2008 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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These reports were prepared for the information of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service personnel, and others interested in imported fire ant control programs. Statements and observations may be based on preliminary or uncompleted experiments; therefore, the data are not ready for publication or public distribution.

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

Compiled and Edited by:

Anne-Marie A. Callcott

April 2009

Available online at the PPQ Imported Fire Ant website:
http://www.aphis.usda.gov/plant_health/plant_pest_info/fireants/index.shtml

2008 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.

- Emphasis on 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 and APHIS approval of all treatments shown to be effective.

OBJECTIVE 2: 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 3: Development and refinement of quarantine treatments for certification of non-traditional or non-specified articles (no work in 2008).

- Emphasis on 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 and APHIS approval of all treatments shown to be effective.

OBJECTIVE 4: Development of survey and detection tools and technologies (no work in 2008).

- 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

TYPE REPORT: Final

PROJECT LEADER/PARTICIPANT(s): Anne-Marie Callcott, Lee McAnally, chemist Connie Ramos

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 analytically 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 Analytical Chemistry section who 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 in 2005. 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 was 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 a 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. Analytically procedures can be obtained from the chemists.

RESULTS:

Results are summarized in Table 1 and Figures 1 & 2. The data 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 0.9 ppm and 3.0 ppm, 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, regardless of mixing method, showed a drop between 0 and 3 months and little change from 3 to 6 months when the trial was terminated. The 25 ppm treatments, regardless of mixing method, showed a similar drop from 0 to 6 months followed by a plateau at 6 and 12 months and another drop at 18 months with little change between 18 and 24 months when the trial was terminated. Overall, this trial indicates that in the first 6 months after mixing, there is an average decrease in bifenthrin detected by chemical analysis of approximately 42% (range of 19-72%) with most of this decrease occurring in the first 3 months after mixing (36% decrease). By 18 months, the decrease in bifenthrin has jumped to 66% of the initial amount (range of 53-81%), with only an additional 2% decrease by month 24 (range 55-83%).

Initial dose rates between mixing methods did vary, but not in any consistent manner. Dose rates over the entire test did vary between media type in the 10ppm trial (Windmill vs. MAFES bulk), but in the 25 ppm trial differences were either not evident or were inconsistent with the 10 ppm trial.

Regardless of mixing type or media type, all treatments in the 10 ppm trial had an average of 4 ppm or greater bifenthrin present at the 6 month time interval. This is amount of bifenthrin is adequate to provide quarantine level efficacy against IFA. In the 25 ppm trial, all treatments had 6.7 ppm or greater at the 24 month time interval, again providing adequate amount of bifenthrin to provide quarantine level efficacy. Wet vs. dry mixing showed differences in the initial dose rate, but these differences leveled out in subsequent monthly analyses.

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	24 mths
MAFES	Bulk	Dry	10 ppm	7.4	5.6	4.8	-	-	-
			25 ppm	25.0	-	15.4	14.7	11.2	11.3
		Wet	10 ppm	14.4	5.3	4.0	-	-	-
			25 ppm	18.8	-	13.5	13.4	8.7	7.9
	Batch	Dry	10 ppm	9.8	6.5	6.7	-	-	-
			25 ppm	46.7	-	15.4	16.2	9.1	8.0
		Wet	10 ppm	11.6	7.2	6.9	-	-	-
			25 ppm	26.6	-	15.4	14.9	9.6	9.7
Windmill	Bulk only	Dry	10 ppm	15.2	11.5	12.3	-	-	-
			25 ppm	26.7	-	16.0	14.2	8.6	6.9
		Wet	10 ppm	13.2	9.0	6.2	-	-	-
			25 ppm	24.2	-	16.7	16.3	5.8	6.7

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.

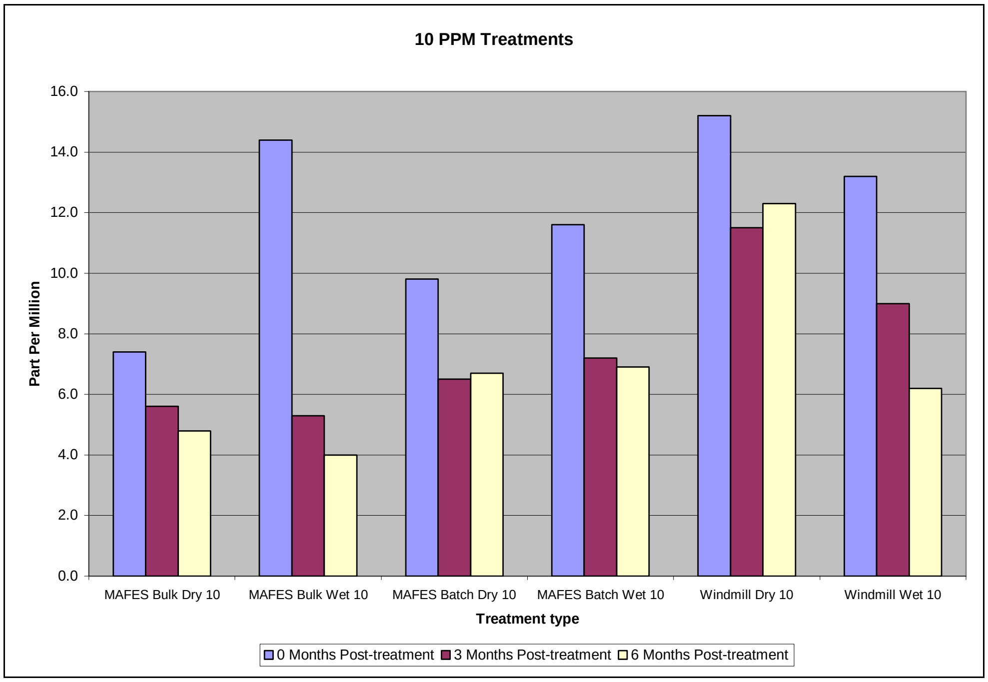
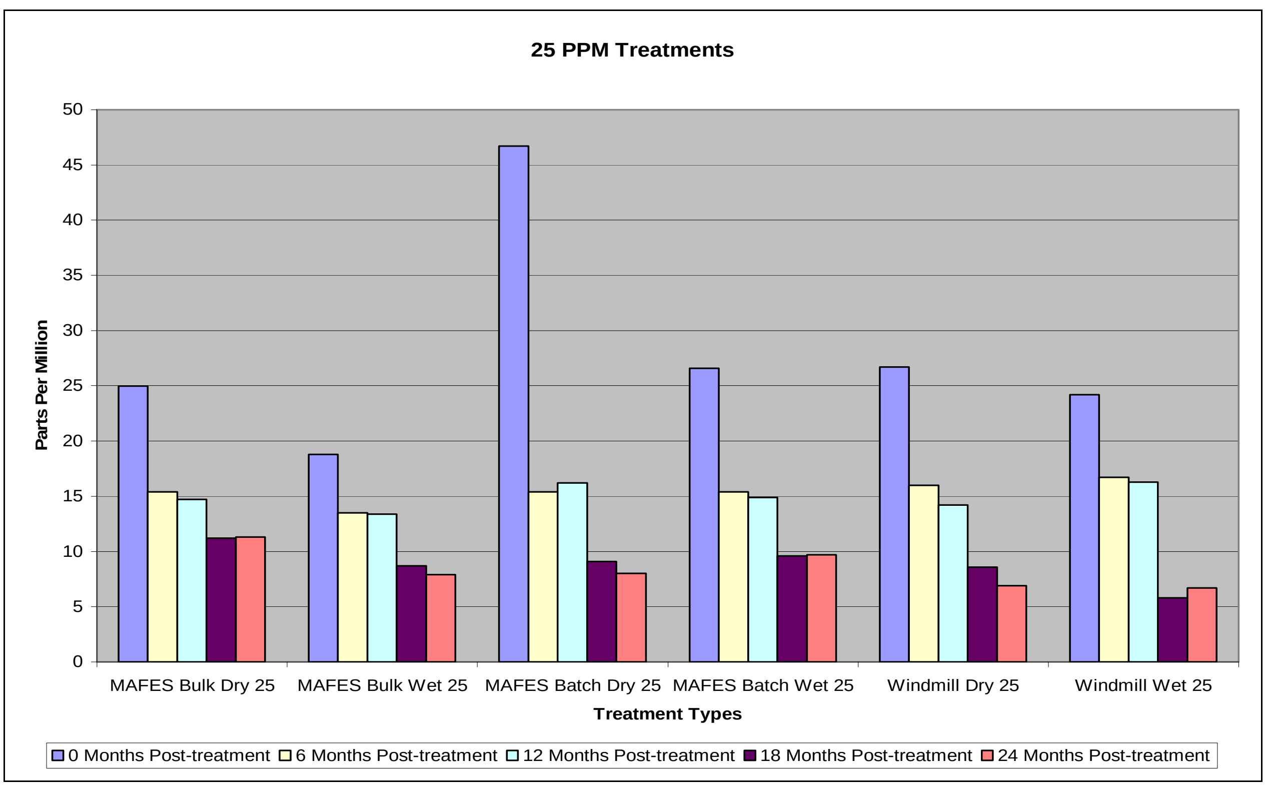


Figure 2. Results of chemical analysis of potting media incorporated with granular bifenthrin at 25 ppm.



CPHST PIC NO: A9F01

PROJECT TITLE: Chemical Degradation of IFA Quarantine Program Insecticides Used for Drench Treatment of Containerized Nursery Stock Potting Media, 2008

TYPE REPORT: Final

PROJECT LEADER/PARTICIPANT(s): Lee McAnally, chemist Connie Ramos

INTRODUCTION:

For certification in the Federal Imported Fire Ant Quarantine (7CFR 301.81), containerized nursery stock can be treated by drenching potting media with insecticide prior to shipment. Various chemical treatments result in various certification periods (e.g., 25 ppm dose rate of bifenthrin provides 180 days 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 drench insecticides focused on the efficacy of the insecticide on the target insect, and no studies were conducted to analytically determine the chemical degradation of the insecticide in potting media. In 2008, a trial was initiated to determine levels of program chemicals detected by chemical analysis over the certification/aging period of the treated media. The chemicals evaluated were bifenthrin and chlorpyrifos applied as a drench onto different potting media. This testing was done in cooperation with the CPHST Gulfport Lab Analytical Chemistry section who 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.

MATERIALS AND METHODS:

Potting media used in this test were: MAFES media (3:1:1 pine bark: sphagnum peat moss: sand with bulk density = 900 lb/cu yd); Windmill media (Windmill Nursery, Folsom, LA with bulk density = 400 pounds per cubic yd). These media where placed in one-gallon capacity plastic nursery pots.

To insure consistency over the quarantine all drench applications are made based on the dry weight bulk density of the media. However, the question on efficacy of bifenthrin and chlorpyrifos and/or adsorption of these chemicals to the media when drenched into thoroughly saturated media or very dry media has been raised. The media were tested in two ways; application to media that was watered to saturation prior to treatment and application to dry media. The bifenthrin treatments were made using both a 2EC and a flowable formulation of the product while chlorpyrifos treatments were made using the 4EC formulation. Both bifenthrin formulations were applied at a theoretical dose rate of 25 ppm while chlorpyrifos was applied at a rate equivalent to 4 fl. oz. 4EC/100 gallons of water (ca. 124 ppm in Windmill media and 56 ppm in MAFES media). The drench solutions where applied to standard 1-gallon nursery pots at a rate of 1/5 the volume of the container (ca. 450ml drench solution) as called for in the quarantine manual. The pots were then placed outdoors and weathered under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week.

Immediately after treatment, 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, 2 weeks, and monthly for 3 months post-treatment for the chlorpyrifos treatments and at 0, 2 weeks, and monthly for 6 months post-treatment for the bifenthrin treatments. Analytical procedures can be obtained from the chemists.

RESULTS:

Results to date are summarized in Table 1 and Figures 1-3. The values 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 0.12 ppm and 0.40 ppm, respectively.

All liquid bifenthrin treatments, regardless of formulation, media type, or wet vs. dry mixing, showed a general trend of decreasing residual over the 6 month trial. At three months after treatment, all bifenthrin data combined (excluding the Windmill dry F due to skewed data) show an average decrease in bifenthrin of 29% (range 19-57%). This is similar to granular bifenthrin degradation data at 3 months. However, at 6 months after treatment, all liquid bifenthrin treatments combined showed an average decrease in bifenthrin of 70% (range 47-91%). Granular degradation data did not approach this number until 18 months after treatment.

The bifenthrin EC formulation was similar within media types whether drenched over wet or dry media with a minimum average of 3.2 ppm bifenthrin present at the end of the 6 month trial. The flowable formulation was much more variable between media types and between wet vs. dry drenching method. However, even the worst case scenario of the Windmill dry drench method still retained an average of 1.38 ppm of bifenthrin at the end of the 6 month trial.

The chlorpyrifos treatments all fluctuated widely over the course of the 3 month trial. Initial dose rates were less than expected, but analyses at subsequent months sometimes showed more insecticide than initial analyses. This treatment is currently approved for use in the IFA quarantine for only a 30 day certification which takes into account the erratic nature of this insecticide.

All treatments were somewhat erratic, however, the bifenthrin treatments showed a general decline in concentration over the evaluation period. The chlorpyrifos treatments were considerably more erratic with no discernable trend. This variation is believed to be caused by variations in how quickly each individual pot drained. The slower a pot drains, the longer the insecticide solution remains in contact with the media, which therefore possibly increases the amount of chemical retained by the media. Because each pot was essentially a separate treatment, it is difficult to determine the true rate of degradation that is occurring. As a result, another trial will be conducted in 2009 using larger pots. Each pot will be one replicate with samples taken from the same pot at each post-treatment interval.

Table 1. Chemical Analysis for Bifenthrin and Chlorpyrifos Drenched onto Various Potting Media and Aged

Media Type	Chemical	Formulation	Media moisture	Initial Theoretical PPM	PPM at Indicated Post-treatment Interval (Mean of 3 samples)							
					0	2 wks	1 mth	2 mths	3 mths	4 mths	5 mths	6 mths
MAFES	Bifenthrin	2EC	Wet	25	19.83	26.33	23.67	18.00	16.00	12.10	16.00	7.90
			Dry	25	23.67	22.33	24.67	15.60	15.67	11.23	10.53	3.20
	Bifenthrin	Flowable	Wet	25	18.36	12.33	10.30	11.33	11.67	5.97	5.60	1.60
			Dry	25	37.90	25.33	25.67	15.30	21.33	27.67	9.77	13.00
	Chlorpyrifos	4EC	Wet	56	22.73	77.33	5.13	96.67	48.33			
			Dry	56	34.77	56.00	17.67	65.33	30.33			
Windmill	Bifenthrin	2EC	Wet	25	15.50	14.67	13.27	15.90	11.67	9.40	6.47	8.23
			Dry	25	14.53	8.90	10.47	5.43	10.67	7.30	3.87	4.33
	Bifenthrin	Flowable	Wet	25	43.23	40.00	34.00	38.67	34.33	25.67	13.67	17.33
			Dry	25	5.93	5.33	5.17	2.53	6.43	2.93	1.91	1.38
	Chlorpyrifos	4EC	Wet	124	69.23	94.67	42.33	61.67	119.00			
			Dry	124	62.47	50.67	22.93	35.00	37.33			

Figure 1. Results of chemical analysis of MAFES potting media drenched with bifenthrin solutions.

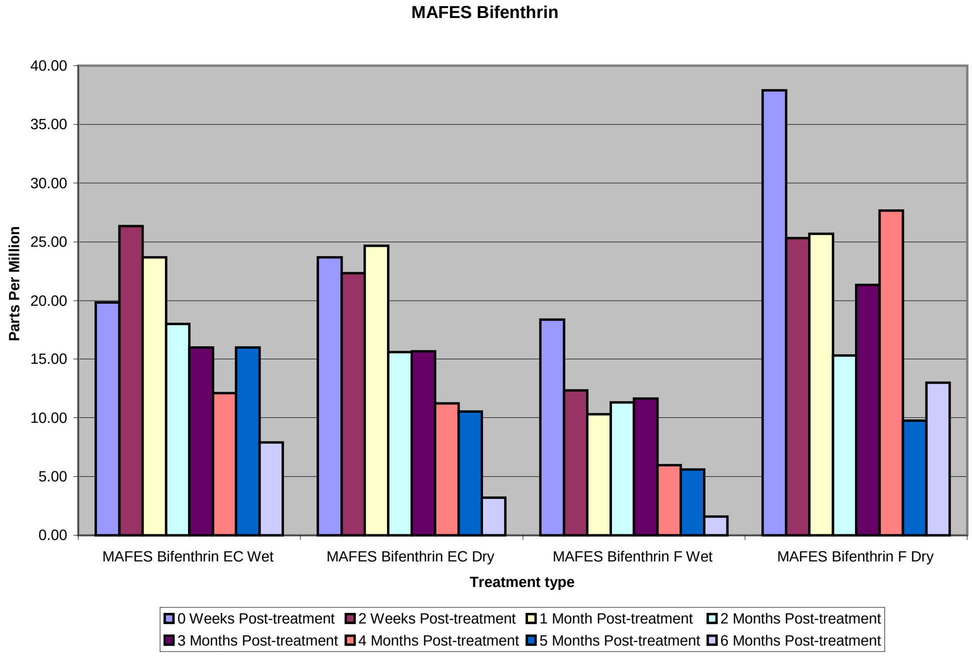


Figure 2. Results of chemical analysis of Windmill potting media drenched with bifenthrin solutions.

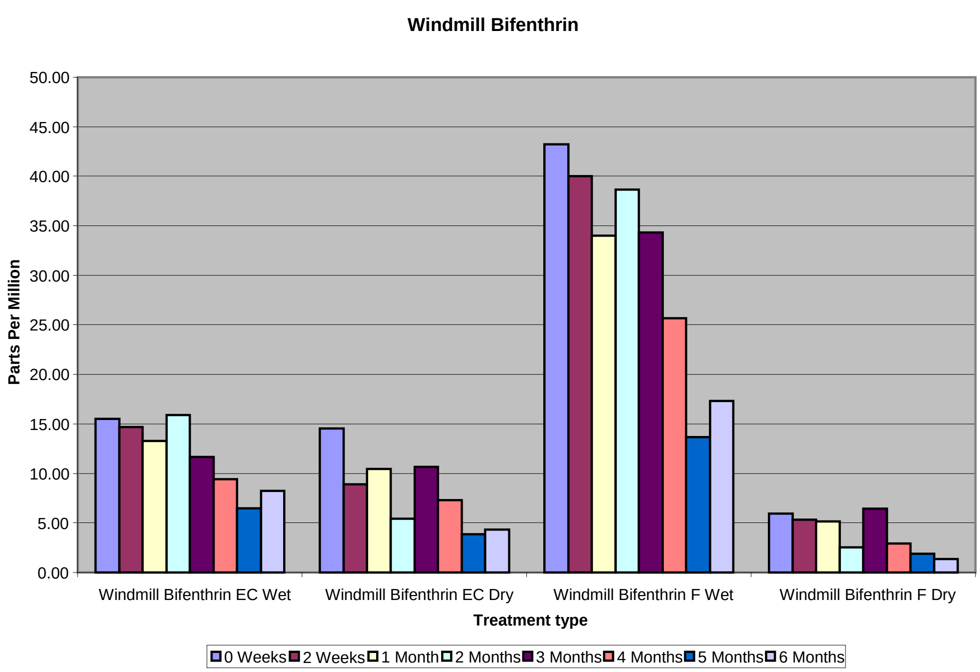
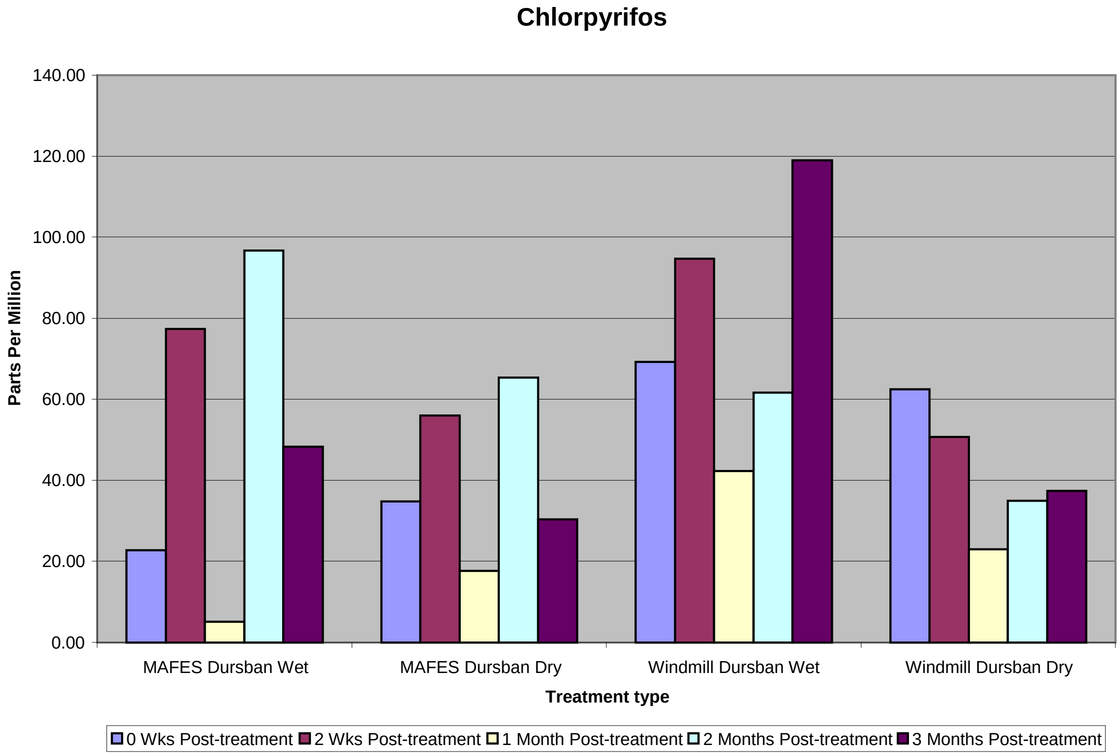


Figure 3. Results of chemical analysis of MAFES and Windmill potting media drenched with Chlorpyrifos solution.



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: Final

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.

As of late 2008, bifenthrin at 0.115 lb ai/100 gal water, is currently undergoing the USDA approval process to be added to the Federal Imported Fire Ant Quarantine as an immersion treatment for B&B nursery stock. The certification period for this rate will be 6 months. Lower rates of bifenthrin evaluated as an immersion treatment for B&B stock, 0.05, 0.025 and 0.0125 lb ai/100 gal water, have been shown to be 99-100% effective for 2 weeks to 2 months after treatment, and have maintained >90% efficacy through 6 months. At 0.006 lb ai/100 gal water, bifenthrin is >90% effective for 2 weeks to 1 month, decreasing to 85% efficacy at 2 months and falling to ca. 75% efficacy at 4 months. Many of the treatments initiated in these trials were started prior to a final decision regarding the 0.115 immersion rate addition to the regulations. Others are an attempt at controlling both IFA and JB, or an attempt to extend the consistent efficacy of lower rates of bifenthrin against IFA.

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.

After treatment, the plants were maintained outdoors 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 female alates bioassays (Fig. 2). 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 (Appendix I).

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.

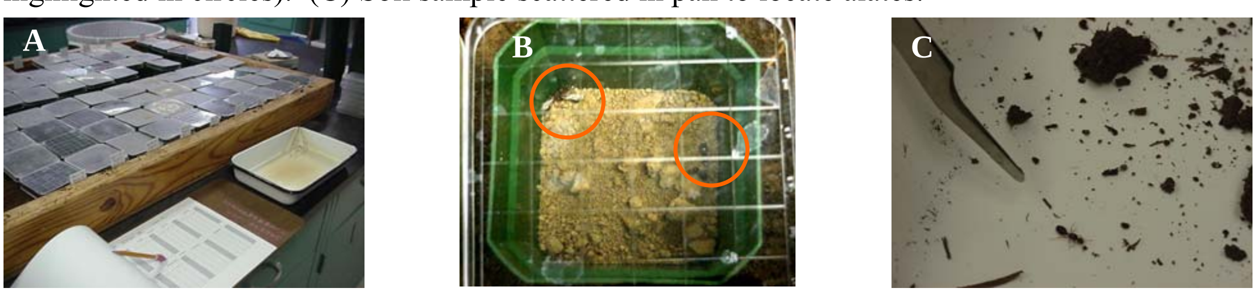


Table 1. List of treatments for 12 inch immersion trial fall 2007 TN

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 fall 2007 TN

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	0.0125 + 0.25	0.71 + 2.37ml
Talstar NF + Dylox 80TO	bifenthrin + dimethyl phosphonate	0.0125+0.25	0.71ml+1.42g
Control	--	--	--

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 100% effective throughout the 6 months of evaluation (Figure 4). Soil samples collected from inner parts of the root balls at month 5 when they were broken up for JB treatment evaluation were also 100% effective against IFA for all three treatments tested.

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.

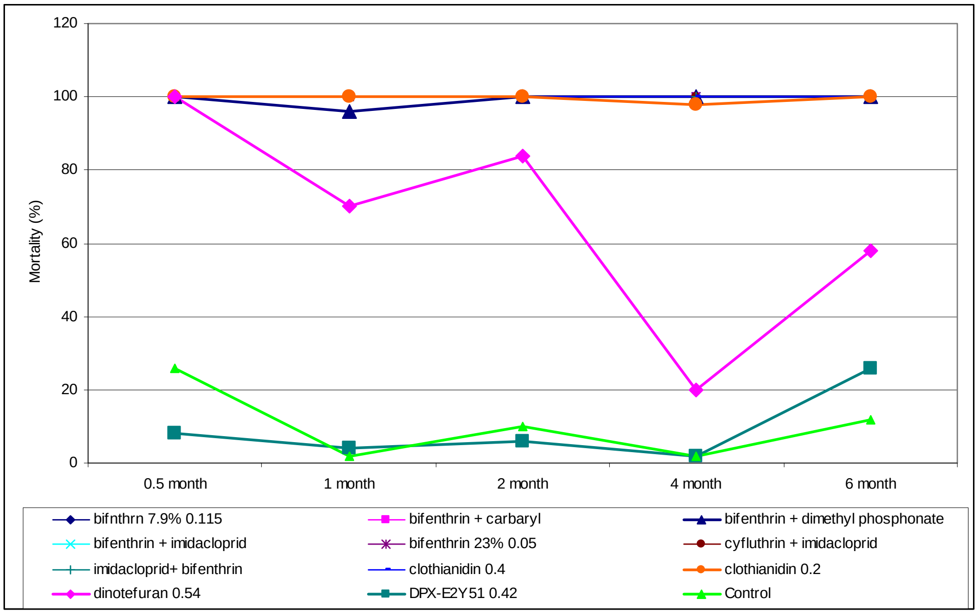
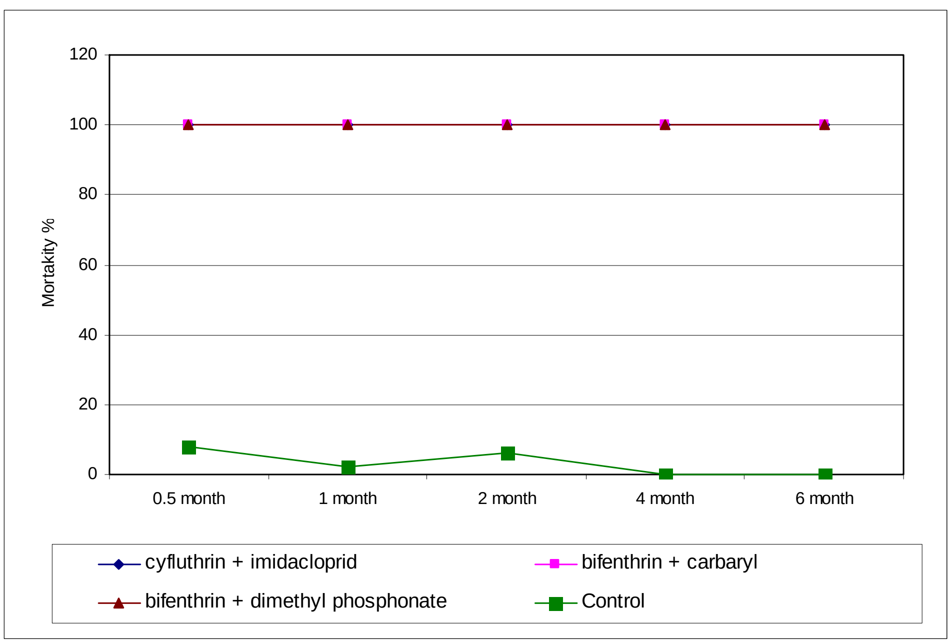


Figure 4. Efficacy of B&B dip treatments against IFA alate females in 24-inch root balls; Tennessee Fall 2007.



PROJECT NO: A1F04

PROJECT TITLE: Alternative Immersion Treatments for Balled-and-Burlapped Nursery Stock for use in the IFA Quarantine, Tennessee, Fall 2008

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.

As of late 2008, bifenthrin at 0.115 lb ai/100 gal water, is currently undergoing the USDA approval process to be added to the Federal Imported Fire Ant Quarantine as an immersion treatment for B&B nursery stock. The certification period for this rate will be 6 months. Lower rates of bifenthrin evaluated as an immersion treatment for B&B stock, 0.05, 0.025 and 0.0125 lb ai/100 gal water, have been shown to be 99-100% effective for 2 weeks to 2 months after treatment, and have maintained >90% efficacy through 6 months. At 0.006 lb ai/100 gal water, bifenthrin is >90% effective for 2 weeks to 1 month, decreasing to 85% efficacy at 2 months and falling to ca. 75% efficacy at 4 months. Many of the treatments initiated in these trials were started prior to a final decision regarding the 0.115 immersion rate addition to the regulations. Others are an attempt at controlling both IFA and JB, or an attempt to extend the consistent efficacy of lower rates of bifenthrin against IFA.

MATERIALS AND METHODS:

Treatment applications were made in March for spring trials and in October for fall trials in 2008 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 Tables 2, 3, and 4. The 24" root balls were immersed using power lifting device (Fig.1 B) in the solution of one of the treatments in Tables 1 and 5. A front-end loader with chains was used to dip root balls individually into a 1,900-liter plastic tank so that roots, soil, and burlap were completely immersed for 2 min (sufficient time for bubbling to cease).

After treatment, the plants were maintained outdoors 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 root balls (five replicates) were collected at 2.5 months (spring 2008) to evaluate 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 female alates bioassays (Fig. 2). 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 (Appendix I).

Figure 1. (A) Workers dip 12" plants in chemical solution for one minute. (B) Front-end loader with chains 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.

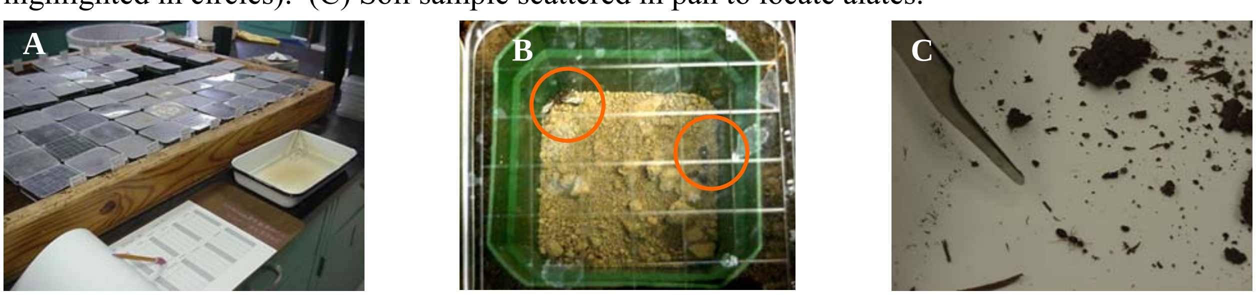


Table 1. List of treatments for 24 inch B&B stock immersion trial in TN spring 2008

Treatment*	Ingredients	Rate #ai	Amount Product/gal of water
Discus	cyfluthrin + imidacloprid	0.25+0.06	36.15 ml
Talstar+Dylox	bifenthrin + dimethyl phosphonate	0.0125+0.25	0.71 ml + 1.42 g
Talstar+Sevin	bifenthrin + carbaryl	0.0125+0.25	0.71 ml + 2.37 ml
Control	--	--	--

* Discus is a formulation with more than one active ingredients.

Table 2. List of treatments for 12 inch B&B stock immersion trial in TN spring 2008

Treatment*	Ingredients	Rate #ai	Amount Product/gal of water
Allectus	imidacloprid+ bifenthrin	0.0625+0.05	5.26 ml
Arena 50 WDG	clothianidin	0.2	1.81 g
Arena 50 WDG	clothianidin	0.4	3.63 g
Discus	cyfluthrin + imidacloprid	0.1875+0.045	27.09
DPX-E2Y51	unknown	0.42	9.25
Onyx 23%	bifenthrin	0.05	0.946 ml
Safari 20 SG	dinotefuran	0.54	12.25
Talstar N F	bifenthrin	0.115	6.53 ml
Talstar+Dylox	bifenthrin + dimethyl phosphonate	0.0125+0.25	0.71 ml + 1.42 g
Talstar+Marathon	bifenthrin + imidacloprid	0.2+0.253	11.36 + 4.79 ml
Talstar+Sevin	bifenthrin + carbaryl	0.0125+0.25	0.71 ml + 2.37 ml
Control	--	--	--

* Allectus and Discus are formulations with more than one active ingredients in the formulation.

Table 3. List of treatments for 12 inch B&B stock immersion trial in TN fall 2008

Treatment	Ingredients	Rate #ai	Amount Product/ gal of water
Allectus	imidacloprid+ bifenthrin	0.0625+0.05	5.26 ml
Allectus	imidacloprid+ bifenthrin	0.125+0.1	10.625 ml
Arena 50 WDG	clothianidin	0.4	3.63 g
Discus	cyfluthrin + imidacloprid	0.1875+0.045	27.09 ml
Onyx 23%	bifenthrin	0.05	0.946 ml
Talstar N F	bifenthrin	0.115	6.53 ml
Talstar N F	bifenthrin	0.23	12.99 ml
Talstar+Dylox	bifenthrin + dimethyl phosphonate	0.00625+0.125	3.55 ml + 0.709 g
Talstar+Marathon	bifenthrin + imidacloprid	0.1+0.1265	5.68 ml + 2.4 ml
Talstar+Sevin	bifenthrin + carbaryl	0.00625+0.125	0.355 ml + 1.18 ml
Control	--	--	--

* Allectus and Discus are formulations with more than one active ingredients in the formulation.

Table 4. List of treatments for 12 inch B&B stock immersion trial: essential oils TN fall 2008

Treatment	Ingredients	Rate #ai	Amount Product/gal of water
Armorex	Rosemary, garlic, clove, white pepper, sesame	12.5	12.5 ml
Armorex + Onyx	Rosemary, garlic, clove, white pepper, sesame + bifenthrin	12.5ml+0.025#	12.5 ml + 0.47 ml
Azatin XL	Azadiractin	17.5 ml	17.5 ml
Azatin + Onyx	Azadiractin + bifenthrin	17.5ml+0.025#	17.5 ml + 0.47 ml
Cinnacure	cinnemaldehyde	12.5 ml	12.5 ml
Cinnacure	cinnemaldehyde	37.5 ml	37.5 ml
Cinnacure + Onyx	Cinnemaldehyde + bifenthrin	12.5 ml + 0.025#	12.5 ml + 0.47 ml
Cinnacure + Onyx	Cinnemaldehyde + bifenthrin	37.5ml+0.025#	37.5 ml + 0.47 ml
Eco-Trol	Rosemary, peppermint	20 ml	20 ml
Eco-Trol + Onyx	Rosemary, peppermint + bifenthrin	20ml+0.025#	20 ml + 0.47 ml
Triact	Neem oil	37.85	37.85 ml
Triact + Onyx	Neem oil + bifenthrin	37.85ml+0.025#	37.85 ml + 0.47 ml
Onyx	bifenthrin	0.025#	0.47 ml
Control	--	--	--

Table 5. List of treatments for 24 inch B&B stock immersion trial in TN fall 2008

Treatment*	Ingredients	Rate #ai	Amount Product/gal of water
Discus	cyfluthrin + imidacloprid	0.25+0.06	36.15 ml
Onyx 23%	bifenthrin	0.05	0.946 ml
Talstar+Dylox	bifenthrin + dimethyl phosphonate	0.125+0.5	0.71 ml + 2.84 g
Talstar+Marathon	bifenthrin + imidacloprid	0.2+0.253	11.36 ml + 4.79 ml
Talstar+Sevin	bifenthrin + carbaryl	0.125+0.5	0.71 ml + 4.73 ml
Control	--	--	--

* Discus is a formulation with more than one active ingredients.

RESULTS AND DISCUSSION:

In the spring 2008 immersion trials with 12- and 24-inch root balls, treatments that had bifenthrin as the major active ingredient (alone or mixed with other chemical, or in a combined formulation) were effective against IFA female alates (Figs 3 & 4).

The combined formulation of bifenthrin + dimethyl phosphonate (Dylox) did not achieve 100% efficacy at 4 and 6 months, which was not consistent with all other bifenthrin related treatments but was consistent with the results of this treatment itself found in 2007 dip trial. The combination formulation of cyfluthrin plus imidacloprid was similar to that of bifenthrin + dimethyl phosphonate in this trial (Fig 3). Again in 2007 trial, the dinotefuran was 100% effective at ½ month, but the efficacy significantly decreased thereafter especially toward the end of the trial. In this 2008 spring trial, we did not have the 0.5 and one month bioassay results because of a sampling error in the early month but the bioassay results from month 2 to 6 showed that dinotefuran was not 100% effective, failing again to show its potential use in IFA quarantine treatment. The experimental DPX product was not different from the control at any evaluation period (Fig 4). Clothianidin at 0.2 and 0.4 lb both were 100% at 2 months but not at 4 and 6 months after final treatment. Bioassay results for the combination formulation of cyfluthrin plus imidacloprid was similar to that of clothianidian (Fig 4). Soil samples collected from inner parts of the 24” root balls at 2.5 months after final treatment, when they were broken up for JB treatment evaluation, were also 100% effective against IFA for all three combination treatments (data not shown).

In the fall 2008 immersion trials the majority of the treatments tested were either bifenthrin alone or bifenthrin combined/mixed with one other chemical. Results of the first two months bioassays showed that they were all 100% effective against IFA in both 12” and 24” dip trials (Figs 5 & 6). The only two non-bifenthrin treatments—the combined formulation of cyfluthrin plus imidacloprid and the clothianidin alone, were also achieving 100% efficacy so far at 2 months after final treatment application for both 12- and 24-inch root balls (Figs 5 & 6).

However, none of the essential oils alone treatments were effective enough against IFA for quarantine treatment purpose except when they were combined with bifenthrin where they all achieved 100% mortality in female alates bioassays (Fig 7).

Figure 3. Efficacy of B&B immersion treatments against IFA female alates in 24-inch root balls; Tennessee spring 2008.

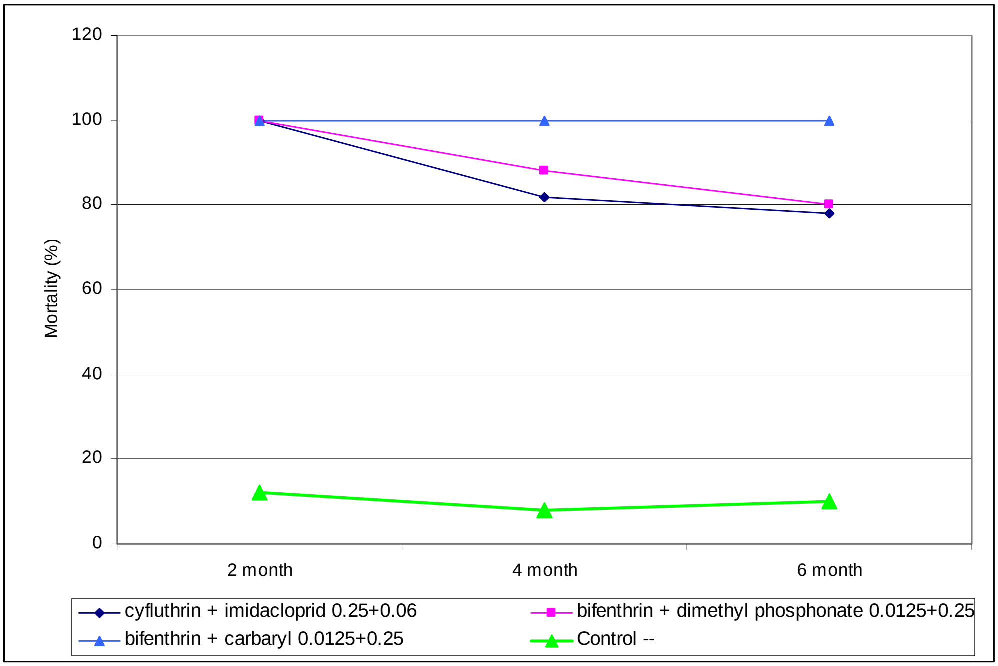


Figure 4. Efficacy of B&B immersion treatments against IFA female alates in 12-inch root balls; Tennessee spring 2008.

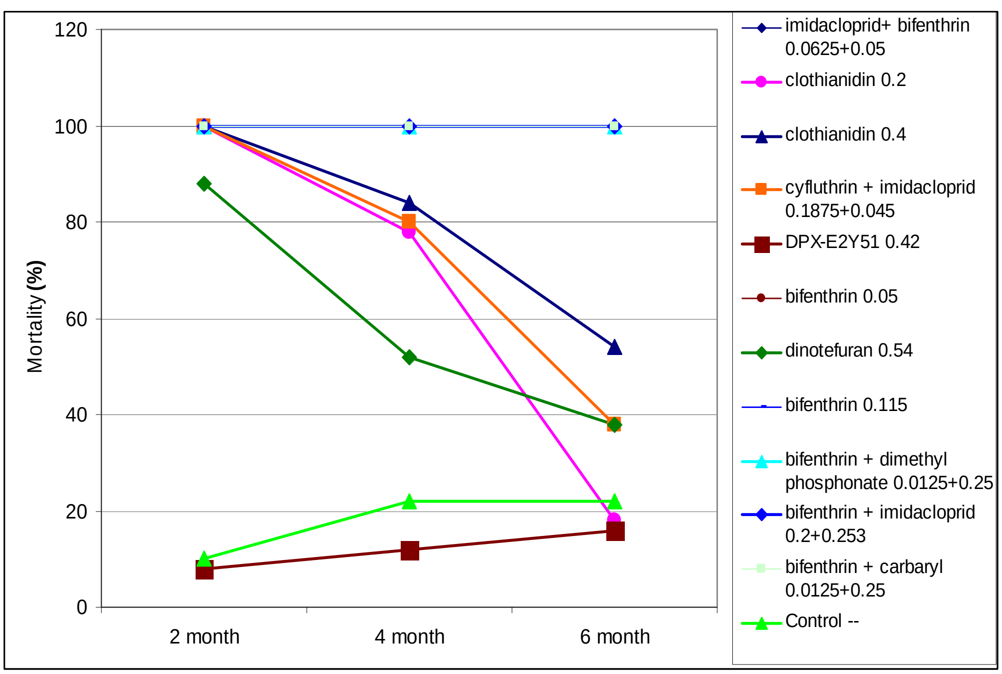


Figure 5. Efficacy of B&B immersion treatments against IFA female alates in 24-inch root balls; Tennessee Fall 2008.

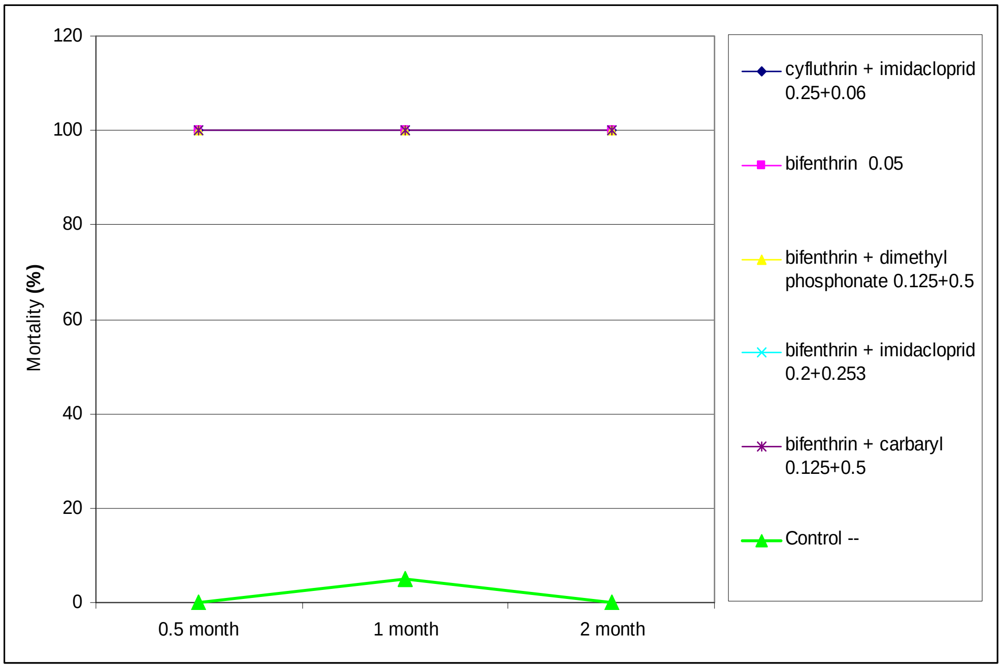


Figure 6. Efficacy of B&B immersion treatments against IFA female alates in 12-inch root balls; Tennessee fall 2008.

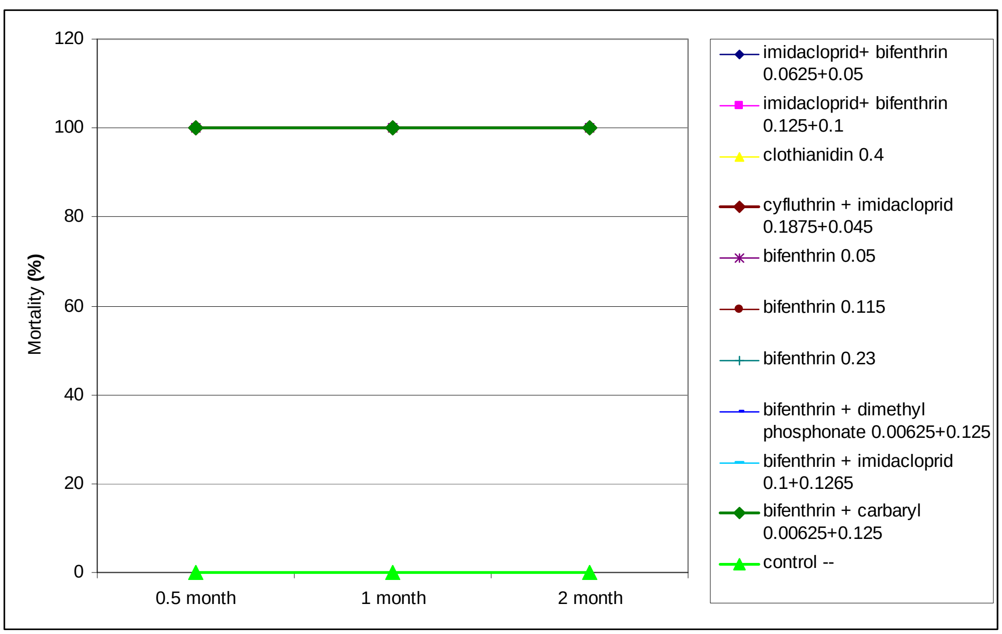
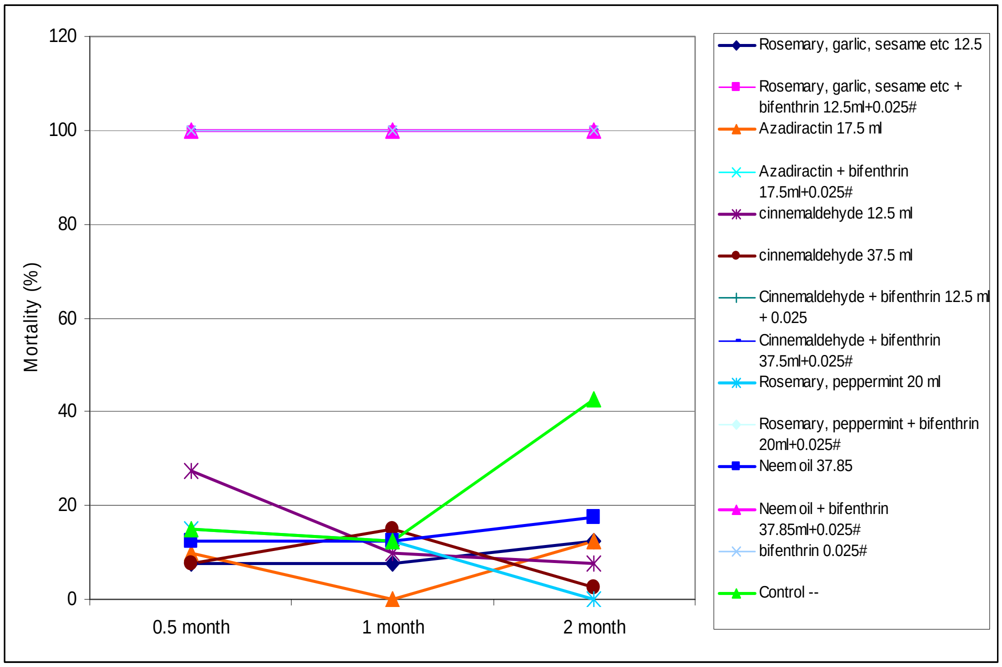


Figure 7. Efficacy of B&B immersion treatments with essential oils against IFA female alates in 12-inch root balls; Tennessee fall 2008.



CPHST PIC NO: A1F04

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

REPORT TYPE: Final

LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Craig Hinton, Lee McAnally; 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 receiving 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 (lb a.i./ 100 gal H_2O)	Handling		
			1F1	2F2	6NF
Lorsban 4E	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.

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 as follows. **1F1:** one drench in the morning; then in the afternoon, flip the trees and drench the other side of the balls. This method requires minimum chemical and days of application for drench treatments. **2F2**: one drench in the morning and another in the afternoon on one side of the root balls. The next day, flip the trees and drench two more times (morning and afternoon) for the other side of the root balls. **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

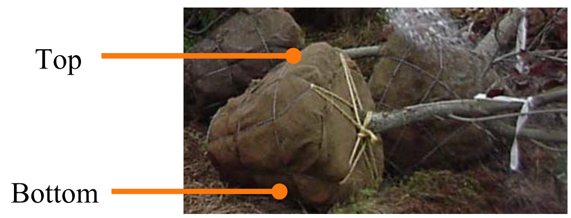


Fig.3. Soil core sample collection sites.

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.

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 (Figs 4 & 5). 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 (Appendix I).

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.

RESULTS AND DISCUSSION:

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. However, the application method 2F2 surprisingly did not do as well as 1F1 in solution coverage. Chlorpyrifos 4E 2.0 (bottom sample at month 2 and 4) and bifenthrin 0.115 (top sample at month 2) applied using 2F2 method both failed to achieve 100% mortality. The reason for this is unknown and is also difficult to explain because 2F2 application method should be at least as good as 1F1 in application coverage and penetration. 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 efficacy. The use of dye in the spray solution could be a good visual aid for assessing the evenness in application coverage for drench treatment.

Soil samples collected from bottom of root balls treated with chlorpyrifos using application method 6NF gave a mortality of only 40-60%, again showing that 6NF is not an effective treatment application method. 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).

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, addition of suitable surfactant in the drench solution should be studied.

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.

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

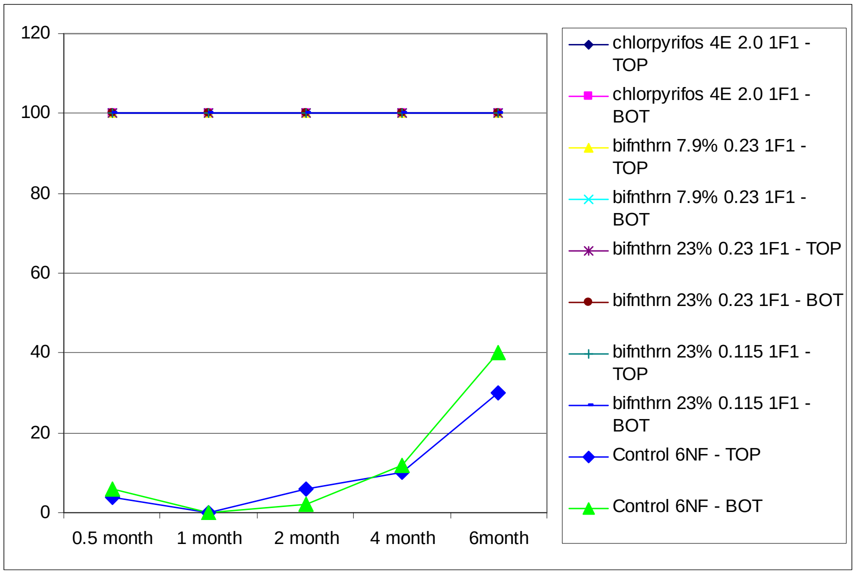


Figure 7. IFA control achieved with three chemicals treated soil samples collected at two surface sites from the application 2F2 regimes at 0.5, 1, 2, 4, and 6 months after final drench application.

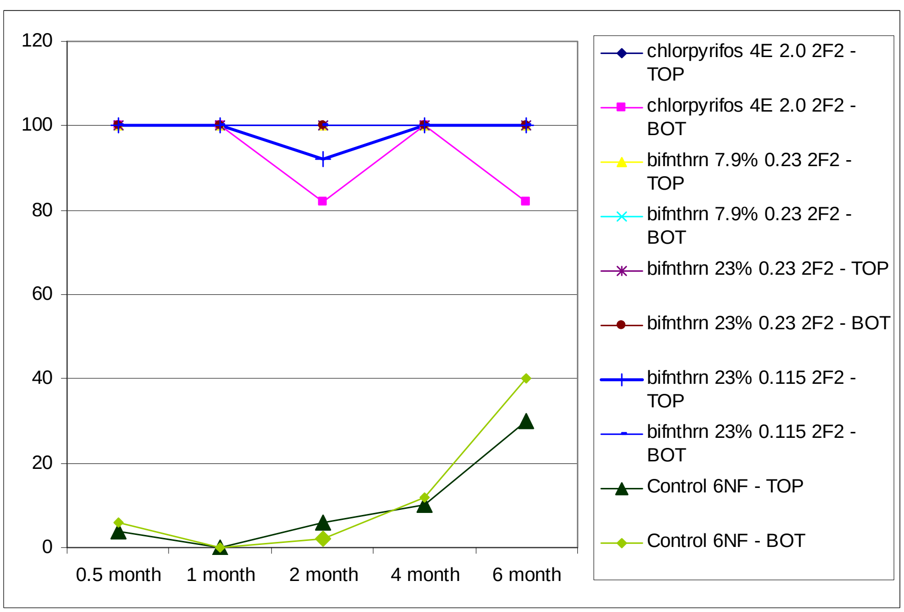


Figure 8. IFA control achieved with chlorpyrifos-treated soil samples collected at two surface sites from various application regimes at 0.5, 1, 2, 4, and 6 months after final drench application.

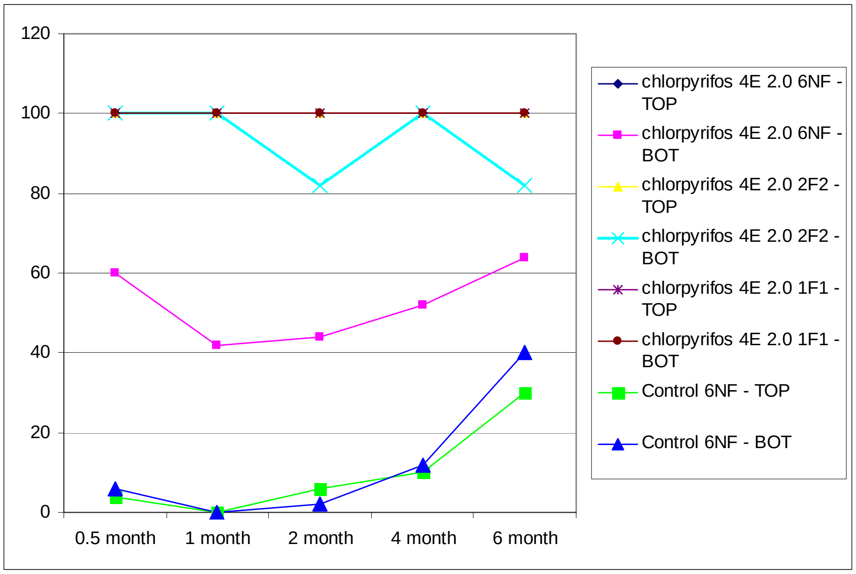


Figure 9. IFA control achieved with bifenthrin flowable (0.23 lb ai/100 gal) treated soil samples collected at two surface sites from 1F1 & 2F2 application regimes at 0.5, 1, 2, 4, and 6 months after final drench application.

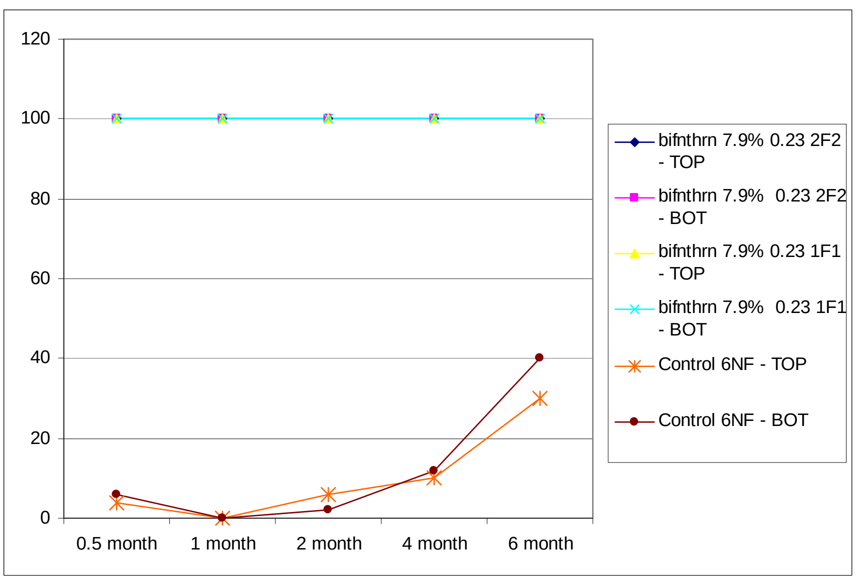
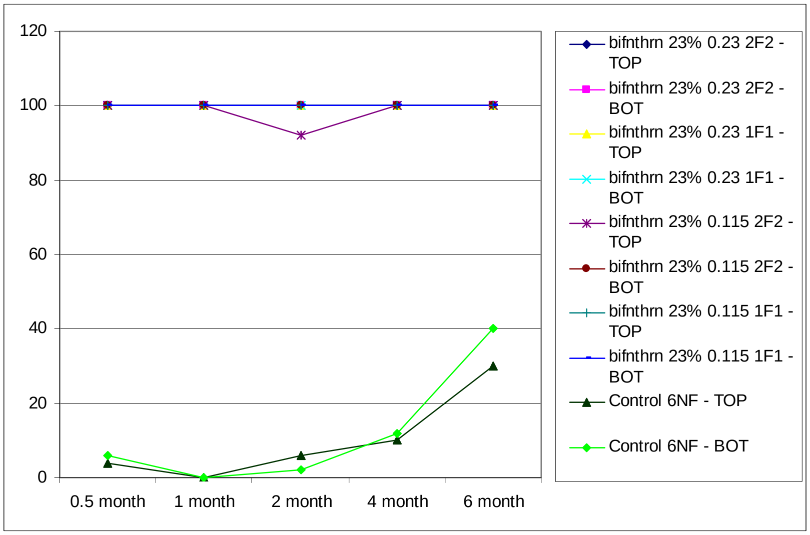


Fig.10. IFA control achieved with two rates of bifenthrin-treated soil samples collected at two surface sites from 1F1 & 2F2 application regimes at 0.5, 1, 2, 4, and 6 months after final drench application.



CPHST PIC NO: A1F04

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

REPORT TYPE: Final

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 (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 receiving 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. 1F1**:** one drench in the morning; then in the afternoon, flip the tree and drench the other side of the balls. This method requires minimum chemical and days of application for drench treatments but it was observed that much of the drench solution ran off the burlap surface while drenching. 2F2: one drench in the morning and in the afternoon on one side of the root ball. Next day, flip the tree and drench two more times (morning and afternoon) for the other side of the root ball. It

was clear from our observation that the second application penetrated better than the first drench application and chemical solution should be able to reach into the balls reasonably well. However, a good portion of the first drench liquid and part of the second drench ran off the rootball surface. Also, treatments still required two days to complete with this method.
6NF: This is the conventional and currently approved method included in the trial as a standard comparison. This method requires applying drenches twice a day for 3 consecutive days without flipping the root balls. This method is not only chemical and time consuming but also has the potential to create a run-off problem.

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. 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 wanted to include a procedure of pre-wetting the root balls with diluted surfactant to reduce run-off problem for better penetration. 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. We called this "1F1 plus".

MATERIALS AND METHODS:

In October 2007, we completed drench applications on B&B plants with 18-inch diameter root balls at the facility of CPHST Lab, Gulfport MS. Drench treatments consisted of two rates for each of the two formulations of bifenthrin (Onyx Pro 23% 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 few 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 drench. 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	#	Active Ingredient	Rate #ai/ 100 gal	Rate ml prod./gal	Water vol./drench	Amount of Insecticide per drench	Total Amount Applied
Bifenthrin Pro 7.9%	1	Bifenthrin	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	Bifenthrin	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. But 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 prepared in 5-gal plastic bucket with polypropylene liners and pumped through a hose attached to a spray nozzle using a battery-powered pump (Figure 1 A). 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 (Figure 1 B). Each root ball received approximately one gallon of drench solution at each drenching.

Figure 1. A: Drench application; B: Flipped over rootballs ready for treatment application to the side that was not directly sprayed on.

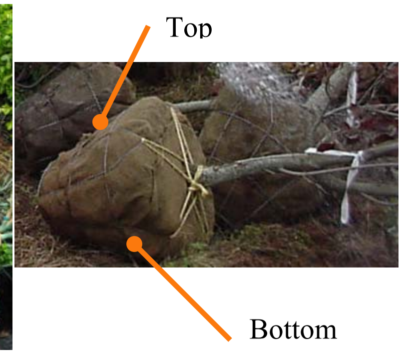


Figure 2. Soil core sample collection sites.

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 and bottom were sampled on each plant to examine evenness of coverage (Figure 2). Soil samples were collected from within the first four inches of soil core depth for bioassay testing against IFA (Figures 3 & 4). 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 (Appendix I).

Figure 3. A tray of alate mortality bioassay cups.

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

RESULTS AND DISCUSSION:

These results showed that bifenthrin treated rootballs were consistent in generating 100% mortality regardless of bifenthrin formulations, application rates tested, and soil core sampling positions (Figures 5). Since there were no differences on efficacy between sampling positions (100% for all samples), top and bottom soil core bioassay results were pooled together in this report. Accordingly, control treatments were also pooled to become one single treatment.

Results also showed that the application method “1F1 plus” achieved a 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 application methods investigated. Run-off should also be at the minimum after pre-wet with surfactant. With both sides of the rootball being well drenched and chemical solution penetrating into the root ball, we could expect the results of this treatment method not much different from that of the 2F2 application method but having the benefit of shortening one day and reduction of run-off problems.

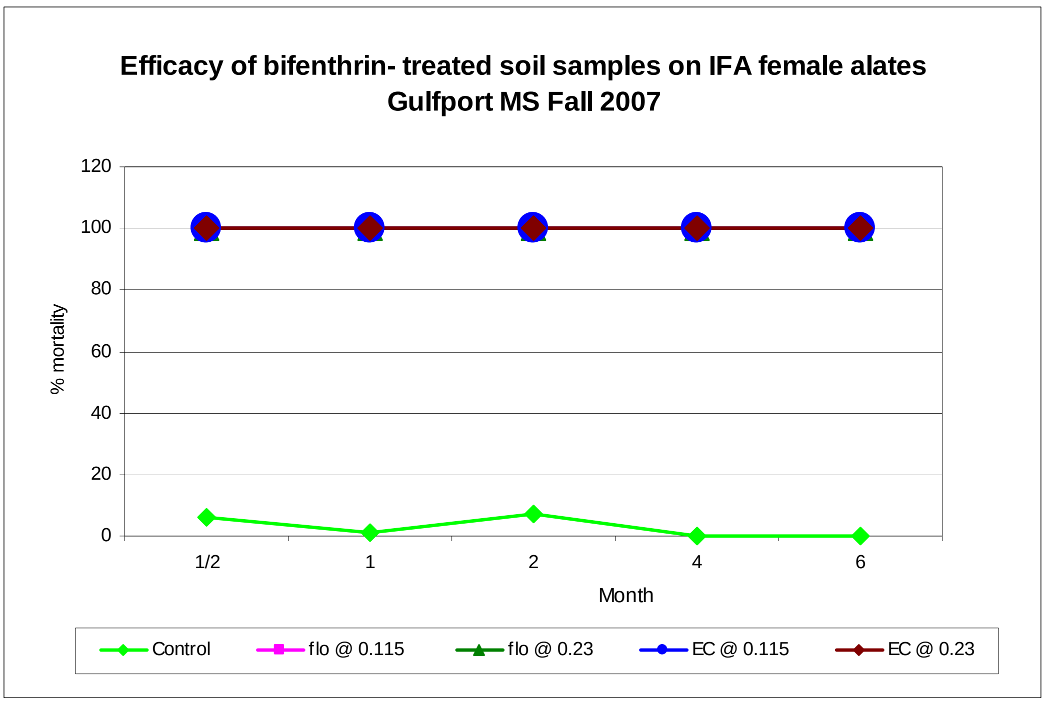


Figure 5. IFA control achieved in bifenthrin treated soil samples collected at two surface sites at various sampling intervals after final drench application. Plants rotated once between 2 drench applications in one day.

CPHST PIC NO: A1F04

PROJECT TITLE: Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Tennessee, Spring and Fall 2008

REPORT TYPE: Interim

LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Craig Hinton, Lee McAnally; 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 receiving 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 from spring 2005 to fall 2007 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. Results of such trials can be found in our annual reports each year from 2005 to 2007. It is clear that rotating root balls during treatment application leads to a uniform coverage of the spray treatment and a consistently effective bioassay results.

2008 drench trials in TN again focused on examining some promising insecticides and plant handling methods for 24" root balls (spring 2008) and 12" root balls (fall 2008). Multiple insecticides and their combinations, application frequencies, and plant handling methods (rotating vs. non-rotating) were investigated.

MATERIALS AND METHODS:

In March 2008 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 three chemical solutions or a water only control. Solutions, final rates, and plant handling which composed the treatments are listed in Table 1 below.

Table 1. List of treatments for 24" drench trial in TN spring 2008

Product	Active Ingredient	Rate (lb a.i./ 100 gal H_2O)	Plant Handling		
			1F1	2F2	6NF
Lorsban 4E	Chlorpyrifos	0.125	X	X	X
OnyxPro 23%	Bifenthrin	0.115	X	X	
OnyxPro 23%	Bifenthrin	0.230	X	X	
Control	----	----			X

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 as follows. **1F1:** one drench in the morning; then in the afternoon, flip the trees and drench the other side of the balls. This method requires minimum chemical solution and days of application for drench treatments. **2F2**: one drench in the morning and another in the afternoon on one side of the root balls. The next day, flip the trees and drench two more times (morning and afternoon) for the other side of the root balls. **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 the need of flipping the root balls. The water control also followed this no-flips (6NF) treatment application method. 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.

Table 2. List of treatments for 12 inch drench trial in TN fall 2008

Product	Active Ingredient	Rate (lb a.i./ 100 gal H_2O)	Handling	
			1F1	2F2
Allectus	imidacloprid+ bifenthrin	0.125+0.1	X	X
Lorsban	chlorpyrifos	0.125	X	X
Onyx 23%	bifenthrin	0.115	X	
Onyx 23%	bifenthrin	0.1	X	X
Talstar+Dylox	bifenthrin + dimethyl phosphonate	0.00625+0.125		X
Talstar+Sevin	bifenthrin + carbaryl	0.00625+0.125		X
Control	--	--		X

Fig.1. TN personnel applied drench treatment to B&B trees with 24" rootballs

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

Fig.3. Top and bottom soil core samples taken from root balls

Fig.4. 12" rootballs grouped for drench

Fig.5. USDA ARS personnel applied drench treatment to 12" B&B rootballs

The fall trial was conducted in October 2008 for 12" rootballs only (Fig 4). The treatments are listed in Table 2 and they were applied using a regular garden watering can (Fig 5). The drench solution was 3.586 gallons to be applied to each treatment. Other than chlorpyrifos and bifenthrin, combinations of bifenthrin and other chemicals were also investigated in this trial. However, only the treatment application methods 1F1 and 2F2 were used in this trial excluding the conventional 6NF application method even for the treatment of chlorpyrifos. This is because we had found over the past few years that flipping the rootballs during treatment application was necessary to achieve an even coverage of chemicals and consistent bioassay results for the drench treatment application method.

For both spring and fall 2008 drench trials conducted in TN, although the total 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.

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 (due to a mistake in sampling, the 0.5 and 1 month soil samples were not taken for this trial). 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 (Figs 6 & 7). 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 (Appendix I).

Figure 6. A tray of alates mortality bioassay cups.

Figure 7. Orange circles indicate the locations of clusters of female alates within this bioassay cup.

RESULTS AND DISCUSSION:

Drench trial spring 2008 on 24" rootballs

Both treatment application methods 1F1 and 2F2 showed promise to become the method of choice for drench treatment because at the end of 6-month weathering naturally, all treatments tested, regardless of chemicals, rates, and surface sampling sites, achieved 100% in mortality except the bottom samples of bifenthrin treatment at 0.23 1F1 which was slightly less than 100% (Figs 8 & 11). However, this slight deviation was most likely an error with sampling or bioassay rather than with the chemical or application method. The application method 2F2 achieved 100% mortality throughout the 6 month period for all treatment tested in this trial (Fig 9). Therefore, it is with confidence to say that the application methods 1F1 and 2F2 both would work well as a drench treatment application method, especially for the bifenthrin.

Figure 8. IFA control achieved with various chemicals treated soil samples collected at two surface sites from the application 1F1 regimes at 2, 4, and 6 months after final drench application Spring 08.

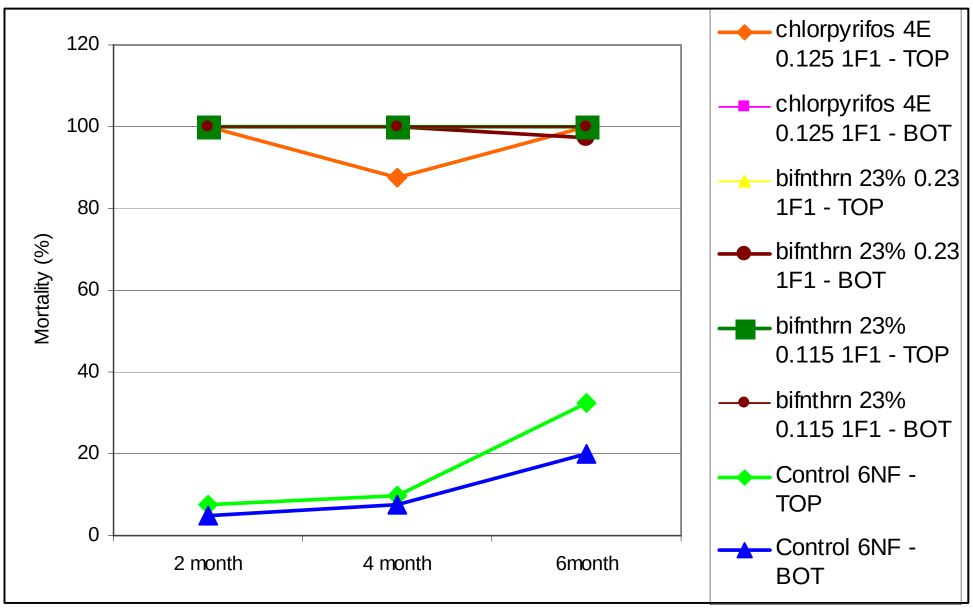
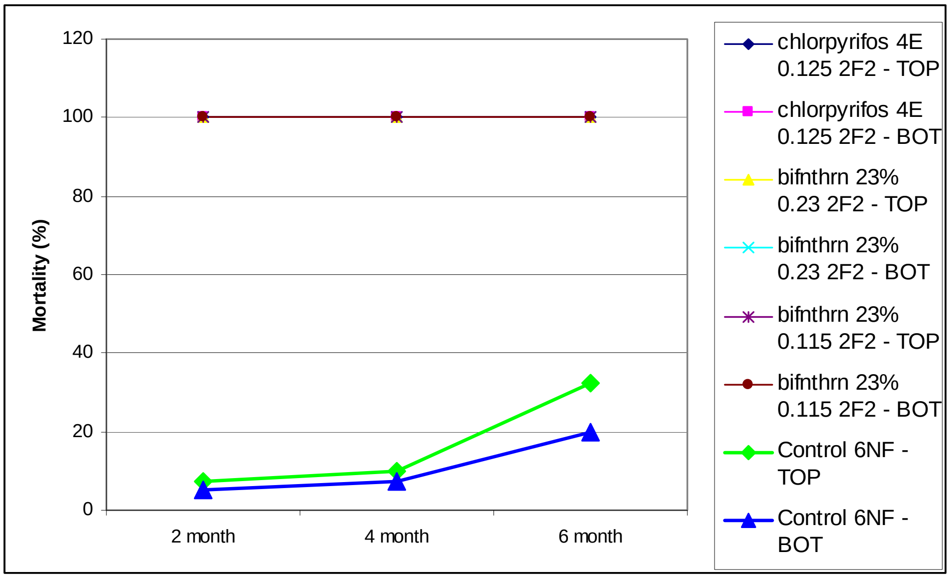


Figure 9. IFA control achieved with three chemicals treated soil samples collected at two surface sites from the application 2F2 regimes at 2, 4, and 6 months after final drench application Spring 08.



Soil sample collected from top of root balls treated with chlorpyrifos 0.125 using application method 6NF gave a mortality of 82% at 4 month, again showing that 6NF is not the most effective application method for drench treatment. Bioassay results from bifenthrin treated root balls were fairly consistent with only one soil sample giving less than 100% mortality at 6 month (Fig 11).

Figure 10. IFA control achieved with chlorpyrifos-treated soil samples collected at two surface sites from various application regimes at 2, 4, and 6 months after final drench application Spring 08.

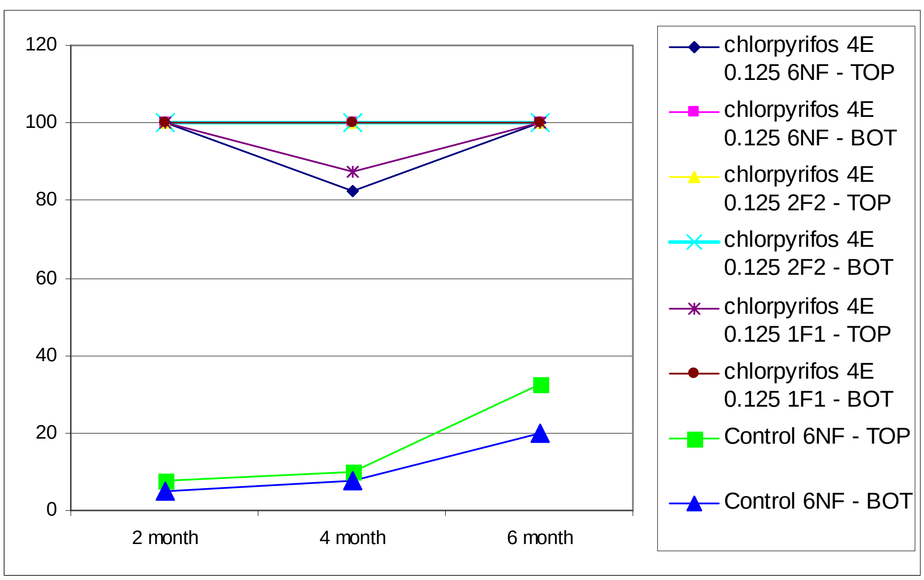
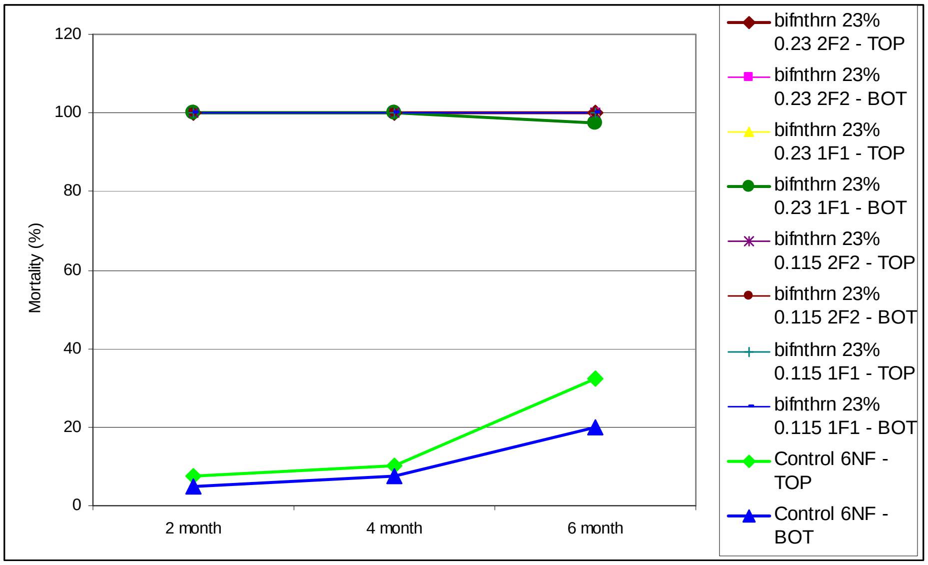


Figure 11. IFA control achieved with two rates of bifenthrin-treated soil samples collected at two surface sites from 1F1 & 2F2 application regimes at 2, 4, and 6 months after final drench application Spring 08.



Drench trial fall 2008 on 12" rootballs

At the time this report was written, there were bioassay data for 0.5 and two months soil samples taken after the final treatment application. These early results showed that all chemicals tested in this trial (see Table 2), regardless of tested rates, application method (1F1, 2F2), and in single chemical or in combination of more than one chemical, achieved a 100% in mortality in the IFA female alates bioassay.

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 Used in the IFA Quarantine, Mississippi, Fall 2008

REPORT TYPE: Final

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 (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 receiving 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. 1F1**:** one drench in the morning; then in the afternoon, flips the tree and drenches the other side of the rootballs. This method requires minimum chemical and days of application for drench treatments but it was observed that much of the drench solution ran off the burlap surface while drenching.

2F2: one drench in the morning and in the afternoon on one side of the root ball. Next day, flip the tree and drench two more times (morning and afternoon) for the other side of the root ball. It was clear from our observation that the second application penetrated better than the first drench application and chemical solution should be able to reach into the balls reasonably well. However, a good portion of the first drench liquid and part of the second drench ran off the burlap surface. Also, treatments still required two days to complete in this method.
6NF: This is the conventional and currently approved method included in the trial as a standard comparison. This method requires applying drenches twice a day for 3 consecutive days without flipping the root balls. This method is not only chemicals and time consuming but also having a major run-off problem.

Results from drench trials conducted in TN and Gulfport Lab in fall 2007 showed that the application method 1F1 was a good method to do drench application. The question now is: how long should we wait before flipping the rootball and drench the other side? Growers would rather not to wait for a few hours before flip the balls and drench the other side. The objective of this trial was to repeat the 1F1 drench and compare the different wait time between the two drenches: half an hour vs. five hours.

MATERIALS AND METHODS:

Drench application

In our previous drench trials in TN and in Gulfport, the application method "1F1" means one drench in the morning; then in the afternoon, flip the trees and drench the other side of the rootballs. Between the two drenches, there was usually a "drying period" of a few hours. Although not specifically designed to have a fixed-length wait period in between but it is a natural way to do drench treatment especially for small scale experiment. However, it was unknown if this drying period between drenches are truly necessary from the standpoint of treatment effectiveness. Nursery growers expressed that they would rather finish the drenches to both sides of the rootballs in a row without having to wait for a few hours before flipping and drenching to the other side of the rootballs. Also, when they do drench application to a large number of harvested trees, it usually will take them quite a while to drench one side before they can come back to flip and drench the other side of the rootballs. Therefore, it would be helpful to find out if waiting for a few hours before flip and drench would make a difference on treatment efficacy. To investigate the effect of this drying period between drenches, we included in our trial a wait period of 0.5 hour (representing drenching in a row) and 5 hours between drenches.

On the day of drench treatment (September 5, 2008), we did one drench in the morning to all the rootballs in the test; it took us about half an hour to finish drenching all the rootballs. We could finish it so quickly because we used a 5-gallon bucket for preparing the solution of each chemical and used a battery-powered pump sprayer to siphon the solution out directly from the bucket while spraying. Then we flipped the rootballs of the 0.5-hour group and drenched them again to the other side. For the rootballs that required a 5-hour wait time, we did the flipping and drenching in the afternoon of that day.

Drench treatments consisted of two chemicals--bifenthrin 23% and chlorpyrifos 44.9% and a water only control. Chemicals used, solutions, final application rates, and handling which composed the treatments are listed in the table below (Table 1).

Table 1. Treatment list for 1F1 drench trial in Gulfport, Mississippi Fall 2008

Material	Active Ingredient	Trt #	Hrs before flip	Rate #ai/ 100 gal	Rate ml prod./gal	Water vol/drench	Amount of Insecticide per drench	Total Amount Applied
Dursban 4E (44.9%)	Chlorpyrifos	1.1	0.5	0.125	1.18	3.5 gal	4.14 ml	8.28 ml
		1.2	5	0.125	1.18	3.5 gal	4.14 ml	8.28 ml
Onyx Pro 23%	Bifenthrin	2.1	0.5	0.05	0.94	3.5 gal	3.3 ml	6.6 ml
		2.2	5	0.05	0.94	3.5 gal	3.3 ml	6.6 ml
	Bifenthrin	3.1	0.5	0.1	1.87	3.5 gal	6.5 ml	13 ml
		3.2	5	0.1	1.87	3.5 gal	6.5 ml	13 ml
Control		4.1	0.5	--	--	3.5 gal	--	--
		4.2	5	--	--	3.5 gal	--	--

*Balls were rotated once between the two chemical drenches

The balled and burlapped plants with 18-inch-diameter root balls were purchased from Deep South Nursery, Lucedale, Mississippi. Five rootballs were used in each treatment. Water volume per drench was determined by measuring the rootball volume (7 gal per ball) and taking 1/5 of the volume (1.4 gal) to be used for total spray volume of each ball. Since this total volume was divided into 2 partial drenches, each partial drench used 0.7 gallons per tree and 3.5 gal per treatment of 5 trees. Insecticidal solutions were prepared in a 5-gal bucket and siphoned through a hose attached to a battery-powered sprayer (Figure 1). Our drench applications showed that this water volume was about right and it reached the point of run-off when finished but without having too much run off to the ground.

Figure 1. Drench application

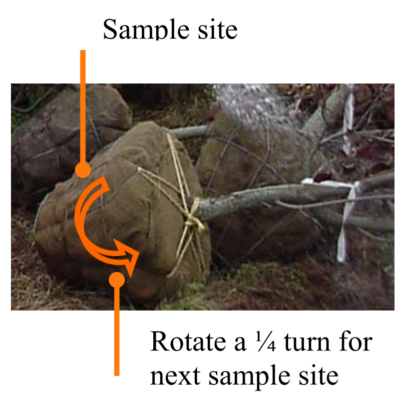


Figure 2. Soil core sample collection sites

Sampling and bioassay:
After final treatment, the plants were maintained outdoors to weather naturally and irrigation schedule was set up to closely simulate outdoors nursery storage conditions. Soil core samples were collected at 0.5, 1, 2, 4, and 6 months post-treatment for laboratory bioassay using female alates. Since we flip-drenched the rootballs, we assumed that they should have received uniform coverage of drench solution all around and there was no need to sample the top and bottom soil core samples like we did in this kind of trial in the past. So we used a different sampling method this year in our MS drench trial. We took only one soil core sample for each rootball from the mid-side area of the ball at the initial bioassay day. On next sample day, we rotated the rootballs for a quarter turn (as shown in Fig 2) and took a soil core from the mid-side of the rootballs at the new location. We rotated the rootballs again for a quarter turn and took the third soil core from the mid-side area and so on. We continued this sampling method until the last set of samples was taken at the end of 6 months post-treatment. This way, we reduced the number of soil samples in half and at the same time, sample sites covered the entire surrounding of a rootball instead of only the top and bottom, which would reflect the coverage of drench treatment better than just sampling two sites of the rootballs. Soil samples were collected from within the first four inches of soil core depth for testing against IFA female alates (Figures 3 & 4). 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 (Appendix I).

Figure 3. A tray of alate mortality bioassay cups.

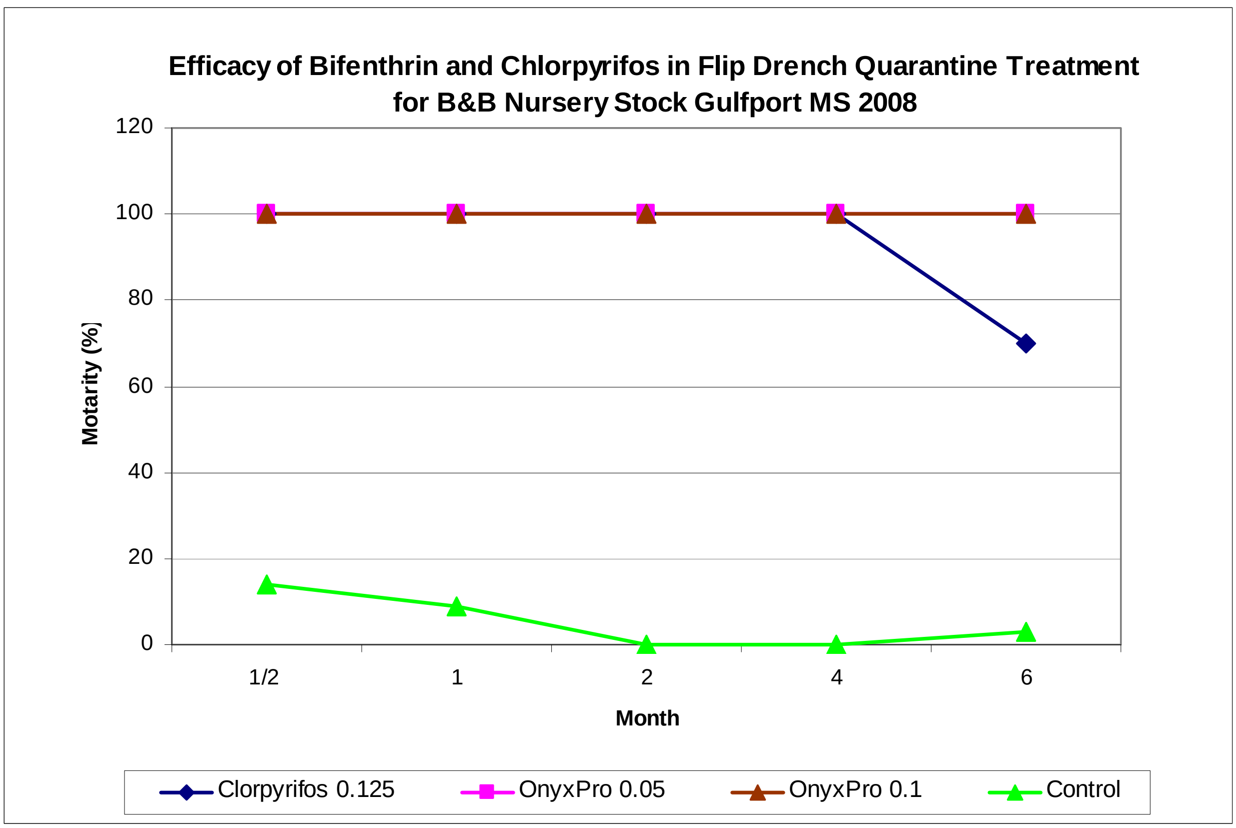
Figure 4. Orange circles indicate the locations of clusters of female alates within this bioassay cup.

RESULTS AND DISCUSSION:

Results showed that bifenthrin treated root balls were consistently generating 100% mortality at both low rates tested, 0.05 and 0.1 lb ai per 100 gal of water, for the entire 6-month trial period (Figure 5). Chlorpyrifos treated at the 0.125 lb ai/100 gal of water also had 100% efficacy during the period of first four months post-treatment when applied with flip drench method. But the 6 month result was only 70% kill of the test female alates, indicating that chlorpyrifos did not last as long as bifenthrin under normal aging in the environment.

However, results from this trial showed that the application method “1F1” resulted in uniform coverage and good penetration of chemicals into the rootballs. Sampling around the rootballs was an adequate method to assess the treatment efficacy for flip drench application method. The different wait time between drenches (0.5 h vs. 5 h) did not generate differences in treatment efficacy, which means that waiting for a few hours before flip and drench did not improve treatment effectiveness; therefore, such delaying was not considered necessary and growers can drench their rootballs in a continuous way-- finish drenching one side of rootballs and then come back to flip and drench the other sides-- if that would fit to their treatment schedule better. Chemical and time requirements for this treatment method are at the minimum of all drench treatment methods investigated. Run-off was also at the minimum. With both sides of the rootball being well drenched and chemical solution penetrating into the root ball, we could expect the results of this treatment method no much different from the 2F2 method but having the benefit of shortening one day and reducing the cost and run-off problems.

Figure 5. IFA control achieved in befenthrin and chlorpyrifos treated soil samples collected at various sampling intervals after final drench application. Plants rotated once between 2 drench applications in one day.



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 Queen Infestation Lab Test 2007-2008

REPORT TYPE: Final

LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Lee McAnally, Craig Hinton; Chemists: Bill Guyton, Richard King and Lisa Mosser

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.

Bifenthrin-treated burlap wrapped over chemogated rootballs may kill newly-mated fire ant queens that land on the rootballs 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 evaluate the suitability of using bifenthrin-treated burlap to prevent newly mated fire ant queen infestation. Specifically, we wanted to find out at normal aging conditions how long the treated-burlap could kill IFA before losing quarantine level efficacy. Our overall goal 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.

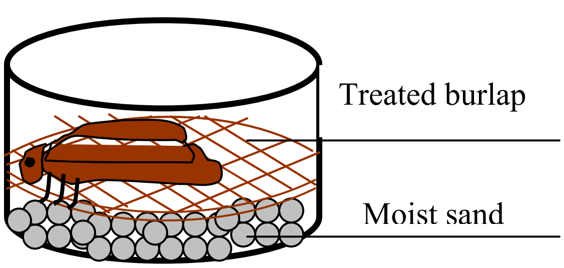
MATERIALS AND METHODS:

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

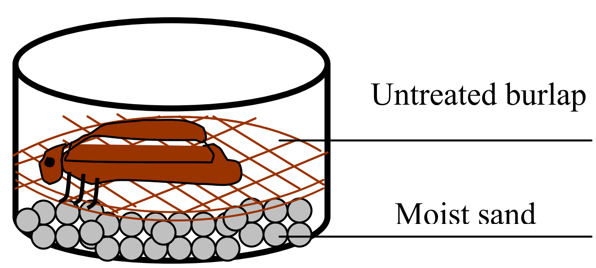
1. Tree-ring chemogation can kill all fire ants residing in the root ball area.
   Trials on tree-ring chemogation will be conducted in a later time, but it is expected that 100% kill will be achieved without much complication through the selection of suitable chemicals (insecticides and/or surfactants), use of proper concentration of the selected chemical(s), and an adequate volume of solution.
2. Bifenthrin-treated burlap will kill fire ant queens that land on the rootballs wrapped with treated burlap.

The second assumption has not been well studied before thus it became the focus of this investigation. Laboratory experiments were conducted for this evaluation. The basic experimental design for the lab study is illustrated bellow. Female alates were placed on bifenthrin-treated burlap that was placed on top of moist sand (see illustrations). Alates had to burrow through the treated burlap barrier to enter into the moist sand. This experiment was designed to answer the following questions: Can alates burrow through treated-burlap? Would bifenthrin-treated burlap kill alates through contact? If so, will they die before or after they enter the moist sand? Can they penetrate the treated burlap, enter into the sand, and survive? A layer of biodegradable erosion control blanket was also used in conjunction with burlap for extra barrier in some treatment. 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 burlap as 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 (7.5 oz) can prevent entry of fire ant queens and kill them
	4	Burlap (10.0 oz)	See if one layer of treated burlap (10.0 oz) can prevent entry of fire ant queens and kill them
Treated burlap + untreated coco blanket	5	Treated burlap (7.5 oz) + untreated coco blanket	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) + untreated coco blanket	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	Treated coco blanket for killing the ants and untreated burlap for wrapping to reduce nursery worker's handling
	8	Untreated burlap (10.0 oz) + treated coco blanket	Treated coco blanket for killing the ants and untreated burlap for wrapping to reduce nursery worker's handling
Untreated burlap + treated burlap	9	Untreated burlap (7.5 oz) + treated burlap (10 oz)	Two layers of burlap, outside layer is untreated to reduce nursery worker's handling
	10	Untreated burlap (10.0 oz) + treated burlap (10 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 make 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 more tightly woven than 7.5 oz one) in the size of 20"x 20" (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" diameter, twelve gallons of water and 52 ml of bifenthrin 23% EC (OnyxPro, FMC Corp) were added to mix into a solution at the rate of 0.23 lb ai/100 gal of water. On Nov. 07, 2007, 24 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 when dried.

On Nov. 14, 2007, the treatment arenas were assembled with the treated burlap with 5 replicates per treatment. To investigate the effects of aging of treated burlap on the efficacy of bifenthrin, experiment units were aged outdoors. 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 (Fig 3). An irrigation schedule was set up to simulate nursery conditions with daily irrigation of 1 cm over a 30 min irrigation period. Experiment units were always stored outdoors except during bioassay dates when they were brought inside for a 48 hours experiment.

Bioassay procedure

Bioassays (Fig 4) were conducted immediately after treatment arena assembly, and then once every two weeks to monitor the aging process. 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. Alates 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).

Chemical analysis of the bifenthrin in water solution and in treated burlap

GC-MS analytical procedures were used to analyze bifenthrin in samples of the insecticide solutions that were used to soak the burlap and in samples of bifenthrin-treated burlap collected immediately after treatment. At the end of the experiment after 9 months of outdoors aging, the aged burlap, coconut fiber, and the sand at the bottom of the treatment units were also analyzed for bifenthrin residues. These analyses were conducted by GC-MS group of CPHST Lab in Gulfport, Mississippi. Detailed analytical methods for these analyses can be obtained from the chemists.

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

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

Fig.3. Treatment units with bifenthrin-treated wrapping materials aging outdoors with exposure to sunlight, rain, and daily irrigation simulating nursery storage

Fig.4. Lab bioassay conducted once every two weeks during a 9-month aging period to evaluate the efficacy of bifenthrin-treated burlap on fire ant female alates.

RESULTS:

Chemical analyses

Chemical analyses were conducted on samples of bifenthrin solutions taken after each use of soaking burlap 24 hours and on different batches of treated burlap that came out of these treatments. Results are shown in Figs. 7 & 8.

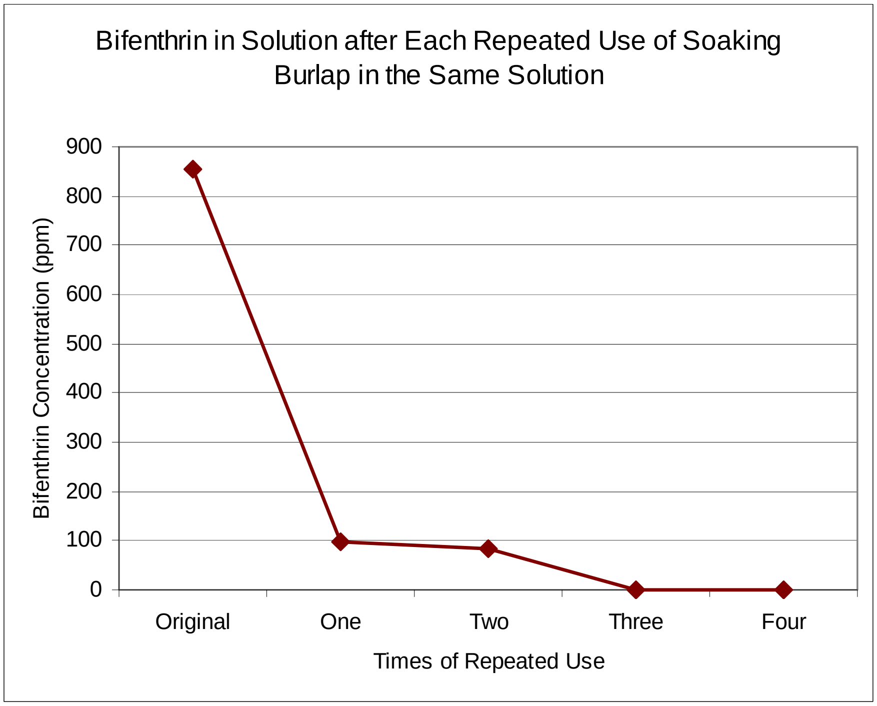


Fig. 7. Bifenthrin left in solution after each use of repeatedly soaking burlap overnight in the same solution.

Bifenthrin in the immersion solution dropped sharply after the first use of soaking burlap for 24 hours (Fig. 7). 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.8). Bifenthrin in the first batch of treated burlap was 5 times higher than that in the second batch which was soaked 24 hours in the same solution after the first batch of burlap was removed.

It is clear that bifenthrin level in the treated burlap of different batches that used the same immersion solution changed markedly (Fig.8). 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 is added to the solution to compensate for the marked drop in bifenthrin level in the solution.

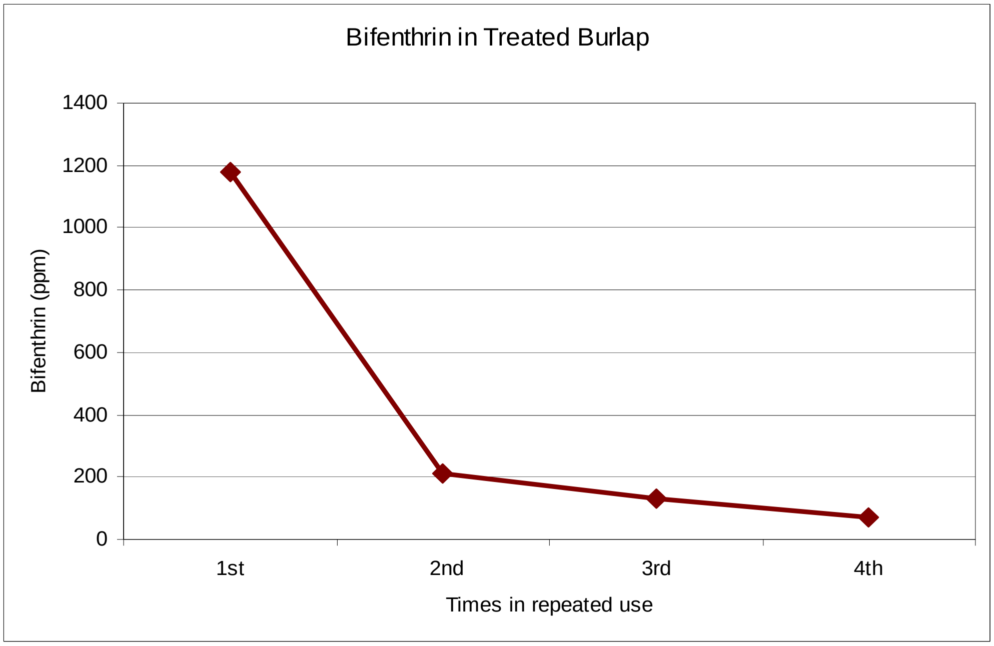


Fig. 8. Change of bifenthrin levels among various batches of treated burlap which repeatedly used the same bifenthrin solution overtime.

Bioassay with female alates

Freshly treated burlap was extremely lethal to fire ant female alates. Alates showed symptoms of intoxication within 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 placed in the second layer (especially when the top layer was the untreated tightly woven 10.0 oz burlap). All treatments achieved 100% mortality within 48 hours and mortality usually occurred within 30 minutes of coming in contact with bifenthrin-treated wrapping materials except the two treatments that had untreated 10-oz burlap on top of the treated materials where there were one or two alates that were really sick but did not die within 48 hours (Figs 9 & 10).

Aged bifenthrin-treated burlap was also toxic to the test alates. After aging outdoors for 6 months, all treatments still achieved 100% mortality with no sign of losing potency. However, the two treatments with the untreated 10-oz burlap on top of the treated materials did not achieve 100% efficacy within 48 hours of exposure especially during the early bioassay dates over the course of the study. As a matter of fact, for those treatments in which the treated materials were placed in the second layer, alates showed symptoms of intoxication sooner after exposure in aged bioassays than in the initial bioassay freshly set up. This is because the untreated top layer burlap got bifenthrin through contamination from the treated layer underneath it during the weathering process. Clear evidence for this explanation can be found from our chemical analysis of aged burlap, coconut fiber and sand conducted at 6 months post-treatment (Fig 11).

In an effort to find out how much longer beyond the 6-month period the residual effect of bifenthrin could last, observational bioassays were continued monthly in two replicates per treatment (the other 3 reps were used for chemical analyses) till 9 months after initial treatment. Bioassay data for these three extra months were not presented in the chart below (Fig. 9) but all treatments were still achieving 100% mortality. Even the sand alone, which was initially added to the bottom of the test arena to provide moisture and hiding place for test alates, killed 100% of test alates in all treatments at 9 months after treatment. Evidently, the sand had picked up enough bifenthrin from the treated materials during the aging process to kill the fire ant alates.

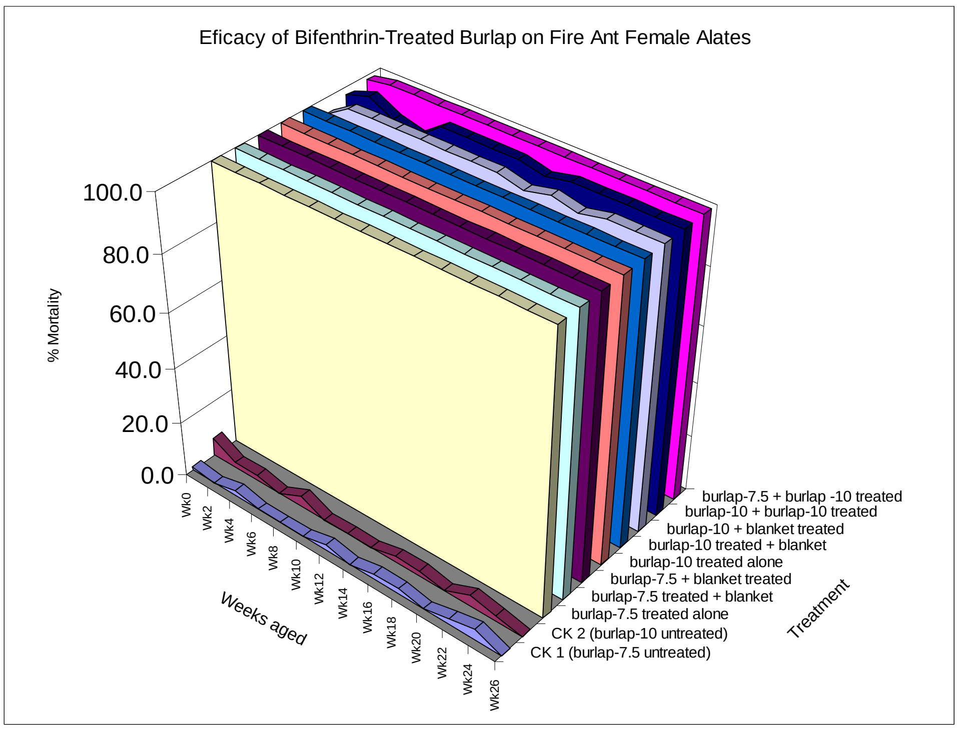


Fig.9. Results of 26 weeks of female alates bioassay with aged burlap materials treated with bifenthrin

Fig.10. Fire ant alates died after they came in contact with the treated burlap

Analyses of residual bifenthrin

Chemical analyses of burlap (treated and not treated), coconut fiber (treated and not treated), and sand (not treated) that were used in the test arena were conducted to estimate the residual (or gained) bifenthrin residue at the end of the experiment after aging for 6 months. Residual bifenthrin concentrations in burlap that was initially treated with bifenthrin remained high (130 ppm and above) after 6 months of weathering outdoors in simulated nursery conditions (Fig 11). Although the residual concentrations were only about 1/10 of the initial level they were still excessively high for the purpose of controlling fire ant infestation because it requires only 2-3 ppm to kill alates with 100% effectiveness. Residual concentrations of bifenthrin in treated coconut fiber also remained high (100 ppm or more) after 6 months of weathering process.

Burlap and coconut fiber that were not treated with bifenthrin initially also picked up bifenthrin through contact with the treated burlap or coconut fiber during the course of the experiment. The average concentrations went as high as nearly 40 ppm and could kill fire ant alates quickly by contact (Fig 11).

Sand was not treated with bifenthrin and was added in the bottom of the test arena to provide moisture and hiding medium for the test alates. However, after 6 months, bifenthrin concentrations in the sand samples were in the range of 2-3 ppm, high enough to kill all alates by contact (Fig 11). In a quick observation, 10 alates were placed on each of the two sand samples from each treatment in the test, and all alates were found dead with 24 hours of exposure.

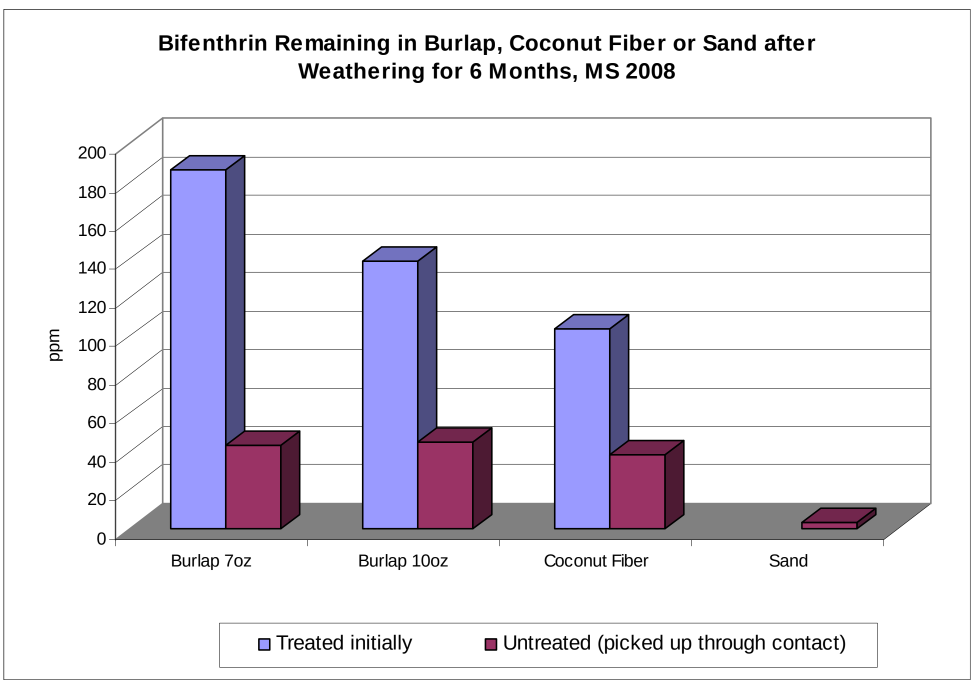


Fig.11. Chemical analysis results of bifenthrin remaining in burlap, coconut fiber or sand after 26 weeks of natural weathering outdoors in simulated nursery conditions

DISCUSSION:

This developing treatment method consists of two parts of treatment: 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 harvesting. 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. These two parts of treatment fit well in the nursery production because most growers do use water to moisten the ground before digging up trees when the ground is hard and this is usually the case in the winter months B&B plants are harvested in major growing areas. Wrapping rootballs with burlap is done by nearly all growers for B&B nursery stock and treating the burlap with bifenthrin does not add too much work to the process. Our original concern was that queens might burrow through coarser burlap (such as 7.5 oz burlap that was used by most growers) too quickly so that they would not have enough time in contact with the bifenthrin-treated burlap to obtain a lethal dose. To address this concern, we included an extra layer of material (biodegradable erosion control blanket or another layer of burlap) in some treatments of our experiment design. It was found from our results that this second layer was not necessary. Adding an extra layer did not increase mortality or the speed of killing the test alates; one layer of bifenthrin-treated burlap, either the tighter woven 10 oz or the coarser 7.5 oz which most growers use, would work just fine for the purpose of preventing newly mated fire ant queens infestation.

The treated-burlap (or treated coconut fiber) lost its killing power in a much slower pace than expected. At the end of 9 months post treatment, all treatment still achieved 100% mortality. This finding is a good boost 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 9 months of aging, however, the untreated burlap or the sand had even gained enough bifenthrin to kill alates effectively through the contact with treated wrapping materials. This was an interesting and beneficial phenomenon because after being wrapped with bifenthrin-treated burlap, the surface soil of the rootballs could become a killing weapon itself, effectively protecting the rootballs from newly mated fire ant queens' infestation. This also eliminates the concern of weather newly mated queens landing on the rootballs can burrow through the treated burlap or not because they certainly will be killed by the surface soil if they do.

Treating burlap involves mixing bifenthrin solution, soaking the burlap 24 hours 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 chemical waste to dispose off? Would the concentrations of bifenthrin in the solution change following each use of immersing burlap in it? Were there any differences in the amount of bifenthrin in the treated burlap of different batches that repeatedly used the same solution? Results of chemical analyses of bifenthrin solutions and bifenthrin-treated burlap clearly answered the above questions. These results could be an important guidance for treating burlap with bifenthrin. 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 if only extra bifenthrin product is added to the solution to compensate the marked drop in bifenthrin level in the solution.

This treatment method hopefully could be one that is effective, easy to do by growers, economical, environmentally safe, 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 tree ring chemogation 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.12).
2. Bifenthrin in treated burlap at the end of six months was high; the treatment rate of bifenthrin could be reduced in half to treat the burlap to begin with. Reducing bifenthrin rate will lower the cost of treatment and be less contaminating to the environment.
3. 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.12. Tree-ring chemogation is an important part of this developing quarantine treatment method for field-grown B&B nursery stock. This photo shows a preliminary tree ring work trial to evaluate how well the chemical penetrate into the ground with the aid of a blue dye.

CONCLUSIONS

1. Burlap tends to accumulate bifenthrin from the bifenthrin solution and can absorb a concentration that is considerably higher than that in the solution.
2. One use of immersing burlap in a bifenthrin solution for 24 hours could sharply reduce bifenthrin level in the solution. Therefore, if repeated use of the same solution is desired, additional bifenthrin product must be added to the solution in order to achieve similar concentrations of bifenthrin in the treated burlap among various batches of treatment.
3. Bifenthrin-treated burlap, using rates of 0.23 lb ai/100 gal water, could kill fire ant alates for a period at least 6 months under normal weathering conditions. Female fire ant alates showed symptoms of intoxication within minutes of contact with the bifenthrin-treated burlap and 100% kill usually occurred within 30 minutes of exposure.
4. Residual bifenthrin concentrations in treated burlap, after 6 months of weathering, dropped to only about 1/10 of the initial level but they were still excessively high for the purpose of controlling fire ant infestation. Lower rates should be studied to determine a suitable rate to treat the burlap.
5. Untreated wrapping materials and the sand used in the test arena effectively gained the killing power by absorbing bifenthrin from the treated materials through contact. This is beneficial to the developing treatment method because after being wrapped with bifenthrin-treated burlap, the surface soil of the rootballs could become a killing weapon itself, effectively protecting the rootballs from newly mated fire ant queens' infestation.

CPHST PIC NO: A1F04

PROJECT TITLE: Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Comparison of Burlap Treatment Methods: Pre-treated vs. Spray-on, for Protecting Intact Root-Ball from Newly-Mated IFA Queens Infestation

REPORT TYPE: Interim

LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Craig Hinton, Lee McAnally; Chemists- Bill Guyton & Richard King

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.

Bifenthrin-treated burlap wrapped on root balls previously treated in the field by chemogation or other means may kill newly-mated fire ant queens that land on the wrapped root balls through contact. The original 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. However, this concern was addressed by the laboratory bioassay results of using treated burlap. Our laboratory bioassay results in 2007-2008 showed that even after aging for 9 months outdoors under simulated nursery stock storage situations, treated burlap (at 0.23 lb ai/100 gal H_2O)would kill fire ant alates soon after they made contact. These lab results led us to believe that our burlap treatment trial was ready to be tested outdoors using real rootballs wrapped with treated burlap.

The objective of this study was to find out if bifenthrin-treated burlap wrapped on rootballs would protect the harvested balled-and-burlapped nursery stock from infestation of newly mated fire ant queens under outdoors conditions. In detail, our objectives of this test were:

1. To compare the two different methods of treating burlap with bifenthrin: pre-treat (immerse burlap in bifenthrin solution 24 hours then dry) vs. spray-on (spray bifenthrin solution onto the burlap after rootballs were harvested).
2. To determine what weight of burlap (10 oz or 7.5 oz) should be recommended for growers to use.
3. To determine the length of time treated burlap would protect root balls from infestation by newly mated fire ant queens.

Our overall goal is 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.

<u>MATERIALS AND METHODS</u>:

Burlap treatment
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" diameter, 12 gallons of water and 52 ml of bifenthrin (FMC Corp. OnyxPro 23% EC) 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 immersed completely in the solution overnight. After 24 hours of soaking in the solution, burlap liners were taken out to dry. Dried burlap was ready to use for wrapping the rootballs during harvesting (Fig. 1) or to be stored for later use.

A local nursery (Deep South Nursery, Lucedale, MS) harvested 40 Japanese boxwood (*Buxus microphylla var. japonica*) plants on April 22, 2008 for our experimental use. According to the treatments in this trial, 16 of the plants were wrapped in bifenthrin-treated burlap (8 rootballs in 10 oz treated burlap and 8 rootballs in 7.5 oz treated burlap) and the other 24 plants were wrapped in plain burlap (16 of them in 10 oz plain burlap and 8 in 7.5 oz plain burlap). We provided the nursery with both bifenthrin-treated and plain burlap for their use. Balled and burlapped plants were transported to the IFA lab and stored under irrigation during the trial. A fall trial was initiated on October 20.

Spray on procedure
Rootballs that were wrapped by the nursery with provided plain burlap (8 rootballs in 10 oz plain burlap and 8 rootballs in 7.5 oz plain burlap) during harvest were assigned to the "Spray-on" treatment group. These rootballs were then treated by spraying bifenthrin solution directly onto the entire burlap wrapping on April 24, 2008. A general purpose pressure sprayer (GardenPlus™ Lawn and Garden Sprayer) was used to spray 2 gal of bifenthrin solution (at the same rate of 0.23 lb ai/100 gal of water) evenly onto the surface of all 8 rootballs in one treatment with each rootball receiving 0.25 gal of solution. This spray-on method

Fig. 1 Bifenthrin-treated burlap was used to wrap the rootball during harvest

was similar to the treatment method of post-harvest drenching for B&B nursery stock, but this method used less amount of liquid, compared with that of drench method, to evenly cover the entire burlap wrapping and root balls were sprayed only once.

Bioassay procedure

To evaluate the residual effect of bifenthrin-treated burlap over a 6-month aging period under outdoors conditions, an effective and easy to do bioassay method was needed. Three different designs were tested at different point during the experiment in our search for a suitable bioassay method (Fig 2: A, B, & C). The apparatus shown at Fig 2 A was first developed and worked well when the outdoors temperatures were cool and mild but did not work when the temperatures were hot because alates placed inside the little basket died within 10 minutes even for the control group at extremely hot outdoors temperatures. Then we designed the apparatus shown in Fig 2 B, and alates placed inside the mesh confined area could tolerate heat better when it was covered with things that provided shade to them. However, the escape of alates from the wire mesh enclosed area was a problem, especially for those in the control treatment. Therefore, a third method (Fig 2 C) was tested which was similar to our standard fire ant alate female bioassay in the lab (Appendix I). Instead of conducting the bioassay on the rootballs stored outdoors, a piece of burlap was cut from each of the rootballs and brought to the lab for efficacy evaluation. The burlap piece was placed in a standard bioassay cup and covered with a clear square dish. A few drops of water were added to moisten the burlap if needed. This method worked well for burlap evaluation in the lab without subjecting ants to the outdoors temperature and thus became the method of choice for our rootball residual efficacy bioassay.

Fig 2. A): Small basket with a wire-meshed bottom allowing alates to contact with the treated burlap underneath for bioassay conducted directly on rootballs. B): wire mesh cage attached directly onto rootballs (without showing the snugly fit Petri dish cover). C): Method of choice--apparatus for bioassay conducted in the lab. D): rootball showing a piece of burlap was removed for bioassay in the lab; soil sample was also collected from where burlap was cut out (within yellow rectangle).

In the fall 2008 trial, in addition to the burlap bioassay, soil samples were also collected from the surface (about 1 cm deep) of the rootball where the piece of burlap was cut out (Fig 2 D) to determine if the soil having close contact with the treated burlap would acquire enough bifenthrin to kill fire ant queens. This lab bioassay method also worked well for soil samples.

To do the bioassay, ten field collected female alates were used for each burlap or soil sample taken from a rootball. Five replicate rootballs per treatment required a total of 50 alates (100 alates if for both burlap and soil samples). Female alates were placed on top of burlap or soil in the bioassay cup and allowed free contact with the material to be tested. Queens were not given food but water was added to moisten the burlap or soil if they were too dry. Mortality data were taken at 2 and 7 days after exposure. To investigate the residual effect of bifenthrin-treated burlap over time, burlap samples (and also soil samples in fall trial) were taken at 0, 0.5, 1, 2, 4, and 6 months to monitor the degradation process. Irrigation schedule for the rootballs was set up to simulate nursery conditions with daily irrigation of 1 cm over a 30 min irrigation period. The treatments in this investigation are listed in Table 1.

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

Fig. 3 Balled & Burlapped nursery stock stored outdoors under simulated nursery storage conditions for more than 6 months.

Table 1. Treatment list and mixing guide for rootball bioassay Gulfport, MS 2008

Treatment (Bifenthrin)	Trt #	Burlap weight	Rate ml prod./gal	Solution volume used	Amount of Insecticide/ treatment
Onyx Pro 23% @ 0.23 lb ai/100 gal of water (*Immersion*)	1	7.5 oz burlap	4.3	12 gal	51.6 ml
	2	10 oz burlap			
Onyx Pro 23% @ 0.23 lb ai/100 gal of water (*Spray-on*)	3	7.5 oz burlap	4.3	2 gal	8.6 ml
	4	10 oz burlap	4.3	2 gal	8.6 ml
Control (*water only spray-on*)	5	10 oz burlap*	--	2 gal	--

* 10 oz burlap was used for control in the spring trial but 7.5 oz burlap was used for control in the fall trial.

RESULTS

Spring 2008 bioassay:

In all burlap bioassays conducted every two weeks from the beginning of the trial through the end of six months post-treatment, all treatments in the test except control obtained a 100% efficacy. There were no differences in efficacy between burlap types (7.5 oz vs. 10.0 oz) or burlap treatment methods (pretreated "immersion" vs. post-harvest "spray on"). Alates in all the bioassays were knocked down within a few minutes after being exposed to the treated burlap and died within 48 hours. Consistent results among various bioassays conducted at different times during the trial showed that bifenthrin remaining in the burlap was potent enough even at the end of the trial, which could also be confirmed by the chemical analysis results shown in Fig. 7.

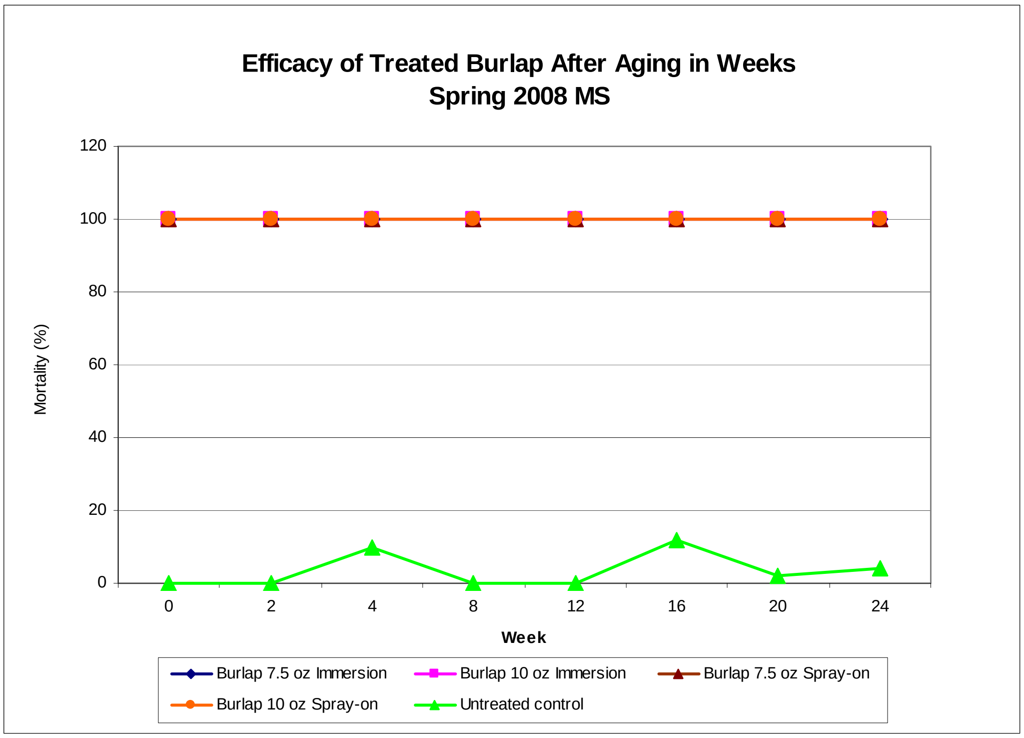


Fig. 4. Efficacy of bifenthrin-treated burlap on female alates of RIFA after aging outdoors under simulated nursery storage conditions.

Fall 2008 bioassay:

At the time this report was written, burlap and soil bioassays for the fall trial were completed for the first 4 months post-treatment. The six month data will be added to this report when data are available. For all the burlap bioassays conducted so far, all treatments in the test except control achieved a 100% efficacy and, similarly to the summer trial, there were no differences in efficacy between burlap types (7.5 oz vs. 10.0 oz) or burlap treatment methods (pretreated "immersion" vs. post-harvest "spray on") (Fig. 5). For all the soil sample bioassays conducted, 100% efficacy was also recorded for all treatments (Fig. 6). A 100% efficacy was achieved even for the soil samples taken from the rootballs wrapped with pre-treated burlap only one day post-harvest, meaning that the soil having direct contact with the bifenthrin-treated burlap gained the killing power soon after it made close contact with the treated burlap. Similar results are expected at the 6 month soil bioassay because longer contact with the treated burlap would make the soil more potent in killing the newly mated fire ant queens.

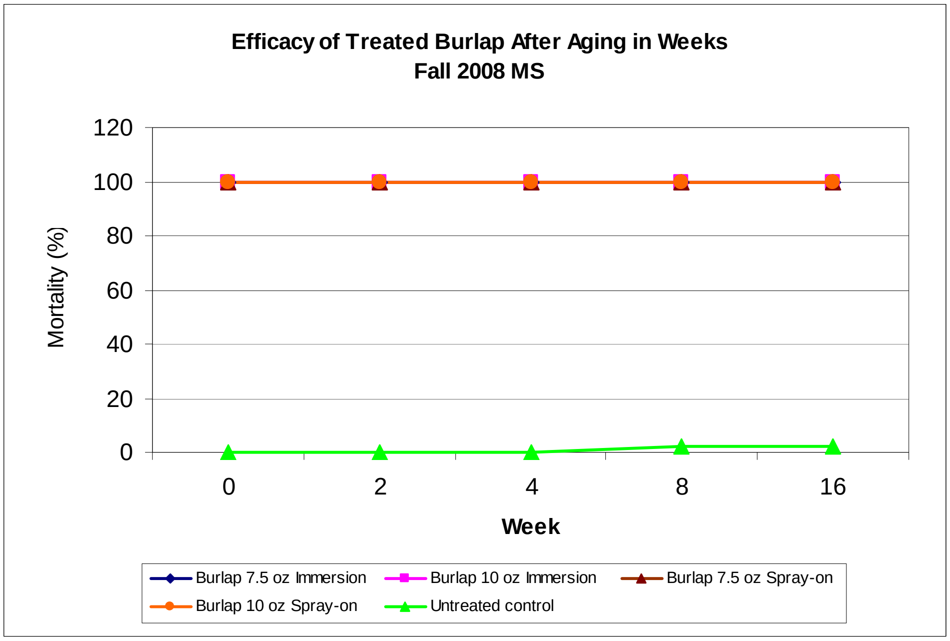


Fig. 5. Efficacy of bifenthrin-treated burlap on female alates of RIFA after aging outdoors under simulated nursery storage conditions.

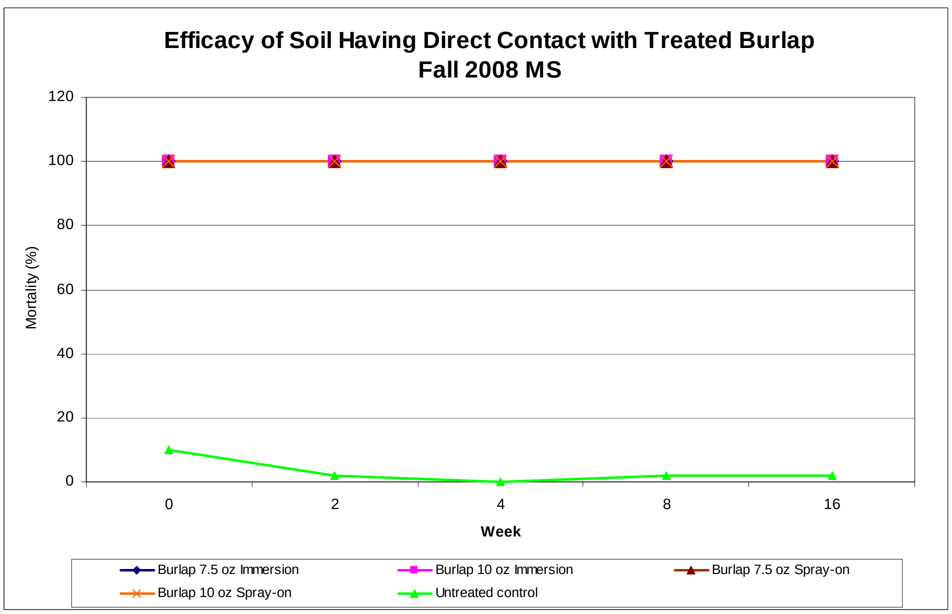


Fig. 6. Bioassays results for surface soil that had close contact with the bifenthrin-treated wrapping burlap after aging outdoors in simulated nursery storage conditions over 2 months.

Chemical analysis results:
When newly treated, burlap immersed in bifenthrin solution overnight (both 10 and 7.5 oz weight burlap) had much higher concentration of bifenthrin (1000 – 1500 ppm) than burlap treated with the spray on method (400 – 600 ppm for 10 and 7.5 oz burlap) (Fig.7). Bifenthrin residues decreased sharply in the first two weeks after treatment (down to only ½ - ⅓ of the week 0 level). After aging for two months, bifenthrin contents in burlap of all four treatments dropped to only ¼ or less of the beginning level, but interestingly, bifenthrin concentrations in 7.5 oz burlap were higher than that in 10 oz burlap in both treatment methods—pre-treated (immersion) and spray-on during the first two months. At 3rd month, there was not much difference in bifenthrin concentrations in the four treatments (all at around 100 ppm levels). At the end of fourth and sixth months, bifenthrin concentrations were still well above 50 ppm for all the treatments (50 - 200 ppm), and laboratory bioassay showed that they killed 100% test alates quickly.

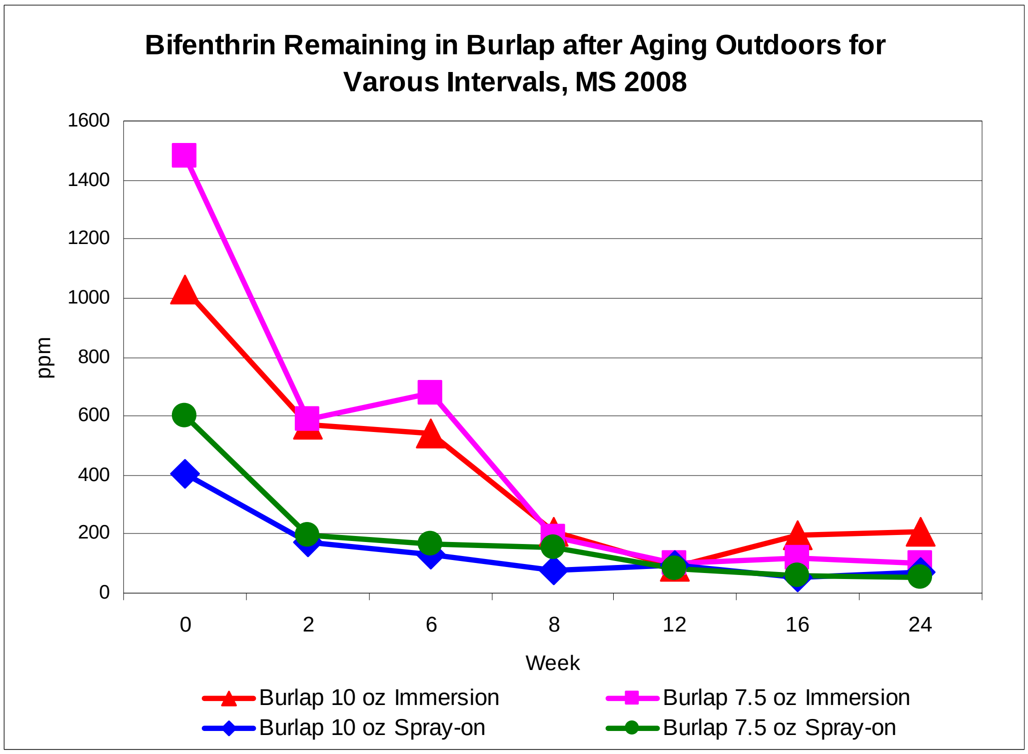


Fig. 7. Bifenthrin in treated burlap at various points of time during the six months aging under simulated nursery storage conditions.

<u>DISCUSSION</u>:

The bifenthrin-treated burlap, either pre-treated before harvesting or sprayed directly onto the rootball after harvest (spray-on), maintained its killing power well during the long-term aging process. It lasted at least for six months under normal outdoors nursery storage conditions, long enough to protect the B&B nursery stock while awaiting shipment. After six months of aging, all treatments are still potent enough to achieve 100% effectiveness.

Since there was no difference found in effectiveness between the two treatment methods of treating burlap with bifenthrin, it will be up to the growers' preference to use either method: pretreated or spray-on. There are advantages for the spray-on method in comparison with the pre-treated method because there will be no soaking the burlap in bifenthrin solution, drying the wet burlap, and disposing the leftover chemical solution at the end. And there will be no such problem with nursery workers handling the insecticide-treated burlap during harvest. However, there will be post-harvest spraying to be done for the spray-on method, and the spray-on treatment has to be applied soon after harvest and has to cover the entire rootball wrapping to be 100% effective. However, this alternative application method only needs to wet the rootball surface and needs to be done only once, which is different from the approved post-harvest drenching quarantine treatment. All it takes is a uniform coverage of bifenthrin solution on the entire surface of the burlap covered rootballs. By doing so, it eliminates the need for pre-treating, storing, and handling the bifenthrin-treated burlap. Plain burlap will be used for wrapping rootballs and after harvesting, spray bifenthrin solution to thoroughly cover the surface of the rootballs, which will require flipping rootballs during the spray application.

Surface soil that had close contact with bifenthrin-treated burlap (as brief as 24 hours) acquired enough bifenthrin to kill newly mated queens. A 100% efficacy was achieved even for soil samples taken from the rootballs wrapped with pre-treated burlap for only one day. This result clearly indicated that the bifenthrin in the pretreated burlap could transfer quickly from burlap to the soil through contact and provided added protection to the rootballs so that coarse burlap (as 7.5 oz weight) would be good enough to prevent fire ant infestation even though queens might burrow through the coarse burlap layer before they finally die.

Results of quantification of bifenthrin degradation and laboratory bioassay clearly showed that at the end of six months, bifenthrin concentrations in burlap were still high and it could well protect the rootballs from infestations of newly mated fire ant queens. Based on our previous data, there is no doubt that much lower bifenthrin doses could still kill 100% test alates.

This developing treatment protocol consists of two parts of treatment 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 or push out all fire ants in the rootball mass and at the same time to moisturize the ground near trees for easy digging. Preliminary results of a tree ring study (not reported in 2008 annual report) showed that chemogation with bifenthrin was effective in killing fire ants in the rootball area before harvesting. 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. Alternatively, growers could choose to use the spray-on method to treat the burlap already wrapped on the harvested rootballs. This treatment protocol 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.

Future study

1. 50 ppm or more of bifenthrin in burlap at the end of six months was very high; the treatment rate of bifenthrin can be reduced in half to treat the burlap to begin with. Reducing bifenthrin rate will lower the cost of treatment and be less contamination to the environment.

2. To visually observe the thoroughness of coverage of spray-on method, especially to determine how deep the spray solution penetrates into the surface soil of rootballs, mixing dye in the bifenthrin solution will provide quick visual aid for coverage assessment.

CONCLUSIONS:

Bifenthrin treated burlap, either pre-treated before use or directly sprayed onto the burlap after rootballs were harvested and wrapped using rates of 0.23 lb ai/100 gal water, could protect harvested B&B nursery stock for at least 6 months from infestation by newly mated fire ant queens. Fire ants were killed soon after they made contact with the treated burlap wrap. Since there was no difference found in the effectiveness between the two treatment methods of treating burlap with bifenthrin, it will be up to the growers' preference to use either method.

Soil that had close contact with bifenthrin-treated burlap (even as brief as 24 hours) acquired enough bifenthrin to kill newly mated queens. This result indicated that the bifenthrin in the pretreated burlap could transfer from burlap to the soil and provided added protection to the rootballs so that even coarse burlap (as 7.5 oz weight) would be good enough to prevent fire ant infestation.

Bifenthrin degraded quickly during the first two weeks of exposure to the environments but still remained potent in killing fire ant queens after 6 months of aging under normal outdoors nursery storage conditions.

CPHST PIC NO: A1F04

PROJECT TITLE: Development of Alternative Quarantine Treatments for Field Grown Nursery Stock – Broadcast Bait plus Block Application of Bifenthrin, Mississippi, Fall 2007-2008

TYPE REPORT: Final

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. In this 2007 trial, we wanted to compare the followings: treatments with bait vs. treatments without bait; single application of contact insecticide in a season vs. two applications in a season; two consecutive applications vs. two non-consecutive applications (2 months between applications); regular spray volume vs. half the regular volume; and bifenthrin EC formulation vs. bifenthrin flowable formulation. The objective was to search for a treatment schedule/method that would best meet the requirement of quarantine treatment for the pre-harvest (in-field) B&B nursery.

MATERIALS AND METHODS:

Fall 2007 Block Trial:
Treatments are shown in Table 1. 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. A minimum of 10 feet apart side to side and end to end was included 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 during the trial period.

Figure 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 nor contact insecticide. Treatments that were included as contact insecticide only without bait did not receive bait broadcast. Contact insecticide application occurred on November 5, 2007 and follow-up contact insecticide treatments on December 4, 2007 and/or February 25, 2008 (Fig.1). 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 150 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.

Table 1. Treatment list of block trial at the Municipal Airport of Hattiesburg, MS, fall 2007-08.

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.

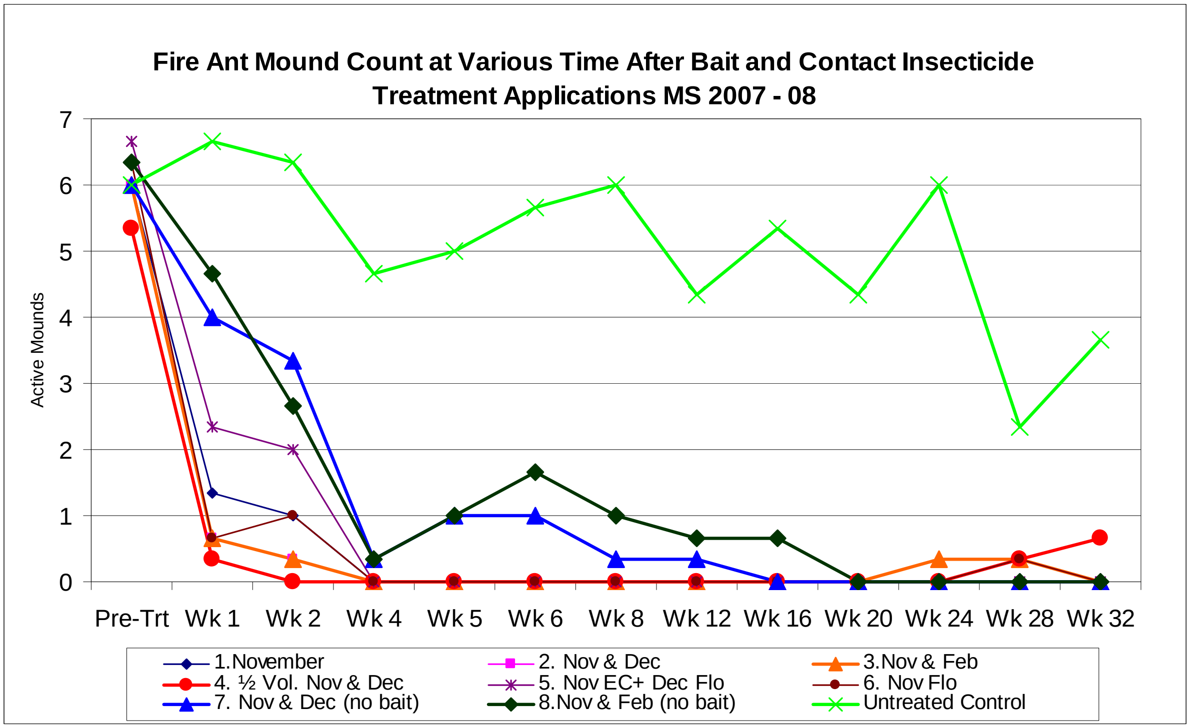
For laboratory bioassay using IFA female alates (Appendix I), one soil core sample was collected from each of the three replicates of a treatment. Soil core samples were not collected on every evaluation date but in a longer interval. Core samples were 2” diameter and 2” in depth and each soil sample was tested with ten female IFA alates.

RESULTS:

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 of 100% control continued through the 20th week evaluation (Figure 2). At the 24th week and later evaluations, however, one or two active mounds began to appear in the treatments of single application at Nov., Nov. & Feb application, and half water volume rate Nov. & Dec application. The treatments of two consecutive applications (made in Nov & Dec) achieved 100% control through 32nd week when we concluded evaluation for the trial. This was the same for both the EC formulation only and EC followed by flowable formulation. Treatments with contact insecticides alone (without prior bait application) experienced a sharp drop in the number of active colonies at around week 4 but remained at a low level after the 4th week evaluation through 20th week. The untreated control plots also experienced some colonies reductions at the beginning but they were still at a level of relatively high numbers except at week 28 evaluation when the numbers of active mounds in all control plots were low.

Figure 2. Fall 2007- 08 trial – Mean colony mortality after a broadcast treatment of bait followed by a block treatment of bifenthrin. Hattiesburg, MS.



Lab soil core bioassay results
At the week 5 bioassay (one week following the second application to some of the treatments), all treatments with bait, except the flowable 0.2 lb ai/Acre @ 45GPA applied in November, provided 100% mortality (Table 2). By week 12, however, the efficacy level has dropped for all treatments. The mortality ranged from 46 to 76%. Efficacy level for all treatment evaluated remained low till the end of soil core bioassay evaluation at week 24 except one non-baited treatment with application scheduled Nov& Feb (100%) and one EC + Flo treatment applied Nov/Dec (93.33%).

Table 2. Results of IFA Alates Bioassay CPHST Lab, Gulfport, MS 2007-08

Treatment	Application Rate	Alate mortality (%)			
		wk 5	wk12	Wk20	wk24
45GPA EC Nov	0.2 lb ai/A	100.0	76.7	50	19.9
45GPA EC Nov/Dec	0.2 lb ai/A	100.0	76.7	46.66	26.5
22.5GPA EC Nov/Dec	0.2 lb ai/A	100.0	70.0	46.67	46.5
45GPA F Nov	0.2 lb ai/A	66.6	46.6	26.67	46.5
45GPA EC + F Nov/Dec	0.2 lb ai/A	100.0	50.0	93.33	19.9
45GPA EC Nov/Feb	0.2 lb ai/A	--	--	66.67	0
45GPA EC NB Nov/Dec	0.2 lb ai/A	--	--	46.66	23.2
45GPA EC NB Nov/Feb	0.2 lb ai/A	--	--	100	43.2
Control	--	0.0	0.0	3.33	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. For bifenthrin, EC formulation and Flowable have no difference in efficacy. Two consecutive applications (made in November and December in this trial) at regular spray volume worked the best; two consecutive applications at 1/2 spray volume worked well but only for 6 months. Treatments with two applications made in November and February do not have advantages over treatments with two applications made in November and December consecutively. In summary, all treatments with bait followed by contact insecticide have provided IFA colony free areas throughout the 20th week evaluation.

Soil core bioassay results revealed that soil core taken after 5th week post-treatment did not achieve 100% mortality on IFA queens. This may mean that the soil in the treated plot could not prevent infestation from newly mated fire ant queens or from new colonies moving into the treated area. The ground soil was not toxic enough to achieve 100% mortality beyond 5 weeks after treatment application in the field; thus they could not prevent new infestations. From this point of view, all treatments tested in this trial were not different from each other. The reason that some treatments (such as the two consecutive application made in Nov & Dec) had longer control than others may be because these treatment schedules could take out all the existing mounds including their queens but other treatments could not. Another reason may be that the light spray application of contact insecticide did not penetrate deep into the soil but only remained in the very top of the ground surface. Much of the soil in the 2” deep samples did not have enough insecticide in it, especially after 5 weeks of degradation in the field. Future soil sample bioassay may need to collect a thin layer of top soil from one square foot and compare with the regular soil core samples. Dye may be used in the spray application to determine the penetration of insecticides in the field.

CPHST PIC NO: Umbrella IFA Quarantine Treatments

PROJECT TITLE: Efficacy of Bifenthrin as a Grass Sod Treatment; Mississippi and Arkansas, Spring 2008

TYPE REPORT: Final

LEADER/PARTICIPANTS: Mississippi – Anne-Marie Callcott, Lee McAnally, Xikui Wei, Craig Hinton; Arkansas – Kelly Loftin (Univ. of Arkansas), John Hopkins and Ricky Corder

INTRODUCTION:

Currently there are two treatments available for sod growers to certify grass sod for movement outside the IFA regulated area: chlorpyrifos applied at 8 lb ai/acre (6 weeks certification after 48 hour exposure) and fipronil applied at a total of 0.025 lb ai/acre applied in two applications ca. 1 week apart (20 weeks certification after a 4 week exposure). In 2008, the only chlorpyrifos labeled product, Dow Dursban® 50W, discontinued the grass sod IFA quarantine rate of application leaving only the fipronil product available for growers.

Bifenthrin was tested as a grass sod treatment over several years ending around 2001. The testing showed that rates of 0.2 lb ai/acre (labeled rates) were not consistent enough to be used as quarantine treatments on grass sod for IFA. Rates of 0.4 lb ai/acre were required for quarantine level efficacy and the liquid formulation was more consistent than the granular formulation (probably due to the granular not being watered in unless natural rainfall occurred). At the time the testing terminated, all liquid bifenthrin labels available for use on grass sod limited the annual application of the product to 0.2 lb ai/acre. Therefore testing was terminated.

In April 2008, conversations with the company with the primary interest in bifenthrin once again included the possibility of using liquid bifenthrin on grass sod. The company is pursuing a new liquid formulation of bifenthrin and new labeling that will include nursery and sod uses. This label, if approved by EPA, may allow us the option of a dual/split application similar to that for fipronil on grass sod. However, we only have one set of data for that type of application and would need additional testing this spring/summer (2008) to insure replication of the data and firm up exposure and certification periods. Unfortunately, bifenthrin is not extremely fast in eliminating IFA and the exposure period will probably be in weeks, not days (as with the chlorpyrifos treatment).

MATERIALS AND METHODS:

The test site for this trial in Mississippi was a working sod farm with fields in several south Mississippi counties. The test site for the spring 2008 trial were fields located in Pearl River County near the community of Henleyfield, MS. The fields were regularly fertilized and mowed by the grower since they would be harvested in the fall. These sites did not have irrigation available. The test site in Arkansas was a commercial sod farm located near Fulton, Arkansas in

Hempstead County. The field used in the study consisted of Centipede grass and was maintained under standard practices for commercial sod production (fertilization, mowing and irrigation).

Plots were 1-acre square in size with a ¼-acre circular efficacy plot in the center. There were 3 plots per treatment and controls. Prior to treatment and at 1, 2, 3 and 4 weeks after treatment and bi-weekly there after, IFA populations in each efficacy plot was evaluated. Due to the weekly evaluations, we used a minimal disturbance method to evaluate the IFA populations. Instead of using a shovel to excavate each colony to determine worker numbers and presence or absence of brood, a stick/rod (ca. ¼-inch diameter and 3 ft. long) was used to "poke" each mound several times to disturb the workers. A rating was then given based on activity; 1= <100 workers, 2=100-1,000 workers, 3=1,000-10,000, 4=10,000-50,000, 5= >50,000 workers.

Mississippi: Treatments included two bifenthrin treatments using the Biflex NCT bifenthrin formulation from FMC; a 23.4% liquid formulation. Treatments were either a single application of 0.4 lb ai/acre or a split application of 0.2 lb ai/acre + 0.2 lb ai/acre, applied a week apart. Applications in Mississippi were made on May 14, 2008 (first 0.2 lb ai/acre application) and May 22, 2008 (second 0.2 lb ai/acre application and the 0.4 lb ai/acre application). There was ca. ½-inch of rainfall after each treatment in Mississippi.

Arkansas: Treatments included one bifenthrin treatment using Biflex NCT 23.4% bifenthrin liquid formulation and an untreated control. Due to restraints in resources only the split application of bifenthrin was made in Arkansas. Applications were made on June 5, 2008 and June 12, 2008.

<u>RESULTS</u>:

Mississippi: Both application methods provided 100% control of IFA within 2-4 weeks of application and maintained that level of control through 20 weeks after application, at which time the trial was terminated due to the grower harvesting the field.

Arkansas: The split application of bifenthrin (0.2 lb.a.i./application) provided 100% control of IFA within 3 weeks of application and maintained that level of control through 20 weeks (140 DALA) after the last application. Temperatures were too cool to obtain an accurate activity rating for the 24 week (168 DALA) evaluation. A final evaluation will be taken in the early spring of 2009 (as soon as temperatures warm sufficiently for an accurate assessment).

<u>DISCUSSION</u>:

The split application in both Mississippi and Arkansas achieved 100% control of IFA within 2-3 weeks after the last application and maintained that control for at least 20 weeks after the last application. Combined with other data previously collected on this split application rate of 0.2 lb ai/acre + 0.2 lb ai/acre of a liquid bifenthrin, recommendations regarding inclusion in the Federal IFA Quarantine as a grass sod treatment will be made. Unfortunately, the bifenthrin treatments do require several weeks to achieve maximum efficacy. Based on all data for split applications, the recommendation will probably include a 4 week exposure period followed by 16 weeks of certification.

Figure 1. Efficacy of bifenthrin as a grass sod treatment for IFA applied at either 0.4 lb ai/acre or in a split application of 0.2 lb ai/acre + 0.2 lb ai/acre – Mississippi, Spring 2008.

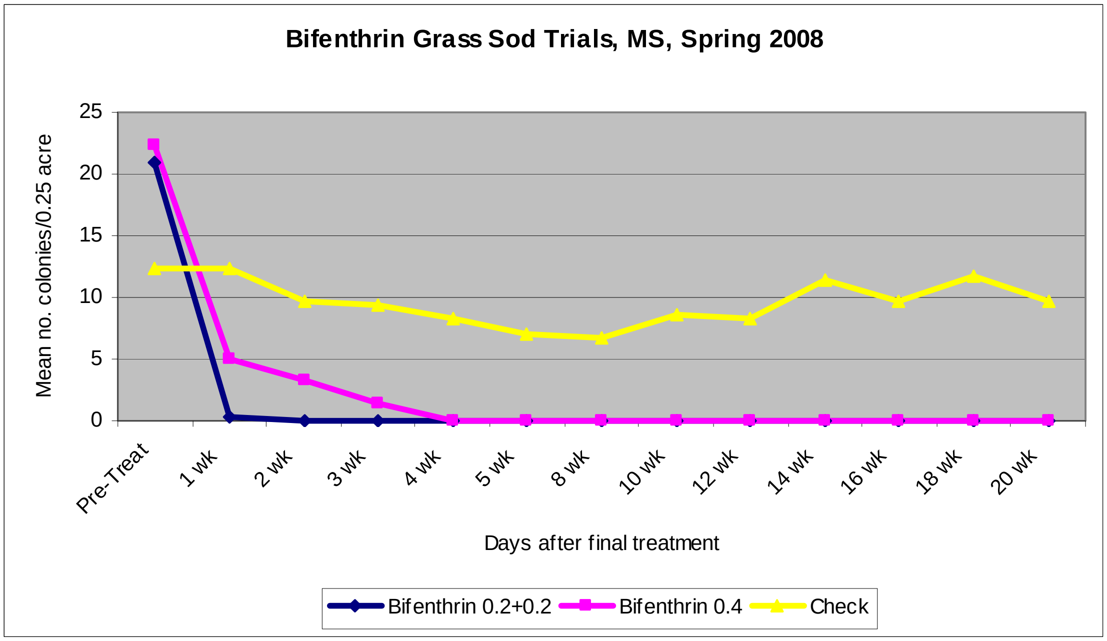
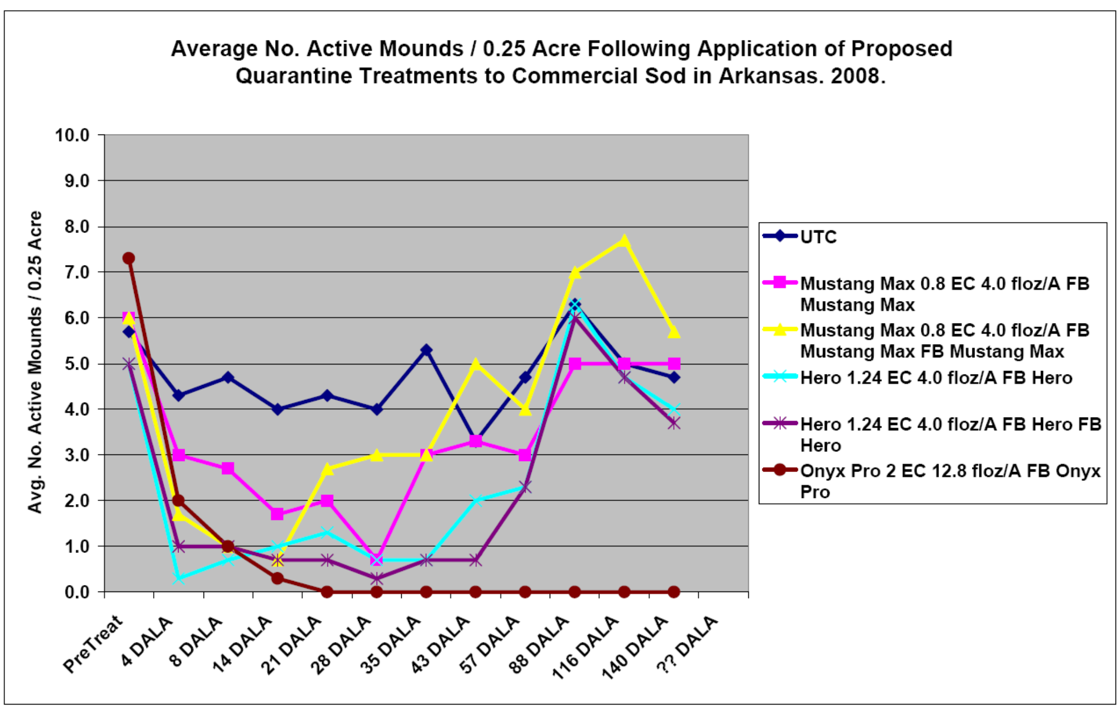


Figure 2. Efficacy of bifenthrin as a grass sod treatment for IFA applied in a split application of 0.2 lb ai/acre + 0.2 lb ai/acre – Arkansas, Spring 2008 (FB=followed by; other data presented on graph discussed in other sod report).



OnyxPro is BiFlex NCT

CPHST PIC NO: Umbrella IFA Quarantine Treatments

PROJECT TITLE: Efficacy of New Candidates as Grass Sod TreatmentS; Mississippi and Arkansas, Spring and Fall 2008

TYPE REPORT: Final

LEADER/PARTICIPANTS: Mississippi – Anne-Marie Callcott, Lee McAnally, Xikui Wei, Craig Hinton; Arkansas – Kelly Loftin (Univ. of Arkansas), John Hopkins and Ricky Corder

INTRODUCTION:

Currently there are two treatments available for sod growers to certify grass sod for movement outside the IFA regulated area: chlorpyrifos applied at 8 lb ai/acre (6 weeks certification after 48 hour exposure) and fipronil applied at a total of 0.025 lb ai/acre applied in two applications ca. 1 week apart (20 weeks certification after a 4 week exposure). In 2008, the only chlorpyrifos labeled product, Dow Dursban® 50W, discontinued the grass sod IFA quarantine rate of application and therefore only one product was available for growers. This product does require 2 applications and a 4 week exposure period, both of which are not cost effective for growers.

MATERIALS AND METHODS:

The test site for this trial in Mississippi was a working sod farm with fields in several south Mississippi counties. The test site for the spring 2008 trial were fields located in Pearl River County near the community of Henleyfield, MS. The fields were regularly fertilized and mowed by the grower since they would be harvested in the fall. These sites did not have irrigation available. The test sites for the fall 2008 trial were fields located in northern Hancock County and had irrigation capabilities. The test site in Arkansas was a commercial sod farm located near Fulton, Arkansas in Hempstead County. The field used in the study consisted of Centipede grass and was maintained under standard practices for commercial sod production (fertilization, mowing and irrigation).

Plots were 1-acre square in size with a ¼-acre circular efficacy plot in the center. There were 3 plots per treatment and controls. Prior to treatment and at 1, 2, 3 and 4 weeks after treatment and bi-weekly there after, IFA populations in each efficacy plot was evaluated. Due to the weekly evaluations, we used a minimal disturbance method to evaluate the IFA populations. Instead of using a shovel to excavate each colony to determine worker numbers and presence or absence of brood, a stick/rod (ca. ¼-inch diameter and 3 ft. long) was used to “poke” each mound several times to disturb the workers. A rating was then given based on activity; 1= <100 workers, 2=100-1,000 workers, 3=1,000-10,000, 4=10,000-50,000, 5= >50,000 workers.

Spring Mississippi: Treatments included 3 rates of zetacypermethrin (Mustang Max™ Insecticide, FMC Corp.), and 3 rates of a bifenthrin plus zetacypermethrin combination (Hero™

Insecticide, FMC Corp). Treatments were made once a week with each set of plots receiving either one (1X), two (2X) or three (3X) applications as noted below.

Trade Name	Active Ingredient	Rate of Application (lb ai/acre)	Date of last application
Mustang Max™	zetacypermethrin	1X = 0.025	5/13/08
		2X = 0.05	5/21/08
		3X = 0.075	5/28/08
Hero™	bifenthrin + zetacypermethrin	1X = 0.0387 total (0.029 bifen + 0.0097 zeta)	5/20/08
		2X = 0.077 total (0.058 bifen + 0.019 zeta	5/27/08
		3X = 0.116 total (0.087 bifen + 0.029 zeta)	6/2/08
Control	untreated		

Unfortunately, in Mississippi we did not stagger treatments to all end on the same date so evaluations are also staggered. In other words, the 1 week evaluation dates are different for most of the treatments.

Spring Arkansas: Due to restraints in resources only the 2X and 3X application rates of Mustang Max™ and Hero™ were conducted. More specifically, treatments included two treatments of Mustang Max™ 0.8% EC (9.6% zeta-cypermethrin), two treatments of Hero™ 1.24 EC (3.75% zeta-cypermethrin and 11.25% bifenthrin) and an untreated control. Applications were made on May 29, 2008, June 5, 2008 and June 12, 2008. Dates and application rates are provided in the table below. Treatments were staggered in Arkansas allowing for all evaluation to take place on the same date.

Arkansas spring 2008 application dates, rates and formulations.

Trt.	5/29/08	6/5/08	6/12/08	Total lb ai/acre (all applications)
1	UTC	UTC	UTC	NONE
2	None	Mustang Max 4 oz./acre	Mustang Max 4 oz./acre	0.05 zeta-cypermethrin = 2X
3	Mustang Max 4 oz./acre	Mustang Max 4 oz./acre	Mustang Max 4 oz./acre	0.075 zeta-cypermethrin = 3X
4	None	Hero 4 oz./acre	Hero 4 oz./acre	0.0775 total (0.058 bifenthrin, 0.019 zeta cypermethrin) = 2X
5	Hero 4oz./acre	Hero 4oz./acre	Hero 4oz./acre	0.11625 total (0.087 bifenthrin, 0.029 zeta cypermethrin) = 3X
6	None	Onyx 12.8 oz./acre	Onyx 12.8 oz./acre	0.4 bifenthrin = 2X Discussed in a separate report

Fall Mississippi: Spring trials were promising but did not reach or maintain adequate control for a regulatory treatment. Therefore in the fall 2008 higher rates of the bifenthrin and zetacypermethrin combination (Hero™ Insecticide) was applied at 2X (2 applications one week apart) and 3X (three applications each one week apart), as well as one rate of another combination formulation of bifenthrin and imidacloprid (Allectus GC SC, Bayer Environmental

Science). These treatments were staggered so that all 1 week evaluations occurred on the same date. Rates are below.

Trade Name	Active Ingredient	Rate of Application (lb ai/acre)	Date of last application
Allectus	bifenthrin + imidacloprid	1X = 0.7 total (0.2 bifen + 0.5 imida)	9/25/08
Hero™	bifenthrin + zetacypermethrin	2X = 0.2 total (0.15 bifen + 0.05 zeta)	9/24/08
		3X = 0.3 total (0.225 bifen + 0.075 zeta)	9/24/08
Control	Untreated		

RESULTS:

Spring Mississippi: All treatments reduced IFA populations compared to the controls however, none of the treatments reached 100% control (Figure 1) nor did any maintain control for any length of time. Maximum control for the zetacypermethrin (Mustang Max) was 73% for the 1X rate of application and 88% for the 3X, and for the bifenthrin + zetacypermethrin combination (Hero) was 55% for the 1X and 68% for the 3X. Unfortunately, there were several counts in Mississippi that were missed due to lack of personnel at the appropriate time. Therefore, no statistics were conducted.

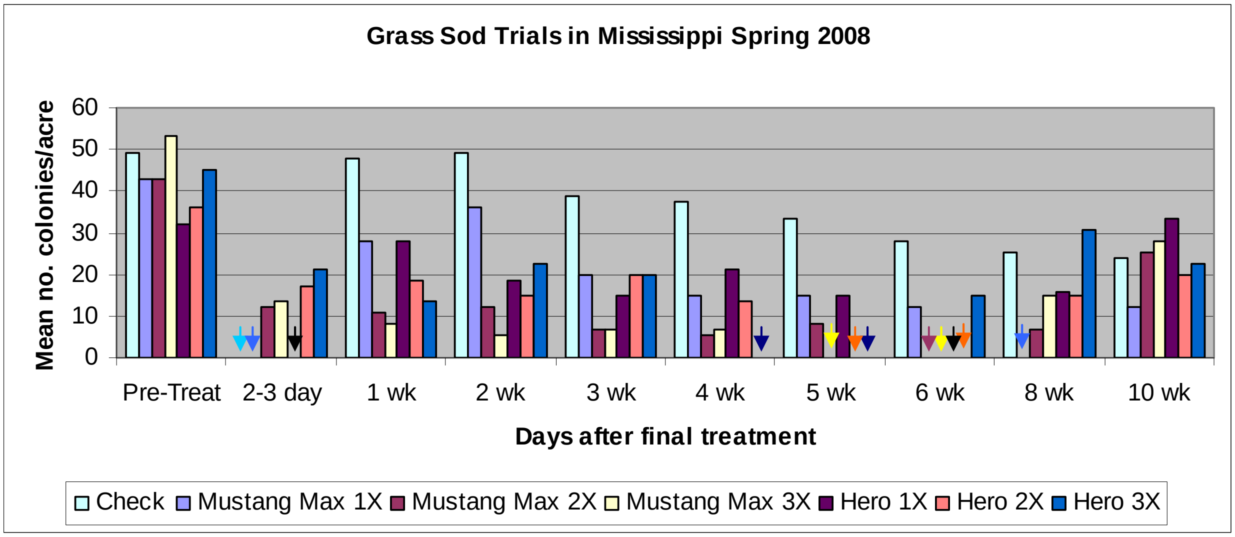
Spring Arkansas: All treatments reduced IFA populations compared to the controls however, as in Mississippi, none of the treatments reached 100% control (Figure 2), nor did any maintain control for any length of time. Maximum control for the zetacypermethrin (Mustang Max) was 88% for both the 2X and 3X rates of application, and for the bifenthrin + zetacypermethrin combination (Hero) was 94% for both the 2X and 3X rates of application. Arkansas figure also includes data for bifenthrin 0.2 lb ai/acre + 0.2 lb ai/acre application that is discussed separately in the "Umbrella Bifenthrin Spring Sod 2008" report.

Fall Mississippi: All treatments dramatically reduced IFA populations compared to the controls (Figure 3). All treatments showed initial decreases over the first 3 weeks, with a slight rebound in colony activity at weeks 4 and 6, followed by additional decreases. The bifenthrin + zetacypermethrin product (Hero) at the 3X rate of application provided the best control, reaching 100% control at 8 weeks and maintaining that level of control through 12 weeks. The 2X rate of the bifenthrin + zetacypermethrin product provided maximum control at 12 weeks after treatment with ca. 97% decrease in colony numbers. The bifenthrin + imidacloprid product (Allectus) was the least effective with a single application, providing a maximum of ca. 76% control at 8 weeks.

DISCUSSION:

All the products tested were effective against IFA, however none provided 100% control within 4 weeks of application, and none provided very long residual at that 100% control rate. The 3X application rate of the bifenthrin + zetacypermethrin product (Hero), equivalent to a total rate of application of 0.225 lb ai/acre of bifenthrin + 0.075 lb ai/acre of zetacypermethrin, was the best product tested in this group, but, as noted above required 8 weeks to reach 100% control and then offered only a 4 week window in which to harvest. Additionally, requiring growers to make 3 trips across a field over a 14 day period, instead of 1 to 2 trips over a 7 day period, significantly increases the costs to the grower as well as increasing the time frame prior to the grower being allowed to cut and ship the sod. Neither the 2X application rate of the bifenthrin + zetacypermethrin product (Hero) nor the bifenthrin + imidacloprid product (Allectus), applied in a one time application at the equivalent of 0.2 lb ai/acre of bifenthrin + 0.5 lb ai/acre of imidacloprid, provided 100% control. However the Allectus product also has a granular formulation that can be applied at a rate of 0.4 lb. ai/acre of bifenthrin + 0.5 lb ai/acre of imidacloprid. This product may be more promising based on previous testing of bifenthrin alone which requires a minimum of 0.4 lb ai/acre to provide 100% control of IFA on sod. The addition of the imidacloprid may speed up the activity of the bifenthrin, which requires up to 4 weeks to reach maximum efficacy of 100%.

Figure 1. Efficacy of grass sod treatments in Mississippi in spring 2008 applied at different rates of application. Arrows indicate where evaluations not conducted due to lack of personnel on specific count dates.



Spring 2X, 3X and fall 2X, 3X rates are not the same. See text for actual rates.

Figure 2. Efficacy of grass sod treatments in Arkansas in spring 2008 applied at different rates of application.

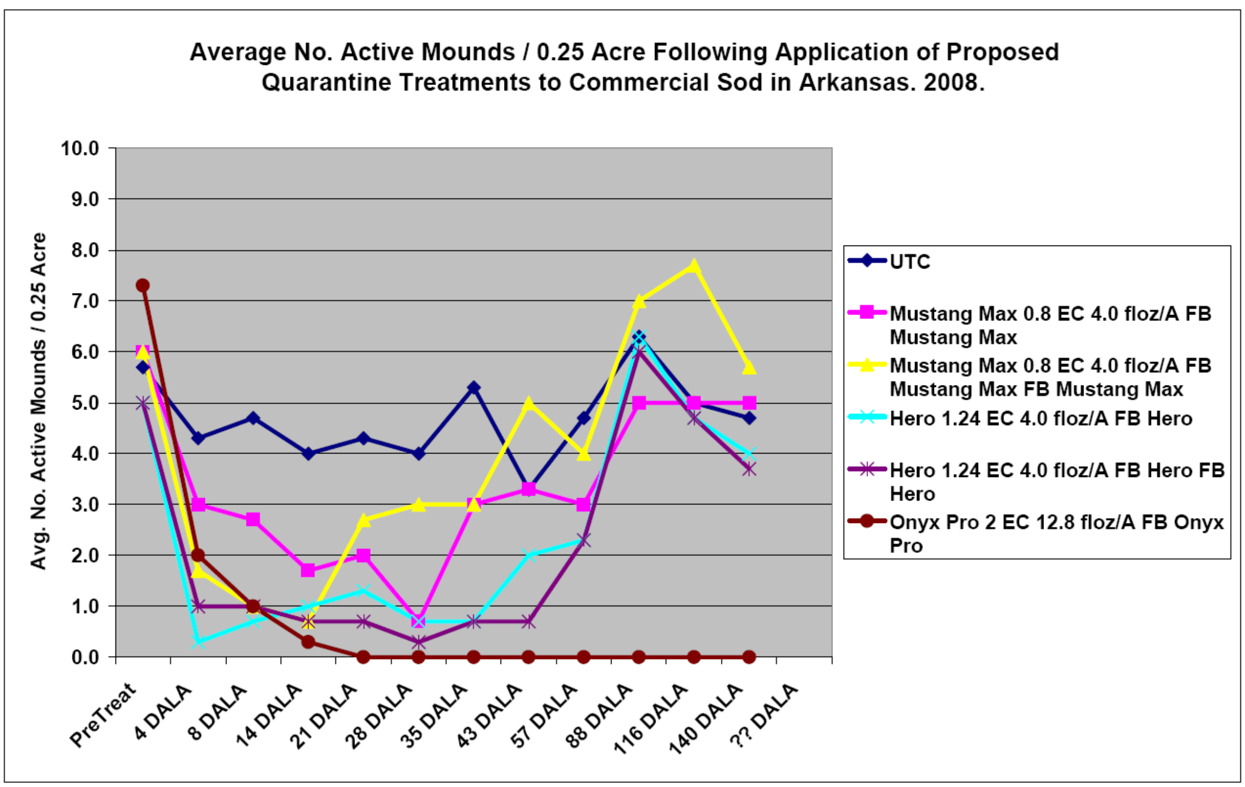
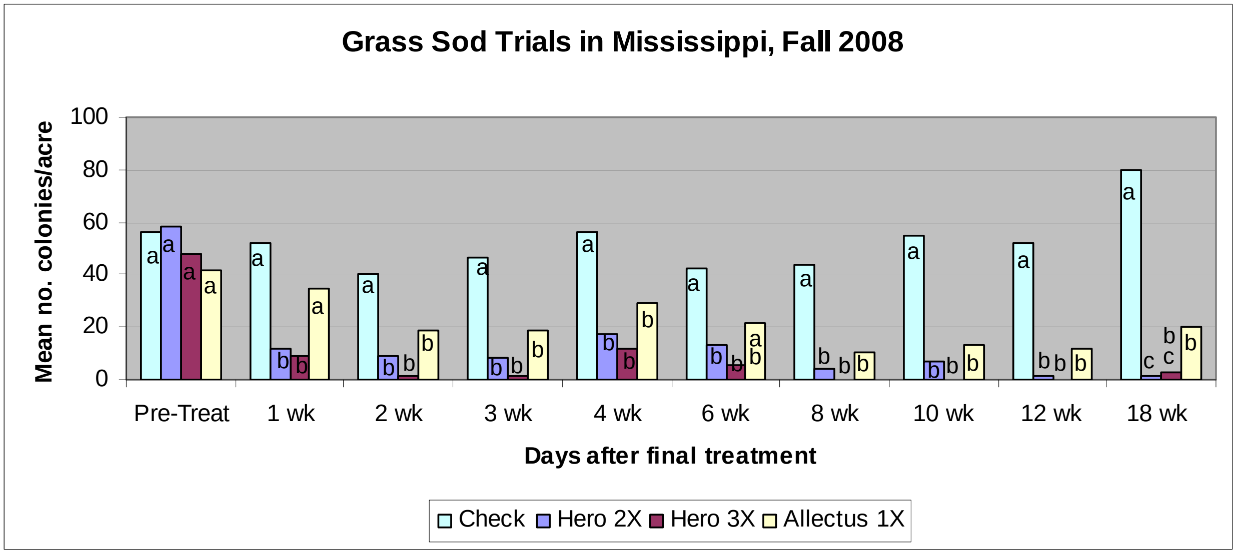


Figure 3. Efficacy of grass sod treatments in Mississippi in fall 2008 applied at different rates of application.



Spring 2X, 3X and fall 2X, 3X rates are not the same. See text for actual rates. Means followed by the same letter are not significantly different. LSD (P=0.05)

CPHST PIC NO: A1F01

PROJECT TITLE: Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing and Release Project, 2008 (*Pseudacteon tricuspis, P. curvatus, P. obtusus*)

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Anne-Marie Callcott, George Schneider and staff at FL DPI, ARS-CMAVE, and State departments of agriculture and their designees

SUMMARY:

The phorid fly rearing and release project is a great success. Since 2002, two species of *Pseudacteon* sp. flies have been released at multiple sites in all imported fire ant quarantined states in the contiguous southeastern states and Puerto Rico (no releases in NM and only one species released in CA) and field releases with a third species began in 2008. From 2002 through 2008 there have been 93 field releases of phorid flies and more than 845,000 potential flies released. Of these 93 releases, 61 were *P. tricuspis,* 30 were *P. curvatus* and 2 were *P. obtusus*. Through APHIS releases, along with other federal and university groups which are also releasing flies, *P. tricuspis* is well established in the southern areas of the IFA regulated area (AL, FL, GA, LA, MS, TX and PR), and moderately established in AR, NC and SC. To date, *P. tricuspis* is not known to be established in CA, OK or TN. The second species, *P. curvatus*, is moderately to well established in all southern IFA regulated states and PR (AL, AR, FL, GA, LA, MS, NC, OK, SC, TN, TX, and PR). *P. curvatus* has not been released in CA. Overwinter establishment of *P. obtusus* has not yet been confirmed. A publication on the known U.S.-wide distribution of *P. tricuspis* and *P. curvatus* is currently being drafted.

INTRODUCTION:

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

MATERIALS AND METHODS:

Preliminary research and rearing techniques have been developed by USDA, ARS for 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 position assigned to the rearing facility was transferred to the cooperative agreement when the position was vacated in early 2008. The position was refilled by one of the FL-DPI qualified and experienced technicians as a promotional opportunity. This position will continue to coordinate the shipment of phorid flies to field cooperators as well as assist in production duties and perform methods development experiments to improve rearing techniques or solve problems as needed. Currently (winter 2008) 3-4 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 is available online from the FL DPI at: http://www.doacs.state.fl.us/pi/methods/fire-phorid.html.

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

Release techniques for the first fly species, *P. tricuspis*, are also labor intensive for the releaser. 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 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.

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.

Release techniques for the third fly species, *P. obtusus*, are utilizing a combination of the above techniques. This fly species parasitizing the largest of the worker ants, and many cooperators are having difficulty collecting enough large workers for a full release. Therefore, if the cooperator can not collect enough large workers, fly pupae (ant heads) are shipped to the cooperator as in the *P. tricuspis* release technique, and upon release of the adult flies, allowing the flies to find the large workers in the field.

Monitoring the success of the fly releases was originally conducted at a minimum annually and 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 had occurred. Our primary focus changed from monitoring release sites and spread from individual sites to determining fly presence by species at the county level. The use of the trap has enabled personnel to monitor many sites in a very short period of time – place the trap and retrieve it 24 hours later. Instructions for making the traps and site selection for monitoring are sent to cooperators involved in the trap monitoring. Traps are usually sent to the Gulfport Lab for fly identification.

<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 4 boxes in 2008. Rearing of *P. tricuspis* was at its peak in 2003 and 2004 with ca. 1.6 million flies being produced annually with production gradually decreased to allow increased production of the *P. curvatus* and *P. obtusus* flies. *P. tricuspis* will continue to be released through 2009 in limited quantities with the aim to phase out production in 2009-2010. *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 with 7 boxes in production and has subsequently decreased as *P. obtusus* production increased. In 2006, the third species, *P. obtusus,* was brought into production. Production has gone well and the first releases of this species were conducted in 2008. Total fly production levels have remained fairly constant in the last several years (Table 1).

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

Releases began in spring 2002. 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. From 2002 through 2008 there have been multiple releases in each of 13 states and Puerto Rico, with a total of 93 field releases and more than 845,000 potential flies released. Of these 93 releases, 61 were *P. tricuspis,* 30 were *P. curvatus* and 2 were *P. obtusus*. The average number of potential flies per release is about 10,000 flies. In 2008, the changing economy had an impact on our cooperators' abilities to conduct releases, and due to lack of resources in many states the number of overall releases in 2008 was significantly less than in previous years. We hope to enlist other resources in 2009 and beyond to conduct releases for this project.

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 118,000 potential flies have been shipped for these varied uses.

Success of the program was originally measured by successful overwintering of fly populations at release sites. 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 were 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 2007 we also realized that we could no longer determine the true source of flies present in an area due to the large number of established and spreading fly populations and so the attempt to determine individual site establishment of flies was abandoned. Since 2007 the use of the phorid fly trap and a new monitoring protocol for surveying for fly presence at the county level has provided a wealth of information regarding establishment and spread of the flies. Through APHIS releases, along with other federal and university groups which are also releasing flies, *P. tricuspis* is well established in the southern areas of the IFA regulated area (AL, FL, GA, LA, MS, TX and PR), and moderately established in AR, NC and SC. To date, *P. tricuspis* is not known to be established in CA, OK or TN. The second species, *P. curvatus*, is moderately to well established in all southern IFA regulated states and PR (AL, AR, FL, GA, LA, MS, NC, OK, SC, TN, TX, and PR). *P. curvatus* has not been released in CA. Overwinter establishment of *P. obtusus* has not yet been confirmed. A publication on the known U.S.-wide distribution of *P. tricuspis* and *P. curvatus* is currently being drafted. Maps are currently being developed for this publication and are not ready for inclusion in this report at this time.

<u>REFERENCES CITED</u>:

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 – 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
tri,cur,obt	2008	2,524,047	80,813	8	10,101.63
	2009				
Total		15,329,276	845,068	93	

* approx. no. potential flies shipped for release
** does not include multiple shipments to LA for initiating their own rearing facility and NM for research purposes, nor multiple shipments to cooperators for educational purposes or small research projects as flies were available
† only tricuspis shipped in 2002
†† only tricuspis and curvatus shipped in 2006 and 2007

USDA-APHIS-PPQ-CPHST-Gulfport Laboratory-Imported Fire Ant Section
Shop-built Granular Spreader for IFA Baits

Traditional broadcast bait treatments for the imported fire ant (IFA) have an application rate of 1 to 1.5 pounds per acre. Accurately applying these materials at this extremely low rate is difficult at best. Herd Seeder Company has a model GT-77 Seeder (http://www.herdseeder.com/special_uses/fireants) specifically designed and calibrated for IFA baits that works well for treating areas where the operator can travel at speeds of 6-10 mph. However, in areas such as nurseries and on rough terrain where lower speeds of 3-4 mph are required, most commercially available spreaders cannot be adjusted to apply at this low speed without causing bait flow problems due to bridging of the material. Bridging is when the material begins to stick together in the area of the opening in the base of the hopper. In addition, research testing requires a higher degree of accuracy than does general use. As a result of this, a shop built spreader was created by the USDA-IFA Lab in Gulfport, MS in the 1980s to meet this need (Fig. 1).

This spreader is essentially a Gandy Cam Gauge Row Applicator (http://www.gandy.net/cam_gauge.php3) that feeds into a modified bottom plate and broadcast fan mechanism from a Herd GT-77, 12 volt seeder. The Gandy hopper has a cam gauge mechanism to meter the flow of bait (figs 2 & 3). Inside the hopper there is a stainless steel rotor (fig. 4) powered by a 12 volt motor (fig 5). Bait is delivered to the modified broadcast fan through Tygon tubes. The bottom section is modified by removing the agitator and calibration mechanisms from a Herd GT-77 seeder and placing a metal plate over the original opening. This plate has two openings that have metal tubes attached to connect to the flexible tubing (figs. 6 & 7). The metal tubes are branched so that one opening is attached to the flexible tubing and the other is open to allow air to enter to aid the flow of bait. The two electric motors are wired to a switch box located on the back side of the hopper box. They are switched separately to allow the hopper motor to run without the slinger motor running. This particular spreader is mounted on a 3-point hitch with modifications to attach the Gandy hopper (fig. 8). However, the Herd GT-77 can be ordered with mounting hardware to fit the specific vehicle that it is to be mounted on. Some modification of any mounting system will have to be done in order to attach the Gandy mechanism.

Calibration is done by determining the required flow rate in grams per minute based on the desired ground travel speed (usually 4 mph). Once the flow rate is determined the hopper is filled with bait and the flexible tubes are detached from the bottom and placed into paper cups to collect the bait as it flows out. The hopper motor is then turned on and allowed to run for one minute. The bait is then weighed to determine the flow rate. Adjustments are made by loosening the wing nuts on the cam gauge and turning it in the required direction to open or close the gates as necessary.

Figure 1. Shop built bait spreader

Figure 2. Cam gauge metering mechanism

Figure 3. Inside view of metering mechanism; removed from hopper.

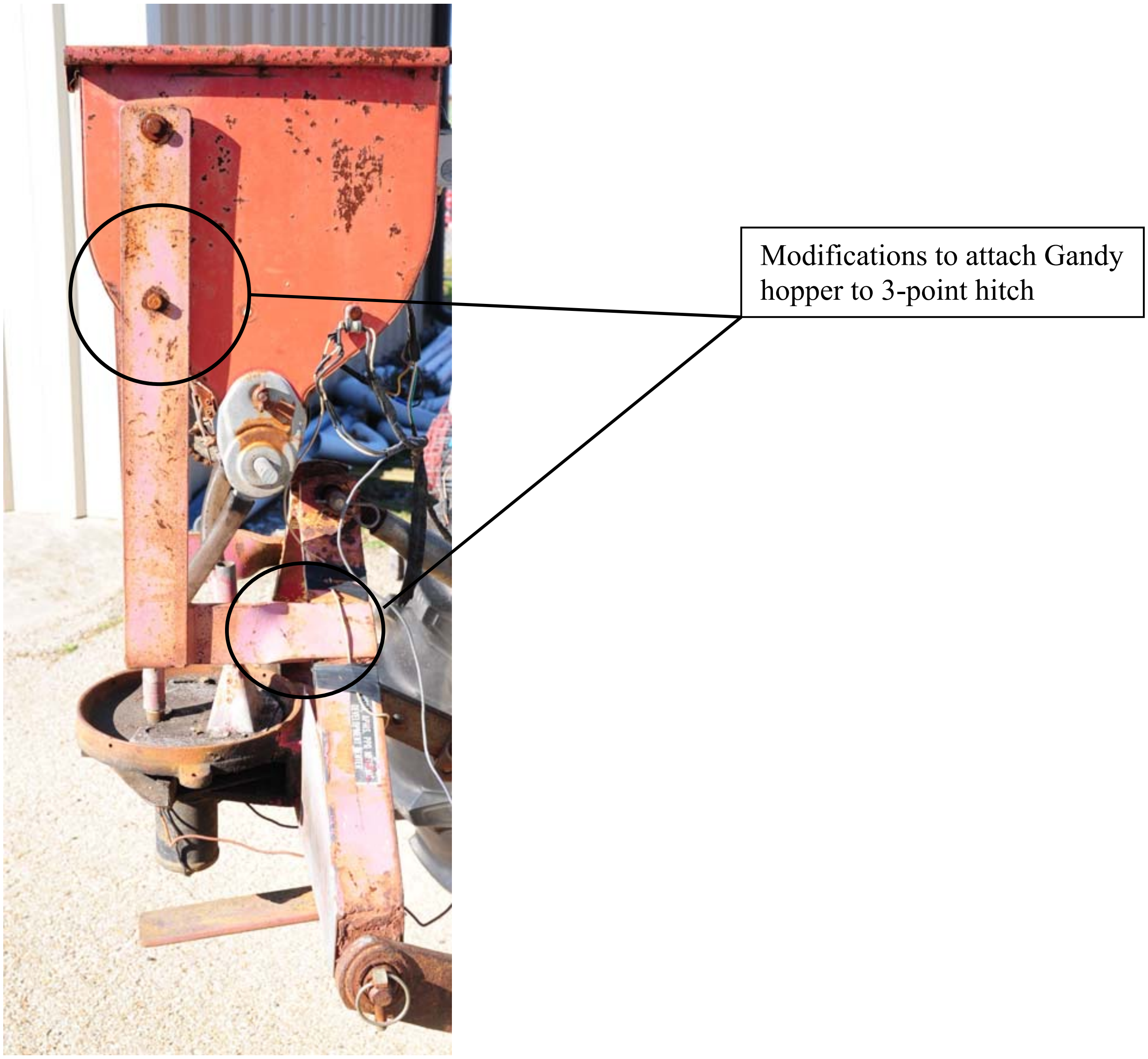

Figure 4. Stainless steel rotor.


Figure 5. Electric drive motor.


Figure 6. Modified Herd seeder bottom

Figure 7. Modified Herd seeder bottom

Figure 8. Modifications to attach the Gandy hopper to a 3-point hitch



2008 Imported Fire Ant Training Workshops for State Inspectors and/or Nursery Growers

Alabama: On February 20, 2008, PPQ headquarters, regional, state and CPHST personnel along with APHIS-IES and Alabama DPI personnel presented a training session on the federal IFA regulatory program to approximately 25 state inspectors. Topics included the biology of IFA, IFA regulations, compliance agreements, quarantine treatments, investigations into violations, etc. A.-M. Callcott presented and X. Wei attended.

Virginia: On March 12, 2008, PPQ headquarters, regional, state and CPHST personnel met with Virginia Department of Agriculture, DPI personnel to discuss the imported fire ant issues in Virginia and to encourage Virginia to initial state/federal IFA quarantines in counties that have established populations of IFA. We anticipate approximately 3 counties in VA to be added to the federal IFA quarantine in late 2008 or 2009. PPQ will provide training to state inspectors in early 2009. A.-M. Callcott presented.

Florida: May 20-23, 2008, CPHST and PPQ Florida personnel presented information on Nursery Fire Ant Management and Quarantine Treatments at the 2008 IPM Update: Nursery Workshops for south Florida. A.-M. Callcott presented in Homestead and Palm Beach; C. Preston (SOSO PPQ-FL) presented in Balm and Apopka.

2008 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 2008. 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 2008, levels of detection (LOD), levels of quantification (LOQ), and range of below quantifiable level (BQL), in ppm, were varied due to changes in the analytical process. January-March 2008 samples were reported at the levels below:

	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

A new analytical process allowed lower levels of detection and a multi-screening were put into action in April through Decmeber 2008 with levels reported below:

	LOD	LOQ	BQL
Bifenthrin	0.12	0.4	0.12 - 0.4
Chlorpyrifos	0.12	0.4	0.12 - 0.4
Diazinon	0.12	0.4	0.12 - 0.4
Fipronil	0.12	0.4	0.12 - 0.4

Overview of sample numbers:

- 307 total samples submitted
  - 226 nursery samples
  - 5 sediment samples collected from around one nursery's treatment area
  - 76 nursery samples submitted from FL due to state lab instrument failure
- 0 samples from potential violations

- 31 blitz samples from NC (blitzes in spring and fall)
- 200 routine samples
    - 20 samples requesting bulk density only
    - 99 samples requesting chemical analysis only (includes sediment)
    - 81 samples requesting chemical analysis and bulk density
- 76 samples from Florida in late December 2008 due to instrument failure at FL State Lab
    - Higher detection levels used (LOD<0.9; LOQ=3.0; bql=0.9-3.0)
    - 37 samples requesting bifenthrin only
    - 39 samples requesting bifenthrin and live insect bioassay

Results:

- 5 sediment samples analyzed for bifenthrin; all less than detectible limit of 0.9 ppm
- 31 blitz samples from NC
    - 24 samples (77%) had detectible levels of program insecticides
- 200 routine samples (some BD only, some chemical analysis only, some both)
    - 102 bulk density samples: range 117-888 lb/cu yd
    - 175 samples analyzed for 1 or more program insecticides (excludes sediment)
        - 167 samples (95%) had detectible levels of program insecticides
        - 8 remaining samples – no program chemicals detected
- 76 Florida samples – unknown circumstances of collection
    - 37 samples requesting bifenthrin only
        - 36 samples (97%) had detectible levels of bifenthrin
    - 39 samples requesting bifenthrin and live insect bioassay
        - 20 samples (51%) had detectible levels of bifenthrin
        - 19 samples (49%) – no program chemicals detected (<0.9 ppm)

APPENDIX I - LABORATORY BIOASSAY PROCEDURE

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

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

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

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

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

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

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

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

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

1) Availability of queens; supply is primarily influenced by season. More time will be spent collecting queens in winter or during extreme droughts.
2) Number of treatments to be evaluated; e.g., if only a single treatment and an untreated check are to be evaluated only 40 queens/month are needed. Conversely, a test involving 4 insecticides at 3 rates of application (12 treatments + untreated check) will require 260 queens monthly for the duration of the test.

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

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

Figure 1. Alate females being removed from nest tumulus.

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

Figure 3. Set up of bioassay test procedure.