23	13.05	4 gal	52.24 ml	104.48 ml
Onyx Pro 23%	3	0.115	2.1	4 gal	8.4 ml	16.8 ml
	4	0.23	4.3	4 gal	17.2 ml	34.4 ml
Control	5			4 gal		

Balls were rotated once between the two chemical drenches.

Four root balls were used in each treatment. Water volume per drench was determined by measuring the root ball volume (7 gal) and taking 1/5 of the volume (1.4 gal) to be used for each ball. This volume turned out to be too much for the already wet root balls to absorb, so it was reduced to 1 gallon per root ball which was sufficient to reach the point of run-off. Insecticidal solutions were mixed in a 60-gal trailer sprayer (Fimco Inc.) with a pistol grip handgun with adjustable nozzle (Figure 1). Chemical solutions were applied once in the morning and again in the afternoon and between these applications, the root balls were rotated or flipped to expose a different side to the direct application. Each root ball received approximately one gallon of drench solution at each drenching.

Figure 1. Drench application

Figure 2. Soil core sample collection sites.



After final treatment, the plants were maintained outside to weather naturally. Soil core samples were collection at 0.5, 1, 2, 4, and 6 months after final treatment for female alates bioassay. Two locations corresponding to top-as-planted (top), and bottom were sampled on each plant to examine evenness of coverage (Figure 2). Samples were collected from within the first four inches of soil core depth for testing against IFA. Each soil sample was tested with ten female IFA alates (Figures 3 & 4).

Figure 3. A tray of alate mortality bioassay cups.

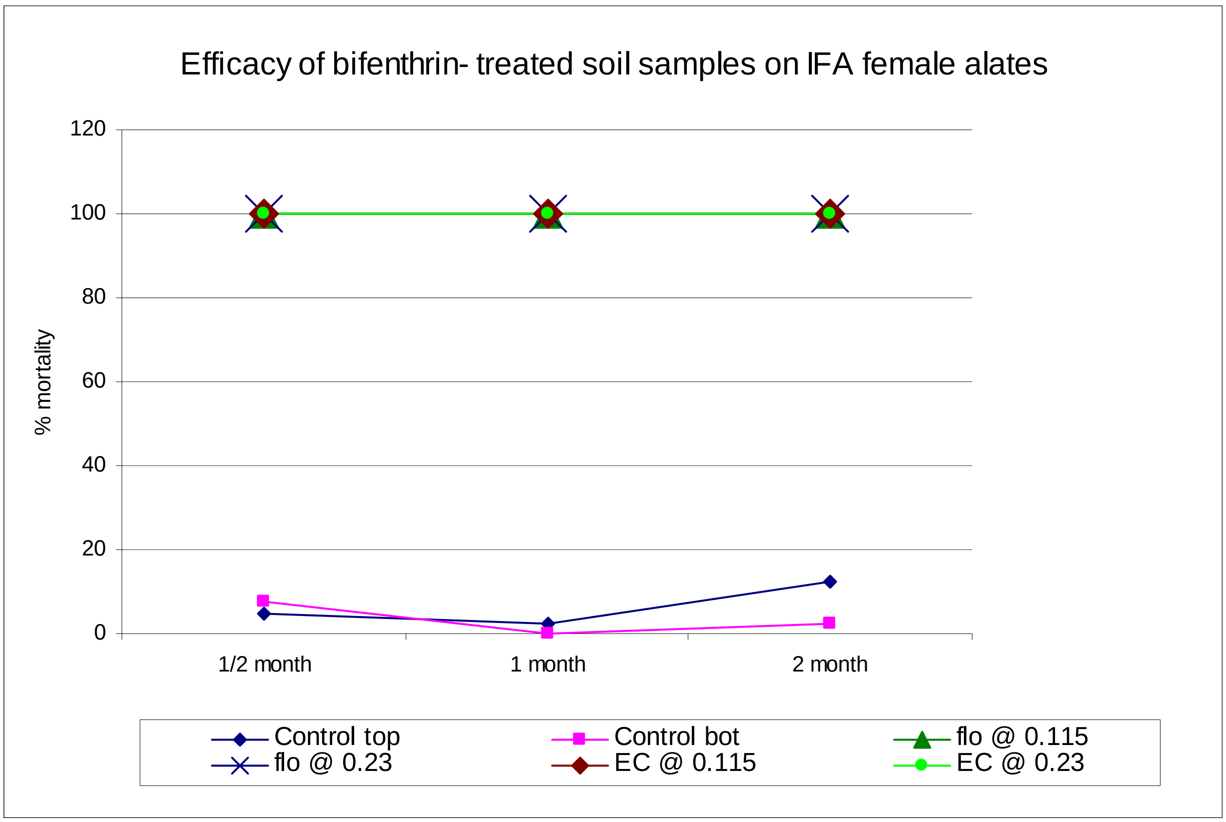
Figure 4. Orange circles indicate the locations of clusters of female alates within this bioassay cup.

<u>RESULTS AND DISCUSSION</u>:

The TN fall 2007 trial used does rates based on amount a.i. per root ball based on the 6NF drench regimen. This resulted in all root balls receiving the same amount a.i. regardless of number of drenches received. In the MS fall 2007 trial, since only the 1F1 drench regimen was conducted, we used a single dose rate based on the labeled rate for treating with the 1F1 regimen. Therefore, the dose rate per root ball in this trial is approximately 1/3 the rate of the corresponding TN fall 2007 1F1 treatment.

These are partial results of the trial because at the time this report was written only the bioassay results of ½ month, one month, and two months have been obtained. However, these partial results showed that bifenthrin treated root balls were consistently generating 100% mortality regardless of formulations, rates tested, and soil core sampling positions (Figures 5). Since there were no difference on efficacy between sampling positions, top and bottom soil core bioassay results were pooled together in this report. If later month results show differences between the two, they will be analyzed and displayed separately.

Figure 5. IFA control achieved in bifenthrin treated soil samples collected at two surface sites at various sampling intervals after final drench application. Plants rotated once between 2 drench applications in one day.



Preliminary results showed that the application method "1F1 plus" resulted in uniform coverage and good penetration of chemical into the root balls. Chemical and time requirements for this treatment method are at the minimum of all drench treatments investigated. Run-off should also be at the minimum after the surfactant pre-wet. With both sides of the root ball being well drenched and chemical solution penetrating into the root ball, we should expect the results of this treatment method not to be much different from the 2F2 method but having the benefit of shortening one day and reduction of run-off problems.

CPHST PIC NO: A1F04

PROJECT TITLE: Comparison of Several Application Techniques for Treating Balled-and Burlapped Nursery Stock (Drip, Drench and Dip), 2006

REPORT TYPE: Final

LEADER/PARTICIPANTS: Tahir Rashid (Univ. of Tennessee) and Shannon James (both resigned so report written by Anne-Marie Callcott based on Rashid and James notes and data)

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 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). Many growers have asked for the option of treating root balls through a drip irrigation system. While most current nursery use insecticide labels do not allow treating through irrigation systems, the efficacy of this application technique has not been investigated for use on IFA.

MATERIALS AND METHODS:

Some details regarding this trial may be sketchy due to the departure of the key personnel involved in the trial. Root balls were treated in Tennessee in spring 2006. Four application techniques were used in this trial and four root balls were treated with each technique and each insecticide rate.

1. Drip application: each treatment solution was prepared in a 50-gal utility tank. A ½ hp Shur-Dri Multipurpose AC Pump was connected to the drain hole of each tank. Insecticide solutions (water in the case of the control) were applied at 2 L/hr drip rate for 25.5 hours. In the water only drip study each root ball in the 2 L/hr drip rate was saturated with an average of 13.5 gal of water.
2. Dip application: each root ball was dipped into a 200 gal container of insecticide solution until bubbling ceased – ca. 3 minutes.
3. Drench application: each treatment was applied with a watering can to a root ball with 2.6L insecticide solution twice a day for 3 consecutive days (4.08 gal total solution applied). (AMC note: I do not believe the root balls were rotated between drenches)
4. Drench with surfactant added application: The Farm World surfactant was added to each insecticide solution at the rate of 8 oz/100 gal. Drench applications applied as above. (AMC note: I do not believe the root balls were rotated between drenches)

Insecticides used and rates of applications are as follows:

Chlorpyrifos 1/2X	0.0625 lb ai/100 gal H_2O
Chlorpyrifos 1X	0.125 lb ai/100 gal H_2O (standard quarantine rate for dip and drench)
Bifenthrin 1/2X	0.0575 lb ai/100 gal H_2O
Bifenthrin 1X	0.115 lb ai/100 gal H_2O
Water control	

Soil samples were taken from the inner core (middle) and the upper side (top) of each root ball at 1, 2 and 4 months after treatment and shipped to the PPQ, CPHST Gulfport laboratory for live insect bioassays. Bioassays were conducted on a modified version of the standard alate female bioassay. Each root ball replicate sample was divided into four sub-samples (bioassay replicate). Two sub-samples were used for an alate female bioassay and two were used in a worker bioassay. Ten alate females were used per alate female bioassay replicate and 20 IFA workers were used per worker bioassay replicate. IFA were confined to the treated soil sample for 14 days, with mortality determined at 7 and 14 days. Fourteen day data is shown here.

RESULTS:

The dip application technique provided the best control of IFA, both workers and queens, regardless of insecticide used and of where on/in the root ball the soil sample was collected from (Figures 1-4). Both chlorpyrifos rates of application provided 1 month of 100% control when the root ball was dipped (consistent with current quarantine treatment), with decreasing efficacy on the top/upper soil samples at 2 months after treatment and decreased efficacy in all samples at 4 months. The bifenthrin 1X application rate provided 100% control through 4 months, while the 1/2X rate of application was effective for 2 months, with decreases in efficacy of the middle/inner samples at 4 months.

The drip application technique was very inconsistent in this trial. At 1 month after treatment, both chemicals at both rates of application showed low efficacy against IFA alates in the soil samples collected from the top/upper part of the root ball. The high rate of bifenthrin was 100% effective at 1 month against workers in both soil samples and against alates in the middle/inner soil samples. At future dates of evaluation, efficacy dropped first in the top/upper samples and then in all soil samples. The 1/2X rate of bifenthrin provided 80 and 100% control of alates and workers, respectively, in the middle/inner samples, but was less effective in the top/upper samples at 1 month, with erratic results throughout the study. Both dripped chlorpyrifos rates of application were erratic throughout the study, but also showed the trend of the middle/inner samples being more effective against IFA than the top/upper soil samples.

No statistics have been done on this data, but trends indicate that the addition of a surfactant does not appear to have a consistent beneficial or adverse affect on insecticide efficacy when applied as a drench treatment. Drench treatments are known to be somewhat inconsistent, and this trial is no different.

DISCUSSION:

The primary purpose of this trial was to investigate the efficacy of applying insecticides to post-harvest root balls through a drip irrigation system. The drip application technique was ineffective using the chemicals and rates of application noted here and in most cases provided inadequate protection against invasion by newly mated queens, with most of the insecticide apparently moving to the middle of the root ball with little insecticide being retained in the top surface. The dip application technique continues to prove to be the most efficacious and most consistent treatment option for control of IFA in harvested balled-and-burlapped nursery stock. Additional drench trials rotating the root balls during insecticide application are underway to improve the efficacy and consistency of this application technique since for many growers it is a viable treatment option.

Figure 1. Efficacy of Drip vs. Dip vs. Drench Application Techniques for Control of IFA on Balled-and-Burlapped Nursery Stock using Chlorpyrifos Applied at the 1X rate (0.125 lb ai/100 gal H_2O). Mid=middle soil sample against alate females, mid-worker=middle soil sample against workers, top=top soil sample against alate females, top-worker=top soil sample against workers.

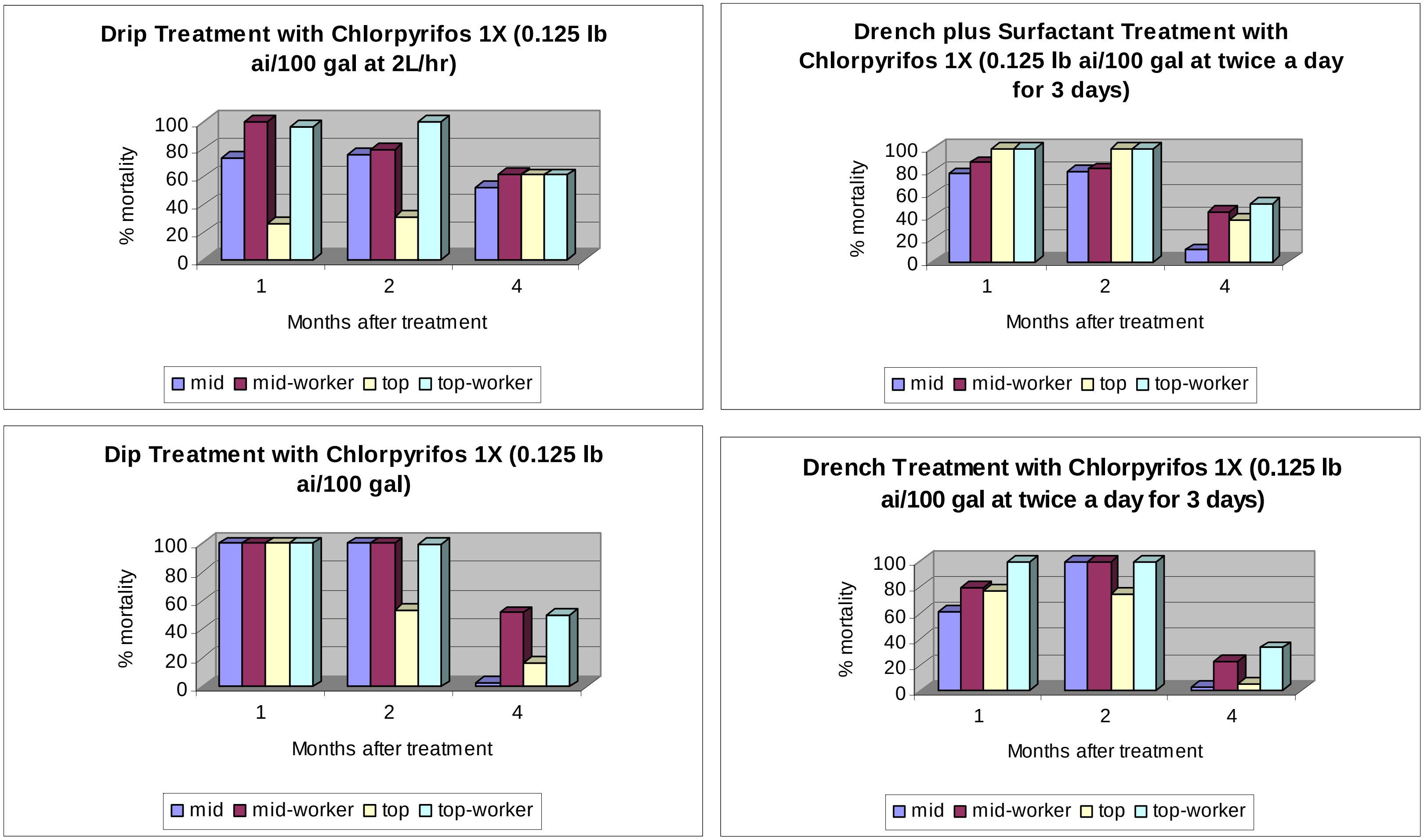


Figure 2. Efficacy of Drip vs. Dip vs. Drench Application Techniques for Control of IFA on Balled-and-Burlapped Nursery Stock using Chlorpyrifos Applied at the 1/2X rate (0.0625 lb ai/100 gal H_2O). Mid=middle soil sample against alate females, mid-worker=middle soil sample against workers, top=top soil sample against alate females, top-worker=top soil sample against workers.

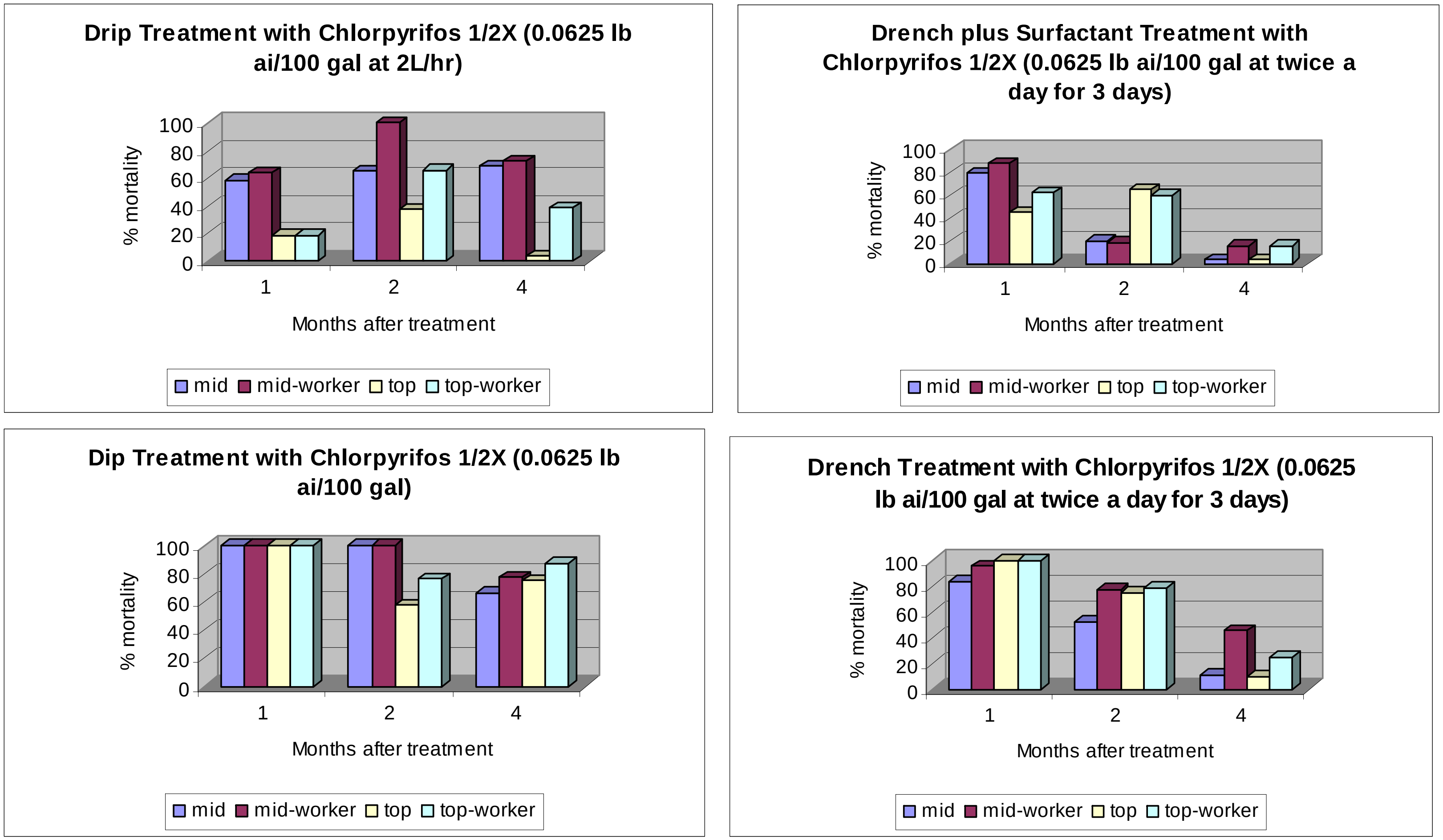


Figure 3. Efficacy of Drip vs. Dip vs. Drench Application Techniques for Control of IFA on Balled-and-Burlapped Nursery Stock using Bifenthrin Applied at the 1X rate (0.115 lb ai/100 gal H_2O). Mid=middle soil sample against alate females, mid-worker=middle soil sample against workers, top=top soil sample against alate females, top-worker=top soil sample against workers.

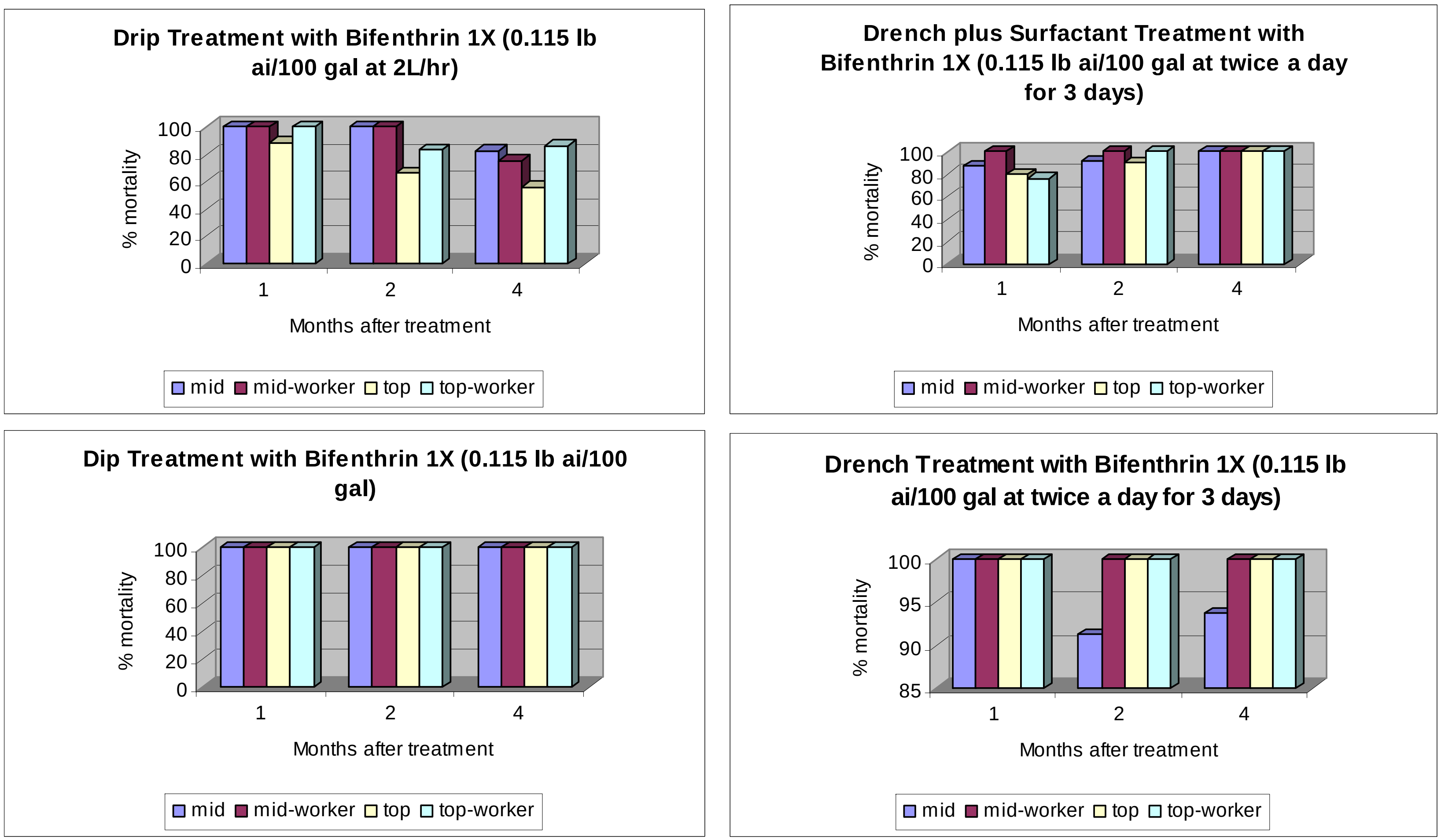
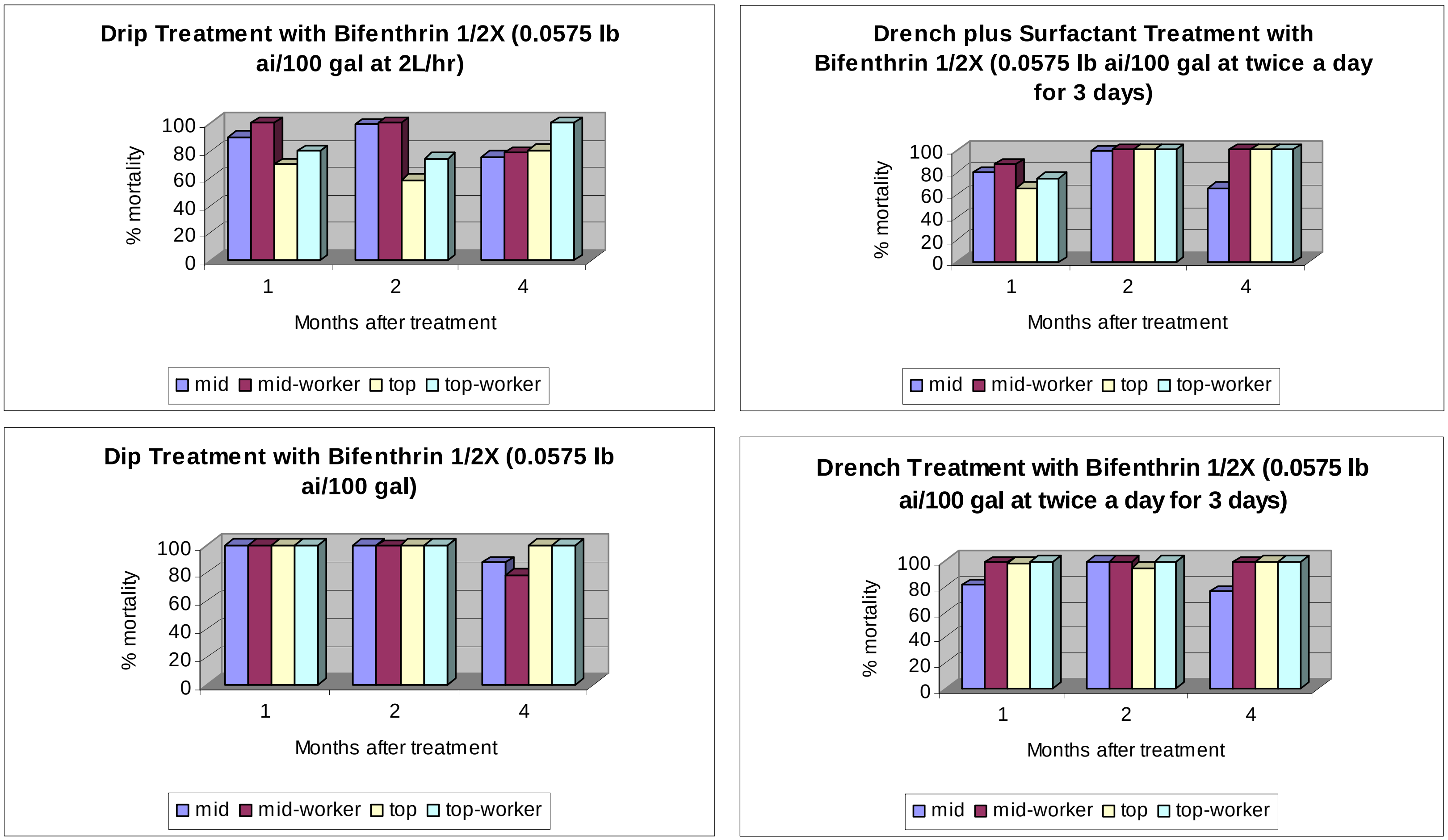


Figure 4. Efficacy of Drip vs. Dip vs. Drench Application Techniques for Control of IFA on Balled-and-Burlapped Nursery Stock using Bifenthrin Applied at the 1/2X rate (0.0575 lb ai/100 gal H_2O). Mid=middle soil sample against alate females, mid-worker=middle soil sample against workers, top=top soil sample against alate females, top-worker=top soil sample against workers.



CPHST PIC NO: A1F04

PROJECT TITLE: Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Using Bifenthrin-Treated Burlap to Wrap Ant-free Root Balls of Nursery Stock for Prevention from Newly-Mated IFA Queens Infestation

REPORT TYPE: Interim

LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Lee McAnally, Craig Hinton

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 not only inefficient but also come with environmental and human health problems. Thus additional treatment methods, as well as additional approved insecticides, are needed to ensure IFA-free movement of this commodity.

The primary objective of a quarantine treatment for field grown nursery stock is to render the plants fire ant free. The currently available pre-harvest (in-field) treatment requires a broadcast 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 method of tree-ring chemogation plus treated burlap has advantages over other methods that are approved for use by nursery industry or that are currently under investigation such as post-harvest dip, drench, and pre-harvest (in-field) band application of contact insecticides following approved bait broadcasting. Tree-ring chemogation may penetrate the entire root ball with chemical solution to achieve results that are similar to the effects of dip treatment but do not require the use of heavy equipment and do not have the problem of disposal of large volume of harmful chemical waste. Compared to post-harvest drench, the tree-ring method requires minimum labor and chemical costs and with little or no run-off problems. Also, this method only selectively treats the trees to be harvested thus avoiding the unnecessary treatment to the entire field. This method could be as effective in killing fire ants as other treatment methods mentioned above.

Study also showed that some surfactants alone can effectively kill fire ants without the addition of toxic insecticides if used in proper concentrations and volume (unpublished data, Jian Chen et al.). In the mean time, the use of surfactants in the chemogation treatment, alone or together with insecticide, may also facilitate penetration of treatment solution to the entire root ball with reduced run-off problem.

Bifenthrin-treated burlap wrapped over the chemogation-treated root balls may kill newly-mated fire ant queens that land on the root balls through contact. One concern was that queens might burrow through coarser burlap too quickly so that they would not have enough time in contact with the bifenthrin-treated burlap to obtain a lethal dose. To be sure they would be killed through contact, an extra layer of materials (such as biodegradable erosion control blanket, another layer of burlap, or any other materials that are inexpensive and suitable for the job) can be included beneath the treated burlap.

The objective of this study was to develop an IFA quarantine treatment method for field grown B&B nursery stock that is effective, easy to do, economical, environmentally friendly, and endanger neither nursery workers nor trees during treatment application.

MATERIALS AND METHODS:

This developing quarantine treatment method for field grown B&B nursery stock is based on the following assumptions:

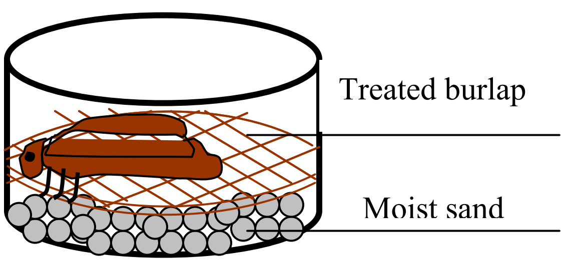
1. Tree-ring chemogation can kill all fire ants residing in the root ball area.

Trials on tree-ring chemogation will be conducted in March 2008, but it is expected that 100% kill will be achieved without much complications through selection of suitable chemicals (insecticides and/or surfactants), use of proper concentration of the selected chemical(s), and adequate volume of solutions.

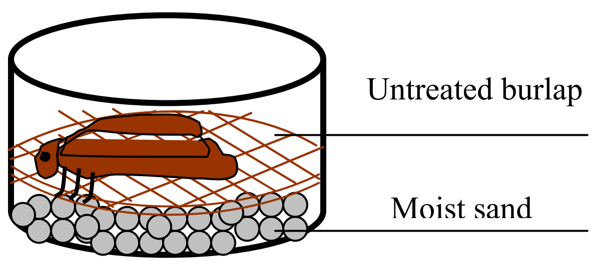
2. Bifenthrin-treated burlap will kill queens landing on and attempting to penetrate the burlap wrapped root balls.

The second assumption has not been well studied before thus it has been the focus of this investigation. Laboratory and outdoor experiments are being conducted for this study.
The basic experimental design for the lab study is illustrated bellow. Female alates are placed on bifenthrin-treated burlap that was placed on top of moist sand (see illustrations). If alates choose to enter into the sand, they have to pass through the treated burlap barrier. This experiment has been designed to answer the following questions: Can alates burrow through treated-burlap? Will bifenthrin kill alates before or after they enter the moist sand? Can they penetrate the treated burlap, enter into the sand, and live without been killed? A layer of biodegradable erosion control blanket can also be used in conjunction with burlap for extra barrier and for protection. The treatments in this investigation are listed in Table 1.

Illustrations for laboratory contact trial:



Treated



Control

Table 1. List of Treatment Combinations and Design Purpose of Each Treatment

Treatment Group	Trt #	Description	Purpose of the Treatment
Untreated Control	1	Burlap (7.5 oz) untreated	See if one layer of untreated burlap (7.5 oz) can prevent entry of fire ant queens into the root balls
	2	Burlap (10.0 oz) untreated	See if one layer of untreated burlap (10.0 oz) can prevent entry of fire ant queens into the root balls
Treated burlap alone	3	Burlap (7.5 oz)	See if one layer of treated burlap can prevent entry of fire ant queens and kill them
	4	Burlap (10.0 oz)	See if one layer of treated burlap can prevent entry of fire ant queens and kill them
Treated burlap + untreated coco blanket	5	Treated burlap (7.5 oz) +…	Extra layer of material to block the entry of ants into root balls thus increase the contact with treated burlap
	6	Treated burlap (10.0 oz) +…	Extra layer of material to block the entry of ants into root balls thus increase the contact with treated burlap
Untreated burlap + treated coco blanket	7	Untreated burlap (7.5 oz) +…	Treated coco blanket for killing the ants and untreated burlap for wrapping to reduce worker's handling
	8	Untreated burlap (10.0 oz) +…	Treated coco blanket for killing the ants and untreated burlap for wrapping to reduce worker's handling
Untreated burlap + treated burlap (10 oz)	9	Untreated burlap (7.5 oz) +…	Two layers of burlap, outside layer is untreated to reduce worker's handling
	10	Untreated burlap (10.0 oz) +…	Two layers of tightly woven burlap for extra protection; outside layer is untreated to reduce worker's handling

Treatment arena description: A plastic drawer organizer (6" x 9" x 2") (Rubbermaid product bought from Wal-Mart) was used as the main apparatus. At the bottom of the organizer, four drainage holes were drilled. Then, a piece of plain burlap (10 oz weight) cut slightly smaller than the bottom of the organizer was used to cover the drainage holes at the bottom to prevent sand from leaking out. Moistened play sand was added to the organizer to fill a 2.5 cm deep layer of sand in the bottom of the plastic box. A layer of wrapping material (burlap alone or burlap plus a layer of coconut blanket or another burlap layer, according to the different treatment combination) was placed over the moist sand. On top of the wrapping material, another plastic organizer of the same kind (with the bottom completely cut out) was pushed against the wrapping material until two organizers snapped tightly together to form an experiment arena (see Figs 1 & 2). The inside wall of the top plastic organizer was coated with diluted Fluon to prevent ant escape.

Treated burlap: Plain burlap of two different weights (7.5 oz and 10 oz per sq. yard, 10 oz weight burlap is tighter woven than 7.5oz one) in the size of 20"x20" (comes as basket liners—a folded rectangular piece of burlap sewn on one side to form a cone shape to fit root ball) was purchased from A.M. Leonard, Piqua, OH. In a metal bucket of 12" high x 36" in diameter, twelve gallons of water and 52 ml of bifenthrin 23% EC (OnyxPro™, FMC Corporation) were added to mix into a solution with bifenthrin at the rate of 0.23 lb ai/100 gal of water. Twenty-four burlap liners (12 in 10 oz and 12 in 7.5 oz) were immerged completely in the solution overnight. After 24 hours of soaking in the solution, burlap liners were taken out to dry. Burlap was ready for use or storage when dried.

Bioassay procedure: Ten field collected female alates were placed on the wrapping material inside the box of the experiment arena. Five replicates were used for each treatment with a total of 50 alates per treatment. Female alates could contact the wrapping freely but could not crawl out of the plastic box because of the Fluon coating on the inner wall. Queens were not given food or water except that the sand was made moist prior to adding to the plastic boxes. After 48 hours of exposure to the treated material, mortality data of the alates were taken. Notes were also taken to record where the alates were when the evaluations were made (in/on the wrapping material or in/on the sand).

To investigate the effects of aging of treated burlap on the efficacy of bifenthrin, experiment units were taken outdoors for aging after each bioassay. Experiment units were placed on top of a weed-blocker on the ground which served the purpose of preventing debris from getting into the units. Irrigation schedule was set up to simulate nursery conditions with daily irrigation of 1 cm over a 30 min irrigation period. Bioassays were conducted once every two weeks to monitor the aging process. Experiment units were always stored outdoors except during bioassay dates when they were brought inside for a 48 hours experiment (see Figs 3 & 4). At the time of this report, the 12th week bioassay was just completed. Bioassay will continue until less than 50% mortality achieved by the bifenthrin-treated burlap within the 48 hrs exposure periods.

Fig.1. One treatment unit setup: a layer of bifenthrin - treated burlap secured in between two plastic boxes with the top box's bottom cut out and wall coated with Fluon.

Fig.2. Similar treatment unit showing an extra layer of coconut blanket attached under the burlap layer. Only one layer of the wrapping materials was treated with bifenthrin when more than one layer was used in the treatment.

Fig.3. Lab bioassay investigating the efficacy of bifenthrin- treated burlap on fire ant female alates.

Fig.4. Treatment units with bifenthrin-treated wrapping materials aging outdoor for exposure to sunlight, rain, and daily irrigation.

Chemical analysis of the bifenthrin in water solution and in treated burlap: GC-MS analytical procedures were used to analyze bifenthrin in samples of water solutions and samples of bifenthrin-treated burlap. These analyses were conducted by GC-MS group of CPHST Lab in Gulfport, Mississippi. Chemists Lisa Mosser, Bill Guyton, and Richard King contributed substantially to the bifenthrin analysis. Detail analytical methods for these analyses can be obtained from the chemists.

RESULTS:

The chemistry in treating burlap: Chemical analyses were conducted on samples of bifenthrin solutions taken after each use of soaking burlap overnight and on different batches of treated burlap that came out of these treatments. Bifenthrin in the dipping solution dropped sharply after the first use of soaking burlap overnight (Fig 5.). Majority of the bifenthrin in the solution was absorbed by the burlap material causing the concentration to drop from originally near 900 ppm to a low level of less than 90 ppm. After additional two repeated uses, bifenthrin in the solution was virtually exhausted. The analysis of bifenthrin in treated burlap confirmed the above findings (Fig.6). Bifenthrin in the first batch of treated burlap is 5 times higher than that in the second batch which was soaked overnight in the same solution after the first batch of burlap was removed.

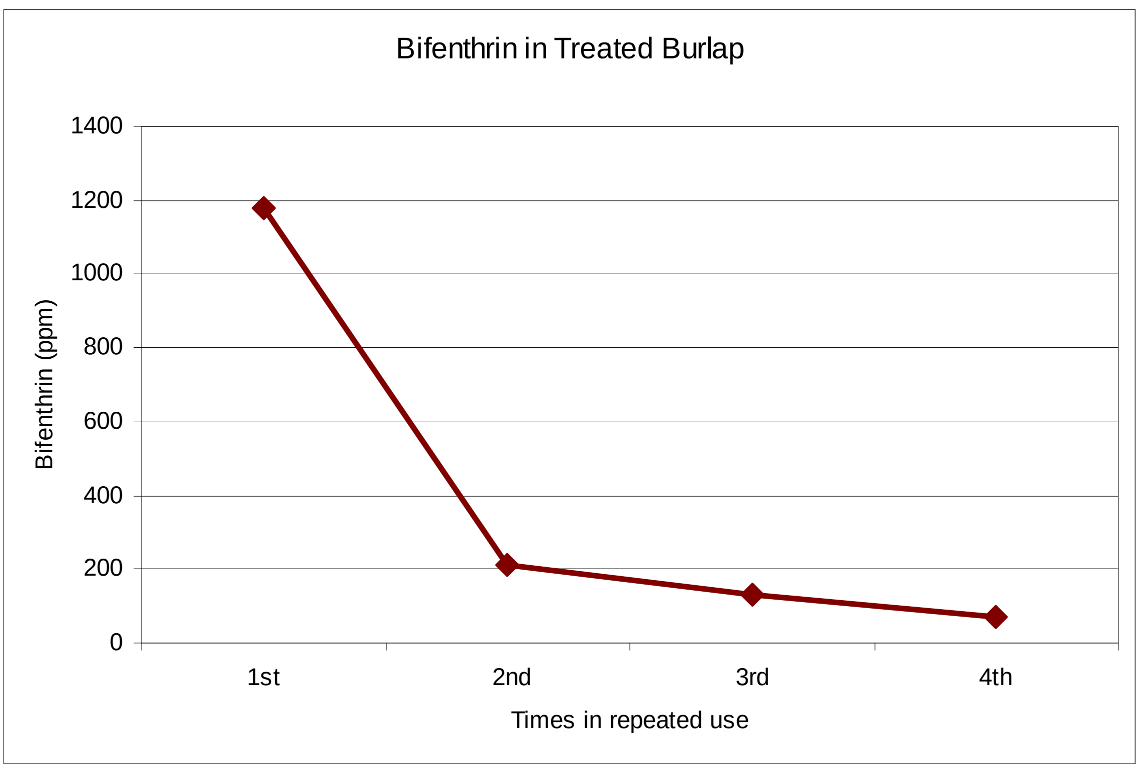


Fig. 5. Bifenthrin in solution after each use of repeatedly soaking burlap overnight in the same solution.

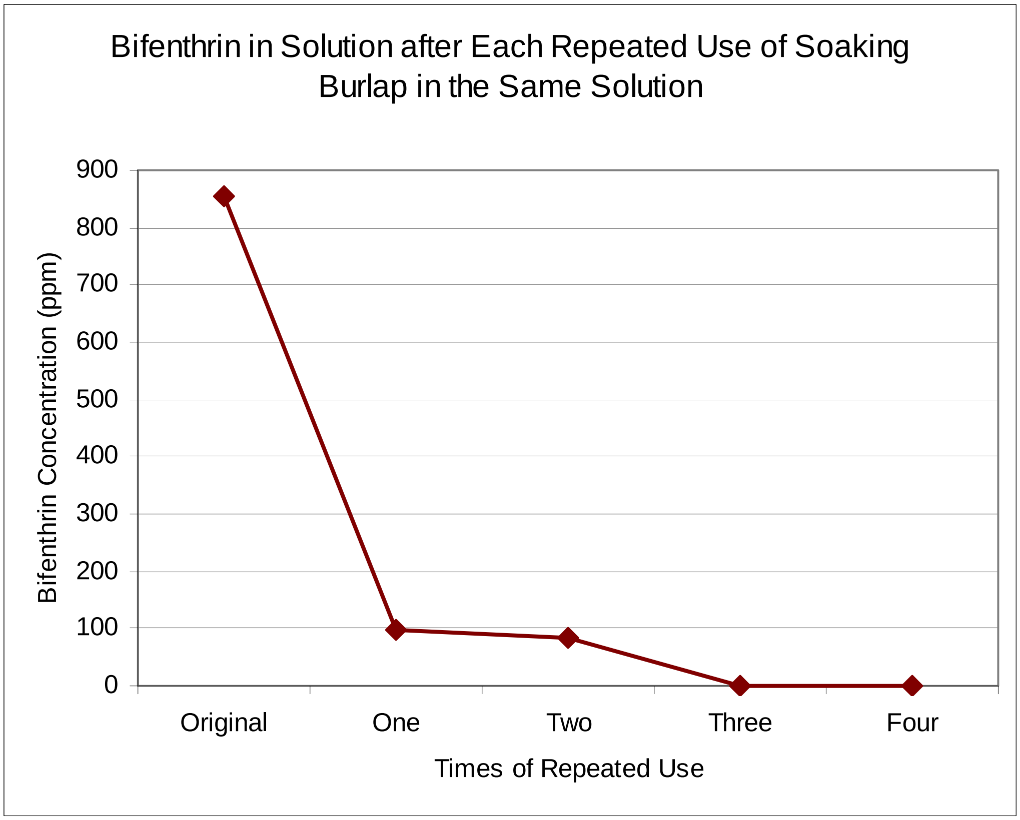


Fig. 6. Change of bifenthrin levels among various batches of treated burlap which repeatedly used the same bifenthrin solution overtime.

Analytical results indicated that bifenthrin level in the treated burlap of different batches changed markedly (Fig.6). Therefore, if repeated use of the same solution is desired, additional bifenthrin product must be added to the solution in order to obtain similar concentration of bifenthrin in the treated burlap among various batches of treatment. Obviously, the same solution could be used repeatedly for treating burlap only if extra bifenthrin product (EC or Flowable formulation) is added to the solution to compensate the marked drop in bifenthrin level in the solution.

Bioassay using fire ant female alates: Freshly treated burlap was extremely lethal to fire ant female alates. Alates showed symptoms of intoxication within a few minutes of contact with the bifenthrin-treated wrapping materials. It took slightly longer for them to show symptoms and to die if the treated material was in the second layer (especially when the top layer was the untreated 10.0 oz burlap).

All treatments achieved 100% mortality (Figs 7 & 8) in 48 hours and mortality usually occurred within 30 minutes of coming in contact with bifenthrin-treated wrapping materials. Aged bifenthrin-treated burlap was also toxic to the alates. Our bioassay with aged burlap has been ongoing for three and half months when this report was written and all treatments still achieved 100% mortality with no sign of losing potency soon. As a matter of fact, for those treatments that placed the treated materials in the second layer, alates showed symptoms of intoxication sooner in aged bioassays than in the first bioassay. This is probably because the untreated top layer got contaminated by the treated layer underneath it during the aging process.

Chemical analysis for bifenthrin in aged burlap also has started and will be conducted every two weeks until the treated burlap loses its killing power. At the time we conclude our aging bioassay and the analytical study of the aged burlap, we will know better how much bifenthrin in the burlap is sufficient to begin with to give an adequate residual protection for the harvested root balls.

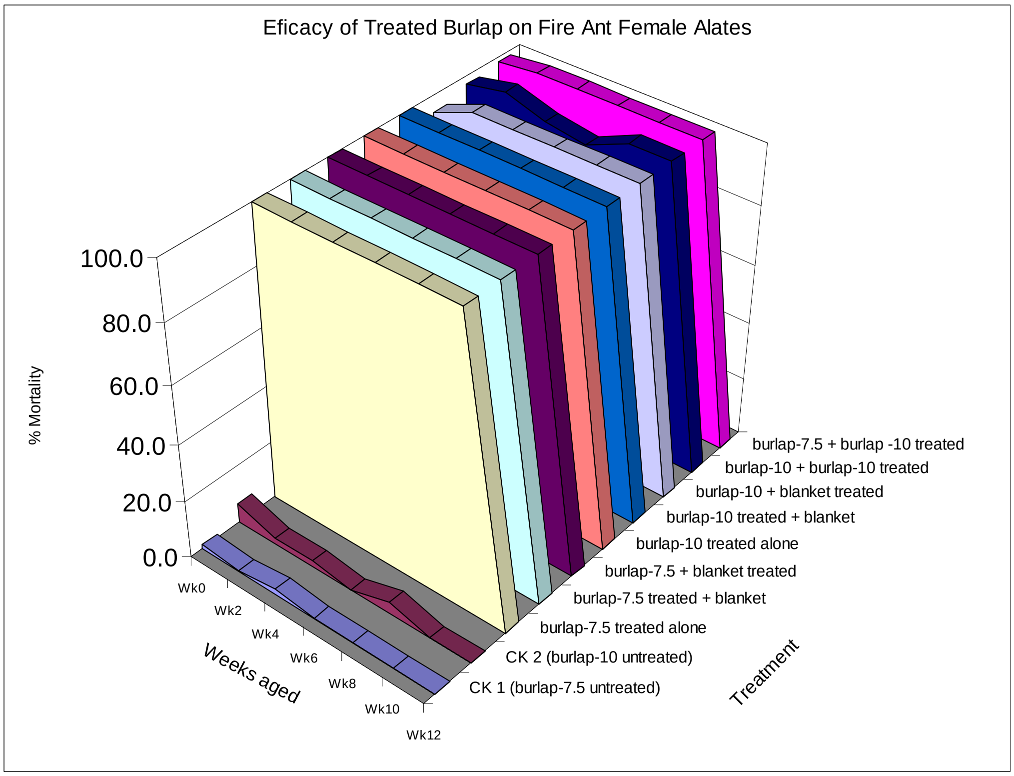


Fig.7. Results of 12 weeks of female alate bioassay with aged treated burlap

Fig. 8. Fire ant alates died after they came in contact with the treated burlap

DISCUSSION:

This developing treatment method consists of two parts that will both fit well in the production: 1) use 10 gal. sized tree-rings (commercially available for slowly watering the ground near trees to irrigate newly planted trees or to facilitate the digging of ready-to-harvest trees) to chemogate the root zone area of the trees before harvesting to kill all fire ants in the root-ball mass and at the same time to moisturize the ground near trees for easy digging. 2) Use bifenthrin-treated burlap to wrap the root balls during harvesting to perform an added function of preventing newly mated fire ant queens from infesting the root balls while stored and during transportation. To enhance the effect of killing fire ant queens through contact with treated burlap, a layer of biodegradable erosion control blanket may be placed between the root ball and the burlap layer either to block the entry of queens physically or to increase their contact with treated burlap. This layer of blanket will also give an extra protection of the root balls in the event of burlap tearing while handling.

Treating burlap involves mixing bifenthrin solution, soaking the burlap overnight in the solution, and at the end disposing the leftover solution. Questions related to this process were: Could the bifenthrin solution be used repeatedly until it is used up so that there is no waste solution to dispose off? Would the concentrations of bifenthrin in the solution change following each use? Were there any differences in the amount of bifenthrin in the treated burlap of different batches? Results of chemical analyses of bifenthrin solutions and bifenthrin-treated burlap clearly answered the above questions. The results could be an important guidance in treating burlap with bifenthrin.

The treated-burlap loses its killing power in a slower pace than expected. This is good for this developing treatment method because the original concern was that bifenthrin would be lost from the treated-burlap fast and may not last long enough for protecting the B&B nursery stock while awaiting shipment. After three and half months of aging and bioassay monitoring, all treatments are still potent enough to achieve 100% mortality.

This treatment method hopefully could be one that is effective, easy to do by growers, economical, environmentally safer, and endanger neither nursery workers nor trees during treatment application. Furthermore, this method fits well in the production process without adding much extra work to the nursery growers.

Investigations to be conducted next:

1. Tree-ring chemogation trial: tree-ring chemogation is an important part of this developing quarantine treatment method for field-grown B&B nursery stock. Study is needed to determine the chemicals (most likely bifenthrin also), concentrations and solution volume necessary to kill all ants in the root ball areas. Since the sole purpose of this treatment is to “clean out” all ants in the root ball areas immediate before harvesting, it is less important to determine the residual effect of the treatment (Fig. 9).
2. Alternative method for treating the burlap. Use of treated burlap to wrap root balls of nursery stock has advantages over other treatment methods but still has room for improvement and refinement to fit different growers’ needs. One way is to spray the

chemical solution directly onto the burlap wrap after harvesting. This is not to be confused with the post-harvest drench method because drenching depends on the chemical solution reaching to the center of the balls to kill all the ants inside. This alternative application method only needs to wet the wrapping surface. All it takes is a uniform coverage of bifenthrin solution on the surface of the root balls. By doing this, it eliminates the need for pre-treating, storing and handling the bifenthrin-treated burlap and therefore there will be no worker handling issue during harvesting. Plain burlap will be used for wrapping root balls and after harvesting, spray bifenthrin solution to thoroughly cover the surface of the root balls mainly the burlap layer.

3. Intact root ball bioassay: This method is an outdoor version of the lab bioassay on treated burlap. Using a real root ball to test it outdoor will be closer to reality. Also, the alternative burlap treatment method (spray on after harvesting) can be tested using intact root ball bioassay. A root ball wrapped with treated burlap will be placed in a container. No moist soil or food will be added in the container. Female alates will be placed the in the container and allowed to have a free contact (or non-contact) with the root ball (Fig.10).
4. Chemical analysis of aged burlap. Analysis will be conducted every two weeks to quantify the degradation of bifenthrin in burlap through aging. This result will provide better understanding to the mortality results obtained from aged burlap bioassay which has been continued for three and a half months. The quantification of bifenthrin degradation and bioassay result together will provide us the basis in determining how much bifenthrin to begin with to treat burlap.
5. Shelf-life determination on bifenthrin-treated burlap. Treated burlap will be stored in a room where temperature is not controlled to simulate storage conditions of most nursery growers. Bioassay will be conducted on the stored burlap using female alates periodically to determine the shelf life of the treated burlap.

Fig.9. Tree-ring chemogation is an important part of this developing quarantine treatment method for field-grown B&B nursery stock.

Fig.10. Intact root ball bioassay: This method is an outdoor version of the lab bioassay on treated burlap. Using a real root ball to test it outdoor will be closer to reality.

CONCLUSIONS:

1. Burlap tends to accumulate bifenthrin from the bifenthrin soaking solution and can absorb a concentration that is considerably higher than that in the soaking solution.
2. One use of soaking burlap in a bifenthrin solution could reduce bifenthrin level in the solution sharply. If repeated use of the same solution is desired, additional bifenthrin product must be added to the solution in order to obtain similar concentration of bifenthrin in the treated burlap among various batches of treatment.
3. Bifenthrin-treated burlap can kill fire ant alates for a period longer than 14 weeks through normal aging. Since this study is still undergoing, the maximum length is yet to be determined.

CPHST PIC NO: A1F04

PROJECT TITLE: Summary of Numerous In-Field Treatment Trials for Nursery Stock 2002-2007

REPORT TYPE: Status Report

LEADER/PARTICIPANTS: Anne-Marie Callcott

INTRODUCTION:

Since 2000, one of the primary focuses of this laboratory has been to find alternative treatments or insecticides for use as imported fire ant quarantine treatments for field grown nursery stock. Current treatments rely solely on the use of the insecticide chlorpyrifos. The infield treatment requires applying a broadcast treatment of a toxic fire ant bait followed in 3-5 days by a granular chlorpyrifos treatment. Alternatively, a post-harvest treatment of the balled-and-burlapped (B&B) stock requires a dip/immersion treatment with a chlorpyrifos solution, or a twice daily for 3 consecutive days drench/watering in treatment with a chlorpyrifos solution. Alternatives are critical to insure continued movement of field grown nursery stock to areas outside the federally regulated imported fire ant (IFA) areas.

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. Tests against individual mounds 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 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, as in the current approved in-field treatment.

There is some evidence of longer residual activity of the contact insecticides during the winter months vs. the spring/summer months. Literature indicates there may be more microbial activity/degradation as well as chemical degradation during the summer months of some insecticides; higher temperatures and moisture contributing to greater biotic and abiotic degradation (Baskaran et al. 1999; Getzin 1981; Tingle et al. 2000). However, the biology of the ant may also be a factor in this phenomenon. Chemical analysis of soil samples collected from treated areas in both spring and fall applications were initiated in 2007. This information is not reported in this summary but is available in the project report in the 2007 Annual Report.

While many people have worked on these projects, the primary leaders of all the trials summarized here are Shannon James (USDA-APHIS-PPQ-CPHST Gulfport resigned) and Jason Oliver (Tennessee State University, McMinnville, TN). Many of these trials were conducted in conjunction with testing of insecticides against Japanese beetle, another soil dwelling pests of regulatory concern to tree growers in Tennessee and other areas in the northern area of the fire ant quarantine area. Not all results or insecticides tested are presented here, but those which show promise or are of interest to the growing community are presented.

MATERIALS AND METHODS:

Specifics can be found in previous annual reports in the individual project trial reports. Trials conducted in the southern region of the IFA quarantined area were conducted in Mississippi (Hancock, Forrest and Oktibbeha Counties). In 2005-2007, trials were conducted in Tennessee in the northern edge of the IFA quarantined area. In Mississippi, all trials were conducted on airports due to the availability of large stretches of land with fire ants. Also, field grown nursery stock is not plentiful in southern Mississippi. While most field grown nursery stock would need to be treated in the fall for winter/early spring shipments, spring treatments may be needed in some areas and those treatments tend to provide us with "worst case" scenario data (more environmental and fire ant pressure in the spring/summer months). Trials in Tennessee were conducted on field production nurseries under a variety of management conditions (weed/grass management between rows) in the McMinnville area. Due to the limited availability of production areas with adequate numbers of IFA, we limited the tests in Tennessee to the most promising insecticide, bifenthrin.

In Mississippi, 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. In Tennessee, plots consisted of rows of nursery stock of varying lengths, but had a minimum of 5 active mounds within a 4-foot wide area with the tree row in the center. Bait applications were made on a determined date, followed in 3-7 days by a contact insecticide treatment. All contact insecticides were applied to a band 3-6 ft. on either side of the tree row (or center line). 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. 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. Treatments consisted of:

Chemical	Formulation	Rate of Application
bifenthrin	flowable	0.2 lb ai/acre
	EC	0.2 lb ai/acre
	granular	0.2 lb ai/acre
	granular	0.4 lb ai/acre
chlorpyrifos	granular	6 lb ai/acre
	granular	3 lb ai/acre
	granular	1 lb ai/acre
	EC	1 lb ai/acre
deltamethrin	granular	0.13 lb ai/acre
	SC	0.13 lb ai/acre
fipronil	granular	0.0125 lb ai/acre
lambda-cyhalothrin	SC	0.13 lb ai/acre
	SC	0.069 lb ai/acre
control	---	---

RESULTS:

Chlorpyrifos: Chlorpyrifos granular applied at 6 lb ai/acre is the current approved treatment (when used in conjunction with a bait application) for infield nursery stock. This treatment provides 12 weeks of certification after a 30 day (4 week) exposure period. Trials in Mississippi have included 1, 3 and 6 lb ai/acre rates of granular chlorpyrifos. Only one trial to date in the fall has been conducted with the 1 lb granular rate and is not included in the graphs below. Both the 6 and 3 lb rates provided 100% control for 16 weeks after the 4 week exposure period when applied in the fall months (Figure 1). Efficacy in the spring was a bit shorter, but still extremely good.

We also tested chlorpyrifos liquid at a rate of 1 lb ai/acre in Mississippi in both the spring and the fall. This rate also required an exposure period to achieve 100% mortality (Figure 2). This lower rate provided 12 weeks of control after a 4 week exposure period when applied in the fall, and 8 weeks of control after the exposure period when applied in the spring.

These results may allow us to decrease the rate of application of the chlorpyrifos component of the current IFA regulatory treatment for field grown nursery stock and allow a smaller area to be treated with the contact insecticide, but will require testing in Tennessee under actual production practices.

Figure 1. Efficacy of granular chlorpyrifos applied after a broadcast bait application at rates of 6 and 3 lb ai/acre to simulated field grown production area in Mississippi.

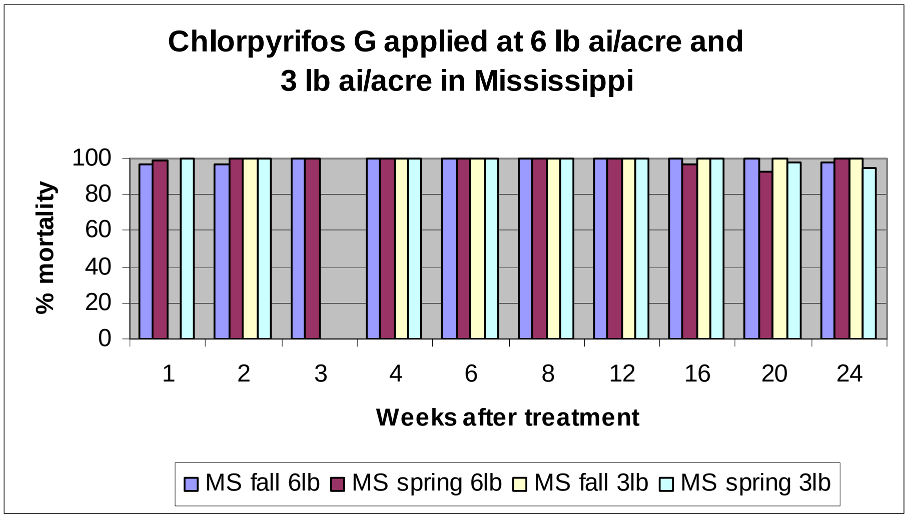
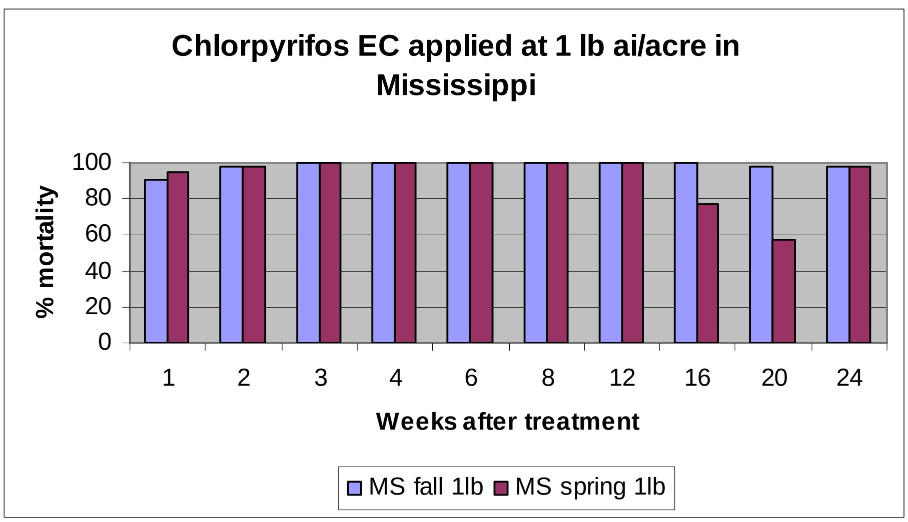


Figure 2. Efficacy of liquid chlorpyrifos applied after a broadcast bait application at 1 lb ai/acre to simulated field grown production area in Mississippi.



Bifenthrin: *Mississippi trials*: Bifenthrin was tested in Mississippi over several years with excellent results (Figures 3 & 4). Granular rate of application was at 0.4 lb ai/acre and flowable rate of application was at 0.2 lb ai/acre (labeled rates). Results indicate longer control when applied during the fall months compared to the spring months. Both rates and formulations after a 4 week exposure period provided excellent control for 16 weeks in the fall and 8 weeks in the spring. One colony in one liquid plot in the fall 2002 trial remained active throughout most of the test period resulting in the average of all the trials presented here not reaching 100% control. This colony was located next to (against) a small concrete structure supporting a wind sock with was located along the center line of the plot. This colony did become significantly less active over time, but it's survival stresses the importance of complete and accurate application of the contact insecticide in this in-field treatment.

Figure 3. Efficacy of granular bifenthrin applied after a broadcast bait application at 0.4 lb ai/acre to simulated field grown production area in Mississippi.

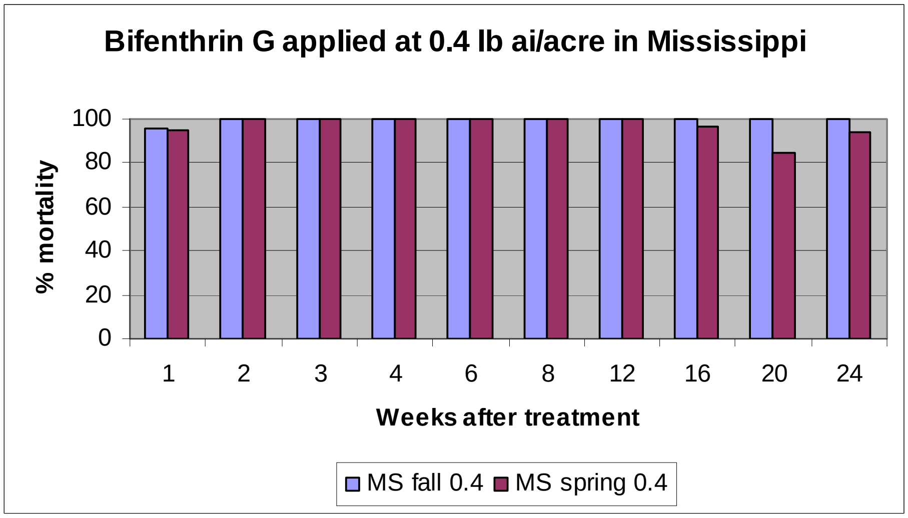
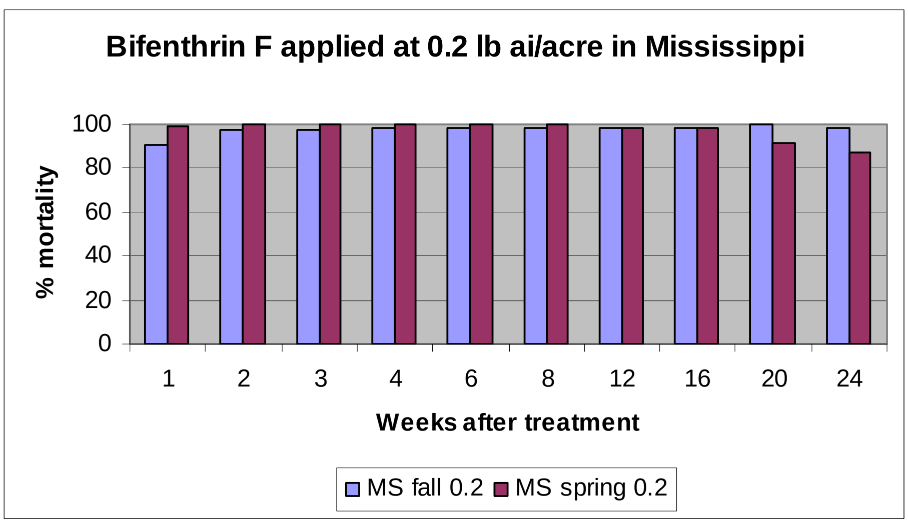


Figure 4. Efficacy of liquid bifenthrin applied after a broadcast bait application at 0.2 lb ai/acre to simulated field grown production area in Mississippi.



Tennessee trials: After successful trials in Mississippi (Figure 5), trials were initiated in Tennessee beginning in 2005 (Figure 6). As stated in the methods, these trials were conducted under actual field grown production systems and only conducted in the fall. After the first year of testing (2005) we were encouraged by the results which indicated the full 4 weeks was needed for exposure to eliminate IFA. However, subsequent years provided less and less control.

Due to our failures in Tennessee with bifenthrin, we will be investigating multiple applications and combination applications of contact insecticides in coming years.

Figure 5. Efficacy of liquid bifenthrin applied after a broadcast bait application at 0.2 lb ai/acre to simulated field grown production area in Mississippi.

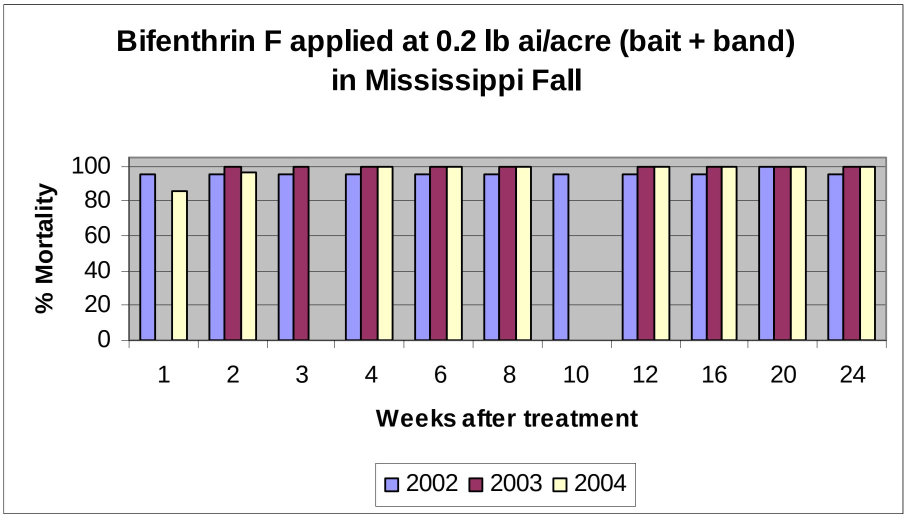
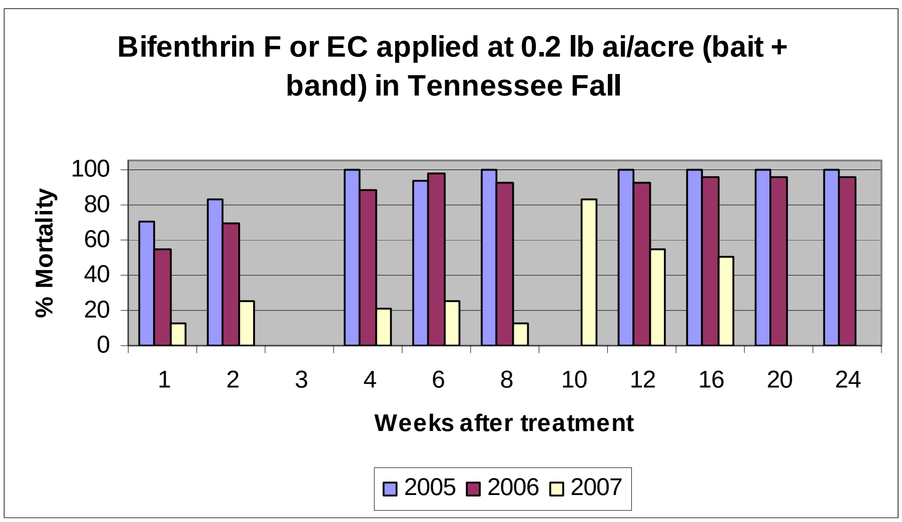


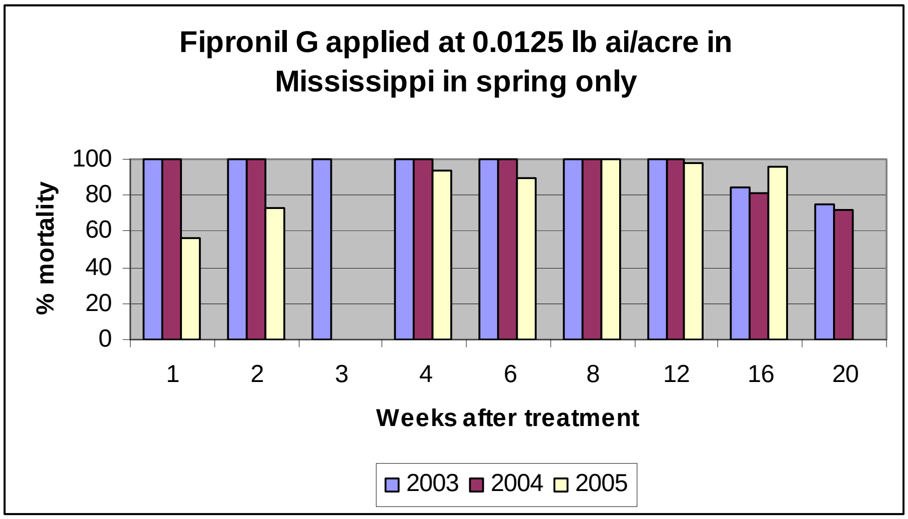
Figure 6. Efficacy of liquid bifenthrin applied after a broadcast bait application at 0.2 lb ai/acre to actual field grown production area in Tennessee.



Fipronil: Fipronil granular is currently approved for use in the IFA quarantine as a treatment for grass sod. The rate of application for grass sod is a total rate of 0.025 lb ai/acre. After a 30 day exposure period, the sod is certified for movement for 20 weeks. We anticipated the addition of a bait treatment would allow for a lower rate of fipronil to be used in the field grown use pattern. Fipronil was tested in 3 spring trials in Mississippi, with the first 2 trials providing excellent results (Figure 7). However, the 3rd trial gave different results, taking 8 weeks to reach 100%

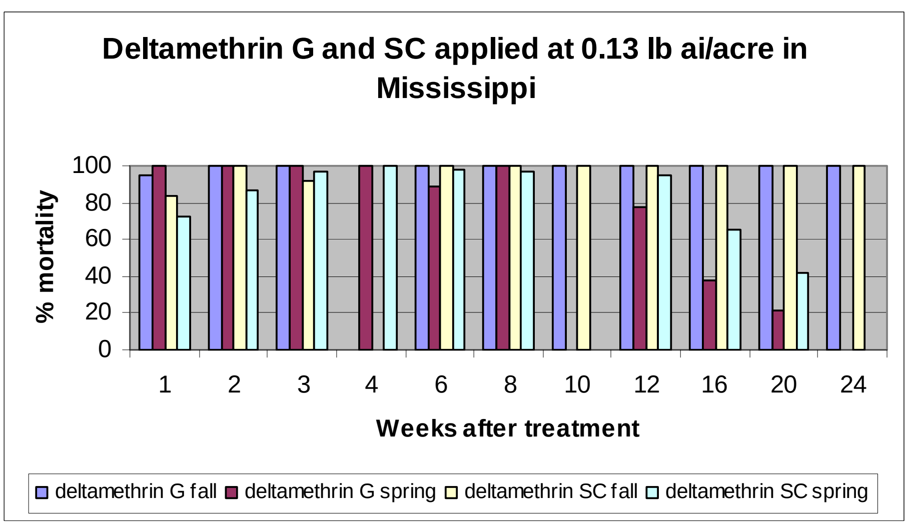
control and only maintaining that 100% control at that one evaluation date. However, when all the data is combined 96-100% control is maintained by fipronil from 4-12 weeks in these spring trials.

Figure 7. Efficacy of granular fipronil applied after a broadcast bait application at 0.0125 lb ai/acre to simulated field grown production area in Mississippi in the spring only.



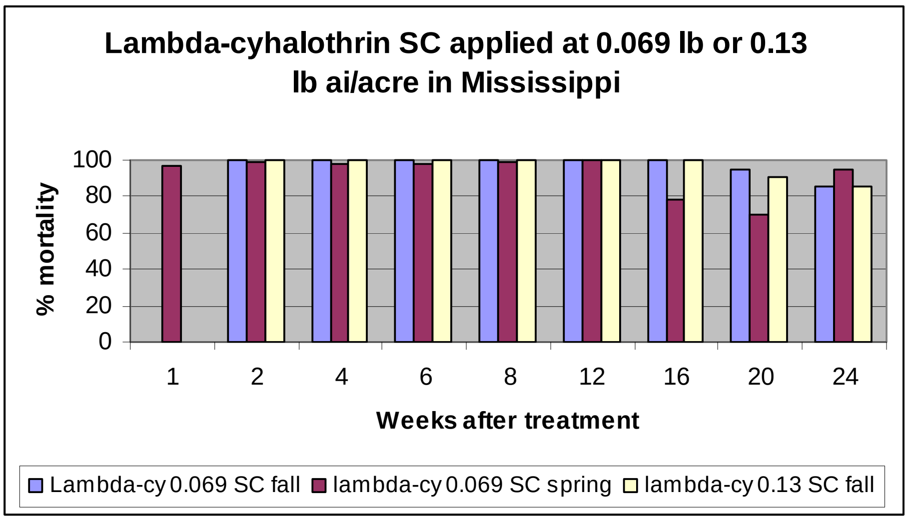
Deltamethrin: Deltamethrin, both granular and liquid applied at 0.13 lb ai/acre, has provided good control in this use pattern, but has not been quite as consistent as the previously mentioned insecticides at the rates under current testing (Figure 8). Only the SC spring application has been replicated at this time.

Figure 8. Efficacy of granular and liquid deltamethrin applied after a broadcast bait application at 0.13 lb ai/acre to simulated field grown production area in Mississippi.



Lambda-cyhalothrin: Lambda-cyhalothrin has provided good results in many use patterns against IFA including its use in combination with a bait treatment in field grown situations. Only the 0.069 lb ai/acre spring application has been replicated at this time; neither fall application rate has been replicated (Figure 9). The spring 0.069 lb rates provided a shorter residual than the fall treatment. Both rates in the fall application were effective within 2 weeks of application, and provided 14 weeks of excellent control. Additional fall tests in Mississippi to replicate these trials need to be conducted as well as trial under actual production conditions in Tennessee.

Figure 9. Efficacy of liquid lambda-cyhalothrin applied after a broadcast bait application at 0.069 and 0.13 lb ai/acre to simulated field grown production area in Mississippi.



DISCUSSION:

Many years have been devoted to finding a substitute for the contact insecticide portion of the IFA in-field regulatory treatment for field grown nursery stock. Decreases in use patterns for chlorpyrifos will continue until the insecticide is no longer available for turf and ornamental uses in the U.S. For many large scale growers the prospect of dipping or drenching all the post-harvest root balls is daunting and economically unfeasible. Our trials with chlorpyrifos indicate it is still an extremely effective treatment, and we may in the future be able to decrease the application rate by 50% or more.

Bifenthrin, which is currently used for containerized nursery stock in the IFA quarantine, was so successful in some areas of the quarantine, but when subjected to Tennessee climatic and environmental conditions as well as actual production sites, proved less effective than we had anticipated. However, we are still trying to determine whether our 2007 extensive failure can be explained. Unfortunately, this failure will not allow us to immediately pursue label changes and the addition of this insecticide to this use pattern in the IFA regulations.

Many other insecticides have been tested for this use pattern. To date our other most promising insecticides are fipronil, lambda-cyhalothrin and deltamethrin. However, testing has not been as extensive on these insecticides and additional work remains to be done to insure efficacy under more strenuous environmental conditions and in actual production systems.

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

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CPHST PIC NO: A1F04

PROJECT TITLE: Development of Alternative Quarantine Treatments for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, Spring and Fall 2006

REPORT TYPE: Final

LEADER/PARTICIPANTS: Shannon James, Lee McAnally, Anne-Marie Callcott, Jennifer Lamont, Shannon Wade, Bill Guyton (chemist)

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. 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. Tests against individual mounds 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 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 (see 2002-2005 IFA Annual Accomplishment Reports). The trials in this report continue to explore alternative insecticides and provide supporting data for those previously seen to perform well.

There is some evidence of longer residual activity of the contact insecticides during the winter months vs. the spring/summer months. Literature indicates there may be more microbial activity/degradation as well as chemical degradation during the summer months of some insecticides; higher temperatures and moisture contributing to greater biotic and abiotic degradation (Baskaran et al. 1999; Getzin 1981; Tingle et al. 2000). However, the biology of the ant may also be a factor in this phenomenon. Chemical analysis of soil samples collected from treated areas in both spring and fall applications were initiated in 2006. Analyses were conducted by the ANPCL-Chemistry Section.

MATERIALS AND METHODS:

Spring 2006 Band Trial:
The Bobby Chain Municipal airport in Hattiesburg, MS (Forrest Co.) was selected as the test location for this fall trial. 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

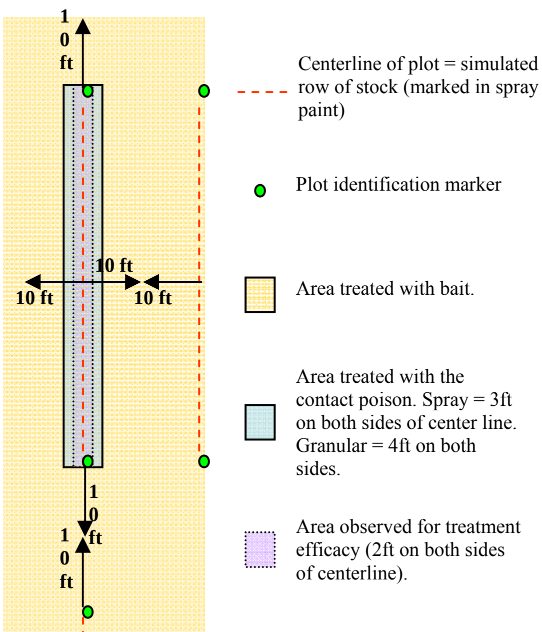


Figure 2. Application of contact insecticide to the plots of simulated stock

On May 12, 2006 hydramethylnon bait was applied at a rate of 1.5 lb/acre through the use of a shop built spreader mounted to a farm tractor. Control plots were not treated with bait. Contact insecticide application occurred on May 16-17, 2006. 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. There were 3 replicates per treatment. Many liquid chemical labels suggest the use of a surfactant or buffer, and in this trial, the adjuvant Indicate® 5 was used for the first time primarily to buffer the water to pH 5. To insure the adjuvant did not have an effect on IFA populations, a set of replicates of this treatment were included. Treatments consisted of the following.

Chemical	Formulation	Rate of Application
chlorpyrifos	granular 2.5%	6 lb ai/acre
chlorpyrifos	granular 2.5%	3 lb ai/acre
lambda-cyhalothrin	SC 9.7%	0.068 lb ai/acre
fipronil	granular 0.0143%	0.0125 lb ai/acre
Indicate 5	liquid	buffered to pH5
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. 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. Temperature was recorded during observation by use of air and soil thermometers.

Additional plots were treated to use for chemical analysis of contact insecticides. These plots, one for each treatment, were separate from those used for IFA evaluation and were treated only with the contact insecticide, not the bait. Five soil core samples were collected from each treated plot and composited for a single sample. Core samples were 2" diameter and 2" in depth. Samples were collected at 2, 4, 6, 8, and 12 weeks and every four weeks thereafter and submitted to the ANPCL-Chemistry Section for analysis. Spring treatments included:

Chemical	Formulation	Rate of Application
chlorpyrifos	granular 2.5%	6 lb ai/acre
chlorpyrifos	granular 2.5%	3 lb ai/acre
chlorpyrifos	liquid 44.8%	1 lb ai/acre
lambda-cyhalothrin	liquid 9.7%	0.069 lb ai/acre
fipronil	granular 0.0143%	0.0125 lb ai/acre
bifenthrin	granular 0.2%	0.4 lb ai/acre
bifenthrin	liquid 7.9%	0.2 lb ai/acre
deltamethrin	liquid 4.5%	0.128 lb ai/acre

Chemical analyses were conducted by the ANPCL-chemistry section. Procedure for the analytical method can be obtained by request.

Fall 2006 Band Trial:
Stennis Airport near Kiln, MS (Hancock 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. Treatments were as follows.

Chemical	Formulation	Rate of Application
bifenthrin	granular 0.2%	0.2 lb ai/acre
chlorpyrifos	granular 2.5%	3 lb ai/acre
chlorpyrifos	granular 2.5%	1 1b ai/acre
chlorpyrifos	liquid 44.8%	1 lb ai/acre
lambda-cyhalothrin	flowable 9.7%	0.069 lb ai/acre
lambda-cyhalothrin	flowable 9.7%	0.137 lb ai/acre
control	---	---

Hydramethylnon bait was applied to insecticide treatment plots on October 26, 2006 at a rate of 1.5 lb/acre through the use of a shop built spreader mounted to a farm tractor. Granular contact insecticide application was done on October 31, 2006. However, due to weather and construction at the airport, the liquid treatments were done on Nov. 13, 2006. The equipment utilized to apply the insecticides in the fall 2006 trial was the same as used in the spring.

Active IFA colonies in each plot's observation area were evaluated and recorded as previously described with observations occurring prior to bait application and at 1, 2, 4, 6, 8, and 12 weeks and every four weeks thereafter.

Additional plots were treated to use for chemical analysis of contact insecticides. These plots, one for each treatment, were separate from those used for IFA evaluation and were treated only with the contact insecticide, not the bait. Five soil core samples were collected from each treated plot and composited for a single sample. Core samples were 2" diameter and 2" in depth. Samples were collected at 2, 4, 6, 8, and 12 weeks and every four weeks thereafter and submitted to the ANPCL-Chemistry Section for analysis. Spring treatments included:

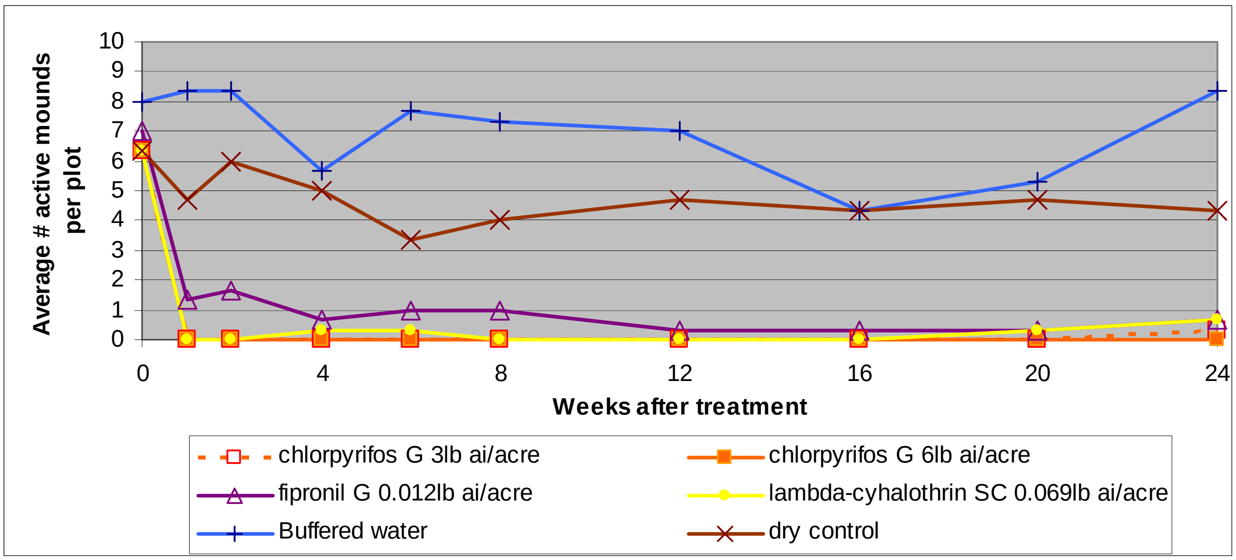
Chemical	Formulation	Rate of Application
chlorpyrifos	granular 2.5%	6 lb ai/acre
chlorpyrifos	granular 2.5%	3 lb ai/acre
chlorpyrifos	granular 2.5%	1 lb ai/acre
chlorpyrifos	liquid 44.8%	1 lb ai/acre
lambda-cyhalothrin	liquid 9.7%	0.13 lb ai/acre
lambda-cyhalothrin	liquid 9.7%	0.069 lb ai/acre
fipronil	granular 0.0143%	0.0125 lb ai/acre
bifenthrin	granular 0.2%	0.4 lb ai/acre
bifenthrin	granular 0.2%	0.2 lb ai/acre
bifenthrin	liquid 7.9%	0.2 lb ai/acre
deltamethrin	liquid 4.5%	0.128 lb ai/acre

Chemical analyses were conducted by the ANPCL-chemistry section. Procedure for the analytical method can be obtained by request.

RESULTS:

Spring 2006 Band Trial:
Both granular chlorpyrifos treatments achieved 100% mortality by the 1 week post-treatment evaluation (Figure 3). The 6 lb ai/acre rate maintained 100% efficacy through the 24 week evaluation period. The 3 lb ai/acre rate maintained 100% efficacy through 20 weeks. Lambda-cyhalothrin at the 0.069 lb ai/acre rate eliminated all colonies by the 1 week evaluation period, but one plot had one mound appear within the evaluation area at weeks 4 and 6, but disappeared again by week 8. Complete control was then maintained through the 16 week evaluation period. Fipronil granular provided 80% control at 1 week, reaching a maximum control of 95% at 12-20 weeks.

Figure 3. Spring 2006 trial – Mean colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide.



Chemical analyses were limited by the extremely low theoretical rates of application. Limits of detection for the analysis were approximately 0.13 ppm. In this initial test on the 2 week samples, the chemist attempted to quantify results for those results falling between the limit of quantification (LOQ = 0.4ppm) and the limit of detection (LOD = 0.13ppm). In subsequent analyses, the results between these limits were captured as below quantifiable limits (BQL = 0.13-0.4ppm). Theoretical initial rates of application (ppm) were determined, but this number is a true best guess, and admittedly not accurate, but it gave a ballpark range for the chemists to look for. Many assumptions were made including bulk density of top soil in the area, and use of a 2-inch acre for calculations. The fipronil initial theoretical was below the detection limit of the analysis and was not detected in any analyses.

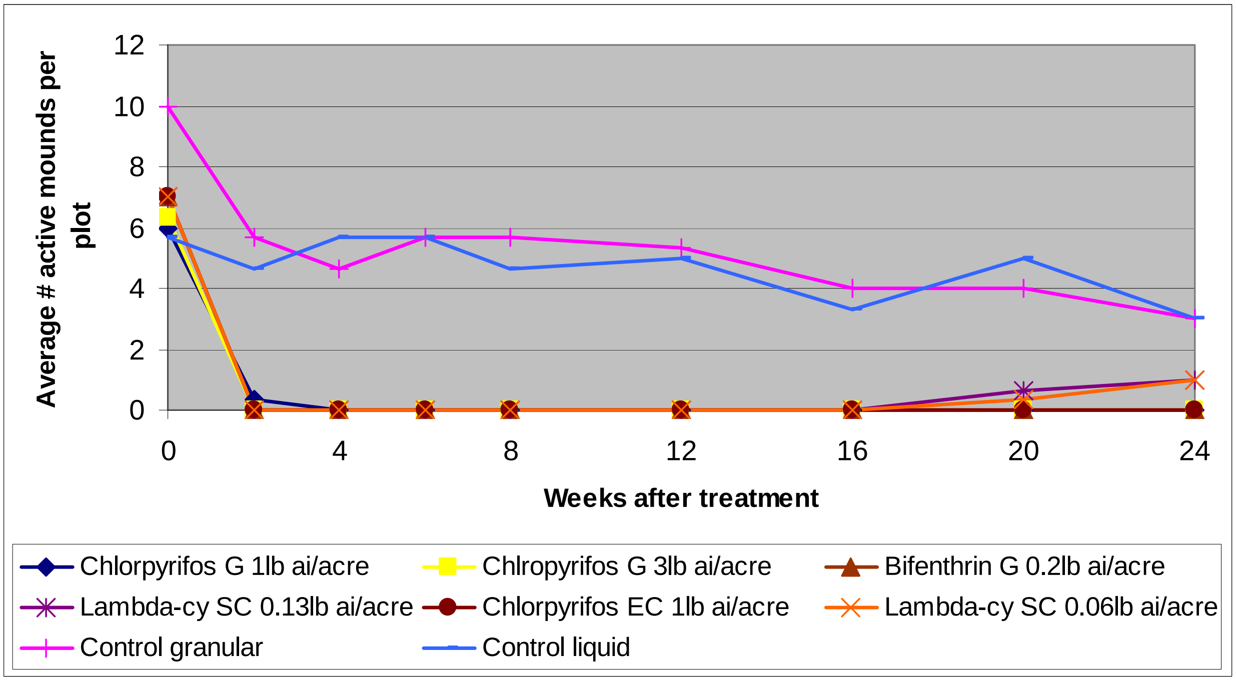
The results of the chemical analyses did detect chemical in all the 2 week samples except the fipronil sample (Table 1). Those chemicals that had multiple rates were detected in proportions similar to the application proportions (1x bifenthrin and 0.5x bifenthrin detected at 4.2 and 2.5 ppm, respectively). However, by 8 weeks all of the spring samples were not detected in the chemical analyses, indicating less than 0.13 ppm present in the samples.

Table 1. Results of chemical analyses of soil samples from spring 2007 band treatments (ND=not detected, or below limit of detection).

Treatment	Application Rate	Theoretical Initial ppm	ppm by chemical analysis at weeks PT				
			2	4	8	12	20
Chlorpyrifos G	6 lb ai/acre	14.66	4.27	0.31	ND	ND	ND
Chlorpyrifos G	3 lb ai/acre	7.33	2.59	BQL	ND	ND	ND
Chlorpyrifos EC	1 lb ai/acre	2.4	0.28	BQL	ND	ND	ND
Lambda-cyhalothrin SC	0.069 lb ai/acre	0.167	0.22	BQL	ND	ND	ND
Fipronil G	0.012 lb ai/acre	0.03	ND	ND	ND	ND	ND
Bifenthrin G	0.4 lb ai/acre	0.97	0.45	BQL	ND	ND	ND
Bifenthrin F	0.2 lb ai/acre	0.49	0.20	BQL	ND	ND	ND
Deltamethrin SC	0.128 lb ai/acre	0.31	0.13	BQL	ND	ND	ND

Fall 2006 Band Trial:
Due to weather and construction at the test site, 1 week evaluations were not conducted. As noted in the materials and methods section, liquid contact treatments were conducted 2 weeks after the granular treatments, therefore the control evaluations were adjusted accordingly. All treatments achieved 100% control of IFA in the evaluation areas by the 2 week evaluation except the chlorpyrifos granular 1 lb ai/acre rate (Figure 4). One plot in this low chlorpyrifos rate had one active mound at the 2 week evaluation, but this colony was no longer present by the 4 week evaluation. Both lambda-cyhalothrin rates showed reinfestation at week 20, but all other treatments were still 100% effective at the termination of the trial at 24 weeks after treatment. Termination was due to changes in airport security and thus accessability.

Figure 4. Fall 2006 trial – Mean colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide.



Chemical analyses were limited by the extremely low theoretical rates of application. Limits of detection for the analysis were approximately 0.1 ppm. The limit of quantification (LOQ) for this trial was 0.4ppm and the limit of detection (LOD) was 0.13ppm. Results between these limits will be captured as below quantifiable limits (BQL = 0.13-0.4ppm). As in the spring 2007 trial, theoretical initial rates of application (ppm) were determined, but this number is a true best guess, and admittedly not accurate, but it gave a ballpark range for the chemists to look for. Many assumptions were made including bulk density of top soil in the area, and use of a 2-inch acre for calculations. The fipronil initial theoretical was below the detection limit of the analysis and was not detected in any analyses.

Presence of all chemicals, except fipronil, was detected by chemical analysis at the 2 week sample date (Table 2). With the exception of the 4 week bifenthrin flowable treatment, all treatments were detected at rates of 0.13ppm or greater through the 8 week sample date. The low rate of lambda-cyhalothrin had fallen to non-detection levels at the 12 week sample date and the low granular chlorpyrifos treatment fell to non-detection levels at the 16 week sample date. The chlorpyrifos granular treatments of 3.0 and 6.0 lb ai/acre have continued to give quantifiable results through the 20 and 24 week sample dates, respectively, unlike in the spring trial where quantifiable results were lost at the 8 week sample date.

Table 2. Results of chemical analyses of soil samples from fall 2007 band treatments (ND=not detected, or below limit of detection; BQL = between 0.13-0.4ppm).

Treatment	Application Rate (lb ai/acre)	Theoretical Initial ppm	ppm by chemical analysis at weeks PT						
			2	4	8	12	16	20	24
Chlorpyrifos G	6.0	14.66	11.6	11.9	15.7	9.3	5.0	6.9	4.8
Chlorpyrifos G	3.0	7.33	14.4	3.5	2.2	3.0	2.1	2.3	BQL
Chlorpyrifos G	1.0	2.4	2.9	3.7	2.0	1.6	ND	0.7	0.5
Chlorpyrifos EC	1.0	2.4	2.6	1.6	BQL	BQL	BQL	BQL	BQL
Lambda-cyhalothrin SC	0.13	0.33	1.8	BQL	BQL	BQL	BQL	BQL	BQL
Lambda-cyhalothrin SC	0.069	0.167	0.54	BQL	BQL	ND	ND	BQL	BQL
Fipronil G	0.012	0.03	ND	ND	ND	ND	ND	ND	ND
Bifenthrin G	0.4	0.97	1.8	0.7	1.3	0.7	0.9	0.6	0.8
Bifenthrin G	0.2	0.49	BQL	0.5	BQL	0.6	BQL	BQL	BQL
Bifenthrin F	0.2	0.49	1.9	ND	BQL	0.6	0.6	0.5	BQL
Deltamethrin SC	0.128	0.31	0.67	0.56	BQL	BQL	BQL	BQL	BQL

DISCUSSION:

The biological results of this trial, both spring and fall data, is supportive of past trials with these chemicals in this use pattern. A compilation of all band trials will be presented in the 2007 IFA Annual Report. Due to limited resources a full discussion of the interpretation of the chemical analyses will not occur in this report, but will be done at a later date. A limited literature search indicates that these results do reflect the normal activity of many insecticides as noted in the introduction; i.e. longer residual under cooler temperatures and drier conditions such as in the winter due to less microbial degradation, photodegradation, hydrolysis, etc.

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CPHST PIC NO: A1F04

PROJECT TITLE: Development of Alternative Quarantine Treatments for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, Spring 2007

REPORT TYPE: Final

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

INTRODUCTION:

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MATERIALS AND METHODS:

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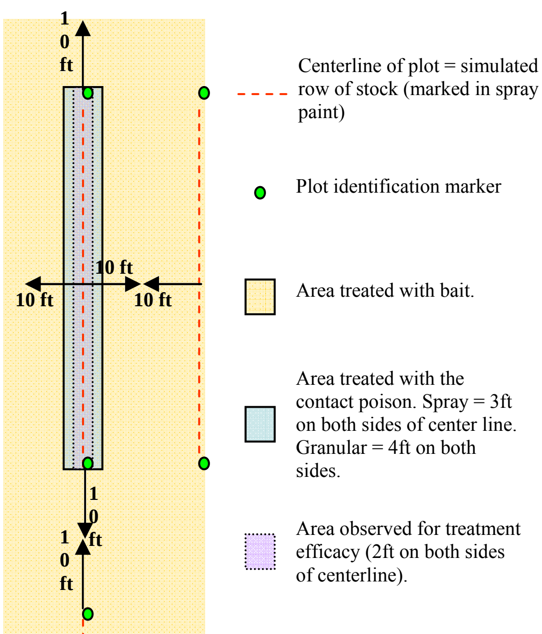

Figure 2. Application of contact insecticide to the plots of simulated stock

On May 11, 2007 hydramethylnon bait was applied at a rate of 1.5 lb/acre through the use of a shop built spreader mounted to a farm tractor. Control plots were not treated with bait. Contact insecticide application occurred on May 14, 2007. 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. There were 3 replicates per treatment. Many liquid chemical labels suggest the use of a surfactant or buffer, and in this trial, the adjuvant Indicate® 5 was used for the first time primarily to buffer the water to pH 5. To insure the adjuvant did not have an effect on IFA populations, a set of replicates of this treatment were included. Treatments consisted of the following.

Chemical	Formulation	Rate of Application
chlorpyrifos	liquid 44.8	1 lb ai/acre
bifenthrin	liquid 7.9%	0.2 lb ai/acre
bifenthrin	granular 0.2%	0.2 lb ai/acre
imidachloprid+cyfluthrin*	liquid 2.94%+0.7%	0.62 lb ai/acre (0.5+0.12)
control	---	---

*Discus™ Nursery Insecticide

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. 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. Temperature was recorded during observation by use of air and soil thermometers.

Additional plots were treated to use for chemical analysis of contact insecticides. These plots, one for each treatment, were separate from those used for IFA evaluation and were treated only with the contact insecticide, not the bait. Five soil core samples were collected from each treated plot and composited for a single sample. Core samples were 2" diameter and 2" in depth. Samples were collected at 1, 2, 4, 8, and 12 weeks and every four weeks thereafter and submitted to the ANPCL-Chemistry Section for analysis. Spring treatments included:

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chlorpyrifos	liquid 44.8%	3 lb ai/acre
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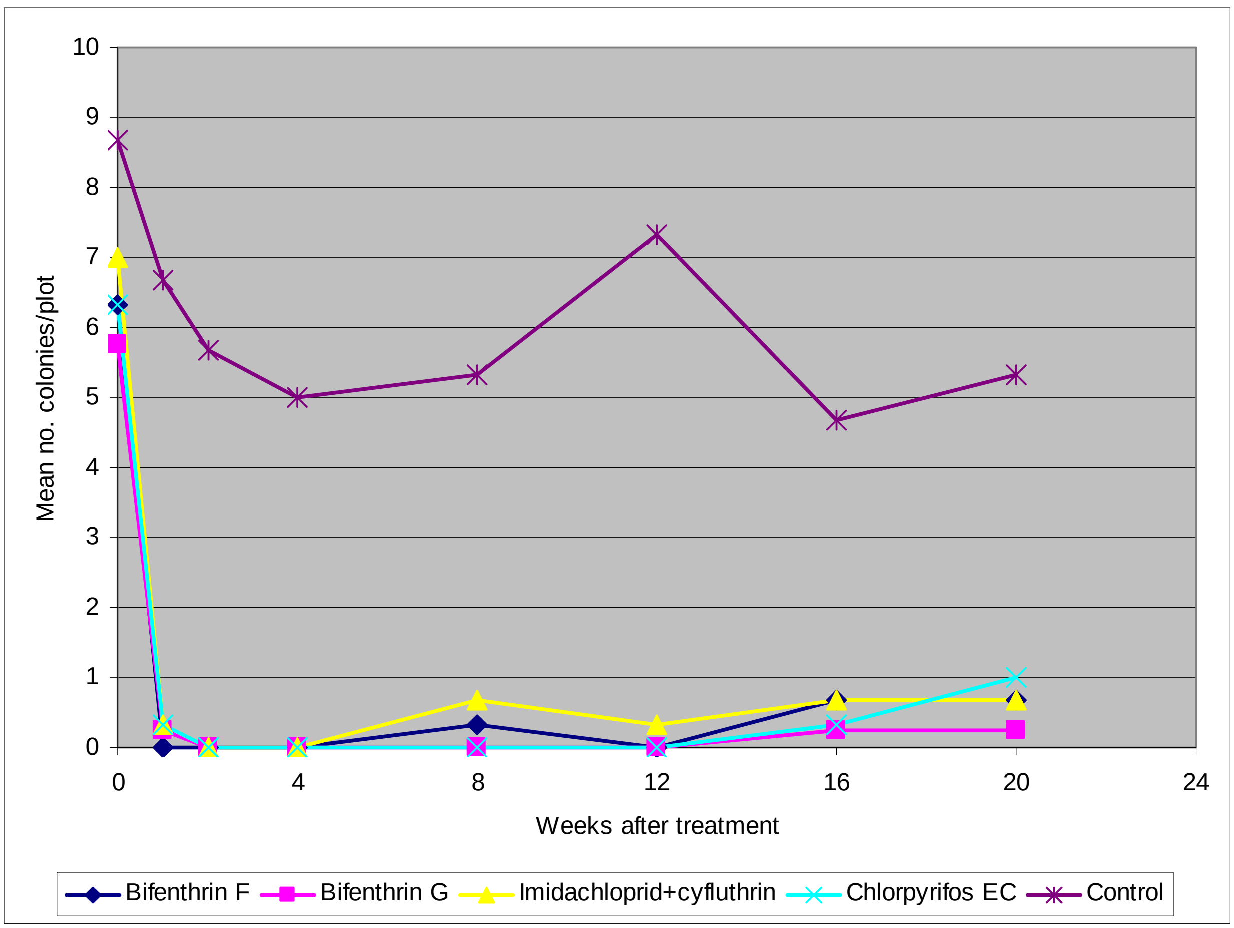
bifenthrin	granular 0.2%	0.4 lb ai/acre
bifenthrin	liquid 7.9%	0.2 lb ai/acre
imidachloprid+cyfluthrin	liquid 2.94%+0.7%	0.62 lb ai/acre

Chemical analyses were conducted by the ANPCL-chemistry section. Procedure for the analytical method can be obtained by request.

RESULTS:

Spring 2007 Band Trial:
The liquid bifenthrin treatment achieved 100% mortality by the 1 week post-treatment evaluation, while all other treatments achieved 100% mortality by the 2 week evaluation (Figure 3). Bifenthrin granular and chlorpyrifos liquid provided 100% control from 2 weeks through 12 weeks. One colony appeared on one bifenthrin flowable plot at week 8 and was gone at week 12, but all bifenthrin flowable plots had at least one mound present at 16 weeks. The combination treatment of imidachloprid/cyfluthrin provided 100% control at 2-4 weeks after treatment, but colonies re-infested these plots at week 8 and remained on the plots throughout the test period. This trial was terminated after the 20 week evaluation.

Figure 3. Spring 2006 trial – Mean colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide.



Chemical analyses were limited by the extremely low theoretical rates of application. Limits of detection for the analysis were approximately 0.1 ppm. The limit of quantification (LOQ) for this trial was 0.4ppm and the limit of detection (LOD) was 0.13ppm. Results between these limits will be captured as below quantifiable limits (BQL = 0.13-0.4ppm). Theoretical initial rates of application (ppm) were determined, but this number is a true best guess, and admittedly not accurate, but it gave a ballpark range for the chemists to look for. Many assumptions were made including bulk density of top soil in the area, and use of a 2-inch acre for calculations. The initial theoretical rate of application of the cyfluthrin portion of the imidachloprid/cyfluthrin treatment was below the quantifiable limit of the chemical analysis and the lower bifenthrin initial rate was at the quantifiable limits of detection.

The results of the chemical analyses did detect chemical in all the 1 week samples (Table 1). Those chemicals that had multiple rates were detected in proportions similar to the application proportions. All treatments were below quantifiable limits (0.5ppm) by 8 weeks after treatment and all except the high rate of bifenthrin were not detected at 12 weeks, although some were detected in low rates at the 16 and 20 week evaluations.

Table 1. Results of chemical analyses of soil samples from spring 2007 band treatments (ND = not detected, or below limit of detection; BQL = between 0.13-0.4ppm).

Treatment	Application Rate (lb ai/acre)	Theoretical Initial ppm	Ppm by chemical analysis at weeks PT						
			1	2	4	8	12	16	20
Chlorpyrifos EC	1.0	2.4	0.75	1.20	ND	ND	ND	ND	ND
Chlorpyrifos EC	3.0	7.3	4.3	6.76	2.01	BQL	ND	BQL	ND
Bifenthrin G	0.2	0.49	BQL	0.57	BQL	BQL	ND	ND	ND
Bifenthrin G	0.4	0.97	0.55	1.77	0.87	BQL	0.55	BQL	BQL
Bifenthrin F	0.2	0.49	BQL	BQL	BQL	BQL	ND	ND	ND
Imidachloprid*	0.5	1.22	0.77	BQL	BQL	BQL	ND	ND	ND
Cyfluthrin*	0.12	0.29	BQL	ND	BQL	ND	ND	BQL	BQL

*applied as Discus™ Nursery Insecticide – a combination treatment of 2 insecticides

DISCUSSION:

Spring band treatments have consistently been less effective with shorter residual activity in south Mississippi than fall treatments. The granular bifenthrin (0.2 lb ai/acre) and the liquid chlorpyrifos (1 lb ai/acre) were 100% effective from 2-12 weeks after treatment, similar to other spring trials. The flowable bifenthrin had a slight decrease in control (95% control-1 mound) at 8 weeks, but weeks 2, 4 and 12 were 100%. The Discus treatment was 100% effective at 2 and 4 weeks, but had no long term residual activity.

The chemical analyses was similar to analyses from spring 2006, with presence of most insecticides decreasing rapidly in the spring/summer months probably due to factors noted in the introduction of this report.

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CPHST PIC NO: A1F04

PROJECT TITLE: Development of Alternative Quarantine Treatments for Field Grown Nursery Stock – Broadcast Bait plus Block Application of Bifenthrin, MS, Fall 2007

REPORT TYPE: Interim

LEADER/PARTICIPANTS: Xikui Wei, Anne-Marie Callcott, Lee McAnally, and Craig Hinton

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. Thus additional treatment methods, as well as additional approved insecticides, are needed to insure IFA-free movement of this commodity.

The primary objective of a quarantine treatment for field grown nursery stock is to render the plants fire ant free. 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. Thus, trials of band-style treatments for large blocks of in-field B&B 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. Tests against individual mounds 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 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 (see 2002-2006 IFA Annual Accomplishment Reports). Unfortunately, when the most promising bifenthrin rate was tested in TN, results were not as consistent or efficacious. Therefore, in 2007 it was decided to apply the insecticides in larger blocks rather than bands.

MATERIALS AND METHODS:

Fall 2007 Block Trial:
The Bobby Chain Municipal Airport in Hattiesburg, MS (Forrest Co.) was selected as the test location for this fall trial. Plots consisted of 50 foot wide pieces of land long enough to contain at least five active fire ant mounds and a minimum of 50 feet long. Plots were a minimum of 10 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 each of the four corners of a plot and Pramitol®, an herbicide, was sprinkled around them to keep the grass from obscuring the stakes. Fluorescent orange spray paint was used to mark the borders of each plot and was repainted as needed.

Fig.1. Application of contact insecticide to the plots

On November 1, 2007, hydramethylnon fire ant bait was applied at a rate of 1.5 lb/acre through the use of a shop built spreader mounted to a farm tractor. Control plots were not treated with bait or contact insecticide. Treatments that were included as contact insecticide only without bait did not receive bait broadcast (see Table 1). Contact insecticide application occurred on November 5, 2007 and follow-up treatments on December 4, 2007 and February 25, 2008 (Fig. 1) after mound evaluations were made. Liquid treatments were applied using a roller pump boom sprayer equipped with seven standard flat spray tips (8015-SS; TeeJet Corp.) to provide a 10’ band spray for each driving pass and the total spray volume equivalent to ca. 45 gal/acre except for one treatment that used only half the volume (22.5 gal/acre). Treatment was applied to the entire plots which varied in length from about 50 feet to 200+ feet depending on the density of ant colonies found in each plot. There were 3 replicates per treatment. Indicate® 5 was used primarily to buffer the water to pH 5 before mixing with insecticides.

Active IFA colonies in each plot were recorded prior to bait application and after contact insecticide application at 1, 2, 4, 5, 6, 8, and 12 weeks and every four weeks thereafter. Mounds were evaluated using as little disturbance as possible, usually through insertion of a plastic rod (5 mm in diameter) into the mound. Mounds were considered active if any workers appeared after disturbance. Temperature was recorded during observation by use of air and soil thermometers.

One soil core sample was collected from each of the three replicates of a treatment for bioassay with female alates in the lab. Soil core samples were not collected on every evaluation date but in a longer interval. Core samples were 2” diameter and 2” in depth.

Table 1. Treatment list of block trial at the Municipal Airport of Hattiesburg, MS, fall 2007.

Chemical	Formulation	Rate of Application	Spray volume (Gal/Acre)	Treatment Timing	Baited First
Bifenthrin	EC 0.23%	0.2 lb ai/A	45 GPA	Nov only	Yes
Bifenthrin	EC 0.23%	0.2 lb ai/A	45 GPA	Nov & Dec	Yes
Bifenthrin	EC 0.23%	0.2 lb ai/A	45 GPA	Nov & Feb	Yes
Bifenthrin	EC 0.23%	0.2 lb ai/A	22.5 GPA	Nov & Dec	Yes
Bifenthrin	EC 0.23% + Flowable 7.9%	0.2 lb ai/A	45 GPA	Nov & Dec*	Yes
Bifenthrin	Flowable 7.9%	0.2 lb ai/A	45 GPA	Nov only	Yes
Bifenthrin	EC 0.23%	0.2 lb ai/A	45 GPA	Nov & Dec.	No
Bifenthrin	EC 0.23%	0.2 lb ai/A	45 GPA	Nov & Feb	No
Control	--	--	45 GPA	Nov only	No

* Bifenthrin EC 0.2 lb/Acre @45 GPA applied in November followed by application of a Flowable 0.2 lb/Acre @45 GPA in December.

<u>RESULTS</u>:

Active mound count in treated plots:
All treatments with the broadcast of bait followed by contact insecticides achieved 100% control of IFA in the evaluation areas by the 4th week evaluation. These results are likely to continue because there have been no new colonies found in the observation areas in these treatments (Figure 2). Treatments with contact insecticides alone experienced a sharp drop in the number of active colonies at around week 4 but remained at a low level through week 12. Even a second bifenthrin application at week 4 (December) did not increase control to 100% when no bait application was made first. The untreated control plots also experienced some colonies reductions at the beginning but they are still at a level of relatively high numbers. All treatments with bait have provided IFA colony free areas throughout the 12 week evaluation. Evaluations and additional (February) applications will continue.

Lab soil core bioassay results
Only plots treated with bait followed by contact insecticide were included in this soil core bioassay evaluation. Results of IFA alates bioassay showed that at the week 5 evaluations, all treatments with bait, except flowable 0.2 lb ai/Acre @ 45GPA applied in November provided

100% mortality (Table 2). However, by week 12, the efficacy level has dropped for all treatments evaluated through soil core alates bioassay. The mortality ranged from 46 to 76%.

Figure 2. Fall 2007 trial – Mean colony mortality after a broadcast treatment of bait followed by a block treatment of bifenthrin.

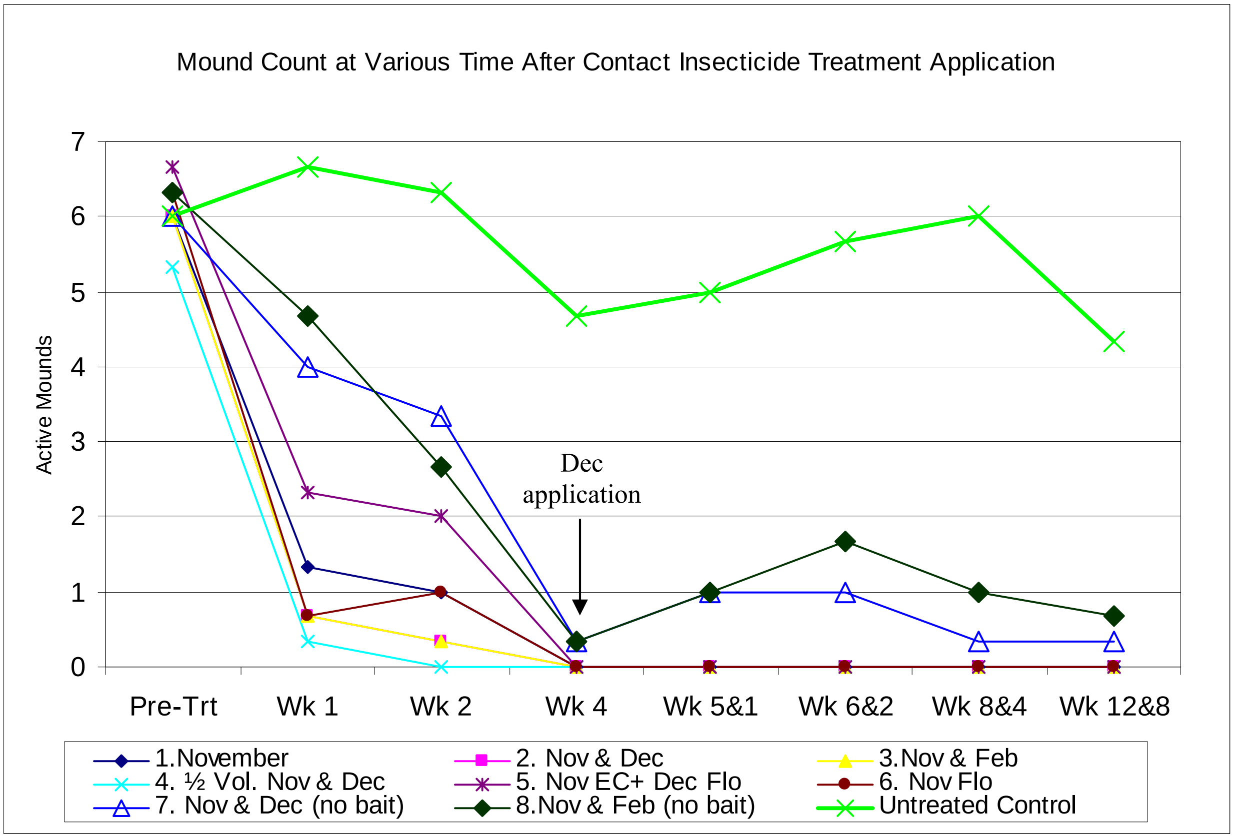


Table 2. Results of IFA alates Bioassay CPHST Lab, Gulfport, MS 2007

Treatment	Application Rate	Queen mortality (%)					
		wk 5	wk12	wk16	wk20		
45GPA EC Nov	0.2 lb ai/A	100.0	76.7				
45GPA EC Nov/Dec	0.2 lb ai/A	100.0	76.7				
22.5GPA EC Nov/Dec	0.2 lb ai/A	100.0	70.0				
45GPA F Nov	0.2 lb ai/A	66.6	46.6				
45GPA EC + F Nov/Dec	0.2 lb ai/A	100.0	50.0				
Control	--	0.0	0.0				

DISCUSSION:

Results of this trial are supportive of past trials with these chemicals in this use pattern. It is clear that contact insecticide for band treatment alone is not likely to achieve the level of quarantine certification. A bait broadcast followed by contact insecticides application is necessary for IFA quarantine treatment. A compilation of all band trials will be presented in the 2007 IFA Annual Report.

CPHST PIC NO: A1F04

PROJECT TITLE: Efficacy of fipronil granular as a small plot treatment (ca. 10'x10')

REPORT TYPE: Final

PROJECT LEADER/PARTICIPANTS: Anne-Marie Callcott, Lee McAnally, Craig Hinton

INTRODUCTION:

Fipronil granular is currently approved for use as a grass sod treatment when used as a broadcast application. Certification period is 20 weeks after a 4-week exposure period when applied at 0.025 lb ai/acre. The use of this type of application in field grown nursery stock has been proposed and the chemical company, Bayer Environmental Science, is pursuing a 24c registration with the product. APHIS is supportive of this labeling and will begin the agency approval process after EPA approved a 24c label. The rates and certification periods for which we have supportive data for broadcast applications are:

0.025 lb ai/acre = 4 weeks exposure; 20 weeks certification
0.01875 lb ai/acre = 6 weeks exposure; 18 weeks certification
0.0125 lb ai/acre = not acceptable as a stand alone treatment

However, due to the significant difference in how grass sod and field grown nursery stock are produced, the definition of broadcast must be refined and determined. Trials for grass sod evaluations were conducted on 1-acre plots with a ¼-acre center circle as the evaluation area (thus a 45' treated buffer around the evaluation area). We do know from previous trials that fipronil is not effective at quarantine levels as an individual mound treatment when treating ca. 3-ft. diameter area around an active mound at rates equivalent to 0.0125-0.025 lb ai/acre. A true in-band or in-furrow treatment applied at 0.05 lb ai/acre was also unsuccessful. A surface band treatment at 0.0125 lb ai/acre applied to a 5'x1000' band (with the simulated plant row in the center of the band), was also unsuccessful, however this was at a lower rate of application that was also not acceptable when broadcast applied on grass sod for quarantine level efficacy.

In the winter of 2007, due to the interest in pursuing the 24c registration for fipronil a trial was initiated to determine the efficacy of fipronil granular applied to very small plots, ca. 12' x 12', at the two rates of application which are of interest.

MATERIALS AND METHODS:

This trial was conducted in Harrison Co., Mississippi. Individual plots/replicates were set up with an active mound in the center of the plot. There were 10 plots/replicates per treatment. We were interested not only in the efficacy of the very small treated plot, but also the efficacy if the application coverage was not consistent due to low limbs on the nursery stock. Therefore, either the entire plot was treated including the mound, or a 2-ft. sq. area around and including the mound were not treated (Figures 1 & 2). Plots sizes for these two treatment regimens were different to accommodate the application equipment. A tractor with a 4-ft. drop spreader was used to apply the granular fipronil. Application rates were 0.025 lb ai/acre or 0.01875 lb ai/acre. Treatments were made on Dec. 6, 2007; a sunny, warm day ca. 55-60F with the last significant rainfall Nov. 25 with ca. 1 inch. Mounds were evaluated with as little disturbance as possible by inserting a probe into several places within the mound to elicit a worker response. Mounds were then rated from 0 (dead, no activity) to 5 modified from the Lofgren and Williams (1982) population rating method, but not using the presence or absence of worker brood.

Figure 1. Treatment area for plots where the IFA mound was not directly treated, but surrounded by granular fipronil treatment. Treated 4 feet around mound leaving 2 ft. sq. area untreated including mound

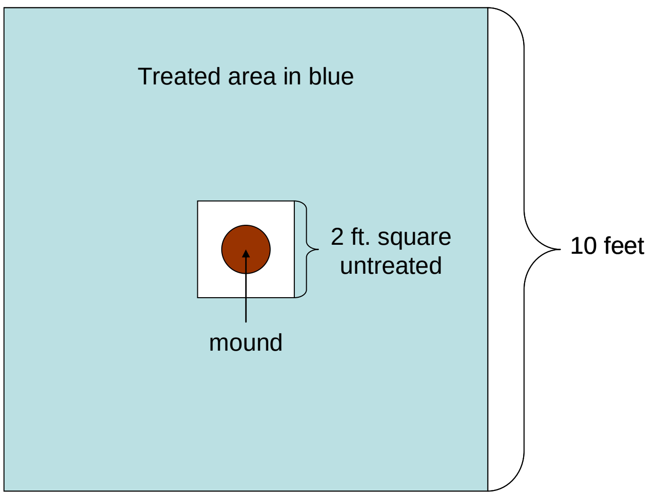
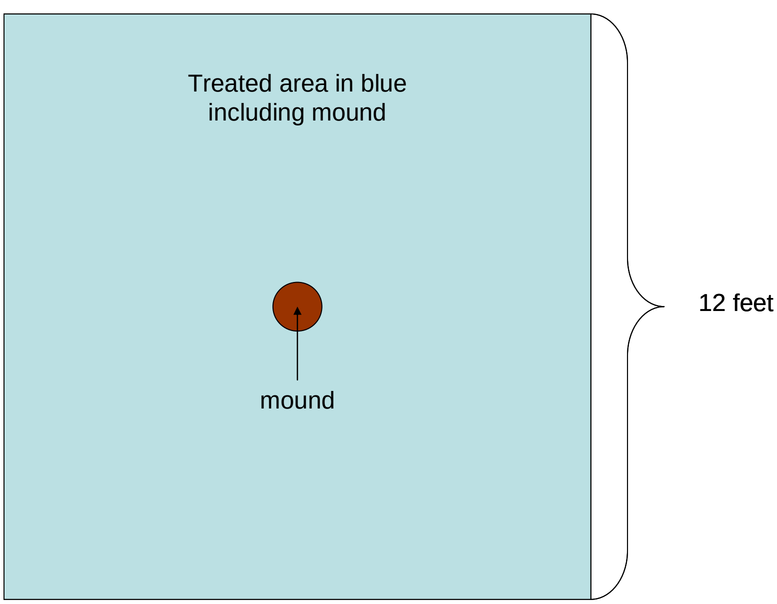


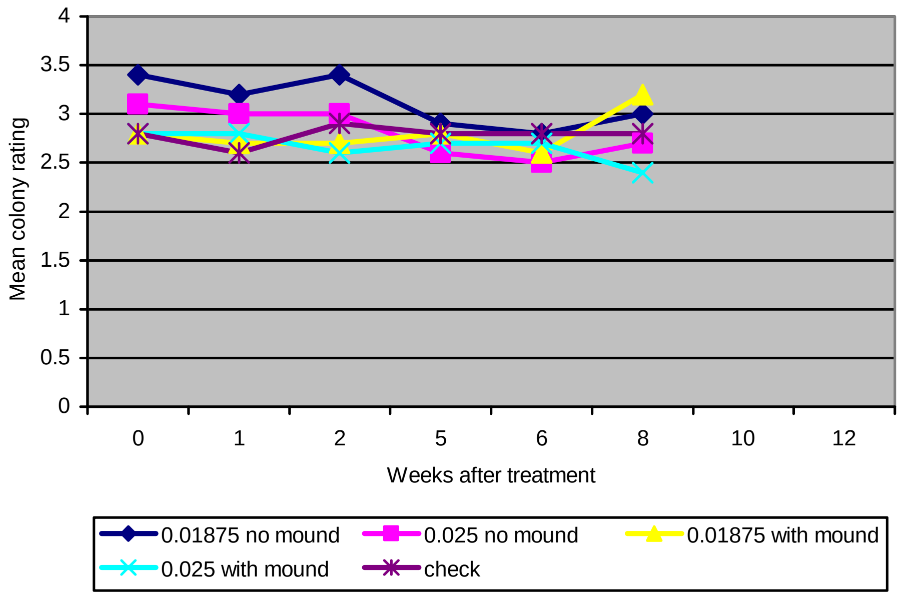
Figure 2. Treatment area for plots where the IFA mound and the surrounding area was treated with granular fipronil; for ease of treatment with 4-foot spreader, treated 12 sq. ft. area with mound



RESULTS:

There was very little rainfall in the first 2 weeks after treatment; 0.02 inches prior to the first evaluation and an additional 0.15 prior to the second evaluation. Due to weather and available personnel the week 4 evaluation was moved to week 5. Prior to the week 5 evaluation there was 4.6 inches of rainfall. As noted in Figure 3, there was very little decline in colony rating and thus in IFA activity in any of the treatments, once again stressing the importance of a broadcast application over a larger area with the fipronil product.

Figure 3. Efficacy of fipronil granular when applied in small plots of 10'x10' or 12'x12' at rates equivalent to 0.01875 lb ai/acre or 0.025 lb ai/acre. Mean colony rating of 10 replicates is shown. (no mound=treatment around mound but not directly over the mound; with mound=treatment includes treating over the mound; see text for details)



DISCUSSION:

This trial confirmed earlier trials in that small area treatments with fipronil granular are not effective. The insecticide requires a large area treatment to be effective. In the spring 2008, additional trials with larger plots will be initiated to determine the smallest area that can utilize this broadcast treatment for fipronil.

Literature Cited:

Lofgren, C. S. and D. F. Williams. 1982. Avermectin B_1a, a highly potent inhibitor of reproduction by queens of the red imported fire ant. Jour. Econ. Entomol. 75: 798-803.

CPHST PIC NO: A1F03

PROJECT TITLE: Exclusion methods for Imported Fire Ants (IFA) in hay-transport operations – Best Management Practices

REPORT TYPE: Final Report: 07-8100-1215-GR

LEADER: Ronald D. Weeks

COLLABORATORS: Paul R. Nester, Program Specialist – IPM, Bart Drees, Extension Specialist – Entomology, and Mike Heimer, County Extension Agent – Montgomery County. Texas AgriLife Extension Service

This report completes a cooperative relationship between Texas A&M University Extension Service and the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) under Notice of Non-competitive Federal Assistance Agreement Award No. 07-8100-1215-GR. It outlines the mission-related goals, objectives, and a copy of the award report prepared by Texas AgriLife Extension Service personnel.

The goal of this cooperative relationship was to evaluate and verify the efficacy of "Best Management Practices" (BMP) methods, developed by scientists in USDA, APHIS, PPQ, CPHST to prevent imported fire ants from infesting commercial hay production systems and being moved to un-infested areas of the U.S. This cooperative relationship supports the APHIS safeguarding mission to develop responsible and effective quarantine treatments against imported fire ants. 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.

Results from this cooperative project support key CPHST derived BMPs. This project demonstrated is the utility of designating ¼ to ½ acre hay storage sites that are treated with broadcast baits to reduce IFA pressure. Results of the chemical barrier applications were not what we expected. Chemical barrier applications of permethrin have been effective at keeping IFA off of baled hay in other CPHST projects (see SIPS Annual Reports 2002 – 2006). However, in this study chemical barrier applications were not significantly different from unprotected hay bales stored in broadcast baited areas. Some IFA control was achieved, whereby a significant decrease in the average number of IFA on protected bales compared to untreated control bales. Results from this project will be combined with concurrent USDA and previous data to develop IFA quarantine methods and develop "Best Management Practices" BMP for hay producers in IFA infested areas.

The approach to the work was similar to previous APHIS research (see SIPS Annual Report 2003 – 2006) that 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. Elements of a BMP for hay include: 1) field level sanitation using broadcast baits, 2) individual IFA mound treatments on nuisance colonies, 3) chemical barrier applications to prevent IFA foraging, 4) protection of hay from chemical contamination, and 5) sampling for IFA to evaluate treatment efficacy at key times during the production process.

Outside of this cooperative agreement, APHIS did not supply equipment or funds to complete this project. The monies transferred to Texas A&M Extension Service and the state cooperator were used by the state to reimburse expendable supplies utilized in this project. Expendable supplies for this project include hay bales, insecticides, ground cover cloth, fuel, and sampling materials. Hay fields and the equipment for harvesting and transporting hay was the responsibility of the state cooperator. Personnel and equipment used for this project were the responsibility of Texas A&M Extension Service and the cooperator. APHIS was not responsible for any equipment, personnel, or supplies used by the cooperator for this project. All work was completed by the cooperator within one-year of issuance of this agreement. This work will be conducted in approved hay production sites. There is no danger of impacting sensitive, natural, or urban environments. All chemicals will be used in a manner consistent with the label directions.

Protecting Harvested Hay from the Red Imported Fire Ant

Paul R. Nester, Program Specialist – IPM
Bart Drees, Extension Specialist – Entomology
Mike Heimer, County Extension Agent – Montgomery County
Texas AgriLife Extension Service

Hay infested with the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) is a regulated item when it is to be shipped out of quarantined areas, refer to Federal Quarantine (7 CFR 301.81) . The Texas Administrative Code (Title 4, Part 1, Chapter 19, Subchapter J, Rule §19.102) does not permit baled hay to be shipped from quarantine counties to non-quarantine counties or to other states due to infestation of the red imported fire ant (http://lamb.sos.state.tx.us/tac/). Currently the only USDA or TDA approved way to have hay or straw approved for shipment from fire ant quarantined counties to non-infested counties is to remove bales from the field immediately after baling and store them in an off ground location. This prevents hay that has been stored in the field in ground contact from being eligible for shipment. There is currently no USDA-APHIS or TDA approved quarantine chemical treatments for assuring that red imported fire ants are not shipped to new locations.

As part of a cooperative effort with Ron Weeks, Entomologist, USDA, APHIS, PPQ, CPHST Lab - Gulfport, MS, a results demonstration was set up in Montgomery County at the Billy Woods Ranch, 5.1 miles south Montgomery, TX on FM 149, with the help of Mike Heimer, CEA-AG/NR, to evaluate best management practices for protecting harvested hay from fire ant infestation. This study will help identify treatment methods which will reduce or eliminate fire ant infestations in round bales.

Material and Methods

Forty-eight round hay bales removed from an on-location hay holding area (Figure 1) were placed in a hay storage area where cattle were excluded with an electric fence. The hay bales were arranged into 12 groups of four bales with each group being considered one experimental unit (Figure 2). Each experimental unit received one of three treatments, Table 1, and each treatment was replicated four times in a randomized block design. Figure 1 shows the size and the approximate placement of the hay bales within the designated hay study storage area, and the location of the hay bales receiving no treatment which was considered the untreated control. The untreated bales were located away from the treated bales because the entire hay storage area was to be treated with a broadcast application of an ant bait product. The entire area had noticeable fire ant activity prior to initial treatment.

On Sept. 29, 2007 Extinguish® Plus fire ant bait (hydramethylnon plus methoprene) was broadcast at 2.0 lb product per acre over the storage area with an ATV (Kawasaki Prairie 700) mounted Herd GT-77 Sure Feed Broadcaster for Fire Ants (Herd Seeder Co., Inc., Logansport, IN (www.herdseeder.com)). The Herd GT-77 was calibrated to deliver 2.0 lb fire ant bait with a 20 ft swath while the ATV traveled at 11 mph. The Herd GT-77 was fitted with a Herd Seeder Co. #0 plate covering the hopper opening. A perimeter broadcast treatment of Extinguish® Plus fire ant bait at 2.0 lb product per acre was made around the on location hay storage area to help minimize the presence of fire ants in hay bales before they were moved to their respective study plots.

On Oct. 19, 2007, GardStar® (permethrin) at the rate of 6 ml/gal @ 6 gal/100 sq ft was broadcast using a spray gun attached to a nurse tank calibrated to deliver 3 gallons per minute to a 12 ft X 12 ft area (Figure 1) in each plot location where hay bales were to be placed on pallets. All 4 ft X 5 ft hay bales were moved into plot areas on Oct. 29, 2007 making a four bale square in each. Final GardStar® perimeter broadcast treatments (3 ft perimeter around hay bales) were applied on Oct. 30, 2007. Beginning 24 hours after final GardStar® perimeter broadcast treatment (Oct. 31, 2007), and each week for 7 weeks following, food lure stations (1/4 inch pieces of hot dog) were placed approximately 4 inches from the ground on each hay bale (Figure 3). The number of foraging red imported fire ant workers on the stations were estimated after 45 minutes of exposure to document presence or absence of the fire ant,

Four weeks after initiation (Oct. 30, 2007), hay bales that showed fire ant activity on the food lure stations were treated using fire ant bait stations (Bell Laboratories', Inc., "Rodent Baiter" stations with 1 oz indoxacarb, Advion®, fire ant bait, Figure 4) placed on the ground close to the food lure station. Fresh Advion bait stations were again placed beside those hay bales showing fire ant activity after the fifth week. The test was concluded after 7 weeks of sampling

Results and Discussion

Table 2 shows the total number of foraging imported fire ant workers associated with four food lure stations placed on 4 round bales per treatment. At the 24 hr and 1 week after treatment (WAT) evaluations, there was a significant difference in fire ant numbers observed on untreated bales versus those bales within other treatments. At 2, 3 and 4 WAT there were no significant differences among insecticide treatments although the bait alone treatment had no foraging fire ants associated with the food lures. Additional treatments using the contact insecticide did not significantly improve the bait only treatment, although foraging fire ant numbers were numerically fewer in treated plots than in untreated plots

On the November 28th evaluations date (4 WAT), we decided to place bait stations containing the fire ant bait Advion® on the ground next to bales with documented fire ant foraging activity. The idea was to use this quick acting bait to remove the fire ants from the bale, as a curative treatment. Table 3 shows that on November 28th, the Advion® bait stations were placed next to 7 hay bales (Figure 5) found to have foraging fire ants. Bait stations remained until the next evaluation date. These hay bales with foraging fire ant activity were in plots treated with fire ant bait followed by application of permethrin (GardStar®) around the perimeter of bales placed on the ground (Premise) and plots treated with fire ant bait followed by permethrin treatment underneath pallets on which bales were placed plus permethrin around the perimeter of bales (Full). It is interesting to note that no Advion® bait stations were placed around bales receiving fire ant bait alone treatment as these had no fire ant activity.

The next evaluation at 5 WAT (December 5th) again showed a significant difference in fire ant numbers observed on bales in the untreated area, versus those bales within treated areas (Table 2). Also, 5 out of the 7 hay bales where the Advion® bait stations were placed showed no fire ant foraging (Table 3). Advion® bait stations with fresh product were placed next to the remaining two hay bales that still had foraging fire ant. All other bait stations were removed.

The December 11th evaluation date also showed a significant difference in fire ant numbers observed on bales in the untreated area, versus those bales within treated areas (Table 2). The Advion® bait stations removed the ants from one of the remaining two hay bales (Table 3), but, foraging fire ants re-appeared in a previously uninfested hay bale. All bait stations were removed at this date, to see if fire ants would return to the hay bales. The final evaluation date on December 19th again showed a significant difference in fire ant numbers observed on bales in the untreated area, versus those bales within treated areas (Table 2). As for the hay bales where the Advion® bait stations were placed, the only foraging fire ants were found in the bales with fire ants on December 11th (Table 3).

Table 4 shows the number of food lure cards of four (rated either present or absent) on which foraging imported fire ant workers were observed on 4 round bales per treatment plot. All treatments were significantly different from the untreated at all evaluations dates, except on Nov. 19 when the Premise treatment did not separate from the untreated plot mean. This data may be misleading. Upon inspection, if the presence of ANY fire ants on a hay shipment going to a non-quarantined area from a fire ant quarantined area is observed that hay shipment is likely to be turned away. Only those treatments that keep hay bales free of foraging fire ants are desired. Oddly, hay bales that were placed on the ground in the bait treated area, and not protected by contact chemicals or elevated on pallets, were IFA free the longest period of time in the study. We do not have an explanation as to why this occurred.

The use of the fire ant bait stations placed next to hay bales with foraging fire ant activity shows promise. It may be better to place bait stations on the individual hay bales instead of placing them on the ground. In this study only hay bales in a treatment area that had an additional permethrin treatment had the additional Advion® bait stations placed where foraging fire ants were observed on the food lure stations. It is not known if any permethrin residue inhibited the foraging fire ants from finding the bait station placed on the ground. Dupont ™ has an ant bait station product, Advion® Ant Bait Arena that should be evaluated as a means for quick removal of fire ant from individual hay bales. This type of ant bait station may also show promise by placing bait stations on the transport trailer, to remove any fire ants that might have been undetected.

Acknowledgements

The authors wish to thank Mr. Billy Woods, Woods Ranch, Montgomery, TX, for allowing us access to the Wood's Ranch property, equipment, and hay bales, Dr. Ron Weeks, Entomologist, USDA, APHIS, PPQ, CPHST Lab - Gulfport, MS, for the USDA-APHIS grant for supplies necessary to complete this study, and Dr. Doug van Gundy, Central Life Sciences for the donation of the Extinguish® Plus fire ant bait used in this study.

Table 1. Treatments tested for fire ant control on hay bales, Montgomery Co. TX, 2007.

Treatment Designation	Broadcast Fire Ant Bait	# product /acre	Contact Insecticide	Insecticides Applied
Bait only	Extinguish® Plus Sept. 29	2	-	Bait
Premise	Extinguish® Plus Sept. 29	2	GardStar® Oct. 30	Bait plus perimeter treatment of GardStar at 6ml/gal
Broadcast plus perimeter	Extinguish® Plus Sept. 29	2	GardStar® Oct. 19	Bait plus perimeter plus broadcast GardStar at 6 gal/100 sq ft
Untreated	None	-	None	-

Table 2. Total number of foraging imported fire ant workers associated with food lure stations placed on 4 round bales per treatment, Montgomery Co., TX, 2007.

	No. foraging ants per four food lure stations[1]							
Treatment	Oct. 31 24 hr	Nov. 6 1 WAT	Nov. 13 2 WAT	Nov. 19 3 WAT	Nov. 28 4 WAT	Dec. 5 5 WAT	Dec. 11 6 WAT	Dec. 19 7 WAT
Untreated	125.0a	131.5a	134.0	99.3	84.0	76.3a	248.5a	207.5a
Bait	0.0b	0.0b	0.0	0.0	0.0	0.0b	21.3b	0.5b
Premise	0.0b	18.8b	52.5	70.0	47.5	2.5b	7.5b	5.0b
Full	0.0b	12.5b	22.5	47.5	20.0	0.25b	23.8b	10.3b
d.f. = 3								
P	0.011	0.010	0.071	0.275	0.074	0.002	0.000	0.000
F	5.832	5.924	3.025	1.462	2.980	9.233	28.166	17,366
Mean Sq.	15,625.0	14904.229	13,731.0	7,015.563	5,298.917	5,680.167	53,565.167	40,968.536

[1] Means followed by the same letter are not significantly different using analysis of variance (ANOVA) with means separated using Tukey's HSD, $P \leq 0.05$ (SPSS 14.0 for Windows).

Table 3. Red imported fire ant infested round bales treated with indoxacarb applied in bait stations, Montgomery Co., TX 2007.

Bale	Ants, 11/28	Ants 12/5	Ants 12/11	Ants 12/19
15, 2a	10*	0	0	0
5, 3b	60*	0	85	40
6, 2b	70*	0	0	0
7, 3b	10*	0	0	0
9, 2b	30*	0	0	0
16, 3d	10*	10*	10	1
15, 2b	80*	5*	0	0
Mean ± S. D.	38.6 ± 30.8	2.1 ± 3.9	13.6 ± 31.7	5.9 ± 15.1

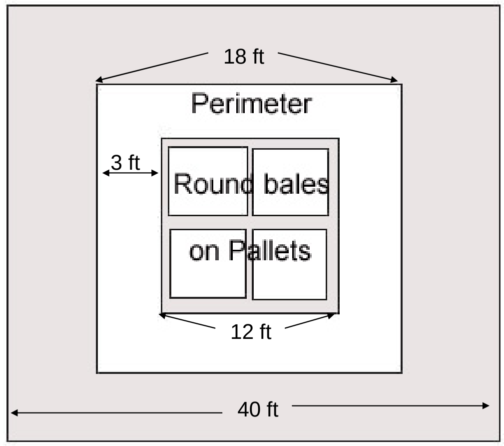
*treated or retreated

Table 4. Number of food lure stations on which foraging imported fire ant workers were observed per treatment, Montgomery Co., TX, 2007.

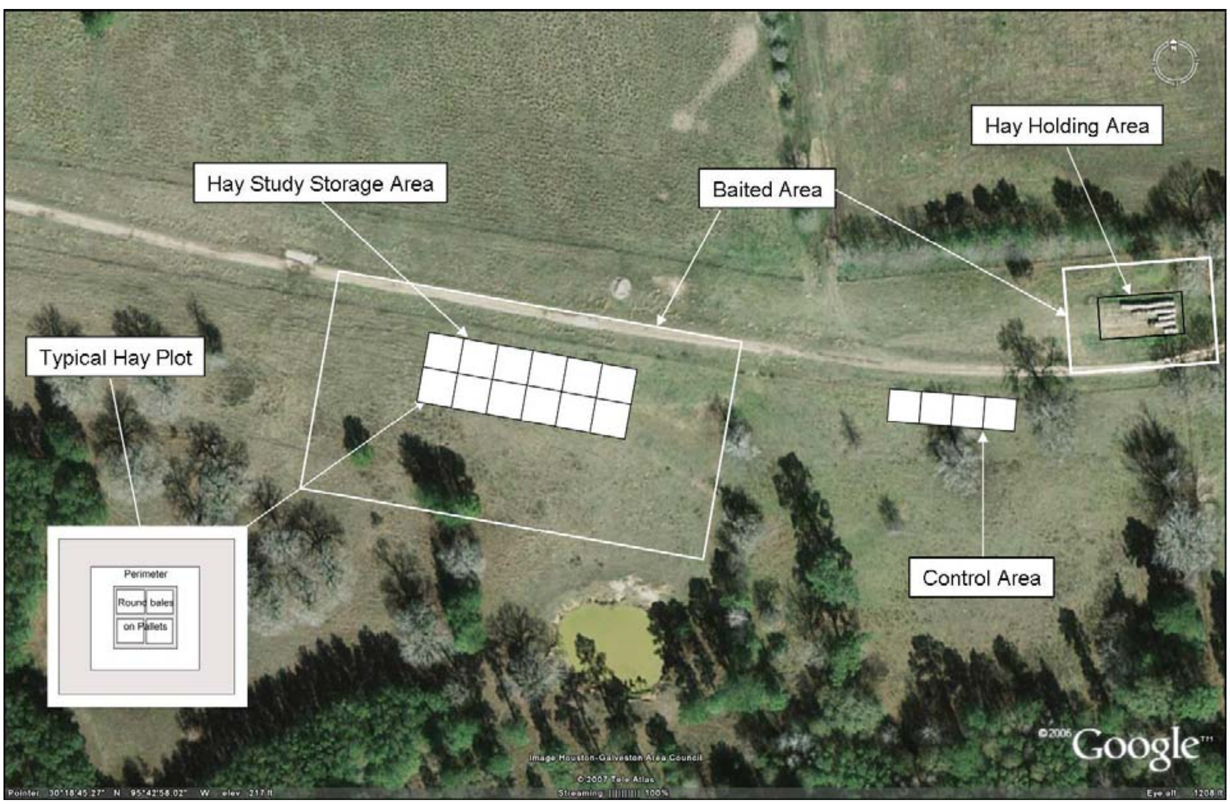
	No. cards/4 attracting foraging ants[12]							
Treatment	Oct. 31 24 hr	Nov. 6 1 WAT	Nov. 13 2 WAT	Nov. 19 3 WAT	Nov. 28 4 WAT	Dec. 5 5 WAT	Dec. 11 6 WAT	Dec. 19 7 WAT
Untreated	2.8a	2.8a	3.3a	2.3a	3.0a	3.0a	4.0a	3.8a
Bait	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.5b	0.3b
Premise	0.0b	0.3b	0.8b	1.3ab	1.0b	0.5b	0.3b	0.3b
Full	0.0b	0.5b	0.8b	0.5b	0.8b	0.3b	0.5b	0.5b
d.f. = 3								
P	0.000	0.000	0.002	0.043	0.000	0.000	0.000	0.000
F	33.0	17.111	9.4390	3.680	16.579	16.130	56.273	43.308
Mean Sq.	7.563	6.417	8.063	3.833	6.563	7.729	12.896	11.729

[1] Means followed by the same letter are not significantly different using analysis of variance (ANOVA) with means separated using Tukey's HSD, $P \leq 0.05$ (SPSS 14.0 for Windows).
[2] Total number of lure food stations per treatment were 16 (1/hay bale or 4/plot)

Figure 1: Plot plan for evaluating insecticidal treatment regiment for preventing round bales from becoming infested with red imported fire ants. The individual plot dimensions are as diagrammed in the expanded plot view. The approximate location of the individual plots is diagrammed in the location view. The expanded plot view illustrates the placement of the bales and those plots with pallets.



Expanded plot view



Location View

Figure 2: Placement of hay bales in four bale squares in hay storage area, Montgomery Co., TX 2007

Figure 3: Placement of food lure stations on hay bales.

Figure 4: Bell Laboratories', Inc., "Rodent Baiter" stations with 1 oz indoxacarb (Advion®) fire ant bait.

Figure 5: Placement of Bell Laboratories', Inc., "Rodent Baiter" stations with 1 oz indoxacarb (Advion) fire ant bait on ground close to food lure station.

CPHST PIC NO: A1F03

PROJECT TITLE: Best Management Practices for Imported Fire Ants (IFA) in Pine Straw Operations, 2007

REPORT TYPE: Interim

LEADER/PARTICIPANTS: Ronald D. Weeks, Lee McAnally

INTRODUCTION:

Baled pine straw has been implicated in the movement of imported fire ants in recent years. Under the Federal Imported Fire Ant Quarantine (7CFR 301.81), baled hay and 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. Further there no approved treatments for killing or evicting IFA colonies from infested pine straw, thereby making it unsuitable for shipment out of the quarantine. Previous experience and research (see Annual Report 2003 – 2006) on baled hay and, to a limited extent, pine straw suggests that Best Management Practices (BMP) that focus on IFA control at the storage site and commodity protection from IFA are effective against IFA. The objectives of this research were to evaluate methods of killing IFA colonies from artificially infested pine straw under two different storage situations.

MATERIALS AND METHODS:

Pine straw experiments were conducted at the CPHST laboratory in Gulfport, MS June – September 2007. We evaluated insecticidal fumigation/fogging methods to kill IFA colonies in artificially infested pine straw bales. Two insecticide application units were compared; 1) a total release fogger, and 2) a fumigator fogger. Home foggers and fumigants are designed to reach hard to treat areas and saturate the environment with pesticide. Total release foggers, also known as "bug bombs," are pesticide products containing aerosol propellants that release their contents at once to fumigate an area. These products are often used around the home to kill cockroaches, fleas, and other pests.

Raid's Hot Shot Roach® household insecticide was evaluated as a total release fogger. Label instructions caution against using more than 1 ounce of product per 1,000 cubic feet of living area. This product is water based and contains 0.2% tetramethrin, 0.4% permethrin. Raid's Fumigator Fumigating Fogger® was used as a fumigating fogger that kills insects on contact. When activated, the 6-ounce fogger fumigates the room with a dry, white cloud of fog. It contains 12.6% permethrin. One unit treats a room of up to 16'x20'. The label recommends keeping treated rooms closed for 3 hours after treatment.

Pine straw bales were purchased from a local hardware/outdoor store, and stored off ground on an 8 x 8 ft trailer until needed. Two storage techniques for commercial pine production were used: 1) pine straw stored on water permeable ground cloth and covered with plastic tarp, and 2) pine straw stored in a portable 8 x 20 ft storage container (fig. 1).

Figure 1. Typical storage container used for baled pine straw operations.

Figure 2. Bioassay pouch comprised of live ants with approximately 10,000 ants including, egg brood, workers, and alates.

Experiment 1# (IFA treatments on individual stacks of pine straw)
Experiments were completed on single stacks of pine straw, comprising 4 bales per stack, covered with plastic tarp. Four treatment stacks and four control stacks were compared. Each pine straw stack was infested with approximately 10,000 ants including, eggs, brood, workers, and alates. Ants were sealed in a screened pouch (i.e. bioassay pouch, fig. 2) and placed in the middle of four pine straw bales stacked 2 x 2 vertically. Each stack was covered with painters plastic and sealed along the edge with wood 2 x4's. A single fogger was "set-off" under each treatment stack and allowed to sit in the covered area for 2hrs. All plastic covers were removed and the stacks vented for 30 minutes per labeled directions. Ants were retrieved from the stacks and scored as alive, impaired, or dead.

Results showed that after 2.5 hours all the ants in the four control pouches were alive and appeared normal. Ants in the four treatment pouches were dead. Visual examinations of ants after 24 hours showed that all ants in the control pouches were alive and all ants in the treated stacks were dead.

Experiment 2# (IFA treatments on a row of stacked pine straw)
Experiments were completed on single row of stack pine straw bales. Four stacks of pine straw, comprising 4 bales per stack, were placed adjacent to each other in a single row and covered with plastic tarp. A control set of four pine straw stacks was set-up for comparative evaluations. Each pine straw stack in the row was infested with approximately 10,000 ants including, egg brood, workers, and alates. Ants were sealed in a screened pouch (i.e. bioassay pouch) and placed in the middle of each stack of four pine straw bales stacked 2 x 2 vertically. The row of stacks was covered with painters plastic and sealed along the edge with wood 2 x4's. A single fogger was "set-off" at one end of treatment row and allowed to sit in the covered area for 2 hrs. All plastic covers were removed and the stacks vented for 30 minutes per labeled directions. Ants were retrieved from the stacks and scored as alive, impaired (i.e. twitching and sporadic leg movements), or dead.

Results showed that after 2.5 hours all the ants in the four control pouches were alive and appeared normal. Ants in the four treatment pouches were either dead or visibly impaired. Specifically, more than 50% of ants were dead while the remaining ants showed signs of impairment. Visual examinations of ants after 24 hours showed that all ants in the control pouches were alive while all ants in the treated stacks were dead.

Experiment 3# (IFA treatments on pine straw in a storage container)
This experiment evaluated the efficacy of using a fumigation fogger to penetrate stacked hay bales in storage container and kill IFA colonies. Live IFA colonies were placed in bioassay pouches and set on top of and in-between stacked pine straw bales. Experiments were completed at the Gulfport facility in a rented 8 x 20 ft storage container, with double sealable doors, plywood floor decking, and passive air vents in each top-corner. Three stacks of pine straw comprising 2 bales each were placed adjacent to each other at the end of the trailer farthest from the double doors. Two bioassay pouches of live ants with approximately 10,000 ants including, egg brood, workers, and alates were placed in each of the stacks; one on top of the stack, and the other in-between the two bales (n = 6 colonies). In front of these, a wall of stacked pine straw was created across the midsection of the container, four bales deep, a width that extended from each side, and a height up to 1 ft. from the top of the container. A single fumigation fogger was "set-off" near the double doors at the front of the container; the doors were closed, and remained closed for 3 hrs. After this waiting period the container doors were opened and the container allowed to vent for 30 minutes per labeled directions. Ants were retrieved from the top and middle of the treatment stacks and scored as alive, impaired (i.e. twitching and sporadic leg movements), or dead. A control of three pine straw stacks was set-up in another storage container on the facility for comparative evaluations. In these controls, each pine straw stack was infested with approximately 10,000 ants including, egg brood, workers, and alates and alates. These ant pouches were placed in each of the stacks; one on top of the stack, and another between the two bales.

Results showed that after 3.5 hours all the ants, both on top and in between bales, in the control stacks were alive and appeared normal. Ants in the bioassay pouches that were placed on top of treated bales were all dead. Ants in the bioassay pouches, that were placed in-between treated bales, showed no visible signs of impairment or death. Subsequent, visual examinations of ants after 24 hours showed that all ants in the control pouches and in the bioassay pouches placed in the middle of treated bales were alive.

Experiment 4# (IFA treatments on pine straw in a storage container using a circulation fan)
This experiment evaluated the efficacy of using a fumigation fogger and a circulation fan to penetrate stacked hay bales in storage container and kill IFA colonies. Experiments were completed at the Gulfport facility in a rented 8 x 20 ft storage container, with double sealable doors, plywood floor decking, and passive air vents in each top-corner. Three stacks of pine straw comprising 2 bales each were placed adjacent to each other at the end of the container farthest from the double doors. Two bioassay pouches of live ants with approximately 10,000 ants including, egg brood, workers, and alates were placed in each of the stacks; one on top of the stack, and another in-between the two bales. In front of these bales, a wall of stacked pine straw was created across the midsection of the container, four bales deep, a width that extended from each side, and a height up to 1 ft. from the top of the container. Three pine straw stacks were set-up in another storage container on the facility for comparative evaluations. Each pine stack was infested with approximately 10,000 ants including, egg brood, workers, and alates and

alates. Ant bioassay pouches were placed in each of the stacks; one on top of the stack, and another in-between the bales. A single fumigation fogger was "set-off" near the double doors at the front of the container; a 300cfm fan was turned-on and placed inside the container, the doors were closed, and remained closed for 3 hrs. After this waiting period the container doors were opened for venting for 30 minutes; per labeled directions. Ants were retrieved from the treatment stacks and scored as alive, impaired (i.e. twitching and sporadic leg movements), or dead.

Results from the control stacks showed that after 3.5 hours all the ants, both on top and in-between bales, were alive and appeared normal. Ants in the treatment stacks that were placed on top of bales were all dead. Ants that were placed in-between bales showed no visible signs of impairment or death. Subsequent visual examinations of ants after 24 hours showed that all ants in the control pouches and ants placed in the middle of treated bales were alive.

Experiment 5# (IFA treatments on pine straw in a storage container and repeated fogging application)

This experiment evaluated the efficacy of using repeated fumigation fogger applications to penetrate stacked hay bales in a storage container and kill IFA colonies. Experiments were completed at the Gulfport facility in a rented 8 x 20 ft storage container, with double sealable doors, plywood floor decking, and passive air vents in each top-corner. Three stacks of pine straw comprising 2 bales each were placed adjacent to each other at the end of the container farthest from the double doors. Two bioassay pouches of live ants with approximately 10,000 ants including, egg brood, workers, and alates were placed in each of the stacks; one pouch on top of the stack, and another in-between two bales (n = 6 ant pouches). In front of these, a wall of stacked pine straw bales was created across the midsection of the container. This wall was four bales deep and extended from each side of the container, with an even height up to 1 ft. from the ceiling of the container. Three pine straw stacks were set-up in another storage container on the facility for comparative evaluations. Each pine stack was infested with approximately 10,000 ants including, egg brood, workers, and alates. Ants were placed in each of the stacks; one on top, and another in-between the bales (n = 6 colonies). A fumigation fogger was "set-off" near the double doors at the front of the container; the doors were closed, and remained closed for 3 hrs. The container doors were opened and vented for 30 minutes; per label directions. A second 3 hour fumigation application and 30 minute venting were repeated. Ants were then retrieved from the treatment stacks and scored as alive, impaired (i.e. twitching and sporadic leg movements), or dead.

Results from the control stacks showed that all the ants, both on top and in-between bales were alive and appeared normal. After the two fumigation treatments the ants in the treatment stacks that were placed on top of bales were all dead. However, ants that were placed in-between bales showed no visible signs of impairment or death. Visual examinations of these ants after 24 hours showed that all ants in the control stacks and the ants in the middle of the treated bales were alive.

DISCUSSION:

The objectives of this research were to evaluate methods of killing IFA colonies from infested pine straw. Initial results using single stacks of pine straw, individually covered with plastic, and treated with a fogger were promising. This method did kill IFA that were placed between two pine straw bales; however, this is may not be a practical or economical method for commercial pine straw producers. Also, this area treated was significantly smaller than the recommended area dosage on the label. Results on bigger area treatments such as a row of stacked pine straw indicate that ants on the surface of pine straw are easily killed if they come in direct contact with the insecticide during the chemically active period of the insecticide. However, ants that are dwelling or foraging inside of pine straw bales are protected from direct contact with the insecticide. Although both the fumigator and fogger units are intended to reach corners and permeate cracks, the active chemical ingredients were not able to penetrate the dense layers of baled pine straw and kill IFA colonies. It is important to remember these are short-lived chemicals with very little residual that are household safe. The use of a circulation fan did not increase the permeability of the chemicals within the pine straw bales. Future research should examine other fumigates that are more dispersive or less sticky than the chemicals used in these evaluations.

CPHST PIC NO: A1F01

PROJECT TITLE: Efficacy of BASF BAS 320 04 I Fire Ant Bait

REPORT TYPE: Final

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

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. In 2006 we tested the BASF BAS 320 fire ant bait (2006 annual report) and got erratic and unusual results that we speculated was due to the unusual drought conditions in 2006. In 2007 we retested BASF BAS 320 04 I fire ant bait on field colonies of imported fire ants.

METHODS AND MATERIALS:

BASF provided BAS 320 04 I fire ant bait. Commercially available Amdro® fire ant bait was used as a standard. Test plots were set up in Forrest county Mississippi at the Bobby Chain Airport, Hattiesburg, MS. 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 as well as 3 replicates for untreated controls. Treatments were applied on May 18, 2007 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 continued until reinfestation was observed. Means subjected to ANOVA and t-test ($\alpha = 0.05$).

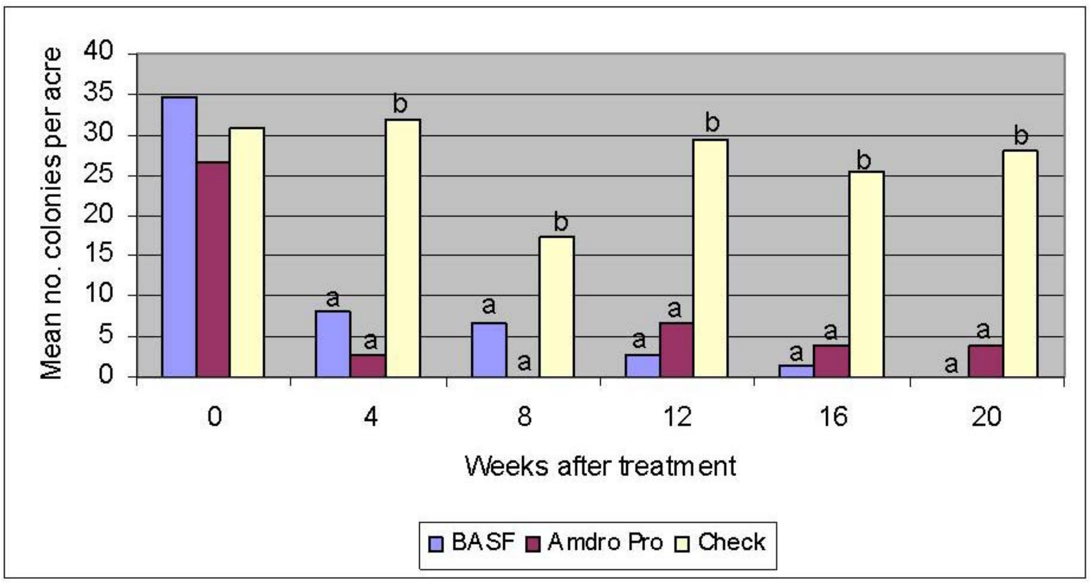
RESULTS:

While it was very hot in 2007, the site received good rainfall over the course of the study (Table 1) unlike much of the south which was under record drought conditions. The BASF bait preformed very well this year, eliminating 80-100% of the colonies (Figure 1) and decreasing the ant population by 90-100% over the course of the study. Comparisons of decreases in number of colonies and decreases in population indices from pre-treatment values show the BASF bait is statistically similar to the Amdro (Student's t test).

Table 1. Rainfall at Hattiesburg Bobby Chain airport May 18, 2007 through Oct. 2, 2007.

Time Frame	Evaluation Date	Rainfall (inches)
May 18-June 13	June 14	2.85
June 14-July 9	July 10	3.61
July 10-Aug. 8	Aug 9	8.43
Aug. 9-Sept 5	Sept. 6	1.93
Sept 6-Oct 2	Oct 3	1.82

Figure 1. Change in mean numbers of colonies per acre in plots treated with BASF BAS 320 04 I and Amdro fire ant baits – initiated May 18, 2007; Hattiesburg Bobby Chain Airport, MS



Means within a time period followed by the same letter are not significantly different (t-test; α = 0.05). Unless noted means are not significantly different.

Table 2. Mean percent change in population index per acre in plots treated with BASF BAS 320 04 I and Amdro fire ant baits – initiated May 18, 2007; Hattiesburg Bobby Chain Airport, MS

Treatment	Pre-treat mean pop. index/acre	Mean change in population index at indicated weeks after treatment				
		(4)	(8)	(12)	(16)	(20)
BASF	540.00a	-91.73a	-95.73a	-97.49a	-97.92a	-100.00a
Amdro	433.33a	-96.46a	-100.00a	-91.59a	-92.98a	-94.71a
Check	480.00a	-12.63b	-46.08b	-17.02b	-17.37b	-26.52b

Means within a column followed by the same letter are not significantly different (t-test; α = 0.05)

<u>REFERENCES CITED</u>:

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

Lofgren, C. S. and D. F. Williams. 1982. Avermectin B_1a, a highly potent inhibitor of reproduction by queens of the red imported fire ant. Jour. Econ. Entomol. 75: 798-803.

CPHST PIC NO: A1F01

PROJECT TITLE: Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing Project, 2007

REPORT TYPE: Interim

LEADER/PARTICIPANTS: Anne-Marie Callcott, Debbie Roberts, 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 and Univ. of Texas, Austin, TX. 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 three species, with others 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: Progress Report of IFA-Phorid Fly Rearing Lab, Gainesville, FL 2007). Currently (winter 2007) 3 attack (rearing) boxes are online producing the first species of fly, *P. tricuspis*, 7 boxes are producing the second species, *P. curvatus* (Formosan biotype), and 4 boxes producing a third species of fly, *P. obtusus*. 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 are 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 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://cphst.aphis.usda.gov/projects/Phorid_rearing or http://cphst.aphis.usda.gov/projects/Phorid_monitoring/.

Release techniques for the second fly species, *P. curvatus*, are somewhat less labor intensive for the releaser, but more intensive for the production facility. 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. Originally, monitoring involved 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 were found at the original release site, the cooperator moved a set distance away from the release site along the four cardinal positions and monitored for flies. Personnel continued moving away from the original release site until no flies were found. In 2007, changes to the monitoring protocols were developed due to the availability of a phorid fly trap and the number of releases that have occurred. The use of the trap will enable personnel to monitor many sites in a very short period of time – place the trap and retrieve it 24 hours later. While we would still like for original release sites to be monitored, our primary focus at this time is to determine fly presence by species at the county level. Explicit instructions for fly monitoring can be found at the same CPHST website mentioned above.

<u>RESULTS</u>:

Rearing data: Rearing was initiated in 2001 for *P. tricuspis*, seeded by flies from the ARS-CMAVE facility. The number of rearing boxes in *P. tricuspis* production has increased from the initial 1-2 boxes in 2001 to a high of ca. 10-12 boxes in 2003 to the current 3 boxes in 2007. Rearing of *P. tricuspis* was at its peak in 2003 and 2004 with ca. 1.6 million flies being produced annually (Table 1). *P. tricuspis* production has been decreased during 2007 to allow increased production of the *P. curvatus* and *P. obtusus* flies. *P. tricuspis* will continue to be released through 2008 in limited quantities with the aim to phase out production in 2009. *P. curvatus* rearing was initiated in late 2002, with the initial 1-2 boxes again seeded by flies from the ARS-CMAVE facility. Production of this species was at its peak in 2006 and 2007 (Table 2) with 7 boxes in production. In 2006, a third species, *P. obtusus* was brought into production. Production has gone well in 2007 (Table 3) and the first releases of this species will be conducted in early 2008. Combined production for all 3 species is shown in Table 4.

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. In most cases, the cooperator made the release at one site, however, in a few cases the cooperator split the release and released flies at more than one site. Also, there are several sites were multiple releases over several years have occurred. We have attempted to capture this information, but “releases” and “release sites” may not match at this time. From 2002 through 2007 there have been 2-11 releases in each of 13 states and Puerto Rico, with a total of 85 field releases (Table 4; Figure 1) and more than 760,000 potential flies released. Of these 85 releases, 59 were *P. tricuspis* and 26 were *P. curvatus*. The number of potential flies per release for *P. tricuspis* has varied between about 5,000-10,000 flies. The number of potential flies per release for *P. curvatus* is significantly higher, with 12,000-17,000 potential flies per release. This is primarily due to the difference in release techniques; the larger number of *P. curvatus* shipped is dependent on the number of worker ants submitted by the state cooperator for parasitization.

In addition to field releases, 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, conducted a few releases and then abandoned rearing flies in 2006-2007 and is now releasing APHIS reared flies only. Over 115,000 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. In 2006 and 2007, 17 and 12 releases were made, respectively. The low number in 2007 was due to a number of contributing factors including the extreme drought in the southeast and cooperator resources.

Success of the program was originally measured by successful overwintering of fly populations. However, resources do not allow all cooperators to conduct the intensive monitoring surveys needed to determine success at this level. Of the 56 releases conducted in 2002-2005, flies have been found after a winter at 27 of these sites, a 48% success rate; 19 *tricuspis* sites (AL, AR, FL, GA, LA, MS, NC, PR, SC, TX) and 8 *curvatus* sites (FL, LA, NC, OK, SC, TX). In fall 2007, as noted in the materials and methods, we introduced a new monitoring technique using a fly trap, and a new monitoring protocol of surveying for fly presence at the county level. Limited data in this format is available at this time and is shown in Figure 1.

Those sites at which flies have not been found have not been abandoned. Cooperators and others studying the flies are finding that it may take 2-4 years for flies to build populations that are easily detected in the field. Unfortunately, this was not known early in this program and many states have conducted multiple releases at the same site when they believed no flies were present a year after a release. As resources allow, all release sites will be monitored 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. 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.

As mentioned previously, APHIS is not the only group rearing and releasing phorid flies in the U.S. Several other federal, state and local groups, including ARS and universities are also rearing and releasing phorid flies. An effort is underway to compile all release and establishment data from all sources into one report. Report A3F02 reports on that effort and shows maps with release and establishment data from numerous sources.

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
P. tricuspis	2002	942,659	58,750	12	4,895.83
	2003	1,625,067	81,450	15	5,430.00
	2004	1,698,942	89,050	9	9,894.44
	2005	1,381,650	91,175	10	9,117.50
	2006	1,079,091	37,600	7	5,371.43
	2007	877,518	35,381	6	5,896.83
Total		7,604,927	393,406	59	

* 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
P. curvatus	2002	7,404	0	0	0.00
	2003	121,316	0	0	0.00
	2004	581,097	39,552	3	13,184.00
	2005	1,383,641	88,638	7	12,662.57
	2006	1,301,738	140,659	10	14,065.90
	2007	1,313,215	102,000	6	17,000.00
Total		4,708,411	370,849	26	

* approx. no. potential flies shipped for release
** does not include one attempted release that was abandoned
*** shipped for all purposes, field release, initiate rearing, education, etc.

Table 3. Rearing and release data for APHIS phorid fly rearing project – *Pseudacteon obtusus*.

Species	Year	No. flies produced	Approx. no. shipped*	No. field releases
P. obtusus	2006†	67,969	0	0
	2007	423,922	0	0
Total		491,891	0	0

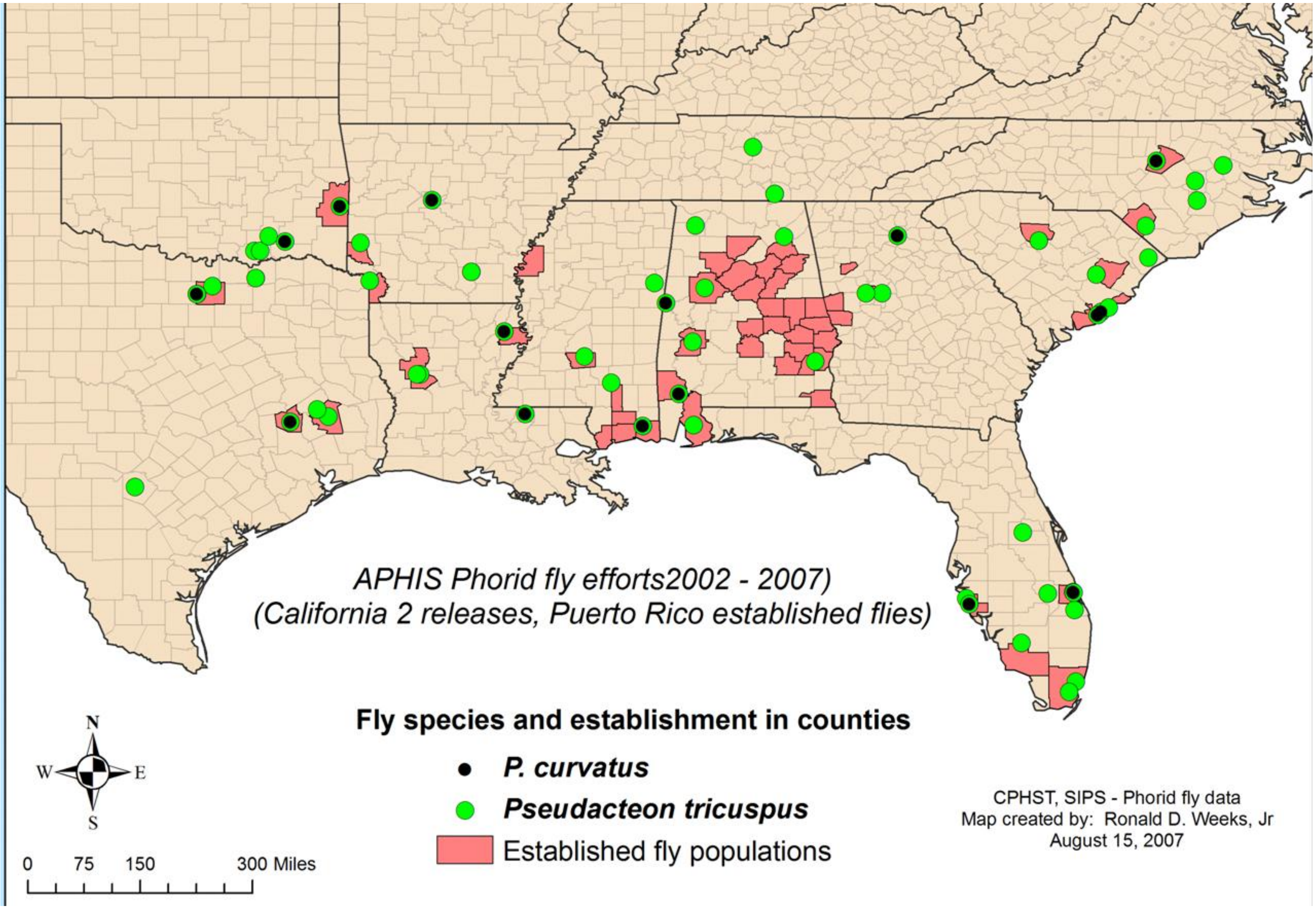
* approx. no. potential flies shipped for release
† rearing initiated in August 2006

Table 4. Rearing and release data for APHIS phorid fly rearing project – all species combined (*P. tricuspis, P. curvatus, P. obtusus*).

Species	Year	No. flies produced	Approx. no. shipped*	No. field releases**	Mean flies/ release
tri,cur	2002†	950,063	58,750	12	4,895.83
tri,cur	2003	1,746,383	81,450	15	5,430.00
tri,cur	2004	2,280,039	128,602	12	10,716.83
tri,cur	2005	2,765,291	179,813	17	10,577.24
tri,cur,obt	2006††	2,448,798	178,259	17	10,485.82
tri,cur,obt	2007††	2,614,655	137,381	12	11,448.42
Total		12,805,229	764,255	85	

* 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.
† only tricuspis shipped in 2002
†† only tricuspis and curvatus shipped in 2006 and 2007

Figure 1. APHIS phorid fly release and establishment efforts 2002-2007. Spread data not complete. California has 2 releases to date with no establishment; Puerto Rico has 3 releases with establishment at all 3 sites.



CPHST PIC NO: A3F02

PROJECT TITLE: Geographic Information Systems (GIS) Program for Monitoring Decapitating Phorid Flies in Imported Fire Ant *Solenopsis* spp. Populations, 2007

REPORT TYPE: Interim

LEADER/PARTICIPANTS: Ronald D. Weeks

INTRODUCTION:

APHIS supports the rearing and distribution of phorid flies to state collaborators for releases in imported fire ant (IFA) infested states and Puerto Rico (see Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing Project A1F01). APHIS, PPQ, CPHST coordinates release activities. USDA, ARS, Center for Medical and Veterinary Entomology (Gainesville, FL) imports flies, develops rearing methods, conducts, preliminary releases and transfers rearing technology to Florida state Division of Plant Industry. Florida DPI rears flies (Gainesville, FL) and ships releases to participating state cooperators.

Considering the vast area that IFA has invaded, a biological control effort of this magnitude requires considerable cooperation and coordination. To this end, a unique consortium of effort and resources has developed among IFA infested states and territories. This group includes 23 federal, state, and university agencies. 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. Data from these organizations and state groups are being shared, complied and organized in this GIS (geographic information systems) project.

GIS is a useful tool that is used to organize and deliver phorid fly release and spread information. Phorid fly release information collected for this project will provide regulatory officials a tool to monitor multiple phorid species releases, establishments, and spread. Also, this tool will enable cooperators to select areas for effective releases and spread.

MATERIALS and METHODS:

A typical release may consist of ca. 5,000-10,000 potential flies (i.e. ant heads with fly pupae or parasitized worker ants) that are shipped to state cooperators. In the case of *P. curvatus*, some worker ants may be removed from colonies in the field and shipped to the laboratory for parasitization and then returned to their respective colonies.

APHIS data for this project is stored in an in-house data management system that organizes paper and email information into an ArcGIS compatible format for basic reporting and data entry functions. Data are maintained using Microsoft Access® and ARCGIS 9 (ESRI®) software. Consortium data includes many federal, state, and university agencies (Table 1).

RESULTS and DISCUSSION:

APHIS has released *Pseudacteon tricuspus and P. curvatus* at more than 84 locations throughout 12 IFA infested states and Puerto Rico between 2002 and 2007 (Fig. 1). Of these releases, 58 were *P. tricuspus* and 26 *P. curvatus*. Over-wintering, a measure of population establishment, has been detected at 40.5% of release sites. A third phorid species, *P. obtusus* is being reared in 3 boxes, with the first releases planned for spring 2008. Data shared to date by consortium groups indicate that more than 126 different locations have received a least one species of flies with 54% of locations recording overwintering fly populations (Fig 2).

As more phorid fly species are released and other organizations become involved, this project will provide regulatory officials a tool to monitor multiple phorid species releases, establishment, and spread. In the future, this GIS-Phorid program may 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.

Table 1. List of consortium partners committing resources to phorid fly releases in the U.S. and Puerto Rico.

Cooperator	**Agency**
Adrain Hunsberger	UF/IFAS, Institute of Food and Agricultural Sciences Miami-Dade Extension
Aixa Ramirez	Departamento De Agricultura, Seccion De Sanidad Vegetal
Anne-Marie Callcott	USDA-APHIS-PPQ-CPHST, Mississippi
Bart Drees	Texas Cooperative Extension, Texas A&M University
Charles Barr	Texas Cooperative Extension, Texas A&M University
Dennis Mudge	University of Florida, IFAS (Institute of Food And Agricultural Sciences)
Fred Santana	University of Florida, IFAS, Sarasota County Extension
Jason Oliver	Tennessee State Univ., Institute for Agric. And Environ. Research
Kathleen Kidd	North Carolina Department of Agriculture and Consumer Services
Kelly Loftin	University of Arkansas, Department of Entomology
Ken Hibbard	Florida Department of Ag. and Consumer Services, DPI
Kimberly Schofield	Texas Cooperative Extension
Kris Godfrey	California Department of Food and Agriculture
Larry Gilbert	University of Texas
Lawrence "Fudd" Graham	Auburn University, Department of Entomology and Plant Pathology
Patrick J. Hogue	University of Florida/Okeechobee County Extension Service
Philip Stansly	UFL, Southwest Florida Research and Ed Center
Rebecca Norris	North Carolina Department of Agriculture and Consumer Services
Sanford D. Porter	USDA-ARS-CMAVE, Florida
Seth Johnson	LSU AgCenter Research and Extension
Tim Davis	Clemson University Extension
Wayne Gardner	University of Georgia, Department of Entomology
Wayne Smith	Oklahoma State University

Figure 1. Map of APHIS phorid fly release efforts (2002-2007).

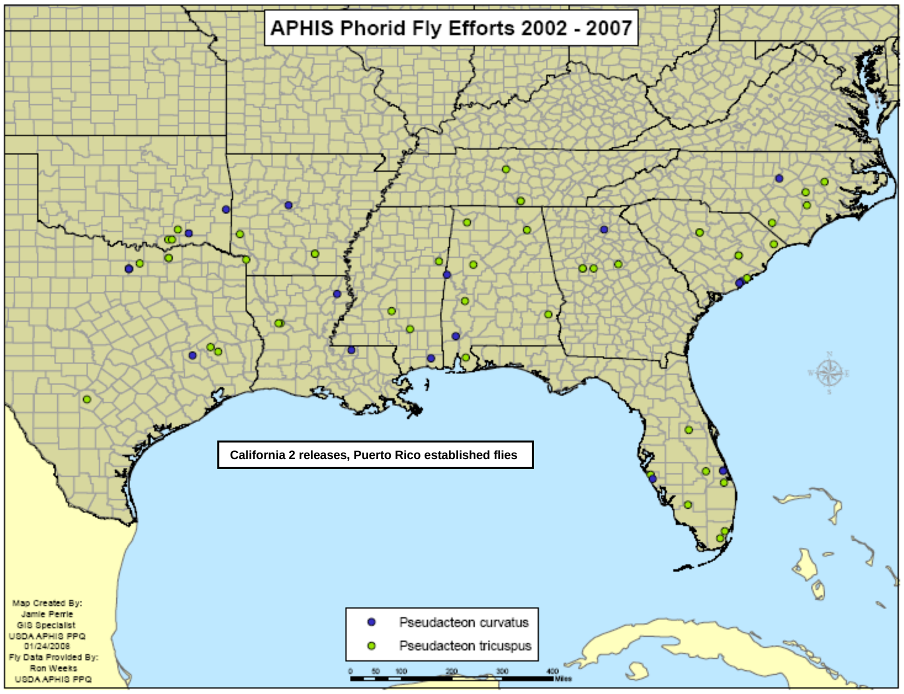
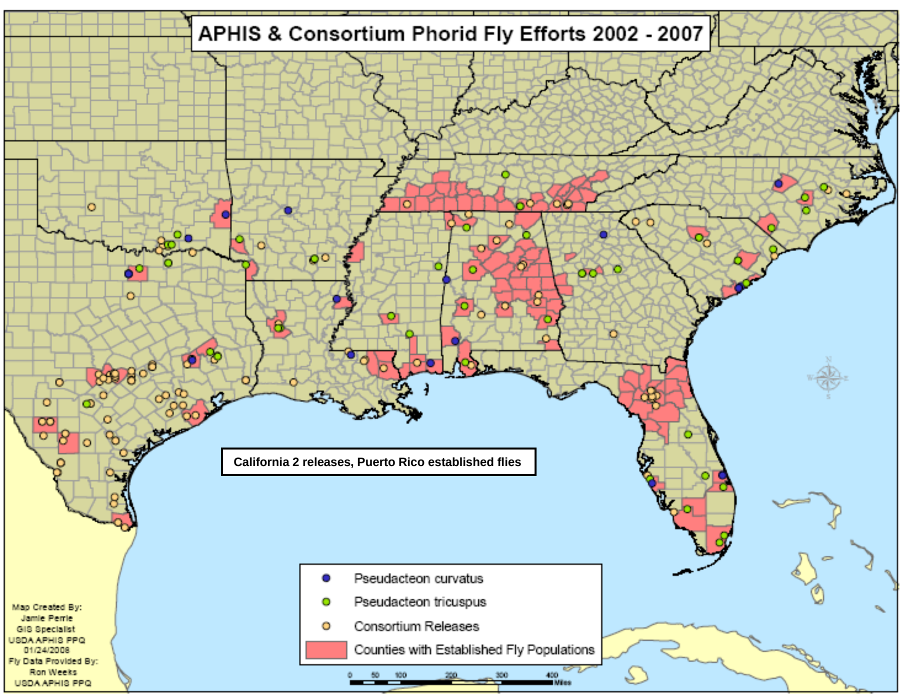


Figure 2. APHIS and Consortium releases and establishments map.



CPHST PIC NO: A1F01

PROJECT TITLE: Progress Report from IFA-Phorid Rearing Lab, Gainesville, FL 2007

REPORT TYPE: Interim

LEADER/PARTICIPANTS: Debbie Roberts, George Schneider and FL-DPI personnel

2007 was an unremarkable year as far as weather conditions here in Florida. Periods of drought and rain did not greatly affect the gathering of fire ants from the field, nor cause any great problems with brood. Weather was a factor in many of the targeted release sites, causing delays or even cancellations of scheduled releases. Strong winds and cooler temperatures in the early spring hampered some of the outside exposures.

The only major change to the system was the decrease in the number of *P. tricuspis* boxes and the increase of *P. obtusus*. In June E box was converted to *P. obtusus*, and in November the conversion of D box was changed from *P. tricuspis* to *P. obtusus.* The fourth box was necessitated by the shutdown of Dr. Porter's *P. obtusus* box. At this time there are three (3) *P. tricuspis*, seven (7) *P. curvatus* and four (4) *P. obtusus* attack boxes in production.

Production numbers

	Species	Production	Shipped
2007	*P. tricuspus*	877,518	45,581
	P. curvatus	1,329,885	110,820
	P. obtusus	423,922	0
2006	*P. tricuspus*	1,079,091	68,190
	P. curvatus	1,301,738	153,344
	P. obtusus	67,969	0
2005	*P. tricuspus*	1,381,650	125,580
	P. curvatus	1,383,641	79,008
2004	*P. tricuspus*	1,698,942	114,800
	P. curvatus	581,097	44,040
2003	*P. tricuspus*	1,625,067	111,810
	P. curvatus	121,316	0
2002	*P. tricuspus*	942,659	59,385
	P. curvatus	7,404	0

Shipments

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

	2007	2006	2005	2004	2003	2002
Field Releases	4 7	7 10	9 7	9 4	15	12
Fairs/Demos	3	3	9	4	6	3
Research Projects	2 1	10 6	4	3	2	-
Box Start-up	2	1	-	1	1	-

(Black=*tricuspis*, Red=*curvatus*, Blue=*obtusus*)

With the continued decrease in the number of *P. tricuspis* boxes comes the problem of diminishing number of available heads for each release. The release numbers for this year were cut to nearly half of those in the past. The reduced number of *P. curvatus* on the other hand, was due to the small amounts of ants being sent to the lab from the field and bad weather at the release sites.

Oklahoma was the sole recipient of parasitized colonies with queens this year. Due to their very dry summer/fall these colonies were not sent until the end of October. This was due partially to a streak of poor weather here in Florida at that time. Splitting the exposure between attack boxes and the outside took longer than the normal time due to the large amount of ants involved. At this time no word has been received on the survival of these two colonies.

Due to lack of regulatory approval for the removal of *P. obtusus* from quarantine status until December 2007, trials on the best way to handle this species were delayed until the spring of 2008. It is hoped that most problems can be worked out quickly, leading to a release in the fall of the year. Collaboration between DPI and Dr. Porter will expedite this process.

Improvements and Maintenance

Repairs to Rubbermaid #6 pan lids: As the program continues, the number of Rubbermaid pan lids that are in need of repair continues to increase as well as having to repair them. Additionally some of the pan bottoms are becoming broken and must be discarded. No satisfactory replacement has been found. The container that Dr. Porter's lab uses is incompatible for the setup here in the lab.

Shipping Containers for *P .curvatus:* Larger Lock and Lock boxes were purchased and converted into shipping containers for the colony shipments. These worked quite well and were a great improvement over the containers used last year.

Collecting Queens: In the past the collection of queens was undertaken by one or two technicians utilizing a large piece of plastic sheeting to spread the mound dirt upon. This was very time consuming and extremely difficult due to all the exposure to the ants. During the time when queens (as well as the colonies w/queens) were being collected, three technicians went out into the field and used fluoned under-the-bed boxes. Each time the queen was spotted within a few minutes by one of the three individuals as the dirt was thinly spread in the boxes, and the technicians were relatively free from ant stings, a vast improvement over the previous technique.

Improvements Updates

Vacuum Lines

The main vacuum system modification has proven to be extremely beneficial.

Collecting Equipment

The modifications to the procedure for collecting *P. curvatus* sized ants have been successful. Releasers were asked to compare it to the old method and they all seemed to like the new equipment; that being the 4 ½" wide piece of PVC pipe cut in to 8 ½" lengths.

Brood Room

Moving the brood room into the main building appeared to have increased brood production initially. Tremendous amounts of effort on the part of the staff out in the IFA trailer has kept the room up and growing, but the purpose for the brood room—to supply large amounts of brood during times of need without acquiring any from the field has not been attained. A collapse of brood room colonies both here and at ARS have been linked to the newly named *Solenopsis invicta* virus. Two strains were first identified and at this time there may be a third strain as yet unidentified that is running through the colonies again.

One improvement within the brood room was placing the sugar tubes within a dish to confine any leaking sugar water to just this basin; cutting back on the clean-up of the pans and the mortality of ants.

Diverse Sized Ants

The need for greater amounts of large ants increases with the addition of every new *P. obtusus* box. Initially there was some concern that more than three attack boxes for this sized ant could not be met, but supplies have been sufficient throughout the phasing-in period.

Summary

The production of the phorids seems to have reached a point where there is little room for improvement. When a system is working well, despite the routines, there is little need to tinker with it. The only way to improve the program at this point would be to have control over the weather…and as this is unobtainable we shall still have stumbling blocks along the way.

Extreme efforts will be made in 2008 to assist all the releasers in any way to help them arrange the releases around their schedules, weather problems or any other unforeseen situations.

2007 Imported Fire Ant Training Workshops for State Inspectors and Nursery Growers

Georgia: On March 20, 2007, PPQ headquarters, regional, state and CPHST personnel along with APHIS-IES and Georgia DPI personnel presented a training session on the federal IFA regulatory program to approximately 25 Georgia state inspectors. Topics included the biology of IFA, IFA regulations, compliance agreements, quarantine treatments, investigations into violations, etc. The session was coordinated and arranged by the PPQ-ER and GA-DPI (Anthony Man-Son-Hing, Bill Kauffman, Philip Bailey (PPQ), Mike Evans and Alan Lowman (GA-DPI)). Federal and state personnel also met with local pine straw distributors to discuss the potential of moving IFA in baled pine straw and possible treatment solutions.

Tennessee: On Aug 1-2, 2007, PPQ headquarters, regional, state and CPHST personnel along with APHIS-IES and Tennessee DPI personnel presented a training session to approximately 15 state inspectors on the federal IFA regulatory program, toured the field-grown stock nursery growing region in middle Tennessee, and held an informational meeting with Tennessee nursery growers in the McMinnville TN area. This area of TN has recently become federally regulated for imported fire ants.

Oklahoma: On October 17-18, 2007, PPQ headquarters, regional, state and CPHST personnel along with APHIS-IES and Oklahoma DPI personnel presented a training session to approximately 5 state plant inspectors on the federal IFA regulatory program, toured the nursery growing region in southeastern Oklahoma (primarily sod farms and containerized production nurseries), and held an informational meeting with Oklahoma nursery growers.

2007 Summary of Imported Fire Ant Samples Submitted to CPHST-Gulfport Laboratory for Chemical Analysis or Bulk Density Determination: Routine, Potential Violation and Blitz Samples

Prior to 2006, IFA samples submitted to the CPHST-Gulfport Laboratory, Chemistry Section for determination of insecticide levels or bulk density probably numbered fewer than 100 samples per year, and were primarily samples collected in response to potential violation incidents. In 2007, the CPHST Gulfport Laboratory, Imported Fire Ant Section began actively encouraging state plant inspectors and through them, individual nurseries, to submit soil samples to insure appropriate amounts of insecticide were present to meet the goals of the IFA quarantine. Some states have their own laboratories conduct analyses, and others submit them to CPHST-Gulfport for analysis. In 2007, the CPHST-Gulfport Laboratory IFA Section began tracking these samples and reported here is a summary of the results of the samples submitted in 2007. Results are reported back to the requesting person, unless they are blitz or potential violation results. Those results are also reported to appropriate SPHD, RPM, and HQ-IFA-PM.

Program insecticides analyzed for include chlorpyrifos, bifenthrin, diazinon, tefluthrin and fipronil. Bifenthrin is the most requested analysis, followed by chlorpyrifos, with a few requesting fipronil. Diazinon can only be used in special circumstances under section 24c labeling, and tefluthrin is not available at this time as a nursery treatment. Fipronil is only used on grass sod, and is applied at levels below the level of detection of the instruments and method currently used (applied below theoretical 0.1 ppm). In 2007, levels of detection (LOD), levels of quantification (LOQ), and range of below quantifiable level (BQL), in ppm, were:

	LOD	LOQ	BQL
Bifenthrin	0.9	3.0	0.9 – 3.0
Chlorpyrifos	0.5	1.67	0.5 – 1.67
Diazinon	0.5	1.67	0.5 – 1.67
Fipronil	0.5	1.67	0.5 – 1.67

Overview of sample numbers:

- 156 total samples submitted
  - 152 nursery samples
  - 4 sediment samples collected from around one nursery's treatment area
- 13 samples from potential violations
  - 5 incidents from AZ resulting in 9 samples
  - 1 incident from DE resulting in 4 samples
- 69 blitz samples from NC (blitzes in spring and fall)
- 70 routine samples
  - 18 samples requesting bulk density only
  - 24 samples requesting chemical analysis only
  - 28 samples requesting chemical analysis and bulk density
    - 3 of these did not contain enough sample to do both analyses; collector was contacted and decided to do bulk density only; therefore only 25 had chemical analysis conducted

Results:

- 4 sediment samples analyzed for bifenthrin; all less than detectible limit of 0.9 ppm
- 13 potential violations
  - 4 samples detected bifenthrin at > 3 ppm
  - 1 sample detected bifenthrin at bql (0.9-3 ppm); detected but below level of quantification
  - 1 sample detected chlorpyrifos at > 1.67 ppm
  - 7 remaining samples – no program chemicals detected
  - Live insect bioassays confirmed analytical results
- 69 blitz samples from NC
  - 49 samples (71%) had detectible levels of program insecticides
- 70 routine samples (some BD only, some chemical analysis only, some both)
  - 46 bulk density samples: range 268-788 lb/cu yd
  - 49 samples analyzed for 1 or more program insecticides
    - 38 samples (77.5%) had detectible levels of program insecticides
    - 11 remaining samples – no program chemicals detected