2009 ACCOMPLISHMENT REPORT

IMPORTED FIRE ANT SECTION

and

ANALYTICAL CHEMISTRY SECTION

GULFPORT LABORATORY

CENTER FOR PLANT HEALTH SCIENCE AND TECHNOLOGY

PLANT PROTECTION AND QUARANTINE

ANIMAL AND PLANT HEALTH INSPECTION STATION

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

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

Compiled and Edited by:

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April 2009

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

The CPHST Gulfport Laboratory in Gulfport, MS, consists of two sections: the Analytical Chemistry section, and the Imported Fire Ant (IFA) section. The analytical chemistry section conducts routine sample analysis for detecting the presence of pesticide residues and toxic substances directly supporting ongoing APHIS Operational and Emergency programs including; Imported Fire Ant, Asian Longhorned Beetle, Boll Weevil, Grasshopper/Mormon Cricket, and Fruit Fly. In addition, the chemistry laboratory supports APHIS projects by providing chemistry based options for PPQ field operatives concerning the identification and detection of prohibited commodities, or the detection of invasive insect species.

The IFA section develops methods and tools for the survey, detection, regulation, and control (both chemical and biological control) of the imported fire ant. Technology developed by the IFA section is utilized by PPQ, State Plant Regulatory Officials (SPROs), the nursery industry, chemical industry, farmers, homeowners, and other stakeholders.

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CPHST PIC NO: A9F01

PROJECT TITLE: Residual Activity of Various Insecticides used as a Containerized Drench Treatment

REPORT TYPE: Final

PROJECT LEADER/PARTICIPANTS: Lee McAnally

INTRODUCTION:

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

MATERIALS AND METHODS:

On June 22, 2009 untreated MAFES media (3:1:1 pine bark: sphagnum peat moss: sand – bulk density = 985 lb/cu yd), and Windmill media (partially decomposed pine bark – bulk density = 475 lb/cu yd) was placed in 1-gallon nursery pots and drenched with 400ml finished solution with various insecticides at the rates indicated in table 1. Twenty-one pots were drenched for each treatment in each media type. The pots were then weathered outdoors under simulated nursery conditions. An overhead irrigation system supplied ca. 1-1½ inches water per week. At 2 weeks and thereafter at monthly intervals for six months, 3 pots of each treatment were composited and sub samples taken. These sub samples were then subjected to standard alate queen bioassay (Appendix I).

Table 1. Various insecticides and rates tested for use as drench treatments

Insecticide	Formulation Tested	Rates of Application (ppm)
lambda-cyhalothrin	Scimitar SC	10, 15, 25
zeta-cypermethrin	Mustang Max EC	10, 15, 25
bifenthrin + zeta-cypermethrin	Hero EC	10, 13.4, 15
imidacloprid + cyfluthrin	Discus EC	25, 50
bifenthrin EC	Bifenthrin EC	10
bifenthrin	Bifenthrin F	10
cyfluthrin	Tempo SC	25, 50

RESULTS:

The results are summarized in table 2. Lambda-cyhalothrin and cyfluthrin were 100% effective in both media types at all rates of application for 6 months. Zeta-cypermethrin in the MAFES media maintained 100% mortality through six months at the 25 ppm rate and 80-100% at the 10 and 15 ppm rate. In the Windmill media zeta-cypermethrin maintained 90-100% at 25 ppm and was erratic at the other rates. The zeta-cypermethrin + bifenthrin product was also 100% effective in the MAFES media at all rates, but erratic in the Windmill media. The bifenthrin formulations at 10 ppm were 100% effective for 6 months in the MAFES, but in the Windmill were effective for 2-3 months, and erratic thereafter. The imidacloprid + cyfluthrin product maintained 85-100% mortality at 50 ppm in the MAFES media but was ineffective at the lower rate and at either rate in the Windmill media.

Historically, we have used the Windmill media as our "worst-case" media when testing insecticides against IFA. In this trial, those formulations that were less than 100% effective had lower mortality rates in the Windmill media than in the MAFES media.

Table 2. Residual activity against IFA of various insecticides used as drench treatments

Media Type	Active Ingredient(s)	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment at 14 days exposure						
			0.5	1	2	3	4	5	6
MAFES	lambda-cyhalothrin	10	100	100	100	100	100	100	100
		15	100	100	100	100	100	100	100
		25	100	100	100	100	100	100	100
	zeta-cypermethrin	10	100	90	100	100	85	100	100
		15	100	100	100	100	95	100	80
		25	100	100	100	100	100	100	100
	bifenthrin + zeta-cypermethrin	10	100	100	100	100	100	100	100
		13.4	100	100	100	100	100	100	100
		15	100	100	100	100	100	100	100
	imidacloprid + cyfluthrin	25	55	80	50	60	65	70	60
		50	85	100	100	100	100	100	90
	bifenthrin EC	10	100	100	100	100	100	100	100
	bifenthrin F	10	100	100	100	100	100	100	100
	cyfluthrin	25	100	100	100	100	100	100	100
		50	100	100	100	100	100	100	100
	Check	--	45	5	5	5	10	10	0
Windmill	lambda-cyhalothrin	10	100	100	100	100	100	100	100
		15	100	100	100	100	100	100	100
		25	100	100	100	100	100	100	100
	zeta-cypermethrin	10	80	75	60	40	25	40	35
		15	85	60	65	50	40	50	65
		25	100	95	90	95	90	100	100
	bifenthrin + zeta-cypermethrin	10	85	100	100	90	70	100	100
		13.4	100	100	100	100	85	100	100
		15	100	100	100	100	65	95	85
	imidacloprid + cyfluthrin	25	35	70	30	20	0	20	25
		50	25	50	75	40	15	30	25
	bifenthrin EC	10	100	100	100	100	75	100	100
	bifenthrin F	10	100	100	100	80	45	100	90
	cyfluthrin	25	100	100	100	100	100	100	100
		50	100	100	100	100	100	100	100
	Check	--	20	45	45	15	25	15	5

CPHST PIC NO: A9F01

PROJECT TITLE: Chemical Degradation of Two Formulations of Bifenthrin Used for Drench Treatment of Containerized Nursery Stock Potting Media, 2009

TYPE REPORT: Final

PROJECT LEADER/PARTICIPANT(s): Lee McAnally

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 suspected 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 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 Chemical Analysis 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.

All treatments in the original trial 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 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 was conducted in 2009 using larger 5-gallon pots. Each pot was one replicate with samples taken from the same pot at each post-treatment interval.

MATERIALS AND METHODS:

Potting media used in this test were: MAFES media (3:1:1 pine bark: sphagnum peat moss: sand with bulk density = 985 lb/cu yd); Windmill media (Windmill Nursery, Folsom, LA with bulk

density = 475 pounds per cubic yd). These media where placed in five-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. The treatments were made using both a 2EC and a flowable formulation of bifenthrin. Both formulations were applied at a theoretical dose rate of 10 ppm and 25 ppm. The drench solutions where applied to standard 5-gallon nursery pots at a rate of 1/5 the volume of the container (ca. 1 gallon drench solution) as called for in the quarantine manual. Three pots were treated for each treatment type with each individual pot being considered a replicate. 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 core samples from each pot (replicate) three such samples per treatment were submitted for analysis. Samples were taken at 0, 2 weeks, and monthly for 6 months post-treatment. For details on the analysis, see the corresponding report in the analytical chemistry section of the 2009 Gulfport Lab annual report.

RESULTS:

Results are summarized in Table 1 and Figures 1 and 2. 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 0.90ppm and 3.0ppm, respectively. Therefore for calculation purposes, 1.95ppm was used as the bql value (bql=below quantifiable level) for any analyses falling between LOD and LOQ, and 0 was used for results of <0.90ppm.

The initial analysis immediately after treatment for both rates of application is significantly lower than the theoretical rates of 10 and 25 ppm with an average of 4.4 and 7.1 ppm, respectively (combining media types and formulations). However, by 2 weeks after treatment, across all media types and formulations, the mean result for the 10 ppm rate is 7.7 ppm and for the 25 ppm rate is 18.8 ppm, 23% and 25% below initial expected theoretical rates. By 3 months after treatment, analyses showed a 54% reduction in initial theoretical dose rate in both the 10 and 25 ppm rates (combined data). At 6 months, the combined data showed a 67-70% reduction in initial theoretical dose rate in both the 10 ppm and 25 ppm rates (combined data).

The 10 ppm rate with both formulation types in the MAFES media provided detectable levels of bifenthrin throughout the 6 month trial. The EC formulation in Windmill media also provided detectable levels of bifenthrin throughout the trial, but at 3-6 months, levels were below quantifiable levels (between 0.9 and 3 ppm). The flowable formulation in Windmill media was detected through 1 month, but was below detectible levels from 2-6 months. At 3 months after treatment, analyses of the MAFES media showed 18% reduction of bifenthrin from initial theoretical dose rates for both formulation types, while the Windmill media showed 90% reduction in bifenthrin levels. At 6 months, the MAFES media showed 50% reduction in initial

theoretical rates (combined formulation types), while the Windmill showed 90% reduction in bifenthrin.

The 25 ppm rate (approved for use in the IFA quarantine for 6 months) in both media types and with both formulations showed acceptable levels of bifenthrin throughout the trial. As with the 10 ppm rate, the MAFES media had higher levels of bifenthrin throughout the trial, while the Windmill media treated with the flowable formulation fell below quantifiable levels at 3-6 months. However, these levels are still high enough to kill IFA. At 3 months after treatment, analyses of the MAFES media showed 18% reduction of bifenthrin from initial theoretical dose rates for both formulation types, while the Windmill media showed 88% reduction in bifenthrin levels. At 6 months, the MAFES media showed 41% reduction in initial theoretical rates (combined formulation types), while the Windmill showed 92% reduction in bifenthrin.

Historically, we have used the Windmill media as our "worst-case" media when testing insecticides against IFA. These chemical analyses support the historical bioassay data that indicated that media type impacts insecticide residual activity. This work documents that bifenthrin is present for 6 months in several media types when used as a 25 ppm container drench treatment. Degradation rates are difficult to determine in this use pattern and are media-dependent, but this data does provide a guide for degradation in two media types.

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

Media Type	Formulation	Rate (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	2EC	10	2.57	9.37	11.77	10.8	12.47	9.73	9.13	7.07
		25	3.04	40.00	18.33	26.33	30.00	20.33	20.00	19.00
	Flowable	10	2.72	7.60	7.53	6.73	3.98	4.85	5.30	3.10
		25	3.06	16.33	17.33	13.33	11.15	10.20	12.67	10.50
Windmill	2EC	10	8.20	10.30	8.10	6.40	1.95	1.95	1.95	1.95
		25	11.67	10.20	7.57	7.63	4.27	4.17	3.63	1.95
	Flowable	10	4.30	3.57	1.95	<0.90	<0.90	<0.90	<0.90	<0.90
		25	10.50	8.90	7.33	5.02	1.95	1.95	1.95	1.95
	Mean	10	4.4	7.7	7.3	9.9	4.6	4.1	4.1	3.03
		25	7.1	18.8	12.6	13.1	11.8	9.2	9.6	8.35

Figure 1. Analytical residue results of bifenthrin EC and F drenched over Windmill and MAFES potting media at 10 ppm.

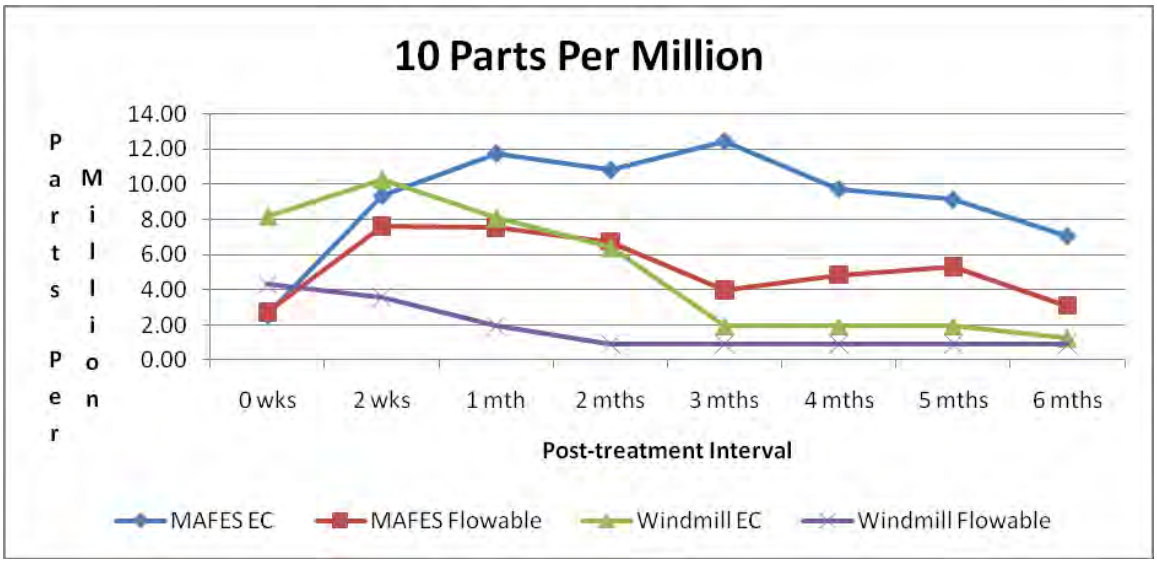
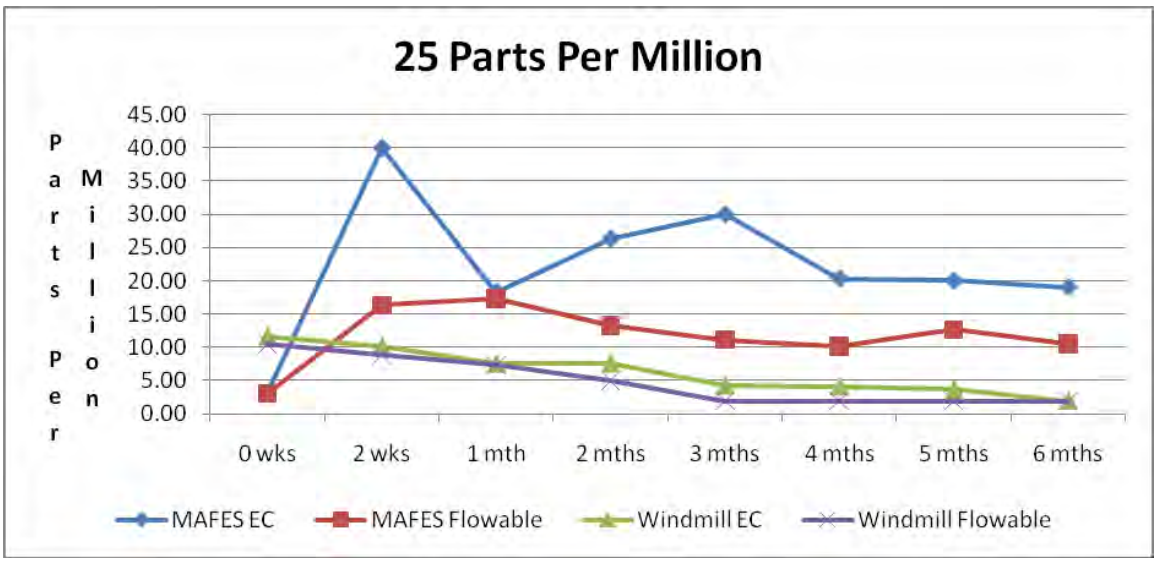


Figure 2. Analytical residue results of bifenthrin EC and F drenched over Windmill and MAFES potting media at 25 ppm.



CPHST PIC NO: A1F04

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

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 elsewhere.

Standard IFA testing of chemical treatments for both dip and drench applications has been conducted through female alate bioassays on soil core samples from the treated root balls. Soil core bioassays for drenches conducted in 2002 and spring 2003 yielded erratic results over time and among replicates within treatments. Results from the same chemicals at equal or lower rates, when applied by immersion, were consistent, thus indicating insufficiency in application of the drench treatments. Doubling the volume of solution in drench application conducted in fall 2003 and spring 2004 failed to eliminate inconsistent results. The search for the cause of the inconsistency problem become narrower and has pointed to coverage and penetration of the drench solutions.

During drenching, B&B normally rests on one side of the root ball throughout the three-day drench process. This was true for all drench treatments done before fall 2004. This drench method possibly restricts treatment coverage on the resting side, while giving the surface of

direct application a higher concentration of chemical and deeper penetration. The 2004 fall drench strongly suggested that rotating root balls during treatment, regardless of application frequency, improved the consistency of bioassay results and could potentially cut the number of days spent applying drenches from three down to one. Trials were repeated 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 spray 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.

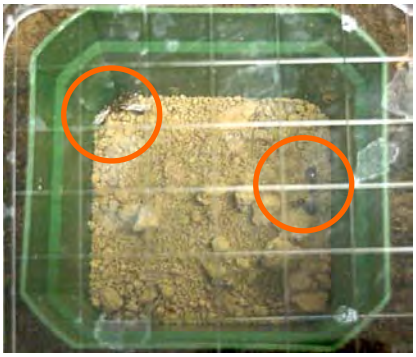


Figure 7. Orange circles indicate the locations of clusters of female alates within this bioassay cup.

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 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.

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.

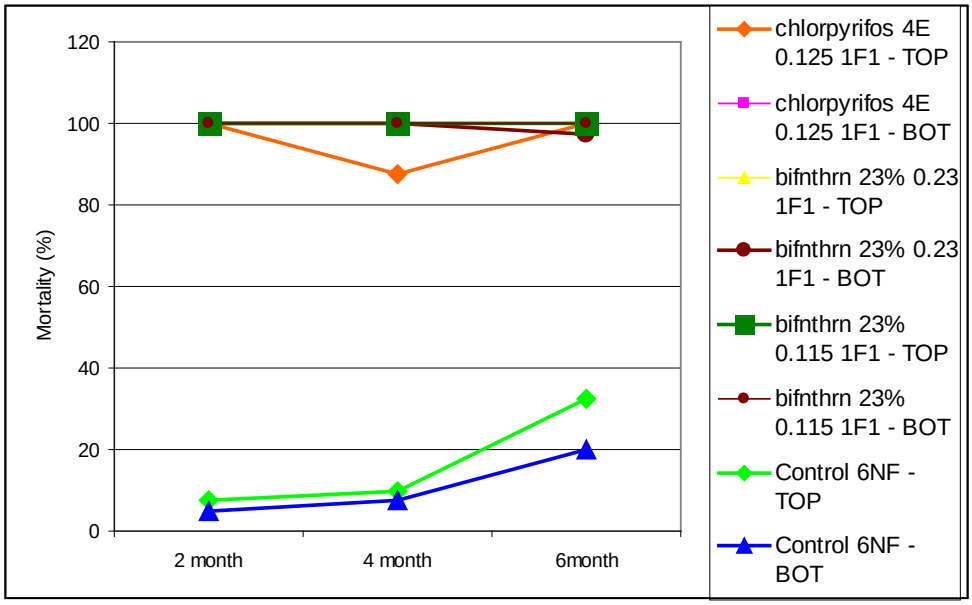
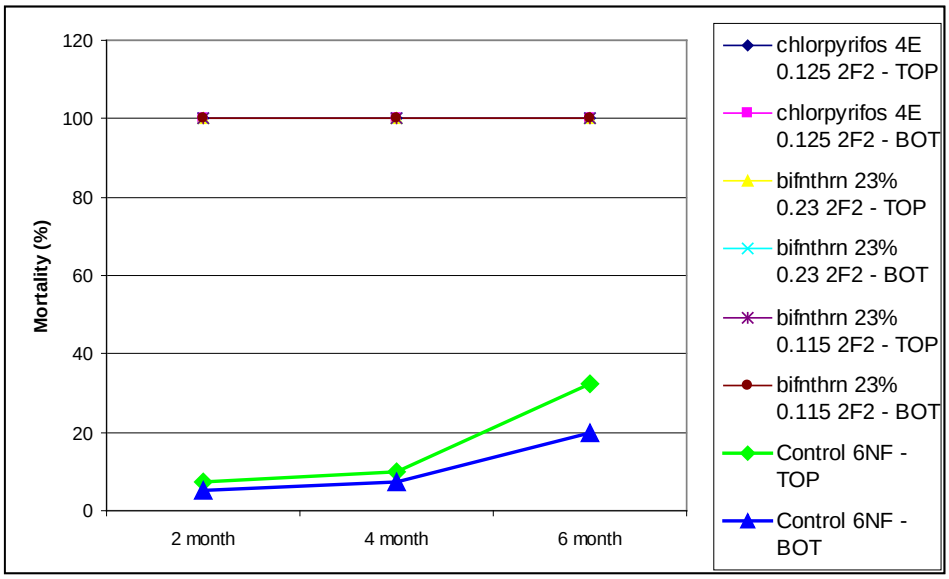


Figure 9. IFA control achieved with three chemicals treated soil samples collected at two surface sites from the application 2F2 regimes at 2, 4, and 6 months after final drench application Spring 08.



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.

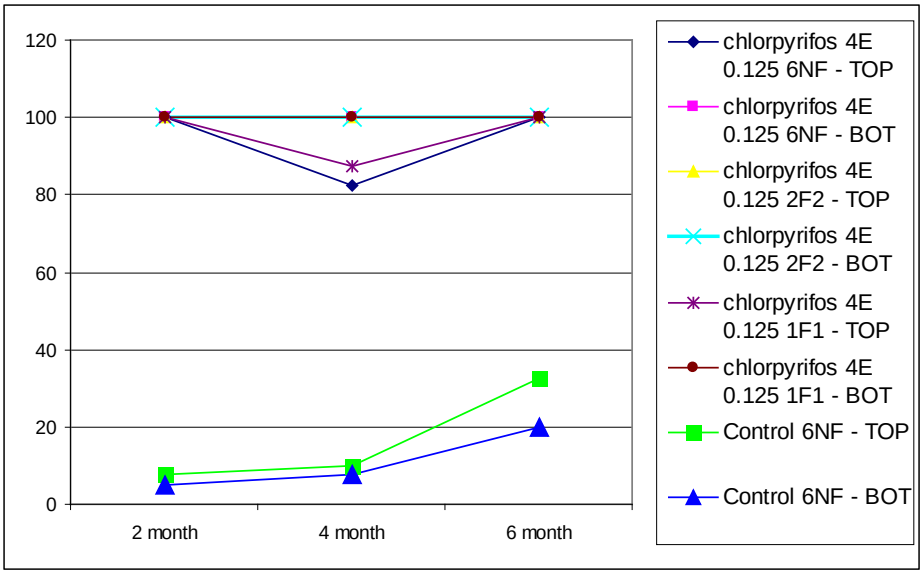
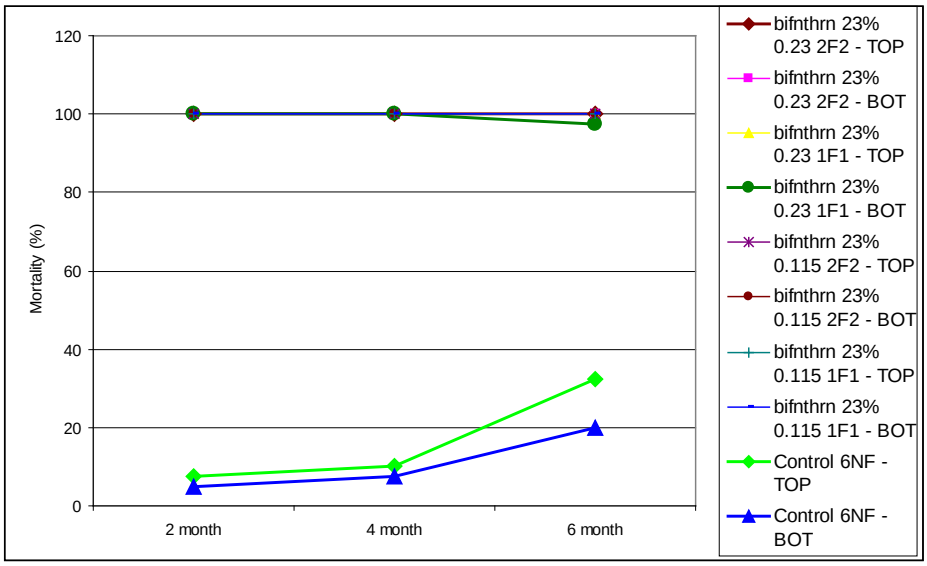


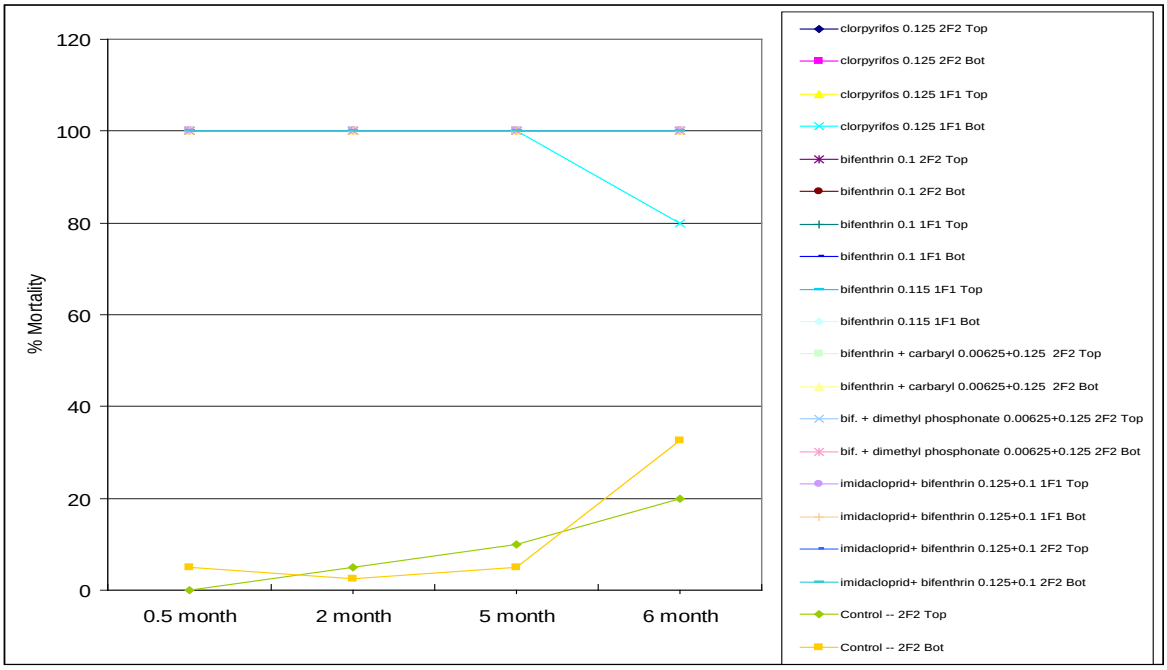
Figure 11. IFA control achieved with two rates of bifenthrin-treated soil samples collected at two surface sites from 1F1 & 2F2 application regimes at 2, 4, and 6 months after final drench application Spring 08.



Drench trial fall 2008 on 12" rootballs

The bifenthrin rates used in the fall trials were only half or less than the spring trial rates, especially in the combination treatments where bifenthrin was as low as 0.00625 lb a.i. per 100 gallons water. Results showed that all treatments tested in this trial (see Table 2), regardless of rates, application method (1F1, 2F2), single chemical treatment or the combination treatment of more than one chemical, achieved 100% in mortality in the IFA female alates bioassay for the entire 6 months except that one bottom sample in the chlorpyrifos treatment at 6 month did not kill which resulted in a control of 80% for that treatment (Fig 12). This trial indicated that bifenthrin at 0.1 lb a.i. per 100 gallon water using the application method 1F1 or 2F2 could achieve IFA quarantine level control requirement. It further confirmed that the application methods 1F1 and 2F2 both are adequate as a drench treatment application method. Since the application method 1F1 could cut the treatment handling in half, it should be the method of choice for the post-harvest drench treatment application method.

Figure 12. IFA control achieved with soil samples treated with bifenthrin or bifenthrin in combination with one other insecticides collected at two surface sites from 1F1 & 2F2 application regimes at 2, 4, and 6 months after final drench application fall 2008.



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

CPHST PIC NO: A1F04

PROJECT TITLE: Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Spring and Fall 2009 in Tennessee

REPORT TYPE: Interim

LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Craig Hinton, Lee McAnally; Jason Oliver and Nadeer Youssef of Tennessee State University; Chris Ranger 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 elsewhere.

Standard IFA testing of chemical treatments for both dip and drench applications has been conducted through female alate bioassays on soil core samples from the treated root balls. Soil core bioassays for drenches conducted in 2002 and spring 2003 yielded erratic results over time and among replicates within treatments. Results from the same chemicals at equal or lower rates, when applied by immersion, were consistent, thus indicating insufficiency in application of the drench treatments. Doubling the volume of solution in drench application conducted in fall 2003 and spring 2004 failed to eliminate inconsistent results. The search for the cause of the inconsistency problem become narrower and has pointed to coverage and penetration of the drench solutions.

During drenching, B&B normally rests on one side of the root ball throughout the three-day drench process. This was true for all drench treatments done before fall 2004. This drench method possibly restricts treatment coverage on the resting side, while giving the surface of

direct application a higher concentration of chemical and deeper penetration. The 2004 fall drench strongly suggested that rotating root balls during treatment, regardless of application frequency, improved the consistency of bioassay results and could potentially cut the number of days spent applying drenches from three down to one. Trials were repeated 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.

2009 drench trials in TN again focused on examining some promising insecticides and plant handling methods for 12" root balls. Multiple insecticides and their combinations, application frequencies, and plant handling methods (rotating vs. non-rotating) were investigated.

MATERIALS AND METHODS:

In March 2009 TSU-NRC and USDA-ARS personnel completed drench applications on B&B plants with 12-inch diameter root balls at the TSU-NRC in Warren Co., TN. Two changes were made to this spring drench trial: 1) conventional 6NF drench method was discontinued in this trial; therefore the control treatment used 2F2 instead of 6NF in the past, and 2) bifenthrin at 0.115 lb ai treatment was "true to the rate" (calculated and applied in each drench at 0.115 lb ai per 100 gallon of water) which is different from the way the bifenthrin at 0.1 rate was calculated and applied (see explanation in Results and Discussion section). Drench solutions, final rates, and plant handling which composed the treatments are listed in Table 1.

Treatments were applied at 0.82 gallons per treatment using a regular garden sprinkler can (Fig 5). Solutions were applied twice daily (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. This plant handling methods are described as 1F1**.** This method requires minimum chemical solution and days of application for drench treatments. The regime 2F2 was to do one drench in the morning and another in the afternoon on one side of the root balls for the first day. The next day, flip the trees and drench two more times (morning and afternoon) for the other side of the root balls. The regime 6NF was not used in this trial but as the currently approved drench application method it requires applying drenches twice a day for 3 consecutive days without flipping the root balls. Each root ball received approximately 0.16 gallons of drench solution at each drenching totaling 0.33 gallons a day (so 1F1 = 0.33 gal solution & 2F2 = 0.66 gal). The amount used per drench application was based on the amount needed to achieve "the point of runoff" required in the IFA quarantine.

The fall trial was conducted in October 2009 for 12" root balls. The treatments for the fall trial are listed in Table 2 and they were also applied using a regular garden sprinkler can (Fig 5). The drench solution was 0.82 gallons to be applied to each treatment. Other than chlorpyrifos and bifenthrin, a combined formulation of bifenthrin with imidacloprid was also investigated in this trial. Similar to the spring trial, only the treatment application methods 1F1 and 2F2 were used excluding the conventional 6NF application method. This is because we had found over the past few years that it is necessary to flip the rootballs during treatment application in order to achieve

an even coverage and consistent bioassay results for the post-harvest drench treatment and the 6NF application method does not provide adequate coverage.

Table 1. List of treatments for 12” drench trial in TN spring 2009

Product	Active Ingredient	Rate * (lb a.i./ 100 gal H_2O)	Plant Handling	
			1F1	2F2
Lorsban 4E	chlorpyrifos	0.125	X	X
OnyxPro 23%	bifenthrin	0.1	X	X
OnyxPro 23%	bifenthrin	0.115*	X	
Allectus	imidacloprid+ bifenthrin	0.25+0.2	X	X
Talstar + Sevin	bifenthrin + carbaryl	0.1 + 0.75		X
Talstar + Dylox	bifenthrin + dimethyl phosphonate	0.1 + 0.5		X
Control	----	----		X

*applied at 0.115 lb ai per 100 gal of water in each drench.

Table 2. List of treatments for 12 inch drench trial in TN fall 2009

Product	Active Ingredient	Rate* (lb a.i./ 100 gal H_2O)	Handling	
			1F1	2F2
Allectus	imidacloprid+ bifenthrin	0.125+0.1	X	X
Allectus	imidacloprid+ bifenthrin	0.25+0.2	X	X
Lorsban	chlorpyrifos	0.125	X	X
Onyx 23%	bifenthrin	0.0575	X	
Onyx 23%	bifenthrin	0.115	X	
Onyx 23%	bifenthrin	0.2	X	
Control	--	--		X

*all treatments applied true to the listed rates without converting to 6NF first.

Fig.4. 12” rootballs grouped for drench treatment

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

In the fall trial, one major change is how treatment rate was calculated for 1F1 and 2F2 application method. In the spring 2009 and previous drench trials, 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. In the fall trial, however, treatment rate was calculated as "true rate" as listed in each drench; therefore, by the conclusion of the final drench, plants applied with 2F2 method received twice as much the amount of chemical and drench solution as the plants with 1F1 method. This change has simplified treatment application and will be easier for nursery growers.

After final treatment, the plants were maintained outdoors 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. 6) for the spring trial. But in the fall 2009 trial, this sampling system was discontinued. Instead, the sampling method used since 2008 for drench trial in Gulfport Lab, MS was followed. In this method, only one soil core sample was taken from the mid-side area of each rootball at the initial bioassay day. On next sample day, we rotated the rootballs for a quarter turn (as shown in Fig 6) 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. Soil samples were collected from within the first four inches of soil depth for testing against red IFA. The soil samples were frozen and sent to the CPHST Lab in Gulfport, MS where they were utilized in female alates bioassays. A single bioassay cup containing 10 female alates was utilized for each soil sample (replicate). Female alate mortality was recorded two times a week during the 14-day exposure period, and dead alates were removed from bioassay cups during these observations (Figs 7 & 8).

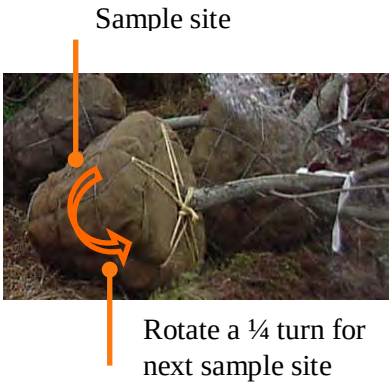


Figure 6. Soil core sample collection sites

Figure 7. A tray of alates mortality bioassay cups.

Figure 8. Orange circles indicate the locations of clusters of female alates within this bioassay cup.

RESULTS AND DISCUSSION:

Drench trial spring 2009

The spring 2009 drench trial focused mainly on bifenthrin and combinations of bifenthrin with one of a few other promising insecticides. As usual, chlorpyrifos was included as a chemical standard because it is the only currently approved insecticide for post-harvest drench and dip treatments of IFA quarantine. The bifenthrin rates used in the spring trial were 0.1 lb ai alone and 0.1 or 0.2 lb ai in combined or tank-mixed treatments. All treatments tested with bifenthrin as the main ingredient achieved 100% mortality throughout the 6-month testing period. The treatment application method 1F1 and 2F2 performed equally well with 100% mortality for all bifenthrin-related treatments for 6 months (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. Since 1F1 could cut the treatment handling in half, it could become the method of choice for the post-harvest drench treatment application method. For chlorpyrifos, however, 1F1 did better than 2F2 in the spring trial, and this may be caused by the fact that 1F1 treatment used solution concentration that is twice as much as 2F2. However, chlorpyrifos 0.125 could only have 2 months of quarantine level control. The top and bottom samples did not make any difference; therefore, only top sample results were pooled together in this report.

Drench trial fall 2009

The fall 2009 drench trial also focused on bifenthrin and a combined formulation of bifenthrin & imidacloprid (Allectus). For the bifenthrin alone treatments, the ½-, 1- and 2-fold of spring trial rate were used. Results available so far (2 months) showed that all bifenthrin-related treatments tested in this trial (see Table 2), regardless of rates, application method (1F1, 2F2), applied alone or in combination, achieved 100% in mortality in the IFA female alates bioassay (Fig 10).

One major change in fall 2009 drench trial in TN was the way treatment rates were calculated. In the past several years when flip drench was first introduced, in order to compare the effectiveness of different handling method (1F1, 2F2, 6NF), we kept the amount of chemical applied to each root ball the same by varying the concentrations of the drench solution so that at the completion of each drenching treatment, each root ball would receive exactly the same amount of chemical regardless how many drenches a root ball received. To achieve this, trees with 1F1 treatment method were drenched with chemical solution that was doubled the rate of trees receiving 2F2 treatment method. As a consequence, trees receiving the same listed treatment rate could be applied with drench solution that is 1/3 or ½ of concentration of other trees, which was not only confusing but also creating other issues of concern. Since fall 2007, drench trial conducted in MS started to use the true listed rate for drench trial, that is, each drench uses the same chemical concentration regardless how many times the rootball received drenches. As a result of this change, rootballs with 2F2 application method were applied twice as much chemical and drench solution as plants with 1F1 application method. This change has dropped the complication and coufusion in rate calculation.

Figure 9. IFA control achieved with treated soil samples collected at 0.5, 2, 4, and 6 months after final drench application in TN Spring 2009.

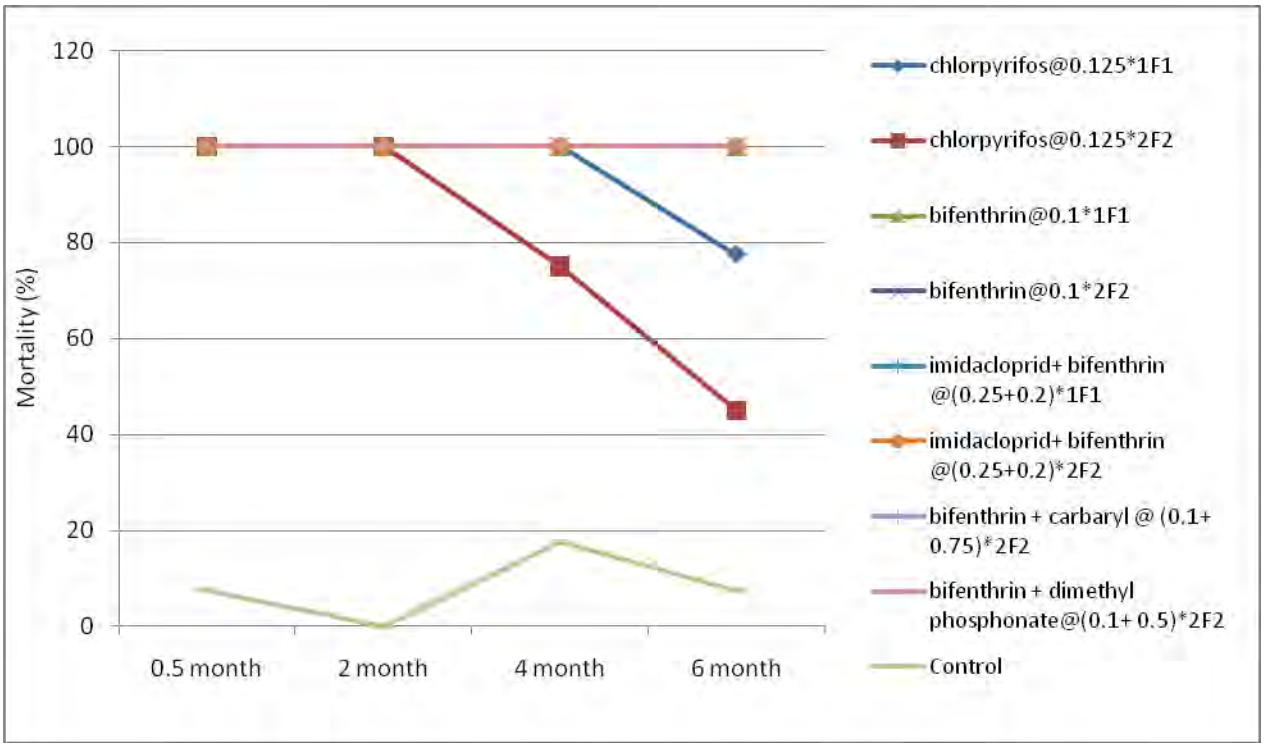
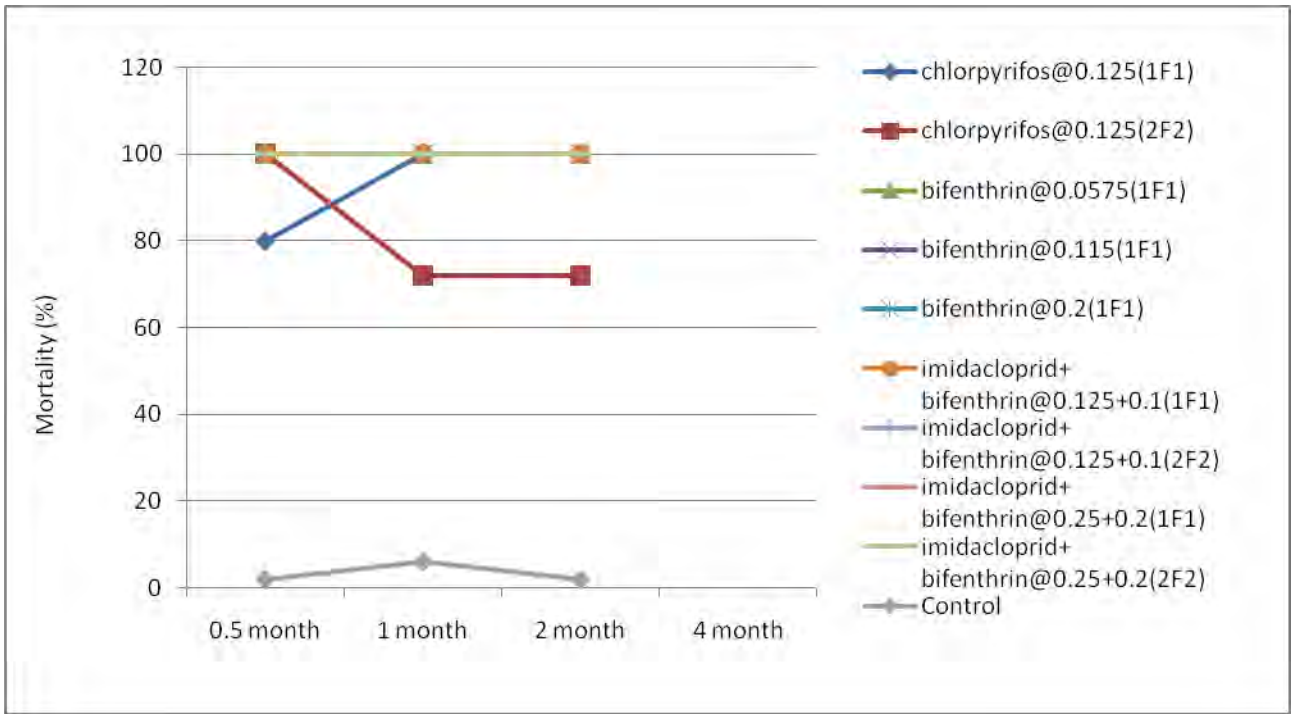


Figure 10. IFA control achieved with soil samples treated with bifenthrin alone or in combination at 0.5, 1, and 2 months after final drench application in TN fall 2009.



CPHST PIC NO: A1F04

PROJECT TITLE: Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Gulfport MS 2009

REPORT TYPE: Interim

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

INTRODUCTION:

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

Current certification options for harvested B&B stock are immersion in a chlorpyrifos solution (dipping) or watering twice daily with a chlorpyrifos solution for three consecutive days (drenching). Standard IFA testing of chemical treatments for both dip and drench applications has been conducted through female alate bioassays on soil core samples from the treated root balls. Soil core bioassays for drenches conducted in 2002 and spring 2003 yielded erratic results over time and among replicates within treatments. The same chemicals at equal or lower rates, when applied by immersion however, gave consistent results, thus indicating insufficiency in either application or the mode of testing for the treatments applied through drench. Drench trials conducted in fall 2003 and spring 2004 determined that doubling the volume of solution applied failed to eliminate inconsistent results.

Until fall of 2004, drenching was done without rotating the root balls and B&B normally rests on one side of the root ball throughout the three-day drench process. This possibly restricts treatment coverage on the resting side of the ball, while giving the surface of direct application a higher concentration of chemical and deeper penetration. The 2004 fall drench strongly suggested that rotating root balls during treatment, regardless of application frequency, improved the consistency of bioassay results and could potentially cut the number of days spent applying drenches from three down to one. Trials were repeated in spring 2005 to examine whether changes in plant handling during application improve penetration and coverage and possibly allow reduction in the number of days required to complete a drench. Fall 2007 trials in TN continued examining the following treatment/plant handling methods for drench application. 1F1**:** one drench in the morning; then in the afternoon, flips the trees and drenches the other side of the rootballs. This method requires minimum chemical and days of application for drench treatments. 2F2: one drench in the morning and in the afternoon on one side of the root ball. 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, treatments still required two days to complete in this method. 6NF: This is the conventional and currently approved method included in this 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 MS in 2007 and 2008 showed that the application method 1F1 was a suitable and effective method for drench application. In 2008 at the Gulfport Lab, we also investigated if "drying period" of 30 minutes versus ca. 5 hours before flipping makes a difference on insecticidal efficacy because nursery growers would rather not wait for hours before flipping the rootballs to complete drenching the other side of the rootballs. Our results indicated that waiting period before flipping did not make any difference on efficacy and therefore a longer waiting period of hours was not considered necessary. The objective of the 2009 trials was to repeat the 1F1 drench treatment method using bifenthrin at lower rates and also added lambda-cyhalothrin to the drench trials.

MATERIALS AND METHODS:

Drench application

The balled & burlapped plants with 18-inch-diameter root balls were purchased from Deep South Nursery, Lucedale, Mississippi. Five rootballs were used in each treatment as 5 replicates. 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 the total spray volume of each ball. Since this total volume of 1.4 gal was divided into 2 drenches, each drench used 0.7 gal per tree and 3.5 gal per treatment of 5 trees (spring trial used 8 trees per treatment; therefore, 5.6 gal was used). 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 drenching but without having too much run off to the ground. Balls were drenched on one side, allowed to rest for 30 minutes, then flipped and drenched on the other side.

Figure 1. Drench application

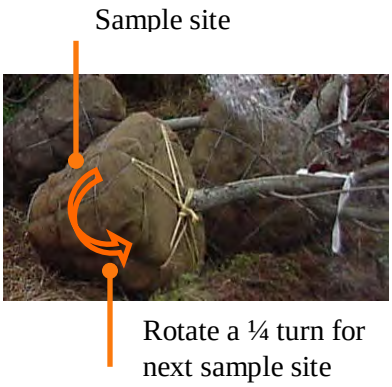


Figure 2. Soil core sample collection sites

Drench treatments consisted of three chemicals: bifenthrin and lambda-cyhalothrin each at two different rates and one rate of chlorpyrifos plus a water only control. Chemicals used, solutions, final application rates, and handling which composed the treatments are listed in the tables below (Tables 1 and 2). The fall trial was a repeat of the spring trial except that the rates of the lambda-cyhalothrin tested were higher than in the spring trial because the spring trial rates of 0.035 and 0.069 # ai/ 100 gal were not effective enough and were raised to 0.069 and 0.138 # ai/ 100 gal in the fall trial.

Table 1. Treatment List for 1F1 Drench Trial in Gulfport, Mississippi Spring 2009

Material	Active Ingredient	Trt #	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	0.125	1.18	5.6 gal	6.61 ml	13.22 ml
Onyx Pro 23%	Bifenthrin	2	0.0125	0.235	5.6 gal	1.32 ml	2.63 ml
		3	0.025	0.47	5.6 gal	2.63 ml	5.26 ml
Scimitar	Lambda-cyhalothrin	4	0.035	1.51	5.6 gal	8.46 ml	16.91 ml
		5	0.069	2.97	5.6 gal	16.63 ml	33.26 ml
Control	--	6	--	--	3.5 gal	--	--

*Balls were rotated once between the two chemical drenches; 8 rootballs were used per treatment but only 5 were used for bioassay.

Table 2. Treatment List for 1F1 Drench Trial in Gulfport, Mississippi Fall 2009

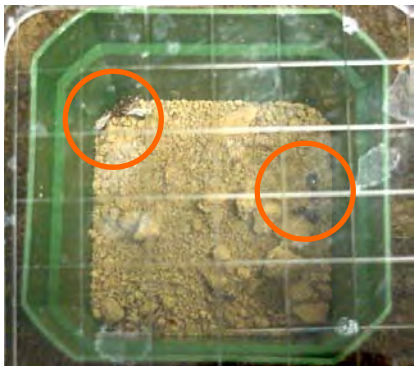
Material	Active Ingredient	Trt #	Rate #ai/ 100 gal	Rate ml prod./gal	Water* vol/drench	Amount of Insecticide per drench	Total Amount Applied
Dursban 4E (44.9%)	Chlorpyrifos	1	0.125	1.18	3.5 gal	4.13 ml	8.26 ml
Onyx Pro 23%	Bifenthrin	2	0.0125	0.235	3.5 gal	0.823 ml	1.65 ml
		3	0.025	0.47	3.5 gal	1.65 ml	3.30 ml
Scimitar	Lambda-cyhalothrin	4	0.069	2.97	3.5 gal	10.4 ml	20.86 ml
		5	0.138	5.94	3.5 gal	20.79 ml	41.58 ml
Control	--	6	--	--	3.5 gal	--	--

*Balls were rotated once between the two chemical drenches; the rate of lambda-cyhalothrin used in the fall trial was doubled from the spring trial rates.

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 this kind of trials in the past. So we used a different sampling method from 2008 in our 2009 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 top and bottom sites of the rootballs. Soil samples were collected from within the first four inches of soil core depth for testing against IFA female alates. A single bioassay cup containing 10 female alates was utilized for each soil sample (replicate) (Figures 3 & 4). 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.



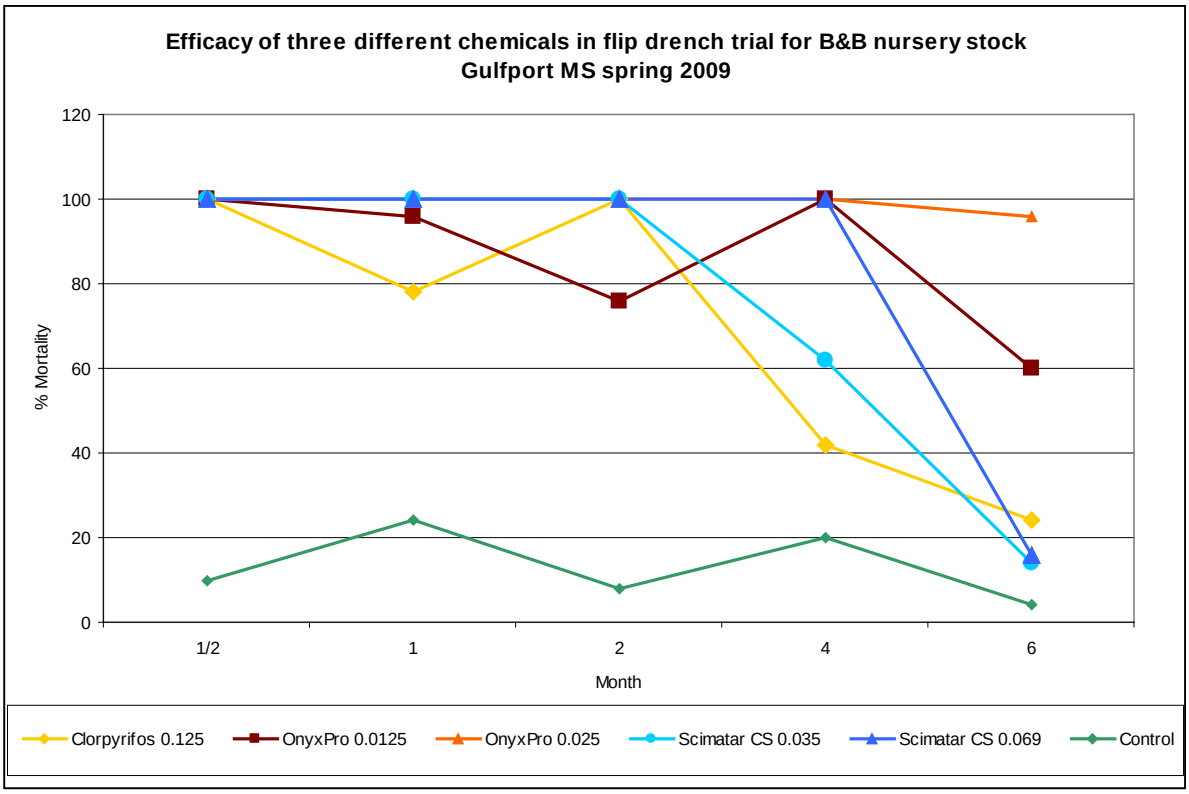
RESULTS AND DISCUSSION:

Spring 2009 flip drench:
Results from our fall 2008 flip drench showed that bifenthrin treated root balls were consistently generating 100% mortality at both rates of 0.05 and 0.1 lb ai per 100 gal of water, for the entire 6-month trial period (see previous report). The further reduced rates of bifenthrin at 0.0125 and 0.025 lb ai per 100 gal of water tested in this trial, however, did not obtain satisfactory results except for the bioassay at 2 weeks after final treatment application in which all chemical treatments had a 100% kill. Bifenthrin at 0.025 were 100% control for the first 4 months but the 0.0125 rate was not consistently achieving 100% control, indicating that the 0.0125 lb ai rate was too low for drench treatment for B&B nursery stock quarantine treatment when using 1F1

application method. The lambda-cyhalothrin lower rate of 0.035 lb ai per 100 gal of water had a 100% control for 2 months and the high rate of 0.069 had a 100% control for 4 months. Since the inconsistency in the results of both chemicals tested at both rates, it is unsuitable to draw any conclusion about the application method 1F1 which was previous tested in TN and in MS for the previous two years and had yielded satisfactory results when used in conjunction with higher bifenthrin concentrations. The inconsistent results generated from this trial may very well be caused by the low rates of chemicals rather than the application method 1F1. 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 in fall 2008 trial. However, the chlorpyrifos treatment at the same rate was inconsistent in the female alates bioassay in this trial, indicating that summer weather could be a factor on the residual effect of the chlorpyrifos.

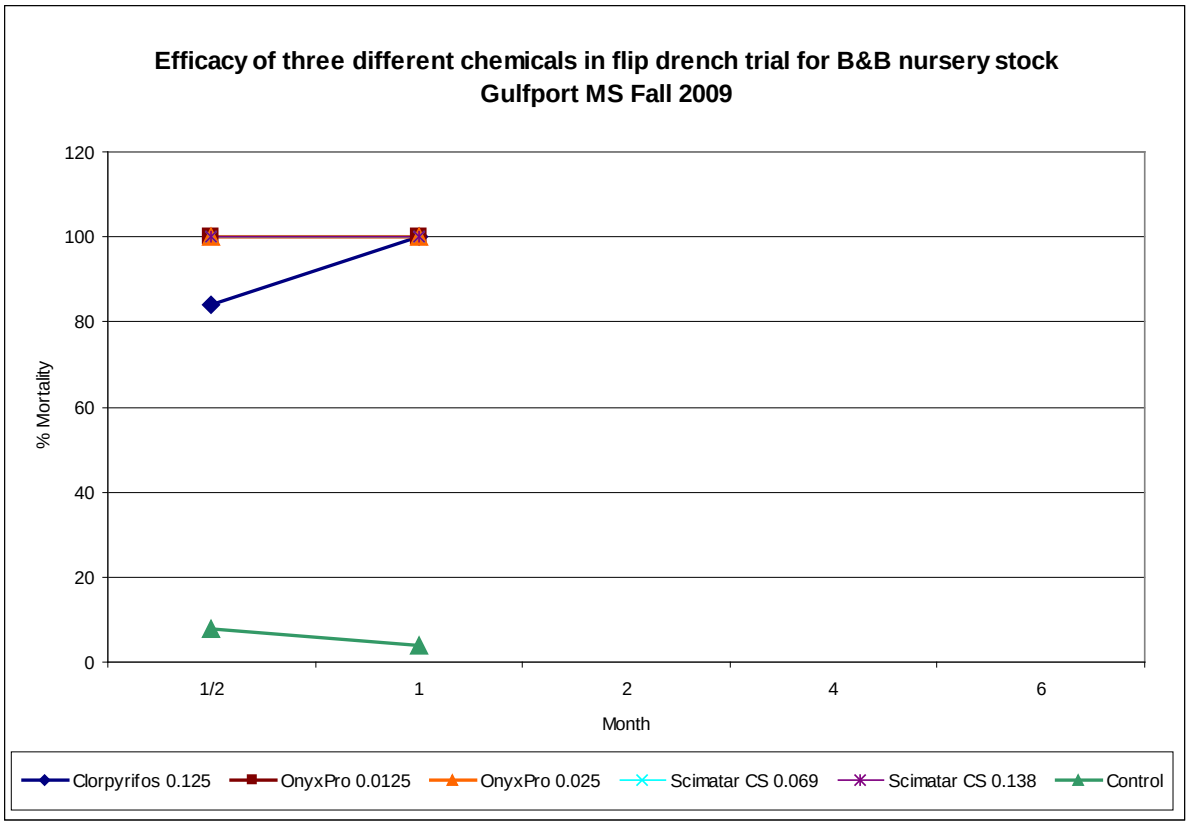
Sampling around the rootballs was started in fall 2008 for taking soil core samples from rootballs after flip drench treatment and has been considered an adequate method to assess the treatment efficacy for flip drench application method. With both sides of the rootball being well drenched and chemical solution penetrating into the root ball, we could expect that the 1F1 treatment application method becomes the method of choice for post-harvest drench treatment with the benefit of shortening treatment time and reducing the cost and run-off problems.

Figure 5. IFA control achieved in bifenthrin, lambda-cyhalothrin, and chlorpyrifos treated soil samples collected at various sampling intervals after final drench application. Plants rotated once between 2 drench applications in one day. Gulfport, MS spring 2009



Fall 2009 flip drench:
At the time this report was written, there were bioassay data for 0.5 and one month soil samples taken after the final treatment application in the fall 2009. These early results showed that both bifenthrin and lambda-cyhalothrin at the two rates tested achieved a 100% control in mortality in the IFA female alates bioassay.

Figure 6. IFA control achieved in bifenthrin, lambda-cyhalothrin, and chlorpyrifos treated soil samples collected at various sampling intervals after final drench application. Plants rotated once between 2 drench applications in one day. Gulfport, MS fall 2009



PROJECT NO: A1F04

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

REPORT TYPE: Final

PROJECT LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Craig Hinton, Lee McAnally 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 2009, 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.

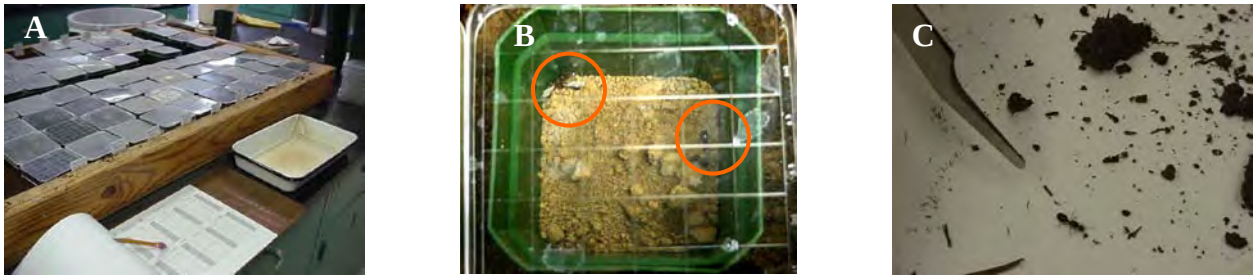


Table 1. List of treatments for 24 inch B&B stock immersion trial in TN spring 2008

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 ingredient.

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 ingredient 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 ingredient.

RESULTS AND DISCUSSION:

In the spring 08 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, failed 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).

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

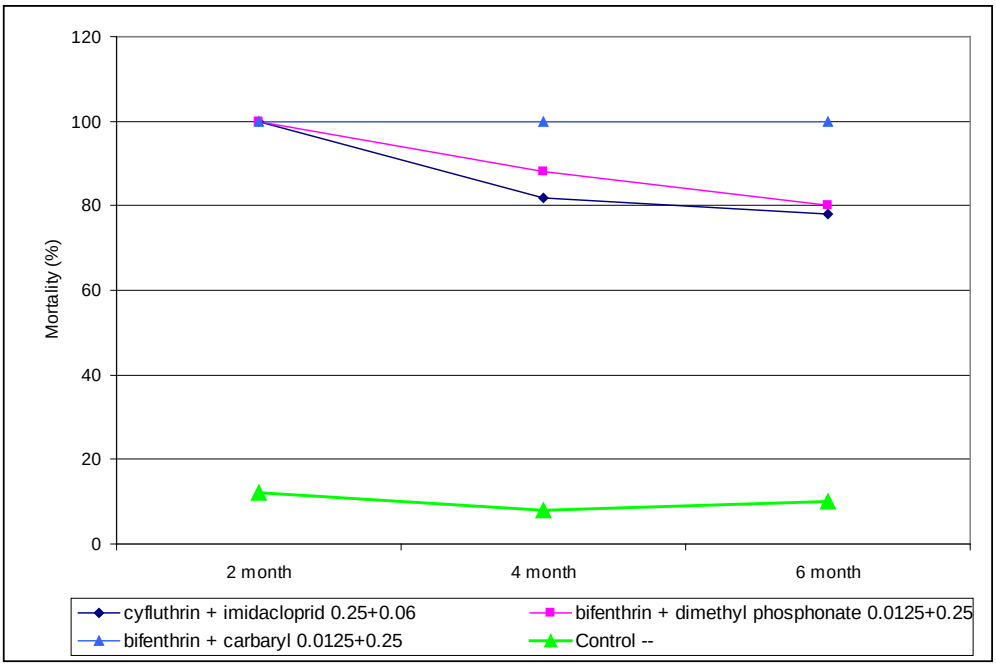
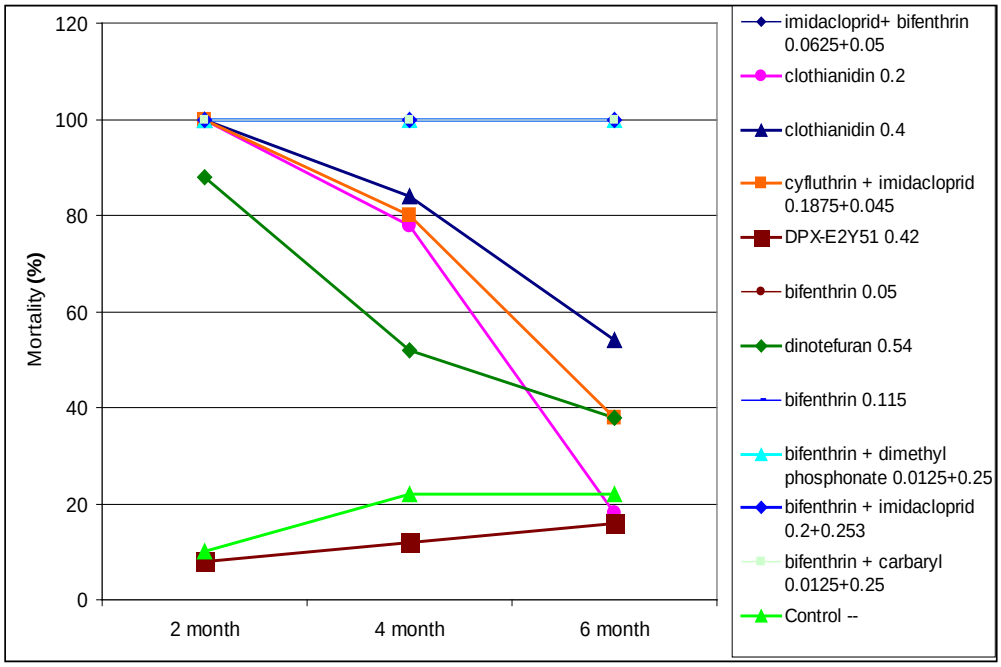


Figure 4. Efficacy of B&B immersion treatments against IFA female alates in 12-inch root balls; Tennessee spring 2008.



In the fall 08 immersion trials with chemical insecticides, majority of the treatments tested were either bifenthrin alone or bifenthrin combined/mixed with one other chemical. Results of the bioassays showed that they were all 100% effective against IFA in both 12" and 24" dip trials (Figs 5 & 6). The combined formulation of cyfluthrin plus imidacloprid also achieved 100% efficacy at 6 months after treatment application for the 24-inch root balls (Fig 5). The other non-bifenthrin treatment, the clothianidin alone used for the 12-inch root balls, achieved 100% efficacy for the first 4 months after final treatment application, but the last evaluation at 6 months post-treatment did not obtain 100% kill (Fig 6).

However, none of the essential oil 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). These results indicated that essential oils alone could not be used as alternative options for IFA quarantine treatment.

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

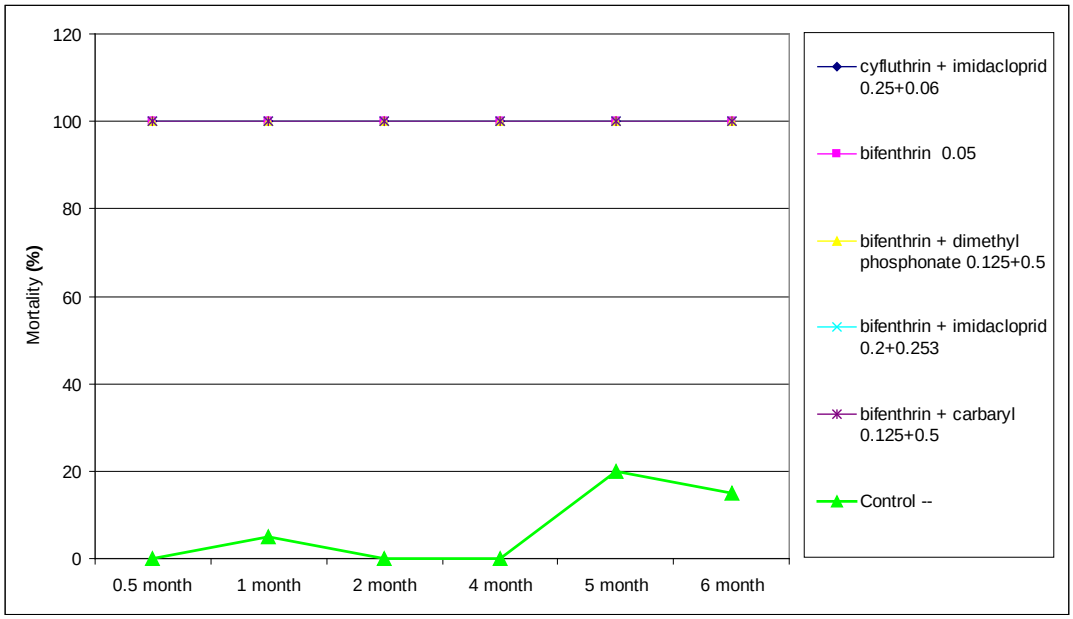


Figure 6. Efficacy of B&B immersion treatments against IFA female alates in 12-inch root balls; Tennessee fall 2008.

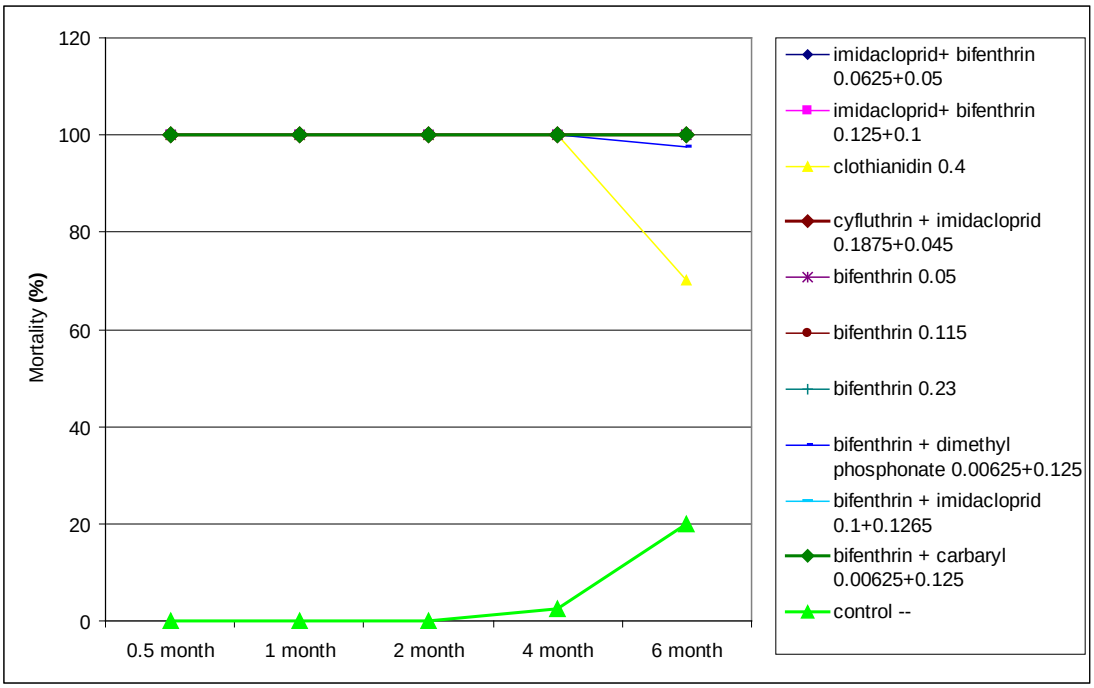
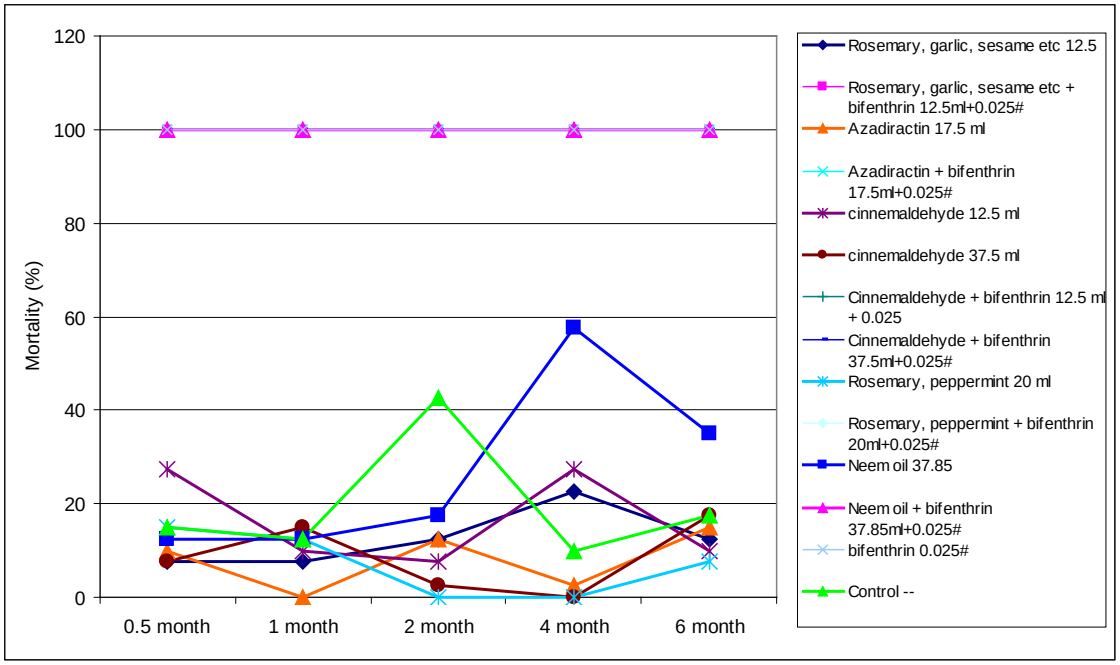


Figure 7. Efficacy of B&B immersion treatments with essential oils against IFA female alates in 12-inch root balls; Tennessee fall 2008.



PROJECT NO: A1F04

PROJECT TITLE: Alternative Immersion Treatments for Balled-and-Burlapped Nursery Stock for Use in the IFA Quarantine, Tennessee Spring & Fall 2009

REPORT TYPE: Interim

PROJECT LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Craig Hinton, Lee McAnally of USDA-APHIS; Jason Oliver and Nadeer Youssef of Tennessee State University; Chris Ranger 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 2009, 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.

The objective of the 2009 dip trials in TN was to repeat the fall 2008 trials (in spring 2009) and initiated new lower rates for dip treatment using mainly bifenthrin or bifenthrin in combination with other chemicals either in combined formulation or in tank mix application. Since the treatment of Talstar + Sevin (bifenthrin + carbaryl) was not performing well in previous trials, it was dropped from the treatment list for the fall 2009 trial.

MATERIALS AND METHODS:

Treatment applications were made in March for spring trials and in October for fall trials in 2009 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. 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).

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.

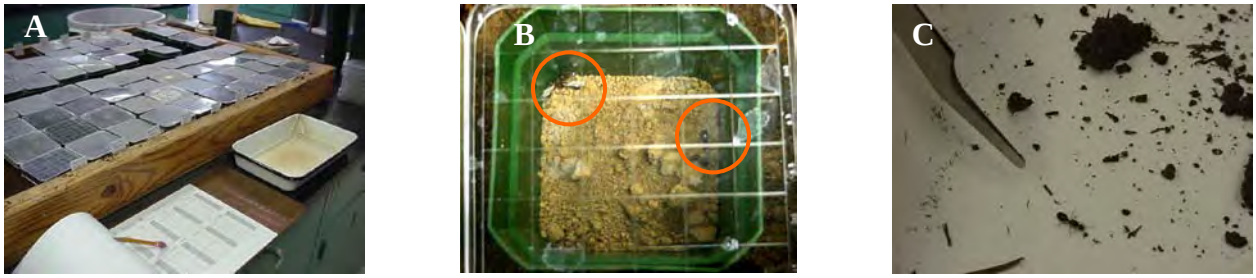


Table 1. List of treatments for 24 inch B&B stock immersion trial in TN spring 2009

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	0.0125+0.5	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+0.5	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 2009

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	10.52 ml
Discus	cyfluthrin + imidacloprid	0.1875+0.045	27.09
Onyx 23%	bifenthrin	0.05	0.946 ml
Talstar N F	bifenthrin	0.115	6.53 ml
Talstar N F	bifenthrin	0.23	13.06 ml
Talstar+Dylox	bifenthrin + dimethyl phosphonate	0.00625+0.125	0.71 ml + 1.42 g
Talstar+Marathon	bifenthrin + imidacloprid	0.1+0.1265	11.36 + 4.79 ml
Talstar+Sevin	bifenthrin + carbaryl	0.00625+0.125	0.71 ml + 2.37 ml
Control	--	--	--

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

After treatment application, 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. A significant change in soil samples collection was that no soil core samples were collected from the middle of root balls which was considered not necessary for the dip treatment application. 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.

Table 3. List of treatments for 12 inch B&B stock immersion trial in TN fall 2009

Treatment	Ingredients	Rate #ai	Amount Product/ gal of water
Allectus	imidacloprid + bifenthrin	0.0156+0.0125	5.26 ml
Allectus	imidacloprid + bifenthrin	0.03125+0.025	10.625 ml
Discus	cyfluthrin + imidacloprid	0.1875+0.045	27.09 ml
Onyx 23%	bifenthrin	0.025	0.946 ml
Onyx 23%	bifenthrin	0.0375	0.946 ml
Onyx 23%	bifenthrin	0.05	0.946 ml
Talstar N F	bifenthrin	0.0575	3.265 ml
Talstar N F	bifenthrin	0.115	6.53 ml
Talstar+Marathon	bifenthrin + imidacloprid	0.05+0.06235	5.68 ml + 2.4 ml
Talstar+Marathon	bifenthrin + imidacloprid	0.1+0.1265	5.68 ml + 2.4 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 spring 2009

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	--	--	--

RESULTS AND DISCUSSION:

In the spring 09 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). However, for the treatments with low bifenthrin rates (at 0.00625 lb ai for 12" dip and 0.0125 lb ai for 24" dip) combined with carbaryl or with dimethyl phosphonate, 100% efficacy was obtained for 4 months after treatment but not for 6 months after, indicating the treatment rates were too low for dip treatment if 6 months quarantine level control is needed.

The combined formulation of bifenthrin + dimethyl phosphonate (Dylox) did not achieve 100% efficacy at 6 months in the spring trial, which was not consistent with all other bifenthrin related treatments but was consistent with the results of this treatment itself found in 2007 and 2008 dip trials (Figs 3&4). It has become clear that bifenthrin concentrations at 0.0125 and below usually do not hold up for 6 months even when combined with other chemicals. Similar to that of bifenthrin + dimethyl phosphonate, the combined formulation of cyfluthrin plus imidacloprid was 100% effective for only two months after treatment in the spring trial (Fig 3&4). Clearly, these two combination formulations failed again to show its potential use in IFA quarantine treatment. Another failed treatment is the tank mix of bifenthrin with carbaryl for the reason that carbaryl did not seem to provide additional efficacy to the control of IFA (Figs 3&4) and therefore, it was not included in the fall 2009 dip trial.

None of the essential oils alone treatments were effective enough against IFA for quarantine treatment purpose (Fig 5). When essential oils were mixed with bifenthrin at 0.025 lb ai rate, some combination treatments achieved 100% mortality in female alates bioassays but others did not. It is questionable if the essential oils contributed to any of the efficacy achieved in IFA control because the bifenthrin alone treatment at 0.025 lb ai had 100% IFA control for 6 months. The addition of essential oils to bifenthrin apparently made some of the combined treatment even less effective than bifenthrin used alone. Therefore, essential oils are not considered potential agents for IFA quarantine treatment and were not included in the fall 2009 immersion trial.

In the fall 09 immersion trials with chemicals, all treatments tested were either bifenthrin alone or bifenthrin combined/mixed with one other chemical with one exception of cyfluthrin plus imidacloprid treatment (at 0.1875 + 0.045) which does not contain bifenthrin. Results of the bioassays for the first two months showed that they were all 100% effective against IFA except that imidacloprid+ bifenthrin (0.0156+0.0125) was 97.5% at one month after treatment (Fig 6). However, at two months after treatment, 100% control resumed for all treatments and it is not yet known at this point if this treatment or any other treatments will achieve 100% control for the rest of the evaluations until 6 months post treatment. However, immersion trial results in the spring 2009 showed that bifenthrin at 0.0125 rate usually did not last for 6 months with 100% control; therefore, it would not be surprising if this imidacloprid+ bifenthrin (0.0156+0.0125) treatment failed to achieve 100% control towards the end of 6 month trial, but we will finalize this report when all bioassay results are available.

Figure 3. Efficacy of B&B immersion treatments against IFA female alates in 24-inch root balls; Tennessee spring 2009.

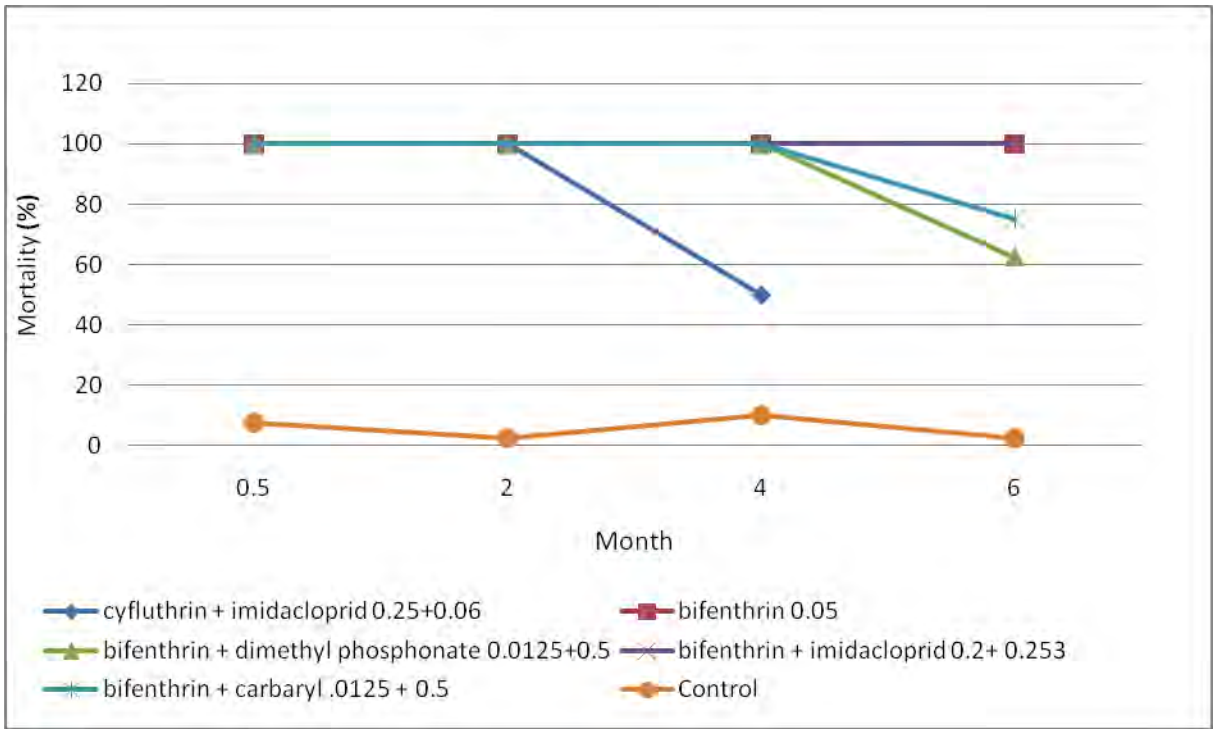


Figure 4. Efficacy of B&B immersion treatments against IFA female alates in 12-inch root balls; Tennessee spring 2009.

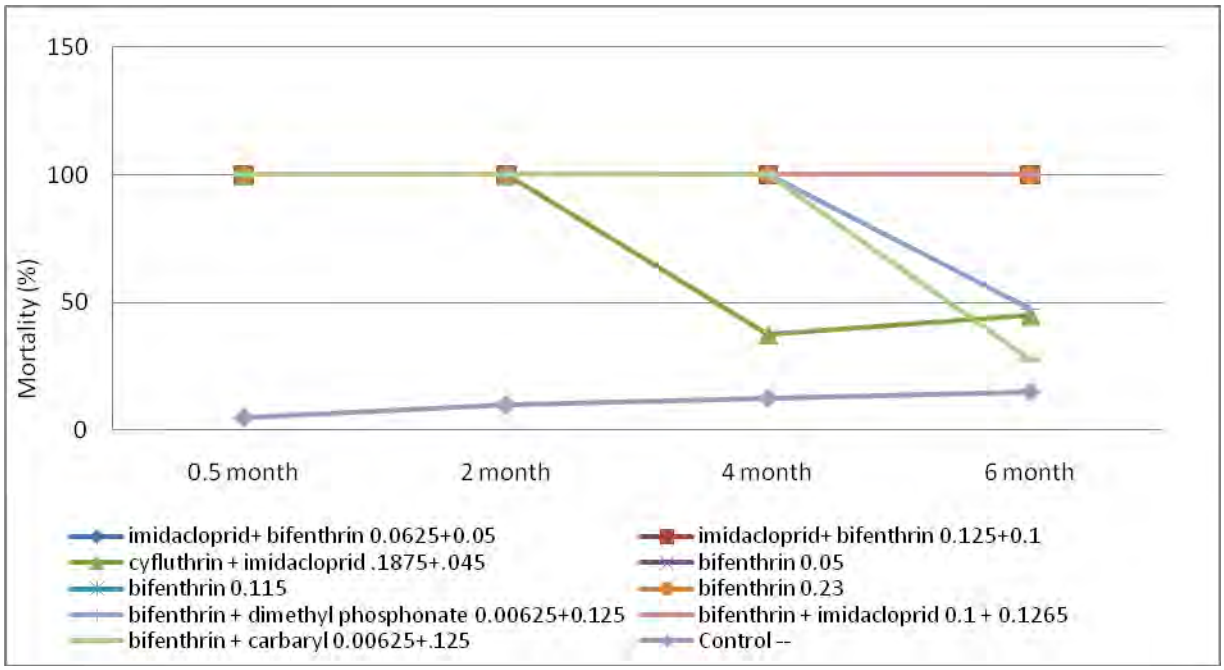


Figure 5. Efficacy of B&B immersion treatments with essential oils against IFA female alates in 12-inch root balls; Tennessee spring 2009.

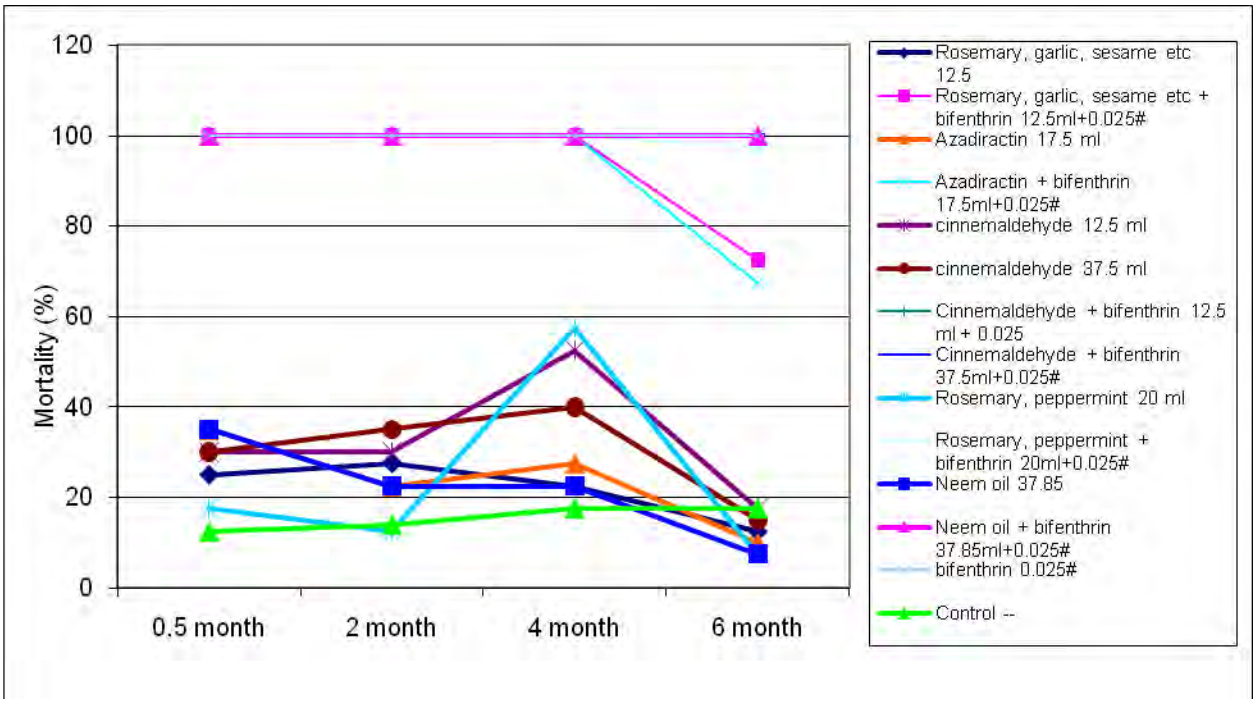
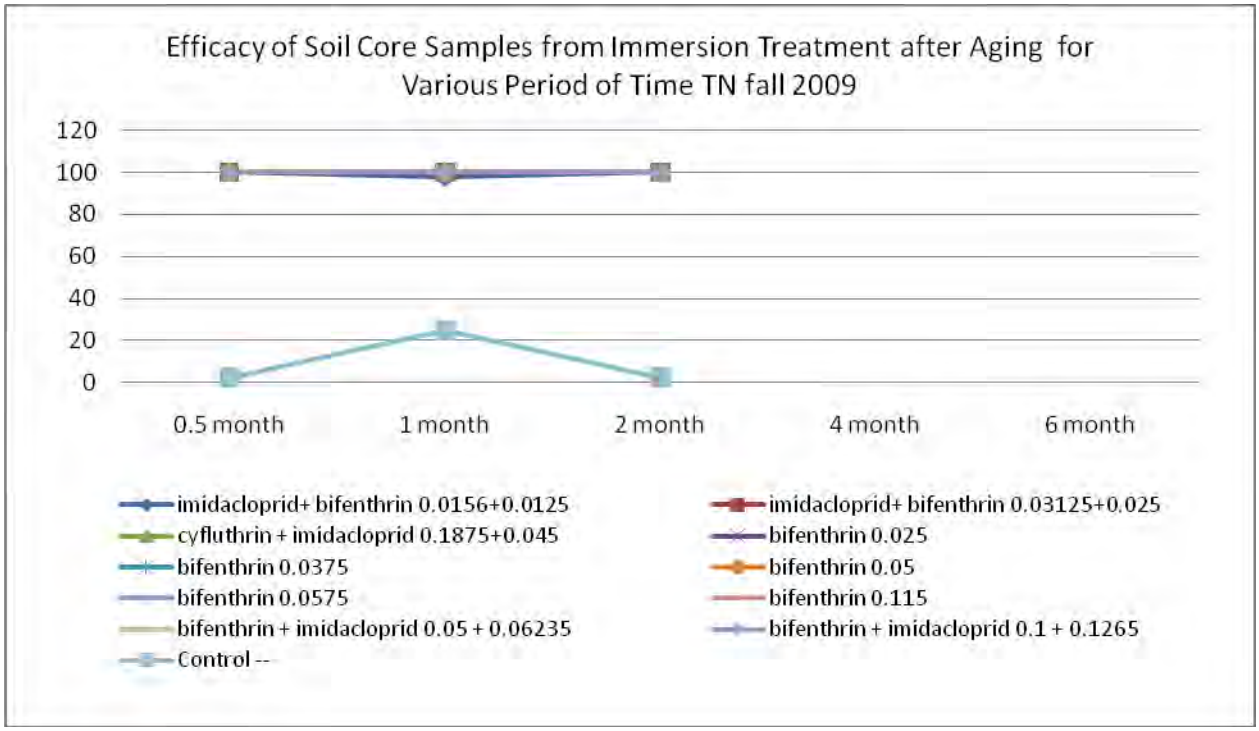


Figure 6. Efficacy of B&B immersion treatments against IFA female alates in 12-inch root balls; Tennessee fall 2009.



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: 2008

REPORT TYPE: Final

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 proved unnecessary 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 would kill fire ant queens 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 overnight 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.

MATERIALS AND METHODS:

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, 2008.

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 was similar to the treatment method of post-harvest drenching for B&B nursery stock, but

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

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 points 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 queens 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 queens 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 queens 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. 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 for 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 in the spring samples. 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 corresponding report in the analytical chemistry section of the 2009 Gulfport Lab report.

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.

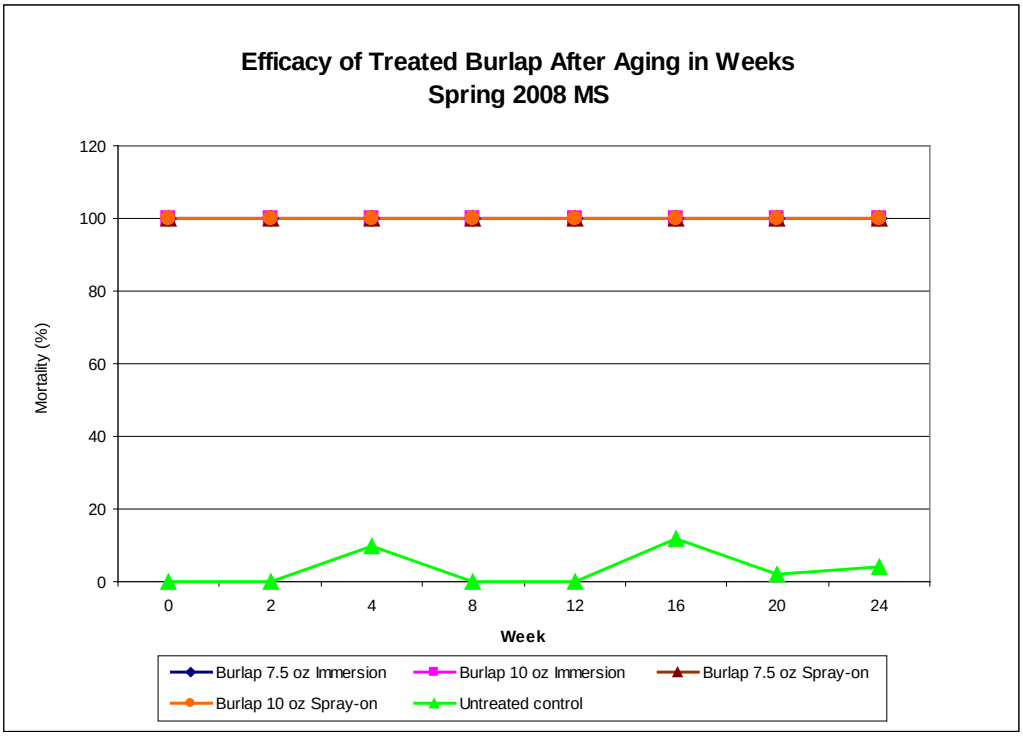


Fig. 4. Efficacy of bifenthrin-treated burlap on female alates of RIFA after aging outdoors under simulated nursery storage conditions.

Fall 2008 bioassay:
All treatments in the test except control achieved 100% efficacy and, similar to the spring 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.

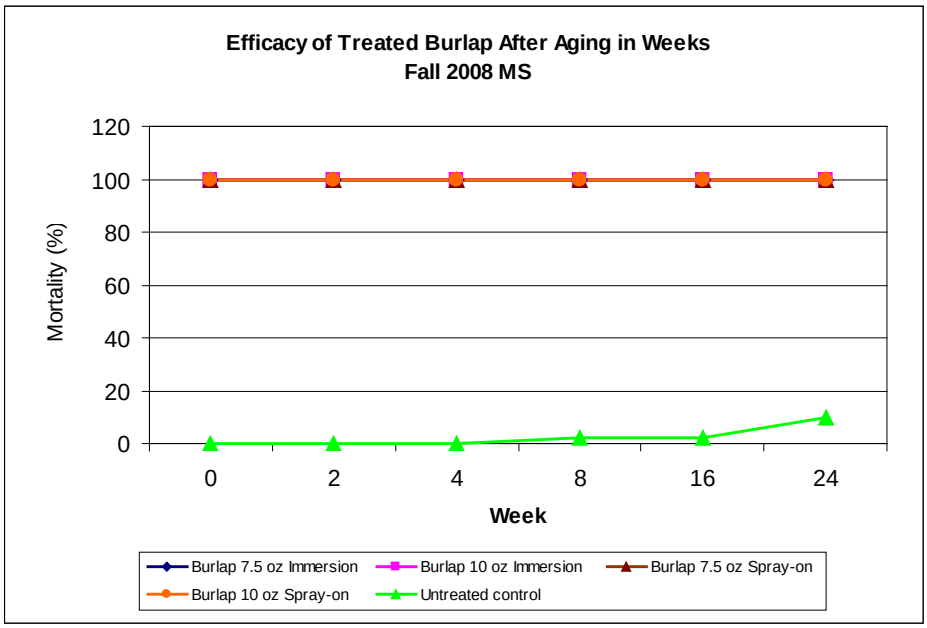


Fig. 5. Efficacy of bifenthrin-treated burlap on female alates of RIFA after aging outdoors under simulated nursery storage conditions.

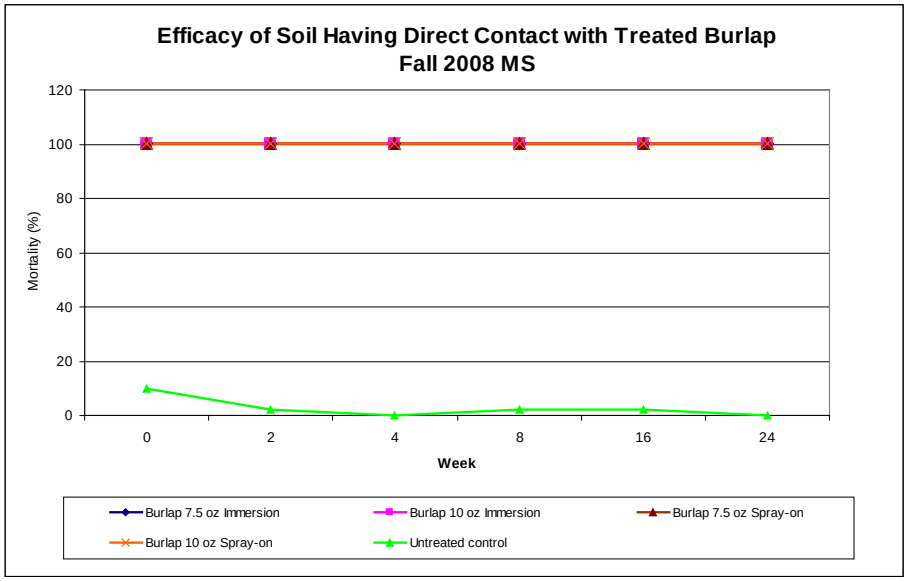


Fig. 6. Bioassays results for surface soil that had close contact with the bifenthrin-treated wrapping burlap after aging outdoors in simulated nursery storage conditions for various length of time during the 6 months post-treatment period.

Chemical analysis results:
When newly treated, burlap immersed in bifenthrin solution for 24 hrs (both 10.0 and 7.5 oz weight burlap treated with 0.23 lb ai/100 gal of water) 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 in burlap decreased sharply in the first two weeks post treatment (decreased by 40 – 66% of the week 0 level). After aging outdoors for two months, bifenthrin contents in burlap of all four treatments decreased by 70 – 90% of beginning level, but interestingly, bifenthrin concentrations in 7.5 oz burlap were higher than that in 10 oz burlap with both treatment methods—pre-treated (immersion) and spray-on, in the first two months. At 3rd month post treatment, 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.

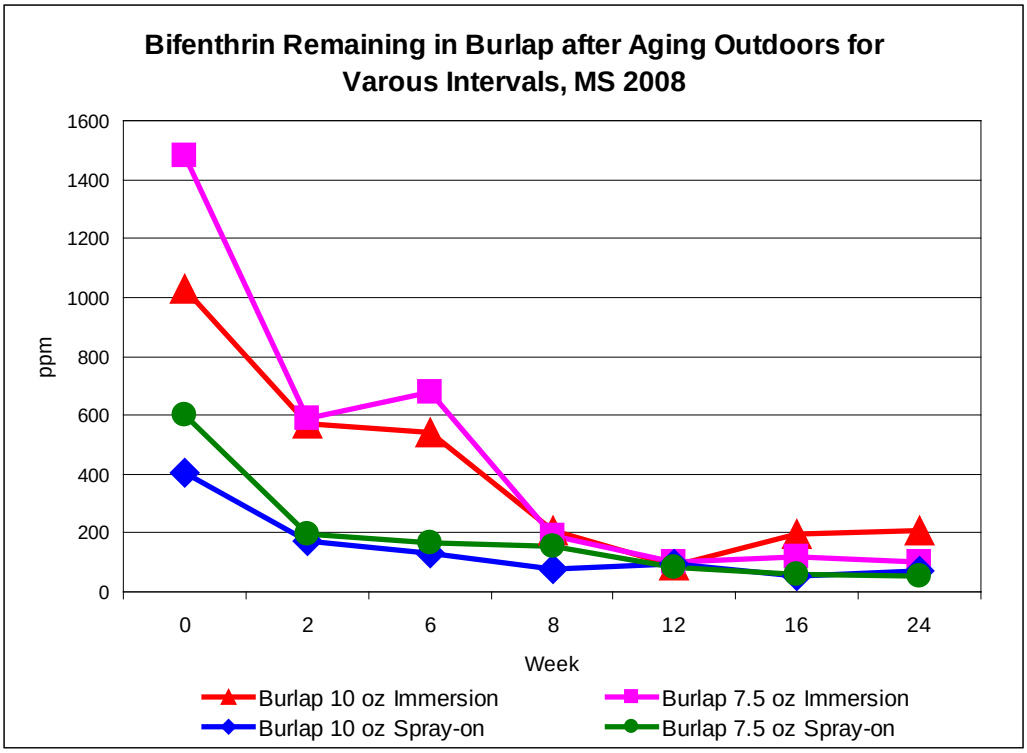


Fig. 7. Bifenthrin in treated burlap at various points of time during the six months aging under simulated nursery storage conditions, spring 2008 trial.

DISCUSSION:

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 may 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 be used.

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 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 month's aging 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 environment 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 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, MS 2009

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 that were previously treated in the field by chemogation or other method of drenching 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 proven unnecessary by the laboratory bioassay using treated burlap. Our laboratory bioassay results in 2007-2008 showed that even after aging for 9 months under simulated nursery stock storage situations outdoors, treated burlap would kill fire ant queens soon after they made the contact.

In the spring and fall of 2008, we took this laboratory experiment to the field and conducted bioassay on intact rootballs wrapped with bifenthrin-treated burlap for a period of 6 months while rootballs were stored outdoors for normal aging. At the 0.23 lb ai rate, bifenthrin-treated burlap killed 100% fire ant queens for at least 6 months after treatment initiation, and the lighter weight burlap (7.5 oz) was found adequate in preventing newly mated fire ant queen infestation. Therefore, further testing will only use 7.5 oz burlap which is commonly used by nursery growers for B&B wrapping. The objective of this study was to evaluate the effectiveness of

using lower rates of bifenthrin (at 0.05 & 0.1 lb ai/100 gal water) for treating burlap for the prevention of newly mated fire ant queen infestation to the harvested nursery stock while in storage outdoors and in transportation. 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.

MATERIALS AND METHODS:

Burlap treatment

Plain burlap of 7.5 oz (7.5 oz in weight per sq. yard) 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, 6 gallons of water and 5.64 ml of bifenthrin (FMC Corp. OnyxPro 23% EC) were added to mix into a solution with bifenthrin at the rate of 0.05 lb ai/100 gal of water. In the 0.1 lb ai rate, 6 gallons of water and 11.22 ml of bifenthrin were used. Ten burlap liners were immersed completely in each of the two solutions. 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 May 11, 2009 for our experimental use. According to the treatments in this trial (Table 1), 16 of the plants were wrapped in bifenthrin-treated burlap (all in 7.5 oz treated burlap) and the other 24 plants were wrapped in plain burlap of 7.5 oz weight. We provided the nursery with both bifenthrin-treated and plain burlap for their use. Balled and burlapped plants were transported to the Gulfport IFA lab and stored under irrigation during the trial. A fall trial was repeated starting on October 26, 2009.

Spray on procedure

Rootballs that were wrapped by the nursery with provided 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 May 12, 2009 for spring trial and October 28, 2009 for fall trial. A general purpose pressure sprayer (GardenPlusTM Lawn and Garden Sprayer) was used to spray 2 gal of bifenthrin solution (at the same rates of 0.05 or 0.1 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 was similar to the treatment method of post-harvest drenching for B&B nursery stock, but this method used less liquid, compared with that of drench method, to evenly cover the entire burlap wrapping and root balls were sprayed only once. Flipping the rootballs while spraying may be needed to get an even coverage.

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

Bioassay procedure
To evaluate the residual effect of bifenthrin-treated burlap over a 6-month aging period under outdoors conditions, the bioassay method developed and chosen in 2008 for this same type of trial was utilized. A piece of burlap was cut from each of the rootballs and brought to the lab for efficacy evaluation (Fig 2 A & B). 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 laboratory.

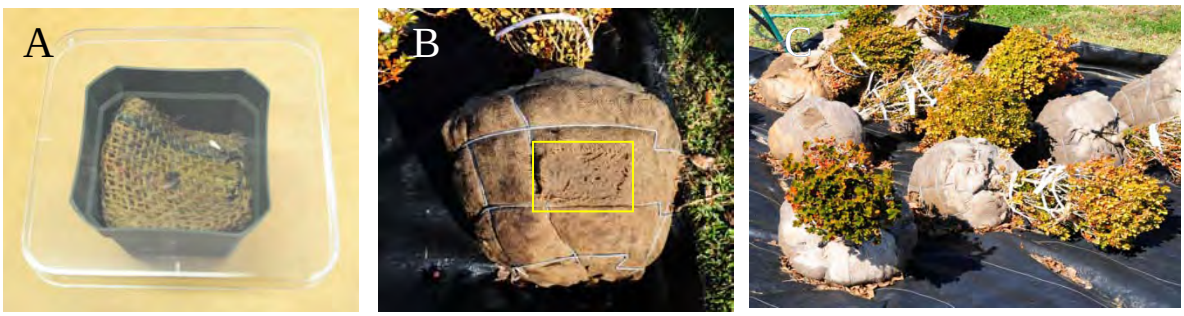


Fig 2. A): Method of choice--apparatus for bioassay conducted in the lab. B): rootball showing a piece of burlap was removed for bioassay in the lab; soil sample was also collected from where burlap was cut out (within yellow rectangle). C): Balled & Burlapped nursery stock stored outdoors under simulated nursery storage conditions for more than 6 months.

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 B) to determine if the soil having close contact with the treated burlap would acquire enough bifenthrin to kill fire ant queens. The bioassay method for the soil samples was the same as for burlap pieces.

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 and soil samples 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 (Fig C). The treatments in this investigation are listed in Table 1.

Chemical analysis of bifenthrin in treated burlap for fall 2009 trial
GC-MS analytical procedures were used to analyze bifenthrin in samples of bifenthrin-treated burlap and soil. 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 found in the corresponding report in the analytical chemistry section of the 2009 Gulfport Lab report.

Table 1. Treatment list of intact rootballs wrapped with bifenthrin-treated burlap or plain burlap with post-harvest spray-on method, Gulfport, MS spring and fall 2009

Treatment*	Trt #	lb ai/100 gal of water	Rate ml prod./gal	Solution volume used	Amount of Insecticide/ treatment
Bifenthrin (*Immersion*)	1	0.05	0.94	6 gal	5.64 ml
	2	0.1	1.87	6 gal	11.22 ml
Bifenthrin (*Spray-on*)	3	0.05	0.94	2 gal	1.87 ml
	4	0.1	1.87	2 gal	3.74 ml
Control (*water only spray-on*)	5	--	--	2 gal	--

*Bifenthrin used was Onyx Pro 23% EC.

RESULTS

Spring 2009 bioassay

Burlap bioassay: Except in month 4 where the lower rate of treatment with both methods did not achieve 100% efficacy, all other treated burlap bioassays conducted from the beginning of the trial through the end of six months post-treatment obtained a 100% efficacy (Fig. 3). There were no differences in efficacy between 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. 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. At month six, the two low rate treatments had 100% kill again, indicating the inconsistent results in month 4 could have resulted from sample or treatment application errors.

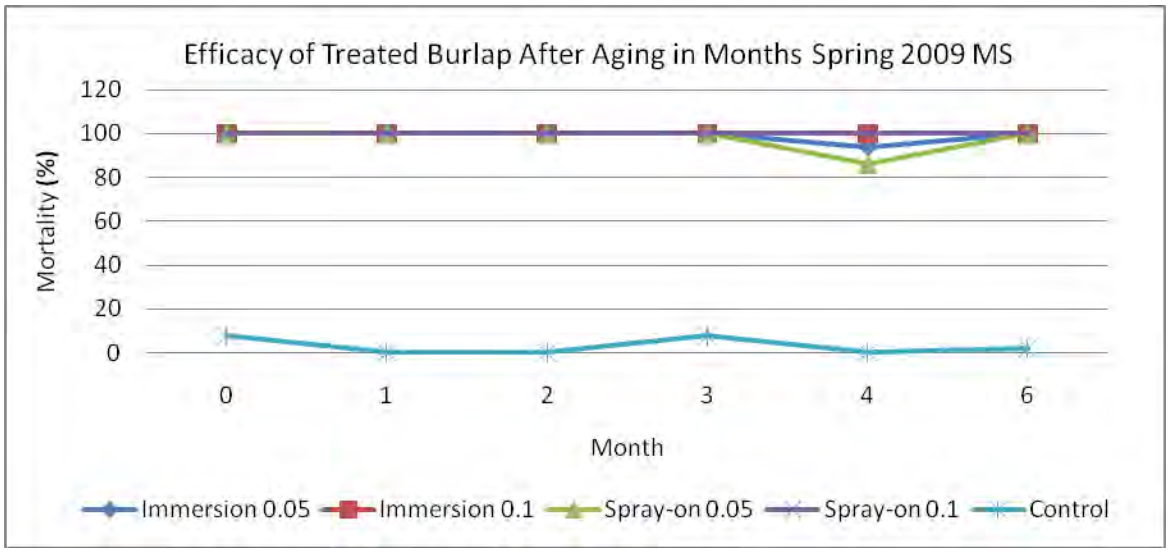


Fig. 3. Efficacy of bifenthrin-treated burlap on female alates of RIFA after aging outdoors under simulated nursery storage conditions.

Soil bioassay: For all the soil sample bioassays conducted, 100% efficacy was also recorded for all treatments except for the immersion treatment of 0.05 rate at month 3 in which there was only one female alate that did not die during the bioassay (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 surface soil having direct contact with the bifenthrin-treated burlap obtained bifenthrin residue and gained the killing power soon after it made close contact with the treated burlap.

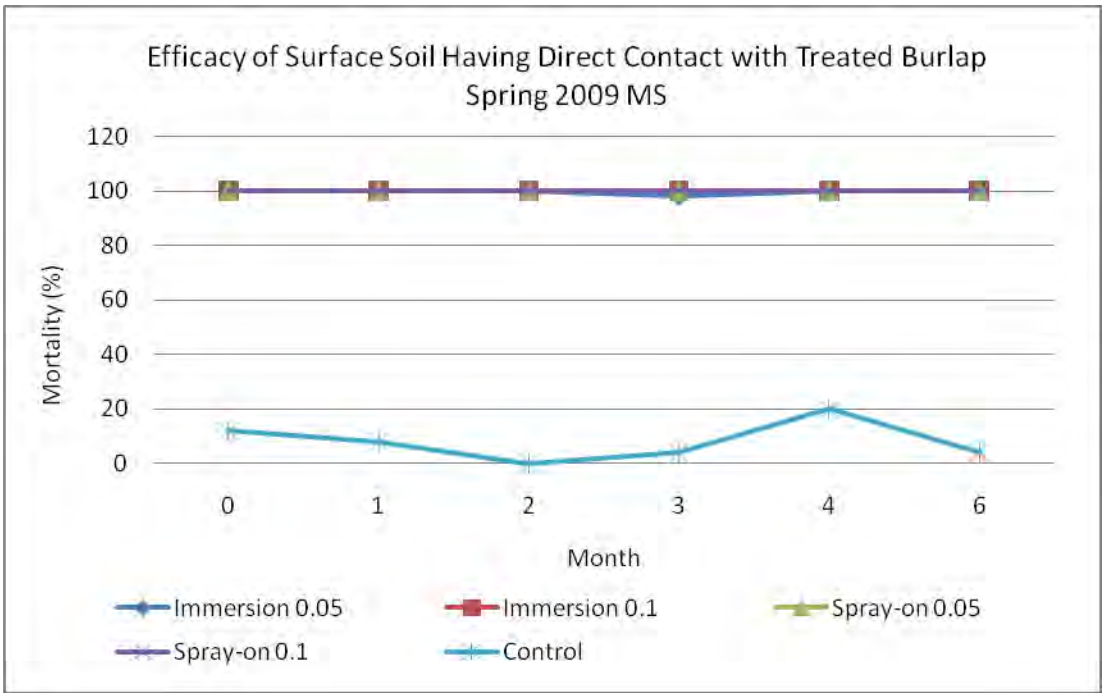


Fig. 4. Bioassays results for surface soil having close contact with the bifenthrin-treated burlap after aging outdoors in simulated nursery storage conditions over 6 months.

Fall 2009 bioassay

At the time this report was written, burlap and soil bioassays for the fall 2009 trial were completed for the first 3 months post-treatment. Data will be added to this report when available. For all the burlap bioassays conducted so far, all treatments in the test except control achieved a 100% efficacy and, similarly to the spring 2009 trial, there were no differences in efficacy between the two burlap treatment methods (pretreated "immersion" vs. post-harvest "spray on"; see Fig. 5). For all the soil sample bioassays conducted, 100% efficacy was also recorded for all treatments (Fig. 6) except for the soil samples collected at the very beginning (24 hrs post wrapping) from the immersion treatments, which did not achieve 100% control but still were extremely high in efficacy (92% for the immersion at 0.05 and 98% for immersion at 0.1) considering the very short period of time of making direct contact with the treated burlap.

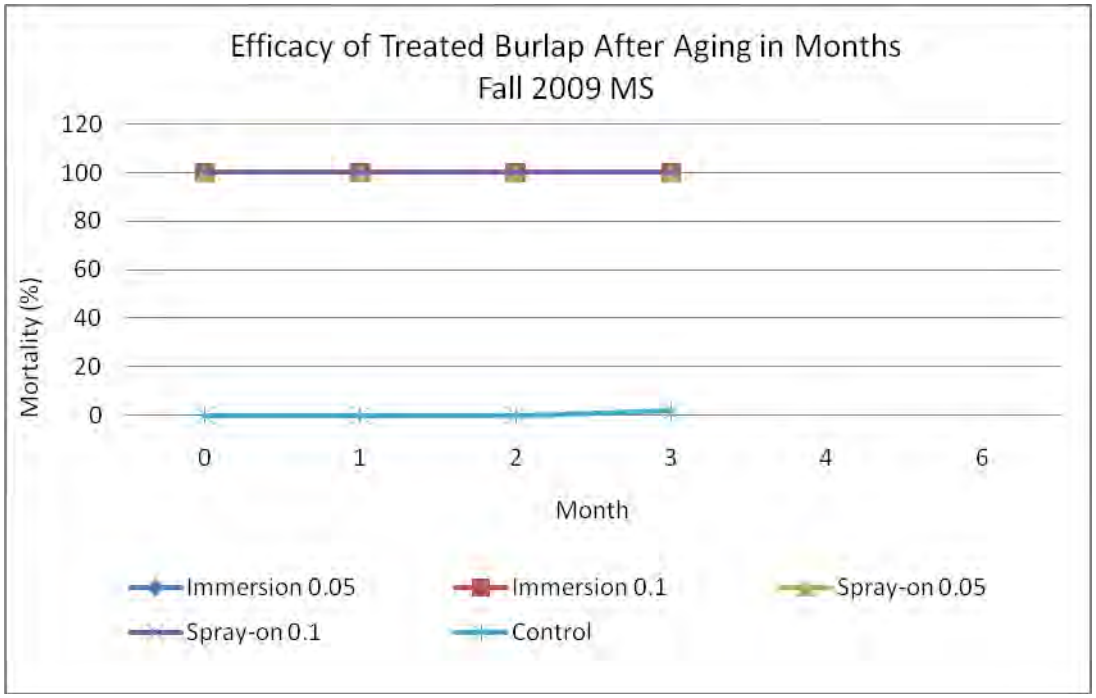


Fig. 5. Efficacy of bifenthrin-treated burlap on female alates of RIFA after aging outdoors under simulated nursery storage conditions.

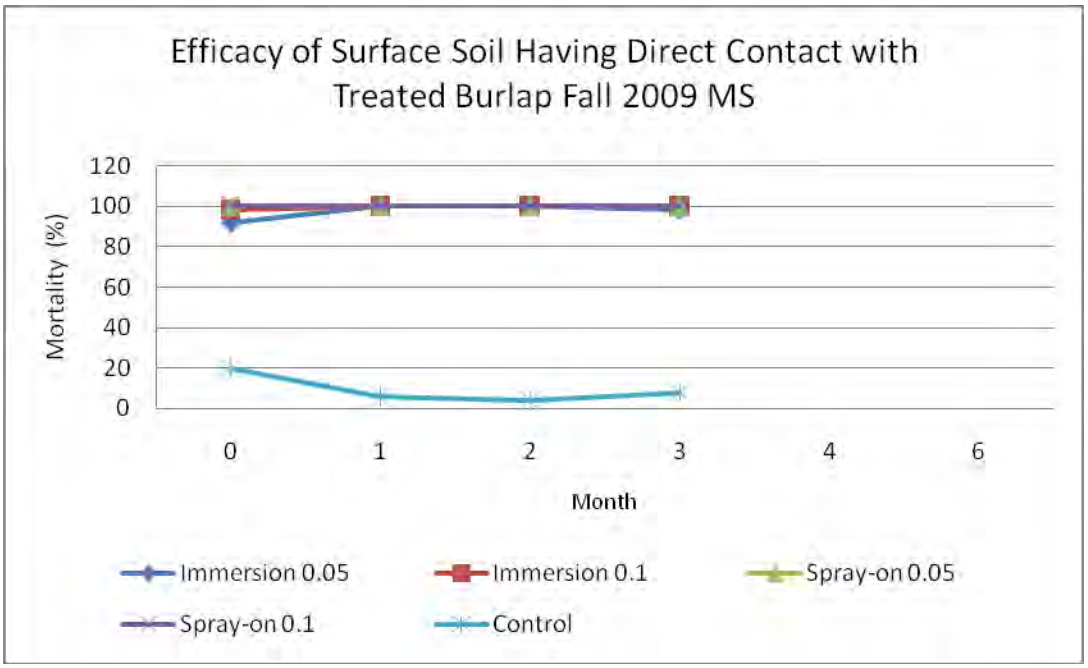


Fig. 6. Bioassays results for surface soil having close contact with the bifenthrin-treated burlap after aging outdoors in simulated nursery storage conditions over 3 months.

Chemical analysis results:

Similar to the previous analysis results of spring 2008, when newly treated, burlap immersed in bifenthrin solution for 24 hrs had much higher concentration of bifenthrin (>500 ppm at 0.1 lb ai rate) than burlap treated with the spray on method (<100 ppm at 0.1 lb ai rate; Fig.7). Similar difference was also found at 0.05 lb ai rate between the two burlap treatment methods. Bifenthrin residues decreased sharply in the first month after treatment (30 – 60% decrease from the week 0 level) in all four treatments. After aging for two months, bifenthrin contents in burlap decreased by 65% or more of the beginning level for the two spray-on treatments and the lower rate of immersion treatment, but for the immersion at 0.1 lb ai rate treatment, bifenthrin concentrations was still at a relatively high concentration of 300 ppm (50% of week 0 level). By 3 months, the maximum decrease in bifenthrin concentrations reached 90 % of the original level with a range of 13 to 95 ppm left in the treated burlap. Results of chemical analysis of bifenthrin in treated burlap for later months will be added to this report when available.

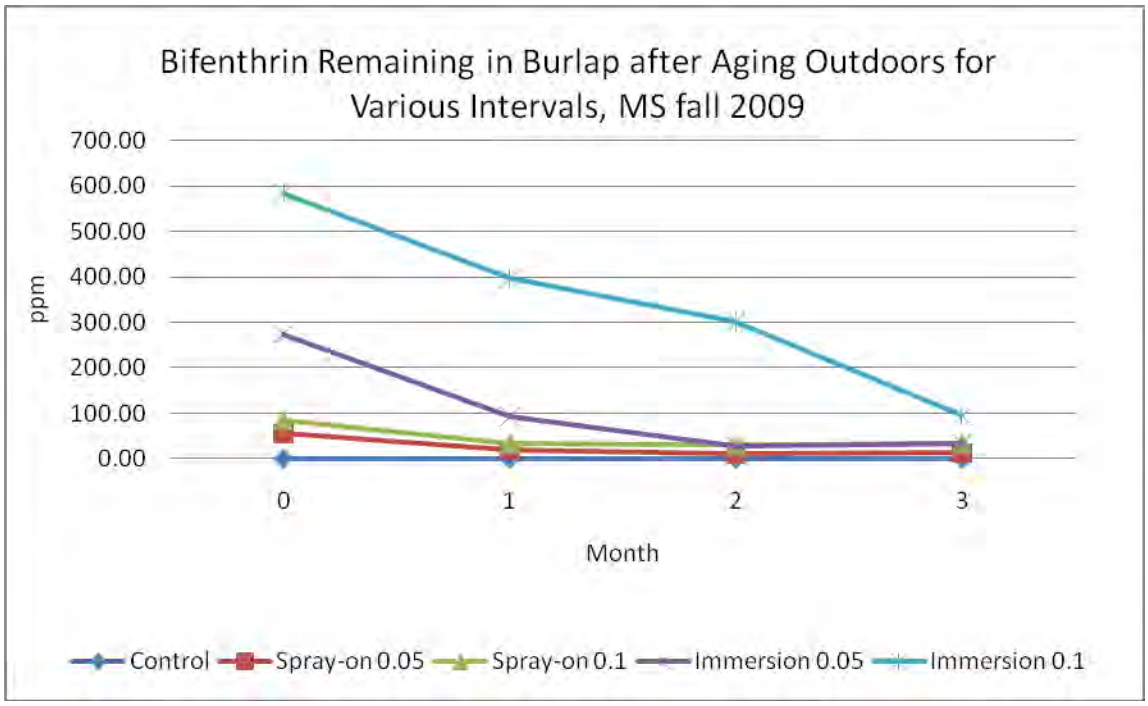


Fig. 7. Bifenthrin in treated burlap at various points of time during the 3 months post-treatment aging under simulated nursery storage conditions.

DISCUSSION:

The bifenthrin-treated burlap, either pre-treated before tree harvesting or sprayed directly onto the rootball after harvest (spray-on) at the lower rates of 0.05 and 0.1, maintained its killing power well during the long-term aging process. It lasted at least for six months under normal outdoors nursery storage conditions in the spring trial, 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. The fall 2009 trial was not completed but the first 3 months result looked strong as well.

Since there was no difference found in effectiveness regarding bioassay results 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 tree 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 currently 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. In this process, plain burlap will be used for wrapping rootballs as usual and after harvesting, spray bifenthrin solution to thoroughly cover the surface of the rootballs, which may 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 or near 100% 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 appropriate 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 (see 2008 annual report) and it could well protect the rootballs from infestations of newly mated fire ant queens. Burlap treated with lower bifenthrin concentrations (½ - ¼ of the 2008 treatment rates) still have a residual concentration of 13 ppm to 90 ppm, way above the required concentration of 3 ppm to kill fire ant queens at 3 months after treatment. Further analytical and bioassay data will determine if the low rate spray-on treatment is adequate for post-harvest burlap treatment.

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 harvesting. Preliminary results of a tree ring study (see 2009 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.

CONCLUSIONS:

Bifenthrin treated burlap, either pre-treated before use or directly sprayed onto the burlap after rootballs were harvested and wrapped using rates of 0.05 and 0.1 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 based on our spring 2009 trial. 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 appropriate to prevent fire ant infestation.

Bifenthrin degraded quickly during the first month 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 Treatment for Field Grown Nursery Stock – Individual Tree Drench Treatment Using Tree Ring or 5-gallon Bucket in Mississippi and Tennessee 2009

REPORT TYPE: Final

LEADER/PARTICIPANT(s): Xikui Wei, Anne-Marie Callcott, Lee McAnally, Craig Hinton; Jason Oliver, and Nadeer Youssef of Tennessee State University

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.

Various drench methods such as tree ring chemogation, multiple bucket drench, or other in-field drench application, coupled with burlap treatment before or after harvest could provide a practical quarantine treatment option in addition to the currently available treatment methods such as post-harvest dip, drench, and pre-harvest (in-field) band application of contact insecticides following approved bait broadcast. Tree-ring chemogation or other pre-harvest drench applications may penetrate the entire root ball with chemical solution to achieve results that are similar to the dip treatment but do not require the use of heavy equipment and do not come with the problem of disposing large volume of harmful chemical waste at the end of the treatment. Compared with post-harvest drench, the tree-ring method could reduce labor and chemical costs and with little or no run-off problem. Also, this method selectively treats the trees to be harvested thus avoiding the unnecessary treatment to the entire field and eliminates the need to wait for a 30-day exposure period before harvesting. Bifenthrin treatment to burlap wrapping before or after harvest may kill newly-mated fire ant queens that land on the rootballs through contact.

The objective of this study was to evaluate an alternative quarantine treatment method that uses various drench methods for individual tree (in-field) treatment combined with bifenthrin

treatment to the wrapping burlap before or after harvest. Specifically, we wanted to find out the effectiveness of various drench methods and also 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

Preliminary trial at Harrison County Farm, MS

Preliminary trial at Harrison County Farm was conducted on Dec. 30, 2008. There were two separate parts of this trial: 1) drench on weedy ground without fire ant mound to simulate individual tree drench treatment and to learn about chemical penetration into the ground (Fig. 3A), 2) drench over active fire ant mound to see if slow drench treatment would kill the colony in its entirety (Fig. 3B). In both drenches, bifenthrin rate was at 0.23 lb ai per 100 gal of water (43 ml of Onyx Pro 23.4% product in 10 gallon water). Tree rings of 10 gal sized was filled with 10 gallon water and mixed thoroughly with the required amount of bifenthrin product and then 750 ml of water-soluble liquid blue dye (Deep Blue Shade from ESCO foods.com) was added to the solution to determine how deep the drench solution penetrates the soil. It took about 2 hours to completely drain the 10 gal water because the original 6 larger drain holes at the bottom of the tree rings were plugged with a glue gun and 6 smaller holes were open for each tree ring to slow down the drench process in order to reduce run-off. Five tree rings were placed on the ground without fire ant mounds and left to drench out completely. One “root ball” of 18” diameter was excavated the next day from the spot directly under the tree ring drench using a manual B&B tree harvester and soil core samples were taken from various depths of the dug balls and also from the holes left on the ground (collected soil sample at top 4” surface soil, in colored range at the middle, and out of colored range at the bottom --3 depths in total) to estimate how deep the toxic zone reaches. Soil samples were brought to Gulfport Lab for female alate bioassay using our regular bioassay method. Ten female alates were used for each soil sample. Since the blue dye was used in the drench, color was clearly seen on the excavated rootballs and also at the dug holes left on the ground. To estimate if bifenthrin travels to the same depth as blue dye does, soil core samples were visually rated for percentage of colored soil in each of the samples before the bioassay was conducted. For the second part of the trial, ten drenches were conducted directly over 10 active fire ant mounds. Because of the irregularity of the mound shapes it usually required some leveling to stabilize the tree ring on top of the mounds. Mounds were examined visually at 24 hours after treatment and evaluated for mortality 7 days after treatment.

Preliminary trial in TN

Preliminary trial in TN was conducted at the Nursery Research Center on a stripe of weedy ground without fire ant mound present on April 15, 2009 (Fig. 4A). Bifenthrin treatment rate was at 0.05 lb ai in 100 gal of water with an application volume of 10 gallon per drench. The procedure of setting up the drench at Harrison County Farm (see above) was followed. Root balls of 2 different sizes (20” and 24” diameter) were excavated from the drenched spots using tree-harvesting machine the next day on April 16, 2009. One “root ball” of 24” diameter was excavated from each of 5 drenched areas directly under the tree ring drench and 20” diameter “root balls” were dug from the other 5 drenches.

Soil core samples were taken from the dug balls and also from the holes that were left on the ground. Details of sample collection are as follows: 2 soil core samples at 6” from the top (weedy surface) in opposite sides of the dug ball, 2 samples at 8” deep on wall of dug holes in opposite sides, and 1 sample at the bottom of each hole. Soil samples were brought to Gulfport Lab for female alate bioassay using the regular laboratory bioassay method. Soil core samples were visually examined and the percentage of colored soil in each of the soil samples was estimated before conducting bioassay.

To understand how this slow drench treatment works on active fire ant mounds in clay soil, ten tree ring drenches were conducted directly over active fire ant mounds at Hawkersmith Nursery, Coffee County, TN (Fig. 4B). Leveling was also needed to stabilize the tree rings on top of fire ant mounds. Drench treatment was applied with 10 gal solution at 0.05 (5 reps) and 0.1 (5 reps) lb ai rates of bifenthrin on April 15, 2009. Two of the treated mounds were dug up next day and examined for the ant colonies and for color deposition in the tunnels but the rest of the 8 mounds were dug and evaluated 7 days after treatment.

Field treatment at Deep South Nursery, Lucedale, MS

Individual tree drench using 5-gal buckets was conducted in a nursery field with rows of Japanese boxwood *(Buxus microphylla japonica)* at Deep South Nursery, Lucedale, MS on September 30, 2009. Trees included in the trial were selected with far enough space in between so that drench solution from one treatment would not contaminate other nearby drenches. Two 5-gal buckets were placed close to but at the opposite sides of the tree with drench holes on the buckets facing toward the base of the tree (Fig. 5 A&B). A water tank mounted on the bed of a pickup truck was used to carry water to the treatment. Buckets were first filled half way full and insecticide and liquid dye were both added to the water and then additional water was added to bring it up to the full 5 gallon mark with each tree receiving 10 gallon drench (see Table 1 for treatment details). Eight trees were used in each treatment with a total of 40 trees in the trial. Control treatment (blue dye solution only) and the lower rate of the chemical treatments were setup and let drain first; then after all draining completed, higher rates of the chemical treatments were setup using the emptied buckets that had hold the same chemical. Since the formulation of Scimitar® CS was thick and sticky and the amount required was small, it was first diluted into 1:10 stock solution and then used 10 x amount of the stock solution to add to the buckets.

Treated trees were dug up the next day on Oct 1, 2009 by the grower with a machine harvester and harvested trees with 18” diameter rootball wrapped in plain burlap of 7.5 oz weight were transported to Gulfport Lab. The burlap on the rootballs was sprayed with bifenthrin at 0.05 lb ai/100 gal of water (0.94 ml Onyx Pro per gallon of water). Two gallon solution was used for 8 trees in each treatment. Then, the trees were stored outdoors under simulated nursery storage conditions for normal aging with a daily irrigation of 1 cm over a 30 min irrigation period. The treatments in this investigation are listed in Table 1. At the time of tree digging, soil samples from the bottom of each dug hole were collected for laboratory bioassay using female alates.

Bioassay method

To evaluate the residual effect of bifenthrin-treated burlap over a 6-month aging period under outdoors conditions, a piece of burlap was cut from each of the rootballs and brought to the lab

for efficacy evaluation (Fig 2). 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 laboratory.

Table 1. Treatment list for individual tree drench application at Lucedale, MS fall 2009

Material	Trt #	Rate # ai/ 100 gal	Rate ml prod./gal	Dye amount per drench	Total volume per tree
Control*	1	--	--	375 ml	--
Onyx Pro 23%	2	0.0125	0.235	375 ml	2.63 ml
	3	0.025	0.47	375 ml	4.73 ml
Scimitar CS 9.7%	4	0.035	1.51	375 ml	15.1 ml
	5	0.069	2.97	375 ml	29.7 ml

*Control treatment used liquid blue dye solution only.

Fig 1. Set up of burlap bioassay conducted in the lab.

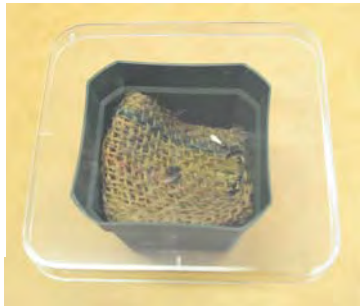


Fig 2. A piece of burlap was removed for bioassay; soil sample was also collected from where burlap was cut out (within yellow rectangle).

Soil samples were also collected from the surface (about 1 cm deep) of the rootball where the burlap was removed (Fig 2) to determine the killing power of the soil. The bioassay method for the soil samples was the same as for burlap pieces.

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 (Fig 1). Queens were not given food but water was added to moisten the burlap or soil if they were not moist enough for the alates. Mortality data were taken at 2 and 7 days after exposure. To investigate the residual effect of bifenthrin-treated burlap over time, burlap and soil samples were taken at 1, 2, 3, 4, and 6 months to monitor the degradation process.

Fig. 3. Tree rings drench on weedy ground (A) and drench on the fire ant mound (B) at Harrison County Farm, Mississippi, spring 2009

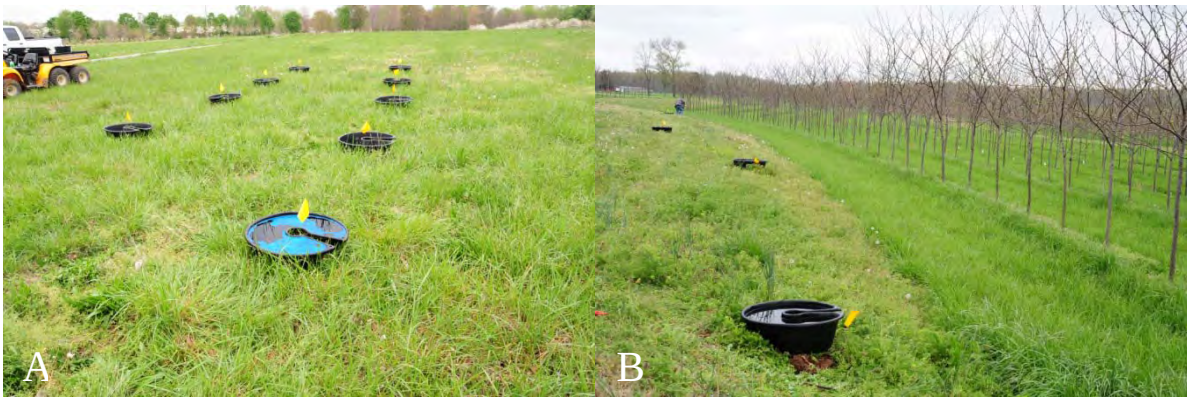


Fig. 4. Tree rings drench on weedy ground (A) at the TSU Nursery Research Center and drench on the fire ant mound (B) at the Hawkersmith Nursery in Coffee County, TN, spring 2009

Fig. 5. Pre-harvest in-field drench using 5-gallon buckets to trees to be harvest in a nursery at Deep South Nursery, Georgia County, Lucedale, MS. A: drench in rows of Japanese Boxwood. B: close look of drench application to a tree.

RESULTS

Results of preliminary trial at Harrison County Farm, MS

From the ground drench experiment, colors were easily seen on the dug soil ball and from the holes left on the ground (Fig. 6 A&B). Bioassay results revealed that all soil samples collected from the balls and from dug holes had 100% kill even for the soil samples with less than 5% coloration (Table 2). This result indicated that colored soil collected from whatever depth will kill, meaning that the dye movement in the soil matches the movement of bifenthrin insecticide in the sandy loam soil.

Drenches over the active fire ant mounds completely eliminated all colonies making them inactive within 24 hours, and the thorough evaluation at 7 days after treatment confirmed this early results (Fig. 7 A&B).

Fig. 6. Colored soil indicating penetration of treatment drench to the entire root ball area. A: Root ball dug up after tree rings drench on weedy ground with blue dye added to the drench solution; B: split in half showing blue dye inside of the ball. Harrison County Farm, Mississippi, spring 2009

Fig. 7. A fire ant nest was eliminated after tree ring drench over the active mound. A: mound was excavated after tree rings drench; B: soil removed from mound showing piles of dead ants. Harrison County Farm, Mississippi, spring 2009

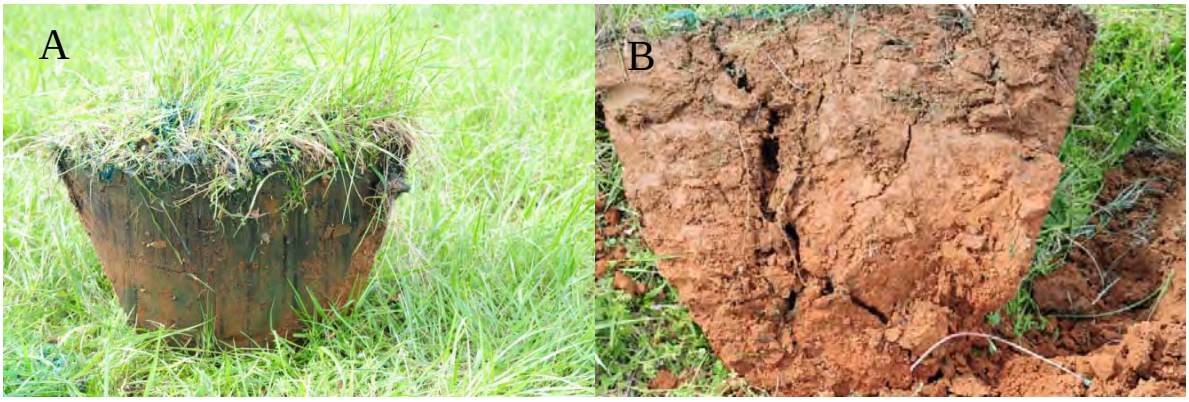


Fig. 8. Tree rings drench on weedy ground at the TSU Nursery Research Center. A: Root ball dug up after tree ring drench on weedy ground with liquid blue dye added to the drench solution; B: split in half showing the inside of the ball; blue color is rarely present.

Results of preliminary trial at TSU Nursery Research Center in TN

The following was found in the preliminary trial in TN.

1. Clay soil in TN is very different from sandy soil in southern MS regarding chemical drench penetration. The tightly packed clay soil was hard for water to penetrate the soil (Fig. 8 A&B). However, the solution went through the tunnels worked by the ant very well (Fig. 10). One of the reasons was that the compact clay soil holds the tunnels in good shape allowing the drench solution to flow freely through the tunnel system inside the mound to the whole structure. This could allow the chemical to reach the queens easier than in mounds in sandy soil. All 10 active fire ant mounds drenched with bifenthrin solution (5 at 0.1 lb ai and 5 at 0.05 lb ai/100 gal) were 100% killed within 24 hours, and the thorough evaluation made at 7 days after treatment confirmed this early result.
2. Bifenthrin could penetrate clay soil well. Bioassays of soil samples collected from dug holes and from rootballs showed that 100% efficacy could be achieved with soil samples in less than 5% coloration (Table 3). This result again indicated that colored soil collected from whatever depth will kill, meaning that the movement of dye matches the movement of bifenthrin insecticide in clay soil, which was similar to that of Mississippi sandy soil.
3. There were mounds larger than the 10-gal sized tree rings could cover; in these cases, the tree ring drench may not kill all the worker ants but may still get the queen killed. This makes using multiple 5-gal buckets be advantageous to tree ring drenches.
4. Multiple buckets are less expensive than tree rings and are readily available to growers. Also, use of multiple buckets could turn out to be a simple solution to covering a large fire ant mound, use on uneven ground, and having the flexibility of drenching more than 10 gal of water and to trees that have large trunks. Extra work was needed to get 10 gal water in one 10 gal sized tree ring on an uneven ground.

Field Treatment at Deep South Nursery, Lucedale, MS

This treatment was an individual tree in-field drench using 5-gal buckets conducted in sandy loam soil in Southern Mississippi. Liquid blue dye penetrated the ground well and colors were easily seen in the soil. Bioassay results from soil samples collected at the bottom of dug holes confirmed our previous preliminary findings which indicated that penetration of the dye in the soil very well matches the vertical movement of bifenthrin in the soil, meaning that colored soil at whatever depth will kill fire ant colonies.

Bioassays on residual effect of the bifenthrin-treated burlap and soil samples collected from the rootballs were conducted for 6 months post-treatment while rootballs were stored outdoors under simulated nursery storage conditions. As it was in previous similar trials, freshly treated burlap was extremely lethal to fire ant female alates. Alates showed symptoms of intoxication within minutes of contact with the bifenthrin-treated burlap. For all the burlap and surface soil bioassays conducted during the entire 6 month post-treatment period, all treatments in the test except control achieved a 100% efficacy. These results showed that burlap treated with bifenthrin at 0.05 lb ai/100 gal water could protect rootballs from newly mated fire ant queens for at least 6 months after treatment under normal weathering outdoors. Soil samples collected from all treatments also achieve 100% efficacy through 6 months. Although it is impossible to separate whether the insecticidal effect was due to the in-field insecticide treatment in the soil or from the acquired chemical residues through close contact with the bifenthrin-treated burlap, all it matters is that the soil portion of the rootballs will add to the killing of newly mated fire ant queens if they land onto the rootballs while in storage outdoors or in transportation. Bioassay results showed that both treated burlap and drenched soil are doing just that.

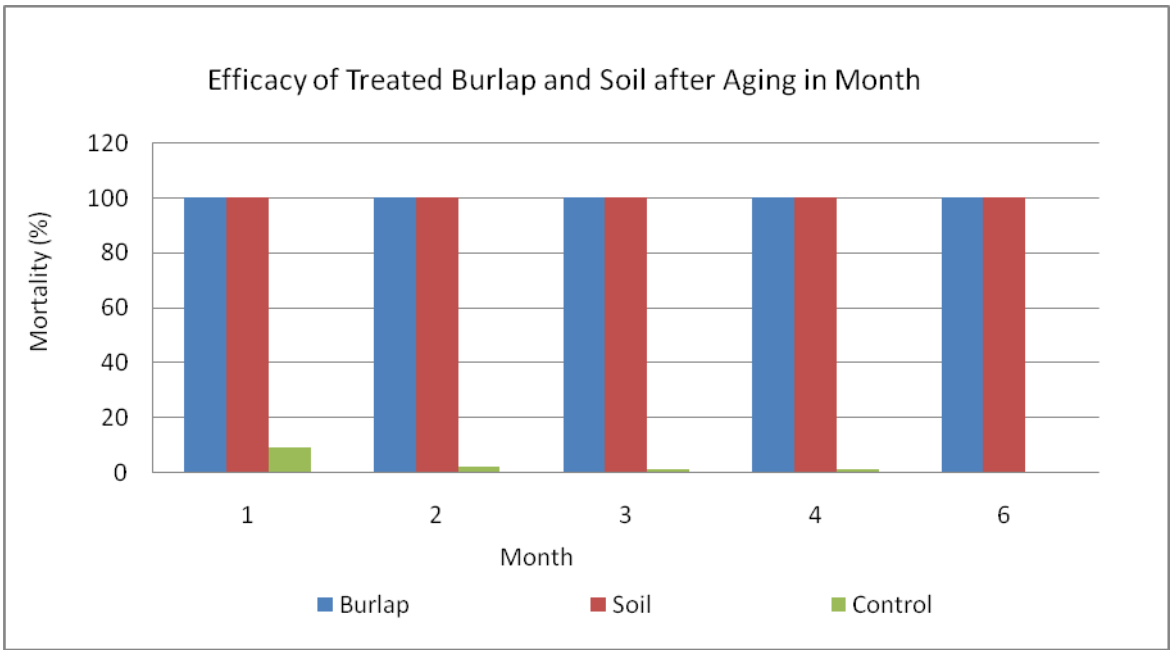


Fig. 9. Efficacy of bifenthrin-treated burlap and bifenthrin- or lambda-cyhalothrin-treated soil on female alates of RIFA after aging for various period of time outdoors under simulated nursery storage conditions

Table 2. Efficacy of soil samples collected from rootballs and dug holes after tree ring drench with bifenthrin (0.23 lb ai/100 gal with 10 gal solution per drench) in a preliminary trial in Harrison County Farm, Mississippi 2009.

Sample Position	**Ball 1**		**Ball 2**		**Ball 3**		**Ball 4**		**Ball 5**	
	%soil colored	% killed (2d)	% colored	% killed (2d)	% colored	% killed (2d)	% colored	% killed (2d)	% colored	% killed (2d)
4" deep hole side*	100	100	100	100	100	100	100	100	100	100
6" deep ball side 1	10	100	70	100	100	100	95	100	30	100
6" deep ball side 2	90	100	95	100	80	100	95	100	65	100
8" deep hole side 1	25	100	5	100	35	100	30	100	40	100
8" deep hole side 2	60	100	<5	100	90	100	<5	100	50	100
Ball bottom (12" d)	70	100	5	100	70	100	90	100	35	100
Hole bottom (>12")	85	100	5	100	5	100	95	100	50	100

* Soil samples were collected 24 hrs after treatment at 4" depth of hole side after soil ball was excavated where 100% colored soil was visually obvious. Five reps of control soil samples taken from untreated spots were 0% killed at 2 days.

Table 3. Efficacy of soil samples collected from rootballs and dug holes after tree ring drench with bifenthrin (0.05 lb ai/100 gal with 10 gal solution per drench) in a preliminary trial at TSU Nursery Research Center, Tennessee 2009*.

Sample Position	Ball 1		Ball 2		Ball 3		Ball 4		Ball 5		Control	
	%soil colored	% killed	% soil colored	% killed	% soil colored	% killed	% soil colored	% killed	% soil colored	% killed	% soil colored	% killed
6" deep ball side 1	10	100	20	100	5	100	5	100	10	100	15	20
6" deep ball side 2	<1	10	15	100	10	100	15	100	5	100	<1	20
8" deep hole side 1	25	100	<1	40	1	50	2	80	4	100	5	10
8" deep hole side 2	10	100	5	100	5	100	3	90	<1	30	2	20
Hole bottom (>18")	15	100	5	100	5	100	2	70	<1	20	10	10

* Rootballs were dug up 24 hrs after treatment from the drenched spots and the size of rootball was 20” in diameter. Soil samples were collected from the balls and from holes after rootballs were excavated. Control was treated with blue dye solution only.

DISCUSSION:

This developing treatment method consists of two parts of application: 1) use one of several individual tree treatment methods such as tree-rings or 5-gal buckets to chemogate the root zone area of the trees, or use garden wand to drench tree bases with chemical solution to kill fire ant in the root-ball mass before harvesting; 2) use bifenthrin at 0.05 lb ai/100 gal water to treat the wrapping burlap before or after harvest to prevent newly mated fire ant queens from infesting the root balls while in storage and during transportation. These two parts of treatment fit well in the nursery production because 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 when B&B plants are mostly 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 is far less work to do than post-harvest dipping or drenching twice daily for 3 consecutive days with chlorpyrifos.

In these trials, both types of holding containers were used for the drench application—tree rings and 5-gal buckets. There are advantages in using 5-gal buckets for drenching treatment. 1. Better suit to the "uneven ground" because getting the tree rings level could sometimes become an issue. As a result, tree rings do not always drain evenly, resulting in some areas underneath the ring not receiving adequate wetting. 2. Some larger ant mounds cannot be covered by one tree ring but can be covered by multiple buckets. 3. Larger tree trunk cannot take the tree rings but all size of trees can use multiple buckets by arranging them around the trees. 4. Tree rings have to do more than one application to drench 10 or more gallon but buckets can drench more than 10 gallons in one fill. 5. Bucket is more economical than tree rings and more readily available to growers.

Blue dye (chosen for its best contract with the soil color) was added to the drench to determine how deep the drench solution penetrates the soil. Since we have already found out from our preliminary trials that bifenthrin travels freely to whatever depth the dye travels in the soil, dye can be used to tell how deep the chemical/toxic zone reaches. Also, we could even use dye in our flip drench trial to visually see if flip drenches, such as 1F1, get good enough coverage or not. If color shows up well on the root balls, we may predict that the root balls are well protected without waiting for 6 months to know the results and without the need of comparing samples taken from bottoms, tops, inners, or outers of the drenched rootballs. We would know or have a pretty good idea about it soon after we do the drench treatment. If color patterns show up as an uneven coverage, we could also adjust factors such as water volume, drench method, flip frequency, etc… to fix the problem without wasting one entire season before knowing it works or not works.

Clay soil in TN is very different form sandy soil in southern MS regarding drench penetration in the soil. The tightly packed clay soil was hard for water to penetrate the soil and the same blue dye used in MS did not show much color on the excavated “root balls” in our TN preliminary trial (Fig. 8). Possibly, a good portion of the drench solution ran away from the drench surface because of the difficulty for water penetration. However, the solution went through the tunnels worked by the ants in the mound very well. One of the reasons was that the clay soil holds the tunnels in good shape even after drench application allowing the solution to flow freely through the tunnel system to the whole structure inside the mound. Color deposition can be seen clearly

surrounding the tunnel holes in the close-up photo (Fig. 10). This could be a positive factor for fire ant control in clay soil because it shows that tunnels in clay soil mounds would allow chemical to reach the entire mound structure easily; thus queens could be killed by individual mound drench easier than in mounds of sandy soil.

Fig. 10. Tightly packed clay soil holds the tunnels inside the mound in good shape allowing drench solution travels easily to the entire structure. This could make reaching the queens easier than in mounds in sandy loam soil. Blue color could be seen surrounding tunnel holes.

CONCLUSIONS

1. In-field chemogation with tree rings or 5-gal buckets could penetrate the rootball area in sandy loam soil. In clay soil, however, penetration was less through in the ground but drench solution could reach the entire tunnel system within the fire ant mounds.
2. When applied at 0.05 lb ai per 100 gal water, bifenthrin-treated burlap and surface soil of rootballs could kill fire ant female alates for 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, greatly reducing the possibility of entering the rootballs by newly mated fire ant queens.
3. Untreated surface soil of rootballs gains killing power by absorbing bifenthrin from the treated burlap through contact, adding to the effective prevention from newly mated fire ant queen infestation.

CPHST PIC NO: A1F04

PROJECT TITLE: Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Individual Tree or Fire Ant Mound Treatment, MS 2009

REPORT TYPE: Final

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

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 disadvantages of his method of treatment are: 1) requires growers to determine which plants to certify more than a month prior to harvest; 2) The bait application must be conducted in weather warm enough for fire ants to actively forage on it, while harvest occurs at temperatures cold enough to ensure dormancy of plants, consequently preventing the treatment of new blocks during harvest and thus preventing any later substitutions from untreated blocks. Therefore, individual tree-style treatments for select in-field plants were initiated to focus on examining efficacy of products other than chlorpyrifos.

Previous individual mound/tree treatment (IMT) experiments conducted in both Mississippi and Tennessee years before focus on treating the immediate surrounding area of nursery stock prior to digging and shipping. Unlike both the current in-field treatment and band treatment, this type of application does not require IFA to forage on bait. Therefore, this would provide an immediate short-term certification (2-6 weeks of quarantine level efficacy) during the harvest season. It would also provide the needed flexibility for growers who ship small numbers of nursery stock outside the quarantined area. Another reason for revisiting the IMT trial now was that in the past three years (2007-2009), band and block trials conducted in TN were not all successful in eliminating mounds in the field with the treatment method of broadcast bait followed by a band application of contact insecticides. The major problem was that 100% of workers in large mounds were difficult to eliminate. There is an urgent need to develop quarantine treatment method for field-grown nursery stock that can take out all mounds in the

field, including those large mounds. Treating the large mounds with IMT application method prior to broadcast application of contact insecticide could be a way to achieve this goal. The objective of this study was to evaluate two IMT application methods-- surface drench and ground injection-- for eliminating large fire ant mounds in the field so that broadcast of contact insecticides could be a successful in-field treatment taking out all fire ant mounds in the field .

MATERIALS AND METHODS:

Treatments were conducted in a wide-open nursery field with naturally infested fire ant colonies at Deep South Nursery, Lucedale, Georgia County, MS on Sept 28, 2009. Mounds were flagged and groups of mounds were randomly assigned to each of the treatments. To facilitate treatment application, mounds in one nursery field were assigned to surface drench treatments and mounds in the adjacent nursery field were used for injection treatments. To mark the treatment area for drench treatments, a 3 ft diameter hula-hoop was used to delineate the treatment area which is 7.069 square feet. The perimeter of the 3 ft diameter circle was marked with paint maker and when applying treatments, the entire amount of chemical solution was uniformly distributed within the hula-hoop delineated area using a sprinkler can. This application method is different from the IMT treatment conducted years before, where 1 gallon was put on mound and 1 gallon around the mound, for the reason that this time we are simulating a ground treatment to evenly cover the entire area. Using a 0.02 lb ai/ 100 gal as our top bifenthrin rate was based on the fact that 1,037 trees (i.e., typical 6 x 7 acre planting) could be treated with a 3 ft diameter circle for each tree without exceeding the 0.2 lb ai / acre maximum rate for a single application (Tables 1 & 2). The total amount of chemical applied around each tree base would not exceed the broadcast (i.e., rates have been adjusted for actual ground area treated, so more chemical can be applied around the tree than the labeled broadcast rate, without exceeding the labeled limit). An Advion fire ant bait treatment was added to the trial for a comparison and was applied at the label rate for individual mound treatment.

Injection treatment was applied using the Soil Injection Probe (B&G Versatool, Model 410, Univar, Indianapolis, IN) connected to a hose attached to a Shur-Dri battery-powered pump (Fig. 1A). In order to make a comparison between the two application methods, injection treatments were applied at the same rates and same volume as the surface drench treatment applications, but the entire volume was injected inside the
mounds (instead of covering a 7.069 square feet area as in surface drench treatment) with the injection tip placed about 6” within the fire ant mound (Fig. 1B).

Evaluations of the treatment results were made 1 week after treatment (WAT) application on Oct 7 by poking the mound with a wire & 3 WAT on Oct 20, 2009 by digging the mound with a shovel to determine if colonies were killed or not.

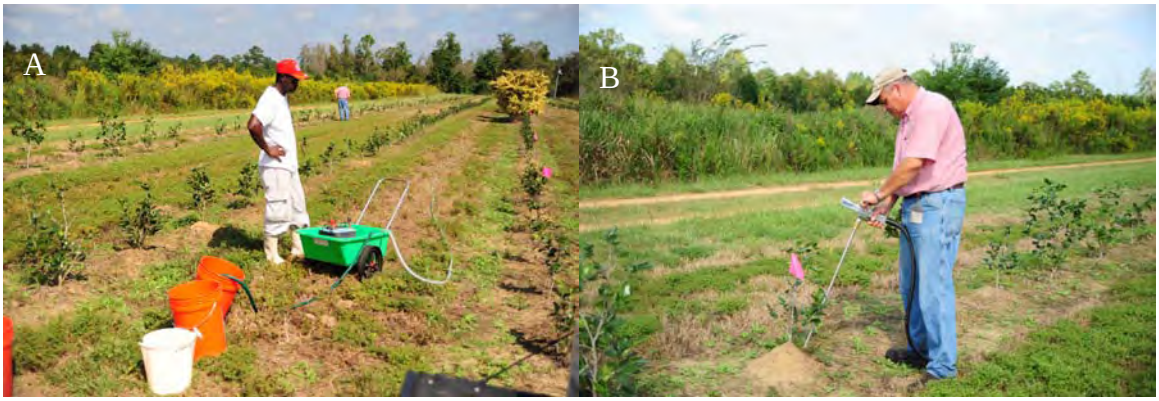


Fig. 1. Treatment application with Soil Injection Probe. A: Injection Probe connected to a battery-powered pump through a garden hose. B: Injection applied to a mound.

RESULTS:

Surface drench

Except for the lowest 0.002 lb ai rate applied at 2 gallon per mound, which was 90% effective, all other treatments tested using surface drench method achieved 100% effectiveness on evaluations at both 1 & 3 WAT. However, evidences were found that small number of colonies moved away from the treated areas and established new mounds nearby, but it was hard to trace all the colonies that moved and we could not really be sure the percentage of colonies that were completely killed by the surface drench treatment and which ones did move away. In this case, the partial colony that moved away may not include the fire ant queen in it but it is uncertain. There was no change in efficacy between the two evaluations made at 1 and 3 WAT. The bait treatment was 70% effective at 1 WAT and 90% at 3 WAT, but failed to achieve 100% effectiveness in the trial.

Injection

The lowest rate bifenthrin treatment (0.002 lb ai per 100 gal of water) and the bifenthrin mixed with lambda-cyhalothrin used in the surface drench treatments were not conducted with the injection method. Except the 0.01 rate applied at 1 gallon per mound, which was 90% effective, all other treatments tested using Injection Probe achieved 100% effectiveness at both evaluations of 1 and 3 WAT. Similar to that of surface drench treatment, evidences were found that small number of colonies may have moved away from the treated mounds and established new mounds nearby. However, it was difficult to trace all the colonies that had moved and we could not be sure what was the percentage of colonies that were killed by the injection treatment and which colonies had moved. It was observed that partial colonies avoided treatment by escaping from the top portion of the mounds while the mounds were receiving injection. This was different from the surface drench treatment where ants were not seen escaping from the top of the well drenched mounds.

Table 1. Effectiveness of surface drench as an individual mound treatment method, Georgia County, MS, fall 2009

Treatment	Rate (lb ai/100 gal)	Drench Vol (gal)	Mound killed (%)	
			1 WAT	3 WAT
bifenthrin	0.002	2	90	90
bifenthrin + lambda cyhalothrin	0.002 + 0.00056	2	100	100
bifenthrin	0.005	2	100	100
bifenthrin	0.010	1	100	100
bifenthrin	0.010	2	100	100
bifenthrin	0.020	1	100	100
Advion Bait	Label rate	2 oz per mound	70	90
Untreated CK	0	0	0	10

Table 2. Effectiveness of ground injection as an individual mound treatment method, Georgia County, MS, fall 2009

Treatment	Rate (lb ai/100 gal)	Injection Vol (gal)	Mound killed (%)	
			1 WAT	3 WAT
bifenthrin	0.005	2	100	100
bifenthrin	0.010	1	90	90
bifenthrin	0.010	2	100	100
bifenthrin	0.020	1	100	100
Advion Bait	Label rate	2 oz per mound	70	90
Untreated CK	0	0	0	10

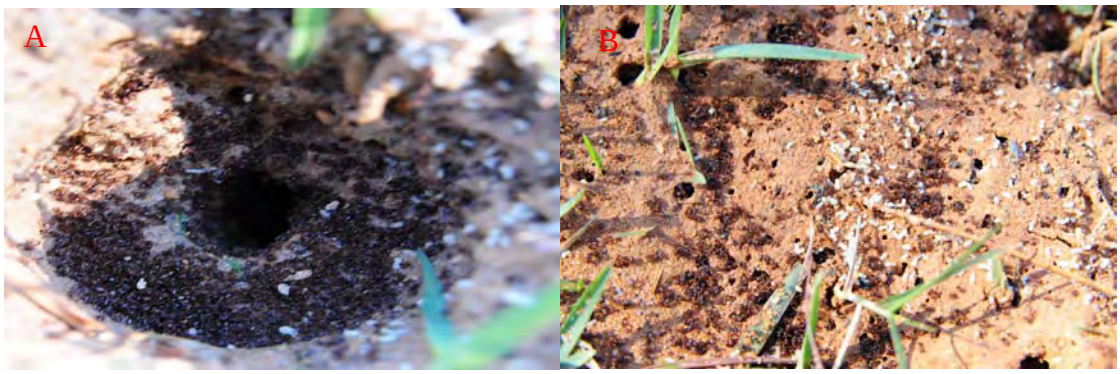


Fig. 2. Ant killed by treatment applications. A: Killed by injection application. B: Ant killed by surface drench application.

DISCUSSION:

The lowest treatment rate of bifenthrin at 0.002 lb ai per 100 gal (3.0 ml Onyx Pro product in 100 gal of water) was so dilute that it was not expected to work, but it did kill the ants and made almost all treated mounds inactive in the surface drench treatment. Piles of dead ants were seen at the base of fire ant mounds in all the treatments (Fig. 2 A & B).

Treatment results represent fire ant mounds that were made inactive at evaluations but did not exclude the mounds that may have possibly moved away from treated mounds. There were obvious signs that some colonies or part of colonies may have moved away to nearby locations but it was hard to tell for sure which colonies may have moved. Colonies relocations were suspected in both treatment methods -- surface drench and injection application. The difference in colony moving between the two treatment methods is that, in the surface drench treatments, colonies moved from the bottom of the mounds but in the injected mounds, colonies moved away from the top portion of the mound that did not receive chemical solution during injection application. Lower rate treatments in both application methods seemed to have more colonies to have moved than that in higher rates treatments. Although all treatments except the lowest concentration made the treated mounds inactive, the highest rates tested (0.02 lb ai per 100 gallons of water) seemed to be more effective for fire ant control using IMT method. Treatments with two gallon solution per mound achieved similar result to the one gallon treatment with concentration doubled. However, since the concentration used was not excessively high, treatment with less water should be more practical for the IMT application because 2 gallons per mound or per tree could mean too much water in field production as well as doubling the amount of water that must be carried to the field.

Some treatment solution ran out of the 3 ft diameter circle in the surface drench application which made the treatment area wider than the 3 ft diameter circle and less solution went into the ground of the mound area, but that was not considered a major problem for this type of treatment application in sandy soil but could become a major problem in clay soil. The fall 2009 TN in-field band trial included a treatment which preceded the broadcast of contact insecticide with a drench application to individual fire ant mounds that were at certain size or larger. We faced a problem of chemical solution not penetrating the mound crest while drenching and we had to poke some holes on the mounds first before applying surface drench treatment. Problem with injection method, however, is that partial colony can escape the treatment from the top of the ant mound that does not receive the chemical solution during injection application.

Individual mound treatment could eliminate mature fire ant mounds with both surface drench and ground injection. The most effective way to do this type of treatment is to have a tool that can do both—surface drench and injection at the same time.

PROJECT TITLE: Summary of In-field Band and Block Treatment Trials for Nursery Stock 2000-2008

TYPE REPORT: Status Report

COMPILED BY: Anne-Marie A. Callcott and Xikui Wei

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INTRODUCTION:

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

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

treatments for large blocks of in-field B&B and individual plant-style treatments for selected plants were initiated to focus on examining efficacy of products other than chlorpyrifos, reduction of treated diameter, and reduction of the exposure time required prior to plant movement.

Preliminary testing initiated in Sept. 2001 assessed several liquid and granular insecticides against individual IFA mounds in the field. Results of this trial indicated promising results with acephate, bifenthrin, and deltamethrin. Tests against individual mounds continue to provide direction for insecticides utilized in the larger scale band treatments. The first two band trials applied in the fall of 2001 and spring of 2002 tested five to six-foot wide bands of bifenthrin and deltamethrin. Both liquid and granular formulations showed promising results but demonstrated that in band treatments contact insecticide alone was not effective enough for use in the IFA quarantine. Subsequent band trials have included a broadcast application of bait 3-5 days prior to the contact insecticide application, as in the current approved in-field treatment.

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

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

MATERIALS AND METHODS:

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

availability of production areas with adequate numbers of IFA, we limited the tests in Tennessee to the most promising insecticide, bifenthrin.

In Mississippi, band trial plots consisted of 800-foot long strips of land containing at least five active fire ant mounds within a 4-foot wide (two feet on both sides of a center line) observation strip that ran the length of the band. Block 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. In Tennessee, plots consisted of rows of nursery stock of varying lengths, but had a minimum of 5 active mounds within a 4-foot wide area with the tree row in the center. Bait applications were made on a determined date, followed in 3-7 days by a contact insecticide treatment. All contact insecticides were applied to a band 3-6 ft. on either side of the tree row (or center line). Active IFA colonies in each plot's observation area were recorded prior to bait application and after contact insecticide application at 1, 2, 4, 6, 8, and 12 weeks and every four weeks thereafter. Mounds were evaluated using as little disturbance as possible, usually through insertion of a wire flag into the mound. Mounds were considered active if any workers appeared. Treatments consisted of:

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

RESULTS**:**

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

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

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

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

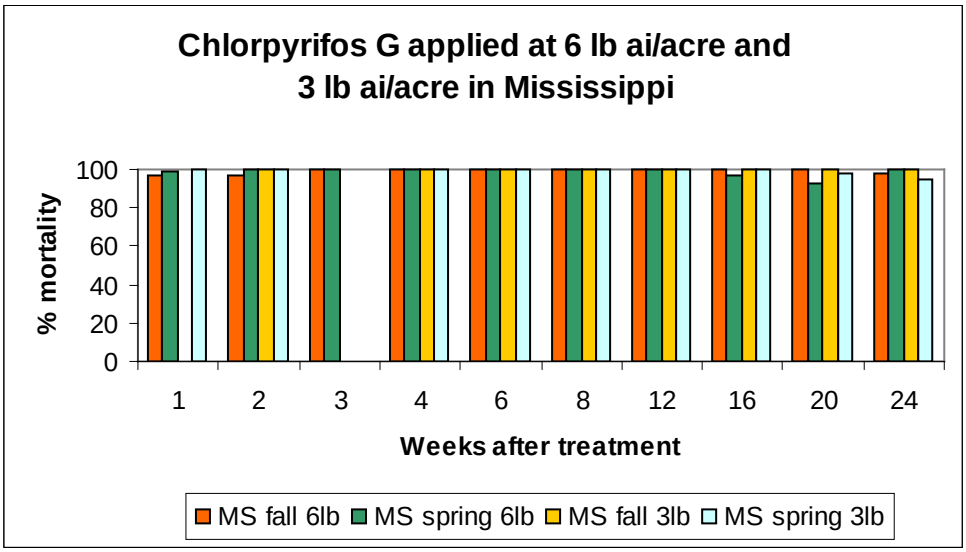
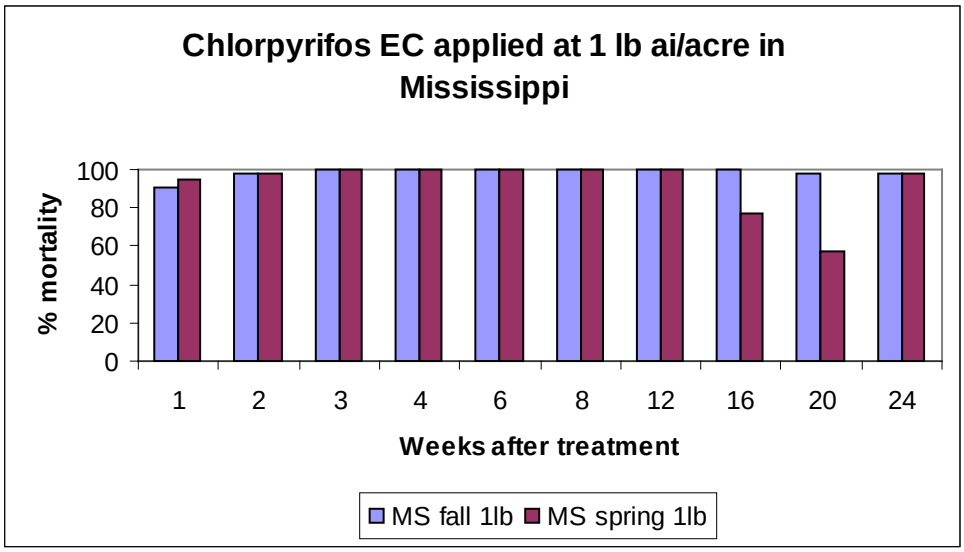


Figure 2. Efficacy of liquid chlorpyrifos applied after a broadcast bait application at 1 lb ai/acre to simulated field grown production area in Mississippi.



Bifenthrin: Mississippi trials: Bifenthrin was tested in Mississippi over several years with excellent results (Figures 3, 4, & 5). Granular rate of application was at 0.4 lb ai/acre and flowable rate of application was at 0.2 lb ai/acre (labeled rates). Results indicate longer control when applied during the fall months compared to the spring months. Both rates and formulations after a 4 week exposure period provided excellent control for 16 weeks in the fall and 8 weeks in the spring. One colony in one EC treated plot in the fall 2002 trial remained active throughout most of the test period resulting in the average of all the trials presented here not reaching 100% control (Figures 4 & 5). This colony was located next to (against) a small concrete structure supporting a wind sock which was located along the center line of the plot. This colony did become significantly less active over time, but its survival stresses the importance of complete and accurate application of the contact insecticide in this in-field treatment.

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

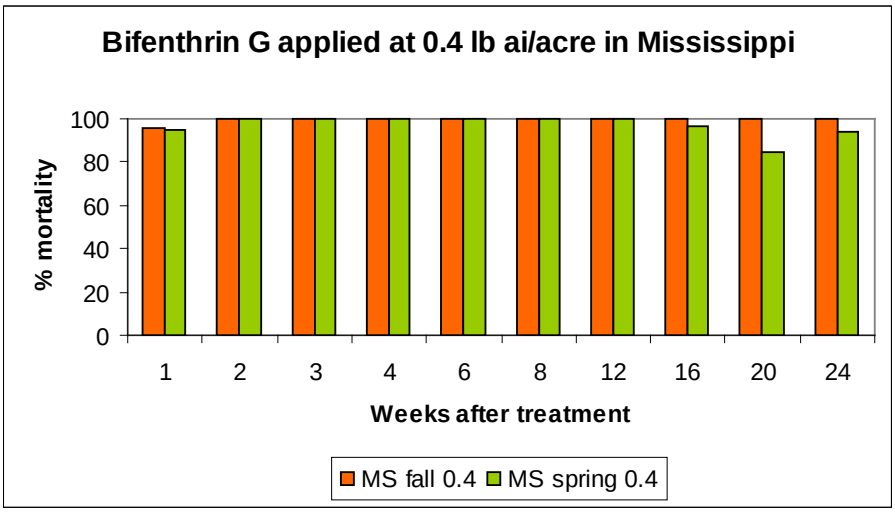


Figure 4. Efficacy of liquid bifenthrin applied at 0.2 lb ai/acre after a broadcast bait application to simulated field grown production area in Mississippi.

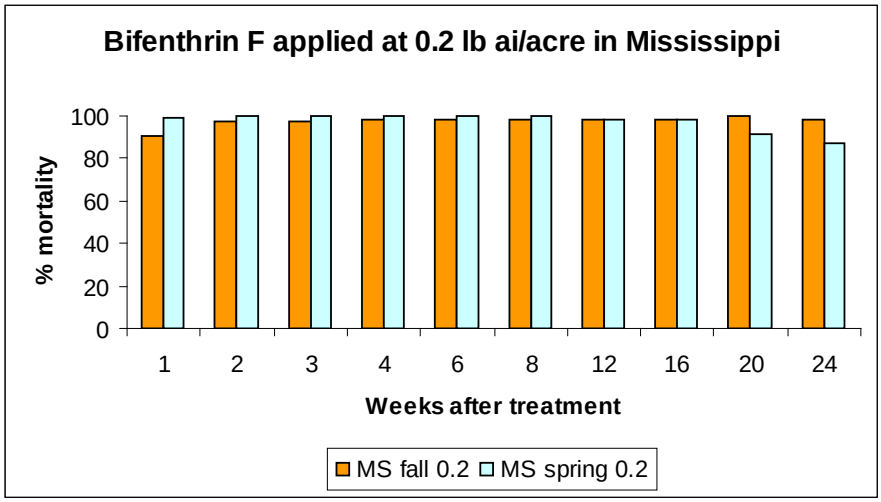
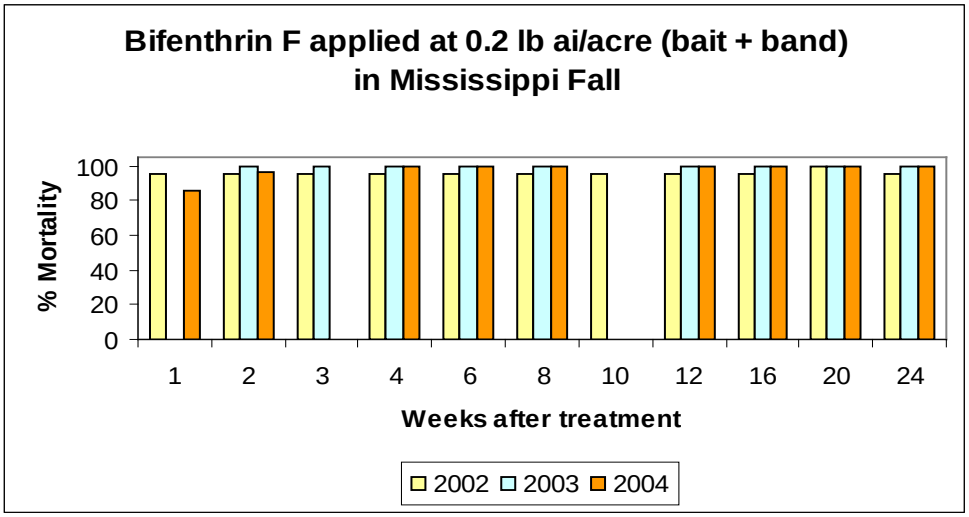
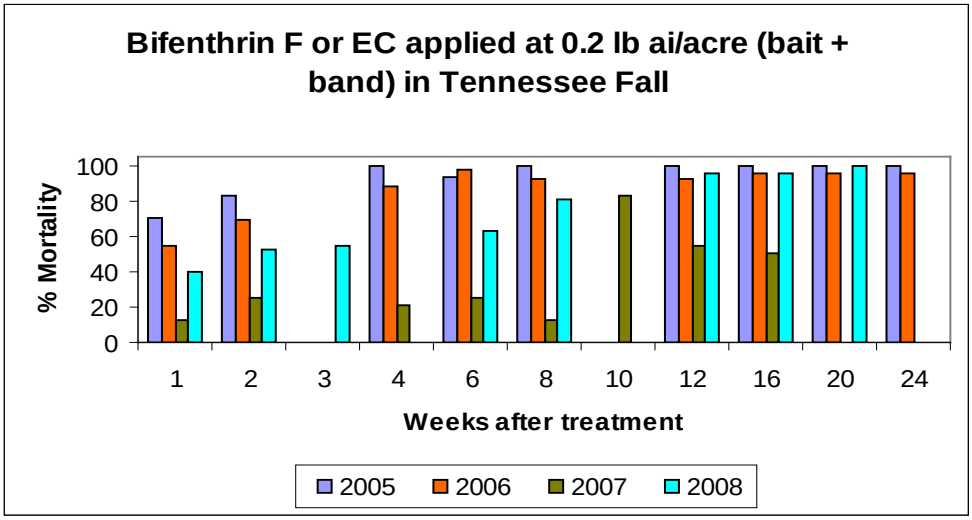


Figure 5. Efficacy of liquid bifenthrin applied at 0.2 lb ai/acre after a broadcast bait application to simulated field grown production area in Mississippi.



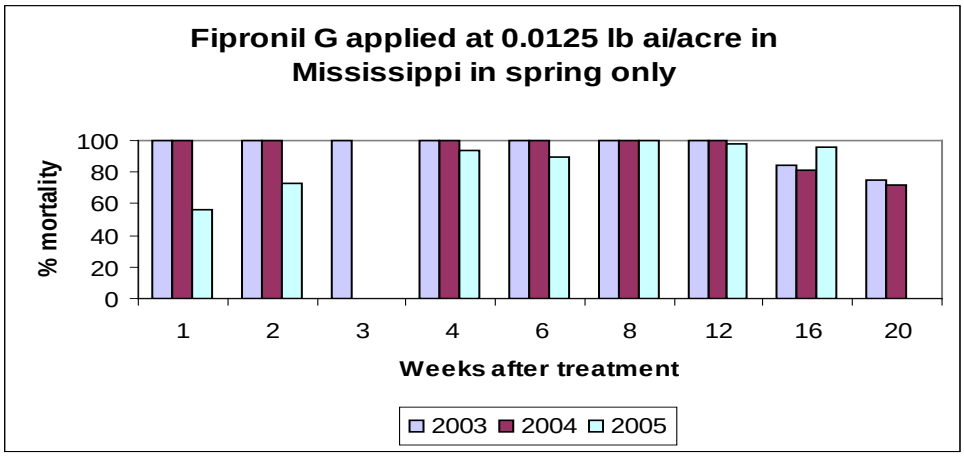
Tennessee trials: After successful trials in Mississippi (Figure 5), trials were initiated in Tennessee beginning in 2005. As stated in the methods, these trials were conducted under actual field grown production systems and only conducted in the fall each year. After the first year of testing (2005) we were encouraged by the results which indicated the full 4 weeks were needed for exposure to eliminate IFA (Figure 6). However, subsequent trials in years 2006, 2007, and 2008 provided less than satisfactory controls even though changes in treatments have been made trying to resolve the problems. Changes we had tried in 2008 included increasing the number of treatment applications in a season, alternating bifenthrin with other insecticides in the multiple application schemes, and even the use of two types of fire ant baits. Using combined formulation that has bifenthrin as a major ingredient and tank-mixing of two insecticides will be tested in the coming years.

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



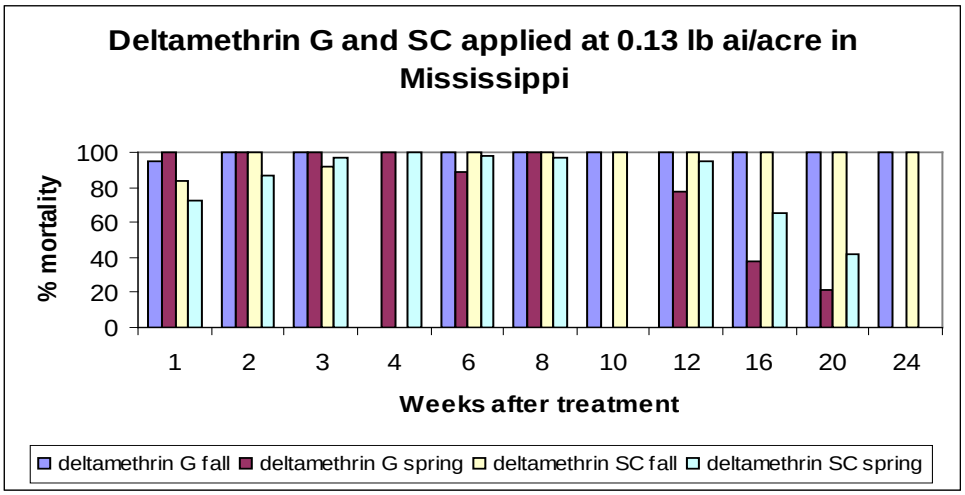
Fipronil: Fipronil granular is currently approved for use in the IFA quarantine as a treatment for grass sod. The rate of application for grass sod is a total rate of 0.025 lb ai/acre. After a 30 day exposure period, the sod is certified for movement for 20 weeks. We anticipated the addition of a bait treatment would allow for a lower rate of fipronil to be used in the field grown use pattern. Fipronil was tested in 3 spring trials in Mississippi, with the first 2 trials providing excellent results (Figure 7). However, the 3rd trial gave different results, taking 8 weeks to reach 100% control and only maintaining that 100% control at that one evaluation date. However, when all the data is combined 96-100% control is maintained by fipronil from 4-12 weeks in these spring trials.

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



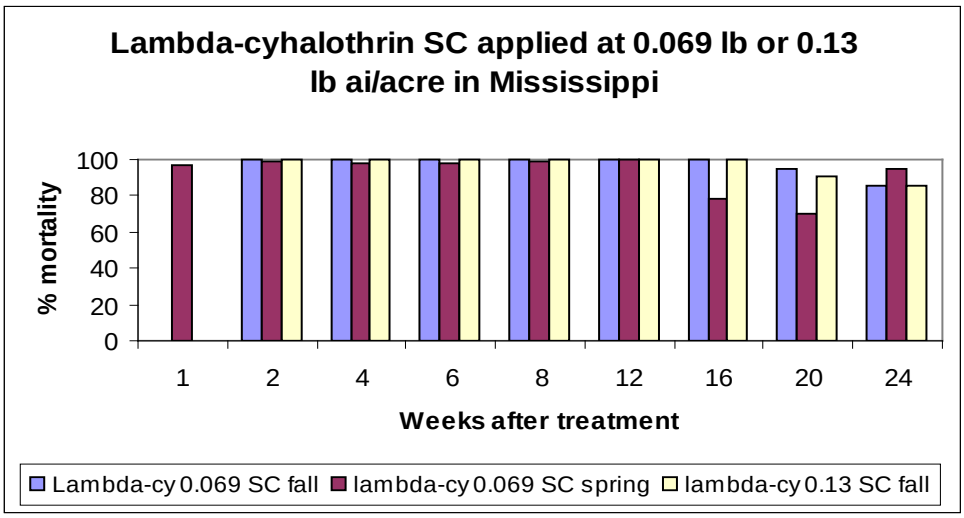
Deltamethrin: Deltamethrin, both granular and liquid applied at 0.13 lb ai/acre, has provided good control in this use pattern, but has not been quite as consistent as the previously mentioned insecticides at the rates under current testing (Figure 8). Only the SC spring application has been replicated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

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



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

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



DISCUSSION**:**

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

Bifenthrin, which is currently used for containerized nursery stock in the IFA quarantine, was so successful in some areas of the quarantine, but when subjected to Tennessee climatic and environmental conditions as well as actual production sites, proved less effective than we had anticipated. However, we are still trying to determine whether our 2007 extensive failure can be

explained. Unfortunately, this failure will not allow us to immediately pursue label changes and the addition of this insecticide to this use pattern in the IFA regulations. To overcome the relative ineffectiveness of bifenthrin band treatment in Tennessee nursery production situation, multiple applications of bifenthrin, bifenthrin in combination with other chemicals either in combined formulation or in tank-mix while treatment application, have been proposed. Tank-mixing with more than one contact insecticides in a spray mixture could be an effective way for this use pattern. The readily available pair of chemicals is bifenthrin and lambda-cyhalothrin, which has been proposed for trials in the fall of 2009 in Tennessee nursery productions.

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

Table 1. Chemicals and Rates Used for In-field Band or Block Trials for Field Grown Nursery Stock

Year	Season	Location	Trial	Bait	Bifenthrin 0.2G	Bifenthrin 7.9%F	Bifenthrin 23.3%EC	Chlorpyrifos 2.32%G	Chlorpyrifos 44.8%EC	Deltamethrin 0.1G; 4.75SC	Fipronil 0.0143G	lambda-cyhalothrin
1999	Fall	Harri. Co Farm, MS	In-band	N/A							0.05 lb ai	
2001	Spring	McNeill, MS	Indivd mound	N/A	0.2 lb; 0.4 lb						0.0125; 0.01875; 0.025	
2001	Summer	Laurel Municipal Airport,MS	Block	distance	0.4 lb	0.2 lb					0.125, blend (13lb bait+ 87 lb g)	
2001	Fall	Camp Shelby, MS	Band	None	0.4 lb	0.2 lb				0.13 lb ai G & SC	0.0125	
2002	Spring	BCMA Hatt	Band	N/A	0.4 lb	0.2 lb				0.13 lb ai G & SC		0.06875
2002	Fall	BCMA Hatt	Band/ Block	Hydram-1.5lb	0.4 lb	0.2 lb				0.13 lb ai G & SC	0.0125	
2003	Spring	Camp Shelby, MS	Band	Hydram-1.5lb	0.4 lb	0.2 lb		6 lb	1 lb	0.13 lb ai G & SC	0.0125	0.06875
2003	Fall	Starkville,MS	Band	Hydram-1.5lb	0.4 lb	0.2 lb		6 lb	1 lb			
2004	Spring	Stennis Airport MS	Band	Hydram-1.5lb	0.4 lb	0.2 lb		3 lb	1 lb		0.0125	0.06875
2004	Fall	Starkville,MS	Band	Hydram-1.5lb	0.4 lb	0.2 lb		6 lb	1 lb			
2005	Spring	BCMA Hatt	Band	Hydram-1.5lb	0.4 lb	0.2 lb		6 lb	1 lb	0.13 SC	0.0125	0.06875
2005	Fall	No trials were conducted because of the Katrina hurricane in the season										
2006	Spring	BCMA Hatt	Band	Hydram-1.5lb				6 lb; 3 lb			0.0125	0.06875
2006	Fall	Stennis Airport MS	Band	Hydram-1.5lb	0.2 lb			3 lb; 1 lb	1 lb			0.069, 0.137
2007	Spring	BCMA Hatt	Band	Hydram-1.5lb	0.2 lb	0.2 lb			1 lb			
2007	Fall	BCMA Hatt	Block	Hydram-1.5lb		0.2; 0.2+0.2	0.2; 0.2+0.2					

Table 2. Trials Conducted in Mississippi for Field-grown Nursery Stock Quarantine Treatment
Bifenthrin 0.2 G; Bifenthrin 7.9% F
(All rates used for flowable were 0.2 lb ai/A; the rates listed in the table were granular only)

Year	Season	Rate (lb ai/A)	location	Trial	Bait	Result
2001	Spring	0.2 & 0.4	McNeill, MS	Spot/ Mound	N/A	90-100% effective for the duration of the trial (8 weeks). No flowable formulation was used.
	Summer	0.4	Laurel, MS	Block	Distance	100% control from 4 -9 weeks and greater than 90% control through 44 weeks.
	Fall	0.4	Harri. Co Farm, MS	Spot/ Mound	N/A	Both formulations had good control for 1 - 4 weeks.
	Fall	0.4	Camp Shelby, MS	Band	N/A	90% control for about 10 weeks but were erratic and not for quarantine efficacy
2002	Spring	0.4	BCMA Hatt	Band	N/A	Could reach 100% control but erratic; single band treatment not sufficient for QT
	Fall	0.4	BCMA Hatt	Band & Block	Hydram. @ 1.5 lb	100% control from 4 -36 weeks for block treatment; 100% control from 2 -24 weeks for band treatment
2003	Spring	0.4	Camp Shelby, MS	Band	Hydram. @ 1.5 lb	100% control for 4 months
	Fall	0.4	Starkville, MS	Band	Hydram. @ 1.5 lb	100% control from 2 – 12 weeks

Table 2 (cont.) Trials Conducted in Mississippi for Field-grown Nursery Stock Quarantine Treatment
Bifenthrin 0.2 G; Bifenthrin 7.9% F
(All rates used for flowable were 0.2 lb ai/A; the rates listed in the table were for granular formulation only)

Year	Season	Rate (lb ai/A)	location	Trial	Bait	Result
2004	Spring	0.4	Stennis Airport MS	Band	Hydram. @ 1.5 lb	100% control for 4 months with granular effective for five months
	Fall	0.4	Starkville, MS	Band	Hydram. @ 1.5 lb	100% control from 4 – 32 weeks for granular, through 28 week for flowable
2005	Spring	0.4	BCMA Hatt	Band	Hydram. @ 1.5 lb	100% control from 2 – 42 weeks for both formulas
	Fall	No trial was conducted because of Hurricane Katrina				
2006	Fall	0.2	Stennis Airport MS	Band	Hydram. @ 1.5 lb	100% control from 2 – 16 weeks
2007	Spring	0.2	BCMA Hatt	Band	Hydram. @ 1.5 lb	100% control from 2 – 12 weeks fro granular; flowable did not provide quarantine level of treatment.
	Fall	(0.2; 0.2+0.2)	BCMA Hatt	Block	Hydram. @ 1.5 lb	No granular was used in this trial; 100% control from 2 – 20 weeks; The treatments of two consecutive applications (made in Nov & Dec) achieved 100% control through 32nd week for both EC and flowable; two consecutive applications of contact insecticides alone (without bait) experienced a sharp drop in the number of active colonies at around week 4 but remained at a low level through 20th week.

Table 3. Trials Conducted in Mississippi for Field-grown Nursery Stock Quarantine Treatment
Fipronil G 0.0143%

Year	Season	Rate (lb ai/A)	location	Trial	Bait	Result
1999	Summer	0.05*	Harri. Co Farm, MS	In-band	N/A	Not effective
2001	Spring	0.0125, 0.01875 & 0.025	McNeill, MS	Spot/ Mound	N/A	Not different from the untreated check, except the high rate at wks 7 & 8
2001	Summer	0.0125	Laurel, MS	Block	Distance	100% control through 8 wks; 100% control through 17 wks for the blend of fipronil bait (1.5 ppm) plus granular (0.0125 lb ai/A).
	Fall	0.0125	Harri. Co Farm, MS	Spot/ Mound	N/A	Not different from the untreated control
	Fall	0.0125	Camp Shelby, MS	Band	N/A	Very slow to eliminate or repel IFA from treatment area
2002	Fall	0.0125	BCMA Hatt	Block	Hydram. @ 1.5 lb	100% control 4 – 26 weeks
2003	Spring	0.0125	Camp Shelby, MS	Band	Hydram. @ 1.5 lb	100% control for 4 months
2004	Spring	0.0125	Stennis Airport MS	Band	Hydram. @ 1.5 lb	100% control through 16 weeks
2005	Spring	0.0125	BCMA Hatt	Band	Hydram. @ 1.5 lb	Did not achieve a quarantine level of control in this trial.
2006	Spring	0.0125	BCMA Hatt	Band	Hydram. @ 1.5 lb	Did not achieve a quarantine level of control in this trial, only 80 - 95% control.

* Granular 0.05% a.i.

Table 4. Trials Conducted in Mississippi for Field-grown Nursery Stock Quarantine Treatment
Chlorpyrifos G 0.2%

Year	Season	Rate (lb ai/A)	location	Trial	Bait	Result
2003	Spring	6.0	Camp Shelby, MS	Band	Hydram. @ 1.5 lb	100% control through 12 wks
	Fall	6.0	Starkville, MS	Band	Hydram. @ 1.5 lb	100% control throughout 12 wks evaluation
2004	Spring	3.0	Stennis Airport MS	Band	Hydram. @ 1.5 lb	100% control through 20 wks.
	Fall	6.0	Starkville, MS	Band	Hydram. @ 1.5 lb	100% control from 4 - 20 wks evaluation
2005	Spring	6.0	BCMA Hatt	Band	Hydram. @ 1.5 lb	100% control from 2 - 32 wks evaluation
	Fall	No trial was conducted because of Hurricane Katrina				
2006	Spring	6.0 &3.0	BCMA Hatt	Band	Hydram. @ 1.5 lb	Chlorpyrifos 6 lb-- 100% control through 24 wks; 3 lb-- 100% control through 20 wks
	Fall	1.0 & 3.0	Stennis Airport MS	Band	Hydram. @ 1.5 lb	Both treatments had 100% control throughout 16 wks evaluation

Table 5. Trials Conducted in Mississippi for Field-grown Nursery Stock Quarantine Treatment
Chlorpyrifos 44.8% EC

Year	Season	Rate (lb ai/A)	location	Trial	Bait	Result
2003	Spring	1.0	Camp Shelby, MS	Band	Hydram. @ 1.5 lb	100% control through 12 wks
	Fall	1.0	Starkville, MS	Band	Hydram. @ 1.5 lb	100% control throughout 12 wks evaluation
2004	Spring	1.0	Stennis Airport MS	Band	Hydram. @ 1.5 lb	100% control through 16 wks.
	Fall	1.0	Starkville, MS	Band	Hydram. @ 1.5 lb	100% control through 20 wks.
2005	Spring	1.0	BCMA Hatt	Band	Hydram. @ 1.5 lb	100% control through 4 - 16 wks
	Fall	No trial was conducted because of Hurricane Katrina				
2006	Fall	1.0	Stennis Airport MS	Band	Hydram. @ 1.5 lb	100% control throughout 16 wks evaluation
2007	Spring	1.0	BCMA Hatt	Band	Hydram. @ 1.5 lb	100% control through 2 - 12 wks

Table 6. Trials Conducted in Mississippi for Field-grown Nursery Stock Quarantine Treatment
Lambda-cyhalothrin 9.7% SC

Year	Season	Rate (lb ai/A)	location	Trial	Bait	Result
2002	Spring	0.06875	BCMA Hatt	Band	N/A	Could reach 100% control but not consistent
2003	Spring	0.06875	Camp Shelby, MS	Band	Hydram. @ 1.5 lb	100% control 1 – 12 weeks
2004	Spring	0.06875	Stennis Airport MS	Band	Hydram. @ 1.5 lb	100% control through 16 wks
2005	Spring	0.06875	BCMA Hatt	Band	Hydram. @ 1.5 lb	Single treatment—8 weeks of ant free control; re-treated plots -- 100% control from 4 – 42 weeks.
2006	Spring	0.06875	BCMA Hatt	Band	Hydram. @ 1.5 lb	100% control 8 – 16 weeks
2006	Fall	0.06875; 0.137	Stennis Airport MS	Band	Hydram. @ 1.5 lb	Both rates 100% control 2 – 16 weeks

Table 7. Trials Conducted in Mississippi for Field-grown Nursery Stock Quarantine Treatment
Deltamethrin G (0.1%); SC (4.75%)
(Rates used for both granular and SC formulations were always the same)

Year	Season	Rate (lb ai/A)	location	Trial	Bait	Result
2001	Fall	0.13	Harri. Co Farm, MS	Spot/ Mound	N/A	Good control with liquid formulation numerically superior to granular
2002	Spring	0.13	BCMA Hatt	Band	N/A	G or SC formulation alone was not good enough control for quarantine treatment
	Fall	0.13	BCMA Hatt	Band	Hydram. @ 1.5 lb	100% control from 2 – 5 weeks through 15 weeks
2003	Spring	0.13	Camp Shelby, MS	Band	Bait unspecified	100% control through 4 wks for granular, SC treatment was not always 100% during the evaluation period.
2005	Spring	0.13	BCMA Hatt	Band	Hydram. @ 1.5 lb	Provided 8 weeks without active mounds on the plot

Table 8. Trials Conducted in Mississippi for Field-grown Nursery Stock Quarantine Treatment
Four Other Chemicals

Insecticide*	Year/Season		Rate (lb ai/A)	location	Trial	Bait	Result
Acephate	2001	Spring	2tsp/ mound	McNeill, MS	Indivd. mound	n/a	90 - 100% effective for 8 weeks
Sevin SL	2002	Spring	1.22	BCMA Hatt	Band	n/a	Did not work
Permethrin	2004	Spring	0.8712	Stennis Airport MS	Band	Hydram. @ 1.5 lb	Did not work
Imidachloprid + cyfluthrin	2007	Spring	0.62	BCMA Hatt	Band	Hydram. @ 1.5 lb	Did not work

* These four insecticides were used one time only and did not get repeated because of lack of efficacy for IFA control.

Table 9. Trials Conducted in Tennessee for Field Grown Nursery Stock Quarantine Treatment
Bifenthrin 0.2 G; Bifenthrin 7.9% F; Bifenthrin 23.4% EC; Lambda-cyhalothrin 9.7%
(Chemicals Used in Tennessee Band Trials)

Year	Season	Chemical and Rate (lb ai/A)	location	Trial	Bait	Result
2005	Fall	Bifenthrin G 0.4	Hwy 111 right-of-way (Sequatchie County)	Band	Amdro Pro @ 1.5 lb	100% control from 8 – 28 weeks for granular
2005	Fall	Bifenthrin F 0.2	Hwy 111 right-of-way (Sequatchie County)	Band	Amdro Pro @ 1.5 lb	100% control from 4 – 28 weeks
2006	Fall	Bifenthrin F 0.2	Spruce / hemlock nursery(Grundy County)	Band	Amdro Pro @ 1.5 lb	Reached 100% control after second Flowable 0.2 application at week 21
2007	Fall	Bifenthrin F 0.2, Lambda-cyhalothrin 0.069, & Bifenthrin EC 0.2	Dogwood nursery (Franklin County)	Band	Award Fire Ant Bait @ 1.5 lb	Bifenthrin F in September followed by Lambda-Cyhalothrin F in October had 100% control from 10 to 30 weeks. Another Onyx September followed by Onyx October had 100% control from 10 to 22 weeks. All other treatments failed to achieve 100% control.
2007	Fall	Bifenthrin F 0.2, & Bifenthrin EC 0.2	Mixed deciduous tree nursery (Coffee County)	Band	Award Fire Ant Bait @ 1.5 lb	No treatments reached 100% control before 20 weeks. Treatments applied late (December and February) were generally better than treatments applied earlier.
2008	Fall	Bifenthrin EC 0.2 & Lambda-cyhalothrin 0.035or 0.069	Coffee & Franklin County	Band	Award or Advion Fire Ant Bait @ 1.25 lb	Only treatments with bait followed by bifenthrin plus a second application of lambda-cyhalothrin reached 100% control from 14- 20 weeks. All other treatments failed to achieve consistent 100% control for extended period of time.

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CPHST PIC NO: Umbrella IFA Quarantine Treatments

PROJECT TITLE: Efficacy of New Candidates as Grass Sod Treatments: Mississippi, Spring and Fall 2009

TYPE REPORT: Interim

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

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 the fipronil 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 2009 trial were fields located in Pearl River Co, just northeast of Poplarville, MS. The fields were regularly fertilized and mowed by the grower but did not have irrigation available. The test sites for the fall 2009 trial were fields located in northern Hancock County and had irrigation capabilities; however supplemental irrigation was not needed due to abundant rainfall in the fall/winter months of 2009.

Plots were 0.52-acre square in size for all contact insecticide only treatments (150’ x 150’). Plots receiving bait plus a contact insecticide were a different size to accommodate different swath widths of the application equipment. Bait treatments were applied to a 0.65 acre area (168’ x 168’). The contact insecticide application on the same plot was applied to the smaller 0.52 acre area within the 0.65 acre area. All plots contained a permanently marked ¼-acre circular efficacy plot in the center. This is the area that was evaluated for active IFA mounds. There were 3 plots per treatment and controls. Prior to treatment and at 1, 2, 3, and 4 weeks after treatment and bi-weekly thereafter, 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 mound 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.

All liquid treatments were applied using an electric diaphragm 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. 27 gal/acre. Granular contact insecticides were applied with a Herd GT-77 granular applicator mounted to a farm tractor. Fire ant bait was applied at a rate of 1.5 lb/acre through the use of a shop built spreader mounted to a farm tractor. Control plots were not treated with bait or contact insecticide. If multiple applications were made on one plot (e.g. bait followed by contact or 3 applications (3X) of Hero™) applications were made approximately one week apart. In the fall 2009 trials, two tank mixes were applied. Both insecticides were mixed in one tank of water and applied simultaneously.

Spring Mississippi rates of application:

Trade Name	Active Ingredient	Rate of Application (lb ai/acre)	Date of last application
Hero™	bifenthrin + zetacypermethrin	2X (2 applications) Total = 0.15 bifen + 0.05 zeta	5/5/09
		3X (3 applications) Total = 0.225 bifen + 0.075 zeta	5/5/09
Hero™ *plus*	bifenthrin + zetacypermethrin	1X Hero + 1X Mustang (1 Hero app followed in 1 wk by 1 Mustang app) Total = 0.075 bifen + 0.025 zeta *plus* 0.025 zeta	5/6/09
Mustang Max™	zetacypermethrin	2X Hero + 1X Mustang (2 Hero apps 1 wk apart followed in 1 wk by 1 Mustang app) Total = 0.15 bifen + 0.05 zeta *plus* 0.025 zeta	5/6/09
F6138-1 ZW	Unknown	1X	5/7/09
		2X (2 apps 1 wk apart)	5/7/09
F6132 0.25G	unknown	1X	5/7/09
Scimitar®	Lambda-cyhalothrin	0.069 lb ai/acre	5/7/09
Scimitar®	Lambda-cyhalothrin	0.13 lb ai/acre	5/7/09
Control	untreated		--

Fall Mississippi rates of application:

Trade Name	Active Ingredient	Rate of Application (lb ai/acre)	Date of last application
Amdro® *plus* Scimitar®	Hydramethylnon *plus* lambda-cyhalothrin	1.5 lb bait/acre *plus* 0.13 lb ai/acre lambda (1 wk apart)	8/25/09
Advion® *plus* Scimitar	Indoxycarb *plus* lambda-cyhalothrin	1.5 lb bait/acre *plus* 0.13 lb ai/acre lambda (1 wk apart)	8/25/09
Amdro® *plus* Bifenthrin (Onyx®)	Hydramethylnon *plus* bifenthrin	1.5 lb bait/acre *plus* 0.2 lb ai/acre bif (1 wk apart)	8/26/09
Advion® *plus* Bifenthrin (Onyx®)	Indoxycarb *plus* bifenthrin	1.5 lb bait/acre *plus* 0.2 lb ai/acre bif (1 wk apart)	8/26/09
Bifenthrin (Onyx®)	bifenthrin	0.2 + 0.2 (1 wk apart)	8/26/09
Bifenthrin (Onyx®) + Scimitar tank mix	Bifenthrin + lambda-cyhalothrin	Tank mix (both insecticides mixed in same tank and applied at same time) = 0.2 bif + 0.069 lambda	8/24/09
		Tank mix = 0.2 bif + 0.13 lambda	8/24/09
Scimitar	Lambda-cyhalothrin	0.13 + 0.069 (apps 1 wk apart)	8/25/09
		0.13 + 0.13 (1 wk apart)	8/25/09
Hero™	bifenthrin + zetacypermethrin	3X = 3 apps 1 wk apart Total = 0.225 bifen + 0.075 zeta	8/24/09
Control	Untreated		--

RESULTS:

Spring Mississippi: There was about 1.5 inches of rainfall between week 2 & 3 evaluations, but no additional measureable rainfall until between weeks 8 & 10. While all the treatments reduced ant populations (Figure 1), only the bifenthrin + zetacypermethrin product (Hero) at the 3X rate of application provided provided 100% control at any time (4 and 5 week evaluations). Four treatments (both lambda-cyhalothrin rates, 1X bifenthrin + zetacypermethrin + 1X zetacypermethrin, and 1X F6138-1 ZW) were not evaluated at the 22 week evaluation since populations had increased significantly at the previous evaluation (thus no bars on the figure for those treatments).

Fall Mississippi: This trial is still underway. These plots had considerable rainfall after all treatments and throughout the test; December has 26 inches of rainfall and no evaluations were conducted during the entire month. While monthly average temperatures were normal through

December, January and February low temperatures were 5 and 9 degrees F below normal lows, respectively.

At 1 week after final treatment, the bifenthrin + zetacypermethrin product, bifenthrin dual application, and three of the four bait plus contact applications provided 100% control (Figure 2). By week 5, all treatments had achieved 100% control, except the bifenthrin + zetacypermethrin product at the 3X rate of application which now had 1 mound present on one replicate (maintained 1 mound on one replicate through 12 weeks). At 12 weeks (late November), four treatments had one small mound on one replicate each. These mounds were no longer present 9 weeks later after prolonged rain and cold temperatures and whether they succumbed to the insecticidal treatment, the cold weather, or a combination of factors, is uncertain. Untreated controls were rebounding well at 26 weeks, with all treated areas maintaining 100% control. Evaluations will continue until reinfestation or until the grower needs the area for harvest.

DISCUSSION:

Over the last two years of testing in Mississippi and Arkansas, most of the bifenthrin + zetacypermethrin product (Hero™) plus zetacypermethrin (Mustang™) combinations have failed to provide quarantine level results (100% control over multiple weeks) and thus in the fall of 2009 we only tested the Hero 3X rate of application. While this rate of application (total of 0.225 lb ia/acre bifenthrin and 0.075 lb ai/acre zetacypermethrin) is very effective at significantly reducing populations of IFA, consistent results with this product over both the spring and fall have not been produced over a two year period. Bifenthrin applied at 0.2 lb ai/acre alone does not provide quarantine level control against IFA and the addition of 0.075 lb ai/acre of zetacypermethrin has not improved that control. Additionally, requiring growers to make 3 trips across a field over a 14 day period, instead of 1 to 2 trips over a 1 to 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.

Past results indicate that most contact insecticides provide longer residual activity in the winter months than the summer months probably due to lower pressure from photo-degradation, microbial degradation, etc. Since all of the fall treatments provided 100% control of IFA at some point in the trial, we will move most of the treatments into a spring 2010 trial to test the treatments under different environmental and insect pressures.

Figure 1. Efficacy of grass sod treatments in Mississippi in spring 2009.

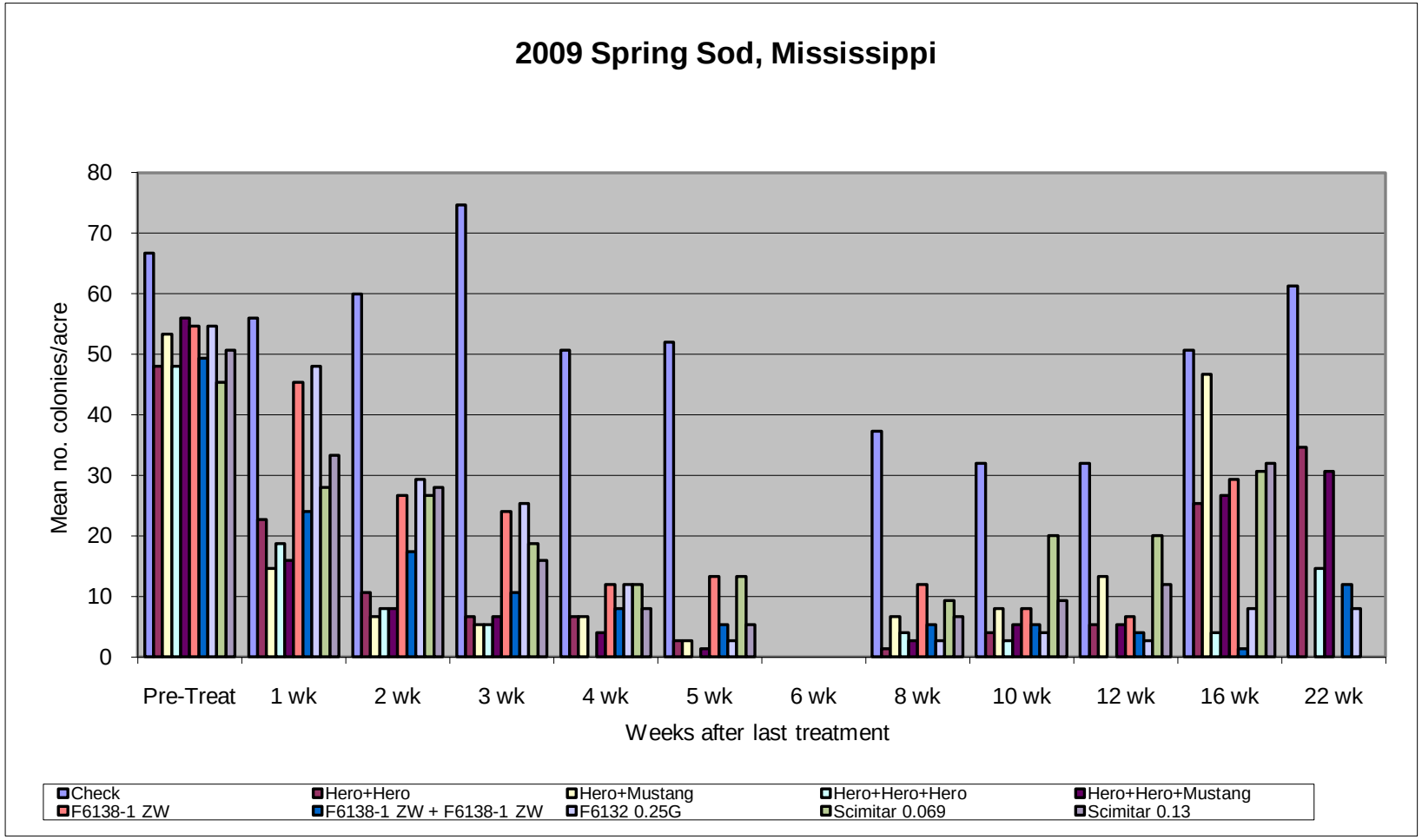
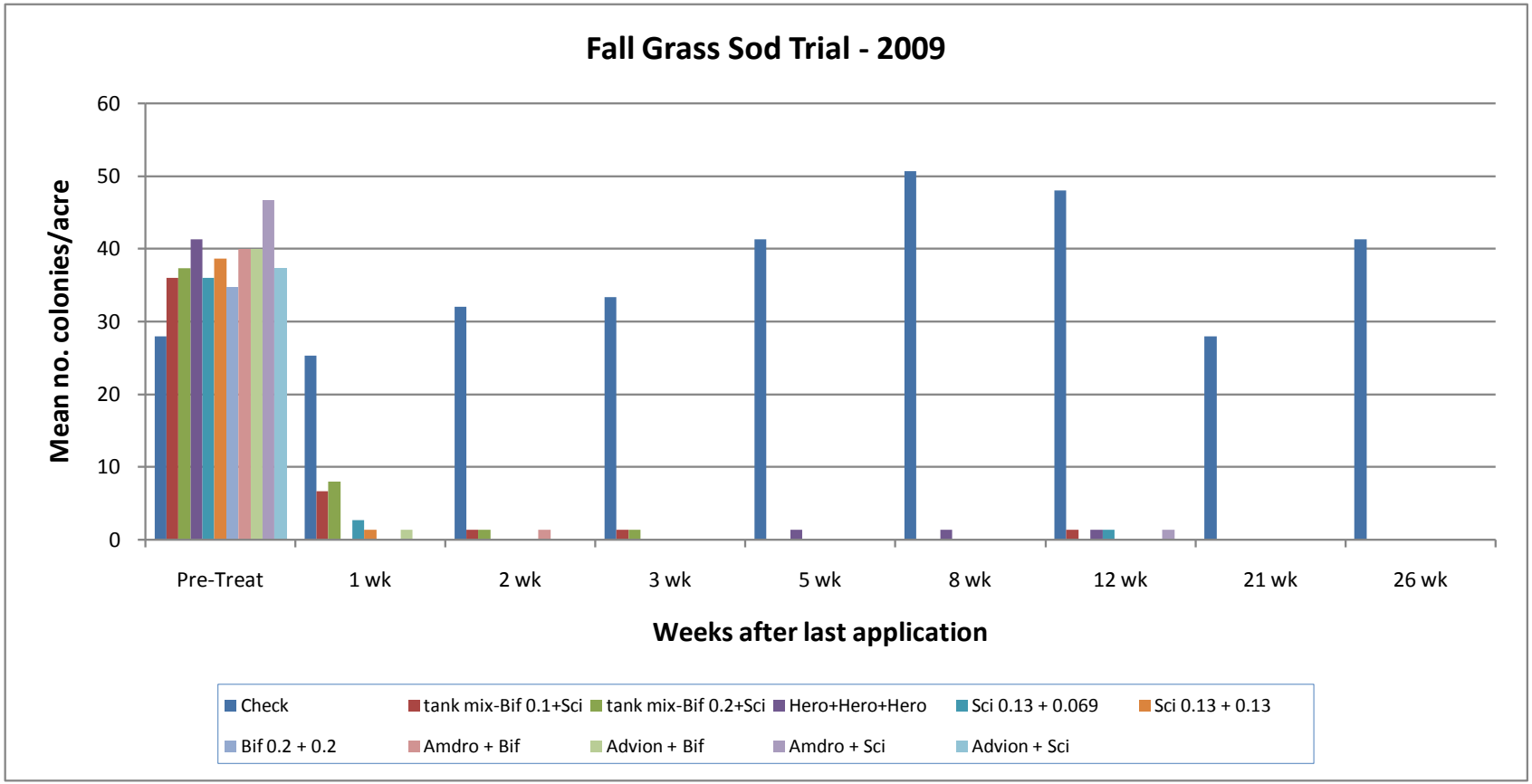


Figure 2. Efficacy of grass sod treatments in Mississippi in fall 2009.



Evaluation of Imported Fire Ant Quarantine Treatments in Commercial Grass Sod: Arkansas 2009

PI – Kelly Loftin, University of Arkansas

08-8100-1325-CA

Introduction

Imported fire ants (IFA) originated from South America and were accidentally introduced into the United States in the early to mid-1900's. IFA are now widespread across the Southeastern United States. Movements of this pest are regulated through a system of Federal and State quarantines. Products regulated by the IFA quarantine include but are not limited to hay, nursery plants and other landscape materials including grass sod.

When treating sod in compliance with Federal and State quarantine regulations, sod producer's options are limited (USDA-APHIS 2006). One option is treatment using the active ingredient chlorpyrifos at a rate of eight pounds of active ingredient per acre. Currently, no products are registered for IFA in sod at that required rate. The other option is to use two separate applications of fipronil at 0.0125 pounds per acre about one week apart. Fipronil can be too expensive to apply and the Environmental Protection Agency has indicated their intention to review the registration and possibly remove it when the 5-year conditional registration expires for broadcast granular products containing fipronil for imported fire ants. The removal of products containing fipronil which are labeled for use against IFA, at the rate required for quarantine, will leave no options for sod producers when selling their products to non-quarantined areas and will also prevent the movement of those products across state lines because of the Federal quarantine regulations.

Because of limited or costly options available to sod producers, a field study was conducted to evaluate the efficacy of other insecticides for use in the IFA quarantine.

Materials and Methods

The study was conducted at the Fulton Grass Farm in Fulton, AR (Hempstead Co.) beginning in June 2009 and ending in September 2009. Plots were square, measured ½ acre in area, and treatments (four treatments and an untreated control) were arranged in a Randomized Complete Block Design (RCBD) with three replications. Each plot used in the study had a minimum of 20 active fire ant mounds per acre when the study began. An active fire ant mound is defined as a mound with 25 or more ants in the colony which is the USDA standard for classifying active mounds. Treatments within the same plot were separated by one week. The treatments were as follows: two separate applications of Hero™ (bifenthrin and zeta-cypermethrin) 1.24 EC at 10.3 fl oz/A followed by one application of Mustang Max™ (zeta-cypermethrin) 0.8 EC at 4 fl oz/A; two separate applications of Hero™ (bifenthrin and zeta-cypermethrin) 1.24 EC at 10.3 fl oz/A; three separate applications of Hero™ (bifenthrin and zeta-cypermethrin) 1.24 EC at 10.3 fl oz/A; one application of Advion™ (indoxacarb) 0.045 GR at 1.5 lb/A followed by one application of Onyx Pro™ (bifenthrin)2 EC at 12.8 fl oz/A. Spray applications were made using a towed boom sprayer applying @ 20 gal/A (15 ft. boom with ten 8003FF nozzles on an 18" spacing at 20 psi and 5.2 MPH). Granular bait applications were made using an Earthway 2750 hand operated seeder calibrated to apply 1.5 pounds per acre. Treatment

numbers, insecticide rates and the total amount of active ingredients applied per acre are provided in Table 1.

The number of active mounds per plot was determined by counting the mounds in a circle at the of the center plot. This circle had a diameter of 58.9 ft which corresponds to a circle with an area of 0.25 acres. The mounds are counted by anchoring one end of a 58.9 ft. rope at the center of the plot and moving the free end along the circumference of the circle. Each mound encountered anywhere along the length of the rope is disturbed by probing with a small rod and estimating the number of imported fire ants exiting the mound within 20 seconds (Jones et al 1998).

The number of active mounds in each plot was determined before any treatments were applied and then at four days and seven days after the last application (DALA) then weekly up to 35 DALA, at which time evaluations were made every 14 days until the study ended.

All data were analyzed using Gylling's Agriculture Research Manager Software (ARM 7.0.3. 2003). An analysis of variance was performed and Least Significant Difference (p=0.05) was used to separate means only when AOV Treatment P(F) was significant at the 5% (ARM 2003).

Results

The data are summarized on Table 2 and Figure 2. Before applying treatments, there were no significant differences in the number of active mounds in any of the plots to be used in the study. All plots used contained at least 20 active mounds per acre. At seven DALA, all treatments had significantly fewer active mounds than did the untreated control and the plots treated with three applications of Hero™ and those treated with Advion™/Onyx Pro™ contained zero active mounds per acre. At 28 DALA there were no longer any significant differences between the untreated control and any of the treated plots, and those plots which previously had zero active mounds had active mounds at 28 DALA or the week before at 21 DALA. The lack of significant differences may have been due to hot, dry weather which lowered the activity of the fire ants in all plots including the control plots. Two applications of Hero™ reduced the number of active mounds to zero by 21 DALA, but active mounds were present the next week.

All insecticide treatments significantly reduced the number IFA colonies in treated plots and at a level acceptable for most IFA management circumstances. However for quarantine treatment, the level of control with a new insecticide option must be 100% to be considered an acceptable insecticide for quarantine treatment. The combination of a bait and residual insecticide treatment into IFA/sod quarantine treatment options appears promising. Future studies evaluating differing treatment regimes incorporating IFA baits with residual contact insecticides are warranted.

References

ARM 7.0.3. 2003. Gylling Data Management, Inc. Brookings, SD.

Jones, D., L. Thompson and K. Davis. 1998. Measuring Insecticide Efficacy: Counting Fire Ant Mounds vs. Bait Station Sampling. In *Proceedings of the 1998 Imported Fire Ant Conference.* Hot Springs, Arkansas. pp. 70-78.

USDA-APHIS. 2006. Imported Fire Ant 2007: Quarantine Treatments for Nursery Stock and Other Regulated Articles. USDA-APHIS Program Aid No. 1904.

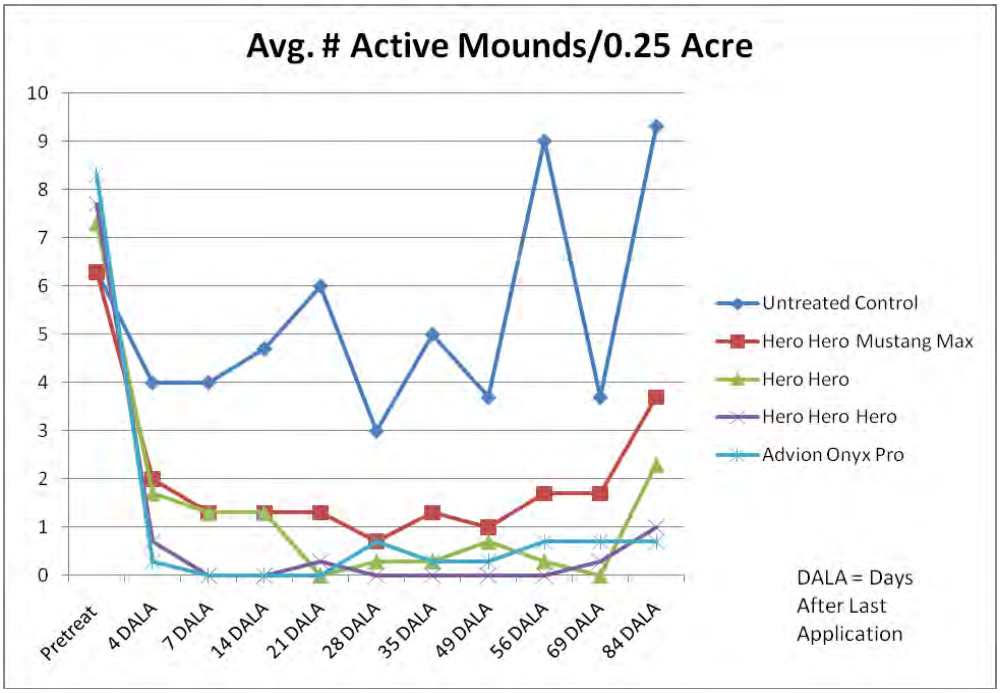
Table 1. Insecticide applications, rates and total amount of active ingredient.

Treatment Number	**Insecticide Application**	**Total active ingredient/acre**
1	None – Untreated Control	None
2	2 applications Hero™1.24 EC @ 10.3 fl oz/A and 1 application Mustang Max™0.8 EC @ 4.0 fl oz/A	0.225 lb/acre total, (0.15 lb bifenthrin & 0.075 lb zeta-cypermethrin)
3	2 applications Hero™ 1.24 EC @ 10.3 fl oz/A	0.2 lb/acre total, (0.15 lb bifenthrin & 0.05 lb zeta-cypermethrin)
4	3 applications Hero™ 1.24 EC @ 10.3 fl oz/A	0.3 lb/acre total, (0.225 lb bifenthrin & 0.075 lb zeta-cypermethrin)
5	1 application Advion™ .045 GR @ 1.5 lb/A and 1 application of Onyx Pro™ 2 EC @ 12.8 fl oz/A	0.2 lb/acre total, (0.2 lb bifenthrin & 0.000675 lb indoxacarb)

Table 2. Avg. # of Active Mounds/0.25 acres for each treatment.

Treatment	Avg. # Active Mounds/0.25 Acre										
	Pretreat	4 DALA	7 DALA	14 DALA	21 DALA	28 DALA	35 DALA	49 DALA	56 DALA	69 DALA	84 DALA
Untreated Control	6.3a	4.0a	4.0a	4.7a	6.0a	3.0a	5.0a	3.7a	9.0a	3.7a	9.3a
Hero Hero Mustang Max	6.3a	2.0a	1.3b	1.3b	1.3b	0.7a	1.3a	1.0a	1.7b	1.7a	3.7b
Hero Hero	7.3a	1.7a	1.3b	1.3b	0.0b	0.3a	0.3a	0.7a	0.3b	0.0a	2.3b
Hero Hero Hero	7.7a	0.7a	0.0b	0.0b	0.3b	0.0a	0.0a	0.0a	0.0b	0.3a	1.7b
Advion Onyx Pro	8.3a	0.3a	0.0b	0.0b	0.0b	0.7a	0.3a	0.3a	0.7b	0.7a	0.7b

Fig. 1. Avg. # of Active Mounds/0.25 Acres for each treatment.



CPHST PIC NO: A1F01

PROJECT TITLE: Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing and Release Project, 2009 (*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 2009 there have been 105 field releases of phorid flies and more than 933,000 potential flies released. Of these 105 releases, 64 were *P. tricuspis,* 34 were *P. curvatus* and 7 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, covering about 50% of the IFA regulated area. 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), covering about 60% of the regulated area. *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 under review for publication.

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 2009) 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.

RESULTS:

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 2010 in limited quantities with the aim to phase out production in 2010 and eliminate rearing of this species totally in 2011. *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). We expect to begin rearing a fourth species, *P. cultellatus*, in 2010.

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 2009 there have been multiple releases in each of 13 states and Puerto Rico, with a total of 105 field releases and more than 933,000 potential flies released. Of these 105 releases, 64 were *P. tricuspis*, 34 were *P. curvatus* and 7 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 less than in previous years. In 2009, we were able to increase our releases from 2008.

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 150,000 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 reviewed for publication. Maps are currently being developed for this publication and are not ready for inclusion in this report at this time.

REFERENCES CITED:

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

Table 1. Rearing and release data for APHIS phorid fly rearing project – 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
tri,cur,obt	2009	3,335,019	88,109	12	7,342.42
Total		18,664,295	933,177	105	

* 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

2009 Imported Fire Ant Training Workshops for State Inspectors and/or Nursery Growers

Arkansas: On January 22, 2009, PPQ headquarters and CPHST personnel presented information on Imported Fire Ant Regulations and Regulatory Treatments to growers in the turf industry and in the nursery industry at their annual trade show. On January 23, 2009, PPQ headquarters, state and CPHST personnel presented a training session on the federal IFA regulatory program to approximately 20 state inspectors. Topics included the biology of IFA, IFA regulations, compliance agreements, quarantine treatments, investigations into violations, etc. A.-M. Callcott presented to all 3 groups.

2009 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 2009. 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 2009, levels of detection (LOD), levels of quantification (LOQ), and range of below quantifiable level (BQL), in ppm, 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

Overview of sample numbers:

- 222 total samples submitted
  - 177 nursery samples
  - 11 sediment samples collected from around one nursery's treatment area
  - 34 blitz samples from NC (blitzes in spring and fall)
- 0 samples from potential violations
- 188 routine samples including sediment
  - 62 samples requesting bulk density only
  - 69 samples requesting chemical analysis only (includes sediment)
  - 57 samples requesting chemical analysis and bulk density

Results:

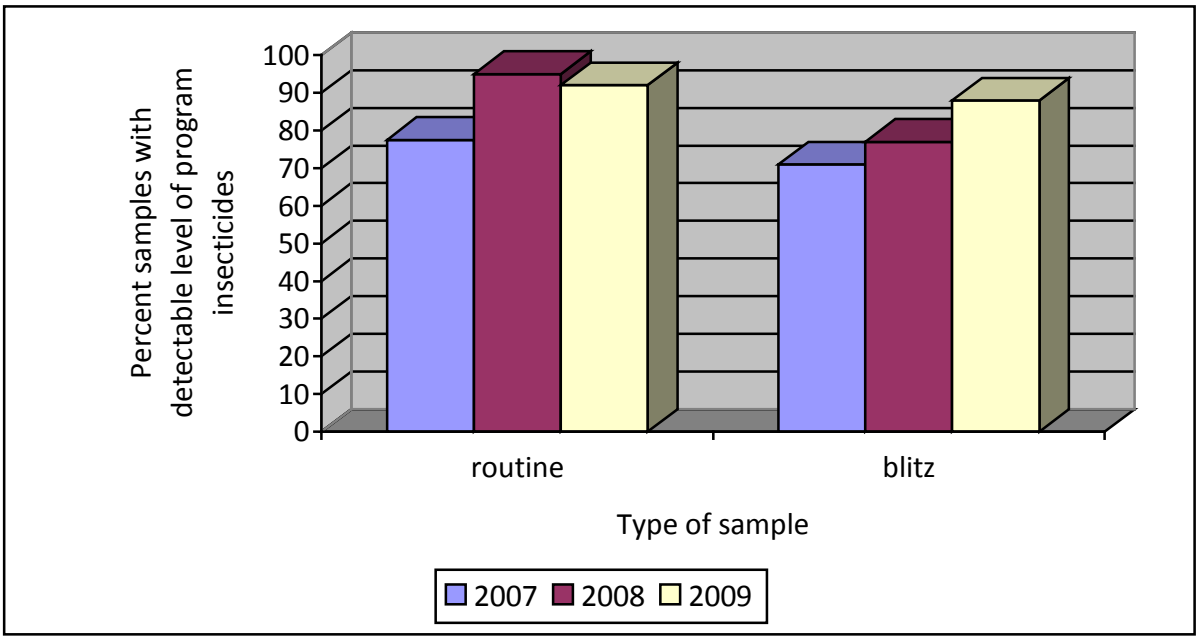
- 11 sediment samples analyzed for bifenthrin
  - 10 less than detectible limit of 0.9 ppm; 1 with 4.7 ppm bifenthrin

34 blitz samples from NC

- 30 samples (88%) had detectible levels of program insecticides
- 28 containerized media (26 with insecticide present; 2 below detectable levels)
- 6 B&B media (4 with insecticide present; 2 below detectable levels)

- 177 routine samples excluding sediment
  - 119 bulk density samples: range 145-758 lb/cu yd
  - 115 samples analyzed for 1 or more program insecticides (excludes sediment)
    - 98 samples (92%) had detectible levels of program insecticides
    - 9 remaining samples – no program chemicals detected

Percent of routine and blitz samples with detectable levels of program insecticides by year from 2007-2009.



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.

PROJECT TITLE: Overview of Analytical Chemistry Routine and Program Support in 2009

TYPE REPORT: Final

LEADER: Robert Smith

INTRODUCTION:

The CPHST-Gulfport Lab analytical chemistry unit is located in Gulfport, MS. The chemistry unit provides a laboratory support service for many APHIS on-going treatment programs. In 2009 the chemistry unit completed multiple support activities spanning a diverse scope of work projects.

So what was the primary objective for CPHST-GL chemistry units in 2009?

Our primary objective was to provide analytical chemistry support services to multiple APHIS nationwide operational and emergency programs that utilize chemical controls. Core programs that submit samples under the scope of "Routine Program Sample Analysis" include:

- Asian Longhorn Beetle Eradication Program (ALB)
- Boll Weevil Eradication Program (BW)
- Rangeland Grasshopper and Mormon Cricket Suppression Program (RGMC)
- Fruit-Fly Attractants and Lures (FF)
- Imported Fire Ant Quarantine (IFA)

So what was our strategy in providing APHIS support?

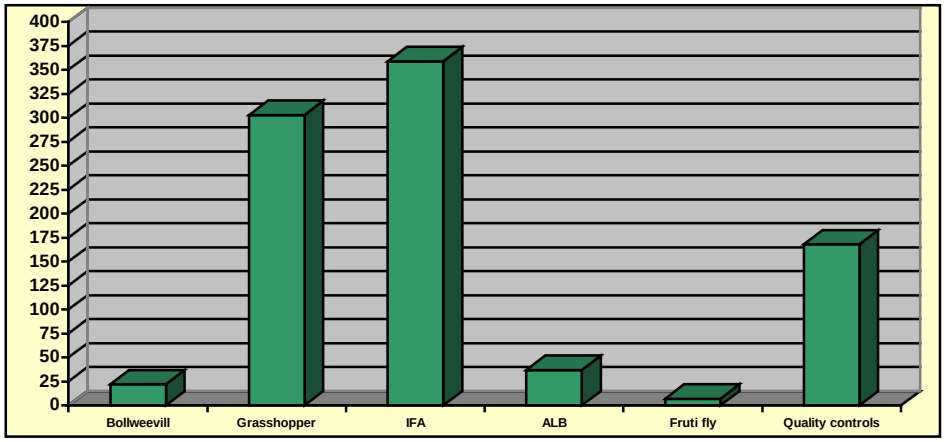
Our strategy was based simply on full commitment to customer support. Our focus was on the following key areas:

- ✓ To provide quality results on submitted field samples within program established time frames.
- ✓ To investigate and provide new scientific options for APHIS consideration in performing Operational and Emergency programs utilizing chemical controls.
- ✓ To actively support APHIS and CPHST projects by providing data and recommendations.

How many program related samples were completed in 2009?

In 2009, CPHST-GL completed work on **926** APHIS program related samples**.** Program related samples are any and all samples analyzed to obtain a valid and high quality determination directly supporting the program. CPHST-GL does not control how many samples are taken, since this is related to program activity.

2009 Sample breakdown by program:



How did we do in delivering sample results back to program leaders?

The laboratory processed all samples as soon as possible, with priority marked samples receiving first preference. Actual delivery time averages across all program samples submitted are listed below.

Program	Program Target Delivery Time (receipt to report)	Actual Average delivery time (receipt to report)
Imported Fire Ant Quarantine (IFA)		
Bulk Density in Soils	21 working days	4.8 working days
Multi-residue in Soils	21 working days	6.7 working days
Boll Weevil Eradication Program (BW)		
Malathion Percent Purity Verification **(ISO certified method)**	3-5 working days	4.2 working days
Asian Long-horned Beetle Program (ALB)		
Imidacloprid in Water	21 working days	5.6 working days
Imidacloprid Percent Purity	21 working days	6.0 working days
Rangeland Grasshopper and Mormon Cricket Suppression Program (RGMC)		
Carbaryl in Dye Cards *First submission of product	21 working days	11.0 working days
Carbaryl Percent in Baits **(ISO certified method)**	21 working days	6.5 working days
Carbaryl tank mix *First submission of product	21 working days	18.0 working days
Dimilin Percent Purity **(ISO certified method)**	21 working days	9.8 working days
Dimilin Formulations (Tank mixes) **(ISO certified method)**	21 working days	9.2 working days
Dimilin in dye Cards	21 working days	7.9 working days
Dimilin in water	21 working days	7.8 working days
Dimilin in vegetation	21 working days	9.2 working days
Fruit-Fly Attractants and Lures (FF)		
FF Methyl eugenol verification analysis	21 working days	4.0 working days
FF Culure verification analysis	21 working days	3.0 working days

***Data source CPHST-GL chemistry Laboratory Information Management System (LIMS).*

How good was our quality?

In 2009**, 98% of all program** samples analyzed were within established CPHST-GL ISO quality guidelines. This means **only 2% of samples failed** to meet the quality expectations of the method applied. All failed samples were either re-analyzed and met the quality objectives of the method applied, or were justified (we explained the issues to the customer, to their satisfaction).

- **ISO 9000 Status:** In 2009 CPHST-GL maintained ISO-9001 certification status.

What other sample work was conducted by CPHST-GL chemistry units in 2009?

In addition to conducting program sample analyses for APHIS chemical control programs, CPHST-GL conducted analysis on **586 samples supporting projects** requested by other APHIS scientists including:

Routine program support work and new Methods Development conducted in 2009:

- ✓ HPLC Imidacloprid analysis methods development and application on honey, bee's and Bee's wax samples. Supporting the Asian Longhorn Beetle Eradication Program (ALB).
- ✓ GC/ECD multi-residue screening studies in soils, methods development. Supporting the Imported Fire Ant Quarantine program (IFA)
- ✓ LC/MS determination of Carbaryl & diflubenzuron, methods development and application on vegetation and soil samples using "QuEeCHers" extraction. Supporting the Rangeland Grasshopper and Mormon Cricket Suppression Program (RGMC)
- ✓ APHIS lure statement of work support, Methods Development for the verification of active ingredients in various lures. (Fruit Fly programs)
- ✓ Methods development and sample analysis for Bifenthrin degradation time study in soils**,** (IFA project support)
- ✓ Methods development and sample analysis for Dursban degradation time study in soils**,** (IFA project support)
- ✓ Methods development and sample analysis for GC/MS based Bifenthrin in burlap and related root ball matrices, (IFA project support)

What are we doing to improve our services to APHIS programs and customers?

- ✓ We provide customers with an annual report at the end of each year, with an accompanying survey to gather their opinions and suggestions.
- ✓ We constantly look for, implement and utilize automated procedures that require less labor, reduce exposure to hazardous materials and reduce hazardous wastes.
- ✓ We are moving toward advanced technical applications using Mass spectral screening technology (GC/MS, LC/MS and ICP/MS). To provide higher quality multi-residue screening options.
- ✓ We actively pursue and investigate simple chemistry based solutions/options for APHIS program leader's consideration for PPQ field applications.

What is the state of our infrastructure?

STAFFING

- As January 1, 2010 we have a staff of 14 (11 scientists and 3 non-scientific support staff).

SCIENTIFIC CORE EQUIPMENT

As January 1, 2010 all of our primary scientific specialized equipment is fully functional including:

- Multiple; Gas Chromatographs (GC) with Flame Photometric Detectors (FPD), Electron Capture Detectors (ECD) and Flame Ionization Detectors (FID).
- Multiple; High Pressure Liquid Chromatographs (HPLC) with UV/Vis and Diode array detectors.
- Gas Chromatograph with Mass Selective detector (GC/MSD quadrupole)
- Liquid Chromatograph with Mass Selective detector (LC/MS ion trap)
- Inductively Coupled Mass Spectrometer (ICP/MS)

GC and LC mass spec systems are nearing the end of their functioning and vendor supported lifecycle.

PROJECT TITLE: IFA-Bifenthrin Degradation in Potting Media Study Annual Report (Chemistry Unit)

TYPE REPORT: Final

LEADER: Connie Ramos

The CPHST-GL laboratory in Gulfport, Mississippi has on-going involvement with the Imported Fire Ant (IFA) study of bifenthrin degradation in nursery soil. The total number of nursery soil samples submitted for 2009 were 172 not including control samples with a total number of 30 (blank composite and 4-LOQ). The technical procedure used to determine bifenthrin pesticide was validated with limit of detection (LOD) of 0.90 ppm and limit of quantification (LOQ) of 3.0 ppm..

Principle: The extraction procedures used are based on a 50 gram mixed nursery soil material. An aliquot is transferred and apportioned into hexane. Bifenthrin extract generated is analyzed by Gas Chromatograph (GC) with Electron Capture Detector (ECD). For soil, an additional sub-sample is taken to determine moisture content which will then be used as a correction factor to calculate the final concentration of bifenthrin.

Process Description

- Sample Custody and Handling
  - A set of nursery soil samples were collected by IFA-unit and submits it to the COC unit for processing. C-O-C unit prepares a packet with sample worksheet, field forms and a technical procedure.

- Sample Preparation
  - Technician or chemist will pick-up the completed packet and nursery soil sample from COC unit to begin weighing sample for extraction procedure.

- Control & Sample Fortification
  - Controls (composite and 4-LOQ) and each soil sample will be fortified with known amount of bifenthrin and process standard (methyl chlorpyrifos) to check its %-recovery.

- Sample Extraction
  - Samples are extracted by adding 70:30 (v/v) Acetonitrile:DI water into the container and capped tightly.
  - Shake sample in a mechanical shaker for 2 hours at low speed.
  - Transfer aliquot extract into separatory funnel, add 5% NaCL solution and Hexane and shake for a minute with venting as needed.
  - Drain extract through funnel plugged with glass wool and NaSO4. Repeat 3 times. Combine all extracts.
  - Extract is ready for GC-ECD analysis.

- Instrumental Analysis (GC-ECD Operating Condition)

Parameters	Specifics
Analytical Instrument	Agilent 6890 GC-ECD
Inlet Mode	Splitless
Inlet Temperature	225 C
Initial Pressure	10.00 psi
Purge Flow	50.0 mL/min.
Purge Time	0.50 min.
Total Flow	65.2 ml/min.
Gas Type	Helium
Primary Column	CLP-1 (30m, 0.53mm id, 0.50um)
Secondary Column	CLP-2 (30m, 0.53mm id, 0.42um)
Oven Temperature Program	150C (1.0min)-9Cmin→300C (10min)
Detector Mode	Constant Flow
Initial Flow	12.5 mL/min
Detector Temperature	300C
Mode	Constant Column + make-up
Combined Flow	60.0 mL/min
Make-up Flow	On
Make-up Gas type	Argon-Methane
Injection Volume	2.0 uL
Syringe Size	10.0 uL
Plunger Speed	Fast

<u>Sample Extract Analysis</u>

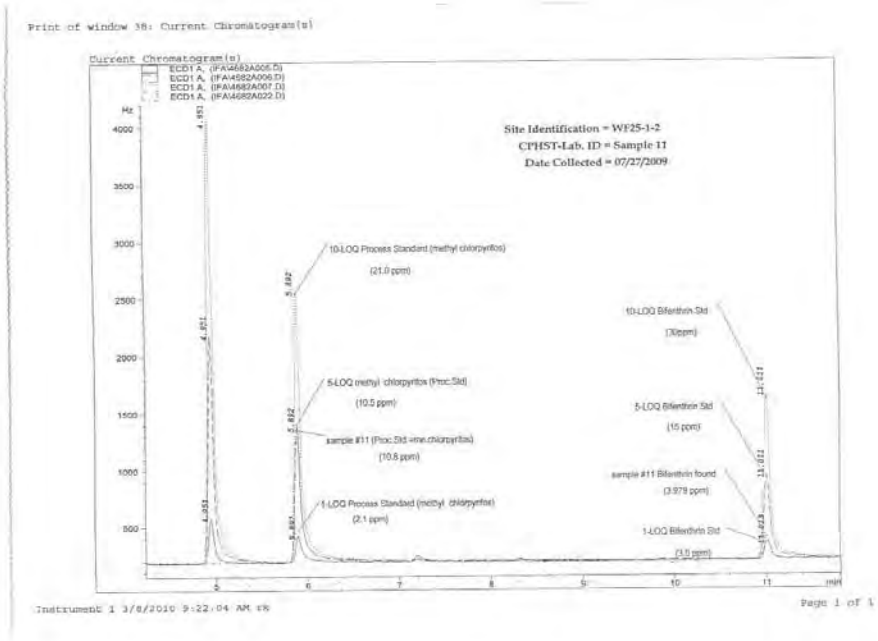
- Fill vials with a set of calibration standard specified in the analytical procedure.
- Fill vials with sample extracts and position them after a set of standards.
- Establish a sequence file w/ corresponding analytical procedure.
- Run system suitability before samples are actually analyzed.
- If suitability "PASSED", the samples are analyzed accordingly as per sequence file.
- The amount of analyte of interest (Bifenthrin) will be automatically calculated as "ppm".
- A chromatographic representation of 1-sample versus 1-set of calibration standard on the last page.

NOTE: Any positive amount found in the samples that is equal or greater than Limit of Quantification (3.0 ppm) must be adjusted using the formula specified on the last page.

Report the results to customer (see IFA Degradation Report for results)

Gas Chromatograph 6890 w/ Electron Capture Detector (ECD)

Gas Chromatograph 6890, monitor (controlled by HP Chem-Station)



Calculations

For Process Standard and 4-LOQ's -

$$\text{Percent Recovered (\%)} = \frac{\text{Amount found (ppm)}}{\text{Amount fortified (ppm)}} \times 100$$

For Targeted Pesticide -------→ See NOTE in text

$$\text{Adjusted amt. bifenthrin found} = \text{Amt. found (ppm)} \times \frac{1}{\text{Dry Weight Factor}} \times \text{dil.factor (if any)}$$

PROJECT TITLE: IFA-Degradation on Burlap Study Annual Report (Chemistry Unit)

TYPE REPORT: Final

LEADER/PARTICIPANTS: Robert Smith and Bill Guyton

The CPHST-Gulfport Laboratory, Chemistry Section has assisted the Imported Fire Ant Section in their study of bifenthrin degradation on burlap after immersion/spraying burlap samples with commercial applications of bifenthrin. The Chemistry section provided data from the analysis of the burlap samples for residual levels of bifenthrin over predetermined time intervals. The samples were analyzed after initial treatment, four weeks, eight weeks and twelve weeks.

Principle: The samples were extracted with acetone. An aliquot was taken from each samples and dried with Na_2SO_4 and filtered through a 0.2µm filter. The extracts were then diluted and analyzed with a gas chromatograph (GC) equipped with a mass selective detector (MSD).

Analytical Process

- Sample Custody
  - Sample sets were submitted to the Chain of Custody section where the samples were stored under frozen conditions until analysis.

- Sample Preparation
  - Each sample was weighed and placed in a 1 liter bottle. A control sample was fortified with a known amount of bifenthrin at this point.
  - Two hundred milliliters of acetone was added to each sample bottle and sealed.
  - The samples were shaken for 2 hours on an orbital platform shaker.
  - An aliquot from each sample was taken from each sample and filtered through a 0.2µm filter filled with ~4 mm of Na_2SO_4 into a culture tube.
  - The extracts were then diluted 1 to 2 with toluene and submitted for CG/MSD analysis.

- Instrumental Analysis
  - The samples were analyzed on an Agilent 6890 GC – MSD
  - A standard spectra tune was performed using perfluorotributylamine (PFTBA).
  - Samples were analyzed using an external standard calibration. A linear calibration curve of concentration vs response was generated and sample unknown concentrations were determined from the generated curve.
  - The analytical conditions are listed in the table below.

GC Parameters
Column HP-5MS 5% Phenyl Methyl Siloxane
dimensions: 30.0 m length x 0.25 mm diameter x 0.25 µm film thickness
flow rate: 1.0 ml/min constant flow
nominal initial pressure: 14.72 psi
carrier gas: helium
Inlet
Mode: pulsed splitless
Initial temp: 210 ° C
pressure: 14.72 psi
pulse pressure: 22.0 psi
pulse time: 0.03 min
purge time: 1.0 min
purge flow: 28.7 ml/min
Oven
initial temp. 175 ° c
initial time: 2.00 min
Ramps:
Rate Final temp Final time
10.00 250 ° C 6.00
25.00 265 ° C 6.00
Run time: 22.1 min
Transfer line 200 ° C
MS conditions
solvent delay 6.00 min
ms source: 230 ° C
ms quad: 156 ° C
low mass: 110.0
high mass 425.0

Discussion:

The fortified control recoveries ranged from 78% to 91% with an average of 85.5% and standard deviation of 5.8%. All samples were analyzed in full scan mode and confirmed via standard spectra comparison and library match. Unknown values ranged from 8.5 parts per million (ppm) to 611 ppm.

Calculations:

$$ppm = \left(\frac{ug\ \det ected}{grams\ sample\ wt} \right) \times\ final\ volume$$

$$\%\ re\cov ery = \left(\frac{ppm\ \ found}{ppm\ added} \right) \times 100$$

```
File        :D:\data\4788-burlap-reinj\007_ctrl-0-2b+spk.D
Operator    :
Acquired    : 10 Mar 2010   9:20     using AcqMethod IFA_BIFENTHRIN_FS.M
Instrument :    Instrument #1
Sample Name: ctrl ck-12-2+130 ppm
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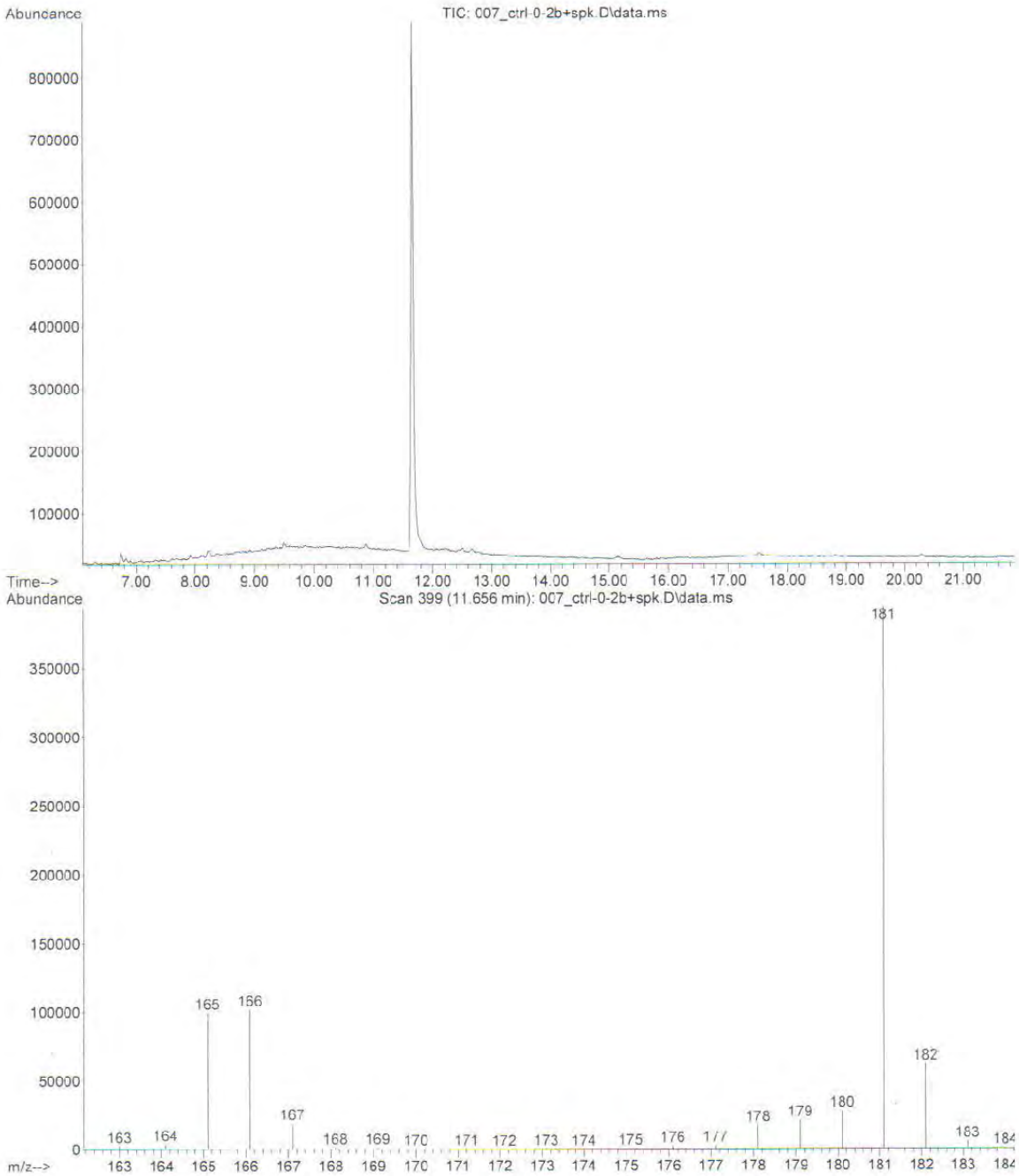


Bifenthrin and peak spectra

PROJECT TITLE: Chemical analysis of Japanese Beetle Lures from Oregon

TYPE REPORT: Interim

LEADERS: Lisa Mosser and Robert Smith

Objective: To verify the percent active ingredients of the Japanese Beetle lures as manufactured by Hercon® (new lures) and Trece® (old lures).

Summary: Analysis of the sex lure component of the two Japanese Beetle lure types received from Oregon was completed in December 2009. We have not completed analysis of the lures for the floral components (3 additional active ingredients) and these will be reported in 2010. Newly produced Hercon® lures were obtained to use in our methods development work and thus we were able to develop an extraction method and verify that method prior to analysis of the Hercon® samples and confirm variability of the sex pheromone component in the new purchased lures compared to the sample lures. We did not obtain newly produced Trece® lures for methods development and thus do not know if variability noted in analyzed samples is comparable to newly purchased lures.

Both lures types theoretically contained 1mg of the sex lure component based on the weight of the lure dispenser at production. However since the Trece® lure dispensers are much heavier than the Hercon® lure dispensers, the % active ingredient (sex pheromone) per lure by weight is different: Hercon® is approximately 0.43% (based on calculations even though the label indicates 0.47%) and Trece® is 0.13%.

Hercon® lures: The mean percent of the sex pheromone component in the 10 Hercon® lures from Oregon was 0.43% (range 0.42-0.47%) with an expected percent of 0.43%. The variability was 3.3%. The mean amount of the sex pheromone component in the Hercon® lures was 1.01mg (range 0.92-1.08mg) with an expected amount of 1.0mg.

Trece® lures: The mean percent of the sex pheromone component in the 10 Trece® lures from Oregon was 0.084% (range 0.05-0.16%) with an expected percent of 0.13%. The variability was 40.2%. The mean amount of the sex pheromone component in the Trece® lures was 0.54mg (range 0.32-1.02mg) with an expected amount of 1.0mg.

Results were reported to Mitchell Nelson, Gary Brown, Roeland Elliston and Andrea Simao

Analytical Procedure:

Extraction of Hercon® and Trece® Lures for the Sex Pheromone of Japonilure

1. Tare a 10 mL centrifuge tube and determine the weight of the sex lure
2. Record the weight of the lure in milligrams on the worksheet
3. Pipette 10 mL of acetone into the tube, cap and vortex for 1 minute
4. Allow samples to sit at room temperature for 2 hours
5. Vortex samples for 1 minute

6. Samples are now ready for GC-FID analysis

Standard Preparation:

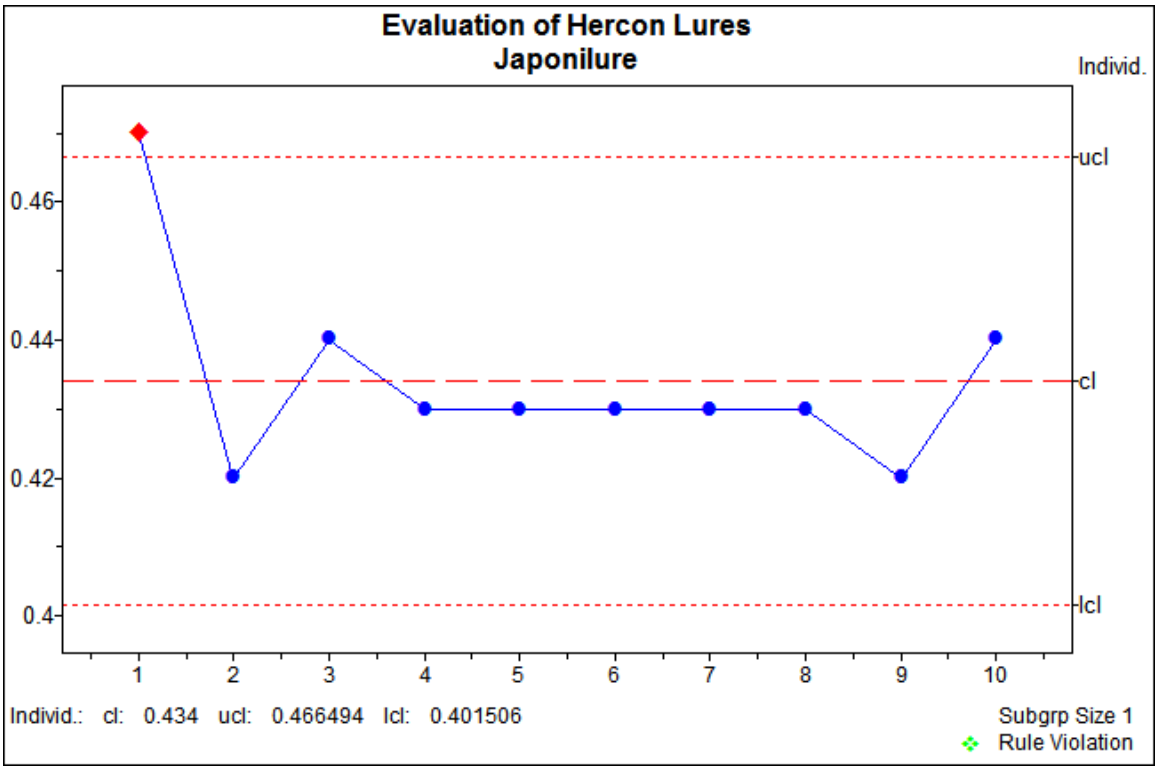
	Level 1	Level 2	Level 3
Japonilure (1000 ng/uL)	20 uL	100 uL	200 uL
Acetone	980 uL	900 uL	800 uL

Note: Standards are prepared in auto sampler vials prior to analysis.

Results:

Hercon® Lures: Expected % Active Ingredient by weight as calculated is 0.43%

	Weight of Lure	Amount of Japonilure	% by Weight
4771-1	230 mg	1.07 mg	0.47 %
4771-2	230 mg	0.96 mg	0.42 %
4771-3	230 mg	1.02 mg	0.44 %
4771-4	250 mg	1.07 mg	0.43 %
4771-5	220 mg	0.94 mg	0.43 %
4771-6	240 mg	1.03 mg	0.43 %
4771-7	240 mg	1.03 mg	0.43 %
4771-8	230 mg	0.98 mg	0.43 %
4771-9	260 mg	1.08 mg	0.42 %
4771-10	210 mg	0.92 mg	0.44 %



Evaluation of Hercon Lures

Japonilure

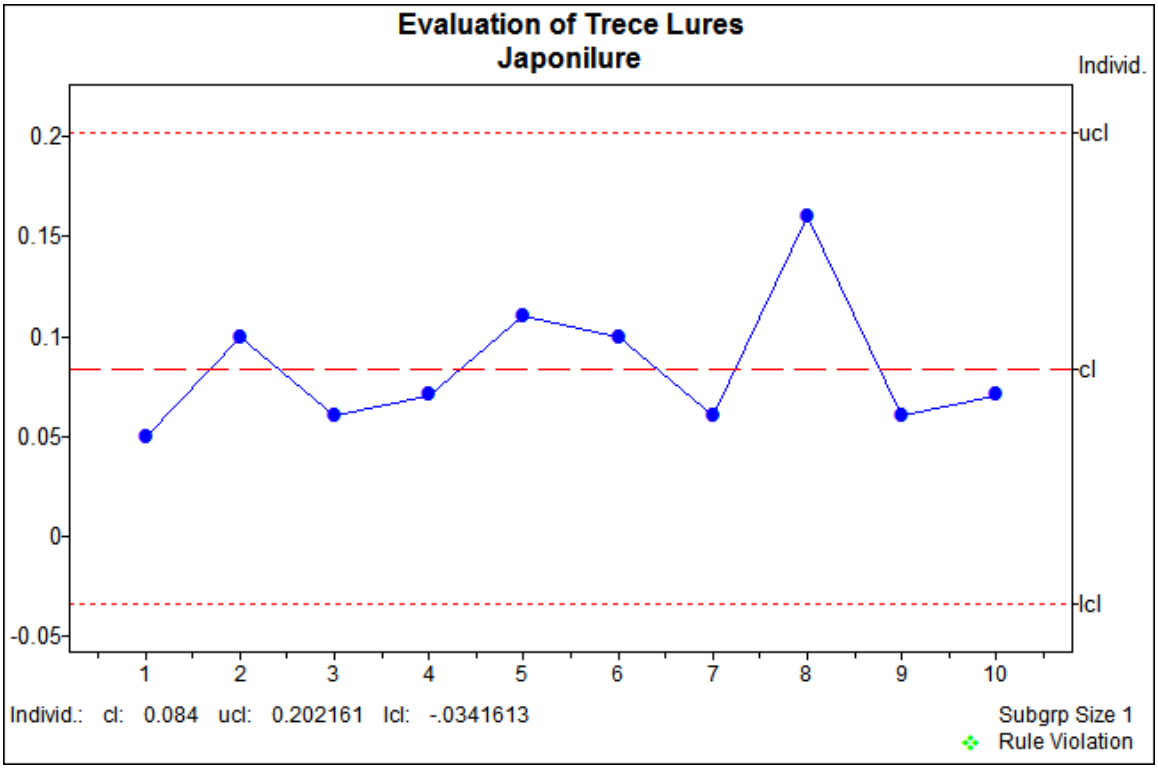
Data Elements (N):	10	Minimum:	0.42
Sum:	4.34	Maximum:	0.47

Arithmetic Mean:	0.434
95% Confidence Interval:	
Upper	0.444229
Lower	0.423771
* Standard Deviation:	0.0142984
* Variance:	0.00020444
Standard Error:	0.0045216
* Coefficient of Variation (%):	3.29456
Harmonic mean:	0.433596
Quadratic mean (RMS):	0.434212
Geometric mean:	0.433795
Third moment about the mean:	4.12800E-06
Fourth moment about the mean:	1.76032E-07
Coefficient of Skewness:	1.65391
Coefficient of Kurtosis:	5.19943

* = Sample Statistics

Trece® Lures: Expected % Active Ingredient by weight as calculated is 0.13%

	Weight of Lure	Amount of Japonilure	% by Weight
4775-1	660 mg	0.32 mg	0.05 %
4775-2	670 mg	0.67 mg	0.10 %
4775-3	650 mg	0.37 mg	0.06 %
4775-4	640 mg	0.42 mg	0.07 %
4775-5	640 mg	0.70 mg	0.11 %
4775-6	620 mg	0.62 mg	0.10 %
4775-7	660 mg	0.42 mg	0.06 %
4775-8	630 mg	1.02 mg	0.16 %
4775-9	660 mg	0.41 mg	0.06 %
4775-10	650 mg	0.48 mg	0.07 %



Evaluation of Trece Lures

Japonilure

Data Elements (N):	10	Minimum:	0.05
Sum:	0.84	Maximum:	0.16

Arithmetic Mean:	0.084
95% Confidence Interval:	
Upper	0.108131
Lower	0.0598692
* Standard Deviation:	0.033731
* Variance:	0.0011378
Standard Error:	0.0106667
* Coefficient of Variation (%):	40.1559
Harmonic mean:	0.0746757
Quadratic mean (RMS):	0.0898888
Geometric mean:	0.0788702
Third moment about the mean:	0.000037848
Fourth moment about the mean:	3.63587E-06
Coefficient of Skewness:	1.15503
Coefficient of Kurtosis:	3.46744

* = Sample Statistics

PROJECT TITLE: Determination of Diflubenzuron in Vegetation

TYPE REPORT: Final

LEADER: Lisa Mosser, Senior Analytical Chemist

OBJECTIVE:
The objective of this project is to develop a cost effective and "green" method for the quantitative determination of diflubenzuron in vegetation.

INTRODUCTION:

In September of 2009, work began on the development of a cost effective and "green" chemical assay for the determination of diflubenzuron in vegetation. Development of this chemical assay was in support of the APHIS Grasshopper Suppression Program. The Grasshopper Program uses the data that is generated from this chemical assay, as a means of fulfilling its commitment to environmental stewardship of its chemical treatment programs. However, in the use of these chemical assays, often the chemical waste generated in determining the amount of the targeted insecticide, has more of a negative impact to the environment than the use of the insecticide itself. Therefore, a chemical assay that shows environmental stewardship was desirable.

Steve Lehotay of the Agricultural Research Service has developed a chemical assay for the "Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Approach for Determining Pesticide Residues" in food matrices. It was the goal of the CPHST-GL to adapt this chemical assay for the determination of diflubenzuron in vegetation. To accomplish this goal, the CPHST-GL Technical Procedure, TPA-17 "Method Validation", was followed.

Results: ATTACHMENT I
Method: ATTACHMENT II

Discussion: Data obtained from the method validation process, shows that the "QuEChERS" method is a chemical assay that can be adapted for the determination of diflubenzuron in vegetation.

Project No._____

TITLE: WIA-TPA17-2 Linearity and Range diflubenzuron

Book No._____

From Page No.___

September 21, 2009

Preparation of Standards

Standard I: $\frac{1\mu l\ (1008\ ng/\mu l)}{1000\mu l} = 1.008\ ng/\mu l$

Standard II: $\frac{5\mu l\ (1008\ ng/\mu l)}{1000\mu l} = 5.04\ ng/\mu l$

Standard III: $\frac{10\mu l\ (1008\ ng/\mu l)}{1000\mu l} = 10.08\ ng/\mu l$

Standard IV: $\frac{20\mu l\ (1008\ ng/\mu l)}{1000\mu l} = 20.16\ ng/\mu l$

Standard V: $\frac{50\mu l\ (1008\ ng/\mu l)}{1000\mu l} = 50.4\ ng/\mu l$

* Recommended
LOD = 0.6 ng/µl
LOQ = 2.0 ng/µl

Document Control Number	WORK INSTRUCTION Analytical and Natural Products Chemistry Laboratory (ANPCL)	Revision Number
WIA-TPA17-2		Original
Effective Date: 05-25-07	Linearity and Range	Page 1 of 1

Discussion:

The Linearity is the ability of the method to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to analyte concentration within a given range. The range is the interval between the upper and lower levels of analyte (inclusive) that have been demonstrated to be determined with precision, accuracy, and linearity using the analytical procedure as written.

Procedure:

1. Establish the range of the analytical procedure to be examined. Generally at the ANPCL the range of the analytical procedure is from the Limit of Quantitation (LOQ) to 10 times LOQ. For purity analysis, the linear range is to be a minimum of 70% - 130% of the test concentration.
2. To demonstrate the linearity across the entire range of an analytical method, prepare five concentrations of the analyte(s) to be examined (see step 1).
3. Analyze each of the concentrations in triplicate.
4. Prepare a regression analysis using Microsoft® Excel (or equivalent software) that includes the following:
   - The variance of the slope of the regression line.
   - The correlation coefficient.
   - The *y* intercept.
   - The residual sum of squares.
   - The range in the same units as the test results obtained by the method.

Document Revision History

Status (Original/Revision/Cancelled)	Document Revision Number	Effective Date	Description
Original	Original	05-25-07	Prepared in accordance to QP-17, Original

Approved by: ANPCL Quality Manager

Signature: *Approved: Signature on File with Original* Date: 5-22-07

Witnessed & Understood by me, Date Filed by Date

Reviewed by

ATTACHMENT I

TITLE WIA-TPA17-5 LOD and LOQ diflubenzuron Project No. _____ Book No. _____ COPY

From Page No. ___

WIA-TPA17-2 "Linearity and Range"
Diflubenzuron
LC/DAD

	ng/uL	Response (Area)
std1	1.008	324.68454
std1	1.008	333.21838
std1	1.008	335.10278
std2	5.04	1334.45496
std2	5.04	1349.30066
std2	5.04	1350.25854
std3	10.08	2084.89307
std3	10.08	2092.63867
std3	10.08	2078.177
std4	20.16	5193.61133
std4	20.16	5237.22021
std4	20.16	5191.24072
std5	50.4	11598.8
std5	50.4	11607.1
std5	50.4	11652.7

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.998005486
R Square	0.99601495
Adjusted R Square	0.995708408
Standard Error	278.1472985
Observations	15

ANOVA

	df	SS	MS	F	Significance F
Regression	1	25137677 9.7	25137677 9.7	3249.1927	5.49788E-17
Residual	13	1005756.956	77365.91969		
Total	14	252392536.7			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	120.9711499	100.4492265	1.204301457	0.2499421	-96.03621021	337.97851	-96.0362102	337.97851
X Variable 1	230.9002154	4.050760861	57.00169013	5.498E-17	222.1490786	239.651352	222.1490786	239.6513522

Document Control Number	WORK INSTRUCTION	Revision Number
WIA-TPA17-5	Analytical and Natural Products Chemistry Laboratory (ANPCL)	Original
Effective Date: 05-25-07	Limit Of Detection and Limit of Quantitation	Page 1 of 1

Discussion:
To determine the Limit of Detection (LOD) and the Limit of Quantitation (LOQ), a technique recommended by the International Conference on Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2B Methodology guideline on analytical method validation will be used (1). The LOD and LOQ will be determined both "in-matrix" and "out-of-matrix". From the statistical data generated, the analyst will determine the LOD and LOQ.

Procedure:
1. Prepare five standards over the desired linear range of the analytical method to be examined. These are the "out-of-matrix" standards.
2. Prepare five final extracts as directed by the analytical procedure.
3. With micoliter amounts, spike the extracts, each at a different level over the desired linear range of the analytical method. **(Note: prepare a spiking solution at a high enough level in order to use micoliter amounts to achieve levels around the five standards prepared in step 1).** Theses are the "in-matrix" standards.
4. Inject the five standards as well as the five spiked extracts in triplicate on the desired instrument.
5. Using Microsoft Excel® (or equivalent software), perform a regression on data acquired from both the "in-matrix" and "out-of-matrix" standards.
6. From the statistics, determine the LOD and LOQ for the analyte(s) of interest using the following equation:

$$LOD = 3.3\ (\sigma/S) \qquad LOQ = 10\ (\sigma/S)$$

Where σ = the standard deviation of the y intercept and S = the slope of the line.

Because the LOD is an estimated value, the LOD will need to be validated by an independent analysis of a suitable number of samples known to be near or prepared at the detection limit. (See WIA-TPA17-4: "Accuracy")

Document Revision History

Status (Original/Revision/Cancelled)	Document Revision Number	Effective Date	Description
Original	Original	05-25-07	Prepared in accordance to QP-17, Original

Approved by: ANPCL Quality Manager

Signature: *Approved; Signature on File with Original* Date: 5-22-07

To Page No

Witnessed & Understood by me | Date | Invented by | Date

Recorded by

ATTACHMENT I

WIA-TPA17-5 "Limit of Detection and Limit of Quantitation"
Diflubenzuron
LC/DAD

Out of matrix

	ng/uL	Response (Area)
std1	1.008	324.68454
std1	1.008	333.21838
std1	1.008	335.10278
std2	5.04	1334.45496
std2	5.04	1349.30066
std2	5.04	1350.25854
std3	10.08	2084.89307
std3	10.08	2092.63867
std3	10.08	2078.177
std4	20.16	5193.61133
std4	20.16	5237.22021
std4	20.16	5191.24072
std5	50.4	11698.8
std5	50.4	11607.1
std5	50.4	11652.7

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.998005486
R Square	0.99601495
Adjusted R Square	0.995708408
Standard Error	278.1472985
Observations	15

ANOVA

	df	SS	MS	F	Significance F
Regression	1	251376779.7	251376779.7	3249.1927	5.49788E-17
Residual	13	1005756.956	77365.91969		
Total	14	252382536.7			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	120.9711499	100.4492265	1.204301457	0.2499421	-96.03621021	337.97851	-96.0362102	337.97851
X Variable 1	230.9002154	4.050760861	57.00169013	5.498E-17	222.1490786	239.6513522	222.1490786	239.6513522

LOD = 3.3(100.4492265/230.9002154) = 1.4 ng/µl
LOQ = 10.0(100.4492265/230.9002154) = 4.4 ng/µl

WIA-TPA17-5 "Limit of Detection and Limit of Quantitation"
Diflubenzuron
LC/DAD

in matrix

	ng/uL	Response (Area)
std1	1.008	319.92657
std1	1.008	321.92892
std1	1.008	320.3515
std2	5.04	1508.88074
std2	5.04	1547.88037
std2	5.04	1489.46387
std3	10.08	2942.27148
std3	10.08	3134.61694
std3	10.08	2934.32422
std4	20.16	4808.64453
std4	20.16	4739.52393
std4	20.16	4781.35889
std5	50.4	13387
std5	50.4	13459.3
std5	50.4	13403.7

maybe out of linear range of DAD

SUMMARY OUTPUT

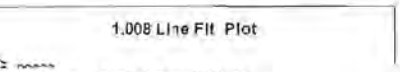
Regression Statistics	
Multiple R	0.9977169
R Square	0.995439013
Adjusted R Square	0.995058931
Standard Error	340.3453118
Observations	14

ANOVA

	df	SS	MS	F	Significance F
Regression	1	303372786.5	303372786.5	2619.01	2.03477E-15
Residual	12	1390019.175	115834.9313		
Total	13	304762805.7			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	70.08402848	131.2571027	0.533944656	0.603131	-215.9006305	356.0686874	-215.9006305	356.0686874
1.008	261.7120198	5.11393415	51.17625923	2.03E-15	250.5697144	272.8543251	250.5697144	272.8543251

LOD = 3.3(131.2571027/261.7120198) = 1.7 ng/µl
LOQ = 10.0(131.2571027/261.7120198) = 5.0 ng/µl



WIA-TPA17-5 "Limit of Detection and Limit of Quantitation"
Diflubenzuron
LC/DAD

in matrix

	ng/uL	Response (Area)
std1	1.008	319.92657
std1	1.008	321.92892
std1	1.008	320.3515
std2	5.04	1508.88074
std2	5.04	1547.88037
std2	5.04	1489.46387
std3	10.08	2942.27148
std3	10.08	3134.61694
std3	10.08	2934.32422

SUMMARY OUTPUT

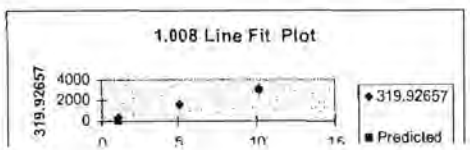
Regression Statistics	
Multiple R	0.99846851
R Square	0.99693936
Adjusted R Square	0.99642925
Standard Error	67.7056751
Observations	8

ANOVA

	df	SS	MS	F	Significance F
Regression	1	8958960.709	8958961	1954.373	8.96988E-09
Residual	6	27504.35063	4584.058		
Total	7	8986465.059			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	24.1309916	46.27633757	0.521454	0.62073	-89.10312698	137.3651102	-89.10312698	137.3651102
1.008	295.649065	6.687638152	44.20829	8.97E-09	279.2850041	312.0131261	279.2850041	312.0131261

LOD = 3.3(46.27633757/295.649065) = 0.5 ng/µl
LOQ = 10.0(46.27633757/295.649065) = 1.6 ng/µl



ATTACHMENT I
Page 1 of 1
Project No.
Book No.
TITLE

TITLE WIA-TPA17-4 Accuracy - dipheniazuron mini validation

Project No. _____ Book No. _____

COPY

From Page No. ___

Document Control Number	WORK INSTRUCTION Analytical and Natural Products Chemistry Laboratory (ANPCL)	Revision Number
WIA-TPA17-4		Original
Effective Date: 05-25-07	Accuracy	Page 1 of 2

Discussion:

Accuracy is the measure of exactness of an analytical method or the closeness of agreement between the conventional, true value or an accepted reference value and the value found. It is measured as the percentage of analyte recovered from spiked samples. At ANPCL, there is a procedure to measure accuracy for residual chromatographic methods and a procedure to measure accuracy for quantitative methods used for formulations and insect pheromone samples.

Procedure:

Accuracy for residual chromatographic methods:

1. The Quality Management Section will prepare a group of blank samples for accuracy determination. The group will be assigned a number and the individual samples will be assigned laboratory accession numbers. The group will consist of a solvent blank, a matrix blank, and at least 3 replicates of the matrix blank spiked at a minimum of five levels (LOD, LOQ, 5LOQ, 7LOQ, and 10LOQ).
2. The Quality Management Section will prepare the necessary fortification standards and spike the matrix blanks.
3. Samples are to be extracted and analyzed using the analytical procedure that is being validated.
4. The percentage recovered is to be calculated by the analyst and results entered into the Laboratory Information Management (LIMs) system.
5. The data is to be sent to the Quality Management Section for statistical interpretation.
6. Upon completion of the statistical analysis, a copy of the report is to be sent to the lead scientist.

Accuracy for quantitative chromatographic methods used for formulations and insect pheromone samples:

1. The Quality Management Section will prepare a group that consists of one solvent blank, three replicates of an 80% sample, three replicates of a 90% sample, three replicates of a 100% sample, and three replicates of a 130% sample. The group is to be assigned a number and the samples will be assignedlaboratory accession numbers.
2. The Quality Management Section is to prepare enough of a typical sample at 80% of the expected concentration for 12 analyses. The unit is also responsible for preparing a spiking solution to bring the concentration to 90%, 100%, and 130%.
3. The analyst assigned the accuracy determination is to prepare three 80% samples, three 80%samples spiked to bring the concentration to 90%, three 80% samples spiked to bring the concentration to 100%, and three 80% samples spiked to bring the concentration to 130%. The analytical procedure that is being examined is to be followed in preparing these samples.
4. The samples are to be analyzed against a calibration curve that covers the range of 70% - 130% of the target concentration.
5. The percentage recovered is to be calculated by the analyst and results entered into the LIMs system.
6. The data is to be sent to the Quality Management Section for statistical interpretation.
7. Upon completion of the statistical analysis, a copy of the report is to be sent to the lead scientist.

Trial I

LOQ = 2.0 ppm for QuEChERS

$\frac{1000\mu l\ (20\ ng/\mu l)}{10{,}000\ mg} = 2.0\ ppm$ (.

$\frac{5000\mu l\ (20\ ng/\mu l)}{10{,}000\ mg} = 10.0\ ppm$ (5.

$\frac{10{,}000\mu l\ (20\ ng/\mu l)}{10{,}000\ mg} = 20.0\ ppm$ (1

$\frac{60\mu l\ (100.8\ ng/\mu l)}{3000\ mg} = 2.016\ ppm$ (2

$\frac{300\mu l\ (100.8\ ng/\mu l)}{3000\ mg} = 10.08\ ppm$ (5.

$\frac{600\mu l\ (100.8\ ng/\mu l)}{3000\ mg} = 20.16\ ppm$ (.

Recommended Std Preparation

$\frac{2ml\ (100\ ng/\mu l)}{100\ ml\ (methanol)} = 2.0\ ng/\mu l$

$\frac{10\ ml\ (100\ ng/\mu l)}{100\ ml\ (methanol)} = 10.0\ ng/\mu l$

$\frac{20\ ml\ (100\ ng/\mu l)}{100\ ml\ (methanol)} = 20.0\ ng/\mu l$

$\frac{1.0\ ml\ (100\ ng/\mu l)}{100\ ml} = 1.0$

$\frac{5.0\ ml\ (100\ ng/\mu l)}{100\ ml} = 5.0$

$\frac{10.0\ ml\ (100\ ng/\mu l)}{100\ ml} = 10.0$

delete LM
10-2-09

To Page No

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ATTACHMENT I

TITLE WIA-TPA17-4 Trial I Book No.

From Page No.

Mini Validation data Results

	% Rec
20QA	42.6
20QB	103
20QC	145
520QA	21.9
520QB	27.4
520QC	27.5
1020QA	33.1
1020QB	32.6
1020QC	31.0

} possible interference of neighboring pk

October 6, 2009 Method Breakdown

I. Investigation of spiking samples pre hydration vs post hydra

pre % recovered = 22% post % recovered 22%

II. Investigation of the addition of 10 minute sonication of pre/po hydration after the addition of MgSO4 Extration tube - prior to centrifugation

pre-sonic % recovered = 19% post-sonic % recovered = 29%

October 7, 2009 Method Breakdown cont'd

III. Investigation of efficiency of initial extraction

- Extract from ~~mini validation~~ previous day that had been spiked
  $\frac{600\,\mu l\ (100.8\ ng/\mu l)}{15{,}000\,\mu l} = 4.032\ ng/\mu l$ in ACN
- 1ml diluted to 4ml in H20 = 1.008 ng/µl 25:75 ACN:H20
- Standard prepared @ 1.008 ng/µl 25:75 ACN:H20 used for comparison

  % recovered = 85%

To Page No.

Witnessed & Understood by me, Date Invented by Date

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ATTACHMENT I

TITLE Method Breakdown cont'd Project No. Book No. COPY

From Page No.

IV. Investigation of dSPE
Extract from B3 taken through dSPE and compared to
% Recovered - Ø

V. Investigation of non-acidified ACN used for extraction solu through dSPE
Pre SPE % Recovered - 74%
Post SPE % Recovered - Ø

VI. Investigation of role of carbon in dSPE
dSPE prepared w/out Carbon 75 mg PSA + 450 mg MgSO4 for a 3mL aliquot.
VI_A % Recovered - 69%
VI_B % Recovered - 71%

Conclusion: dSPE w/out Carbon - redo mini validation w/ this change

* for 6mL aliquot 900 mg MgSO4 + 150 mg PSA

delete LM 10-16-09

To Page No.

Witnessed & Understood by me, | Date | Invented by | Date
Recorded by

ATTACHMENT I

TITLE WIA-TPA17-4 Accuracy - diflubenzuron - mixl (Trial 11) Project No. _____ Book No. _____

From Page No. ___

Document Control Number	WORK INSTRUCTION	Revision Number
WIA-TPA17-4	Analytical and Natural Products Chemistry Laboratory (ANPCL)	Original
Effective Date: 05-25-07	Accuracy	Page 1 of 2

Discussion:

Accuracy is the measure of exactness of an analytical method or the closeness of agreement between the conventional, true value or an accepted reference value and the value found. It is measured as the percentage of analyte recovered from spiked samples. At ANPCL, there is a procedure to measure accuracy for residual chromatographic methods and a procedure to measure accuracy for quantitative methods used for formulations and insect pheromone samples.

Procedure:

Accuracy for residual chromatographic methods:

1. The Quality Management Section will prepare a group of blank samples for accuracy determination. The group will be assigned a number and the individual samples will be assigned laboratory accession numbers. The group will consist of a solvent blank, a matrix blank, and at least 3 replicates of the matrix blank spiked at a minimum of five levels (LOD, LOQ, 5LOQ, 7LOQ, and 10LOQ).
2. The Quality Management Section will prepare the necessary fortification standards and spike the matrix blanks.
3. Samples are to be extracted and analyzed using the analytical procedure that is being validated.
4. The percentage recovered is to be calculated by the analyst and results entered into the Laboratory Information Management (LIMs) system.
5. The data is to be sent to the Quality Management Section for statistical interpretation.
6. Upon completion of the statistical analysis, a copy of the report is to be sent to the lead scientist.

Accuracy for quantitative chromatographic methods used for formulations and insect pheromone samples:

1. The Quality Management Section will prepare a group that consists of one solvent blank, three replicates of an 80% sample, three replicates of a 90% sample, three replicates of a 100% sample, and three replicates of a 130% sample. The group is to be assigned a number and the samples will be assignedlaboratory accession numbers.
2. The Quality Management Section is to prepare enough of a typical sample at 80% of the expected concentration for 12 analyses. The unit is also responsible for preparing a spiking solution to bring the concentration to 90%, 100%, and 130%.
3. The analyst assigned the accuracy determination is to prepare three 80% samples, three 80%samples spiked to bring the concentration to 90%, three 80% samples spiked to bring the concentration to 100%, and three 80% samples spiked to bring the concentration to 130%. The analytical procedure that is being examined is to be followed in preparing these samples.
4. The samples are to be analyzed against a calibration curve that covers the range of 70% - 130% of the target concentration.
5. The percentage recovered is to be calculated by the analyst and results entered into the LIMs system.
6. The data is to be sent to the Quality Management Section for statistical interpretation.
7. Upon completion of the statistical analysis, a copy of the report is to be sent to the lead scientist.

Extraction of Diflubenzuron from Vegetation (QuEChERS)

1. 1. Allow sample to come to room temperature.
2. Pour contents of foil bag into deep pan and mix with about 50/50 sample/crushed or powdered dry ice (ice can be prepared using Grist Mill).
3. Allow sample to set for 20-30 seconds. Use more ice if the sample is wet.
4. Place ice/sample mixture in Robot coupe blender and blend into a fine powder.
5. Dump contents of blender onto foil.
6. Allow ice to sublime and transfer sample back to the foil bag.
7. Samples are ready for extraction
8. Store sample in cryogenic freezer if extraction is not planned within the next two days.
9. Clean laboratory equipment between samples.
10. Transfer 3 g of homogenized vegetation sample to a 50 mL PTFE centrifuge tube.
11. Add 15 mL of deionized water, 15 mL 1% acetic acid in acetonitrile plus contents of dispersive SPE (dSPE) Mg_2SO_4 Extraction Tube (55234-U, Sigma-Aldrich)
12. Shake vigorously for 1 minute and centrifuge at 4000rpm for 5 minutes
13. Transfer 6 mL of acetonitrile layer to dispersive SPE (dSPE) PSA CleanUp tube (55228-U, Sigma-Aldrich) and vortex for 1 minute.
14. Centrifuge at 4000rpm for 5 minutes.
15. Transfer 2 mL into a 15mL centrifuge tube and evap to dryness and reconstitute to 1 mL with methanol
16. Sample is now ready for analysis. (0.5 mg/mL sample size)

Matrix	Vegetation	Analyte	DIMILIN	Group #:

General Worksheet
QA Revision Nov-01

Storage Spike

Process Standard

Fortification Standard diflubenzuron @ 100.8 ng/µL

SFP _____ 2LQ _____ 4LQ _____

Fortifier L. Hasser Date 10-13-09

Observer God

Sample Number	Description	Observations
	SC	
	COMP	
	LOQ-A	60µL (3.016 ppm)
	LOQ-B	
	LOQ-C	
	5 LOQ-A	300µL (10.08 ppm)
	5 LOQ-B	
	5 LOQ-C	
	10 LOQ-A	600µL (20.16 ppm)
	10 LOQ-B	
	10 LOQ-C	

	$\bar{x}$	SD	%CV
10Q	92%	13	14
5LOQ	77%	1.5	1.9
10LOQ	80%	0.58	0.73

Witnessed & Understood by me

ATTACHMENT I

ATTACHMENT II

CPHST-GL Chemistry Laboratory

3505 25th Avenue
Gulfport, MS 39501

Approved by: CPHST-GL Quality Manager

Signature: _____ Date: _____

Approved by: CPHST-GL Technical Manager

Signature: _____ Date: _____

Approved by: CPHST-GL Laboratory Director

Signature: _____ Date: _____

Training Provided by:

Signature: _____ Date: _____

Document Revision History

Status (Original/Revision/Cancelled)	Document Revision Number	Effective Date	Description
Original	Original		Newly developed Technical Procedure

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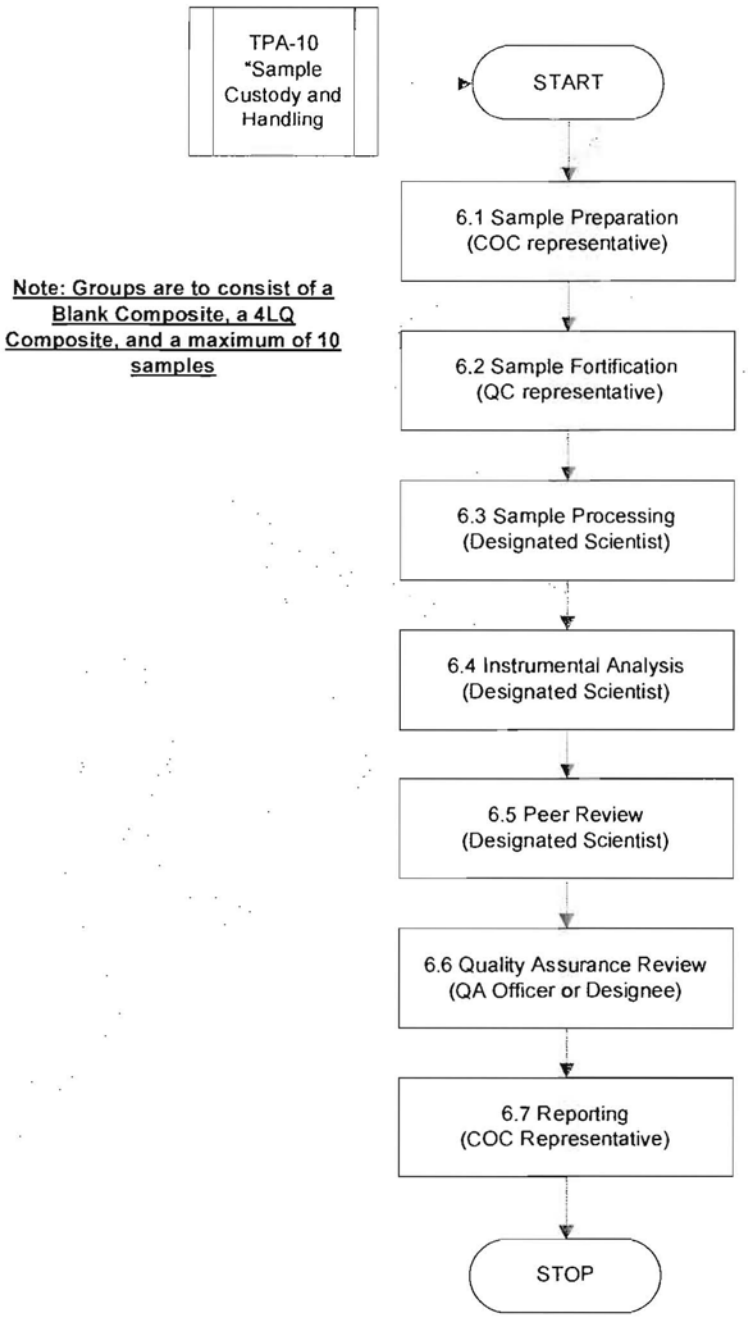
Process Map



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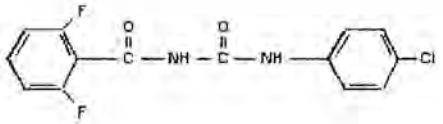
1.0 Scope

This method is a QuEChERS extraction that is applicable to the quantitative determination of Diflubenzuron in vegetation. The method has a validated limit of detection (LOD) of 0.6 ppm and a limit of quantitation (LOQ) of 2.0 ppm and has been validated over a concentration range of 2.0 ppm – 20.0 ppm. This method generates approximately 12 mL of organic waste per sample.



Diflubenzuron

2.0 Principle

A 3 gram sample is extracted with acetonitrile. The extract is cleaned-up by Dispersive Solid Phase Extraction (dSPE), a 2 mL aliquot is concentrated to dryness and reconstituted to 1.0 mL of 0.025% TFA methanol. The resulting solution is analyzed by High Pressure Liquid Chromatography (HPLC) with UV detection at 254 nm wavelength.

3.0 Safety Precautions

Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Disposal of reagents and solvents must be in compliance with local, state, and federal laws and regulations.

Volatile and/or flammable organic solvents such as acetone and hexane must be used in well-ventilated areas away from ignition sources.

Personal protective equipment and eye protection should be worn when handling all reagents.

4.0 Measurement of Uncertainty

The measurement of uncertainty for this method is to be determined.

5.0 Method Deviations

The "Control of Nonconforming Work" (QP-4), "Corrective Action" (QP-5), and "Preventive Action" (QP-6) processes will be used to document deviations of this method.

6.0 Process Description

6.1 Sample Preparation

6.1.1 Apparatus and Reagents

- Analytical Balance ($\pm$ 0.1 gram)
- 50 mL disposable centrifuge tube (VWR cat#: 21008-242)
- Robot Coupe Blender
- Dry Ice
- Foil

6.1.2 Mixing and Preparation of Samples

- Place entire sample and dry ice (50/50) into Robot Coupe Blender and blend into a fine powder
- Dump contents of blender onto foil
- Allow ice to sublime and transfer back into the original sample bag
- Store sample in cryogenic freezer if extraction is not planned within the next two days
- Samples are ready for extraction
- Clean laboratory equipment between samples

We have performed the operations stated in this section.
Initial_____ **Date** _____

Custody of Samples and paperwork is relinquished to the designated scientist for processing.

6.1.3 Weighing of Sub-Samples

- Verify balance suitability and record appropriate documentation in balance logbook.
- Place a 50 mL disposable centrifuge tube into a beaker and tare the balance.
- Transfer 3 grams of sample into the 50 mL centrifuge tube.
- Record activity on sample worksheet.
- Notify QC for sample fortification.

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.2 Sample Fortification

6.2.1 Apparatus and Reagents

- 2mL, 4mL volumetric pipettes
- 100 mL volumetric flasks
- Brown bottles
- Acetonitrile
- 0.025% TFA in Methanol (1vial of TFA in 4L Methanol)
- 1 mg/mL Diflubenzuron stock solution in acetonitrile
- 100 ng/µL Diflubenzuron intermediate standard in acetonitrile

6.2.2 Preparation of Fortification Solution

- Using a Class A volumetric pipette, pipette 2 mL of a 1 mg/mL stock of diflubenzuron into a 100 mL volumetric flask and dilute to the mark with acetonitrile. Concentration should be approximately 20 ng/uL diflubenzuron.
- Transfer solution into the appropriate size brown bottle and label.
- Record activity in QC logbook.

6.2.3 Preparation of Standard Check

- Using a Class A volumetric pipette, pipette 4 mL of a 100 ng/uL diflubenzuron solution into a 100 mL volumetric flask and dilute to the mark with 0.025% TFA in Methanol. Concentration should be approximately 4 ng/uL diflubenzuron.
- Transfer solution into the appropriate size brown bottle and label.
- Record activity in QC logbook.

6.2.4 Determination of Percent Recovery of Targeted Pesticides

- To the 4LQ composite, spike 1 mL of the fortification solution.
- Record activity on the sample worksheet and fortification logbook.

$$\text{Amount Spiked of Fortification Standard (ppm)} = \frac{1000\ \mu L \times 20\ ng/uL}{3000\ mg\ sample}$$

Amount Spiked of Fortification Standard (ppm) = 6.7 ppm Diflubenzuron

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.3 Sample Processing

6.3.1 Apparatus and Reagents

- 1% (v/v) Acetic Acid in Acetonitrile (10mL acetic acid + 1000mL Acetonitrile)
- HPLC Water
- Magnesium Sulfate Extraction tube (Sigma-Aldrich, 55234-U)
- Dispersive SPE (dSPE) PSA CleanUp Tube (Sigma-Aldrich, 55228-U)
- 15 mL disposable centrifuge tubes
- 0.025% TFA in Methanol (1vial of TFA in 4L Methanol)
- 1mL, 2mL, 6mL pipettes
- N-Evap
- Vortex
- Centrifuge

6.3.2 Sample Extraction

1. To each sample add 15mL of HPLC water.
2. Cap vortex for 1 minute
3. To the sample, add 15mL of 1% (v/v) acetic acid in acetonitrile.
4. Add contents of magnesium sulfate tube (Sigma-Aldrich, 55234-U) to the sample.
5. Cap and shake vigorously for 1 minute.
6. Centrifuge sample at 4000 rpm for 5 minutes.

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.3.3 Sample Clean-up

1. Transfer 6mL of extract to dispersive SPE (dSPE) PSA CleanUp tube (Sigma-Aldrich, 55228-U) and vortex for 1 minute.
2. Centrifuge at 4000 rpm for 5 minutes.
3. Transfer 2mL of cleaned extract into a 15mL disposable centrifuge tube.
4. Concentrate to dryness on a N-Evap with a water bath set at 40°C
5. Using a pipette, pipette 1mL of 0.025% TFA in Methanol into the 15mL centrifuge tube and vortex.

We have performed the operations stated in this section.
Initial_____ **Date** _____

Custody of Samples is relinquished to COC and paperwork along with extracts is relinquished to the designated scientist for analysis.

6.4 Instrumental Analysis

6.4.1 Apparatus and Reagents

- HP1100 Liquid Chromatograph equipped with a diode array detector
- Pre-column, Betasil C18, 10 x 3 mm
- Analytical column, Thermo Hypersil Green ENV 150 x 4.6 mm, 3um
- 0.025% TFA in HPLC water (1 vial of TFA in 4L of water)
- 0.025% TFA in acetonitrile (1 vial of TFA in 4L acetonitrile)
- 1, 5, 8,and 10 mL pipettes
- 100mL volumetric flasks
- Brown bottle and labels
- Autosampler vials
- Acetonitrile
- 0.025% TFA in methanol

6.4.2 Typical Operating Conditions

Parameter			
Instrumentation	Agilent 1100 Series HPLC with UV detection		
Column	Hypersil Green Env 150mm x 4.6mm 3um		
Pre Column	Betasil C18 10 x 3 mm		
Column Temperature	35°C		
Mobile Phase	Solvent A : 0.025%TFA in water		
	Solvent B: 0.025%TFA in acetonitrile		
Gradient	**Time**	**%Solv A**	**%Solv B**
	0.00	70.0	30.0
	30.00	0.00	100.0
Post Time	3.00 min		
Flow Rate	1.00 mL/min		
Injection Volume	100.00 uL		
UV Wavelength	254 nm		
Run Time	30.00 min		

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.4.3 Preparation of Bench Standards

- Using Class A volumetric pipettes, pipette 8 mL of a 1 mg/mL stock of diflubenzuron into a 100 mL volumetric flask and dilute to the mark with acetonitrile. Tranfer to the appropriate size brown bottle and label. Concentration should be approximately 80 ng/uL diflubenzuron. Shelf life is to be 6 months. This solution is the bench intermediate. Prep Date: _____

Working Standard	Initial Concentration	Amount added	Solvent Amount	Solvent	Final Concentration	Target Concentration	Prep Date
LOQ Std Diflubenzuron	_____ (ng/µL)	**1mL Bench Intermediate**	**99mL**	**0.025% TFA In Methanol**	_____ (ng/µL)	**0.8 ng/uL**	
5LOQ Std Diflubenzuron	_____ (ng/µL)	**5mL Bench Intermediate**	**95mL**	**0.025% TFA In Methanol**	_____ (ng/µL)	**4 ng/uL**	
10LOQ Std Diflubenzuron	_____ (ng/µL)	**10mL Bench Intmediatee**	**90mL**	**0.025% TFA In Methanol**	_____ (ng/µL)	**8 ng/uL**	

$$\text{ppm} = \frac{\text{ng/µL (Bench Standard)}}{\text{0.4 mg/µL (Final Sample Size)}}$$

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.4.4 System Suitability

Criteria	Expected	Analyst Review
Peak Height @ LOQ of 1st 3 standards On reporting column only	≤ 5% RSD for requested compounds	□ Y □ N
Standard curve at 1,5, and 10 X LOQ On reporting column only	r = 0.995 for requested compounds	□ Y □ N
Standard check analyte requested On reporting column only	(+/-) 4% of expected value as noted On reporting column	□ Y □ N

- Suitability is not met unless all criteria have been checked "Yes"
- If suitability is not met follow TPA-13, "*Maintenance*" until suitability has been achieved
- Do not proceed to section 6.4.5 until suitability has been achieved

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.4.5 Analysis

- A standard of the analyst's choice is to be placed after every 5 samples and at the end of the analysis
- Samples found to be outside of the calibration curve, are to be diluted appropriately and the dilution factor applied to the final amount.
- Calculation results are to be recorded on the corresponding chromatogram and/or spreadsheet
- Confirmations of values at or equal to LOQ are done by LC/MS.

6.4.6 Calculations

- To determine percent recovered of Process Standard and Fortified Targeted Pesticides use the following calculation:

$$\text{Percent Recovered} = \frac{\text{Amount found (ppm)}}{\text{Amount Spiked (ppm)}} \times 100\%$$

- To determine the amount of targeted pesticide, use the following calculation:

Amount of Targeted Pesticide (ppm) = Amount Found (ppm) x Dilution Factor

LIMS Entry (Consult Team Leader if Network is Down)

- Double click on "Labworks Enterprise ES" icon on designated computer desktop
- Enter in the appropriate fields, laboratory assigned user ID and password
- Click OK
- From Labworks desktop, double click on "Results"
- Enter the group number and click OK
- Click on "All Samples"
- In the appropriate field enter the results found in 2 significant figures and click "Save"

- Click "Exit"
- Click "Exit" from the Labworks desktop to complete data entry

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.4.8 Control Charting (Consult Team Leader if Network is Down)

- Double click on the "NWA Quality Analyst 5.1" icon on the designated laboratory computer
- Click on the folder identified as "Open an Existing Data Set"
- Under "Drives", click the drop down arrow and select "l:[\\msgus 10\LAB300]
- Under "Directories", select "Control Charts" and double click to open
- Select the appropriate year's charts and double click to open
- Under "Filename", select the file name that corresponds to the appropriate technical procedure and double click to open
- Complete the required fields and click on the disk icon to save
- Exit NWA by clicking on the "X" in the upper right hand corner

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.4.9 Data Package Assembly <u>(Each Data Package is to include the following in this order)</u>

- A copy of the sample worksheet and accompanying field forms
- A copy of the appropriate technical procedure
- A printout of the sequence file used for analysis
- A printout of the calibration table for the reporting column and confirmation column if applicable
- A printout of the extended statistics of the peak height for the targeted pesticides
- A printout of chromatograms supporting system suitability (3 LOQ standard injections, linearity curve, and standard check)
- A printout of chromatograms supporting sample analysis
- A printout of calculation spreadsheet if applicable
- A printout of report if applicable

We have performed the operations stated in this section.
Initial_____ **Date** _____

Peer Review

Criteria	Expected	Peer Review
System Suitability Met	See Section 6.4.4 for criteria	□ Y □ N
4LQ Composite	TBD	□ Y □ N
Analyst Standard at end of sample set	Within 10% of expected value	□ Y □ N _____ %
Confirmation @ LOQ and above	By LC/MS	□ Y □ N
Calculations & Dilutions	Completed & Checked	□ Y □ N All Calculations

LIMS Entry	Units and value as determined	□ Y □ N All
NWA Entry	Completed	□ Y □ N All
Failed samples	Identified and submitted to QM and Deputy for Re-check consideration	□ Y □ N All

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.5 Quality Assurance Review

Criteria	Expected	QA Review
System Suitability Met	See Section 6.4.4 for criteria	□ Y □ N
4LQ Composite	TBD	□ Y □ N
Analyst Standard at end of sample set	Within 10% of expected value	□ Y □ N _____ %
Confirmation @ LOQ and above	By LC/MS	□ Y □ N
Calculations & Dilutions	Completed & Checked	□ Y □ N _____ %
LIMS Entry	Units and value as determined	□ Y □ N All
NWA Entry	Completed	□ Y □ N All
Failed samples	Identified and submitted to COC for Re-check	□ Y □ N All

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.6 Reporting

- After QA acceptance of data, the data package is to be forwarded to the COC representative for reporting
- The COC representative transmits data to the appropriate designee, see WIA-TPA-10-1, "*Grouping, Data Package Assembly & LIMS Entry and Report*"
- After transmission, the data package is forwarded for archiving, see WIA-QP2-1, "*Maintenance of ANPCL Records*"

We have performed the operations stated in this section
Initial_____ Date_____

7 Records

Data Package

PROJECT TITLE: Method Validation Results for Imidacloprid in Honey Group # 4801

TYPE REPORT: Final

LEADER/PARTICIPANTS: Lisa Mosser, Senior Analytical Chemist
Joseph Dawson, Physical Science Technician
Marsha Lowe, Physical Science Technician
Gene Bohannon, Physical Science Technician
Tim Bond, Physical Science Technician
Bich Tran, Chemist

OBJECTIVE: The objective is to validate TPA-406, "Determination of Imidacloprid in Honey" for general laboratory use.

METHOD:

- WIA-TPA17-4, "Accuracy" (A 3 x 3 mini-validation was conducted due to expected sample submission from the Asian Longhorn Beetle Program)
- TPA-406, "Determination of Imidacloprid in Honey" (Draft Attached)

RESULTS:

Sample Description	Actual Amt (ppm)	Expected Amt (ppm)	% Recovered
LOQ-A	0.2195	0.2030	108%
LOQ-B	0.2072	0.2030	102%
LOQ-C	0.2321	0.2030	114%
5LOQ-A	1.0183	1.0149	100%
5LOQ-B	1.0260	1.0149	101%
5LOQ-C	1.0545	1.0149	104%
10LOQ-A	2.0533	2.0298	101%
10LOQ-B	2.0817	2.0298	103%
10LOQ-C	2.0764	2.0298	102%

DISCUSSION:

Statistical analysis of the data generated from group 4801, revealed a mean of 104%, a standard deviation of 4.5, and a percent coefficient variation of 4.3%. The positive bias of the recoveries is most likely due to an adjacent peak located within 0.1 minutes of the peak of interest, resulting in skewed integration. Based on the statistical analysis, it is recommended that the data be deemed acceptable and a recovery acceptance criteria be set at 70% - 120% until more control points can be gathered.

CPHST-GL Chemistry Laboratory

3505 25th Avenue
Gulfport, MS 39501

Approved by: CPHST-GL Quality Manager

Signature: _____ Date: _____

Approved by: CPHST-GL Technical Manager

Signature: _____ Date: _____

Approved by: CPHST-GL Laboratory Director

Signature: _____ Date: _____

Training Provided by:

Signature: _____ Date: _____

Document Revision History

Status (Original/Revision/Cancelled)	Document Revision Number	Effective Date	Description
Original	Original		Newly developed Technical Procedure

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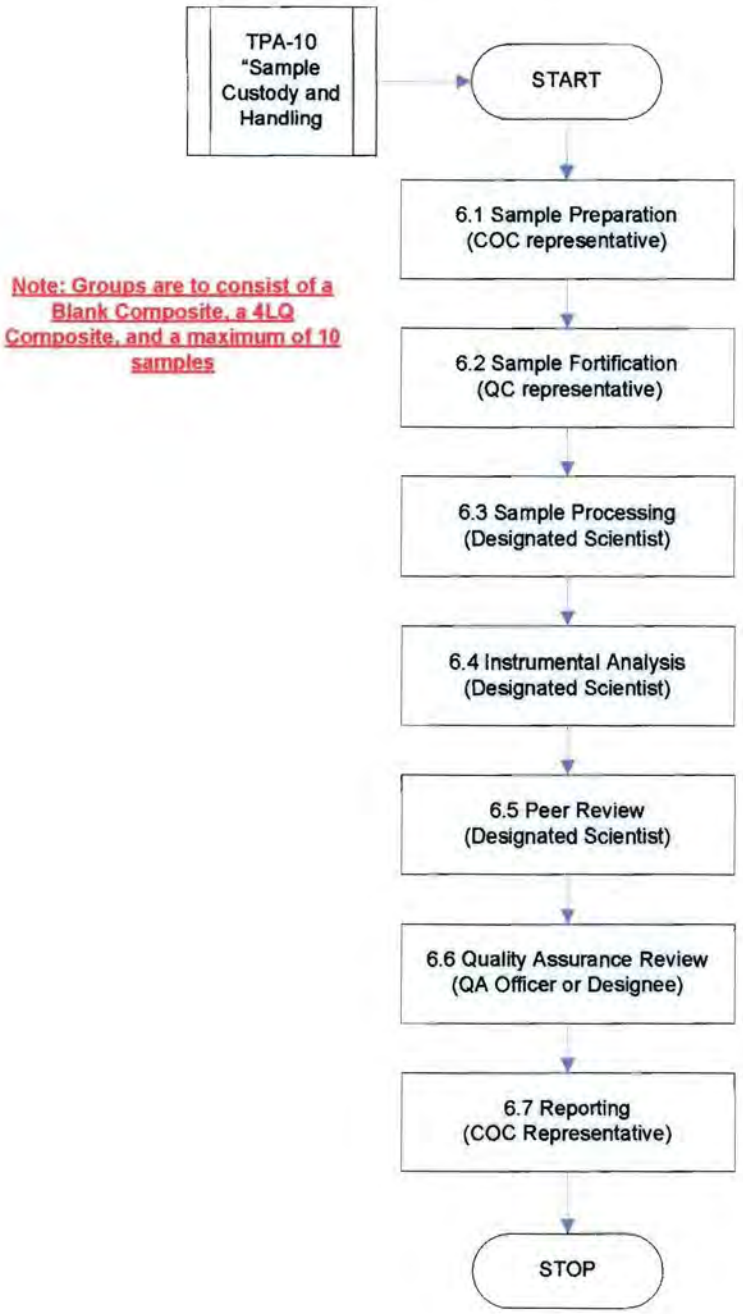
Process Map



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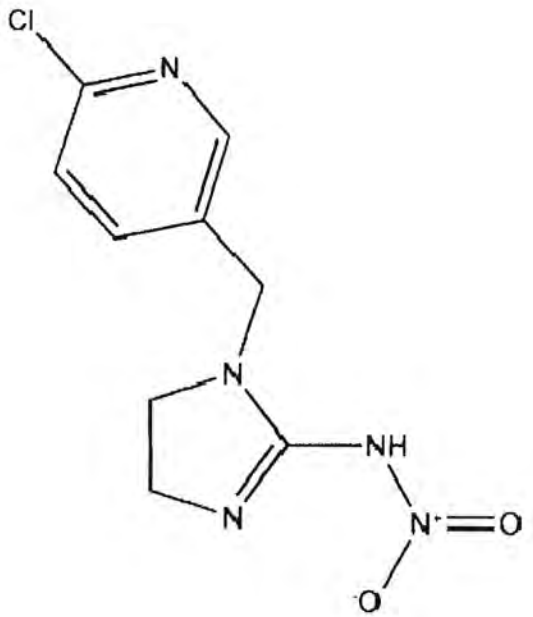
1.0 Scope

This method is a modified QuEChERS extraction that is applicable to the quantitative determination of Imidacloprid in Honey. The method has a validated limit of detection (LOD) of 0.06 ppm and a limit of quantitation (LOQ) of 0.2 ppm and has been validated over a concentration range of 0.2 ppm – 2.0 ppm. This method generates approximately 12 mL of organic waste per sample.



Imidacloprid

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2.0 Principle

A 15.0 gram sample is extracted with acetonitrile. The extract is cleaned-up by Dispersive Solid Phase Extraction (dSPE), concentrated to dryness and reconstituted in 3.0 mL of 0.025% trifluroacetic acid (TFA) in methanol. The resulting solution is analyzed by High Pressure Liquid Chromatography (HPLC) with UV detection at 272 nm wavelength.

3.0 Safety Precautions

Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Disposal of reagents and solvents must be in compliance with local, state, and federal laws and regulations.

Volatile and/or flammable organic solvents such as acetone and hexane must be used in well-ventilated areas away from ignition sources.

Personal protective equipment and eye protection should be worn when handling all reagents.

4.0 Measurement of Uncertainty

The measurement of uncertainty for this method is to be determined.

5.0 Method Deviations

The "Control of Nonconforming Work" (QP-4), "Corrective Action" (QP-5), and "Preventive Action" (QP-6) processes will be used to document deviations of this method.

6.0 Process Description

6.1 Sample Preparation

6.1.1 Apparatus and Reagents

- Analytical Balance ($\pm$ 0.1 gram)
- 50 mL disposable centrifuge tube (VWR cat#: 21008-242)

6.1.2 Mixing and Preparation of Samples

- Samples are manually mixed by inverting the field container several times.

We have performed the operations stated in this section.
Initial_____ **Date** _____

Custody of Samples and paperwork is relinquished to the designated scientist for processing.

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6.1.3 Weighing of Sub-Samples

- Verify balance suitability and record appropriate documentation in balance logbook.
- Place a 50 mL disposable centrifuge tube into a beaker and tare the balance.
- Transfer 15 grams of sample into the 50 mL centrifuge tube.
- Record activity on sample worksheet.
- Notify QC for sample fortification.

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.2 Sample Fortification

6.2.1 Apparatus and Reagents

- 10 mLvolumetric pipette
- Eppendorph pipette capable of accurately delivering 120uL
- 100 mL volumetric flasks
- Brown bottles
- Acetonitrile
- 0.025% trifluroacetic acid (TFA) in 75:25 Water:Methanol
- 1 mg/mL Imidacloprid stock solution in acetonitrile

6.2.2 Preparation of Fortification Solution

- Using a Class A volumetric pipette, pipette 1 mL of a 1 mg/mL stock of imidacloprid into a 100 mL volumetric flask and dilute to the mark with acetonitrile. Concentration should be approximately 10 ng/uL imidacloprid.
- Transfer solution into the appropriate size brown bottle and label.
- Record activity in QC logbook.

6.2.3 Preparation of Standard Check

- Using a Class A volumetric pipette, pipette 1 mL of a 100 ng/uL imidacloprid solution into a 100 mL volumetric flask and dilute to the mark with 0.025% trifluroacetic acid (TFA) in 75:25 Water: Methanol. Concentration should be approximately 1 ng/uL imidacloprid.
- Transfer solution into the appropriate size brown bottle and label.
- Record activity in QC logbook.

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6.2.4 Determination of Percent Recovery of Targeted Pesticides

- To the 4LQ composite, spike 1 mL of the fortification solution.
- Record activity on the sample worksheet and fortification logbook.

$$\text{Amount Spiked of Fortification Standard (ppm)} = \frac{1000\ \mu L \times 10\ ng/\mu L\ \text{Imidacloprid}}{15000\ mg\ \text{sample}}$$

Amount Spiked of Fortification Standard (ppm) = 0.67 ppm Imidacloprid

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.3 Sample Processing

6.3.1 Apparatus and Reagents

- 1% (v/v) Acetic Acid in Acetonitrile:10mL acetic acid + 1000mL acetonitrile
- Dispersive SPE (dSPE) PSA CleanUp Tube (Sigma-Aldrich, 55228-U)
- 15 mL disposable centrifuge tubes
- 0.025% trifluroacetic acid (TFA) in 75:25 Water:Methanol: 1 vial of TFA + 3000 mL Water + 1000 mL Methanol
- 3mL, 6mL, 10mL, 15mL pipettes
- N-Evap
- Vortex
- Centrifuge

6.3.2 Sample Extraction

1. To each sample add 15mL of 1% acetic acid in acetonitrile.
2. Cap and shake vigorously for 1 minute.
3. Centrifuge samples at 5000 rpm for 5 minutes.

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.3.3 Sample Clean-up

- Transfer 6mL of extract to dispersive SPE (dSPE) PSA CleanUp tube (Sigma-Aldrich, 55228-U) and vortex for 1 minute.
- Centrifuge at 5000 rpm for 5 minutes.
- Transfer 3mL of cleaned extract into a 15mL disposable centrifuge tube.
- Concentrate to dryness on a N-Evap with a water bath set at 50°C
- Using a pipette, pipette 3mL of 0.025% trifluroacetic acid (TFA) in 75:25 Water:Methanol into the 15mL centrifuge tube and vortex.
- Store samples in refrigerator until analysis

We have performed the operations stated in this section.
Initial_____ **Date** _____

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Custody of Samples is relinquished to COC and paperwork along with extracts is relinquished to the designated scientist for analysis.

6.4 Instrumental Analysis

6.4.1 Apparatus and Reagents

- HP1100 Liquid Chromatograph equipped with a diode array detector
- Pre-column, Betasil C18, 10 x 3 mm
- Analytical column, Thermo Hypersil Green ENV 150 x 4.6 mm, 3um
- 0.025% TFA in HPLC water: 1 vial of TFA + 4000mL of HPLC water
- 0.025% TFA in acetonitrile: 1 vial of TFA + 4000mL of acetonitrile
- 1, 2, 5,and 10 mL pipettes
- 100mL volumetric flasks
- Brown bottle and labels
- Autosampler vials
- Acetonitrile
- 0.025% TFA in 75:25 water : methanol: 1 vial of TFA + 3000 mL Water + 1000 mL Methanol

6.4.2 Typical Operating Conditions

Parameter			
Instrumentation	Agilent 1100 Series HPLC with UV detection		
Column	Hypersil Green Env 150mm x 4.6mm 3um		
Pre Column	Betasil C18 10 x 3 mm		
Column Temperature	35°C		
Mobile Phase	Solvent A : 0.025%TFA in water		
	Solvent B: 0.025%TFA in acetonitrile		
Gradient	**Time**	**%Solv A**	**%Solv B**
	0.00	90.0	10.0
	30.00	0.00	100.0
Post Time	5.00 min		
Flow Rate	1.00 mL/min		
Injection Volume	60.00 uL		
UV Wavelength	272 nm		
Run Time	30.00 min		

We have performed the operations stated in this section.
Initial_____ **Date** _____

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6.4.3 Preparation of Bench Standards

- Using Class A volumetric pipettes, pipette 2 mL of a 1 mg/mL stock of imidacloprid into a 100 mL volumetric flask and dilute to the mark with acetonitrile. Tranfer to the appropriate size brown bottle and label. Concentration should be approximately 20 ng/uL imadicloprid. Shelf life is to be 6 months. This solution is the bench intermediate. Prep Date: _____

Working Standard	Initial Concentration	Amount added	Solvent Amount	Solvent	Final Concentration	Target Concentration	Prep Date
LOQ Std Imadacloprid	_____ (ng/µL)	1mL Bench Intermediate	99mL	0.025% TFA In 75:25 Water : Methanol	_____ (ng/µL)	0.2 ng/uL	
5LOQ Std Imadacloprid	_____ (ng/µL)	5mL Bench Intermediate	95mL	0.025% TFA In 75:25 Water : Methanol	_____ (ng/µL)	1 ng/uL	
10LOQ Std Imadacloprid	_____ (ng/µL)	10mL Bench Intmediatee	90mL	0.025% TFA In 75:25 Water : Methanol	_____ (ng/µL)	2 ng/uL	

$$\text{ppm} = \frac{\text{ng/µL (Bench Standard)}}{\text{1 mg/µL (Final Sample Size)}}$$

We have performed the operations stated in this section.

Initial_____ **Date** _____

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6.4.4 System Suitability

Criteria	Expected	Analyst Review
Peak Height @ LOQ of 1st 3 standards On reporting column only	≤ 5% RSD for requested compounds	□ Y □ N
Standard curve at 1,5, and 10 X LOQ On reporting column only	r = 0.995 for requested compounds	□ Y □ N
Standard check analyte requested On reporting column only	(+/-) 4% of expected value as noted On reporting column	□ Y □ N

- Suitability is not met unless all criteria have been checked "Yes"
- If suitability is not met follow TPA-13, "*Maintenance*" until suitability has been achieved
- Do not proceed to section 6.4.5 until suitability has been achieved

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.4.5 Analysis

- A standard of the analyst's choice is to be placed after every 5 samples and at the end of the analysis
- Samples found to be outside of the calibration curve, are to be diluted appropriately and the dilution factor applied to the final amount.
- Calculation results are to be recorded on the corresponding chromatogram and/or spreadsheet
- Confirmations of values at or equal to LOQ are done by LC/MS.

6.4.6 Calculations

- To determine percent recovered of Process Standard and Fortified Targeted Pesticides use the following calculation:

$$\text{Percent Recovered} = \frac{\text{Amount found (ppm)}}{\text{Amount Spiked (ppm)}} \times 100\%$$

- To determine the amount of targeted pesticide, use the following calculation:

$$\text{Amount of Targeted Pesticide (ppm)} = \text{Amount Found (ppm)} \times \text{Dilution Factor}$$

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6.4.7 LIMS Entry (Consult Team Leader if Network is Down)

- Double click on "Labworks Enterprise ES" icon on designated computer desktop
- Enter in the appropriate fields, laboratory assigned user ID and password
- Click OK
- From Labworks desktop, double click on "Results"
- Enter the group number and click OK
- Click on "All Samples"
- In the appropriate field enter the results found in 2 significant figures and click "Save"
- Click "Exit"
- Click "Exit" from the Labworks desktop to complete data entry

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.4.8 Control Charting (Consult Team Leader if Network is Down)

- Double click on the "NWA Quality Analyst 5.1" icon on the designated laboratory computer
- Click on the folder identified as "Open an Existing Data Set"
- Under "Drives", click the drop down arrow and select "I:[\\msgus 10\LAB300]
- Under "Directories", select "Control Charts" and double click to open
- Select the appropriate year's charts and double click to open
- Under "Filename", select the file name that corresponds to the appropriate technical procedure and double click to open
- Complete the required fields and click on the disk icon to save
- Exit NWA by clicking on the "X" in the upper right hand corner

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.4.9 Data Package Assembly (Each Data Package is to include the following in this order)

- A copy of the sample worksheet and accompanying field forms
- A copy of the appropriate technical procedure
- A printout of the sequence file used for analysis
- A printout of the calibration table for the reporting column and confirmation column if applicable
- A printout of the extended statistics of the peak height for the targeted pesticides
- A printout of chromatograms supporting system suitability (3 LOQ standard injections, linearity curve, and standard check)
- A printout of chromatograms supporting sample analysis
- A printout of calculation spreadsheet if applicable
- A printout of report if applicable

We have performed the operations stated in this section.
Initial_____ **Date** _____

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6.5 Peer Review

Criteria	Expected	Peer Review
System Suitability Met	See Section 6.4.4 for criteria	□ Y □ N
4LQ Composite	TBD	□ Y □ N
Analyst Standard at end of sample set	Within 10% of expected value	□ Y □ N _____ %
Confirmation @ LOQ and above	By LC/MS	□ Y □ N
Calculations & Dilutions	Completed & Checked	□ Y □ N All Calculations
LIMS Entry	Units and value as determined	□ Y □ N All
NWA Entry	Completed	□ Y □ N All
Failed samples	Identified and submitted to QM and Deputy for Re-check consideration	□ Y □ N All

We have performed the operations stated in this section.
Initial_____ **Date** _____

6.6 Quality Assurance Review

Criteria	Expected	QA Review
System Suitability Met	See Section 6.4.4 for criteria	□ Y □ N
4LQ Composite	TBD	□ Y □ N
Analyst Standard at end of sample set	Within 10% of expected value	□ Y □ N _____ %
Confirmation @ LOQ and above	By LC/MS	□ Y □ N
Calculations & Dilutions	Completed & Checked	□ Y □ N _____ %
LIMS Entry	Units and value as determined	□ Y □ N All
NWA Entry	Completed	□ Y □ N All
Failed samples	Identified and submitted to COC for Re-check	□ Y □ N All

We have performed the operations stated in this section.
Initial_____ **Date** _____

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6.7 Reporting

- After QA acceptance of data, the data package is to be forwarded to the COC representative for reporting
- The COC representative transmits data to the appropriate designee, see WIA-TPA-10-1, "*Grouping, Data Package Assembly & LIMS Entry and Report*"
- After transmission, the data package is forwarded for archiving, see WIA-QP2-1, "*Maintenance of ANPCL Records*"

We have performed the operations stated in this section
Initial_____ Date_____

7 Records

Data Package

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