2010 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

U.S. DEPARTMENT OF AGRICULTURE

IMPORTED FIRE ANT SECTION

ANNE-MARIE CALLCOTT	Supervisory Entomologist/Laboratory Director
CRAIG HINTON	Biological Science Technician
LEE McANALLY	Agriculturalist
XIKUI WEI	Entomologist

ANALYTICAL CHEMISTRY SECTION

ROBERT D. SMITH	Supervisory Chemist
GENE BOHANNON	Physical Science Technician
TIM BOND	Physical Science Technician
JAMES BRADLEY	Laboratory Worker
MARY COLLINS	Administrative Support Assistant
JOE DAWSON	Physical Science Technician
MARIE DUBRA	Physical Science Technician
BILL GUYTON	Chemist
RICHARD KING	Chemist
MARSHA LOWE	Physical Science Technician
BARBARA MOFFETT	Warehouse Clerk
LISA MOSSER	Chemist
CONNIE RAMOS	Chemist
BICH TRAN	Chemist

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

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

Compiled and Edited by:

Anne-Marie A. Callcott

April 2010

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.

TABLE OF CONTENTS

IMPORTED FIRE ANT SECTION

QUARANTINE TREATMENTS FOR CONTAINERIZED NURSERY STOCK

PROJECT NO	*TITLE*	*PAGE*
A9F01	Residual Activity of Various Insecticides used as a Containerized Drench Treatment……..	1

QUARANTINE TREATMENTS FOR FIELD GROWN NURSERY STOCK

PROJECT NO	*TITLE*	*PAGE*
Status Report	Summary of Flip Drench Trials for Post-Harvest Balled-and-Burlapped Nursery Stock 2004-2009…….	3
A1F04	Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Spring and Fall 2009 in Tennessee……	12
A1F04	Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Gulfport MS, 2009……..	18
A1F04	Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Fall 2010 in Tennessee…….	24
A1F04	Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Gulfport MS, Fall 2010……	28
A1F04	Alternative Immersion Treatments for Balled-and-Burlapped Nursery Stock for use in the IFA Quarantine, Tennessee Spring And Fall 2009……	32
A1F04	Alternative Immersion Treatments for Balled-and-Burlapped Nursery Stock for use in the IFA Quarantine, Tennessee Spring 2010……..……	39

PROJECT NO	*TITLE*	*PAGE*
A1F04	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 Queen Infestation, 2009	43
A1F04	Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Individual Tree Drench Treatment using 5-gallon Bucket in Mississippi, Fall 2010	52

QUARANTINE TREATMENTS FOR GRASS SOD

PROJECT NO	*TITLE*	*PAGE*
Umbrella	Efficacy of New Candidates as Grass Sod Treatments; Mississippi, Spring and Fall 2009	59
Umbrella	Efficacy of New Candidates as Grass Sod Treatments; Mississippi, Spring and Fall 2010	65

BIOLOGICAL CONTROL AND BIODIVERSITY

PROJECT NO	*TITLE*	*PAGE*
A1F01	Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing and Release Project, 2010 (*Pseudacteon tricuspis, P. curvatus, P. obtusus*)	71

MISCELLANEOUS

PROJECT NO	*TITLE*	*PAGE*
	2010 Summary of Imported Fire Ant Samples Submitted to CPHST-Gulfport Laboratory for Chemical Analysis or Bulk Density Determination: Routine, Potential Violation and Blitz Samples	76
Appendix I	Protocol for Bioassay of Insecticide Treated Potting Media/Soil With Alate IFA Females	78

ANAYLTICAL CHEMISTRY SECTION

PROJECT NO	*TITLE*	*PAGE*
	Overview of Analytical Chemistry Routine and Program Support in 2010	81
	Impact of Surfactant on Diflubenzuron Tank Mixes	86
	Determination of Emamectin Benzoate in Leaves	88
	Identification of Signature Compounds found in Rutaceae Bonsai	90
	Determination of Active Ingredient in Hercon® BW Xtra Lure	98
	Determination of LBAM Pheromome Blend Content in Three Lure Types: Hercon DISRUPT BIO-TIE® LBAM, ISCA SPLAT, and ISOMATE-LBAM PLUS	99

Page left blank intentionally

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 September 21, 2010 untreated MAFES media (3:1:1 pine bark: sphagnum peat moss: sand – bulk density = 700 lb/cu yd), and Windmill media (partially decomposed pine bark – bulk density = 500 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. Eighteen pots were drenched for each treatment in each media type. Check pots were drenched with an equal volume of plain water. The pots were then weathered outdoors under simulated nursery conditions. An overhead irrigation system supplied ca. 1-1½ inches water per week. 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

Formulation Tested	Rates of Application (ppm)
Scimitar SC	10
Bifenthrin EC	10
Bifenthrin F	10
Tempo SC	10, 15, 25

RESULTS:

The results are summarized in table 2. Through 6 months, Scimitar was 100% effective in both media types. Bifenthrin formulations were also 100% effective through 6 months in the MAFES media, and through 3 months in the Windmill media. Tempo at 10 ppm was 95-100 % effective in MAFES media and 75-100% effective in the Windmill media. The 15 ppm rate was 95-100% effective in MAFES media and 100% effective in Windmill media. The 25 ppm rate was 100% effective in both media.

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

Media Type	Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment at 14 days exposure					
			1	2	3	4	5	6
MAFES	Scimitar SC	10	100	100	100	100	100	100
	Bifenthrin EC	10	100	100	100	100	100	100
	Bifenthrin F	10	100	100	100	100	100	100
	Tempo SC	10	95	100	100	100	95	95
		15	95	100	100	100	100	100
		25	100	100	100	100	100	100
		Check	15	5	15	5	10	5
Windmill	Scimitar SC	10	100	100	100	100	100	100
	Bifenthrin EC	10	100	100	100	70	95	80
	Bifenthrin F	10	100	100	100	85	85	50
	Tempo SC	10	100	80	90	90	80	75
		15	100	100	100	100	100	100
		25	100	100	100	100	100	100
		Check	15	5	5	5	5	0

CPHST PIC NO: A1F04

PROJECT TITLE: Summary of Flip Drench Trials for Post-Harvest Balled-and-Burlapped Nursery Stock 2004-2009

Type Report: Status Report

LEADER/PARTICIPANTS: Xikui Wei

INTRODUCTION:

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

Similar to fire ant quarantine treatment requirement in B&B, the current treatment for Japanese beetle (*Poppillia japonica* Newman), another soil dwelling pests of regulatory concern to tree growers in Tennessee and other northern area of the fire ant quarantine area, 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.

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 bioassay results for the same chemicals at equal or lower rates, when applied by immersion, were consistent, thus indicating insufficiency in either application or the mode of testing for the drench applied treatments. Drench trials conducted in fall 2003 and spring 2004 determined that doubling the volume of applied solution failed to eliminate inconsistent results.

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

the number of days required to complete a drench. The 2004 fall drench strongly suggested that rotating root balls during treatment, regardless of frequency, improved the consistency of bioassay results and could potentially cut the number of days spent applying drenches from three down to one.

This report summarized the flip drench trials conducted in Mississippi and Tennessee from 2004 – 2009. Many people have worked on these projects; among them was Shannon James (USDA-APHIS-PPQ-CPHST Gulfport resigned) who was the primary leader for B&B drench project before 2007. Some of these trials were conducted in conjunction with testing of insecticides against Japanese beetle. The JB testing portion of these trials were planned and conducted by TSU-NRC, McMinnville, TN (Jason Oliver as primary leader) and the USDA-ARS Horticultural Insects Research Laboratory in Wooster, OH, and they report the details and results for that portion of these trials. Not all results or insecticides tested are presented here, but those which show promise or are of interest to the growing community are presented.

MATERIALS AND METHODS:

Specifics can be found in previous annual reports in the individual project trial reports. Root balls were obtained from various nurseries in both Mississippi and Tennessee for testing and ranged in size from 12-inch to 24-inch diameter. Insecticides were tested at rates listed on Table 1 with special mixing procedures described in the *rate* section below. Root balls were drenched according to various handling methods. After drenching root balls were then stored outdoors for aging. Usually irrigation was set up to simulate nursery storage conditions. At specific intervals, soil samples were collected using a soil corer. Samples were taken either from the middle/core of the root ball, or from the surface/top of the root ball (also see notes below for changes on sampling method). There were generally 5 replicates (number of root balls) per treatment in each trial, and soil samples were generally collected at 0.5, 1, 2, 4, and 6 months after treatment, although there was some variation in sampling. Testing was initiated in both fall and spring months. Details on chemicals used, mixing rate, plant handling, and sampling method are provided below.

Chemicals: Non-flip drench trials conducted before fall 2004 indicated that several of the insecticides tested did not show promising results against IFA at the rates tested. These included acephate, carbaryl, and imidachloprid. Therefore, insecticides used in the flip trials were focused on those chemicals or formulations that show promising for IFA quarantine treatment. Chlorpyrifos was always included in the trials as a chemical standard because it is still the only insecticide approved for IFA quarantine treatment for field-grown nursery stock. In recent years (2008-09), tank mix of more than one insecticide (bifenthrin + carbaryl; bifenthrin + trichlorfon) or a combined formulation with bifenthrin as a main ingredient (Allectus: imidacloprid + bifenthrin) have been explored in TN as an effort on enhancing control effectiveness in the flip drench method (Table 1).

Rate: When we initially started the flip application trials, in order to compare the effects of various drench methods, we decided that each treated root ball should receive the same amount of chemical no matter how we apply drenches to the root balls. To achieve this, we adjusted the amount of chemical in the drench solution so that by the conclusion of the final drench, all

treated root balls within the same chemical and rate group would have received equal amount of that chemical. As a result, the chemical solution used for the 1F1 application regime (total of 2 drenches) was two times the concentration for 2F2 drench method (total of 4 drenches), even though both application methods used the same treatment rate of a chemical. Likewise, the concentration in solution for 3F3 or 6 NF was even lower compared with that of 2F2 because it requires a total of 6 drenches to complete instead of 4 for the 2F2 method. In another words, although the volume of solution applied increased as the number of days drenched increased, the amount of chemical in the solution was adjusted so that within a single chemical group, regardless of the number of drench days, each plant was exposed to the same total amount of pesticide by the conclusion of its final drench. This reasoning was perfectly sound and was necessary to begin with, but this also has caused confusion even among researchers not to mention nursery growers. From fall 2007 in MS and from fall 2009 in TN, this rate calculation method was replaced by the "true" rate, that is, no matter how many drenches a plant receives, concentration of chemical solution in each drench remains the same. Obviously, by the end of drench treatments, trees in 2F2 regime will have received twice as much chemical as trees in the 1F1 regime. Besides eliminating rate confusion, this method was to determine, at a pre-set rate of a chemical, how many times (1F1 or 2F2) we have to drench B&B stock to achieve quarantine level of control, or to determine what treatment rate it should be if we want to use a particular application method, such as 1F1, in our quarantine treatment. This method will allow ease of application for growers.

Insecticidal solutions were prepared either in 30-gal drums with polypropylene liners or in 5 gal bucket according the total volume of solution needed. Drench solution was pumped through a hose attached to a shower-headed nozzle using a Shur-Dri battery-powered pump (Figure 1). 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.

Handling: To determine application methods that are both more economical to apply and uniform coverage over the whole root ball, various application handling regimes was investigated over the past several years. Table 1 below shows the treatment application handlings of flip drench treatments over the years for each of the chemicals tested. The application method 1F1 means: one drench in the morning, then in the afternoon flip the trees and drench the other side of the root balls. This method requires minimum amount of chemical and days in treatment application. 2F2: one drench in the morning and another in the afternoon to the same side of the root ball. Next day, flip the tree and drench two more times (morning and afternoon) to the other side of the root ball. It was observed that the second drench application penetrated better than the first and chemical solution should be able to penetrate into the balls reasonably well. However, a portion of the first drench liquid and part of the second drench ran off the rootball surface. Also, treatments required two days to complete with this method. 6NF: This is the conventional and currently approved method included in some of the early trials as a standard comparison. This method requires applying drenches twice a day for 3 consecutive days without flipping the root balls. This method not only is chemical and time consuming but also has a major run-off problem during treatment application.

The 1F1flip drench in our trials in Mississippi and in Tennessee as mentioned above was completed in one day. Between the two drenches, there was usually a "drying period" of a few hours. Although not specifically designed to have a fixed-length "wait period" in between but it is a natural way of doing drench treatments especially for small scale experimental treatments. However, it was unknown if this drying period between drenches was truly necessary from the standpoint of treatment efficacy. Nursery growers expressed that they would rather finish the drenches to both sides of rootballs in a row without having to wait for a few hours before flipping and drenching to the other side. Also, when they do drench application to a large number of harvested trees, it usually will take them quite a while to drench one side before they can come back to flip and drench the other side of the rootballs. Therefore, in the fall 2008 we did an investigation to see if waiting for a few hours before flip and drench would make a difference on treatment efficacy. We included in our trial a wait period of 0.5 hour (representing drenching in a row) and 5 hours between two drenches.

Table 1. Insecticides tested for use as imported fire ant quarantine drench treatment for balled-and-burlapped nursery stock from 2004 – 2009 in Mississippi and Tennessee.

Product	Active Ingredient	Rate* (lb a.i./ 100 gal H_2O)	Handling				Year tested
			1F1	2F2	3F3	6NF	
Dursban TNP	chlorpyrifos	0.125	x	x	x	x	2004-09
		2.000†	x	x		x	2005, 07
Flagship 25WG	thiamethoxam	0.260	x	x	x	x	2004, 05
Talstar Lawn & Tree Flowable	bifenthrin	0.115	x				2006,07
		0.230	x	x	x	x	04, 05, 07
Onyx Pro	bifenthrin	0.0125	x				2009
		0.025	x				2009
		0.05	x				2008
		0.0575	x				2009
		0.1	x	x			2008, 09
		0.115	x	x			07,08,09
		0.2	x				2009
		0.23	x	x			2007,08
Allectus	imidacloprid + bifenthrin	0.125 + 0.1	x	x			2008, 09
		0.25 + 0.2	x	x			2008, 09
Talstar + Sevin	bifenthrin + carbaryl	0.1 + 0.75		x			2008, 09
Talstar + Dylox	bifenthrin + trichlorfon	0.1 + 0.5		x			2008,09
Scimitar	lambda-cyhalothrin	0.035	x				2009
		0.069	x				2009
		0.138	x				2009

* See the *Rate* section under Materials and Methods for special notes on mixing rate for drench solution of various drench application methods.

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

Sampling: In the early years of drench trials, soil core samples for alate bioassay were taken from four locations corresponding to the four sides of a root ball: top-as-planted (top), lateral side 1 (S1), lateral side 2 (S2), and the lateral side the plant rested on at the first drench application (base), to explore evenness of coverage. Later, sample locations were reduced to top (the up-facing, direct application side) and bottom (the resting side) of the root balls. This method of sampling was continued for some time when flip drench trials were conducted. However, since the introduction of flipping during drench application, root balls were no longer resting on one side for the entire treatment process; it became hard to distinguish the top or bottom location. Also, since we flip-drenched the root balls, we assumed that they should have received uniform coverage of drench solution all around and there was no need to take the top and bottom soil core samples like we did this kind of trials in the past. Therefore, taking soil samples from top and bottom would no longer reflect coverage of drench application but just add extra work in sample taking and also double the number of soil samples for bioassay. So we dropped the top and bottom sampling method and instead took only one soil core sample from a root ball on each sample date. In fall 2008, 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 application better than just sampling two sites of the rootballs. Soil samples were collected from within the first four inches of soil core depth for testing against IFA female alates.

All IFA bioassays were conducted in Gulfport, MS at the APHIS-PPQ-CPHST laboratory. The soil samples collected from TN trials were frozen and sent to the Gulfport lab where they were utilized in alate female bioassays (Appendix I – Standard Laboratory Bioassay). Field collected IFA alate females were subjected to the soil samples and mortality was accessed at 14 days after continued confinement to the soil sample. While the number of females per sample varied due to changes in the protocol and resources, a minimum of 10 alate females per replicate, and thus a minimum of 40 alate females per treatment per sampling interval were always used.

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

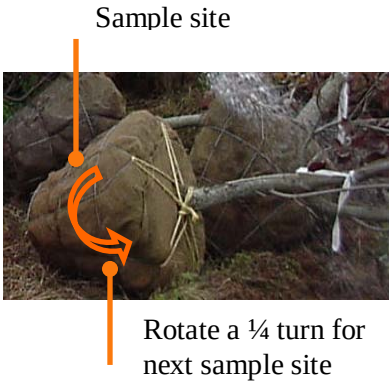


Fig.2. Soil core sample collection sites

RESULTS:

Flip-drenched treatments initially conducted in fall 2004 suggested that rotating root balls during treatment application, compared to the ones that remained stationary, improved the consistency of bioassay results and could potentially cut the number of days spent applying drenches from three down to one. Since then, drench trials were focused on using application method 1F1 and 2F2 instead of 6 NF (see *Handling* section in Material and Method for definitions), with a clear objective of reducing the amount of time and chemical needed for post-harvest drench treatment to achieve quarantine level IFA control. Comparison of the treatment application method 1F1 with 2F2 was made in TN in trials from 2007 – 2009 with bifenthrin, chlorpyrifos, and a combined formulation of bifenthrin with imidacloprid as major insecticides (Table 2).

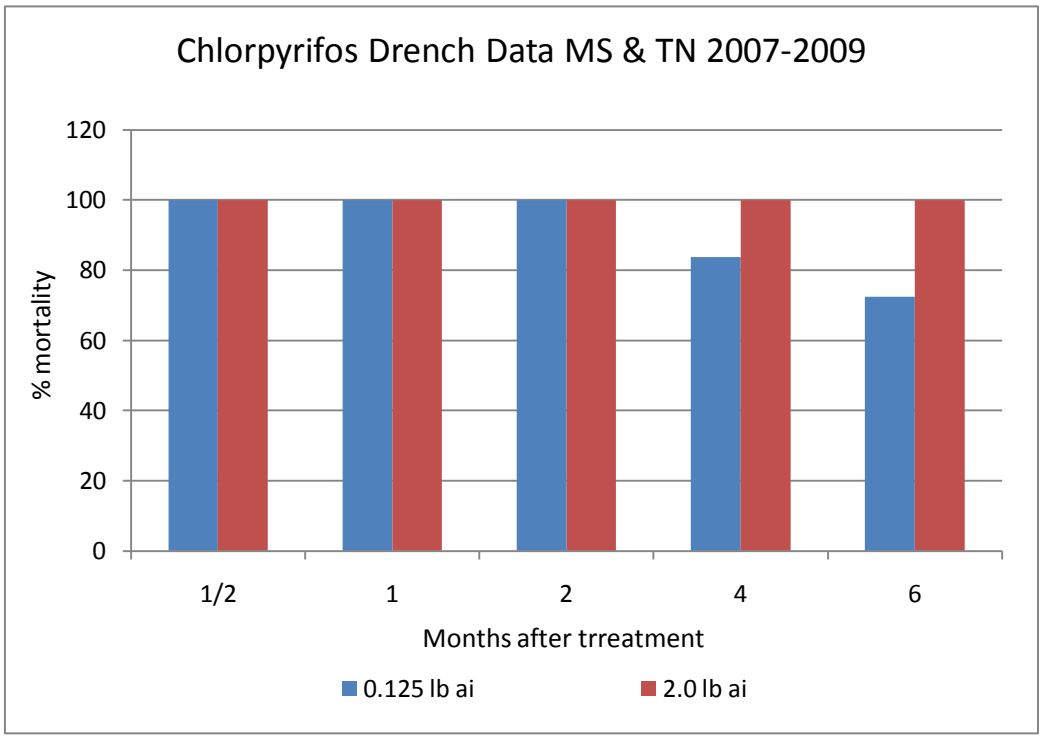
Table 2. Bioassay results achieved with application methods 1F1 and 2F2 Tennessee 2007 - 2009

Season	Chemicals	Rate (lb ai/100 gal water)	**1F1**			**2F2**		
			month 2	month 4	month 6	month 2	month 4	month 6
2007 fall	chlorpyrifos	2.000	100	100	100	91	100	91
	bifenthrin	0.115	100	100	100	96	100	100
	bifenthrin	0.230	100	100	100	100	100	100
2008 fall	chlorpyrifos	0.125	100	100	90	100	100	100
	bifenthrin	0.100	100	100	100	100	100	100
	Imidacloprid + bifenthrin	0.125 + 0.1	100	100	100	100	100	100
2009 spring	chlorpyrifos	0.125	100	100	78	100	76	43
	bifenthrin	0.100	100	100	100	100	100	100
	Imidacloprid + bifenthrin	0.25 + 0.2	100	100	100	100	100	100

In the three years these two application methods were used with the three insecticides at different rates, there is no obvious difference in the bioassay results between the two application methods (Table 2). As a matter of fact, 1F1 achieved more 100% control than 2F2 which indicated 1F1 is an effective and suitable method for post-harvest drench treatment application. The reason that 1F1 had better result may be partially because of the way the treatment solution was mixed (see *rates* in Material and Method for detail explanation), resulting higher concentration in the 1F1 treatment solution than that in the treatment solution for 2F2.

Chlorpyrifos: Currently, chlorpyrifos is the only insecticide that can be used to support the IFA regulatory drench treatment for B&B nursery stock. It is mixed at a rate of 0.125 lb ai/100 gal water and provides a certification period of 30 days. Because of this, it has been used as a standard chemical for comparison for our drench tests and was included in nearly all trials of post-harvest B&B drenches. Our numerous repeated testing confirms this rate and certification period (Figure 3). Because of the refinement in our treatment application method and chemical mixing procedures over the years, results from the more recent years (2007-2009) are thought better representatives of the efficacies of this insecticide; therefore, Figure 3 included test results from 2007-2009. The 2.0 lb ai rate was also tested in some of the years (2005 and 2007) because it is the current rate of application to certify movement of B&B nursery stock for the Japanese beetle (JB) Harmonization Plan and some growers must treat for both pests. The high JB rate of drench treatment provided excellent control of IFA for at least 4 months.

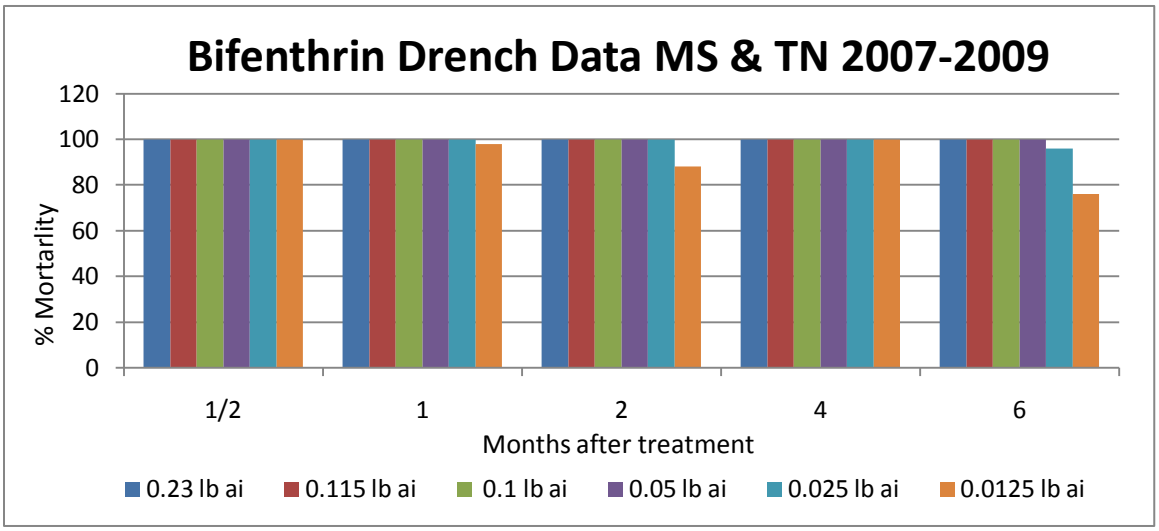
Figure 3. Efficacy of chlorpyrifos as a drench treatment for balled-and-burlapped nursery stock. Compilation of data from numerous trials with the treatment application method 1F1 in MS and TN from 2007-2009.



Bifenthrin: Bifenthrin tested as a drench treatment for balled-and-burlapped nursery stock provided consistent and long-term control against imported fire ants. Rates as low as 0.1 lb ai/100 gal water provided 100% control for 6 months after treatments in numerous trials in both Mississippi and Tennessee during both spring and fall applications (Figure 4). Bifenthrin treatment rate of 0.05 lb ai/100 gal water was near the lowest rate at which it generally obtained

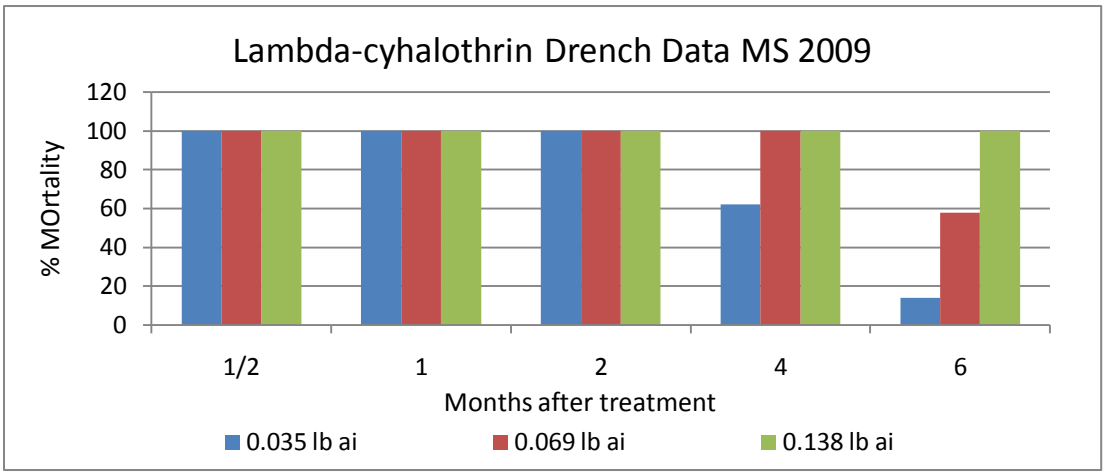
100% efficacy for 6 months. However, rates tested that were lower than 0.05 lb ai/100 gal water (0.025 lb and 0.0125 lb) in the drench treatment usually did not obtain 6 months of quarantine level control on IFA (Figure 4).

Figure 4. Efficacy of bifenthrin as a drench treatment for balled-and-burlapped nursery stock. Compilation of data from 1F1 flip drench trials in MS and TN from 2004-2009.



Lambda-cyhalothrin: Lambda-cyhalothrin was tested for drench treatment in MS in spring 2009 (at both 0.035 and 0.069 lb ai per 100 gal water) and fall 2009 (at higher rates than spring: 0.069 and 0.138 lb ai per 100 gal water). The reason that the rate was increased in the fall trial was that the lower rate of 0.035 did not achieve quarantine level control in the spring trial (Figure 5). However, due to the label change on this insecticide, it is not likely to be a single treatment insecticide for IFA quarantine drench treatment and therefore lambda-cyhalothrin was not included in our 2010 drench trial.

Figure 5. Efficacy of lambda-cyhalothrin as a drench treatment for balled-and-burlapped nursery stock. Data from 1F1 flip drench trials conducted in spring and fall 2009, Mississippi.



Imidachloprid + bifenthrin: This combination of insecticides is currently available in the labeled product Allectus™ (Bayer Environmental Science). Several tests at a single rate of application (0.125 – 0.25 imidachloprid + 0.1 – 0.2 bifenthrin) have been conducted in TN since fall 2008 with this combination insecticide, and imidacloprid + bifenthrin combination has been 100% effective against IFA for 6 months after drench treatment. Further testing to determine a suitable rate for efficacy at 6 months was continued in fall 2010 and testing at lower rates of application (0.0625 imidachloprid + 0.025 bifenthrin) was under investigation in MS. The test at a lower rate (with bifenthrin at 0.025 lb ai) would determine whether or not a combination of the two insecticides would have synergistic effect on fire ant control because past data have shown that bifenthrin alone at 0.025 lb ai per 100 gal water did not achieve 100% control 6 months after treatment.

Thiamethoxam: This insecticide was included in the initial tests in fall 2004 and spring 2005 in MS. Over the course of the sample dates thiamethoxam in the 1F1, 2F2, and 6NF regimes lost significant efficacy by the six-month sample date. Soil from the thiamethoxam treated balls provided inconsistent results regardless of application technique throughout the 4 months of the trial. This chemical was not effective on fire ant control and was not included in drench treatments after 2005 trials.

Bifenthrin mixed with other insecticides: Bifenthrin mixed with carbaryl (Sevin, at 0.1 + 0.75) or with trichlorfon (Dylox, at 0.1 + 0.5) were tested in TN in fall 2008 and spring 2009. Both treatments achieved 100% control of IFA for 6 months but these results did not provide much additional efficacy information because of the bifenthrin rate (0.1 lb ai per 100 gal water) used in the treatments. Since bifenthrin alone at 0.1 lb ai usually had 100% control for the entire 6 months, these two treatments did not reveal additional information whether mixing a second insecticide could result in any synergistic effect on IFA control. Therefore, mixing bifenthrin with another insecticide at the rates tested may not justify for the extra chemical cost. These tank mix application may be worth investigating for JB control purpose.

CONCLUSION

1. Flipping of rootballs while applying the drench treatment improves chemical coverage uniformity on rootballs thus improving consistence of treatment efficacy.
2. The treatment handling method 1F1 is adequate for drench treatment application for post-harvest B&B nursery stock for IFA quarantine treatment.
3. No differences were found in treatment efficacy with different intervals between the two drench applications of the 1F1 treatment method; flip and drench to the other side of root balls could be followed by drenching the other side of the root balls without a waiting period at the completion of first drench.
4. Bifenthrin performed consistently in treatments with rates at or above 0.05 lb ai per 100 gal water. Rates lower than 0.05 lb ai per 100 gal water usually did not maintain quarantine level control for 6 months after drench treatment.
5. Combination formula with bifenthrin as a major ingredient such as Allectus (imidacloprid + bifenthrin) provide good control against IFA; therefore, this combination formulation may be worth further testing.

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: Final

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 (*Popillia japonica* Newman) in B&B requires dipping in chlorpyrifos. Since both imported fire ants (IFA) and Japanese beetle (JB) are a concern for the Tennessee field-grown nursery industry, the trials detailed in this report were conducted in cooperation with the Tennessee State University Nursery Research Center (TSU-NRC) with the goal of determining treatments useful against both pests. The JB testing portion of this trial was planned and conducted by TSU-NRC and the USDA-ARS Horticultural Insects Research Laboratory in Wooster, OH, and they report the details and results for that portion of these trials.

Standard IFA testing of chemical treatments for both dip and drench applications has been conducted through female alate bioassays on soil core samples from the treated root balls. Soil core bioassays for drenches conducted in 2002 and spring 2003 yielded erratic results over time and among replicates within treatments. Results from the same chemicals at equal or lower rates, when applied by immersion, were consistent, thus indicating insufficiency in application of the drench treatments. Doubling the volume of solution in drench application conducted in fall 2003 and spring 2004 failed to eliminate inconsistent results. The search for the cause of the inconsistency problem became 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 (Figs. 1&2). 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 apply 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 2). 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.1. 12” rootballs grouped for drench treatment

Fig.2. USDA ARS personnel applied drench treatment to 12” B&B rootballs

In the fall trial, one major change was 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 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 3) 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 4 & 5); (Appendix I – Standard Laboratory Bioassay).

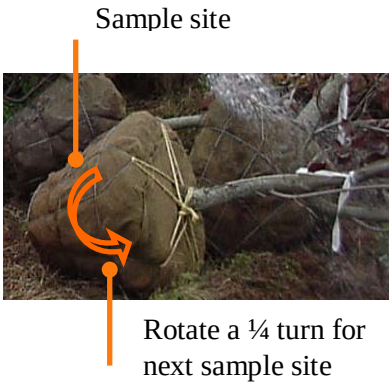


Fig. 3. Soil core sample collection sites

Fig. 4. A tray of alates mortality bioassay cups.

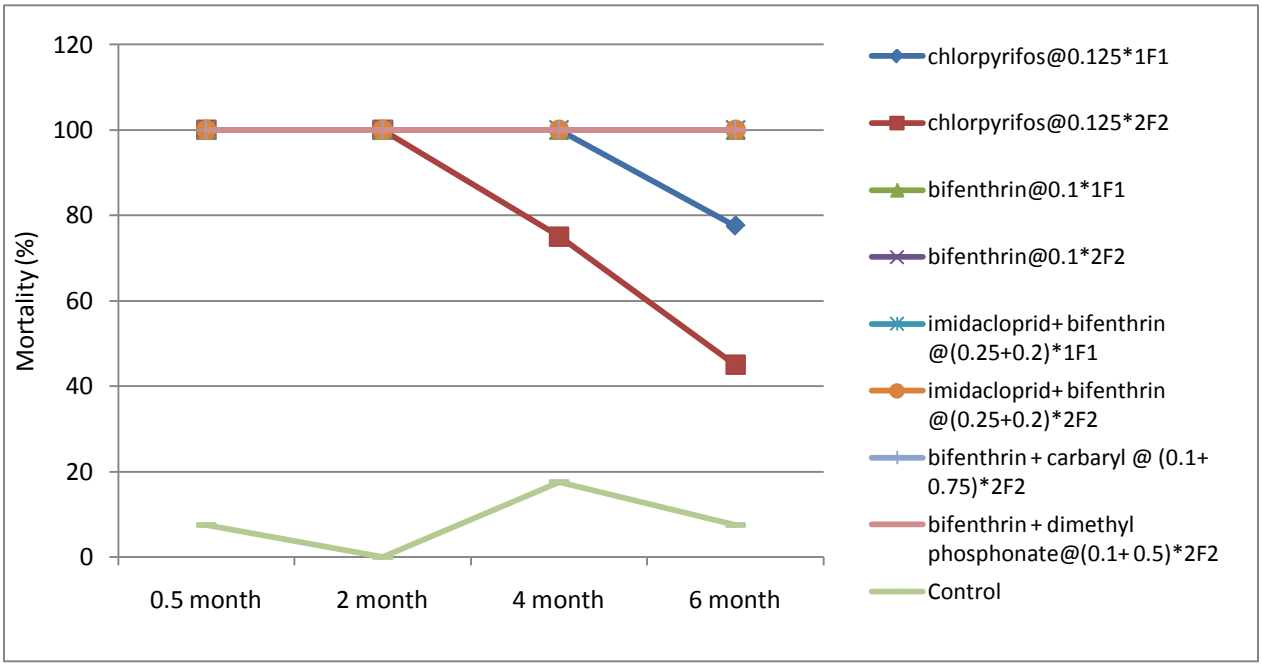
Fig. 5. Orange circles indicate the locations of clusters of female alates within this bioassay cup.

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 6). 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.

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

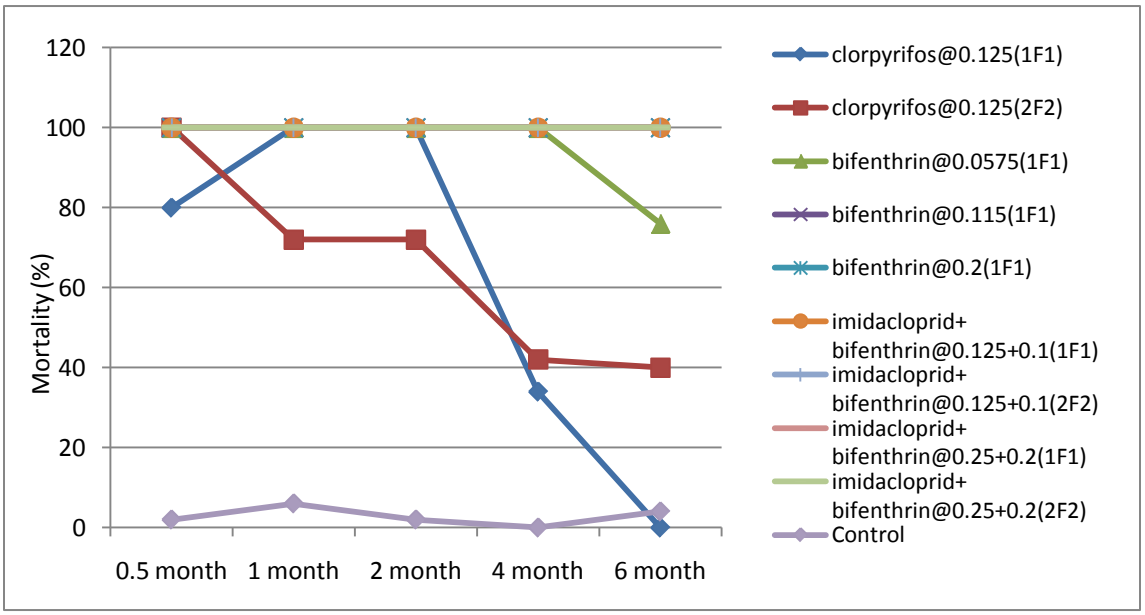


Drench trial fall 2009

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 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 for the first 4 months after the final treatment (Fig 7). The 6 month bioassay results remained the same as the previous four months except the bifenthrin at 0.0575 lb ai treatment which was at 76% kill rather than 100%. However, since our 0.05 lb ai rate tested in MS in fall 2008 drench trials maintained 100% control for entire 6 months after treatment, this result could be either an exception or due to the poor penetration of the TN clay soil.

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.

Fig. 7. IFA control achieved with soil samples treated with bifenthrin alone or in combination at 0.5, 1, 2 and 4 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: Final

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

INTRODUCTION:

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

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

Until fall of 2004, drenching was done without rotating the root balls and B&B normally rests on one side of the root ball throughout the three-day drench process. This possibly restricts treatment coverage on the resting side of the ball, while giving the surface 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 chemical and time consuming but also has run-off issues.

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

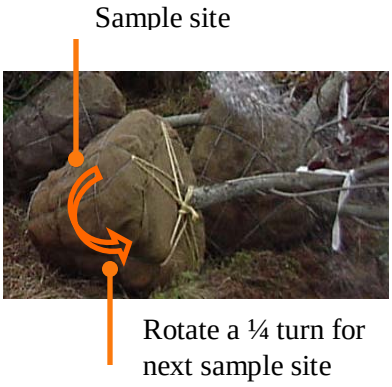


Figure 2. Soil core sample collection sites

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 H_2O	Rate ml prod./gal H_2O	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

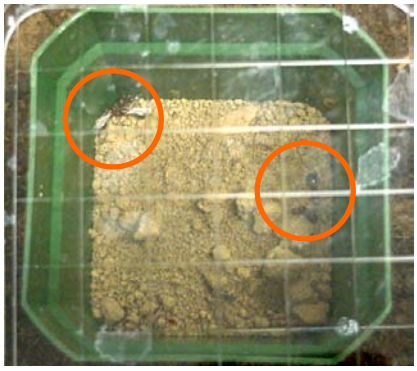
Material	Active Ingredient	Trt #	Rate #ai/ 100 gal H_2O	Rate ml prod./gal H_2O	Water* vol/drench	Amount of Insecticide per drench	Total Amount Applied
Dursban 4E (44.9%)	Chlorpyrifos	1	0.125	1.18	3.5 gal	4.13 ml	8.26 ml
Onyx Pro 23%	Bifenthrin	2	0.0125	0.235	3.5 gal	0.823 ml	1.65 ml
		3	0.025	0.47	3.5 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 in this kind of trial 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-Standard Laboratory Bioassay).

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.



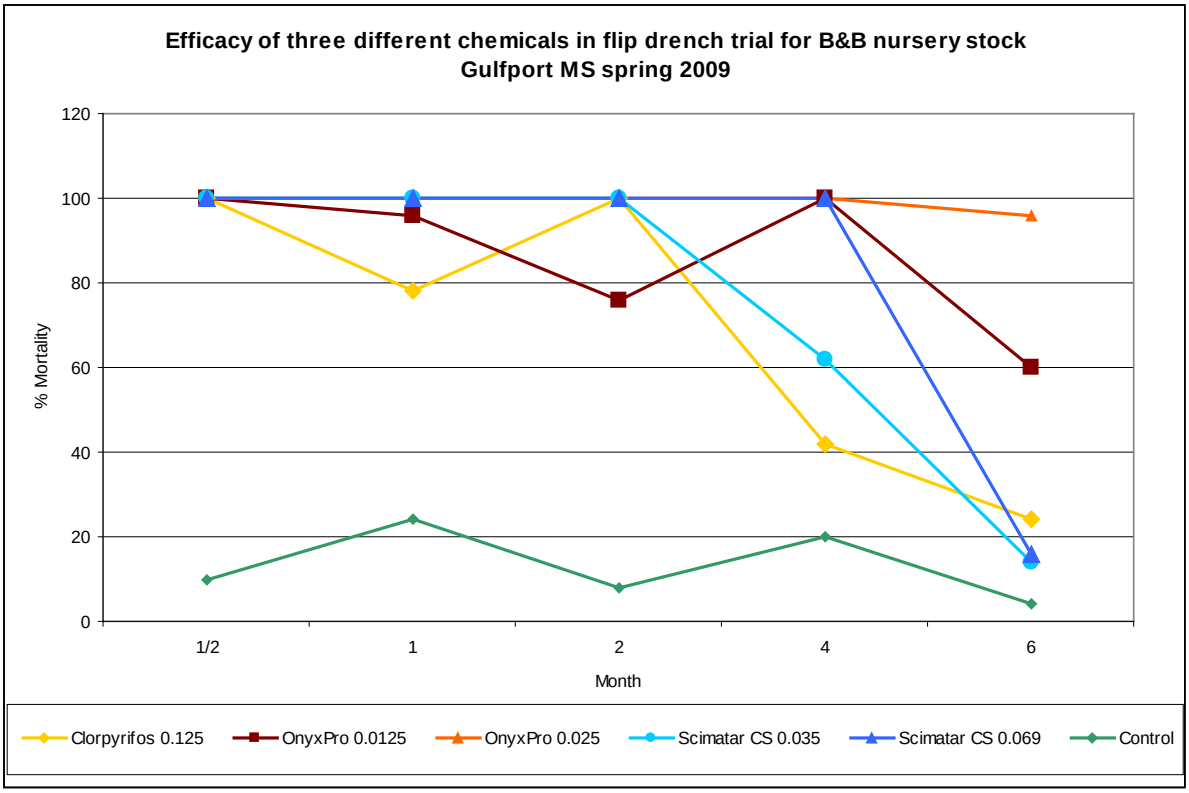
RESULTS AND DISCUSSION:

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 there is 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.

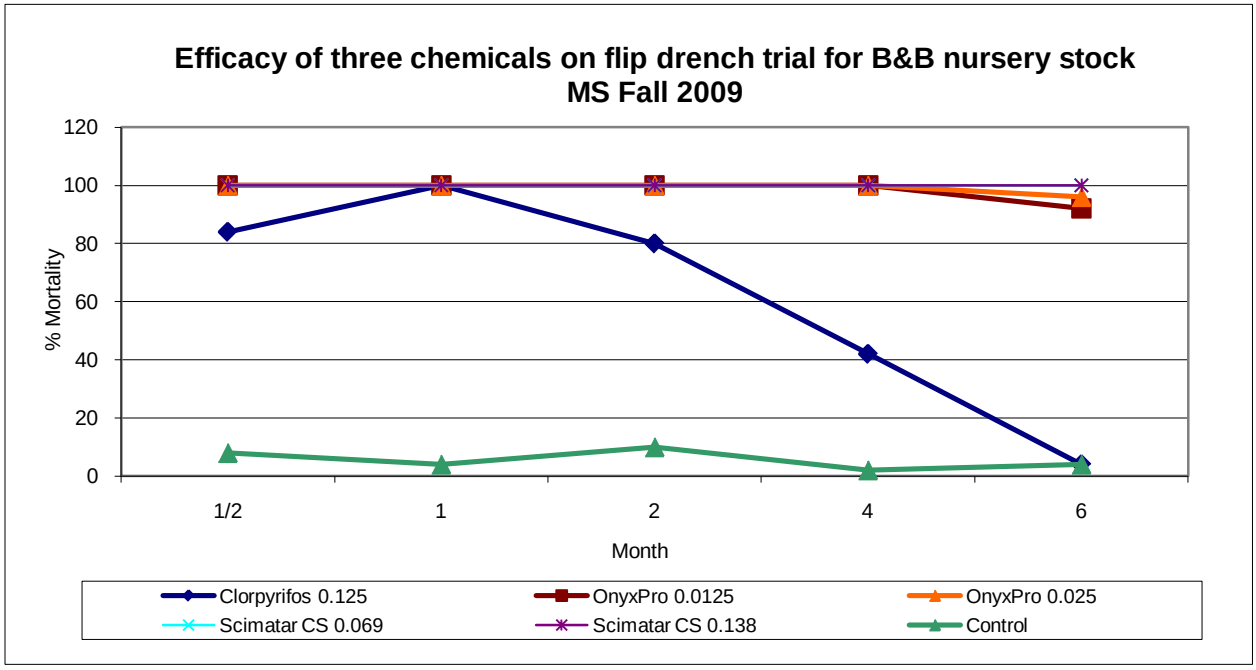
Sampling around the rootballs was started in fall 2008 for taking soil core samples from rootballs after flip drench treatment and has been considered an adequate method to assess the treatment efficacy for flip drench application method. With both sides of the rootball being well drenched and chemical 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:
The lambda-cyhalothrin test rates were raised to 0.069 and 0.138 # ai/ 100 gal in the fall trial from the rates of 0.035 and 0.069 # ai/ 100 gal in the spring trial which did not maintain quarantine level control of IFA for 6 months after treatment. These two treatments achieved 100% in mortality in the IFA female alates bioassays for 6 months. Similarly to spring trial, the bifenthrin treatments at 0.025 lb ai per 100 gal of water had a 100% control only till 4 months after the final treatment. Obviously, to obtain quarantine level control on IFA for 6 months post-treatment in flip drench treatment, bifenthrin rate must be higher than 0.025 lb ai per 100 gal of water.

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



CPHST PIC NO: A1F04

PROJECT TITLE: Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Fall 2010 in Tennessee

REPORT TYPE: Final

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, 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 (*Popillia japonica* Newman) in B&B requires dipping in chlorpyrifos. Since both imported fire ants (IFA) and Japanese beetle (JB) are a concern for the Tennessee field-grown nursery industry, the trials detailed in this report were conducted in cooperation with the Tennessee State University Nursery Research Center (TSU-NRC) with the goal of determining treatments useful against both pests. The JB testing portion of this trial was planned and conducted by TSU-NRC and the USDA-ARS Horticultural Insects Research Laboratory in Wooster, OH, and they report the details and results for that portion of these trials.

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

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

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

The fall 2010 drench trials in TN again focused on examining some promising insecticides and plant handling methods for 12" root balls. The insecticide bifenthrin and a combined formulation of bifenthrin with another insecticide, application rates, and plant handling methods (1F1 vs. 2F2 for some treatments) were investigated.

MATERIALS AND METHODS:

In October 2010 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. The treatments for the fall trial are listed in Table 1. Other than chlorpyrifos and bifenthrin, a combined formulation of bifenthrin with imidacloprid (available in the labeled product of Allectus®) was also investigated in this trial.

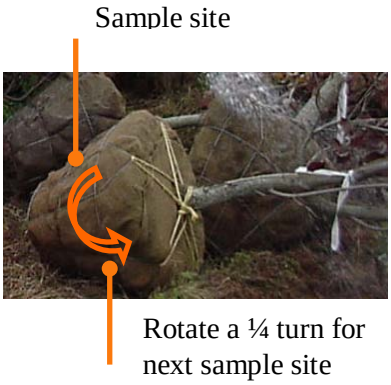
Treatments were applied at 0.82 gallons per treatment using a regular garden sprinkler can (Fig 1). Solutions were applied twice daily (once in the morning and again in the afternoon) and between these two applications the root balls were rotated or flipped to expose a different side to the direct application. This plant handling method was described as 1F1**.** This method requires minimum chemical solution and days of application for drench treatments. The regime 2F2 requires 2 days to complete the treatment application: one drench in the morning and another in the afternoon to one side of the root balls on the first day. The next day, flip the trees and drench two more times (morning and afternoon) to the other side of the root balls. The 6NF treatment regime was not used in this trial but as the currently approved drench application method it requires applying drenches twice daily 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 (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.

After final treatment, the plants were maintained outdoors to weather naturally. Five replicate root balls were used in each treatment and soil core samples were taken at 0.5, 1, 2, 4, and 6 months after final treatment. One soil core sample was taken from the mid-side area of each rootball at the initial bioassay day. On next sample day, the rootballs were rotated 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. The rootballs were rotated again for a quarter turn and took the third soil cores 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 after collection and then sent to CPHST Lab in Gulfport,

MS where they were utilized in female alate 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 3 & 4); (Appendix I – Standard Laboratory Bioassay).

Fig.1. USDA ARS personnel applied drench treatment to 12" B&B rootballs

Figure 2. Soil core sample collection sites



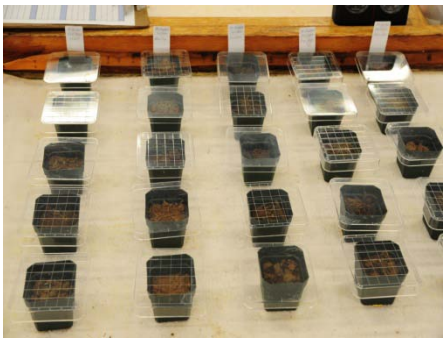


Figure 3. A tray of alates mortality bioassay

Figure 4. Single bioassay cup showing clusters of female alates inside.

Table 1. List of treatments for flip drench trial on 12 inch root balls in TN fall 2010

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	0.0575	X	
Onyx 23%	bifenthrin	0.115	X	
Control	--	--		X

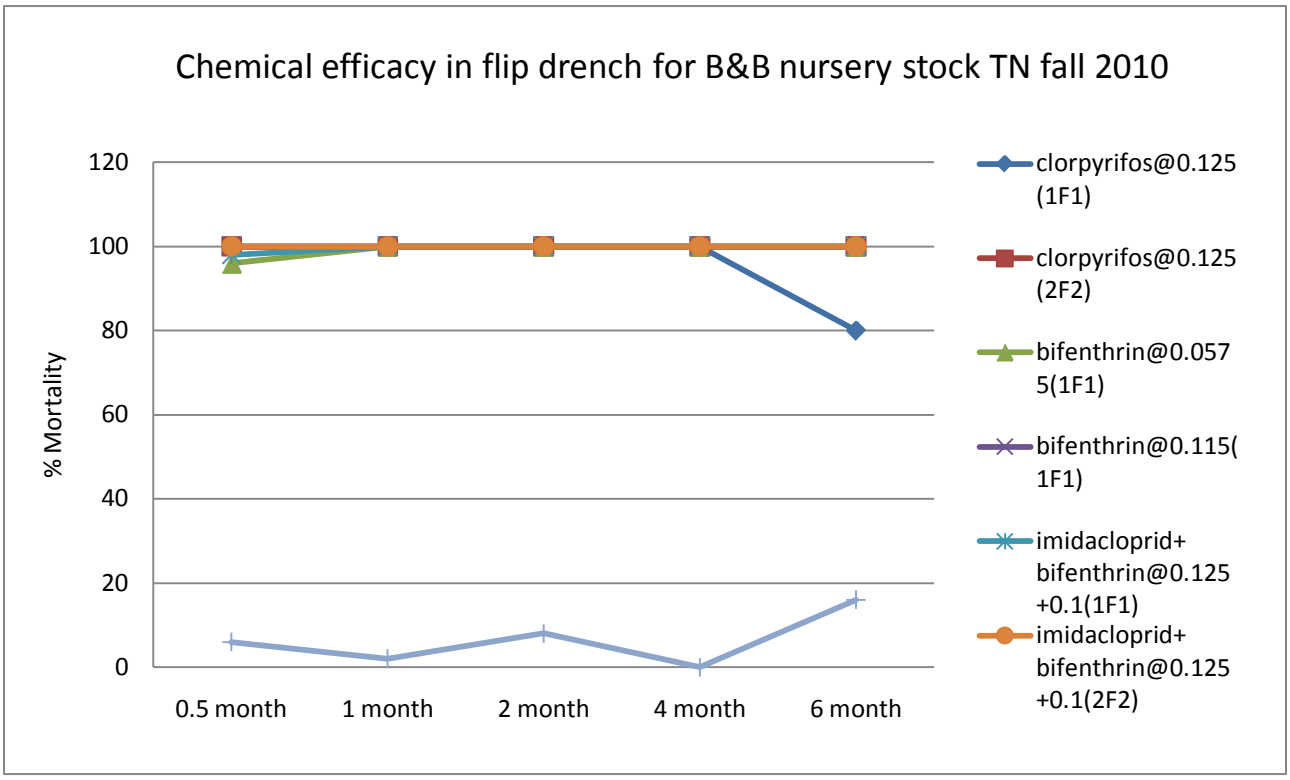
*all treatments applied true to the listed application rates.

RESULTS AND DISCUSSION:

Drench trial fall 2010

The TN fall 2010 drench trial focused on bifenthrin and a combined formulation of imidacloprid & bifenthrin. 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. Chlorpyrifos at 0.125 lb ai usually provides one or two months of quarantine level control of IFA. But this treatment achieved a 100% control at the first four months of bioassay with the 1F1 and for the entire 6 months with the 2F2 application method in this trial, which is better than normally expected of this insecticide (Fig 5). For the bifenthrin alone treatments, the rates of 0.0575 and 0.115 lb ai per 100 gal water were used. Results showed that these two bifenthrin treatments achieved 100% in mortality in the IFA female alate bioassay for the entire trial period of 6 months after the final treatment. The treatments with combination insecticides (imidacloprid + bifenthrin) also obtained 100% efficacy in bioassays for 6 months in either 1F1 or 2F2 treatment application method. However, these results were easily predictable with the rates used in the treatments because when used alone at a rate of 0.1 lb ai, bifenthrin normally would also achieve 100% control against IFA. The application methods 1F1 and 2F2 both performed well in this trial and it confirmed our findings in trials of previous years. It is the conclusion in our research summary on flip drench trials from 2004-2009 (can be found in the 2010 annual report) that the application method 1F1 could be a method of choice for post-harvest drench treatment of B&B nursery stock for IFA quarantine treatment.

Figure 5. IFA control achieved with soil samples treated with selected chemicals in flip drench application in TN fall 2010.



CPHST PIC NO: A1F04

PROJECT TITLE: Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Gulfport MS Fall 2010

REPORT TYPE: Final

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

INTRODUCTION:

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

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

Until fall of 2004, drenching was done without rotating the root balls and B&B normally rests on one side of the root ball throughout the three-day drench process. This possibly restricts treatment coverage on the resting side of the ball, while giving the surface 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 chemical and time consuming but also has a run-off issue.

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 2010 trials was to repeat the 1F1 drench treatment method using bifenthrin at 0.05 and 0.1 lb ai rates and also added a combination formulation containing bifenthrin (Allectus) 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 root ball 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. 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 (referred as 1F1).

Figure 1. Drench treatment application.; blue dye was added to the solution for coverage indication.

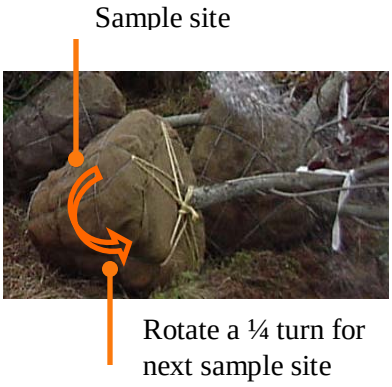


Figure 2. Soil core sample collection sites

Details of the drench treatments in this trial can be found in Table 1.

Table 1. Treatment List for 1F1 Drench Trial in Gulfport, Mississippi Fall 2010

Material	Active Ingredient	Trt #	Rate #ai/ 100 gal H_2O	Rate ml prod./gal H_2O	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	0.05	0.95	3.5 gal	3.325 ml	6.65 ml
		3	0.1	1.89	3.5 gal	6.65 ml	13.30 ml
Allectus	Imidacloprid+ bifenthrin	4	0.0625 + 0.025	5.26	3.5 gal	18.4 ml	36.8 ml
Control	--	5	--	--	3.5 gal	--	--

*Balls were rotated once between the two chemical drenches.

Sampling and bioassay:
After final treatment, the plants were maintained outdoors to weather naturally and irrigation schedule was set up to closely simulate outdoor nursery storage conditions. Soil core samples were collected at 1, 2, 3, 4, 5 and 6 months post-treatment for laboratory bioassay using female alates. One soil core sample for each rootball was taken from the mid-side area of the ball at the initial bioassay day. On the next sample day, rootballs were rotated 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. 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 – Standard Laboratory Bioassay).

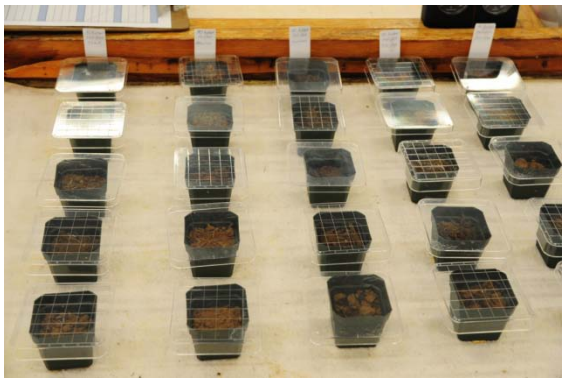


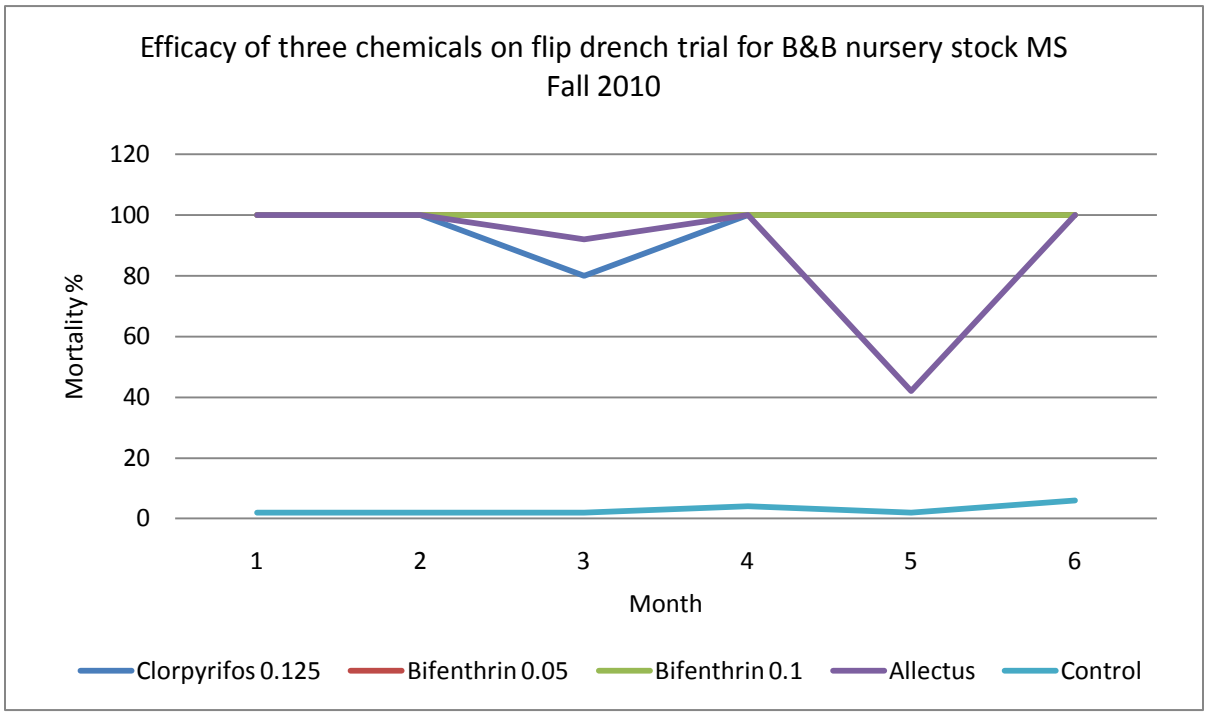
Figure 3. A tray of alate mortality bioassay cups.

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

RESULTS AND DISCUSSION:

The two rates of bifenthrin treatments (0.05 and 0.1 lb ai per 100 gal of water) achieved 100% in mortality in the IFA female alates bioassays conducted for the entire 6 months post-treatment. The results again confirmed findings from flip drench trials in the past in MS and TN at these rates. The combination formula of imidacloprid and bifenthrin (found in Allectus) was tested at the rate which contains 0.025 lb ai of bifenthrin. This rate was chosen because we found in our prior trials (see 2009 annual report on MS drench trials) that bifenthrin used alone at 0.025 lb ai usually could achieve 100% control of IFA for the first four months but not at the 6th month after treatment. Therefore, we had hoped that by having another insecticide in the formulation, quarantine level of IFA control for the entire 6 months post treatment could be achieved without increasing bifenthrin concentration higher than 0.025 lb ai. However, since the inconsistence results were obtained from month 3 to month 6, this would seem unlikely to happen (Fig. 6). But this combination formulation could still be worth investigating because it could be effective or has synergistic effect for the Japanese beetle control, another soil dwelling insect pest requiring quarantine treatment for nursery stock in Tennessee.

Figure 6. IFA control achieved by insecticide treated soil samples collected at various sampling intervals after final drench application. Plants were rotated once between the two drench applications made in one day. Gulfport, MS fall 2010



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: Final

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

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 Table 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.

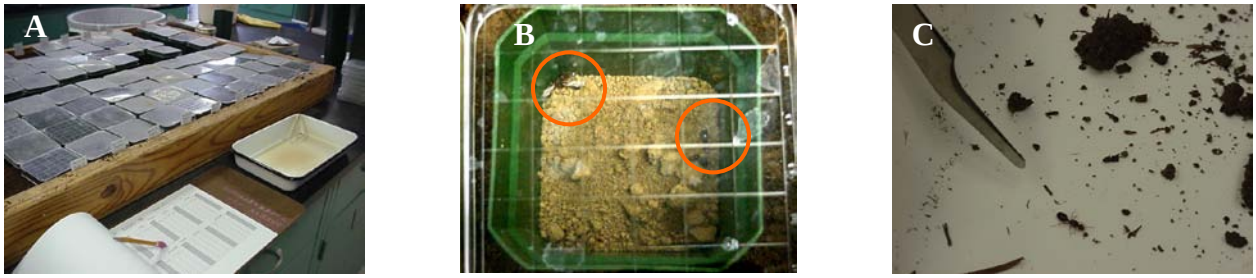


Table 1. List of treatments for 24 inch B&B stock immersion trial in TN spring 2009

Treatment*	Ingredients	Rate #ai/100 gal H2O	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/100 gal H2O	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 (Appendix I – Standard Laboratory Bioassay).

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

Treatment	Ingredients	Rate #ai/100 gal H2O	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/100 gal H2O	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.

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

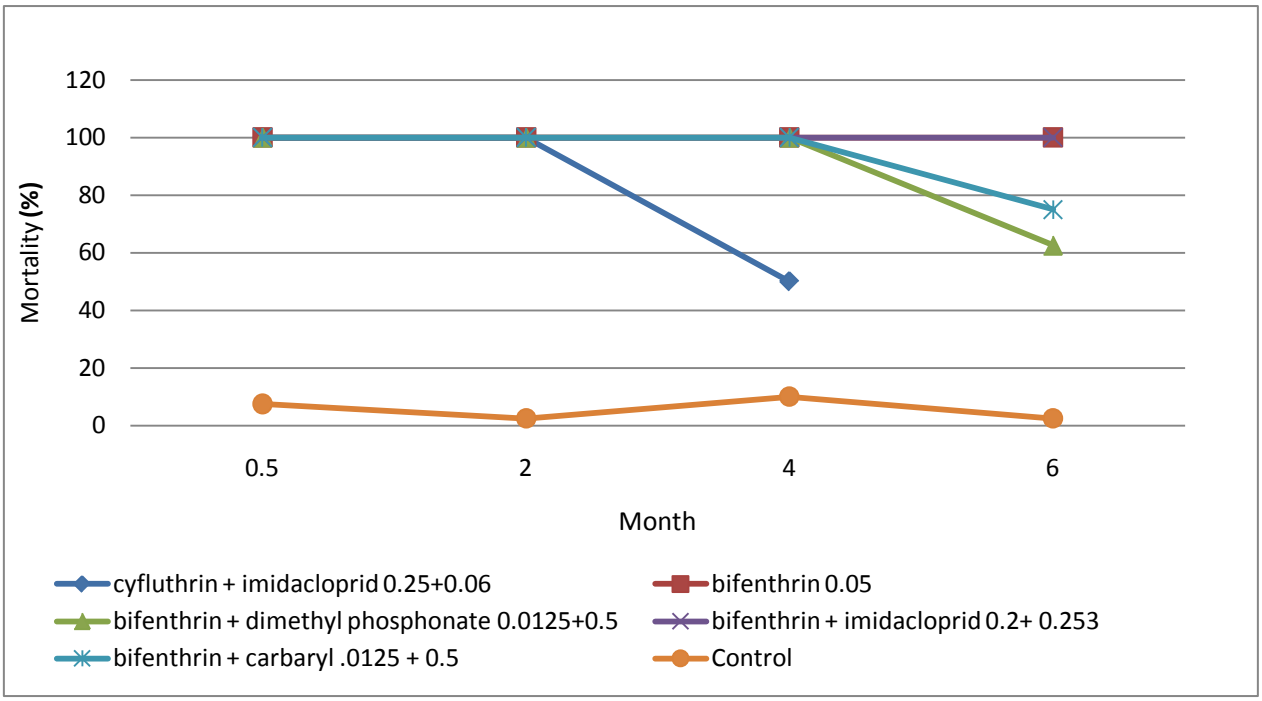
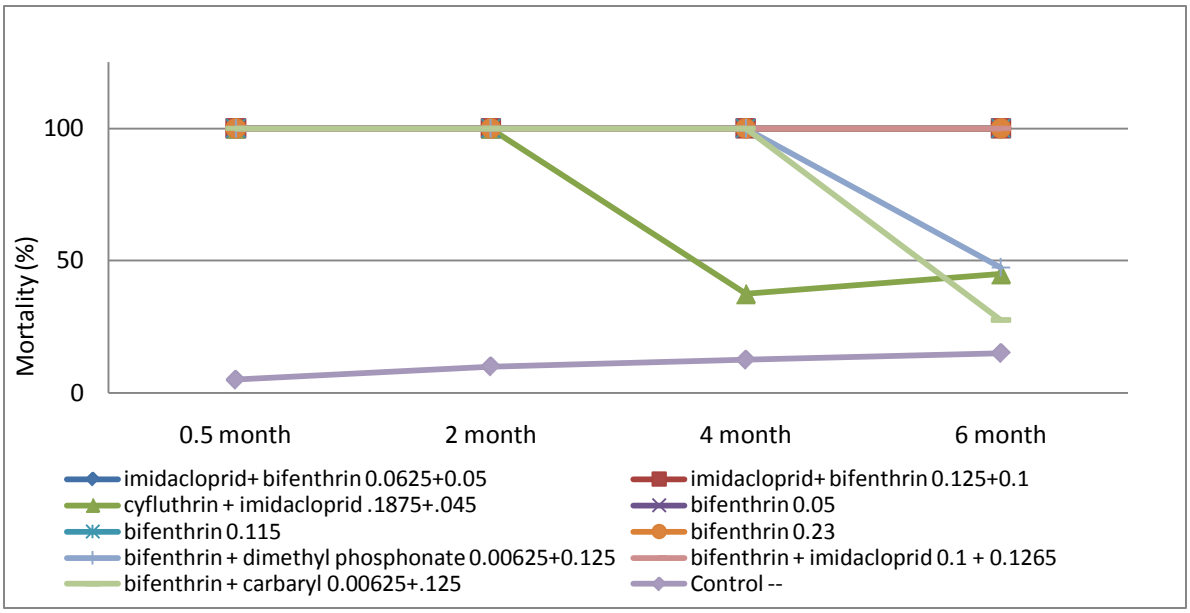
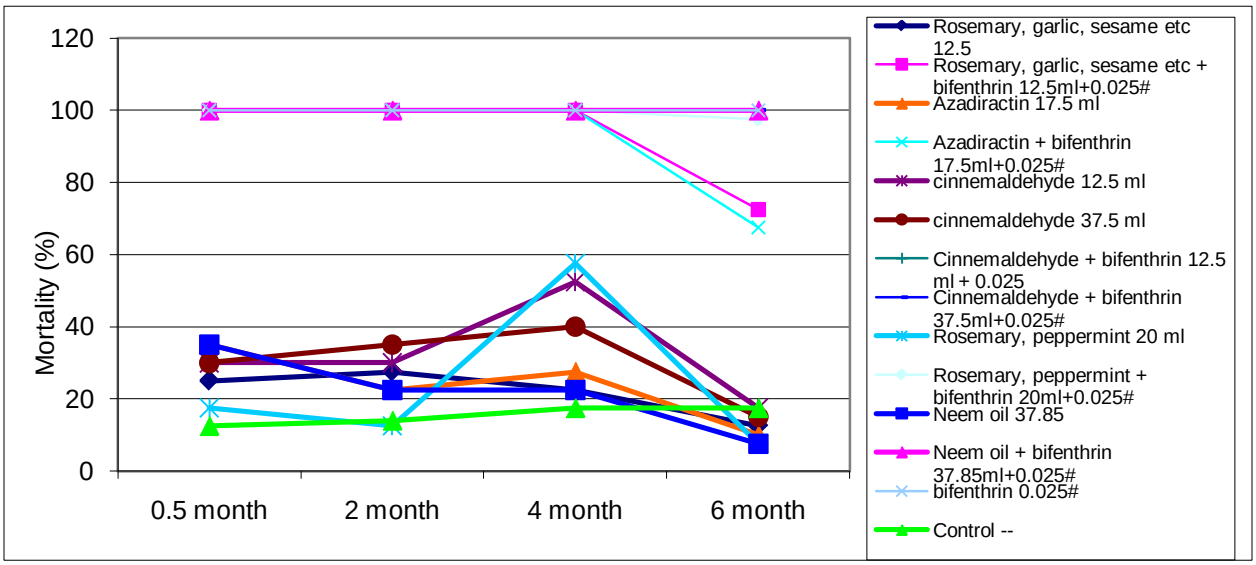


Figure 4. Efficacy of B&B immersion treatments against IFA female alates in 12-inch root balls; Tennessee spring 2009.



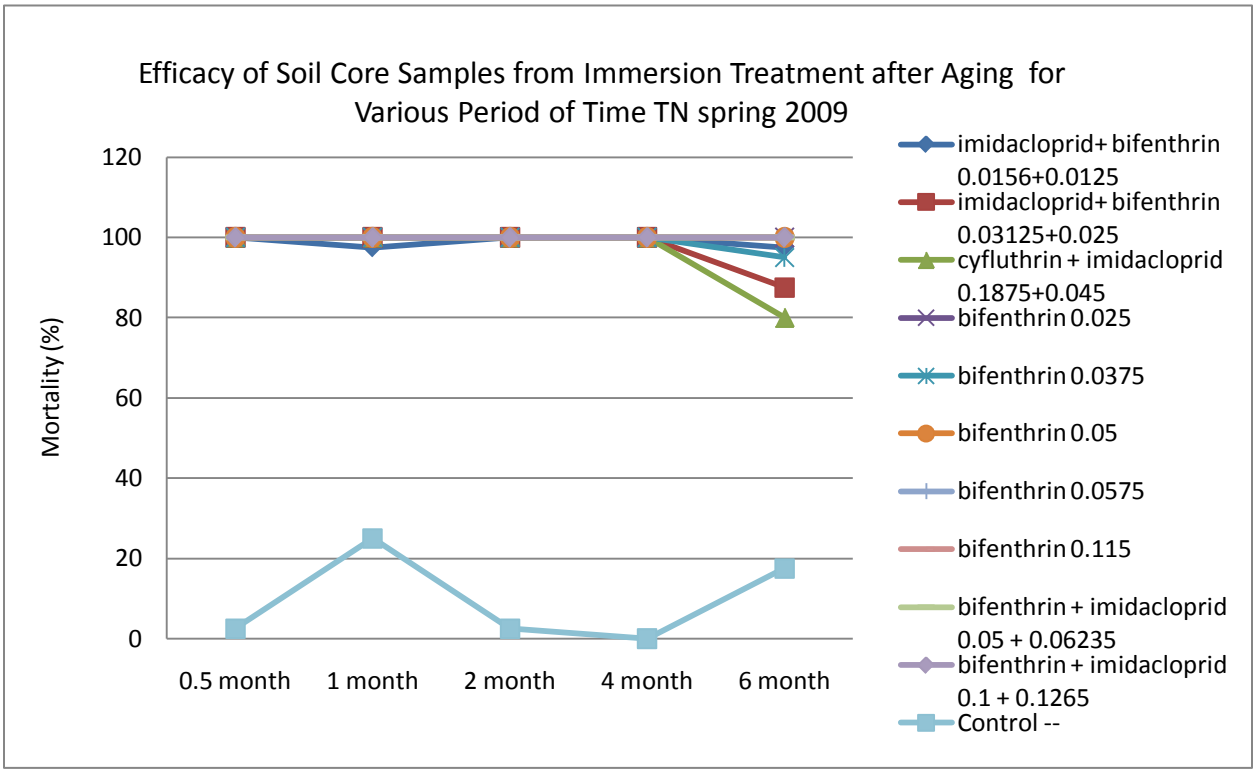
None of the essential oils alone treatments were effective enough against IFA for quarantine treatment purpose (Fig 5). When essential oils were mixed with bifenthrin at 0.025 lb ai rate, some combination treatments achieved 100% mortality in female alates bioassays but others did not. It is questionable 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.

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



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 showed that they were all 100% effective against IFA for the first four months after treatment except that imidacloprid+ bifenthrin (0.0156+0.0125) was 97.5% at one month (Fig 6). However, at 6 months after treatment, 100% control generally was not achieved by treatments with bifenthrin rates that were lower than 0.05 lb ai used either alone or in combination/mixed with another insecticide. This confirmed our results of the spring 2009 immersion trials in which bifenthrin at a rate of 0.0125 lb ai per 100 gal of water or lower, used in combination with another insecticide, did not last for 6 months with 100% control of the IFA.

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



PROJECT NO: A1F04

PROJECT TITLE: Alternative Immersion Treatments for Balled-and-Burlapped Nursery Stock for Use in the IFA Quarantine, Tennessee Spring 2010

REPORT TYPE: Final

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 also effective for Japanese beetle (JB), was initiated with the Tennessee State University Nursery Research Center (TSU-NRC) and the USDA-ARS Horticultural Insects Research Laboratory, Wooster, OH. Trials conducted in past few years indicated several chemicals could potentially be used in addition to chlorpyrifos in treatment of B&B nursery stock.

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

The objective of the spring 2010 dip trials in TN was to repeat the fall 2009 trials on new lower rates for dip treatment using mainly bifenthrin or bifenthrin in combination with other chemicals either in combined formulation or with tank mix application.

MATERIALS AND METHODS:

Treatment applications were made in March for spring 2010 trials at the Nursery Research Center by personnel from TSU-NRC and USDA-ARS. A commercial grower in Warren Co., TN provided plants with 12 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) that consisted of one of the treatments in Table 1.

Figure 1. Workers dip plants with 12" root-balls in chemical solution for one minute.

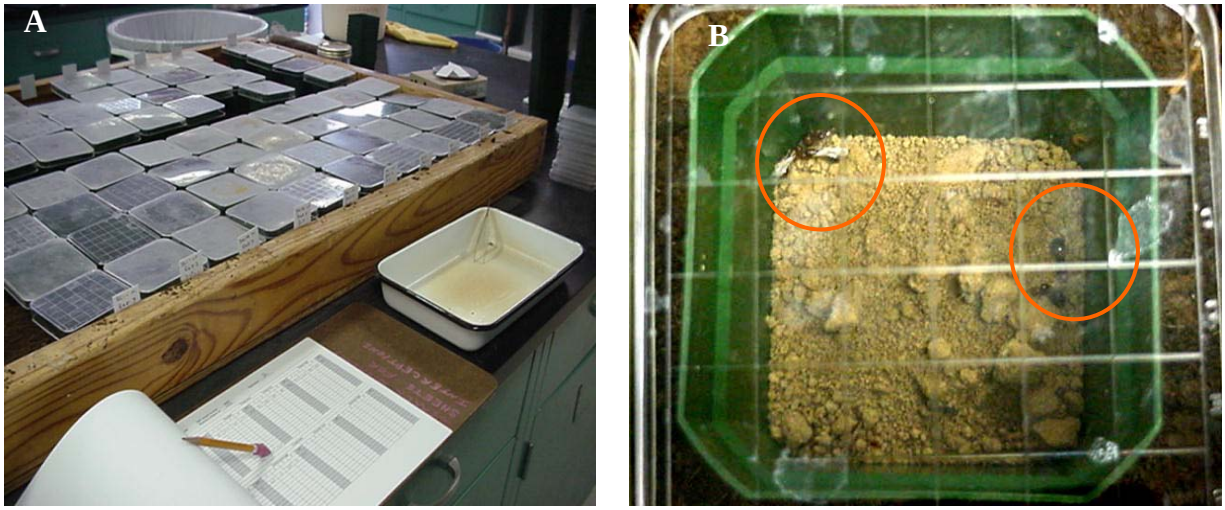


Figure 2. (A) General laboratory set up of bioassays. (B) A single bioassay cup (visible alates highlighted in circles).

Table 1. List of treatments for 12 inch B&B stock immersion trial in TN spring 2010

Treatment*	Ingredients	Rate #ai/100 gal H2O	Amount Product/gal of water
Allectus	Imidacloprid + bifenthrin	0.0156+0.0125	1.32 ml
Allectus	Imidacloprid + bifenthrin	0.03125+0.025	2.63 ml
Discus	cyfluthrin + imidacloprid	0.1875+0.045	27.09 ml
Onyx 23%	bifenthrin	0.025	0.473 ml
Onyx 23%	bifenthrin	0.0375	0.709 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	2.84 + 1.18 ml
Talstar+Marathon	bifenthrin + imidacloprid	0.1+ 0.1265	5.68 + 2.4 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. 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 – Standard Laboratory Bioassay).

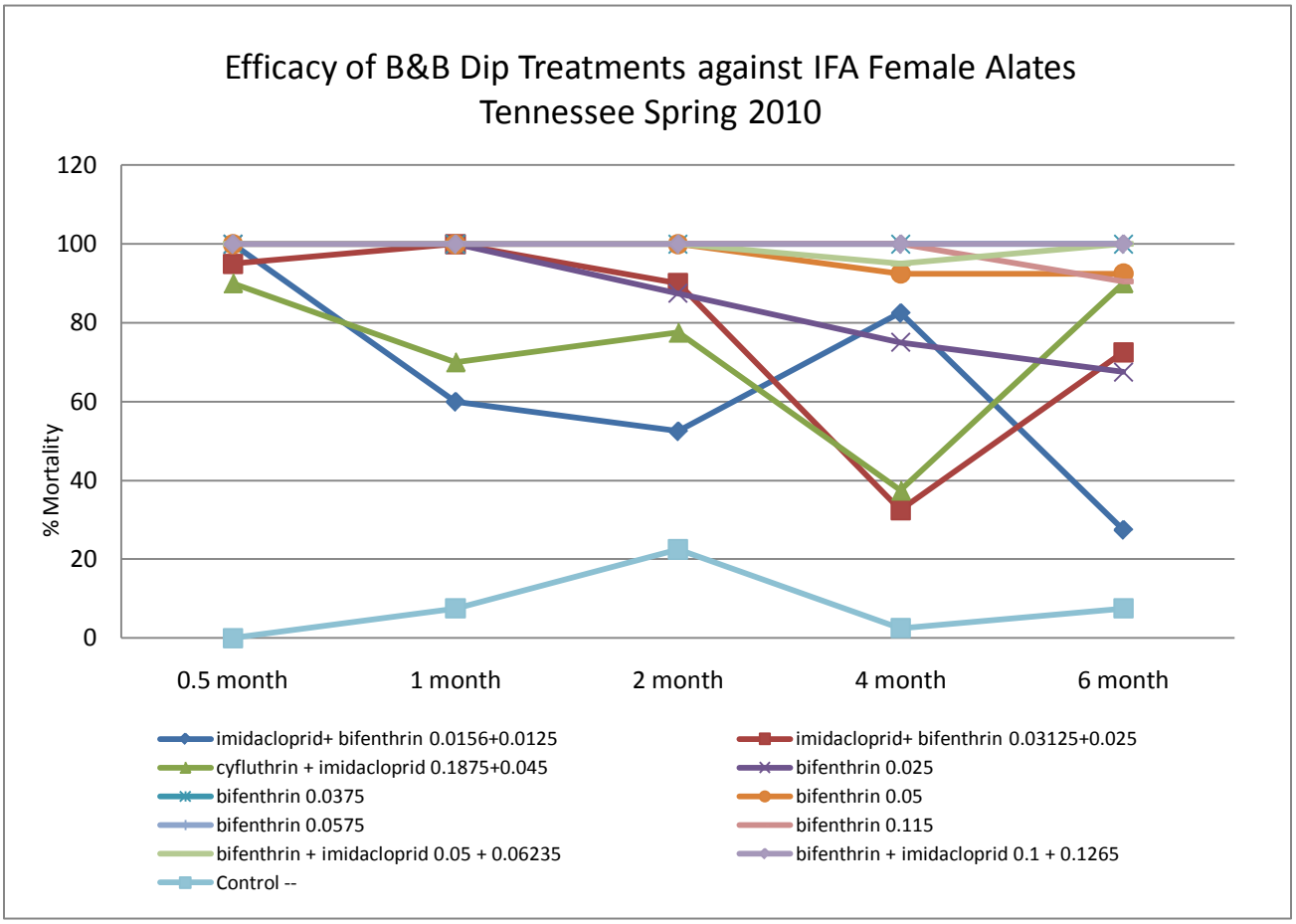
RESULTS AND DISCUSSION:

In the spring 2010 immersion trials with 12-inch root balls, treatments were mainly focused on the insecticide bifenthrin. One treatment was a combination formula of cyfluthrin and imidacloprid (available in the product Discus), which never reached 100% efficacy on IFA control anytime in the entire 6 month trial period at the rate tested. All the remaining treatments had bifenthrin as the major active ingredient (used either alone or mixed with imidacloprid, or in a combined formulation of the two insecticides). Since new lower rates of bifenthrin were used in this trial (0.025 lb ai per 100 gal water or lower, beginning in fall 2009 trial), some treatments failed to obtain quarantine level of control on IFA at one month or after the first month post-treatment. With bifenthrin rate at 0.025 lb ai used alone, for example, 100% efficacy was obtained only during the first month after treatment (Fig 1), indicating this treatment rate was too low for post-harvest dip treatment if 6 months quarantine level control is needed. Bifenthrin rates of 0.025 lb ai per 100 gallon water or lower with the addition of imidacloprid (in a formulated combination product Allectus) did not increase the efficacy for IFA control; the results were comparable to the treatments with bifenthrin used alone at those rates. Obviously, adding the

insecticide imidacloprid, through tank-mix application or formulated into a combined product, did not increase the efficacy of bifenthrin on IFA control. Clearly, bifenthrin concentrations at 0.025 and below do not hold up for 6 months even when combined with imidacloprid at tested rates.

In this trial, bifenthrin used alone at 0.0375 lb ai achieved 100% control for 6 months but the higher rate of bifenthrin at 0.05 did not obtain 100% control at 4 or 6 months. This certainly was an atypical result because the 0.05 lb ai rate has routinely provided 100% efficacy for the entire 6 months, even with the less consistent post-harvest drench application method. Rates above 0.05 lb ai of bifenthrin used alone or in combination with imidacloprid achieved 100% efficacy as expectedly for entire trial of 6 months post treatment. This trial confirmed the results of our previous immersion trials in which bifenthrin at a rate of 0.025 lb ai per 100 gal of water or lower, used in combination with another insecticide, did not last for 6 months with 100% control of the IFA.

Figure 1. Efficacy of B&B immersion treatments against IFA female alates in 12-inch root balls; Tennessee spring 2010.



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: 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 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.

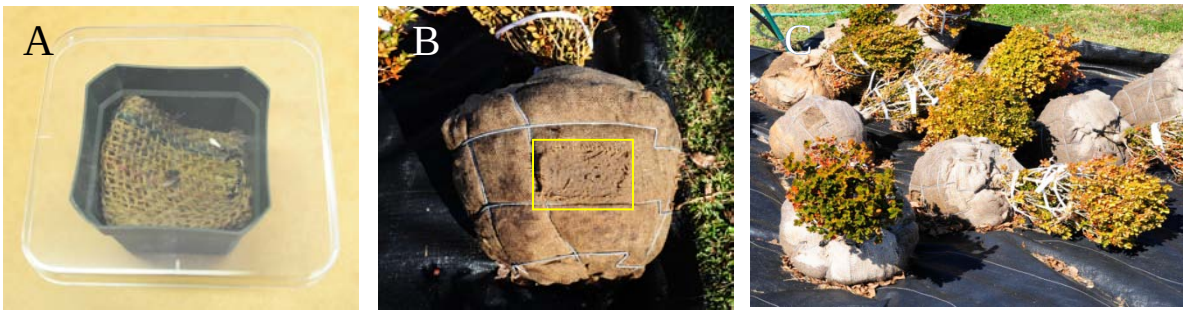


Fig 2. A): Method of choice--apparatus for bioassay conducted in the lab. B): rootball showing a piece of burlap was removed for bioassay in the lab; soil sample was also collected from where burlap was cut out (within yellow rectangle). C): Balled & Burlapped nursery stock stored outdoors under simulated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

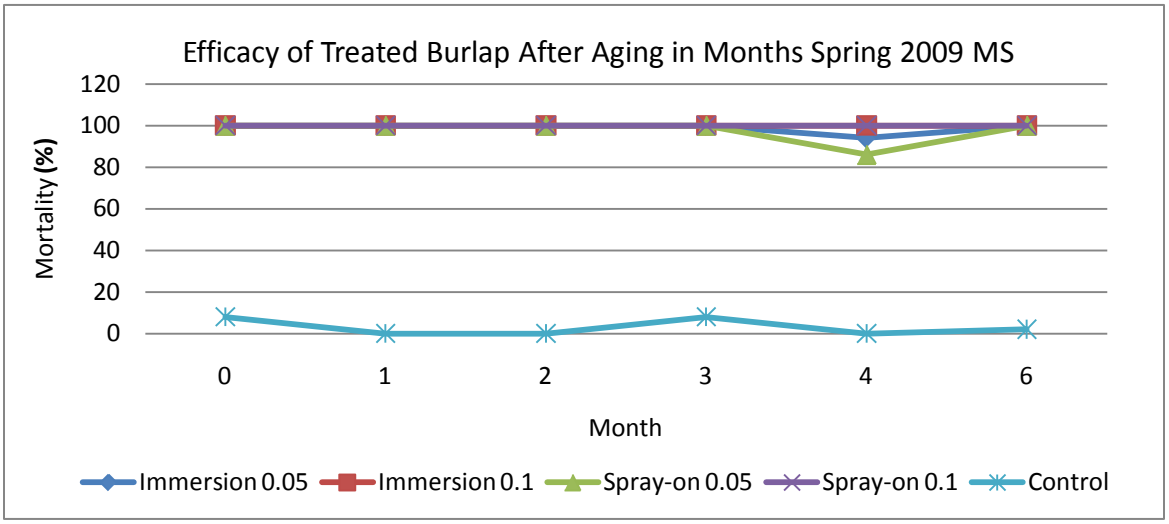


Fig. 3. Efficacy of bifenthrin-treated burlap on female alates of RIFA after aging outdoors under simulated nursery storage conditions.

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.

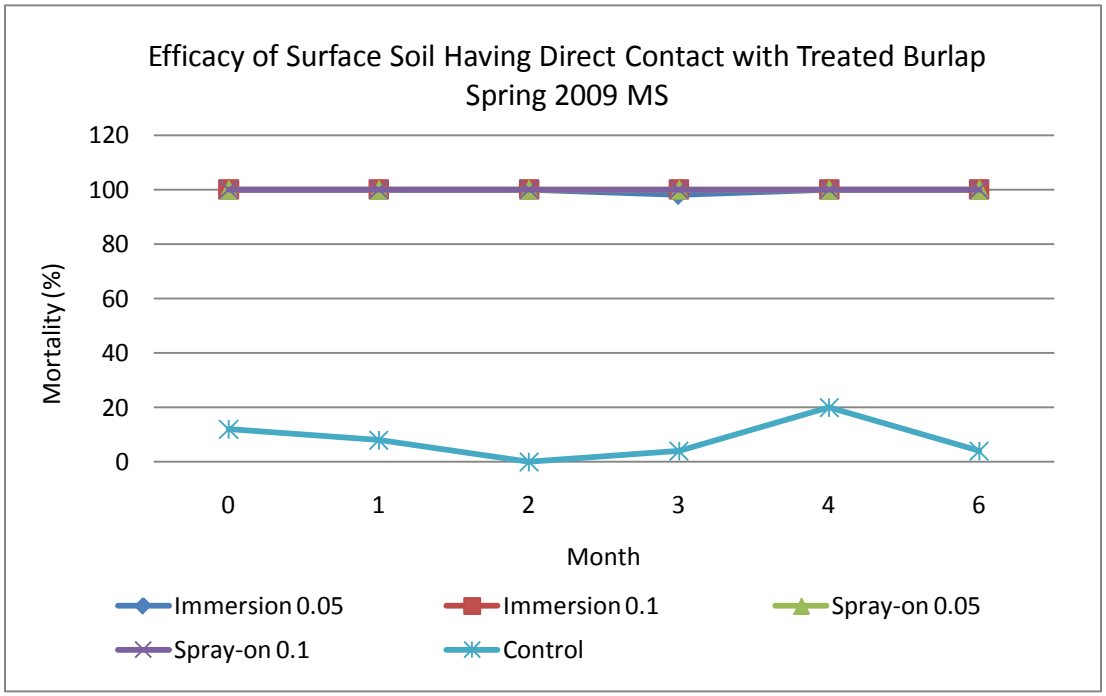


Fig. 4. Bioassays results for surface soil having close contact with the bifenthrin-treated burlap after aging outdoors in simulated nursery storage conditions over 6 months.

Fall 2009 bioassay

All treated burlap bioassays conducted from the beginning of the trial through the end of six months post-treatment obtained a 100% efficacy (Fig. 5). Similarly to the spring 2009 trial, there were no differences in efficacy between burlap treatment methods (pretreated "immersion" vs. post-harvest "spray on"). Consistent results among various bioassays conducted at different times during the 6 month trial showed that bifenthrin remaining in the burlap was lethal enough for fire ant alates even at the end of the trial.

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.

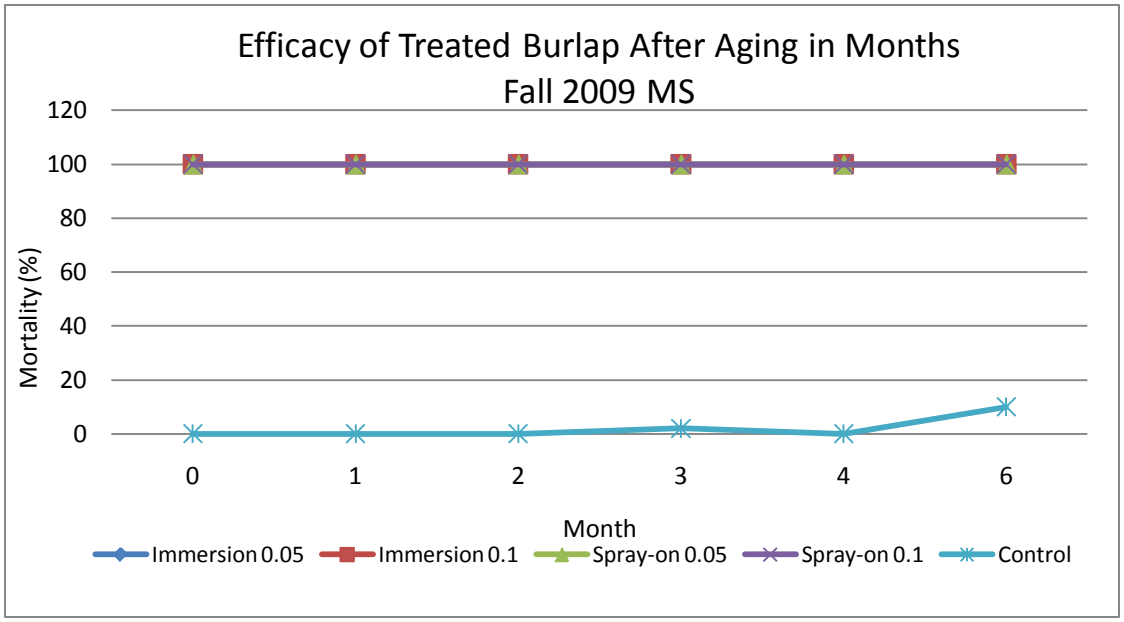


Fig. 5. Efficacy of bifenthrin-treated burlap on female alates of RIFA after aging outdoors under simulated nursery storage conditions.

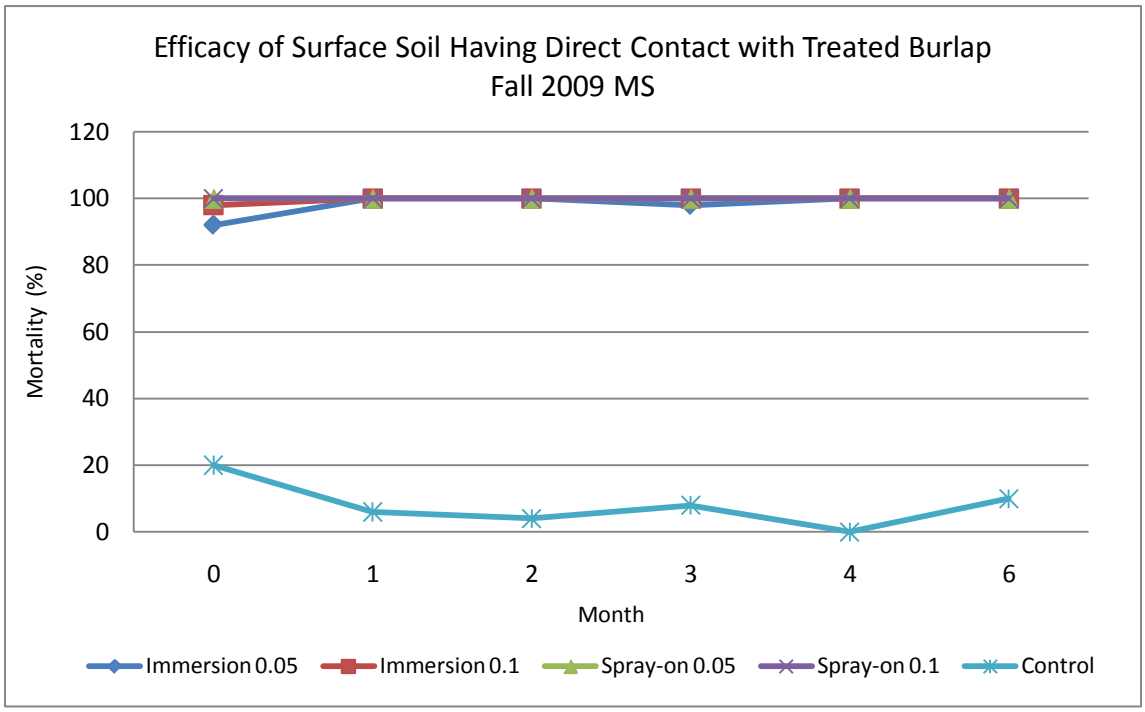


Fig. 6. Bioassays results for surface soil having close contact with the bifenthrin-treated burlap after aging outdoors in simulated nursery storage conditions over 3 months.

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). Comparable difference was also found at 0.05 lb ai treatment 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. Changes in bifenthrin concentrations for the following 3 months were slower, with remaining concentration of 7 ppm or greater being found at the end of the 6-month trial which were still quite potent in killing the fire ant alates used in bioassays.

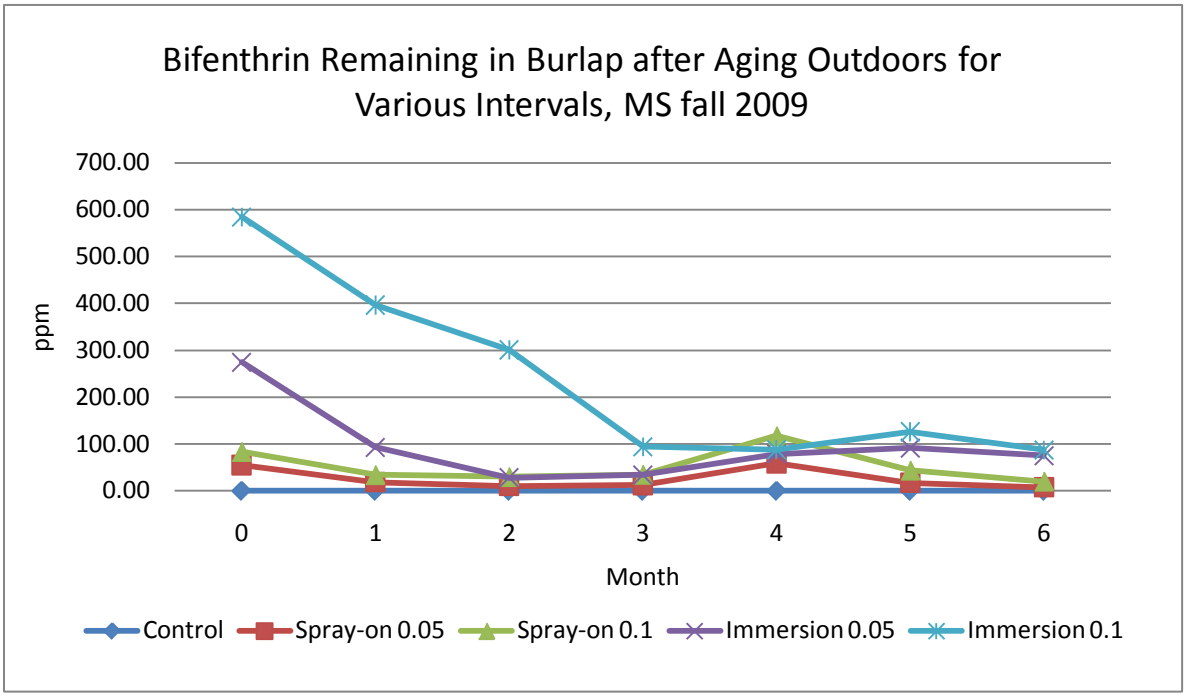


Fig. 7. Bifenthrin in treated burlap at various points of time during the 6 months post-treatment aging under simulated nursery storage conditions.

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 trials, long enough to protect the B&B nursery stock while awaiting shipment. The results of fall 2009 trial confirmed the findings of the spring

trial. After six months of aging, all treatments are still potent enough to achieve 100% effectiveness in both seasons.

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 also 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 residual concentrations of 7 ppm to 90 ppm at 6 months after treatment, way above the required concentration of 3 ppm to kill fire ant queens.

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) or two 5 gallon buckets (see 2010 annual reports) 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 and fall 2009 trials. 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 lethal for 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 5-gallon Bucket in Mississippi Fall 2010

REPORT TYPE: Final

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

INTRODUCTION

APHIS is responsible for developing treatment methodologies for certification of regulated commodities, such as field grown balled-and-burlapped nursery stock, 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 5-gal bucket drench treatment method 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

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 April 7, 2010. Trees included in the trial were selected with 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 trees with drench holes on the buckets facing toward the base of each tree (Fig. 1 A&B). A water tank mounted on the bed of a pickup truck was used to carry water to the treatment field. Buckets were first filled half way full and insecticide and liquid dye (included to help visualize penetration of drench solution in the soil) were both added to the buckets 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). Five trees were used in each of the list treatments with 10 additional trees were utilized in both the 0.025 lb ai rate of bifenthrin treatment and the control treatment for the purpose of being used for whole colony bioassay; a total of 45 trees being treated in this trial. At application, 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 for each 5-gal bucket, 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 on April 9, 2010 by the nursery grower with a machine harvester and harvested trees with 18” diameter root ball wrapped in plain burlap of 7.5 oz weight were transported to Gulfport Lab. The burlap on the root balls was sprayed with bifenthrin at 0.05 lb ai/100 gal of water (0.94 ml Onyx Pro per gallon of water) except trees in the control group. This same rate of bifenthrin solution was sprayed to all chemically treated trees regardless what chemical and rates the trees had received at the previous bucket-drench in the field. Two and a half (2.5) gallon solution was used for every 10 trees. 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.

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 root balls 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 (Fig 3). 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 spring 2010

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.

Soil samples were also collected from the surface (about 1 cm deep) of the rootball where the burlap was removed (Fig 2) to determine if the soil that has direct contact with the treated burlap would also kill the ant as the burlap does. The bioassay method for the soil samples was the same as that for burlap pieces.

To do the bioassay, ten field collected female alates were used for each burlap or soil sample taken from a root ball. Five replicate root balls 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.

Intact root ball tested with whole colony:
At both 3 and 6 months after the final treatment, intact root balls were subjected to a whole colony bioassay to evaluate if the aged treated burlap could withstand the pressure of IFA invasion while in storage outdoors with direct ground contact. The test plants were part of the 45 box woods dug up after bucket-drench treatment in the field. Five control plants and five bifenthrin-treated plants (bucket-drench with 0.025 lb ai and burlap sprayed with 0.05 lb ai) were used at each bioassay. Plants at the time of testing were kept in 26” diameter by 7” deep (66 x 18 cm) plastic Plantainer™ pans (Mac Court, Denver, CO) which were painted on the inside surface with Fluon (AGC Chemicals Americas Inc., Bayonne, NJ) to prevent ant escape (Figure 4). Wild collected IFA were removed from their nest soil by dripping, and at infestation approximately a 1/3

the plant stem shaken to agitate any live IFA in the soil. If workers appeared within a minute of this disturbance, the plant was considered to have an active infestation. Plants that did not show active infestation at day 14 were split open and searched for live IFA.

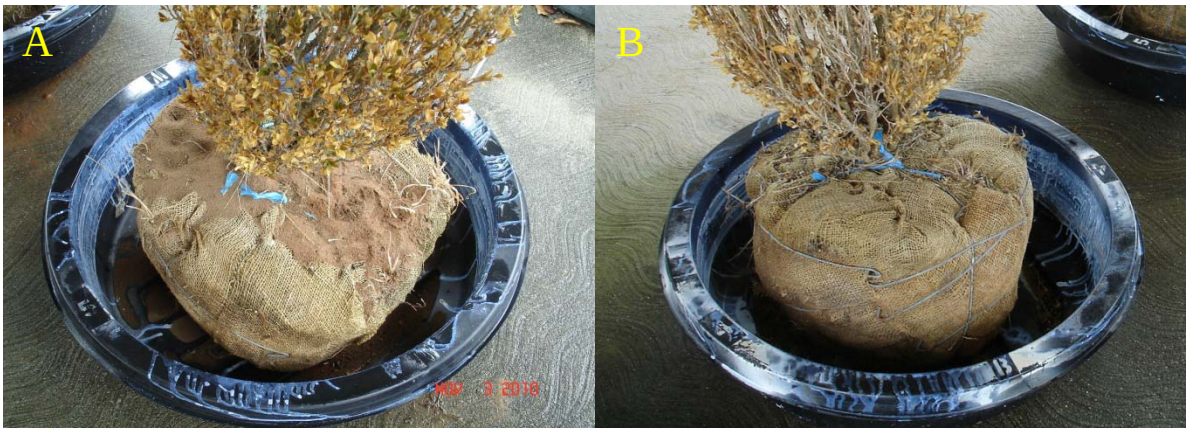


Fig. 1. Pre-harvest in-field drench using 5-gallon buckets to trees to be harvest in a nursery at Deep South Nursery, George County, Lucedale, MS. A: drench in rows of Japanese Boxwood. B: close look of drench application to a tree.

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

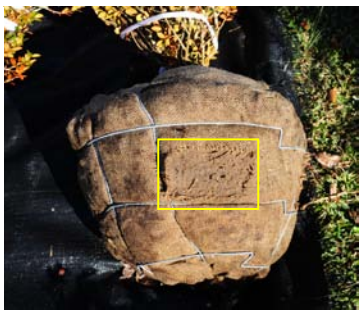


Fig 3. Set up of burlap bioassay conducted in the lab.

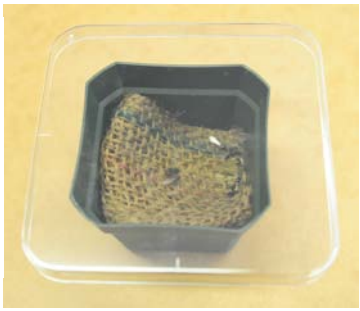


Figure 4. Intact root ball bioassay at 6 months after treatment. A: an infested root ball at observation with mound soil build-up on top of the root ball indicating an active infestation. B: an un-infested root ball at observation with no ant activity seen.

RESULTS

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 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.

As it was in previous 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 the burlap bioassays conducted monthly in the 6 month post-treatment period, 100% efficacy was achieved in each of the bioassays (Fig. 5). These results showed that burlap sprayed with bifenthrin at 0.05 lb ai/100 gal water could protect root balls from newly mated fire ant queens for at least 6 months after treatment under normal weathering outdoors. Surface soil samples collected from root balls in all treatments also achieved mostly 100% efficacy through 6 months (Fig. 6) which is an additional enhancement to the protections provided by the treated burlap wraps.

The results of whole colony bioassay with intact root balls at both 3 and 6 months after the final treatment indicated that the aged burlap could withstand the pressure of IFA invasion while in storage outdoors with direct ground contact. IFA colonies in the test made nests in each of the untreated control root balls but failed to start an infestation on any of the treated root balls (Fig. 4 A&B). Clearly, the bifenthrin-treated burlap can protect the root ball from IFA invasion while in storage outdoors with direct ground contact through 6 months after final spray treatment.

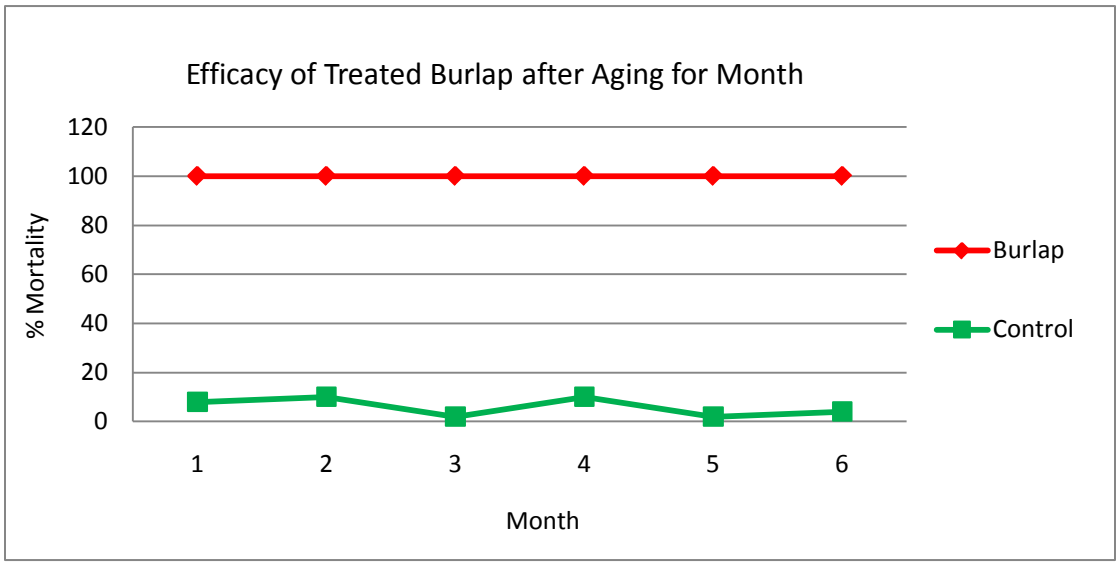


Fig. 5. Efficacy of bifenthrin-treated burlap on female alates of RIFA after aging for various period of time outdoors under simulated nursery storage conditions, fall 2010, Mississippi

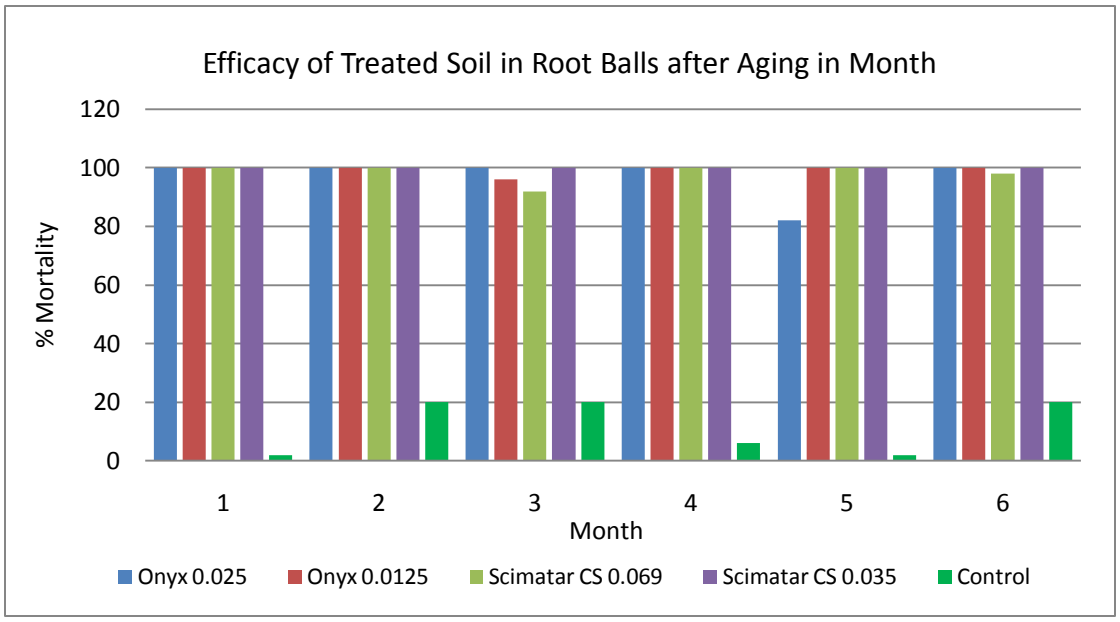


Fig. 6. Efficacy of bifenthrin- or lambda-cyhalothrin-treated soil on female alates of RIFA after aging for various period of time outdoors under simulated nursery storage conditions, fall 2010, Mississippi

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 root balls 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.

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 that bifenthrin travels freely to whatever depth the dye travels in the soil in our previous trials, dye can be used to tell how deep the chemical/toxic zone reaches.

Although it is impossible to separate whether the insecticidal effect of the surface soil was due to the in-field insecticide treatment or from the chemical residues acquired through close contact with the bifenthrin-treated burlap, all it matters is that the soil portion of the root balls will add to the killing of newly mated fire ant queens if they land onto the root balls while in storage

outdoors or in transportation. Bioassay results showed that both treated burlap and surface soil were doing just that.

The results of whole colony bioassay with intact root balls confirmed our laboratory bioassay which indicated that the aged burlap could withstand the pressure of IFA invasion while in storage outdoors with direct ground contact. Clearly, the bifenthrin-treated burlap can protect the root ball from IFA invasion, either by newly mated queens landing on the root balls or by IFA whole colony moving into the treated root balls, while in storage outdoors with direct ground contact for at least 6 months after final spray treatment.

CONCLUSIONS

1. In-field chemogation with 5-gal buckets could penetrate the root ball area in sandy loam soil.
2. When applied at 0.05 lb ai per 100 gal water, bifenthrin-treated burlap and the resulting toxin surface soil of the root balls could kill IFA whole colony including female alates for at least 6 months under normal weathering conditions.

CPHST PIC NO: Umbrella IFA Quarantine Treatments

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

TYPE REPORT: Final

LEADER/PARTICIPANTS: Mississippi – 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 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: These plots had considerable rainfall after all treatments and throughout the test; December 2009 had 26 inches of rainfall and no evaluations were conducted during the entire month. While monthly average temperatures were normal through December 2009, January and February 2010, 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. The last reliable evaluation was conducted at 33 weeks after treatment, at which time two of the bait + contact treatments, as well as the lowest rate of the bifenthrin and lambda-cyhalothrin tank mix treatment, had active colonies reinfesting the plots. At 39 weeks after treatment, the owner had begun harvesting sod from some of the plots, therefore reliable data was not available. However, of the plots that were able to be evaluated, several treatments were still effective: the dual application bifenthrin liquid, the 3X Hero, the highest rate of the tank mix, and two of the bait + contact treatments.

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.

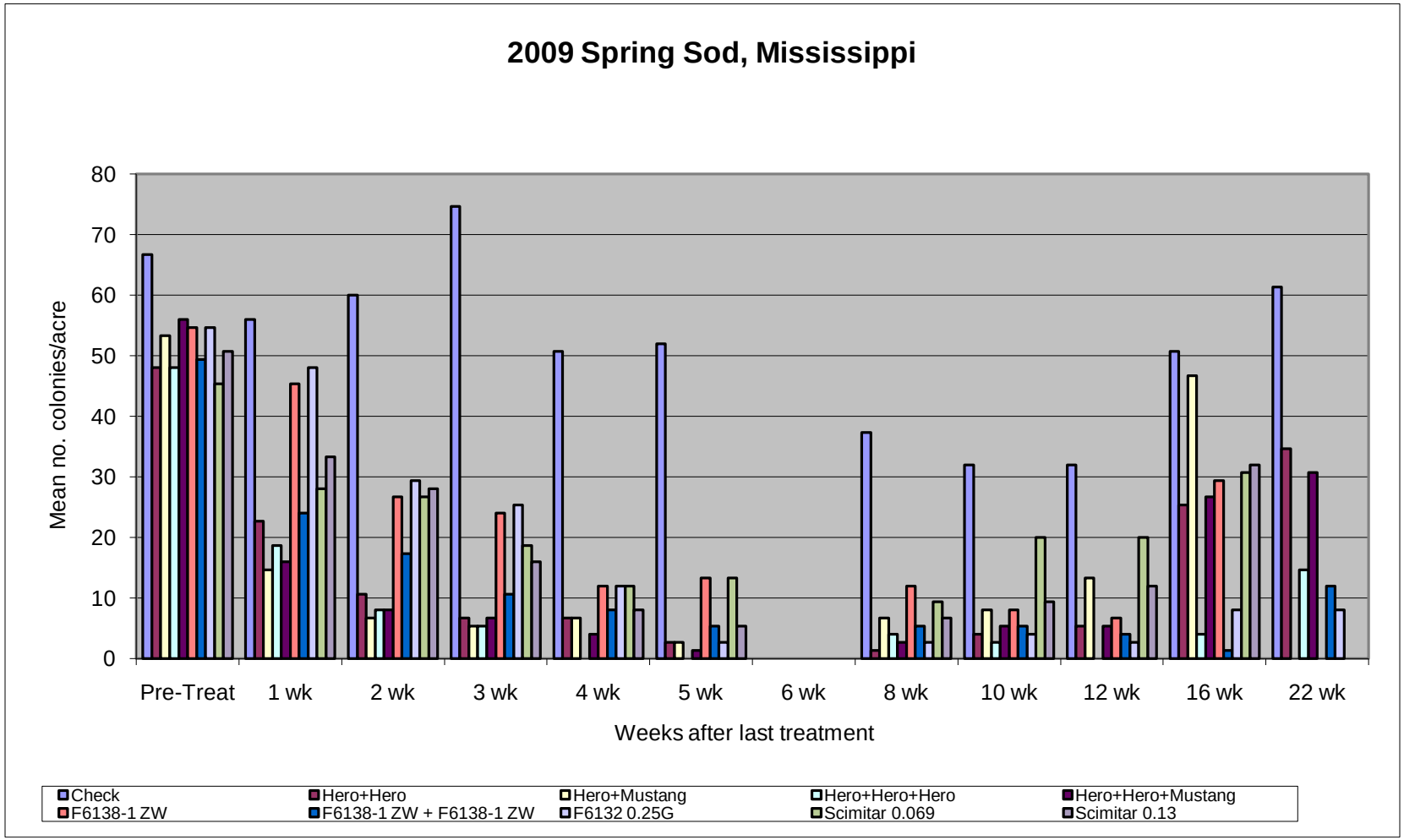
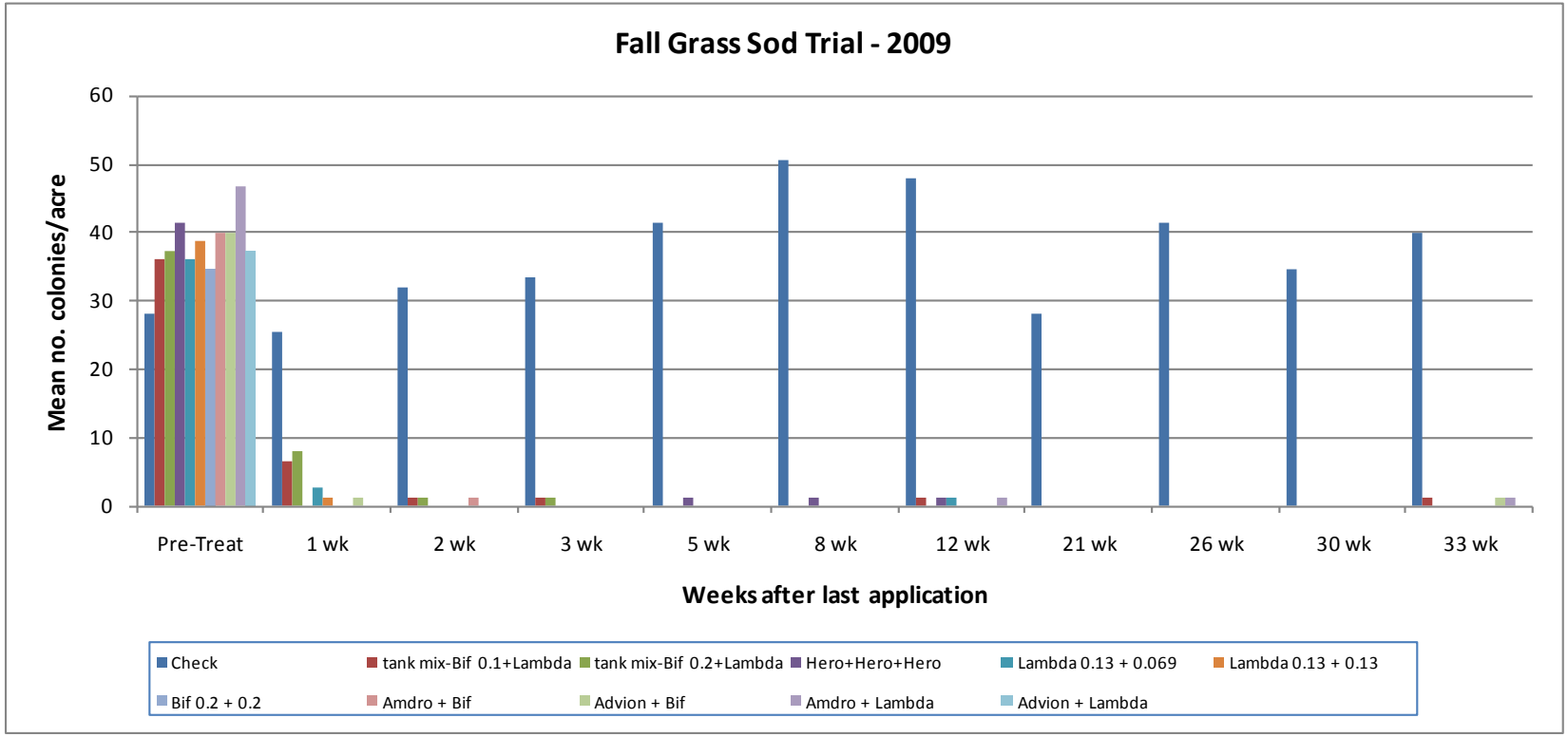


Figure 2. Efficacy of grass sod treatments in Mississippi in fall 2009.



CPHST PIC NO: Umbrella IFA Quarantine Treatments

PROJECT TITLE: Efficacy of New Candidates as Grass Sod Treatments: Mississippi, Spring and Fall 2010

TYPE REPORT: Fianl

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

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 2010 trial were fields located in Pearl River Co, near the community of Henleyfield, MS. The fields were regularly fertilized and mowed by the grower but did not have irrigation available. The test sites for the fall 2010 trial were fields located in Pearl River Co. northeast of Poplarville, MS. The fields were regularly fertilized and mowed by the grower but did not have irrigation available.

Plots were 0.52-acre square in size for all treatments (150’ x 150’). On plots receiving bait plus a contact insecticide, the bait was applied to 147’ x 150’ of the plot to accommodate the bait spreader we use. The contact insecticide application on the same plot was applied to the full 0.52 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 or monthly 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. 32.5 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 baits or contact insecticides. If multiple applications were made on one plot (e.g. bait followed by contact or 2 applications of lambda-cyhalothrin) applications were made approximately one week apart. In the spring 2010 trials, two tank mixes were applied. Both insecticides were mixed in one tank of water and applied simultaneously.

Spring trials were initiate in early May, 2010, with the first part of treatments (baits or first contact application of a dual application) applied the week of May 6, 2010 and the second part of the treatments applied the week of May 13, 2010. Treatments and rates are listed below.

Spring Mississippi rates of application:

Trade Name	Active Ingredient	Rate of Application (lb ai/acre)	Date of last application
Amdro® *plus* Bifenthrin EC (Onyx®Pro)	Hydramethylnon *plus* bifenthrin	1.5 lb bait/acre *plus* 0.2 lb ai/acre bif (1 wk apart)	5/13/10
Amdro® *plus* Bifenthrin G	Hydramethylnon *plus* bifenthrin	1.5 lb bait/acre *plus* 0.2 lb ai/acre bif (1 wk apart)	5/13/10
Advion® *plus* Scimitar®	Indoxycarb *plus* lambda-cyhalothrin	1.5 lb bait/acre *plus* 0.13 lb ai/acre lambda (1 wk apart)	5/13/10
Bifenthrin EC (Onyx®Pro) + 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	5/14/10
		Tank mix = 0.2 bif + 0.13 lambda	5/14/10
Scimitar®	Lambda-cyhalothrin	0.13 lb ai/acre + 0.13 lb ai/acre	5/14/10
Control	untreated		--

Due to a change in the allowed labeled rates of lambda-cyhalothrin, we have dropped this insecticide from our grass sod trials. We were approached by Arysta Life Sciences to include their clothianidin + bifenthrin product, Aloft®, in our fall 2010 and spring 2011 grass sod trials. Also, the bifenthrin G product of Talstar® changed in mid-2010 to a combined bifenthrin/zetacypermethrin product. Fall trials were initiated in late August-early September, 2010, with the first part of treatments (baits) applied the week of August 25, 2010 and the second part of the treatments applied the week of August 31, 2010. Treatments and rates are listed below.

Fall Mississippi 2010 rates of application:

Trade Name	Active Ingredient	Rate of Application (lb ai/acre)	Date of last application
Amdro® *plus* Bifenthrin EC (Onyx® Pro)	Hydramethylnon *plus* bifenthrin	1.5 lb bait/acre *plus* 0.2 lb ai/acre (1 wk apart)	9/1/10
Amdro® *plus* Bifenthrin F (Talstar® Select)	Hydramethylnon *plus* bifenthrin	1.5 lb bait/acre *plus* 0.2 lb ai/acre (1 wk apart)	9/1/10
Amdro® *plus* Bifenthrin/Zeta G (Talstar® Xtra)	Hydramethylnon *plus* bifenthrin+ zetacypermethrin	1.5 lb bait/acre *plus* 0.2 bif + 0.05 zeta (1 wk apart)	8/31/10
Amdro® *plus* Aloft® GC SC	Hydramethylnon *plus* clothianidin+ bifenthrin	1.5 lb bait/acre *plus* 0.2 lb ai/acre bif (1 wk apart)	9/2/10
Bifenthrin/Zeta G (Talstar® Xtra)	bifenthrin+zeta cypermethrin	0.4 bif + 0.1 zeta	8/31/10
Aloft® GC SC	clothianidin+ bifenthrin	0.2 cloth + 0.1 bif	9/2/10
		0.4 cloth + 0.2 bif	9/2/10
Control	Untreated		--

RESULTS:

Spring Mississippi: All treatments decreased IFA populations compared to the control, but only the bait + bifenthrin EC (0.2 lb ai/acre rate) provided 100% control (Figures 1&2). This treatment achieved 100% control by 2 weeks after the contact insecticide treatment and maintained that control until reinfestation was noted at 17 weeks after treatment. The bait + bifenthrin G and bait + lambda-cyhalothrin treatments both reduced populations significantly between 2 and 6 weeks, but never acquired 100% control, and both had reinfestation occurring at 9 weeks, and thus were not evaluated after 14 weeks.

The contact only treatments were not as effective as the bait plus contact insecticide treatments (Figure 2). The lambda-cyhalothrin dual treatment provided 45-68% control between 2 and 4 weeks after treatment with reinfestation noted at 6 weeks. The low rate tank mix achieved 79% control by 6 weeks, but reinfestation was noted at subsequent evaluations. The high rate tank mix achieved 85% control by 9 weeks, with reinfestation noted in the following evaluation. Due to reinfestation in all contact only plots, this portion of the trial was terminated after the 14 week evaluation.

Figure 1. Efficacy of bait + contact insecticide grass sod treatments in Mississippi in spring 2010 (the bifenthrin 0.2G and lambda-cyhalothrin 0.13 treatments were not counted at week 17 or 20 due to high rates of reinfestion at week 14).

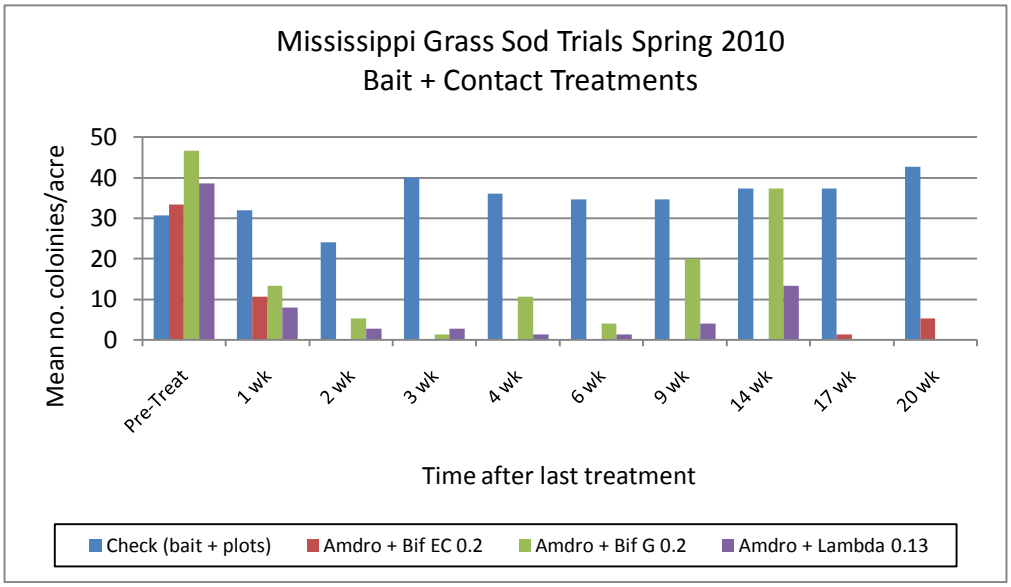
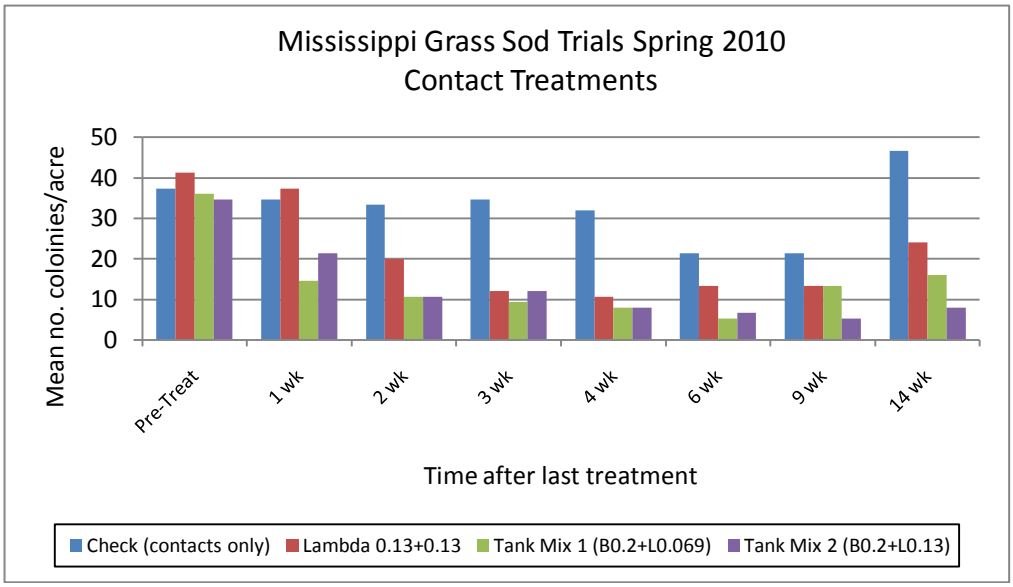


Figure 2. Efficacy of contact insecticide grass sod treatments in Mississippi in spring 2010.



Fall Mississippi: We were not able to conduct evaluations at weeks 3 and 5. All bait + contact insecticide treatments significantly reduced IFA populations (Figure 3). Both the bait + bifenthrin F and the bait + clothianidin/bifenthrin product (Aloft) achieved 100% control by 2 weeks after treatment and maintained that control through 33 weeks, except for 1 small mound on 1 replicate of each treatment at week 11. The bait + bifenthrin EC maintained 86-97% control from week 1 through week 28, falling to 80% control at week 33. The bait + bifenthrin G

provided >90% control from week 1 through week 11, finally reaching 100% control at week 15 and maintaining that control through week 33. The trial was discontinued after the week 33 evaluation due to the grower's needs.

The contact only treatments were slower to reduce IFA populations than the bait + contacts treatments (Figure 4). The high rate of the clothianidin/bifenthrin product did achieve 95% control by week 4 and maintained >89% control through 28 weeks. The lower rate was more erratic with control. The bifenthrin/zetacypermethrin product was still very slow acting and the addition of the zetacypermethrin did not appear to enhance the efficacy of the bifenthrin. The trial was discontinued after the week 33 evaluation due to the grower's needs.

Figure 3. Efficacy of bait + contact insecticide grass sod treatments in Mississippi in fall 2010.

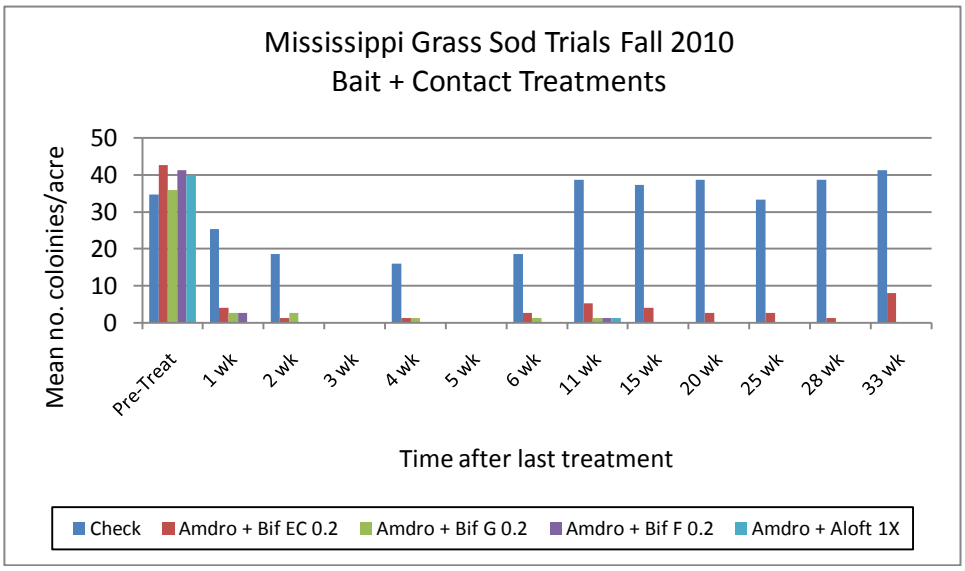
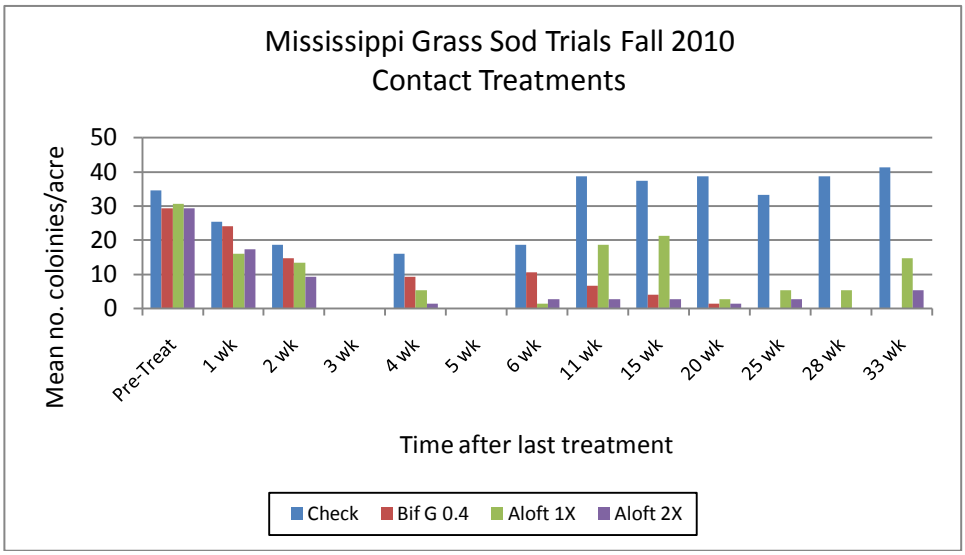


Figure 4. Efficacy of contact insecticide grass sod treatments in Mississippi in fall 2010.



DISCUSSION:

Temperatures were somewhat below average for the 2010-2011 winter. The actual December 2010 and January 2011 average lows were 34.7°F and 35.4° F, 6°F and 3°F degrees below the normal, respectively. Numerous below average low temperatures between weeks 11 and 20 may have impacted IFA mortality in weak colonies during that period.

The use of a bait in conjunction with a contact insecticide is generally providing better control of IFA at lower rates of application in grass sod than contact insecticides alone. All of these treatments, except the low rate of clothianidin/bifenthrin alone, will be repeated in spring 2011. There will also be a summary of grass sod trials from 2008-2011 produced for the 2011 annual report.

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 117 field releases of phorid flies and more than 933,000 potential flies released. Of these 117 releases, 67 were *P. tricuspis,* 39 were *P. curvatus* and 11 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 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 65% of the regulated area. *P. curvatus* has not been released in CA. Overwinter establishment of *P. obtusus* has been confirmed. A publication on the known U.S.-wide distribution of *P. tricuspis* and *P. curvatus* is currently under review for publication in the Journal of Insect Science. This publication has an excellent history of the APHIS program, other federal and state/university release programs, maps depicting distribution in 2008 and expected distribution in 2011, and a discussion of the future of new species releases.

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. This has decreased our average number of potential flies for each release.

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 reared through 2011 in limited quantities with the aim to phase out production in 2011 and eliminate rearing of this species totally in 2012. *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 and 11 releases to date. In 2010, rearing was initiated on the fourth species, *P. cultellatus*, with the first releases anticipated in

2011. Except for 2009 when production levels were above 3,000,000, total fly production levels have remained fairly constant in the last several years (Table 1).

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

Releases began in spring 2002. In most cases, the cooperator made the release at one site, however, in a few cases the cooperator split the release and released flies at more than one site. Also, there are several sites were multiple releases over several years have occurred. From 2002 through 2009 there have been multiple releases in each of 13 states and Puerto Rico, with a total of 117 field releases and more than 1,000,000 potential flies released. Of these 117 releases, 67 were *P. tricuspis*, 39 were *P. curvatus* and 11 were *P. obtusus* (Table 1). 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 and maintain that level through 2010.

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 225,000 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 also well established in all southern IFA regulated states and PR (AL, AR, FL, GA, LA, MS, NC, OK, SC, TN, TX, and PR), and appears to be better suited to life in the U.S. than the *P. tricuspis*. *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 in the Journal of Insect Science with publication expected in early 2011. 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, P. cultellatus*).

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
tri,cur,obt,cul	2010†††	2,571,357	76,221	12	6,351.75
Total		21,235,652	1,009,398	117	8,594.44

* approx. no. potential flies shipped for release
** does not include multiple shipments to LA for initiating their own rearing facility and NM for research purposes, nor multiple shipments to cooperators for educational purposes or small research projects as flies were available
*** shipped for all purposes, field release, initiate rearing, education, etc.
† only tricuspis shipped in 2002
†† only tricuspis and curvatus shipped in 2006 and 2007
††† only tricuspis, curvatus and obtusus shipped in 2010

2010 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 2010. 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 EDP.

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 2010, 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:

- 150 total samples submitted (chemistry unit counts 233 samples as explained below)
  - 124 nursery samples
  - 26 blitz samples from NC (blitzes in spring and fall) (these are counted as 2 samples each for the chemistry unit since the multiple analysis requires splitting the sample to move down 2 separate paths and therefore must be tracked separately)
- 0 samples from potential violations
- 124 routine samples
  - 20 samples requesting bulk density only
  - 47 samples requesting chemical analysis only
  - 57 samples requesting chemical analysis and bulk density (these are counted as 2 samples each for the chemistry unit since chemical analysis and bulk density move down separate paths and therefore must be tracked separately)

Results:

26 blitz samples from NC

22 of 26 total samples (85%) had detectible levels of program insecticides

25 containerized media (21 with insecticide present; 4 below detectable levels)

1 B&B media (1 with insecticide present)

124 routine samples

77 bulk density samples: range 182-731 lb/cu yd

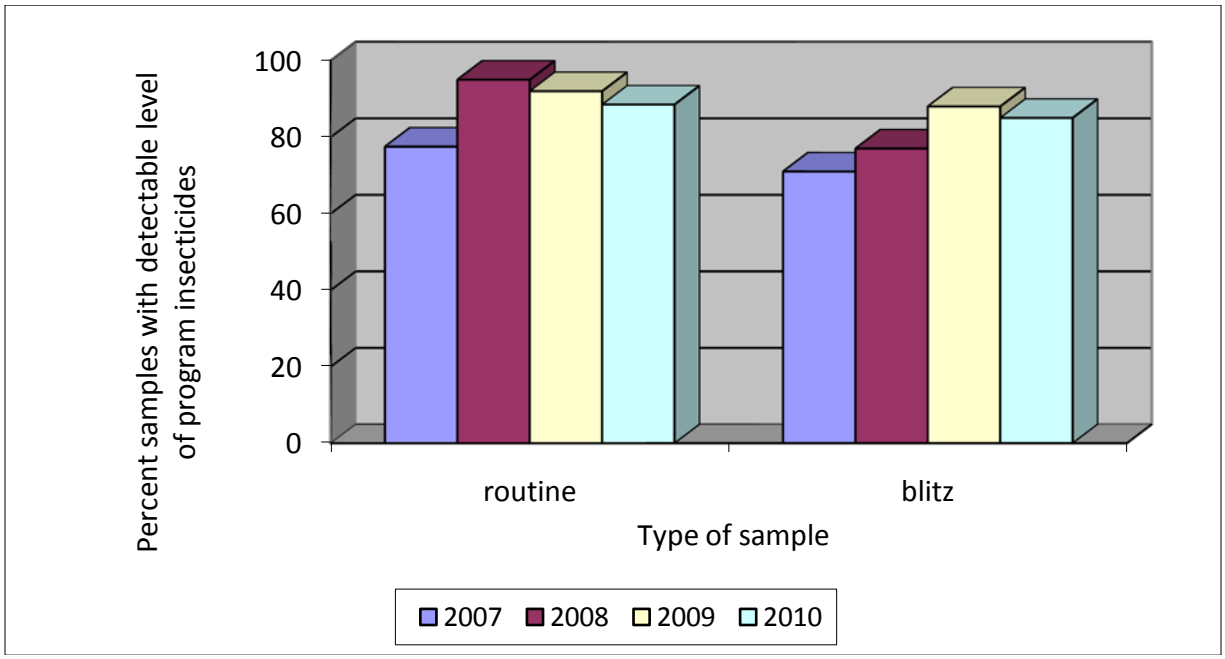
104 samples analyzed for 1 or more program insecticides

102 container media: 2 field/B&B media

92 samples (88.5%) had detectible levels of program insecticide(s)

12 remaining samples – no program chemicals detected

Percent of routine and blitz samples with detectable levels of program insecticides by year from 2007-2010.



APPENDIX I - LABORATORY BIOASSAY PROCEDURE

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

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

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

Test chambers: Test chambers are 2.5" x 2.5" plastic flower pots which have been equipped with a Labstone® bottom. Labstone is generally available through dental supply firms such as Nowak Dental Supplies, 8314 Parc Place, Chalmette, LA 70043 (800-654-7623). The labstone bottom prevents the queens from escaping through the drain holes in the bottom of the pot and

also serves as a wick to absorb moisture from an underlying bed of wet peat moss. Ants are susceptible to desiccation so humidity/moisture levels must be optimized. Pots should be soaked in water to moisten the labstone prior to placing potting media in the pots. The peat moss bed should be watered as needed to maintain a constant supply of moisture to the test chamber. Plastic petri dishes are inverted over the tops of the pots to prevent escape from the top of the test chambers (Figure 2). Prior to placing queens in the test chamber, 50 cc of treated potting media is placed in the bottom of each pot. Each test chamber with test media and queens is placed in a tray with a bed of wet peat moss (Figure 3). Due to possible pesticide contamination, test chambers are discarded after use.

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

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

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

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

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

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

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

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

Figure 1. Alate females being removed from nest tumulus.

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

Figure 3. Set up of bioassay test procedure.

PROJECT TITLE: Overview of Analytical Chemistry Routine and Program Support in 2010

TYPE REPORT: Final

LEADER: Robert Smith

INTRODUCTION:

The CPHST-GL chemistry unit is located in Gulfport, MS. The chemistry unit provides a laboratory support service for many APHIS on-going treatment programs and researchers. In 2010 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 2010?

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

Asian Longhorn Beetle Eradication Program (ALB).
Asian Citrus Psyllid (ACP) * new program in 2010
Boll Weevil Eradication Program (BW)
Rangeland Grasshopper and Mormon Cricket Suppression Program (RGMC)
Fruit-Fly Attractants and Insect Lures (FF)
Imported Fire Ant Quarantine (IFA)

So what was our strategy in providing APHIS support?

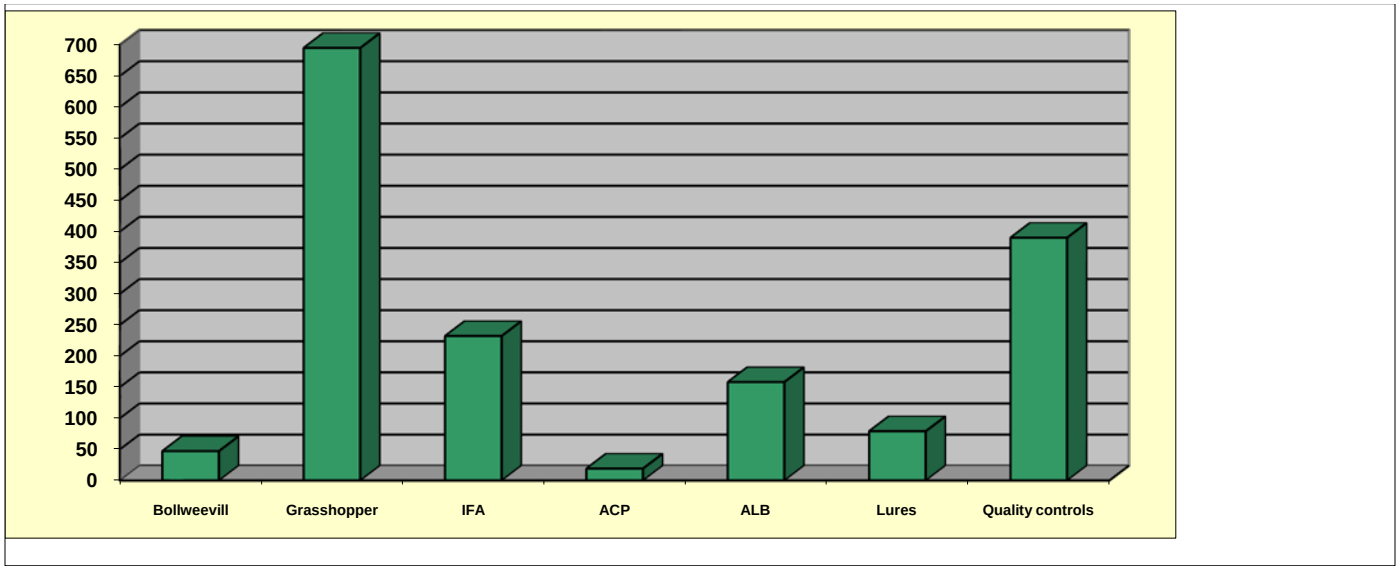
Our strategy was based simply on full commitment to customer support. Our focus was on the following key areas:

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 2010?

In 2010, CPHST-GL completed work on **1,620 APHIS program related samples.** Program related samples are any and all samples analyzed to obtain a valid high quality determination, directly supporting the program. *** CPHST-GL does not control how many samples are taken, since this is related to program activity.

2010 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 on the following page.

2010 delivery times by program.

Program	Groups / samples run (includes required QA)	Actual Average delivery time (receipt to report)
Imported Fire Ant Quarantine (IFA)		
Bulk Density in Soils	40 groups / 74 samples	3.77 days
Multi-residue in Soils (Bifenthrin, dursban, diazinon)	43 groups / 309 samples	6.19 days
Boll Weevil Eradication Program (BW)		
Malathion Purity/tank mix Verification **(ISO certified method)**	15 groups / 55 samples	3.47 days
Malathion in Vegetation	2 groups / 9 samples	3.22 days
Malathion in Fish	1 group / 3 samples	7 days
Malathion in Water	1 group / 3 samples	7 days
Asian Long-horned Beetle Program (ALB)		
Imidacloprid in Water	5 groups / 43 samples	6.02 days
Imidacloprid in Vegetation	2 groups / 63 samples	10.28 days
Imidacloprid in Soil	6 groups / 37 samples	8.51 days
Imidacloprid in Honey * new request	3 groups / 30 samples	8.67 days
Imidacloprid in Bees * new request	3 groups / 30 samples	8.30 days
Asian Citrus Psyllid (ACP)		
Imidacloprid	1 group / 12 samples	10 days
Chlorpyrifos (dursban)	1 group / 11 samples	10 days
Rangeland Grasshopper and Mormon Cricket Suppression Program (RGMC)		
Carbaryl in Dye Cards	4 groups / 34 samples	10.38 days
Carbaryl Percent in Baits **(ISO certified method)**	6 groups / 19 samples	6.02 days
Carbaryl Neat & Tank mix verification	5 groups / 30 samples	5.67 days
Carbaryl in Water * new request	1 group / 4 samples	16.0 days
Carbaryl in Vegetation * new request	1 group / 9 samples	4 days
Dimilin Neat & Tank mix verification **(ISO certified method)**	39 groups/ 259 samples	5.11 days
Dimilin in Soil * new request	6 groups/ 48 samples	6.96 days
Dimilin in Dye Cards	28 groups/ 368 samples	13.65 days
Dimilin in Water	6 groups / 30 samples	7.83 days
Dimilin in Vegetation * new request	8 groups/ 52 samples	9.56 days
Lures Evaluation and Fruit Fly programs		
Methyl eugenol verification	1 group / 2 samples	11 days
Ammonium Acetate lure verification * new request	2 groups/ 14 samples	33 days
Putrescine Lure verification * new request	2 groups / 14 samples	33 days
Trimethylamine Lure verification * new request	2 groups / 14 samples	33 days
Lineatine Flex Lure verification * new request	1 group / 5 samples	10 days
Trimedlure Plugs verification	7 groups / 35 samples	9.3 days
Trimedlure Liquid verification	2 groups/ 4 samples	6 days

***Data source CPHST-GL sample log books and chemistry Laboratory Information Management System (LIMS).*

How good was our quality?

In 2010**, 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 9001 Status: In 2010 CPHST-GL maintained ISO-9001 certification status.

What other sample work was conducted by CPHST-GL chemistry units in 2010?

In addition to conducting routine program sample analyses for APHIS chemical control programs, CPHST-GL conducted analysis on ~ **1,412 samples supporting projects** requested by other APHIS scientists and program leaders including:

Project/ Experiment	Groups / samples run (includes required QA)
Routine Program Support Work	
Imidacloprid in Honey method Validation	2 groups / 22 samples
Diflubenzuron in Vegetation method Validation	2 groups / 28 samples
APHIS/CPHST Experiments and Projects	
GC/MS Bifenthrin in Burlap (degradation study)	6 groups/ 102 samples
Bifenthrin in Soil (degradation study)	5 groups/ 77 samples
LC/MS Dimilin in Tank mix field trials	1 group / 11 samples
LC/MS Dimilin in Tank mix (RAATS)	1 group / 11 samples
Diflubenzuron in Dye Cards field trial	1 group / 6 samples
Vegetation pesticide screen	1 group / 16 samples
Carbaryl Bait field trial	1 group / 3 samples
GC/MS & GC/FID Khapra Beetle Lure	3 groups/ 33 samples
LC/MS Orange Grandlure	1 group / 5 samples
LC/MS White Grandlure	1 group / 5 samples
LC/MS Yellow Grandlure	1 group / 5 samples
Lure comparison for OTIS	2 groups/ 8 samples
CPHST-GL Methods Development (conducted by lead chemists)	
New Lure Methods development (extraction, instrumental analysis and emission rate studies)	~200 samples run during development process
Lure comparison and evaluation for CPHST-OTIS	~30 samples run during MD & analysis
Chlor-Tetracycline in insects for CPHST-Phoenix	~30 samples run during MD & analysis
Diflubenzuron in Vegetation "QUECHERS" method adaptations	~60 samples run during development process
LC/MS Emamectin Benzoate Methods Development and sample analysis	~250 sample run during development process
Carbaryl in Vegetation & soil Methods Development	~60 samples run during development process
Grasshopper program Field spray mix studies & related MD	~50 samples run during development process
Methods development for Imidacloprid in Bees, Honey and Wax	~150 samples run during development process
Z-Nose use in Identification of Signature Compounds	~250 samples run during development process

***Data source CPHST-GL sample log books and lead chemist's experimental logs.*

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, 2011 we have a staff of 14 (11 scientists and 3 non-scientific support staff).

SCIENTIFIC CORE EQUIPMENT

As January 1, 2011 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 Diode array and Fluorescence 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) * relocating to new building

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

If you have any specific questions concerning this report or you require additional information, please contact:

Robert David Smith, Supervisory Chemist
1-228-822-3112 (laboratory)
Robert.d.smith@aphis.usda.gov

TITLE: Impact of Surfactant on Diflubenzuron Tank Mixes

CPHST Staff: Lisa Mosser, chemist
Bich Tran, chemist
Nelson Foster, Phoenix, entomologist

OBJECTIVE:

To determine if the nonionic surfactant R-11® will negatively impact the analysis of diflubenzuron in tank mixes.

PROCEDURE:

Samples were submitted from the Grasshopper Suppression Program for analysis of diflubenzuron that had been prepared with R-11®. CPHST-GL Technical Procedure TPA-351, "Determination of Diflubenzuron in Technical Formulations and Tank Mixes" was used to conduct the analysis.

RESULTS:

Group # 4791(RAATs)
¾ fl oz Dimilin 2L, 0.1875 (3/4 of ¼ fl oz In-Place), 0.25% R11, up to 12 fl oz with water per acre

Sample ID	**ppm**
1	13600
2	13300
3	13500
4	13600
5	13800
6	13500
7	13400
8	13800
9	13700
10	13900

Group # 4792 (Traditional)
1 fl oz Dimilin 2L, ¼ fl oz In-Place, 0.25% R11, up to 12 fl oz with water per acre

Sample ID	**ppm**
1	19100
2	19100
3	19200
4	19200
5	18800
6	19400
7	19100
8	20000
9	18900
10	19300

CONCLUSIONS:

Based on chromatography of samples as well as data results there is no interferences that would impact the quantification of dimilin with the introduction of the nonionic surfactant R-11® in the preparation of tank mixes.

The expected ppm for the RAATS treatment is calculated to be approximately 13750 ppm. Data shows that the mean of the actual concentration is determined to be 13610 ppm. This is within 1% of the expected.

Based on the replicate data, a standard deviation of 191 ppm and a coefficient of variation of 1.4% were calculated. Data reflects that proper mixing and sub-sampling was achieved for the RAATS treatment.

The expected ppm for the Traditional treatment is calculated to be approximately 18333 ppm. Data shows that the mean of the actual concentration is determined to be 19210 ppm. This is within 4.7% of the expected.

Based on the replicate data a standard deviation of 328 ppm and a coefficient of variation of 1.7% were calculated. Data reflects that proper mixing and sub-sampling was achieved for the Traditional treatment.

Submitted and Reviewed by:

Bich Tran, Chemist
Lisa Mosser, Senior Analytical Chemist

TITLE: Determination of Emamectin Benzoate in Leaves

CPHST Staff: Dr. Phillip Lewis, Program Leader Insecticide and Applied Technologies Section
Lisa Mosser, Senior Analytical Chemist

COOPERATORS: Dr. Deb McCullough, Dept. of Entomology & Dept. of Forestry Michigan State University

OBJECTIVE:

To develop a validated chemical assay for the determination of Emamectin Benzoate in Leaves and analyze leaves over four sampling periods for pesticide content.

METHOD:

TPA-17, "Method Validation"
"Determination of Emamectin Benzoate in Leaves" (Attached)

RESULTS:

Method Validation Data:

Sample Description	Actual Amt (ppm)	Expected Amt (ppm)	% Recovered
LOQ-A	0.10589 PPM	0.1501 PPM	71%
LOQ-B	0.10758 PPM	0.1501 PPM	72%
LOQ-C	0.12504 PPM	0.1501 PPM	83%
5LOQ-A	0.56871 PPM	0.7507 PPM	76%
5LOQ-B	0.50909 PPM	0.7507 PPM	68%
5LOQ-C	0.49081 PPM	0.7507 PPM	65%
10LOQ-A	0.74114 PPM	1.5014 PPM	49%
10LOQ-B	0.86739 PPM	1.5014 PPM	58%
10LOQ-C	0.87451 PPM	1.5014 PPM	58%

Chemical Assay of Leaves:

Site	Tree ID	Block	Treatment	6/13/2007 - 6/14/2007	7/10/2007 – 7/11/2007	8/1/2007 – 8/2/2007	8/14/2007 – 8/15/2007
7N	3	A	E	1.6 ppm	2.8 ppm	1.7 ppm	0.82 ppm
7N	7	A	C	Non-detect			
7N	12	B	E			0.85 ppm	0.87 ppm
7N	19	C	E	2.0 ppm	2.4 ppm	2.1 ppm	1.8 ppm
7N	26	D	E			0.99 ppm	0.95 ppm
7N	32	E	E	3.5 ppm	1.7 ppm	0.77 ppm	0.48 ppm
7N	39	F	E			0.73 ppm	0.69 ppm
7N	45	G	E	1.9 ppm	1.6 ppm	1.2 ppm	1.1 ppm
7N	48	G	C	Non-detect			
W	1	A	C	Non-detect			
W	6	A	E	1.3 ppm	2.4 ppm	0.90 ppm	3.9 ppm
W	14	B	E			1.7 ppm	1.2 ppm
W	18	C	E	2.2 ppm	1.9 ppm	1.1 ppm	Non-detect
W	26	D	E			1.3 ppm	1.0 ppm
W	32	E	C	Non-detect			
W	34	E	E	1.4 ppm	1.9 ppm	1.8 ppm	0.98 ppm
W	39	F	E			1.0 ppm	0.53 ppm
IS	7	A	E	0.55 ppm	0.98 ppm	0.67 ppm	1.1 ppm
IS	18	B	E			0.88 ppm	0.69 ppm
IS	29	C	E	4.1 ppm	4.3 ppm	2.4 ppm	3.3 ppm
IS	39	D	E			2.0 ppm	1.5 ppm
IS	60	E	C	Non-detect			
IS	62	E	E	3.7 ppm	2.8 ppm	3.6 ppm	3.5 ppm
IS	68	F	E			2.1 ppm	2.8 ppm
IS	84	G	E	3.1 ppm	1.8 ppm	1.3 ppm	3.6 ppm
IS	86	H	E			2.6 ppm	3.0 ppm
IS	107	I	E	0.65 ppm	0.83 ppm	0.35 ppm	0.68 ppm
IS	109	J	E			2.1 ppm	3.8 ppm
IS	130	K	E	1.4 ppm	0.77 ppm	1.0 ppm	0.89 ppm
IS	124	L	E			0.51 ppm	0.91 ppm
IS	142	L	C	Non-detect			

DISCUSSION:

Statistical analysis of validation data shows a mean of 67%, a standard deviation of 10.4 and a coefficient of variation of 15%. During the validation process a limit of detection (LOD) of 0.05 ppm and a limit of quantification (LOQ) of 0.15 ppm were determined. Prior to analysis, samples were stored at -40ºC.

Submitted by: Lisa Mosser, Senior Analytical Chemist

TITLE: Identification of Signature Compounds found in Rutaceae Bonsai

CPHST Staff: Amy Roda, PhD Entomologist
Lisa Mosser, Senior Analytical Chemist

OBJECTIVES:

1. Obtain analytical standards of compounds tentatively identified and compare Kovats indices to those obtained from the citrus bonsai.
2. Verify signature compounds in authentic samples against the z-nose library file.
3. Purchase and analyze on the z-nose various citrus essential oils and compare with the z-nose library file as well as comparing chromatograms against authentic samples.

EXPERIMENTAL DESIGN:

Objective 1:

1. Purchase standards from Sigma Aldrich of Pinene and Myrcrene.
2. Analyze standards on z-nose and determine Kovats Indices.
3. Prepare a standard mix and analyze on z-nose to determine acceptable separation.

Objective 2:

1. Purchase Citrus plants from local Home Depot.
2. Analyze samples on z-nose and compare against z-nose library file.

Objective 3:

1. Purchase citrus essential oils.
2. Analyze citrus essential oils on z-nose and compare against z-nose library file.
3. Compare chromatograms of citrus essential oils with authentic samples.

RESULTS AND DISCUSSION:

Objective 1: From work done the week of March 1, 2010, a list of tentative compounds of interest were identified. The list is as follows:

Peak	Indice	Potential Constituent	Odor Characteristic
1	968	β- mycrene	balsamic, must, spice
2	968	β-pinene	pine, resin, terpentine
3	1331	σ-elemene	wood
4	1473	τ-muurolene	oil
5	1370	copaene	wood, spice
6	1413	caryophyllene	wood, spice
7	1446	α-caryophyllene	wood, spice

Only the mycrene and pinene were available and therefore purchased from Sigma Aldrich. Prior to analysis, an alkane standard of C6-C14 was analyzed on the z-nose to tune the instrument. (Figure 1.1) All analysis was conducted under the same conditions with the exception of pump time. (Figure 1.2) Pump time was only altered so that the detector would not be saturated.

Figure 1.1-Alkane Standard

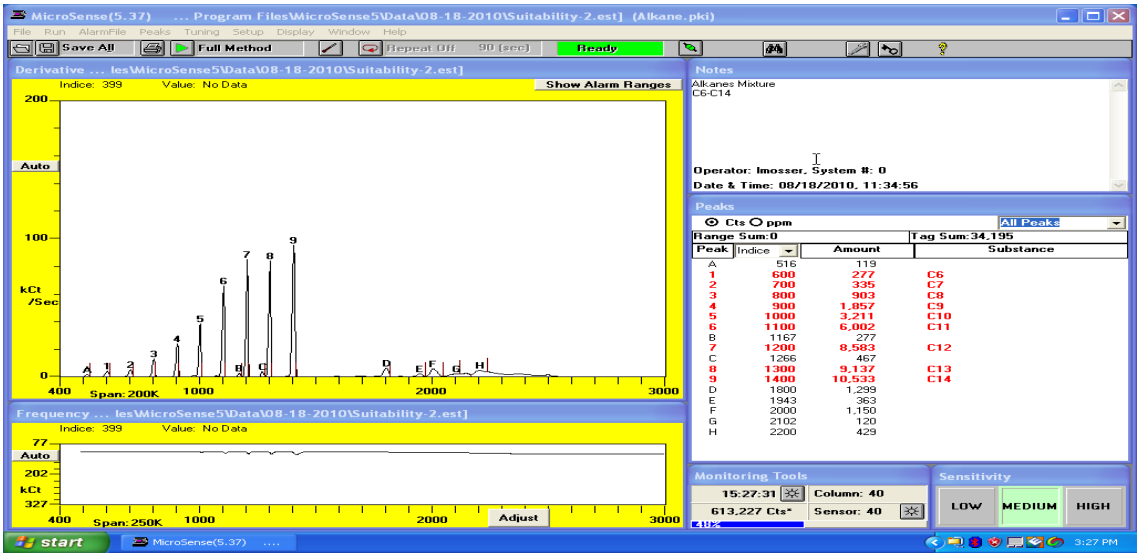
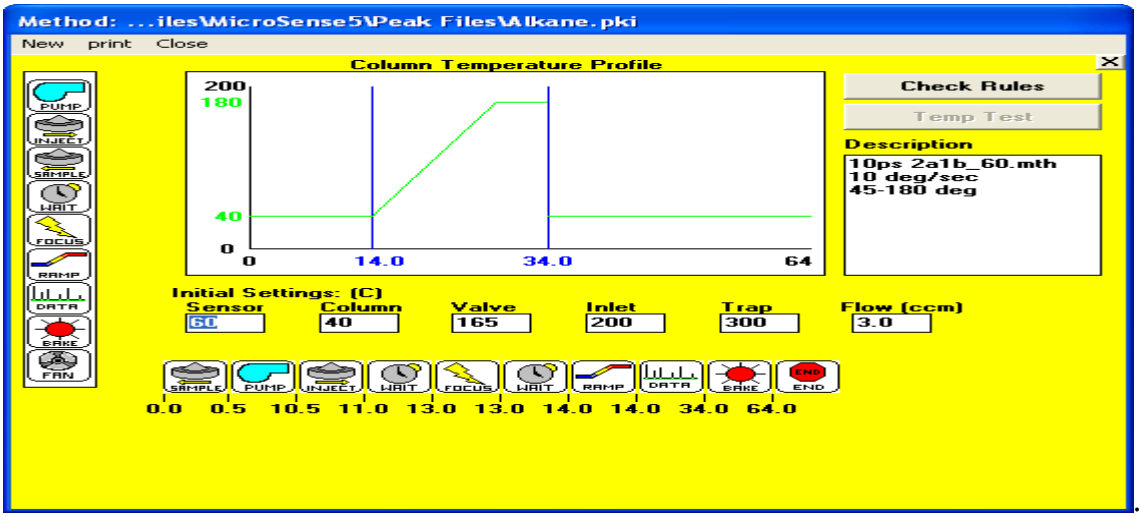


Figure 1.2-Instrumental Parameters



The standards β-pinene and β-mycrene was analyzed and kovats indices were determined. (Figure 1.3 and Figure 1.4 respectively) The kovats indices of β-pinene and β-mycrene was

determined to be 942 and 1005 respectively. Also, a standard mix was prepared with a drop of hexane to see if there was any shift. (Figure 1.5) Shifting was observed and can be corrected in the MicroSense software.

Figure 1.3-β-Pinene

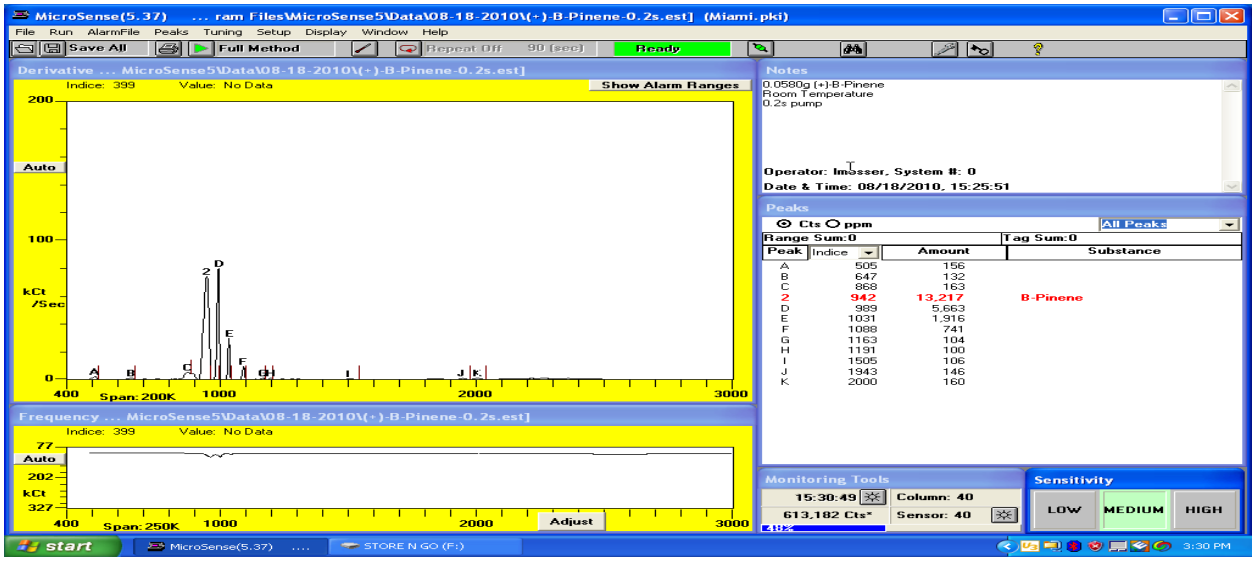


Figure 1.4-β-mycrene

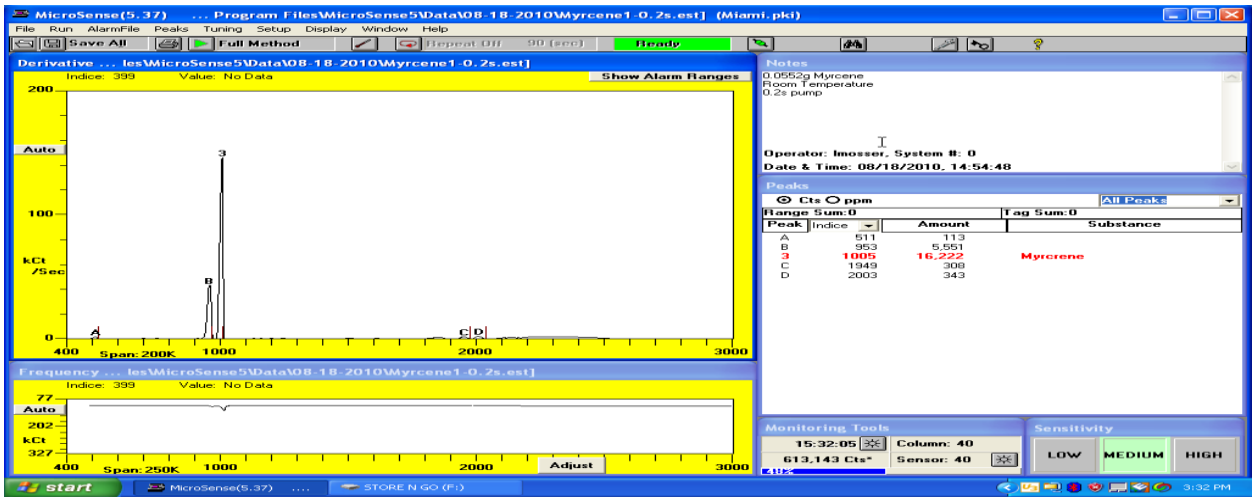
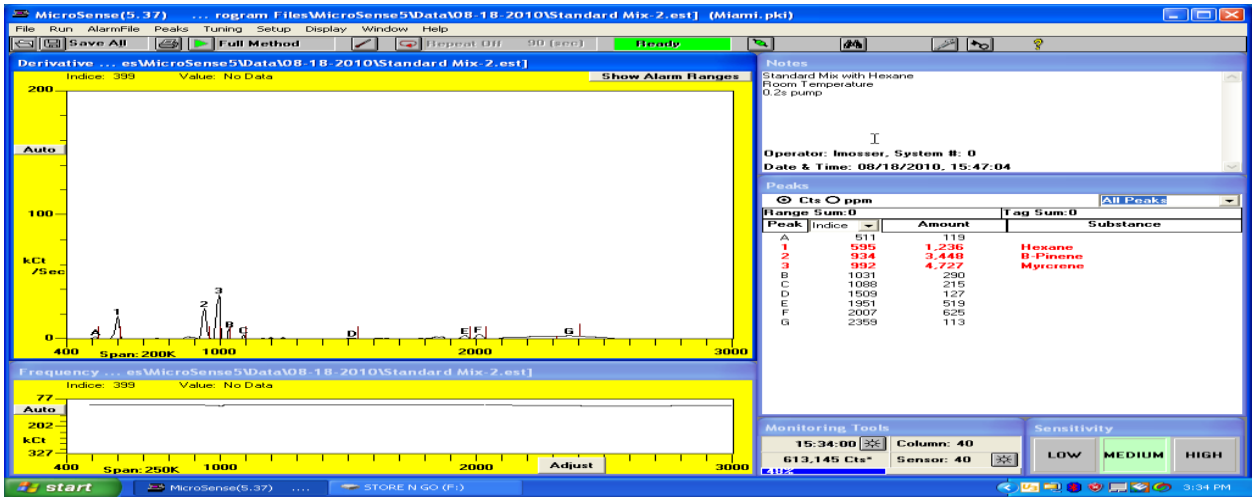


Figure 1.5-Standard Mix with Hexane



Objective 2: The following citrus plants were purchased from Home Depot:
Meiwa Sweet Kumquat
Owari Satsuma
Thornless KeyLime

Bark peels from each were placed in a 40mL vial and allowed to equilibrate for 10 seconds. Samples were analyzed on the z-nose and compared to the z-nose library file.

Figure 2.1-Meiwa Sweet Kumquat

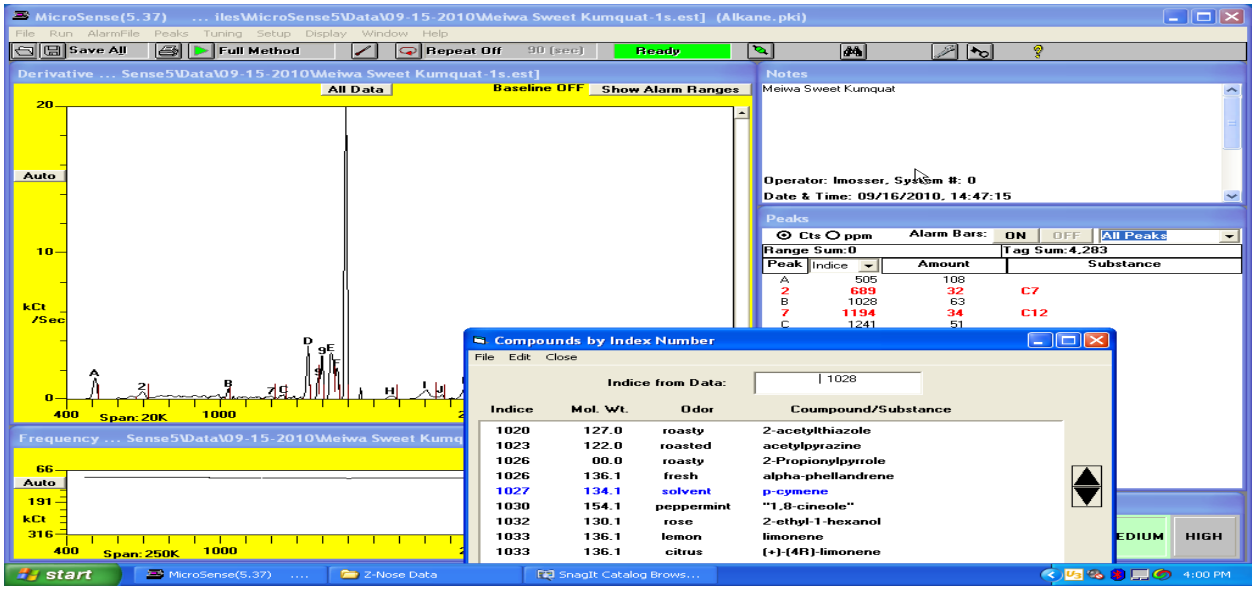


Figure 2.2-Owari Satsuma

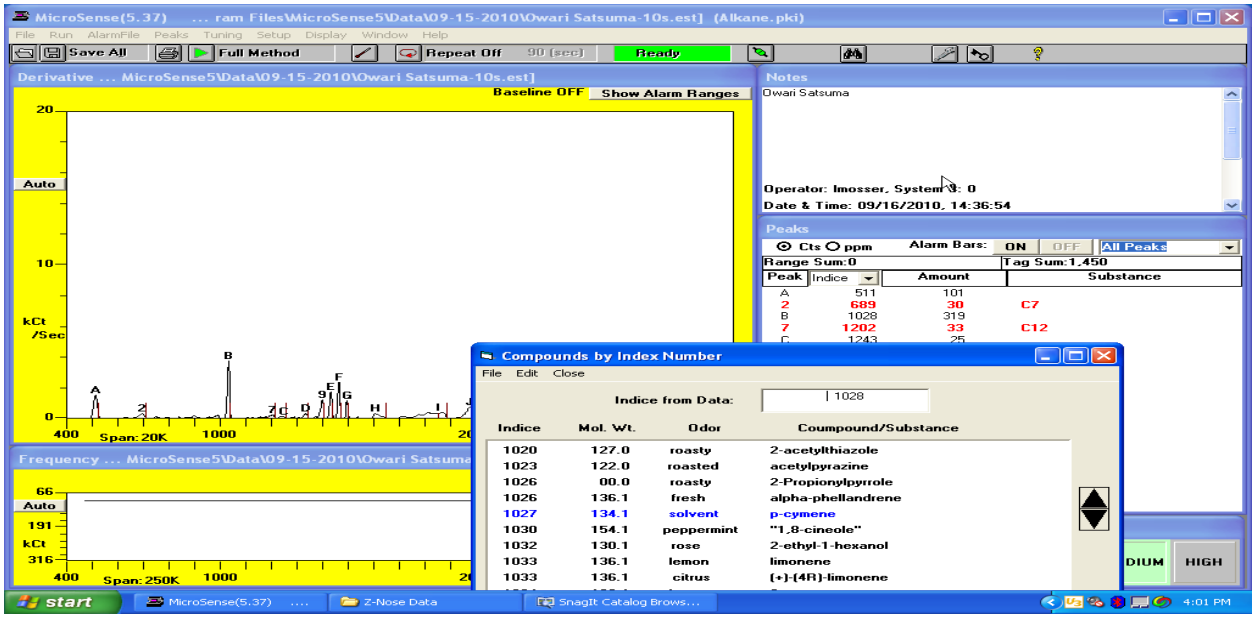
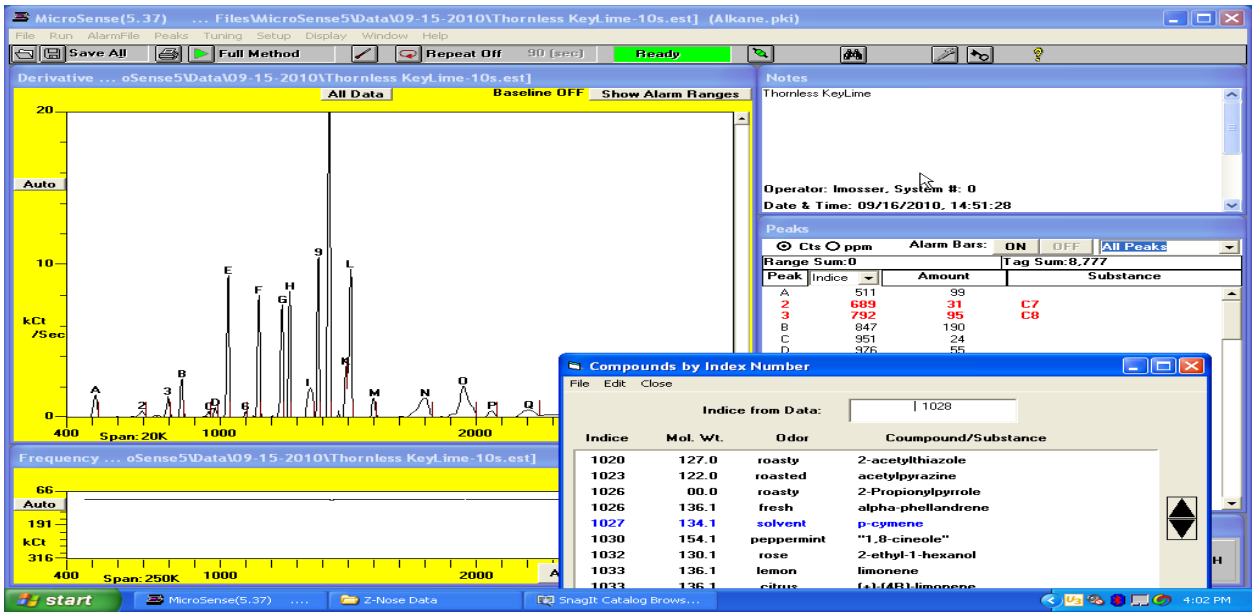


Figure 2.3-Thornless KeyLime



Observe that all three samples had p-cymene which is an odorant with a characteristic of solvent, gasoline, and citrus based on literature research. The other peaks when compared to the library were compounds associated with bark. These "bark" results are very similar to the data acquired on March 1, 2010.

Objective 3: Various citrus essential oils were purchased from Essential Oil University and analyzed on the z-nose. Peaks were compared to the z-nose library and compounds such as alpha-terpinene (lemon odor), limonene (lemon odor) and (+)-(4R)-limonene (citrus odor) were identified to name a few. It was also observed that there was no difference if the citrus oil had been extracted from the leaf or the peel. This may mean that no significant difference can also be determined from citrus compounds found in the twigs or branches. A comparison of the Meiwa Sweet Kumquat, the Owari Satsuma, and the Thornless KeyLime was done using the waterfall capabilities of the MicroSense software. It was observed that the inherent citrus peaks of the citrus oils did not visually match the peaks in the authentic samples. The conclusion here is that most of the peaks in the samples are inherent odorants associated with bark or wood. The citrus peaks may be there, but are not as sensitive.

Figure 3.1-Meiwa Sweet Kumquat against Essential Oil Waterfall

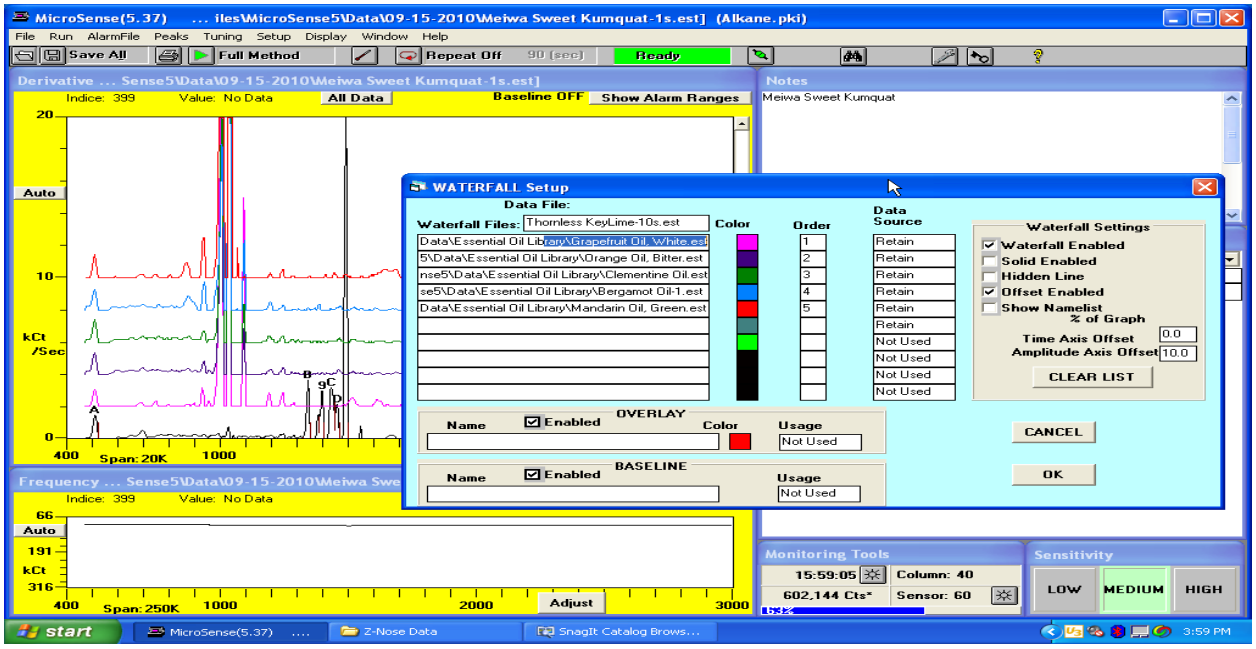


Figure 3.2-Owari Satsuma against Essential Oil Waterfall

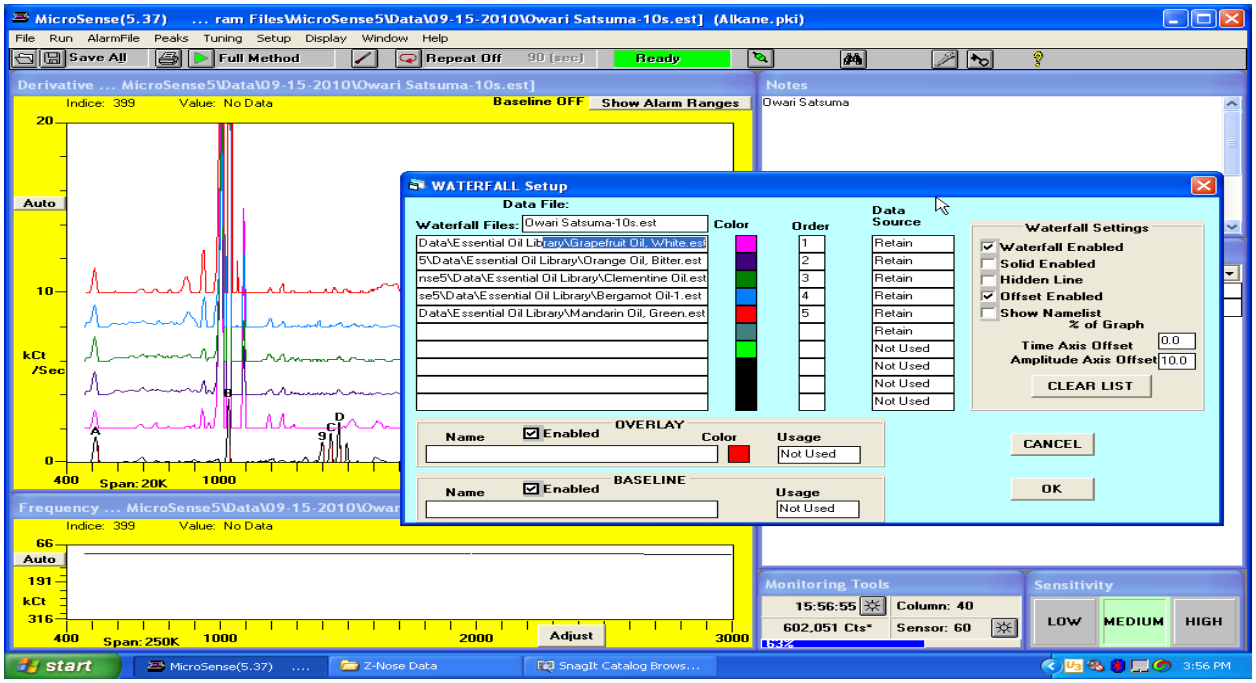
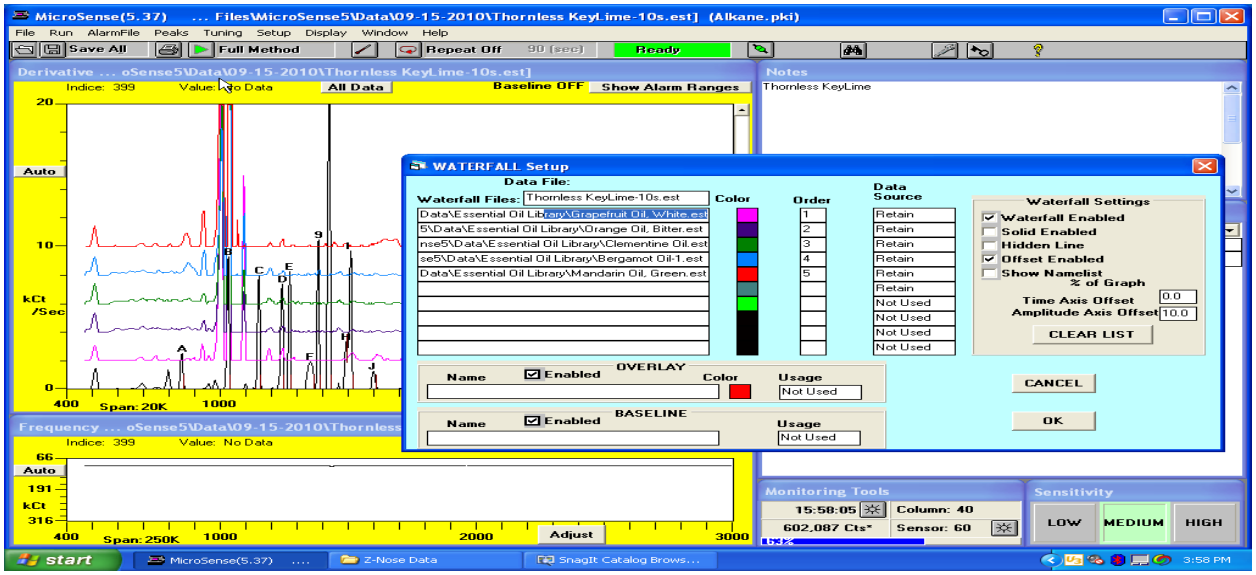


Figure 3.3-Thornless KeyLime against Essential Oil Waterfall



Action Items:

1. Purchase compounds that are known to be citrus specific and prepare a "citrus" library for peak comparison of unknowns. This library will be specific for the z-nose system. Keep in mind that the Kovats Indice is based

on the retention time of the analyte which is column and condition specific. Most Kovats Indices found in literature are based on 15, 30 or 60 meter columns under various instrumental parameters. The z-nose is equipped with a DB-5 which is 1 meter in length. Creating a library under the system conditions to be used in the field would ensure reproducibility. There are 29 compounds that are unique to citrus and are available by Sigma Aldrich.

2. Analyze various samples and compare against the "citrus" library.
3. If needed, determine a simplistic method to extract citrus compounds out of plant shavings.
4. Develop an Analysis Protocol.

Submitted by: Lisa Mosser, Senior Analytical Chemist

TITLE: Determination of Active Ingredient in Hercon® BW Xtra Lure

CPHST Staff: Lisa Mosser, Senior Analytical Chemist

OBJECTIVE:

To quantitatively determine the amount of grandlure and eugenol in Hercon® Xtra Lure manufactured by Hercon® Environmental

METHOD:

- Hercon® Environmental Analytical Method 100718

RESULTS:

Sample Number	Description	Amount Grandlure	Amount Eugenol
5037-1	Orange	18 mg	25 mg
5037-2	Orange	17 mg	26 mg
5037-3	Orange	16 mg	24 mg
5037-4	Orange	16 mg	24 mg
5037-5	Orange	17 mg	25 mg
5038-1	Yellow	19 mg	25 mg
5038-2	Yellow	19 mg	26 mg
5038-3	Yellow	20 mg	28 mg
5038-4	Yellow	20 mg	26 mg
5038-5	Yellow	20 mg	28 mg
5039-1	White	20 mg	30 mg
5039-2	White	21 mg	31 mg
5039-3	White	21 mg	32 mg
5039-4	White	20 mg	29 mg
5039-5	White	21 mg	32 mg

DISCUSSION:

Analysis of data shows that the orange lures had an average of 17 mg grandlure and 25 mg eugenol. The yellow lures had an average of 20 mg grandlure and 27 mg eugenol. The white lures had an average of 21 mg grandlure and 31 mg eugenol. Expected active ingredient amounts have been defined as 25 mg grandlure and 30 mg eugenol by Hercon® Environmental. Therefore, grandlure was determined to be 16% - 32% less than expected and eugenol was determined to be 0 – 17% less than expected. The analytical method used in this analysis was not validated in-house by the CPHST Gulfport laboratory. It is assumed that Hercon® Environmental validated their method and that the extraction procedure produces acceptable

recoveries of actives and criteria for reproducibility and repeatability of results are statistically sound.

TITLE: Determination of LBAM Pheromome Blend Content in Three Lure Types: Hercon DISRUPT BIO-TIE® LBAM, ISCA SPLAT, and ISOMATE-LBAM PLUS

CPHST Staff: Lisa Mosser, Senior Analytical Chemist

OBJECTIVE:

To quantitatively determine the amount of LBAM pheromone blend consisting of E-11-tetradecenyl acetate, E,E-9,11-tetradecadienyl acetate and Z-11-tetradecenyl acetate.

METHODS:

- Hercon® Environmental Analytical Method gc.100835.rtx225
- CPHST-GL modification of Hercon Environmental Analytical Method gc.100835.rtx225 (ISCA and Isomate lures)

RESULTS AND DISCUSSION:

Hercon Disrupt Bio-tie

Sample	E11-14Ac	EE9,11-14Ac	Z11-14Ac
A	9.1%	0.57%	2.6%
B	0.24%	1.7%	0.08%
C	0.30%	1.6%	0.11%

Results are located in Data Package labeled Group # 5053. Analysis of data shows a high variability among lures. Expected value for E11-14Ac is 14.3% and EE9, 11-14Ac is 0.71%. The lures are also to be absent of the Z11-14Ac. However, data results are based on retention time match of chromatographic conditions compared to known standards. Confirmation would be absolute in comparing mass spectra data of samples and standards. The analytical method used in this analysis was not validated in-house by the CPHST Gulfport laboratory. It is assumed that Hercon® Environmental validated their method and that the extraction procedure produces acceptable recoveries of actives and criteria for reproducibility and repeatability of results are statistically sound.

ISCA Splat

Sample	Sample Mass	E11-14Ac	EE9,11-14Ac	Z11-14Ac
A	1.0678 g	9.7%	0.48%	None Detected
B	1.4246 g	9.7%	0.48%	None Detected
C	1.1233 g	10%	0.52%	None Detected
D	1.1907 g	10%	0.52%	None Detected
E	1.3705 g	10%	0.50%	None Detected

*Results are located in Data Package labeled Group # 5053. Analysis of data shows a mean of 9.9%, a standard deviation of 0.16, and a %CV of 1.6 for E11-14Ac. Data also shows a mean of 0.50%, a standard deviation of 0.02, and a %CV of 4 for EE9, 11-14Ac. Expected value for E11-14Ac is 9.5% and EE9, 11-14Ac is 0.5%. The lures are also to be absent of the Z11-14Ac. However, data results are based on retention time match of chromatographic conditions compared to known standards. Confirmation would be absolute in comparing mass spectra data of samples and standards. The analytical method used in this analysis was not validated in-house by the CPHST Gulfport laboratory.

Isomate LBAM Plus

Sample	E11-14Ac	EE9,11-14Ac	Z11-14Ac
A	64.2%	3.5%	24.5%
B	63.9%	3.4%	24.4%
C	63.7%	3.4%	24.2%
D	64.3%	3.4%	24.5%
E	64.0%	3.5%	24.5%

Results are located in Data Package labeled Group # 5057. Analysis of data shows a mean of 64.0%, a standard deviation of 0.24, and a %CV of 0.38 for E11-14Ac. Data shows a mean of 3.4%, a standard deviation of 0.05, and a %CV of 1.5 for EE9, 11-14Ac. Data shows a mean of 24.4%, a standard deviation of 0.13, and a %CV of 0.53 for Z11-14Ac. Expected value for E11-14Ac is 67.15% and EE9, 11-14Ac is 2.74%. The lures are also to be absent of the Z11-14Ac. However, data results are based on retention time match of chromatographic conditions compared to known standards. Percentages of isomers were calculated by dividing response of isomers by the total response of isomers plus impurities. Confirmation would be absolute in comparing mass spectra data of samples and standards. The analytical method used in this analysis was not validated in-house by the CPHST Gulfport laboratory.