2003 ACCOMPLISHMENT REPORT

SOIL INHABITING PESTS LABORATORY

ANALYTICAL AND NATURAL PRODUCTS CHEMISTRY LABORATORY

CENTER FOR PLANT HEALTH SCIENCE AND TECHNOLOGY

PLANT PROTECTION AND QUARANTINE

U.S. DEPARTMENT OF AGRICULTURE

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

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

Compiled and Edited by:

Anne-Marie A. Callcott

May 2004

2003 IMPORTED FIRE ANT OBJECTIVES

SOIL INHABITING PESTS LABORATORY
GULFPORT, MS

OBJECTIVE 1: Development and refinement of quarantine treatments for certification of regulated articles.

- Emphasize development of quarantine treatments for containerized nursery stock.
- Evaluate candidate toxicants, formulation, and dose rates for various use patterns.
- Test and evaluate candidate pesticides for use on grass sod and field grown nursery stock.
- Assist in registration of all treatments shown to be effective.

OBJECTIVE 2: Advancement of technology for population suppression and control.

- New product/formulation testing and evaluation.
- Conduct label expansion studies.
- Evaluation of non-chemical biocontrol agents, including microbial, nematodes, and predaceous arthropods.

OBJECTIVE 3: Preparation/distribution of technical information on control, quarantine procedures, new technology, biological hazards, etc., to state agencies, the media, and the public.

- Provide training to state regulatory agencies and nursery associations.
- Publish and distribute informational aids for state agencies, nursery associations, PPQ personnel, and other interested stakeholders.

OBJECTIVE 4: Determine impact of IFA on biodiversity of various ecosystems.

- Provide technical support and assistance to other research organizations such as ARS, Universities, Mississippi Nature Conservancy, etc. to expedite ecological studies on the impact of IFA on T&E species.
- Conduct bait transects and compare current myrmecofaunal records with similar surveys done in the past to determine impact of IFA on other ant species.

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MISCELLANEOUS

PROJECT NO: A9P01

PROJECT TITLE: Residual Activity of TopPro Specialties' Formulation of Bifenthrin, 2002

REPORT TYPE: Interim

PROJECT LEADER/PARTICIPANTS: Lee McAnally, Shannon James

INTRODUCTION:

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

TopPro Specialties, Micro Flo Company has begun the manufacture of bifenthrin in both granular (0.2%) and liquid flowable (7.9%) formulations. The granular formulation is produced on two different carriers, Sand and DG Lite. In August 2002 a study was initiated to determine the efficacy of TopPro bifenthrin. Each formulation was set up in treatment rates equivalent to those specified in the quarantine treatment manual for durations corresponding to the certification periods for each treatment rate.

In 2003, TopPro Specialties returned production and distribution of these bifenthrin formulations to BASF.

MATERIALS AND METHODS:

Granular Incorporation Treatment:

Both formulations (carriers) of granular TopPro were blended into the MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 850 lb/cu yd) at rates of 10, 12, 15, and 25 ppm. A portable cement mixer (2 cu ft capacity) was use to blend the toxicant into the potting media, and was operated for 15 minutes per batch to insure thorough blending. Treated media was then poured into one-gallon capacity plastic nursery pots and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week. At monthly intervals, sub samples were taken from 2 pots of each treatment and composited and subjected to standard alate queen bioassay (Appendix I).

Drench Treatment:

Untreated media was placed in 1-gallon nursery pots and drenched with 400ml finished solution at a rate of 25 ppm. The pots were then placed under the same conditions and tested in the manner described above.

RESULTS:

Results are summarized in Table 1. The TopPro Specialties flowable bifenthrin formulation in the MAFES media is as effective as the FMC formulation, providing 6 months of residual activity, indicating acceptability as a product to be used in the IFA quarantine. The granular product has provided acceptable efficacy for 6 months at 10ppm, and at least 8 months of activity at 12, 15, and 25 ppm rates. Testing of these rates will continue for 12 to 24 months, depending on quarantine certification periods. To ensure efficacy across media types, additional testing in other media types will be initiated with both the liquid and granular formulations.

Table 1. Residual activity of TopPro bifenthrin.

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)							
		1	2	3	4	5	6	7	8
DG lite carrier	10	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	***	***
	12	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)
	15	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)
	25	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)
Sand carrier	10	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	***	***
	12	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)
	15	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)
	25	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	100(1)	100(3)
Drench	25	100(1)	100(3)	100(1)	100(3)	100(3)	100(1)	***	***
	Check	0	0.	0	0	0	0	0	0

Table 1. (cont.) Residual activity of TopPro bifenthrin.

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)							
		9	10	11	12	13	14	15	16
DG lite carrier	10	***	***	***	***	***	***	***	***
	12	100(1)	100(3)	100(3)	100(1)	***	***	***	***
	15	100(1)	100(3)	100(3)	100(1)	100(3)	100(3)	100(1)	100(3)
	25	100(1)	100(3)	100(3)	100(1)	100(3)	100(3)	100(1)	100(3)
Sand carrier	10	***	***	***	***	***	***	***	***
	12	100(1)	100(3)	100(3)	100(1)	***	***	***	***
	15	100(1)	100(3)	100(3)	100(1)	100(3)	100(3)	100(1)	100(3)
	25	100(1)	100(3)	100(3)	100(1)	100(3)	100(3)	100(1)	100(3)
Drench	25	***	***	***	***	***	***	***	***
	Check	0	5	10	5	0	0	5	5

PROJECT NO: A9P01 - GPPS00-01

PROJECT TITLE: Further Testing of Chlorfenapyr as an Imported Fire Ant Quarantine Treatment (2000)

REPORT TYPE: Interim

PROJECT LEADER/PARTICIPANT(s): Lee McAnally

INTRODUCTION:

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

Chlorfenapyr is an experimental insecticide-miticide under development by American Cyanamid (Princeton, NJ). The product is active against many pests, and works as a broad spectrum contact and stomach poison. Previously we tested a liquid formulation to determine whether the product showed significant activity against IFA in containerized nursery stock. In August 1997, we began testing a 0.5G granular formulation as an incorporated treatment (FA01G097).

In August 1999, we initiated an expanded test of chlorfenapyr using a 2SC liquid formulation as a drench treatment, as well as 1G, 1.5G and 2G formulations each on two different carriers (clay and corn cob grit) as incorporated treatments. All of these treatments were applied to three different potting media (FA01G019).

In August 2000, the trial reported here was initiated using the 1G and 1.5G formulations on the grit carrier.

MATERIALS AND METHODS:

Incorporated Treatments:
Granular treatments included 1% and 1.5% products formulated on a corn cob grit carrier. Each of the granular formulations was blended into the MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 785 lb/cu yd) at rates of 50, 75, 100 and 200 ppm. A portable

cement mixer (2 cu ft capacity) was use to blend the toxicant into the potting media, and was operated for 15 minutes per batch to insure thorough blending. Treated media was then poured into one-gallon capacity plastic nursery pots and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week. At monthly intervals, subsamples were taken from 3 pots of each treatment and composited and subjected to standard alate queen bioassay. The 1.0G formulation was mixed on August 28 and the 1.5G formulation was mixed on August 29, 2000.

RESULTS:

All rates are producing 100% mortality in 12 days exposure or less through 27 months post-treatment (Table 1), showing excellent potential as a preplant incorporation treatment for the IFA quarantine. Additional trials in various media types would need to be completed to ensure efficacy does not decrease in other media types. Discussion with the company will precede any future testing and current trial will run through 30 months and then be terminated.

Table 1. Residual activity of chlorfenapyr 1.0G and 1.5G.

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)							
		1	2	3	5	6	7	8	9
1.0G	50	100(6)	100(8)	100(11)	100(10)	100(10)	100(8)	100(13)	100(8)
	75	100(5)	100(7)	100(7)	100(9)	100(7)	100(7)	100(6)	100(7)
	100	100(4)	100(7)	100(8)	100(9)	100(6)	100(7)	100(7)	100(7)
	200	100(3)	100(7)	100(5)	100(6)	100(4)	100(3)	100(8)	100(6)
1.5G	50	100(6)	100(8)	100(11)	100(11)	100(10)	100(9)	100(7)	100(9)
	75	100(6)	100(8)	100(8)	100(11)	100(7)	100(7)	100(7)	100(7)
	100	100(6)	100(4)	100(7)	100(9)	100(7)	100(7)	100(6)	100(7)
	200	100(4)	100(4)	100(5)	100(6)	100(6)	100(7)	100(6)	100(7)
	Check*	15	10	5	5	5	5	30	5

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)							
		10	11	12	13	14	15	17	18
1.0G	50	100(10)	100(6)	100(9)	100(11)	100(12)	100(7)	100(9)	100(10)
	75	100(10)	100(6)	100(8)	100(11)	100(11)	100(7)	100(6)	100(6)
	100	100(7)	100(6)	100(7)	100(8)	100(7)	100(7)	100(6)	100(6)
	200	100(7)	100(4)	100(7)	100(6)	100(5)	100(4)	100(6)	100(4)
1.5G	50	100(10)	100(6)	100(8)	100(8)	100(11)	100(8)	100(8)	100(7)
	75	100(7)	100(4)	100(7)	100(7)	100(7)	100(7)	100(6)	100(7)
	100	100(6)	100(6)	100(7)	100(7)	100(6)	100(7)	100(6)	100(5)
	200	100(10)	100(3)	100(6)	100(5)	100(5)	100(4)	100(6)	100(4)
	Check*	10	10	5	10	5	0	0	0

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)							
		19	20	21	22	23	24	25	26
1.0G	50	100(11)	100(11	100(10)	100(10)	100(7)	100(7)	100(11)	100(10)
	75	100(10)	100(10)	100(7)	100(7)	100(7)	100(6)	100(8)	100(7)
	100	100(6)	100(6)	100(5)	100(5)	100(6)	100(6)	100(8)	100(6)
	200	100(6)	100(6)	100(5)	100(5)	100(5)	100(6)	100(4)	100(5)
1.5G	50	100(11)	100(10)	100(10)	100(7)	100(7)	100(6)	100(9)	100(7)
	75	100(10)	100(10)	100(7)	100(6)	100(6)	100(6)	100(8)	100(7)
	100	100(6)	100(6)	100(7)	100(5)	100(6)	100(6)	100(8)	100(5)
	200	100(5)	100(5)	100(5)	100(4)	100(4)	100(2)	100(4)	100(5)
	Check*	5	0	10	5	10	10	5	10

Formulation Tested	Rate of Application (ppm)	Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality)							
		27							
1.0G	50	100(11)							
	75	100(10)							
	100	100(6)							
	200	100(5)							
1.5G	50	100(10)							
	75	100(11)							
	100	100(11)							
	200	100(11)							
	Check*	10							

*Check mortality is shown at longest exposure time

PROJECT NO: A9P01

PROJECT TITLE: Residual Activity of Granular Deltagard G Incorporated into Nursery Potting Media, 2002

REPORT TYPE: Final

PROJECT LEADER/PARTICIPANTS: Shannon Wade, Lee McAnally

INTRODUCTION:

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

Bayer Corp. has developed Deltagard 0.1G for testing as a granular incorporation treatment. On May 21, 2002, we initiated a test to determine residual activity as a soil incorporation treatment.

MATERIALS AND METHODS:

The formulation was blended into the MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 850 lb/cu yd) at rates of 25, 50, and 75 ppm. A portable cement mixer (2 cu ft capacity) was use to blend the toxicant into the potting media, and was operated for 15 minutes per batch to insure thorough blending. Treated media was then poured into one-gallon capacity plastic nursery pots and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week. At monthly intervals, sub-samples were taken from 3 pots of each treatment and composited and subjected to standard alate queen bioassay (Appendix I).

RESULTS:

The 25 ppm rate provided 100% control in 14 days exposure or less through 10 months post-treatment (Table 1). Both 50 and 75 ppm rates provided 100% control through 12 months. Due to the higher rates of application necessary to achieve 10-12 months of residual activity this product may not be cost effective for quarantine use. However, we will discuss the economics as well as continued interest with the company prior to any future testing.

Table 1. Residual Activity of Deltagard G as an Incorporated Treatment

Rate of Application (ppm)	Mean % mortality to alate females at indicated post-treatment interval								
	1 mth	2 mths	3 mths	4 mths	5 mths	7 mths	8 mths	9 mths	10 mths
25	100	100	100	100	100	100	100	100	100
50	100	100	100	100	100	100	100	100	100
75	100	100	100	100	100	100	100	100	100
check	10	0	0	5	20	5	0	5	5

Rate of Application (ppm)	Mean % mortality to alate females at indicated post-treatment interval								
	11 mth	12 mths	14 mths	15 mths	16 mths	17 mths			
25	50	45	10	40	35	5			
50	100	100	80	85	65	75			
75	100	100	75	100	85	70			
check	50	25	10	40	5	25			

PROJECT NO: A1P04

PROJECT TITLE: Traditional In-field Treatments for Field Grown Nursery Stock: Bait plus Broadcast Contact Insecticide, 2002

TYPE REPORT: Final

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

INTRODUCTION:

Current quarantine treatments for field grown nursery include a bait treatment followed in 3-5 days by a granular chlorpyrifos treatment. This treatment allows for 12 weeks of certification after a 30-day exposure period. Most field grown nursery stock is harvested during the winter months. With the potential loss of chlorpyrifos and the reliance on one contact insecticide, we initiated a trial to replace the chlorpyrifos portion of this treatment regimen with more available chemicals.

MATERIALS AND METHODS:

The bait used was hydramethylnon. The contact insecticides were bifenthrin flowable and granular and fipronil granular. Rates of application are shown below.

Insecticide	Trade Name	Formulation	Rate of Application
hydremethylnon	Amdro®	bait	1.5 lb/acre
bifenthrin G	Talstar®	0.2G	200 lb/acre (0.4 lb ai/acre)
bifenthrin F	Talstar®	Flowable	40 oz/acre (0.2 lb ai/acre)
fipronil	Top Choice®	0.0143G	87 lb/acre (0.0125 lb ai/acre)

The test site was located at the Hattiesburg Municipal Airport in Hattiesburg, MS. As with many airport sites, the upkeep and accessibility is superior, but ant populations tend to be somewhat low. The bait was applied on September 18, 2002 with a shop built applicator mounted on a farm tractor. Air temperature was 85°F and the soil temperature was 83°F. The contact insecticides were applied on September 23-24, 2002 with air temperatures of 79-81°F and soil temperatures of 80°F. Granular material was applied with a Herd™ spreader mounted on a farm tractor. Liquid material was applied with a roller pump boom sprayer equipped with five TKSS tips with provided a 10 ft. swath. The system was operated at 50 psi providing ca. 50 gallons of finished spray per acre. There were three replicates per treatment, and all test plots were 1.0 acre in size. A ¼-acre circular efficacy plot was established in the center of each 1.0 acre test plot. Prior to bait application and at 2 and 4 weeks after final treatment (June 26), IFA populations in each efficacy plot were evaluated using the population index system developed by Harlan et al. (1981), and later revised by Lofgren and Williams (1982). Treatments were evaluated at 4 week intervals thereafter. Using this data, both colony mortality and decrease in pretreatment population indices were calculated. Experimental data were statistically analyzed using analysis of variance, and treatment means were separated using the LSD test (P=0.05) for each posttreatment rating interval.

RESULTS:

Due to adverse weather conditions, the 2 week evaluation was not done. The first evaluation was done at 4 weeks after treatment, at which time all treatments provided 100% control of IFA (Tables 1 and 2). Excellent control by all treatments continued through the 26 week evaluation. Bifenthrin granular continued at 100% control through 36 weeks. At 30 weeks, late March, some re-infestation was noted on one and two bifenthrin flowable and fipronil replicates, respectively. The early June evaluation was conducted after several weeks of early hot, dry weather, with mounds becoming hard to locate as evidenced by the control numbers. By 42 weeks after treatment, all treatments had evidence of reinfestation and the trial was terminated.

The current in-field treatment using chlorpyrifos, has a 12 week certification after a 30 day exposure. All of these treatments in this trial exceeded that by providing at least 22 weeks of 100% control after a 30 day exposure. In a previous trial (in 2001), using these same products at the same rates of application, results were similar for the bifenthrin granular treatment (30+ weeks of 100% control after 30 day exposure). As always with IFA, results vary from trial to trial, and multiple duplication of results is necessary to verify potential quarantine treatments.

References Cited:

Harlan, D. P., W. A. Banks, H. L. Collins, and C. E. Stringer. 1981. Large area tests of AC217,300 bait for control of imported fire ants in Alabama, Louisiana, and Texas. Southwest. Entomol. 8: 42-45.

Lofgren, C. S. and D. F. Williams. 1982. Avermectin B1a, a highly potent inhibitor of reproduction by queens of the red imported fire ant. J. Econ. Entomol. 75: 798-803.

Table 1. Bait followed by broadcast contact insecticide treatment - Decrease in colony numbers.

Treatment	Mean no. colonies/acre – pretreat	% decrease in no. pretreat colonies at indicated wks. after treatment							
		-4-	-9-	-12-	-20-	-26-	-30-	-36-	-42-
Bait + Talstar F	32.0	100.0a	100.0a	100.0a	100.0a	100.0a	89.3a	96.3a	89.3a
Bait + Talstar G	33.3	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	90.5a
Bait + Fipronil G	29.3	100.0a	100.0a	100.0a	100.0a	100.0a	96.7a	96.7a	95.2a
Check	34.7	0.0b	11.8b	7.0b	20.3b	4.8b	11.1b	47.6b	28.8b

LSD test (P=0.05) means within a column followed by the same letter are not significantly different

Table 2. Bait followed by broadcast contact insecticide treatment - Change in population indices.

Treatment	Mean pop. index/acre - pretreat	% change in pretreat population indices at indicated wks. after treatment							
		-4-	-9-	-12-	-20-	-26-	-30-	-36-	-42-
Bait + Talstar F	422.7	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-93.3a	-97.5a	-88.3a
Bait + Talstar G	418.7	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-91.9a
Bait + Fipronil G	460.0	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-98.1a	-98.1a	-96.7a
Check	437.3	56.2b	-1.7b	-1.6b	-44.8b	4.4b	-11.0b	-49.6b	-25.3b

LSD test (P=0.05) means within a column followed by the same letter are not significantly different

PROJECT NO: A1P04

PROJECT TITLE: Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, Fall 2002

TYPE REPORT: Final

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

INTRODUCTION:

APHIS is responsible for developing treatment methodologies for certification of regulated items, such as field grown/balled-and-burlapped (B&B) nursery stock in the Imported Fire Ant Quarantine. Current treatments for field grown stock, as described below, are labor intensive, cumbersome and expensive. The only in-field treatment utilizes chlorpyrifos, the future of which is uncertain at best. Thus additional treatment methods are needed to insure movement of this type of material. Imported fire ants are slowly moving into areas of Tennessee where many producers of field grown nursery stock are located. This has prompted renewed interest in development of new treatments for this stock. New regulatory treatment methods for field grown/B&B nursery stock are needed to insure that nursery growers can compile with the Federal IFA Quarantine.

Currently the Federal Imported Fire Ant Quarantine (7CFR 301.81) has three treatment regimens for certification of field-grown and B&B nursery stock. The in-field treatment requires a broadcast application of an approved bait followed in 3-5 days by a broadcast application of granular chlorpyrifos. After a 30-day exposure period, plants are certified for 12 weeks. In 1999, PPQ allowed for a second application of the granular chlorpyrifos to extend the certification period for an additional 12 weeks. For harvested B&B stock, there are two certification methods: immersion in a chlorpyrifos solution or watering twice daily with a chlorpyrifos solution for 3 consecutive days.

The in-field treatment currently requires that the treatment must extend at least 10 feet from the base of each plant to be certified. This virtually means that the treatments must be applied broadcast to the entire nursery block. Numerous granular formulations of common insecticides such as diazinon, chlorpyrifos, acephate, and others are labeled for spot treatment of imported fire colonies. Imported fire ant colonies readily respond to any insecticide application made directly to the nest by relocating the colony (Collins & Callcott 1995, Hays et al. 1982, Franke 1983, Williams & Lofgren 1983). This insecticide induced movement is usually over a relatively short distance (1.5 to 3.0 meters), but can be greater (AMC, personal observation). The primary objective of a quarantine treatment for field grown nursery stock is to render the plants fire ant free. Therefore, it does not matter if colonies are killed outright by the treatment or simply induced to move away from the area around each individual plant intended for harvest.

Preliminary testing was initiated in Sept. 2001 by testing several liquid and granular insecticides against individual IFA mounds in the field. Results of this trial indicated promising results with acephate, bifenthrin, and deltamethrin (see GPPS01-02). A second preliminary trial, taking selected products to the field and testing in band applications was initiated in fall 2001 and spring 2002 in Mississippi. Using band widths of 5' to 6', bifenthrin and deltamethrin, both liquid and granular formulations showed promising results (see GPPS02-01; GPPS02-02). These trials, while promising, showed that contact insecticide treatments alone were not effective enough for use in the IFA quarantine. Therefore, the following trial was initiated applying a broadcast bait application followed by the contact insecticide applied as a band treatment.

MATERIALS AND METHODS:

Bait treatments were applied at Hattiesburg Municipal Airport, Mississippi on October 31, 2002 using a shop built spreader on a farm tractor calibrated to apply 1.25 lbs/acre. Due to excessive rainfall over the weeks following the bait application, the contact insecticides were not applied until November 25, 2002. Granular band treatments were applied using a Gandy 48" granular drop spreader attached to a farm tractor. Liquid band treatments were applied using a roller pump boom sprayer equipped with two standard flat spray tips (8015-SS; TeeJet Corp.) to provide various a 36" band spray and a total spray volume equivalent to ca. 76 gal/acre. Band widths for the preliminary trial were 36" bands on either side of the "stock" for a treated width of 6' for the liquid applications and 48" bands on either side of the "stock" for a treated width of 8' for the granular treatments. Treatments were applied to plots x feet wide (depending on swath width tested) and 800' long (long enough to include a minimum of 5 IFA active mounds per plot). Active mounds within two-feet of the center line of the treatment were counted, leaving a treated buffer area outside the area that would normally be harvested with B&B stock. There were 3 replicates per treatment, including an untreated check. Prior to treatment and at 1, 2, 4, 6 and 8 weeks after final treatment, active IFA colonies in each plot were enumerated. Treatments were evaluated at 4-week intervals thereafter. Mounds were evaluated using as little disturbance as possible, usually a wire flag inserted into the mound. Mounds were considered active if any workers appeared. Using this data, colony mortality was calculated and experimental data was statistically analyzed using analysis of variance, and treatment means separated using the LSD test (P=0.05) for each posttreatment rating interval. Original plans were to include more contact insecticides than the four listed below, but excessive rainfall flooded areas of the test site and treatments could not be made.

Chemical	Formulation	Rate of Application
bifenthrin	Talstar® G (0.2%)	200 lb/acre (0.4 lb ai/acre)
deltamethrin	DeltaGard® G (0.1%)	131 lb/acre (0.13 lb ai/acre)
bifenthrin	Talstar® F (7.9%)	40 oz/acre (0.2 lb ai/acre)
deltamethrin	DeltaGard® SC (4.75%)	39 oz/acre (0.13 lb ai/acre)

RESULTS:

Both granular products provided 100% control of IFA in the evaluation area within 2 weeks of the contact insecticide application and maintained that control through 24 weeks (Table 1 and Figure 1). Deltamethrin liquid reached 100% control at 5 weeks and maintained it through 24 weeks. The bifenthrin liquid reached 95% control the first week and maintained that control through 24 weeks. One colony in one bifenthrin liquid plot remained active throughout the test period, however it became significantly less active over time, with very few workers appearing at the 15 week evaluation, none at 20 weeks and less than 25 workers at 24 weeks. This colony was located next to (against) the small concrete structure supporting a wind sock and apparently our application did not completely saturate this area. This lone remaining colony, although weakened, stresses the importance of complete and accurate application of the contact insecticide in this "dual" treatment technique.

This trial was terminated after the 24 week evaluation. Additional trials duplicating this treatment method will be conducted in 2003-2004 to substantiate results.

References Cited:

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

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

Hays, S.B., P.M. Horton, J.A. Bass and D. Stanley. 1982. Colony movement of imported fire ants. J. Georgia Entomol. Soc. 17: 266-272.

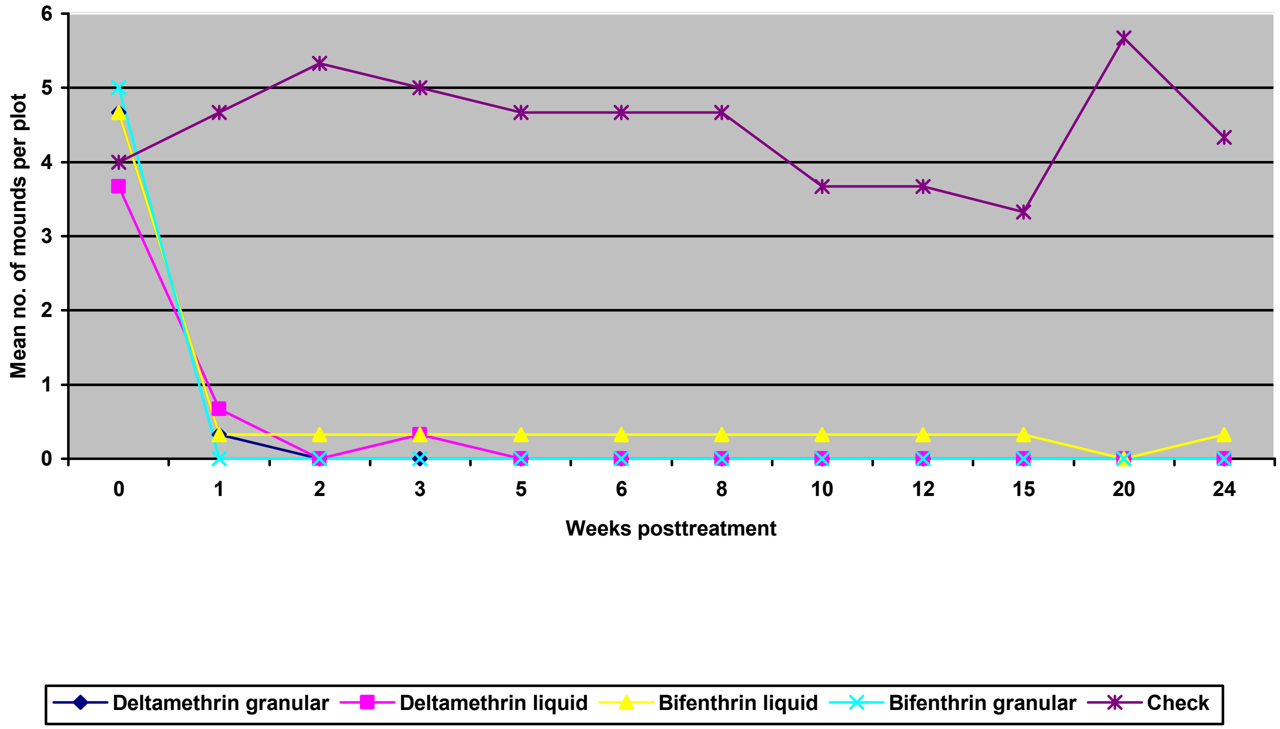
Williams, D.F. and C.S. Lofgren. 1983. Imported fire ant control: evaluation of several chemicals for individual mound treatments. J. Econ. Entomol. 76: 1201-1205.

Table 1. Efficacy of broadcast bait plus band contact insecticide treatments in eliminating IFA colonies from a specific area.

Treatment	Mean no. mounds pretreat*	Mean % change in no. active mounds present from pretreatment nos. at indicated weeks posttreatment										
		1	2	3	5	6	8	10	12	15	20	24
Deltamethrin Granular	4.67a	-94.4a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a
Deltamethrin Liquid	3.67a	-83.3a	-100.0a	-91.7a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a
Bifenthrin Liquid	4.67a	-95.2a	-95.2a	-95.2a	-95.2a	-95.2a	-95.2a	-95.2a	-95.2a	-95.2a	-100.0a	-95.2a
Bifenthrin Granular	5.00a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a
Check	4.00a	36.1b	58.3b	41.7b	25.0b	33.3b	25.0b	-2.8b	-2.8b	-11.1b	80.6b	33.3b

Means within a column followed by the same letter are not significantly different (LSD, P<0.05)

Figure 1. Colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide.



PROJECT NO: A1P04

PROJECT TITLE: Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, 2003

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Shannon James, Lee McAnally, Bob Jones, Tim Lockley, Shannon Wade, Ron Weeks, Anne-Marie Callcott

INTRODUCTION:

APHIS is responsible for developing treatment methodologies for certification of regulated items, such as field grown/balled-and-burlapped (B&B) nursery stock in the Imported Fire Ant Quarantine. Current treatments for field grown stock, as described below, are labor intensive, cumbersome and expensive. The only in-field treatment utilizes chlorpyrifos, the future of which is uncertain at best. Thus additional treatment methods are needed to insure movement of this type of material. Imported fire ants are slowly moving into areas of Tennessee where many producers of field grown nursery stock are located. This has prompted renewed interest in development of new treatments for this stock. New regulatory treatment methods for field grown/B&B nursery stock are needed to insure that nursery growers can comply with the Federal IFA Quarantine.

Currently the Federal Imported Fire Ant Quarantine (7CFR 301.81) has three treatment regimens for certification of field-grown and B&B nursery stock. The in-field treatment requires a broadcast application of an approved bait followed in 3-5 days by a broadcast application of granular chlorpyrifos. After a 30-day exposure period, plants are certified for 12 weeks. In 1999, PPQ allowed for a second application of the granular chlorpyrifos to extend the certification period for an additional 12 weeks. For harvested B&B stock, there are two certification methods: immersion in a chlorpyrifos solution or watering twice daily with a chlorpyrifos solution for 3 consecutive days.

The in-field treatment currently requires that the treatment must extend at least 10 feet from the base of each plant to be certified. This virtually means that the treatments must be applied broadcast to the entire nursery block. Numerous granular formulations of common insecticides such as diazinon, chlorpyrifos, acephate, and others are labeled for spot treatment of imported fire ant colonies. Imported fire ant colonies readily respond to insecticide applications made directly to the nest by relocating the colony (Collins & Callcott 1995, Hays et al. 1982, Franke 1983, Williams & Lofgren 1983). This insecticide induced movement is usually over a relatively short distance (1.5 to 3.0 meters), but can be greater (AMC, personal observation). The primary objective of a quarantine treatment for field grown nursery stock is to render the plants fire ant free. Therefore, it does not matter if colonies are killed outright by the treatment or simply induced to move away from the area around each individual plant intended for harvest.

Preliminary testing was initiated in Sept. 2001 by testing several liquid and granular insecticides against individual IFA mounds in the field. Results of this trial indicated promising results with acephate, bifenthrin, and deltamethrin (see GPPS01-02). A second preliminary trial, taking selected products to the field and testing in band applications was initiated in fall 2001 and spring 2002 in Mississippi. Using band widths of 5' to 6', bifenthrin and deltamethrin, both liquid and granular formulations showed promising results (see GPPS02-01; GPPS02-02). These trials, while promising, showed that in band treatments contact insecticide alone was not effective enough for use in the IFA quarantine. Therefore, in the fall of 2002, a trial was initiated whereby a broadcast application of a bait was done 3-5 days prior to the contact insecticide band treatments (Project No: A1P04; Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, Fall 2002). In this trial excellent results were achieved with all treatments (liquid and granular bifenthrin, liquid and granular deltamethrin).

MATERIALS AND METHODS:

Spring 2003 Band Trial:
Hydramethylnon bait treatments were applied at Camp Shelby Hagler Field, Mississippi on May 22, 2003 using a shop built spreader on a farm tractor calibrated to apply 1.5 lbs/acre. The following liquid and granular treatments were applied during May 27-30, 2003.

Chemical	Formulation	Rate of Application
bifenthrin	Talstar® G (0.2%)	200 lb/acre (0.4 lb ai/acre)
bifenthrin	MicroFlo® F (7.9%)	40 oz/acre (0.2 lb ai/acre)
chlorpyrifos	Dursban® 2.32G (2.32%)	260 lb/acre (6 lb ai/acre)
chlorpyrifos	Dursban® 4E (44.8%)	32 oz/acre (1 lb ai/acre)
deltamethrin	DeltaGard® G (0.1%)	131 lb/acre (0.13 lb ai/acre)
deltamethrin	DeltaGard® 5SC (4.75%)	39 oz/acre (0.13 lb ai/acre)
fipronil	Topchoice® G (0.0143%)	87 lb/acre (0.0125 lb ai/acre)
lambda-cyhalothrin	Scimitar® (9.7%)	10 oz/acre (0.06875 lb ai/acre)

Granular band treatments were applied using a Gandy 48" granular drop spreader attached to a farm tractor. Liquid band treatments were applied using a roller pump boom sprayer equipped with two standard flat spray tips (8015-SS; TeeJet Corp.) to provide a 36" band spray and a total spray volume equivalent to ca. 76 gal/acre. Band widths for the preliminary trial were 36" bands on either side of the "stock" for a treated width of 6' for the liquid applications and 48" bands on either side of the "stock" for a treated width of 8' for the granular treatments. Treatments were applied to plots x feet wide (depending on swath width tested) and 800' long (long enough to include a minimum of five IFA active mounds per plot).

Active mounds within two-feet of the center line of the treatment were counted, leaving a treated buffer area outside the area that would normally be harvested with B&B stock. There were 3 replicates per treatment, including an untreated check. Prior to treatment and at 1, 2, 3, 4, 6, and 8 weeks after final treatment, active IFA colonies in each plot were enumerated. Treatments were evaluated at 4-week intervals thereafter until 24 weeks after treatment or until two of the three plots per treatment displayed resurgence in IFA colonies. Mounds were evaluated using as little disturbance as possible; usually a wire flag was briefly inserted into the mound. Mounds were considered active if any workers appeared. Using this data, colony mortality was calculated and experimental data was statistically analyzed using analysis of variance, and treatment means separated using the LSD test (P=0.05) for each post treatment rating interval.

Fall 2003 Band Trial:
Bryan airport in Starkville, MS was selected as the test location for the fall trial. The majority of B&B is harvested in cold weather when trees are dormant, so it was important to initiate testing in colder weather than that found in south Mississippi. The more northern location proved difficult to locating sufficient numbers of mounds per plot, resulting in the test consisting of the following four treatments and a control.

Chemical	Formulation	Rate of Application
bifenthrin	Talstar® G (0.2%)	200 lb/acre (0.4 lb ai/acre)
bifenthrin	Talstar® F (7.9%)	40 oz/acre (0.2 lb ai/acre)
chlorpyrifos	Dursban® 2.32G (2.32%)	260 lb/acre (6 lb ai/acre)
chlorpyrifos	Dursban® 4E (44.8%)	32 oz/acre (1 lb ai/acre)

Treatments were applied as previously described with bait application occurring November 4, 2003 and contact insecticide application the following week on the 12th. Plot observation thus far has occurred prior to treatment and at 2, 3, 4, 6, 8, and 12 weeks post-treatment.

RESULTS:

Spring 2003 Band Trial:
At one week after the contact insecticides were applied, all treatments except the deltamethrin liquid achieved 100% control of IFA within the evaluation area (Table 1 and Figure 1). Apparent control was maintained by all products except the deltamethrin formulations for approximately four months after treatment. Bifenthrin granular was the longest lived treatment with week 20 showing its first indication of reinfestation. The deltamethrin spray treatment had one active mound on the same plot at both the one and three week observations, and at week eight a second plot had activity. Deltamethrin granular provided 100% control for a month after treatment; by week six there were two mounds on one of the plots for this treatment. Deltamethrin treatments have shown better results in other trials previous to this. More than seven inches of rain (rain gauge only measures 7 inches) fell previous to both weeks three and six which may have affected the longevity of the treatments. The granular treatments were most likely watered in by 1.5 inches of rain between application and the first observation a week later.

All previous trials were conducted in the fall and winter months when IFA populations are declining and/or at their lowest levels. There is also very little pressure from newly mated queens establishing new colonies during this time frame. During the spring and summer months, IFA colonies are very active, with many newly mated queens coming into areas as well as mature colonies relocating. These pressures may have impacted the longevity of the treatments during this trial compared to previous fall/winter trials.

Fall 2003 Band Trial:
All four chemical treatments displayed 100% mortality by the first observation at two weeks after treatment (Figure 2). No active mounds have appeared on any of the treated plots as of the 12 week observation. The number of active mounds on the control plots has declined over this period. The control plots in this trial are located in close proximity to the chemically treated plots and some foraging on bait may have occurred. Weather at this site has been persistently wet with frequent reports of standing water in some of the plots. The granular treatments were most likely watered in by 1.8 inches of rain between application and the first observation two weeks later. Observations for this trial will continue will continue every four weeks until reinfestation occurs on all treatments.

References Cited:

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

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

Hays, S.B., P.M. Horton, J.A. Bass and D. Stanley. 1982. Colony movement of imported fire ants. J. Georgia Entomol. Soc. 17: 266-272.

Williams, D.F. and C.S. Lofgren. 1983. Imported fire ant control: evaluation of several chemicals for individual mound treatments. J. Econ. Entomol. 76: 1201-1205.

Table 1. Sping 2003 trial – Efficacy of bait plus contact insecticide band treatments in eliminating IFA colonies from the observed plot area.

Treatment	**Mean no. mounds pretreat**	**Mean % change in no. active mounds present from pretreatment nos. at indicated weeks post treatment**								
		1	**2**	**3**	**4**	**6**	**8**	**12**	**16**	**20**
Bifenthrin F	6.33	-100 b	-100 b	-100 b	-100 b	-100 b	-100 b	-95.8	-94.4 d	-75 cd
Bifenthrin G	6.33	-100 b	-100 b	-100 b	-100 b	-100 b	-100 b	-100 b	-100 d	-87.8 d
Chlorpyrifos E	6.33	-100 b	-100 b	-100 b	-100 b	-100 b	-100 b	-100 b	-70.3 bcd	-62.8 cd
Chlorpyrifos G	6.67	-100 b	-100 b	-100 b	-100 b	-100 b	-100 b	-100 b	-89.7 cd	-84.9 d
Deltamethrin SC	8.00	-97.4 b	-100 b	-97.4 b	-100 b	-100 b	-94.4 b	-90.8 b	-43.8 abc	-42.0 bc
Deltamethrin G	6.00	-100 b	-100 b	-100 b	-100 b	-88.9 b	-100 b	-77.8 b	-37.6 ab	-21.7 ab
Fipronil G	6.33	-100 b	-100 b	-100 b	-100 b	-100 b	-100 b	-100 b	-84.7 bcd	-75.0 cd
Lambda-cyhalthrin	7.33	-100 b	-100 b	-100 b	-100 b	-100 b	-100 b	-100 b	-86.7 cd	-88.9 d
Check	10.67	6.7 a	-37.0 a	1.5 a	19.0 a	-3.5 a	-17.3 a	36.6 a	1.7 a	11.5 a

Means within a column followed by the same letter are not significantly different (LSD, P<0.05)

Figure 1. Spring 2003 trial - Colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide.

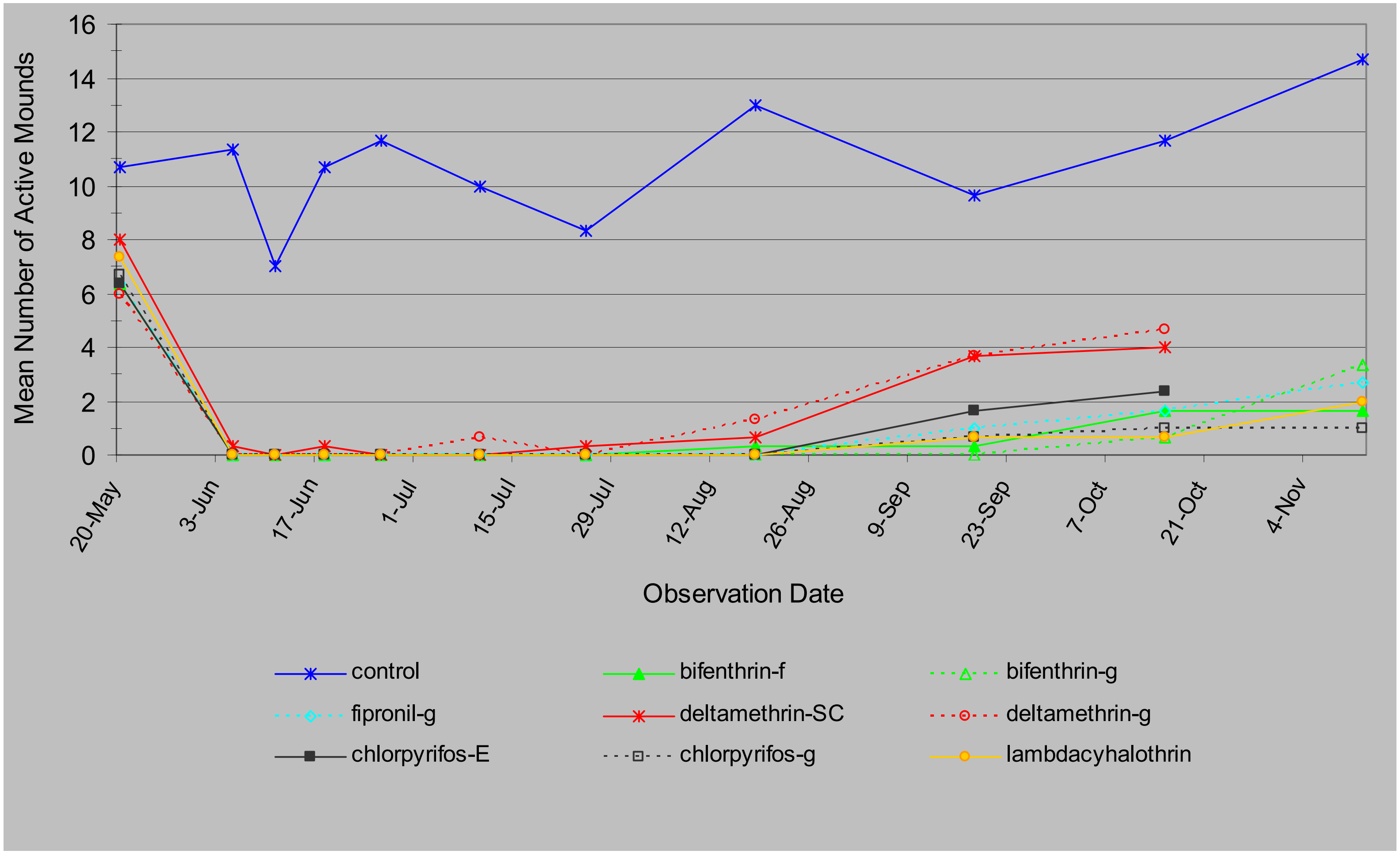
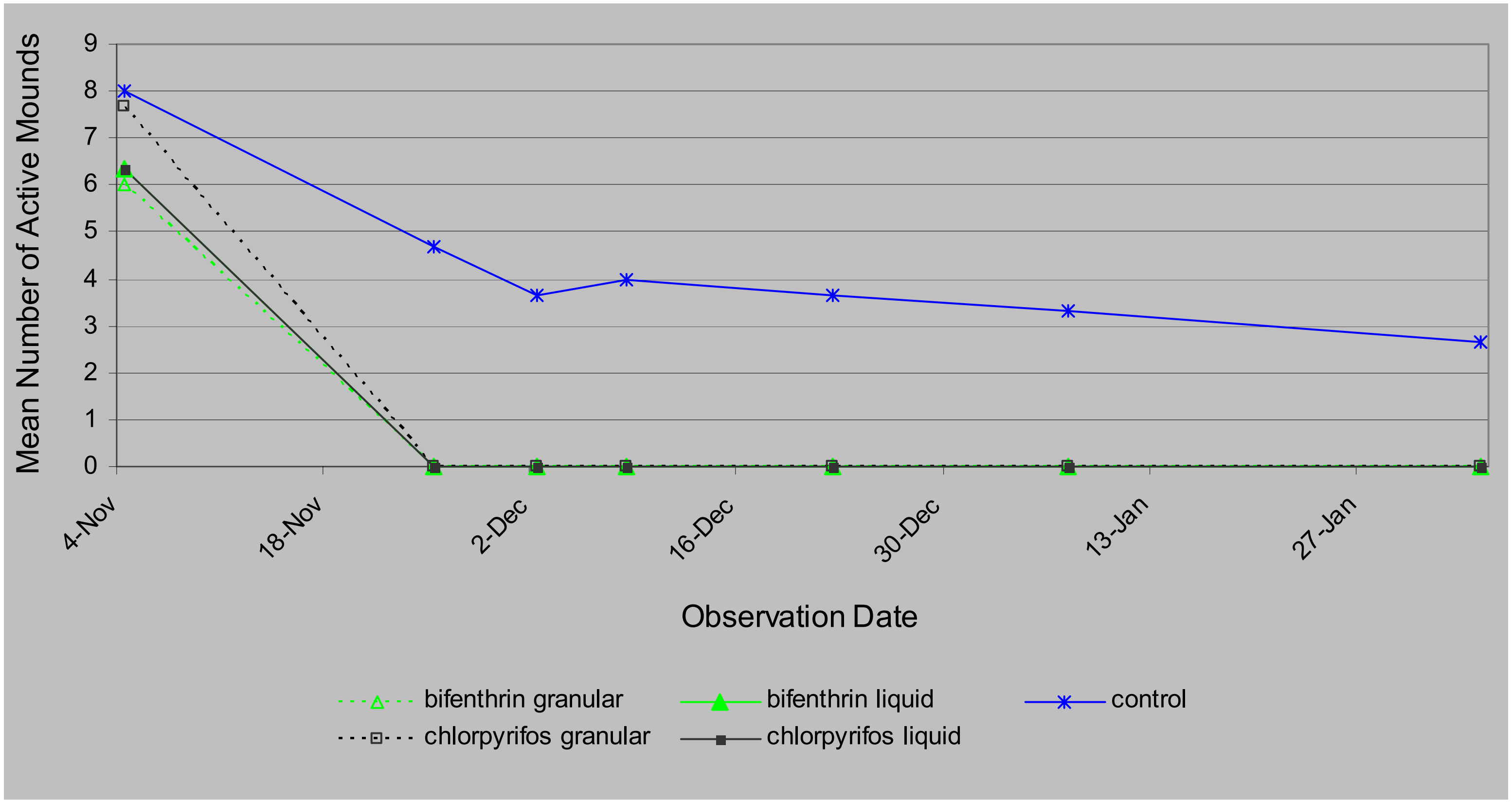


Figure 2. Fall 2003 trial – Colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide.



PROJECT NO: A1P04 - GPPS02-02

PROJECT TITLE: Development of Alternative Quarantine Treatment for Field Grown Nursery Stock – individual in-field tree treatment, 2003 (Winter/Spring)

TYPE REPORT: Final

LEADER/PARTICIPANTS: Shannon James, Anne-Marie Callcott, Lee McAnally, Tim Lockley, Shannon Wade, and Ron Weeks

COOPERATORS: Jason Oliver[1], Nadeer Youssef[1], and Karen Vail[2] .

[1] Tenn. State Univ., Nursery Crop Research Station, McMinville, TN
[2] Univ. of Tenn., Knoxville, TN

INTRODUCTION:

APHIS is responsible for developing treatment methodologies for certification of regulated items, such as field grown/balled-and-burlapped (B&B) nursery stock in the Imported Fire Ant Quarantine. Current treatments for field grown stock, as described below, are labor intensive, cumbersome and expensive. Furthermore, the only in-field treatment relies on a single contact insecticide. Thus additional treatment methods are needed to insure movement of this type of material. Imported fire ants are slowly moving into areas of Tennessee where many producers of field grown nursery stock are located. This has prompted renewed interest in development of new treatments for this stock. New regulatory treatment methods for field grown/B&B nursery stock are needed to insure that nursery growers can comply with the Federal IFA Quarantine.

Currently the Federal Imported Fire Ant Quarantine (7CFR 301.81) has one treatment regimen for certification of in-field and two treatment regimens for harvested B&B nursery stock. The in-field treatment requires a broadcast application of an approved bait followed in 3-5 days by a broadcast application of granular chlorpyrifos. After a 30-day exposure period, plants are certified for 12 weeks. In 1999, PPQ allowed for a second application of the granular chlorpyrifos to extend the certification period for an additional 12 weeks.

Numerous formulations of common insecticides such as diazinon, chlorpyrifos, acephate, and others are labeled for spot treatment of imported fire ant colonies. Imported fire ant colonies readily respond to any insecticide application made directly to the nest by relocating the colony (Collins & Callcott 1995, Hays et al. 1982, Franke 1983, Williams & Lofgren 1983). This insecticide-induced movement is usually over a relatively short distance (1.5 to 3.0 meters), but can be greater (AMC, personal observation). The primary objective of a quarantine treatment for field grown nursery stock is to render the plants fire ant free. Therefore, it does not matter if colonies are killed outright by the treatment or simply induced to move away from the area around each individual plant intended for harvest.

The Federal Imported Fire Ant Quarantine in-field treatment of B&B nursery stock currently requires that the treatment must extend at least 10 feet from the base of each plant to be certified. This virtually means that the treatments must be applied broadcast to the entire nursery block. Furthermore, the 30-day required exposure period following treatment application also precludes growers from making any additions or substitutions from previously untreated blocks. Consequently all plants required for orders must be anticipated more than a month in advance. Thus, trials of band-style treatments for large blocks of in-field B&B and individual plant-style treatments for select in-field plants were initiated to focus on examining efficacy of products other than chlorpyrifos, reduce treated diameter, and reduce the time of exposure required prior to plant movement.

MATERIALS AND METHODS:

Preliminary trials initiated in MS in January and September 2001 assessed several liquid and granular insecticides against individual IFA mounds in the field. Results of this trial indicated promising results with acephate, bifenthrin, and deltamethrin (see GPPS01-02 report). A similar trial conducted that February in Tennessee by TSU cooperators indicated that lower temperatures experienced at that test site might have hindered the performance of some treatments. Since B&B nursery products are generally harvested in the winter when the trees are dormant, product performance in low temperatures is of concern. Thus treatments were applied and observation initiated January 16, 2003 in Mississippi and February 5, 2003 at the Franklin and Sequatchie Co., TN sites. A small scale warm weather trial was also initiated at the end of March in Grundy Co., TN by Karen Vail.

Mississippi: Test plots in this trial consisted of individual IFA mounds and the surrounding ground that fit within a 36”-diameter circle. This size plot represents the smallest commonly harvested root ball size, 12” diameter, plus a 12” treated buffer zone surrounding the area to be harvested. One hundred twenty IFA mounds in a pasture in Harrison Co., MS were marked for use and divided into fifteen replications for each treatment. Mound activity was determined by poking a wire flag in the mound and observing IFA response. Mounds with ten or more ants appearing within ten seconds of mound disturbance were considered active. Wooden stakes labeled with the plot identification number were planted in close proximity to each plot to aid in visually locating the plot and attributing results to the appropriate treatment. Hula-hoops with a 36” diameter were utilized in conjunction with orange spray paint to uniformly mark the treatment areas around each plot.

Liquid treatments were applied using a roller pump-powered 55-gallon spray tank with a garden nozzle set on shower-pattern attached to the tank by a garden hose. Two gallons of the liquid treatments including the water control were applied on each of their respective plots. Preweighed amounts of the granular treatment were packed in individual zip-close bags and sprinkled over its respective plots by hand. The treatments in this trial are as follow:

Product	Active Ingredient	Rate of Application
Talstar GC - granular	bifenthrin	0.20 lb a.i./acre
Talstar GC - flowable	bifenthrin	0.20 lb a.i./acre

Scimitar (low rate)	lambda-cyhalothrin	0.88 lb a.i./acre
Scimitar (high rate)	lambda-cyhalothrin	1.76 lb a.i./acre
DeltaGard 5SC	deltamethrin	0.13 lb a.i./acre
Sevin SL	carbaryl	4.00 lb a.i./acre
Wet Control	water	Water only
Dry Control	----	No application

Observations of mound activity after application were conducted weekly until seventeen weeks had passed or failure of treatment. Weather conditions prevented observations at week seven after treatment. Temperature data throughout the duration of the trial was collected using a StowAway® data logger and accessed using BoxCar® 3.6 (Onset Computer Corp., Bourne, MA) and manual readings of air and soil temperatures were taken at the times of observation. A rain gauge located in the pasture was also checked manually.

Tennessee – Franklin and Sequatchie Counties: Jason Oliver and Nadeer Youssef set up fifteen replicates in Franklin Co. and an additional two replicates at a high elevation (~2000 ft above sea level) site in Sequatchie Co. Tennessee. Plot selection and set up were as described in the Mississippi trial. Applications were conducted similar to Mississippi treatments, with the exception that liquid applications were applied using 2-gallon watering cans. Two plots were lost at the Franklin Co. site prior to chemical treatment, so bifenthrin flowable and deltamethrin only had fourteen replicates at this site. The treatments applied to these sites were as follow:

Product	**Active Ingredient**	**Rate of Application**
Talstar GC - granular	bifenthrin	0.20 lb a.i./acre
Talstar GC - flowable	bifenthrin	0.20 lb a.i./acre
Scimitar (low rate)	lambda-cyhalothrin	0.88 lb a.i./acre
DeltaGard 5SC	deltamethrin	0.13 lb a.i./acre
Sevin SL	carbaryl	4.00 lb a.i./acre
Wet Control	water	Water only
Dry Control	----	No application

Sites were observed weekly from February 12, 2003 through May 28, 2003. Weather data was collected by data logger after the first week of the trial through the trial's end.

Tennessee – Grundy County: Karen Vail followed methods similar to those in the other TN trial; however due to scarcity of IFA mounds five plots each were allocated to the chemical treatments and four plots each for the wet and dry controls. Activity was determined using a trowel to open the mounds. Treatments applied at this site were as follow:

Product	**Active Ingredient**	**Rate of Application**
Talstar GC - flowable	bifenthrin	0.20 lb a.i./acre
Scimitar (low rate)	lambda-cyhalothrin	0.88 lb a.i./acre
DeltaGard 5SC	deltamethrin	0.13 lb a.i./acre
Wet Control	water	Water only
Dry Control	----	No application

RESULTS:

Mississippi: The deltamethrin, both rates of lambda-cyhalothrin, and the bifenthrin flowable treatments delivered fast knock down in their respective plots (Figures 1 and 2). All colonies in the deltamethrin and high rate of lambda-cyhalothrin were inactive by two weeks after treating and remained inactive until week 10. Bifenthrin flowable and the lower rate of lambda-cyhalothrin displayed 100% inactivity in their plots in weeks 4-10 and 5-8 observations, respectively. There was no evidence of product efficacy in the granular bifenthrin treatments until after rains between observations on weeks four and five (Figure 3). It appears that the granular bifenthrin treated plots may have been prone to reinfestation while still maintaining some insecticidal activity as a different plot was infested each week after week six. Likewise, when renewed activity was seen in the flowable bifenthrin and high rate lambda-cyhalothrin treatment plots, it was never the same plot twice. At two months after application the carbaryl treatment had three plots that never went inactive and one that was reinfested. This treatment was only slightly more effective at removing active fire ant colonies than water. Carbaryl at week 8 was determined to be inadequate to quarantine needs and further observation of those plots was terminated. When the trial was terminated, 17 weeks after treatment application, the untreated dry control had only lost activity in one of its plots. The water treated control demonstrated a relatively slow descent to a minimum of five active plots but rebounded to seven by the trial's end.

Tennessee – Franklin County: Bifenthrin flowable and deltamethrin treatments both provided a quick kill with 100% inactivity at weeks two and three respectively (Figure 4). Periods with no IFA activity for bifenthrin flowable and deltamethrin treated plots lasted from weeks 2-9 and 3-8 respectively, but mounds did not remain active in consecutive weeks until around weeks 13 and 14. Lambda-cyhalothrin also provided a fairly quick kill, but had a lingering colony through week five. After week 5, no activity was noted in weeks 6-16. Granular bifenthrin displayed a slightly delayed kill, but dropped down to one active plot at three weeks and maintained one or two through the rest of the trial. The somewhat harsher weather is probably the cause for synchronous drops in activity in plots across both control treatments and the carbaryl treatment in the period from week 5 through week 7. Carbaryl maintained several active colonies throughout the trial.

Tennessee – Sequatchie County: Both controls died out before the end of the test at this higher elevation (Figure 5). Dry controls were inactive at week 10 and one of the wet plots was destroyed by week 2 and the other wet plot was inactive at week 6. The deltamethrin treated plots at this elevation were inactive from week three through the end of the trial. One of the bifenthrin flowable treated plots remained active at least through week seven at this site. Bifenthrin granular also had a lingering active mound here. One lambda-cyhalothrin treated mound was intermittently active through week 6. Both carbaryl plots appear to have survived.

Tennessee – Grundy County: Bifenthrin flowable attained control of IFA by week six and no activity was recorded in those plots through week 18. Deltamethrin and lambda-cyhalothrin treatments showed no activity in their plots at weeks 3 and 4 respectively, but periodically activity was recorded in plots afterwards. The water control plots had a better survival than the dry control plots; most likely this was due to the dry hot weather that occurred during this trial.

Plots were unable to be checked at weeks 8-10 and at week 11 mounds were hard to find due to vegetation height.

DISCUSSION:

Data from these single mound plot trials will be used in conjunction with that from band trials to determine new treatment regimes that meet quarantine standards and at least some of the goals mentioned previously. At this point the cold-weather tests in both Mississippi and Tennessee indicate that chemical applications of deltamethrin, lambda-cyhalothrin, and flowable bifenthrin hold promise as potential additions to the quarantine treatments. The Tennessee application of granular bifenthrin demonstrated control of IFA populations much sooner than the Mississippi application. The Tennessee site however had wet soil at the time of application and received rainfall after application sooner than the Mississippi site. The next trial with this product will include watering in directly after application. Carbaryl was insufficient in both of the trials and even out survived the two controls that were tested in Sequatchie Co., TN.

The results from the high altitude plots may indicate control issues at higher altitudes, but with so few plots tested at this elevation no conclusions can be drawn. Likewise, with so few plots in the Grundy Co., TN trial it is hard to determine true efficacy of treatments, but differences due to warmer weather are indicated. Trials of single mound plot treatments using at least 15 replicates are scheduled for both winter and spring of 2004 to examine these potential differences.

References Cited:

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

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

Hays, S.B., P.M. Horton, J.A. Bass and D. Stanley. 1982. Colony movement of imported fire ants. J. Georgia Entomol. Soc. 17: 266-272.

Williams, D.F. and C.S. Lofgren. 1983. Imported fire ant control: evaluation of several chemicals for individual mound treatments. J. Econ. Entomol. 76: 1201-1205.

Figure 1. Efficacy of individual mound treatments – Mississippi.

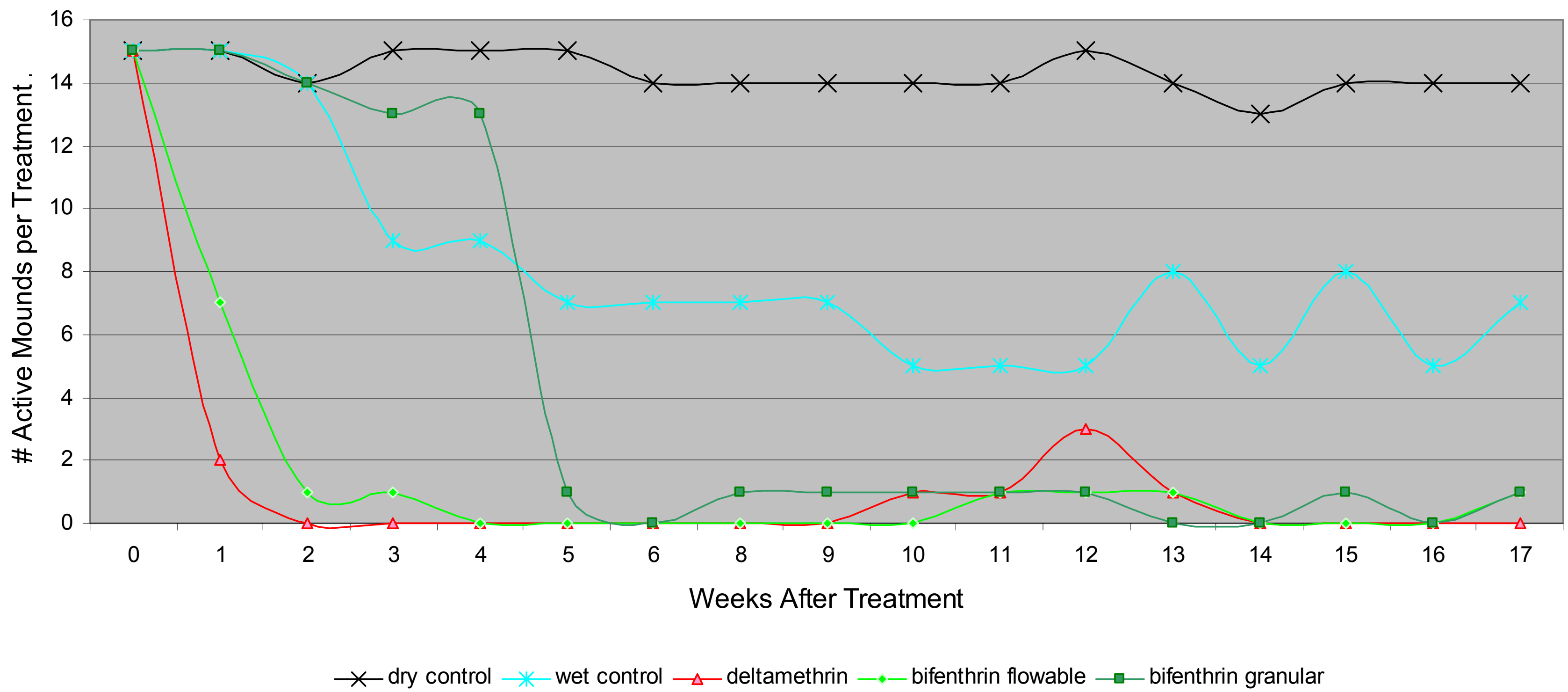


Figure 2. Efficacy of individual mound treatments – Mississippi.

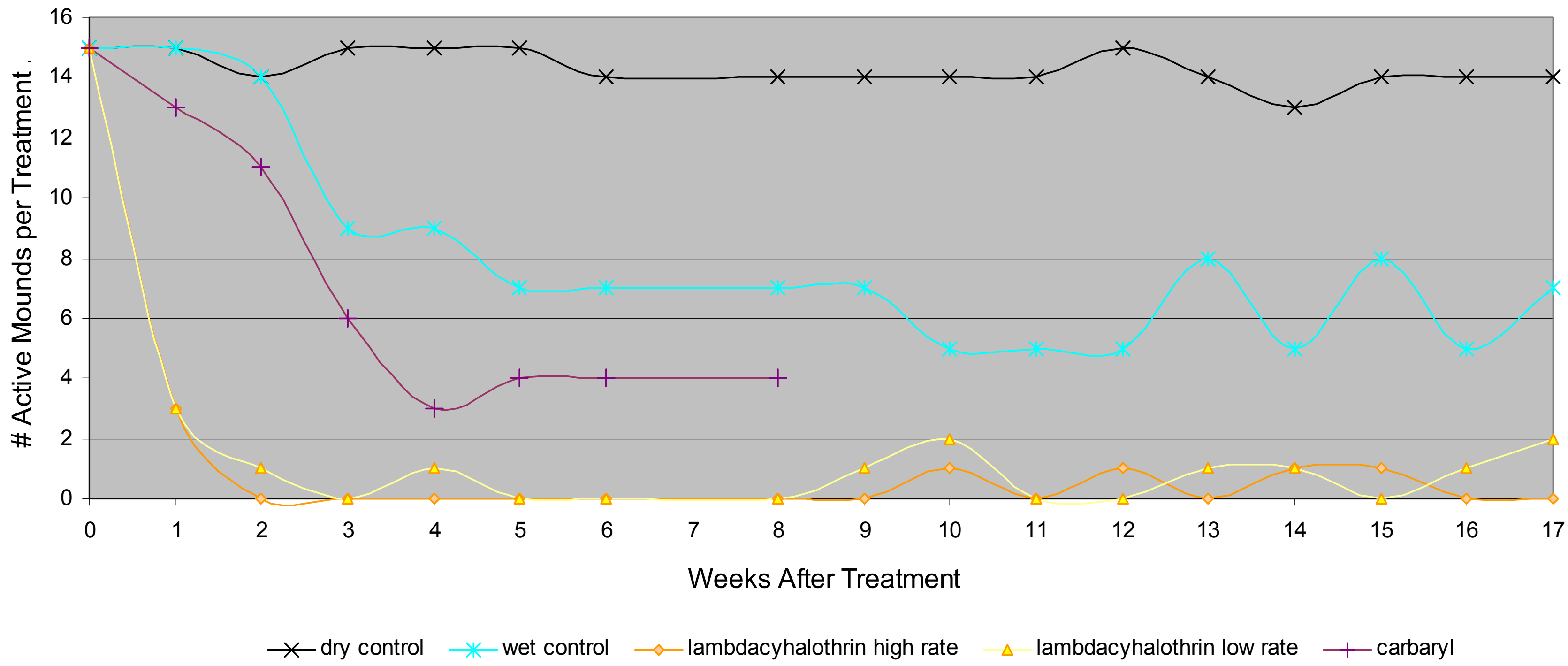


Figure 3. Weather measured at the Mississippi trial site through the duration of the test.

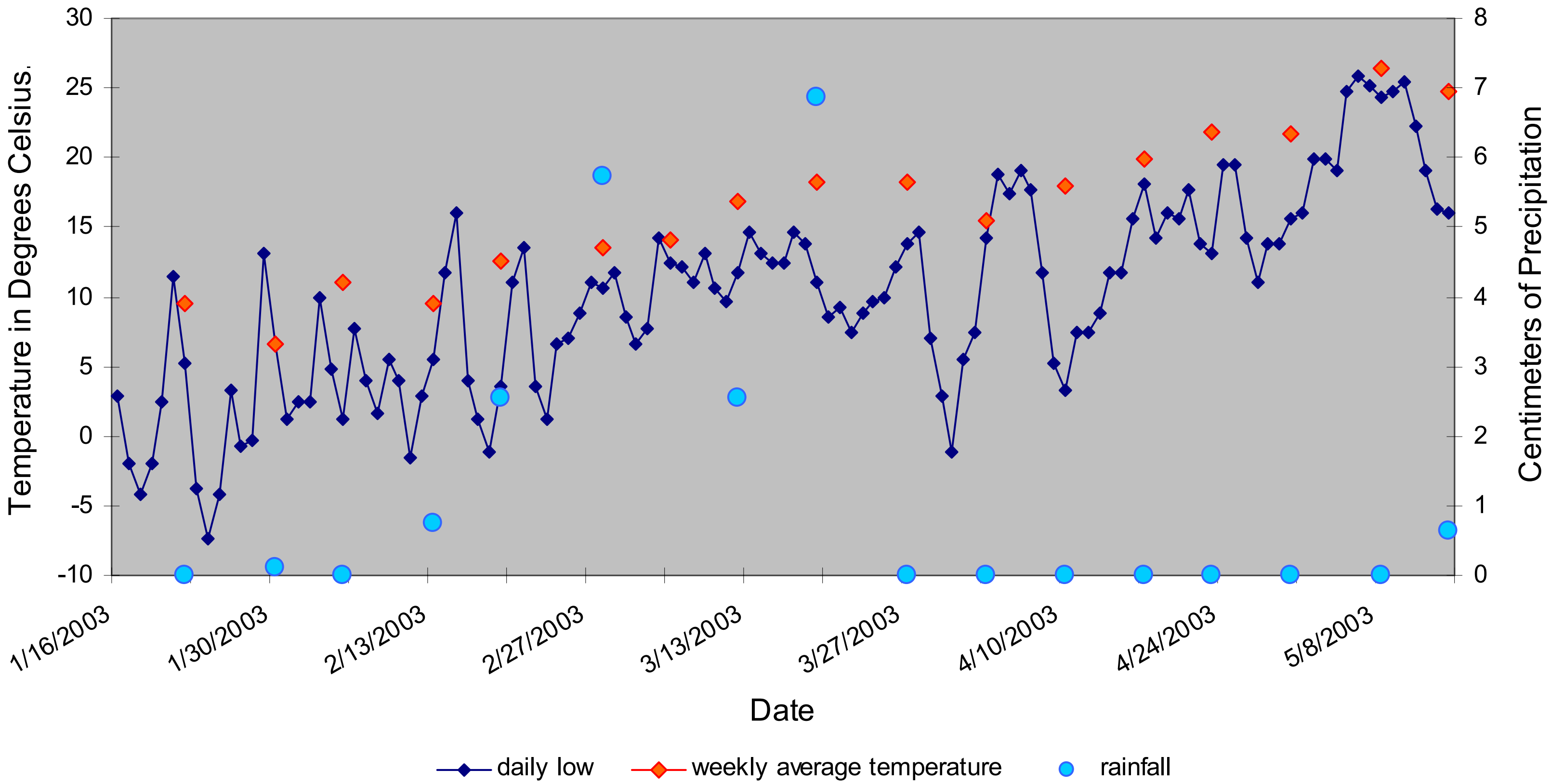


Figure 4. Efficacy of individual mound treatments – Franklin Co., Tennessee

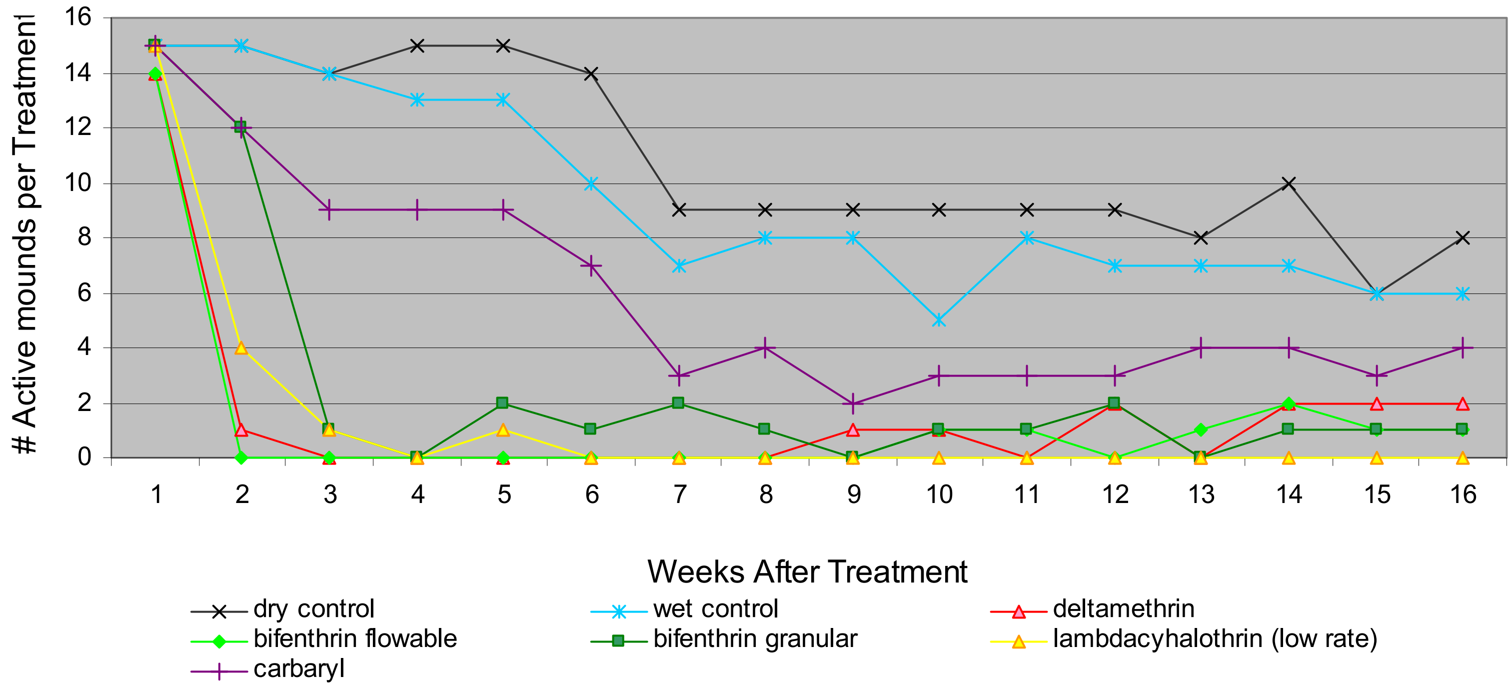


Figure 5. Efficacy of individual mound treatments – Franklin & Sequatchie Co., Tennessee

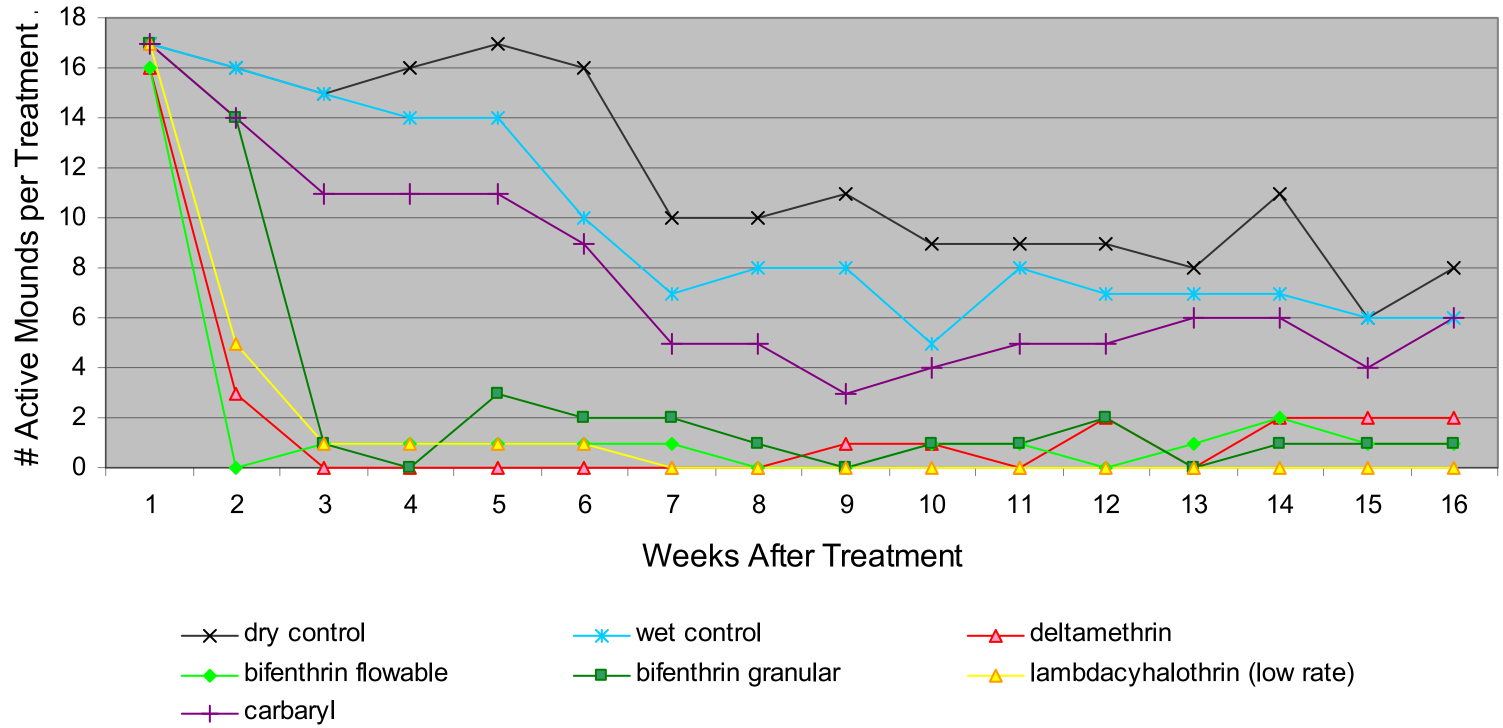
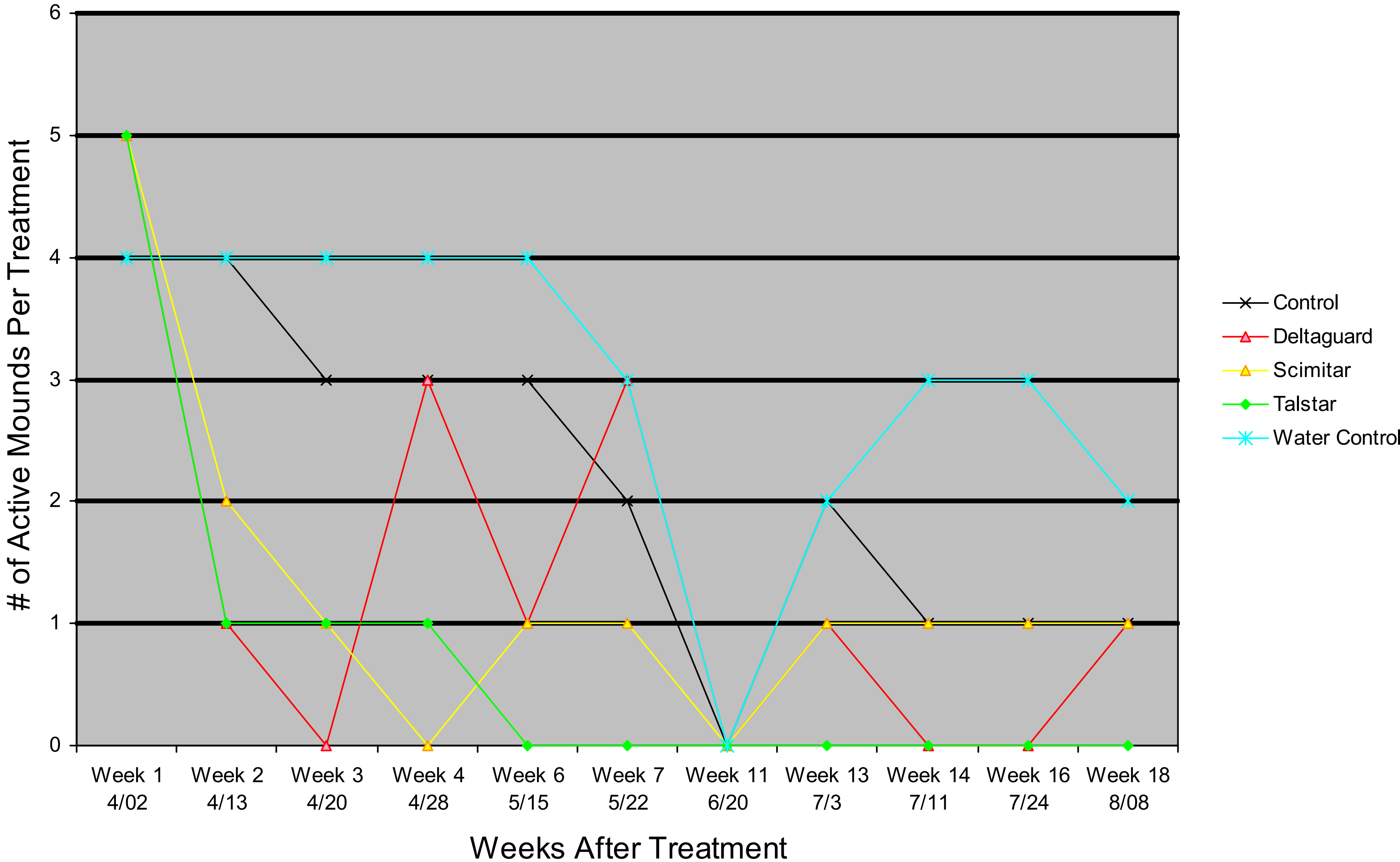


Figure 6. Efficacy of individual mound treatments – Grundy Co., Tennessee



PROJECT NO: A1P04

PROJECT TITLE: Balled-and-Burlapped (B&B) Drench Treatments: Efficacy of Single Drench Treatments of Harvested Balls, 2001-2002.

REPORT TYPE: Final

PROJECT LEADER/PARTICIPANT(s): 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 balled and burlapped plants include immersion in a chlorpyrifos solution or twice daily drenches for three consecutive days with a chlorpyrifos solution. Adding additional chemical options to these use patterns will give growers more products to choose from. We also intend to investigate the efficacy of single drenches on balls instead of the labor intensive 3-day drenching schedule.

Initial trials were conducted to determine what pesticides might be good candidates for root ball drenches. Once pesticides were selected they were subjected to actual root ball drench trials.

MATERIALS AND METHODS:

Initial Container Trials:
Three gallon nursery containers were filled with sandy clay topsoil from southern Mississippi and placed on a brick in 12" x 18" x 5" plastic pan. The sides of the pans were painted with fluon to prevent escape. Three replicates per treatment were utilized. Field collected colonies were then separated from their nest tumulus by placing them in 2' x 8' x 5" separation trays and spreading the nest tumulus out. Artificial nests were provided for the colonies to move into. Once colonies moved into the artificial nests they were removed and placed into a 3 gallon bucket, anesthetized with CO_2 and 100cc of workers and brood were added to each soil filled pot. The ants were then allowed 24 hours to acclimate. Each pot was then treated with an amount of insecticidal solution equal to 1/5 the volume of the pot. Treatment rates were as follows:

Talstar F (bifenthrin)	0.1 lb ai/100 gal H_2O	45.2 ppm (1X)
Talstar F (bifenthrin)	0.05 lb ai/100 gal H_2O	22.6 ppm (½X)
Deltagard SC (deltamethrin)	0.08 lb ai/100 gal H_2O	35.5 ppm (1X)
Deltagard SC (deltamethrin)	0.04 lb ai/100 gal H_2O	17.75 ppm (½X)
Scimitar CS (lambda-cyhalothrin)	0.14 lb ai/100 gal H_2O	59.7 ppm (1X)
Scimitar CS (lambda-cyhalothrin)	0.07 lb ai/100 gal H_2O	29.85 ppm (½X)
Demon EC (cypermethrin)	0.07 lb ai/100 gal H_2O	50 ppm
Demon EC (cypermethrin)	0.14 lb ai/100 gal H_2O	100 ppm
Platinum (thiamethoxam)	0.07 lb ai/100 gal H_2O	50 ppm
Platinum (thiamethoxam)	0.14 lb ai/100 gal H_2O	100 ppm

Pots were checked daily for mortality for 7 days or until 100% mortality. Once 100% mortality was achieved the pots were then placed in a simulated can yard with overhead irrigation. At intervals of 1 week, 2 weeks, 1 month, 2 months, and 3 months one pot from each treatment was placed in the separation trays mentioned above, along with a check pot with untreated media (option test). A field collected colony was then placed in the tray. The pots were checked daily to determine which pot the colony moved into.

Root Ball Drenches:
Balled and burlapped plants 15 to 18 inches in diameter were placed in a 2'x 4'x 6" tray lined with black plastic sheeting (three root balls per treatment (one per tray). The upper walls of the trays were coated with fluon to prevent escape. Field collected colonies were then separated from their nest tumulus using the floatation method (Banks et al. 1981) and 100cc of ants and brood were placed on the root ball and allowed 24 hours to move into the root balls. Three replicates per treatment were used. The root balls were then drenched with 1 gallon of solution, corresponding to the lb ai/100 gal rate of the containerized test, by pouring the solution very slowly over the top of the root ball. Root balls were observed daily for mortality. Once 100% mortality was achieved root balls were moved outside to weather naturally. Soil core samples were taken monthly and subjected to alate queen bioassay (Appendix I).

<u>RESULTS:</u>

Initial Container Trials:
All treatments except the Platinum treatment provided 100% mortality within 24 hours. Platinum treatments did not appear to have any mortality and thus was excluded from the option test. Results of the option test are summarized in Table 1. All ants moved into the available control container in all choice tests through 2 months. At 3 months, ca. 100 workers did iinfest the high rate deltamethrin container, but all others continued to exclude infestation.

Root Ball Drenches:
Three trials were initiated; bifenthrin (Talstar) ½X, deltamethrin (DeltaGard) ½X, and lambda-cyhalothrin (Scimitar) 1X. All rates provided 100% mortality of colonies infesting the root ball. In alate female bioassays, the Talstar ½X drench rate produced 100% mortality through 4 months and 85% or better through 6 months. The Deltagard ½X drench rate produced 100% mortality through 6 months. The Scimitar 1X drench rate has produced 100% mortality through 6 months. Results of alate female bioassays are summarized in Table 2. Test was terminated after this due to other root ball drench and dip tests being initiated in Tennessee and Mississippi.

References Cited:

Banks, W.A., C.S. Lofgren, D.P. Jouvenaz, C.E. Stringer, P.M. Bishop, D.F. Williams, D.P. Wojcik and B.M. Glancey. 1981. Techniques for collecting, rearing, and handling imported fire ants. USDA, ARS, Science and Education Administration, Advances in Agricultural Technology, Southern Series, No. 21.

Table 1. Drench Option Test (number of ants moved into indicated pot after 7 days exposure*)

Treatment	Post-Treatment Interval									
	1 wk		2 wks		1 month		2 months		3 months	
	treated	check	treated	check	treated	check	treated	check	treated	check
Talstar 45.2 ppm	0	5000	0	5000	0	5000	0	5000	0	5000
Talstar 22.6 ppm	0	5000	0	5000	0	5000	0	5000	0	5000
Deltagard 35.5 ppm	0	5000	0	5000	0	5000	0	5000	100	4900
Deltagard 17.75 ppm	0	5000	0	5000	0	5000	0	5000	0	5000
Scimitar 59.7 ppm	0	5000	0	5000	0	5000	0	5000	0	5000
Scimitar 29.85 ppm	0	5000	0	5000	0	5000	0	5000	0	5000
Demon 50 ppm	0	5000	0	5000	0	5000	0	5000	0	5000
Demon 100 ppm	0	5000	0	5000	0	5000	0	5000	0	5000

*Assumes 5000 ants per tray

Table 2. Residual Activity of Various Drenches as Root ball Drenches

Treatment	% Mortality After 7 Days Exposure at Indicated Post-treatment Interval														
	1 Month					2 Months					3 months				
	Treated Replicate			AVG	Check	Treated Replicate			AVG	Check	Treated Replicate			AVG	Check
	1	2	3												
Talstar ½ label	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
Deltagard ½ label	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
Scimitar 1X label	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0

Treatment	% Mortality After 7 Days Exposure at Indicated Post-treatment Interval														
	4 Months					5 Months					6 months				
	Treated Replicate			AVG	Check	Treated Replicate			AVG	Check	Treated Replicate			AVG	Check
	1	2	3												
Talstar ½ label	100	100	100	100	0	100	55	100	85	10	100	95	100	98.333	5
Deltagard ½ label	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0
Scimitar 1X label	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0

PROJECT NO: A1P04

PROJECT TITLE: Alternative B&B Immersion or Drench Treatments for use in the IFA Quarantine, 2002

REPORT TYPE: Final

PROJECT LEADER/PARTICIPANT(s): Lee McAnally, Shannon James; Jason Oliver and Nadeer Youssef of Tennessee State University

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 balled and burlaped (B&B) plants include immersion in a chlorpyrifos solution or twice daily drenches for three consecutive days with a chlorpyrifos solution. Several trials were initiated in conjunction with the Tennessee State University Nursery Crop Research Station to identify other dip or drench treatments that could be used concurrently for both the IFA Quarantine and Japanese Beetle programs.

MATERIALS AND METHODS:

Spring 2002 Dip Trial:
Due to limitations of resources, all treatments were conducted at the Japanese Beetle rates of application. Treatments were conducted in March 2002 at LanTenn Nursery (Belvidere, TN) by personnel from the Tennessee State University Nursery Crop Research Station. B&B plants were dipped in insecticidal solutions in large garbage cans lined with plastic. Treated plants were placed in a nursery environment. Core type soil samples were taken and shipped to the CPHST-ANPCL soil Inhabiting Pests Lab where they were subjected to standard alate queen bioassay (Appendix I). Samples were collected at 60, 120 and 180 days post-treatment. Candidate treatments and rates were as follows:

Treatment	Common Name	Rate (lb ai/100 gal H2O)
Mach 2 2SC	Benzoic acid	1.50 (1X)
		0.75 (1/2X)
Orthene 75WSP	Acephate	.075 (1X)
Talstar Nursery F	Bifenthrin	0.23 (1X)
		0.12 (1/2X)
Marathon 60WP	Imidachloprid	0.30 (1X)
		0.15 (1/2X)
Dylox 80 T&O	Dimethyl phosphonate	8.00 (1X)
		4.00 (1/2X)
Sevin SL	Carbaryl	8.00 (1X)
		4.00 (1/2X)
Flagship 25WG	Thiamethoxam	0.13 (1X)

		0.07 (1/2X)
Deltagard GC 5S	Deltamethrin	0.13 (1X)
Dursban TNP	Chlorpyrifos	2.00 (1X)
Control		------

Fall 2002 Dip and Drench Trials:
Again, rates of application were based on Japanese beetle rates, due to the dual nature of this testing. Fall dip trials were conducted in the same manner and location as above in late October 2002. Additionally, B&B Dogwood trees were drenched in the field twice a day for 3 consecutive days. Approximately 35 gallons of drench solution was mixed in a 55 gal drum lined with polyethylene drum liner. Solutions were applied by means of a hand operated transfer pump fitted with a garden hose and a spray wand with a showerhead type spray nozzle. Approximately 1/6 gal of solution was applied to each root ball at each application time for a total of 1 gallon of solution per ball. At 2 weeks, and then at monthly intervals core type soil samples were taken from four replicates of each treatment and shipped to Gulfport where they were subjected to standard alate queen bioassay. Samples from the dipped plants were taken from the top of the root ball. Samples from the drenched plants were taken from the top, middle and bottom of the root ball as it was lying in the field.

Treatment	Common name	Rate (lb ai/100 gal H2O)
Talstar Nursery F	Bifenthrin	0.23 (1X)
		0.12 (1/2X)
Flagship 25WG	Thiamethoxam	0.13 (1X)
		0.07 (1/2X)
Deltagard GC 5S	Deltamethrin	0.13 (1X)
Marathon 60WP	Imidachloprid	0.40 (1X)
Dursban TNP	Chlorpyrifos	2.00 (1X)
Scimitar SC	Lambda-cyhalothrin	0.034 (1X)
Control		------

RESULTS:

Spring 2002 Dip Trial:
Both full and half rates of Talstar F were 100% effective against IFA alate females for 6 months after treatment (Figure 1). Dursban and the full rate of Flagship were very effective for 4 months. DeltaGard and the half rate of Flagship were effective for 2 months.

Fall 2002 Dip and Drench Trials:
Dip: Only samples from the top of the ball were evaluated for dip treatments, assuming that because the balls were completely immersed in the treatment solution all parts of the ball should be equally treated. At 3 months after treatment, all treatments and rates have been 100% effective. Through 2 months all rates provided 100% control within 6 days of exposure, while at 3 months, both Flagship rates required up to 14 days to provide 100% control (still acceptable). At 4 months after treatment both Flagship rates had fallen below acceptable control and were removed from further testing. Marathon fell below 100% in month 4 below 80% in month 5 and was back up to 100% in month 6. All other treatments provided 100% control through 6 months.

Drench: In this trial, the top of the root ball was considered that area which received the watering in or drench treatment, and the bottom was that area opposite the "top", usually the side in contact with the ground. All products and rates from media collected from the top of the root balls provided excellent control of IFA alate females through 3 months (Figure 3). Media collected from the middle of the balls was very ineffective at 2 weeks and was not collected after that time. However, in hindsight, this should have been done since many of these products appeared to penetrate the media over time (as shown in the bottom data) and provide better control later in the evaluation period, therefore a 6 month bioassay was set up and all treatments except Flagship(not tested) and Scimitar attained 95-100% control. Media collected from the bottom of drenched root balls provided interesting and erratic information for many of the products (Figure 5). However, the high rate of Talstar consistently provided >90% control of alate females through 4 months and became erratic after that. The Dursban was less effective at 2 weeks than at any other time, and provided 100% control at 1 month, dropping to around 90% at months 2-4 and declined after that, verifying current treatment certification period of 30 days.

The most promising treatments were replicated in spring 2003 in Tennessee and additional trials in Mississippi, using IFA rates were applicable, were also conducted in 2003.

Figure 1. Efficacy of various immersion/dip treatments for B&B nursery stock – spring 2002.

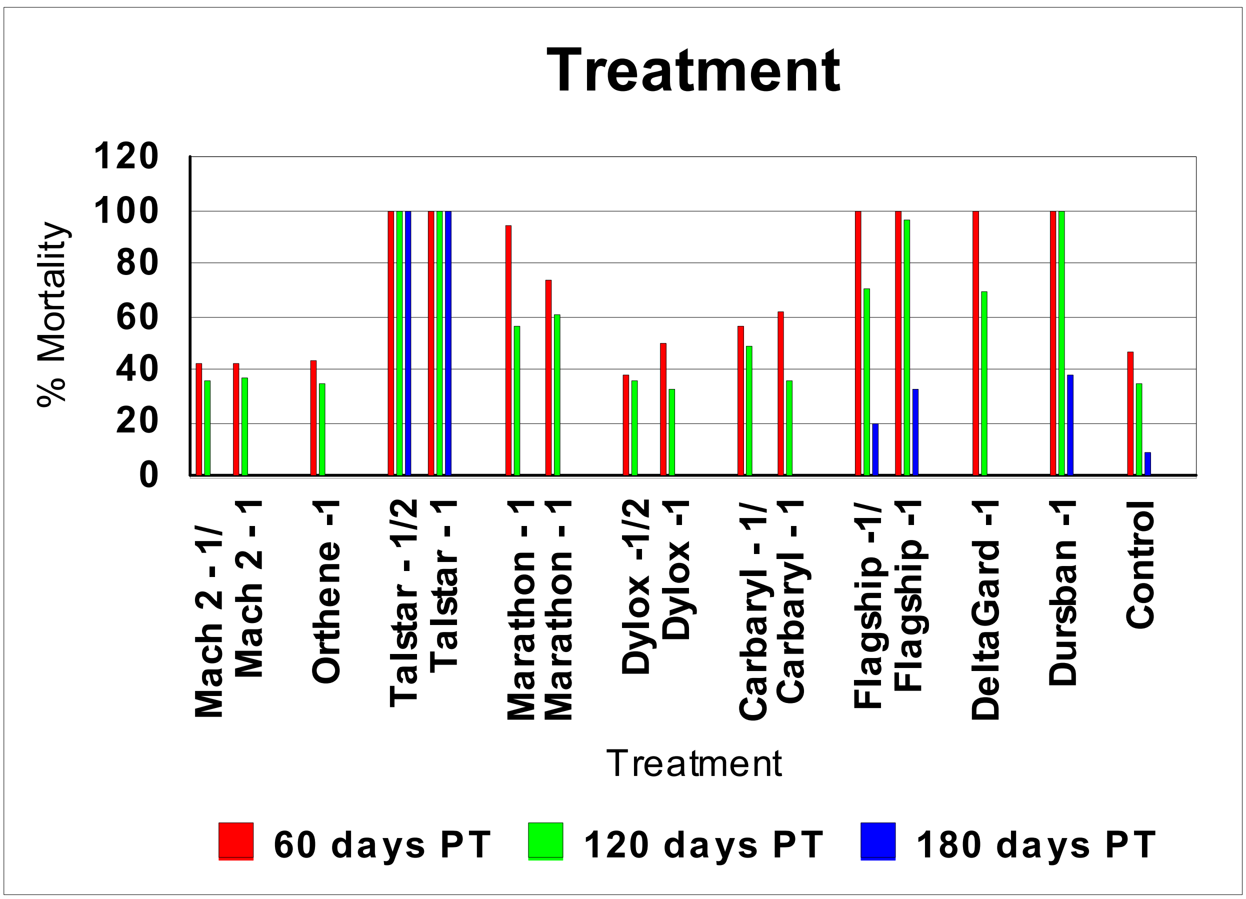


Figure 2. Efficacy of various immersion/dip treatments for B&B nursery stock – fall 2002.

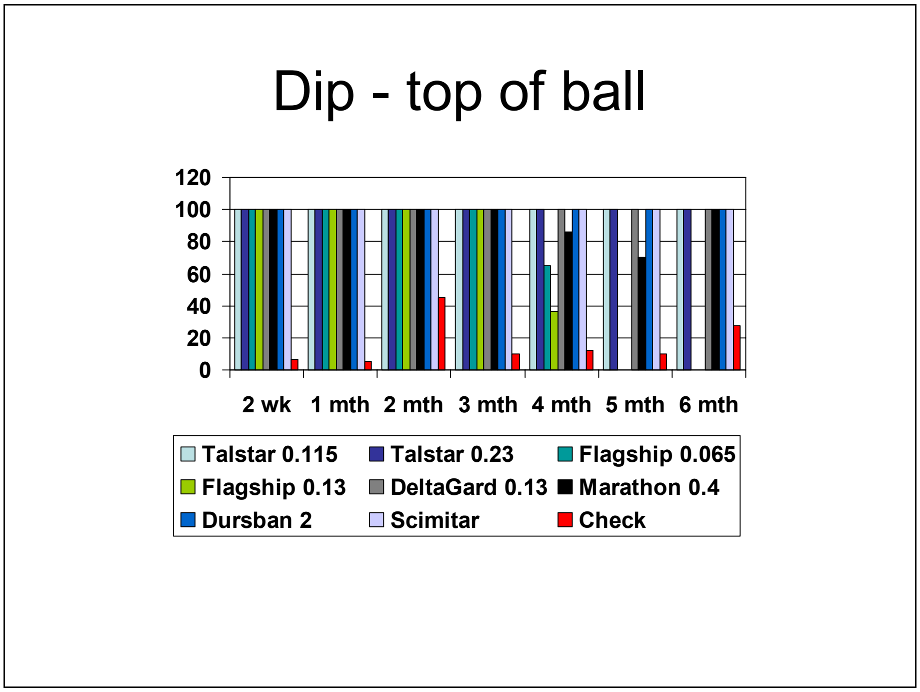


Figure 3. Efficacy of B&B drenches (2X/day on 3 consecutive days) testing media collected from various parts of the root ball – top of ball.

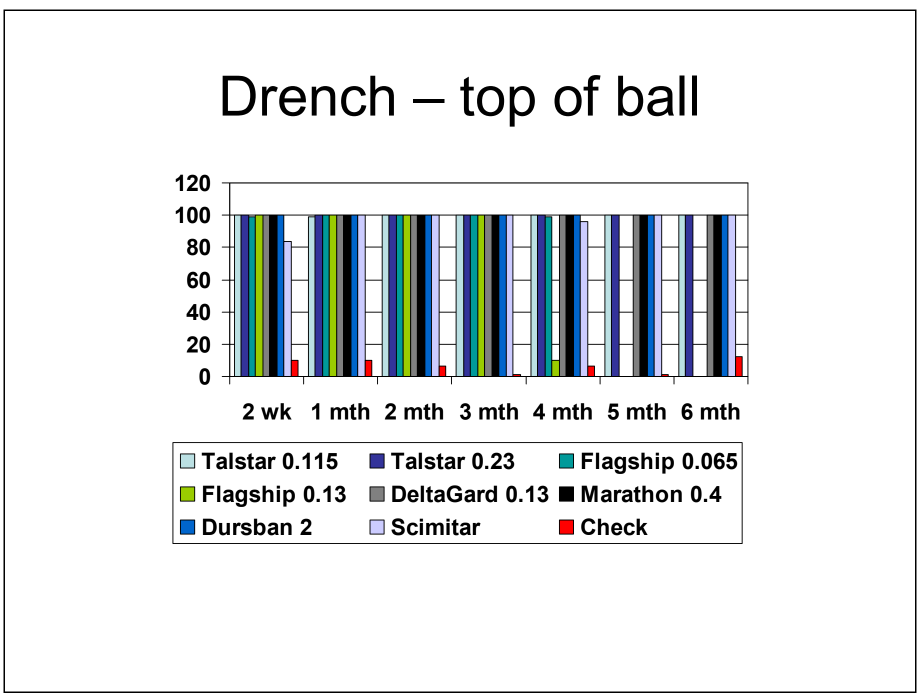


Figure 4. Efficacy of B&B drenches (2X/day on 3 consecutive days) testing media collected from various parts of the root ball – middle of ball.

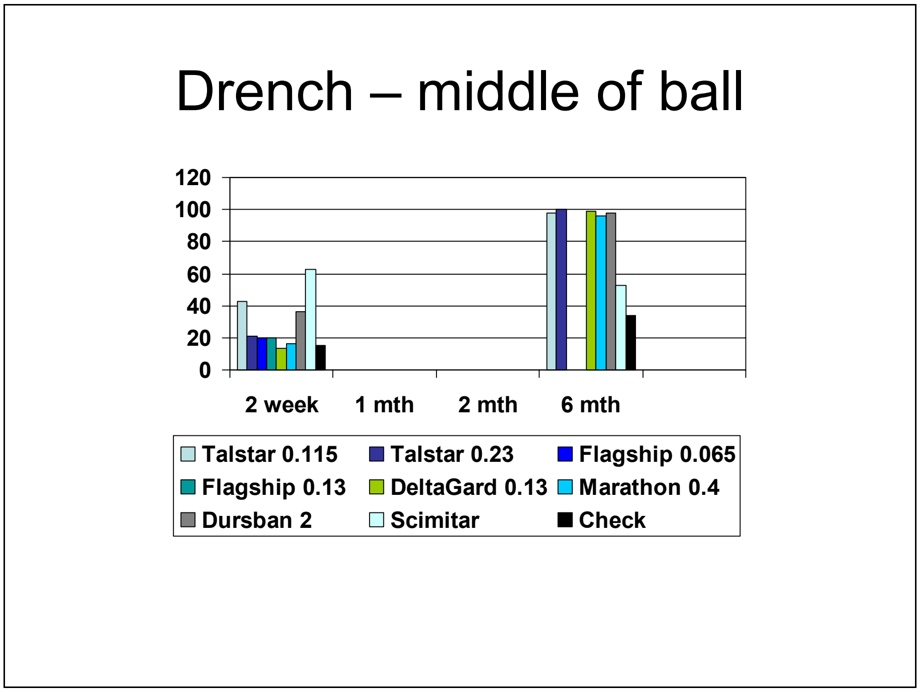
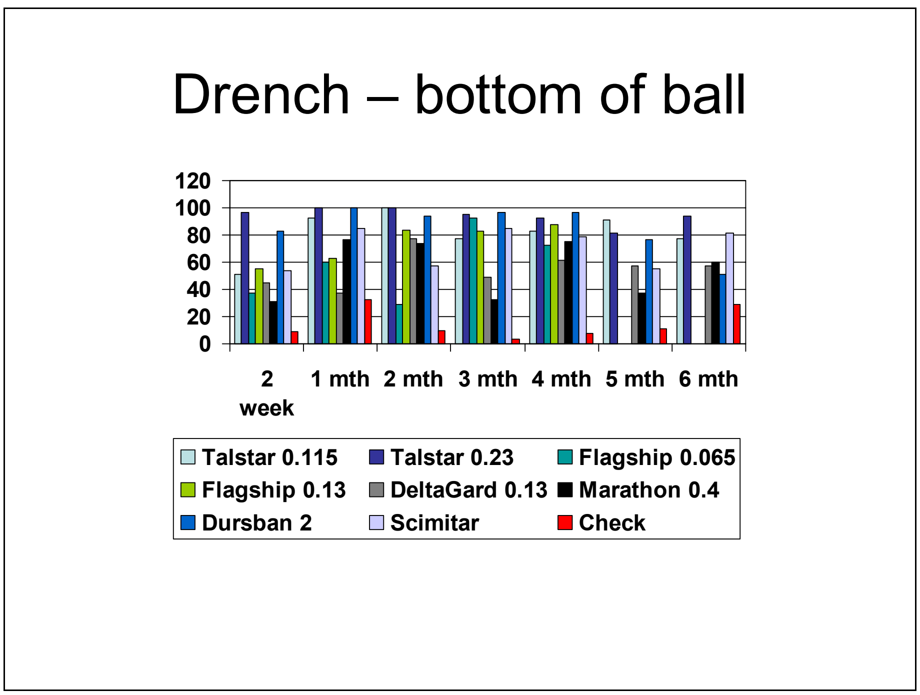


Figure 5. Efficacy of B&B drenches (2X/day on 3 consecutive days) testing media collected from various parts of the root ball – bottom of ball.



PROJECT NO: A1P04

PROJECT TITLE: Alternative B&B Immersion Treatments for use in the IFA Quarantine, Spring 2003

REPORT TYPE: Final

PROJECT LEADER/PARTICIPANT(s): Lee McAnally, Shannon James; Jason Oliver and Nadeer Youssef of Tennessee State University; Mike Klein and Jim Moyseenko

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 balled and burlaped (B&B) plants include immersion in a chlorpyrifos solution or twice daily drenches for three consecutive days with a chlorpyrifos solution. Several trials were initiated in conjunction with the Tennessee State University Nursery Crop Research Station to identify other dip or drench treatments that could be used concurrently for both the IFA Quarantine and Japanese Beetle programs.

MATERIALS AND METHODS:

Spring 2003 Dip Trial:
Treatments were conducted in Spring 2003 at LanTenn Nursery (Belvidere, TN) by personnel from the Tennessee State University Nursery Crop Research Station. On March 25, 2003 B&B plants were dipped in insecticidal solutions in large garbage cans lined with plastic. Treated plants were placed in a nursery environment. Core samples were taken and shipped to the CPHST-ANPCL soil Inhabiting Pests Lab where they were subjected to standard alate queen bioassay (Appendix I). Core samples from the center of the root ball were taken at 60, 90, 120 and 180 days post-treatment and surface samples were taken at 60 days post-treatment. Candidate treatments and rates were as follows:

Product	Active Ingredient	Rate (lb a.i./100 gal H20
Flagship 25WG	Thiomethoxam	0.065
Flagship 25WG	Thiomethoxam	0.13
Marathon 60WP	Imidicloprid	0.3
Sevin SL	Carbaryl	8.0
Deltagard GC 5SC	Deltamethrin	0.13
Dursban TNP	Chlorpyrifos	0.125
Dursban TNP	Chlorpyrifos	2.0
Scimitar GC	Lambda-cyhalothrin	0.017
Scimitar GC	Lambda-cyhalothrin	0.034
Talstar Lawn & Tree Flowable	Bifenthrin	0.115
Talstar Lawn & Tree Flowable	Bifenthrin	0.23

RESULTS:

Results summarized in Table 1 are the average of the four replicates used in each treatment at 7 and 14 day exposure intervals. Deltagard and both rates of Talstar provided 100% control through 180 days post treatment. The high rates of Dursban and Scimitar both provided 100 % control through 90 days post-treatment.

Table 1. Efficacy of B&B immersion treatments.

Formulation tested	Mean % mortality to alate females at indicated months post-treatment									
	60 Days (surface)		60 Days (center)		90 Days (center)		120 Days (center)		180 Days (center)	
	7day	14day	7day	14day	7day	14day	7day	14day	7day	14day
Flagship (0.065)	26.25	51.25	65	96.25	23.75	83.75	21.25	70	45	76.25
Flagship (0.13)	56.25	82.5	73.75	97.5	41.25	100	16.25	97.5	33.75	68.75
Marathon	78.75	80	60	93.75	63.75	100	15	62.5	32.5	62.5
Sevin	13.75	31.25	5	22.5	***	***	***	***	***	***
Deltagard	100	100	100	100	100	100	100	100	100	100
Dursban (0.125)	88.75	90	86.25	92.5	43.75	53.75	28.75	32.5	8.75	40
Dursban (2.0)	100	100	100	100	100	100	75	80	58.75	70
Scimitar (0.017)	100	100	97.5	100	90	97.5	80	88.75	60	73.75
Scimitar (0.34)	100	100	100	100	100	100	88.75	98.75	77.5	91.25
Talstar (0.115)	100	100	100	100	100	100	100	100	100	100
Talstar (0.23)	100	100	100	100	100	100	100	100	100	100
Check	16.25	41.25	22.5	35	17.5	37.5	6.25	20	20	32.5

PROJECT NO: A1P04

PROJECT TITLE: Alternative B&B Immersion or Drench Treatments for use in the IFA Quarantine, Mississippi and Tennessee 2003

REPORT TYPE: Final

PROJECT LEADER/PARTICIPANT(s): Shannon James, Lee McAnally, Anne-Marie Callcott, Shannon Wade, Ron Weeks, Tim Lockley

INTRODUCTION:

Imported fire ants are slowly moving into areas of Tennessee where many producers of field grown nursery stock are located. Approximately 80% of this nursery stock ships outside the Federal IFA Quarantine zone. This has prompted renewed interest in development of new treatments for this stock. 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 harvested balled and burlapped (B&B) plants include immersion in a chlorpyrifos solution or twice daily drenches for three consecutive days with a chlorpyrifos solution. Both treatment solutions are mixed at a rate of 4 fl. oz. of a 4EC formulation per 100 gal. water. Restrictions on chlorpyrifos use patterns within the past several years may foreshadow loss for B&B uses: furthermore, producers of B&B frequently must treat stock for multiple quarantined pest species prior to shipment. Thus, a cooperative research effort to screen other liquid insecticides for inclusion in IFA quarantine treatments for B&B, with special deference to products effective for Japanese beetle, was initiated with Jason Oliver of the Tennessee State University Nursery Crop Research Station. Trials conducted in 2002 in cooperation with the Tennessee State University Nursery Crop Research Station indicated several chemicals which could be used in addition to chlorpyrifos in this type of nursery stock.

MATERIALS AND METHODS:

Mississippi Trials

Immersion Trial:

Treatments were conducted on site July 8-10, 2003. Six B&B plants with 16”-diameter root balls were submerged per treatment in insecticidal solutions in large plastic garbage cans until cessation of bubbling. Treated root balls were placed on pallets to prevent cross contamination by run off from other treatments and stored outside on these pallets through the duration of testing. Soil core samples were taken from the top surface of three of the root balls in each treatment at monthly intervals over the first three months. These soil samples were then used in standard alate queen bioassays (Appendix I) to determine efficacy of the treatment. At three months these root balls were then split open for a sample from the center of each ball as well as the regular top sample. The remaining three balls in each treatment were sampled for the following three months (4-6 months PT) and split for a center sample at 6 months PT. Treatments and rates were as follow:

Treatment	Active Ingredient	Rate (lb ai/100 gal H_2O)	
		High rate	Low rate
Talstar Nursery F	Bifenthrin	0.05	0.025
Deltagard GC 5S	Deltamethrin	0.04	0.02
Scimitar	Lambda-cyhalothrin	0.034	0.017
Dursban 4E	Chlorpyrifos	0.125	------
Control	Water	------	------

Drench Trials:
Six B&B trees with 16"-diameter root balls per treatment were drenched twice a day for three consecutive days from June 24th through the 26th for a total of 6 drench applications per ball. Solutions were applied by means of a roller pump powered spray tank fitted with a garden hose and a showerhead type spray nozzle. Approximately 0.63 L (0.17 gal) of solution was applied to each root ball at each application time for a total of 3.78 L (1 gal) of solution per ball (Appendix II). At monthly intervals soil core samples were taken from three replicates of each treatment and subjected to standard alate queen bioassays. Soil samples from the drenched plants were taken from the top, middle and bottom of the root ball as determined by ball position during treatment application. The top of the root ball was considered that area which received the watering in or drench treatment, and the bottom was that area opposite the top, usually the side in contact with the ground. At three months the sampled balls were split for a sample from the center of the ball. This soil sampling method was repeated over the following three months with the remaining balls. Treatments and rates were as follow:

Treatment	Active Ingredient	Rate (lb ai/100 gal H_2O)	
		High rate	Low rate
Talstar Nursery F	Bifenthrin	0.10	0.05
Deltagard GC 5S	Deltamethrin	0.04	0.02
Scimitar	Lambda-cyhalothrin	0.034	0.017
Dursban 4E	Chlorpyrifos	0.125	------
Control	Water	------	------

Tennessee Trials
Immersion Trials:
Due to limitations of resources and the dual nature of this project, all treatments were conducted at the Japanese Beetle rates of application. Treatments were performed October 20-22, 2003 at a commercial nursery in Warren Co., TN by personnel from the Tennessee State University Nursery Crop Research Station.

Thirty plants with 12”-diameter root balls were immersed per treatment for a minute in a dip tank that consisted of one of the following treatments:

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

Soil samples are being collected from the surface and center of each of four replicates used for red imported fire ant studies at 15, 30, 60, 120, and 180 days post-harvest.

Drench Trials:
Twenty harvested B&B plants with 25-inch root balls were drenched twice daily over three consecutive days for each treatment in this trial for a total of 6 drench applications. Insecticidal solutions were prepared in 30-gal drums with polypropylene liners and pumped through a hose attached to a shower-headed nozzle using a Shur-Dri battery-powered pump. Pesticide treatments were applied at the same rate in one of two water volume treatments, including 2.56 L (0.68 gal) water per root ball per application (1X) (equivalent to 1/30 the total ball volume and sufficient to achieve runoff as described by Fire Ant Quarantine) or 5.12 L water per root ball (2X) (Appendix II; used larger 25-inch root ball dimensions). All products, with the exception of Marathon which was tested only at the 2X rate, were tested at both the 1X and 2X rates. Products and rates used are as follow:

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

Four of the twenty balls from each treatment are being sampled at three locations for red imported fire ant bioassays. Sample locations are top-surface, bottom-surface, and middle. The two surface samples are collected from within the first four inches and the middle sample is collected at depths between four and eight inches. The “top” of the ball is determined as the upper most surface during treatment; this was marked with orange spray paint after treatment was complete. Sample dates for the drench trial are the same as the immersion trial. The samples for both trials are frozen until arrival at the CPHST-ANPCL Soil Inhabiting Pests Lab where the samples are utilized in standard alate queen bioassays. At this time samples have been collected and bioassayed for 15, 30, and 60 days post-treatment.

RESULTS:

Mississippi Trials

Immersion Trial:

Chlorpyrifos and both bifenthrin rates produced 100% mortality throughout the six-month duration of the trial (Figure 1). The three and six month center samples for chlorpyrifos and bifenthrin also matched the performance of the regular top surface soil samples (Figure 2). Lambda-cyhalothrin at its high rate produced 95% to100% mortality in all sample periods except at two months. Center samples for lambda-cyhalothrin at the higher rate were lower than the regular soil samples taken at three months, but both center and regular soil from the balls sampled at six months had 100% mortality. Both the high rate of deltamethrin and the low rate of lambda-cyhalothrin killed better than 95% of the ants in the first month after treatment and dropped off in the following two months. However, when the second set of balls was sampled for these two treatments ant mortality was high again. Center samples at both three and six months for the high rate of deltamethrin produced results similar to their regular top surface sample counterparts. Both the three and six month center samples for the low rate of lambda-cyhalothrin were slightly more lethal than the corresponding regular sample results. The low rate of deltamethrin did not perform well except in the fifth month of testing.

Drench Trial:

Bifenthrin at the higher rate was the only treatment to yield 100% mortality across all three external sample locations; top, middle and bottom (Figures 3, 4, and 5). This treatment maintained a mortality of 90% or higher through five months in the top and middle samples and through three months in the bottom samples. Chlorpyrifos and the low rate of bifenthrin were the only other treatments to affect a 90% or higher control in more than one sample location. Adequate control at the top and middle locations was achieved for the first two months and the first month for the low rate of bifenthrin and the chlorpyrifos treatments respectively. It is worth noting at this point that through out the trial enough rain water to saturate the base of the balls periodically collected in the wading pools the balls were stored in. This situation may have affected results of samples taken from ball bottoms. The high rate of lambda-cyhalothrin demonstrated control at higher than 90% in the first two months and again in the first sampling of the second set of balls at four months but only in top samples. Top samples of the high rate of deltamethrin yielded 90% or better in the first two months and in month five. Both of the low rates of lambda-cyhalothrin and deltamethrin demonstrated such low results that, after evaluating the first samples from the second set of balls at four months post-treatment, further sampling from these two treatments was discontinued.

Even coverage as demonstrated by comparative treatment efficacy at 90 days post-treatment was only seen in the two bifenthrin treatments (Figure 6). Center core sample results were all lower than external samples at 90 days indicating lack of treatment penetration. Results from center core samples of both bifenthrin treatments collected at 180 days post-treatment were higher than external samples and much higher than center core sample results at 90 days post-treatment

(Figure 7). This may indicate some inward migration of bifenthrin through the soil. Evenness demonstrated by both bifenthrin treatments at 90 days was diminished by 180 days.

Tennessee Trials

The results reported here are only the red imported fire ant portions of a much larger project. Japanese beetle and chemical degradation information will be reported along with the completed fire ant results at a later date. Samples have yet to be collected and tested for the 120-day and 180-day (four and six month respectively) sample dates.

Immersion Trial:

Thus far at all sample dates and in both the surface and center locations of sampling, Scimitar, Dursban, Talstar, and DeltaGard yield 100% control (Figures 8 and 9). Flagship provided 100% mortality at all sample dates in its center samples and at the fifteen day sample for the surface. Results from the surface samples on the other two sample dates are above 80% mortality for this treatment. Marathon appears to yield uneven coverage of control or sampling of cold and hot spots of treatment. Both at the fifteen day and one month sample dates results for the surface samples and the center samples did not closely match, but both times one would yield results in the 90% or higher range. The results from the two month sample however both reached 100%. All queens died in both locations of samples for Dylox collected at fifteen days. Results for both sample locations for the remaining dates however declined to levels below 70% mortality. The highest mortality induced by the Sevin treatment occurred in the center samples collected at fifteen days. All samples for that treatment on following dates dropped to levels similar to the untreated control. Orthene, with the exception of a spike in mortality at the surface location on the most recent sample date, has maintained levels of mortality consistent with the control.

Drench Trial:

Due to the complex comparisons available by volume rate, sample location, and date, results for the drench trial at this point in time are broken down by product used.

Dursban treatments at both volume rates yielded equivalent control at 100% or in the 90's in the top and center samples across all collection dates (Figures 10 and 11). Comparisons of the bottom sample results indicate inconsistency in the 2X volume treatment or sampling of a cold spot (Figure 12). The 1X volume treated samples maintained 100% mortality in the first two sample dates but dropped to the high 70's at the 60-day sample. Results from the 2X treated samples from the base, however, were relatively low at two weeks, 100% mortality at one month, and then higher than 1X at two months.

Talstar treatments at both volumes provided complete control at the top sample location at all sample dates. The 2X volume treatment maintained control either at 100% or in 90's in the other sample locations. Results for the 1X volume treatment were at 100% at all locations of sampling on both the two week and two month sample dates. The one month results at this volume rate however dipped for the center and bottom surface samples.

Flagship at the 1X volume rate produced high levels of mortality, 90% or greater, except on the first sample date at the bottom sample location. The 2X volume rate was not as consistent in its results as the 1X and was not consistent across sample dates and locations.

Scimitar treatments at both volume rates had control at 100% or in the high 90's across all sample locations for the day fifteen collected samples. Results from the top surface remained high while results from bottom-surface and center samples fell. Scimitar applied at the 2X volume was either equal or slightly better than the 1X volume treatment in ability to control the ants.

The Marathon treatment had its highest results for each sample date at the top sample site. Results from the center samples indicate possible movement of the treatment into the ball over the two month period. Overall, results for this treatment are not sufficient for quarantine purposes.

DISCUSSION:

Multiple candidate treatments are indicated by the results of the immersion method of treatment. Further trials are expected to refine rates and define potential duration of certification periods for B&B treated in this manner. The drench applications at rates similar to the immersions did not yield equivalent results. The Mississippi portion of this trial indicated only the higher rate of bifenthrin was adequate over an extended period of time when applied in the twice-daily-over-three-days style of drench. The bottom samples may have had product leached out by the rain soaked situation previously mentioned in the Mississippi trials. Mississippi trials of drench applications to be conducted in 2004 will be moved out of runoff containment immediately after the end of treatment and stored on pallets to prevent cross contamination. A variety of drench application regimes will be examined in the coming year in both Mississippi and Tennessee to determine application methods that are both more economical to apply and provide even control over the whole root ball. A comparison of the Mississippi results with the Tennessee results will be conducted at the conclusion of the Tennessee trial.

Figure 1. Efficacy of various chemicals and rates used in immersion treatments for B&B nursery stock: top surface of ball - Mississippi 2003.

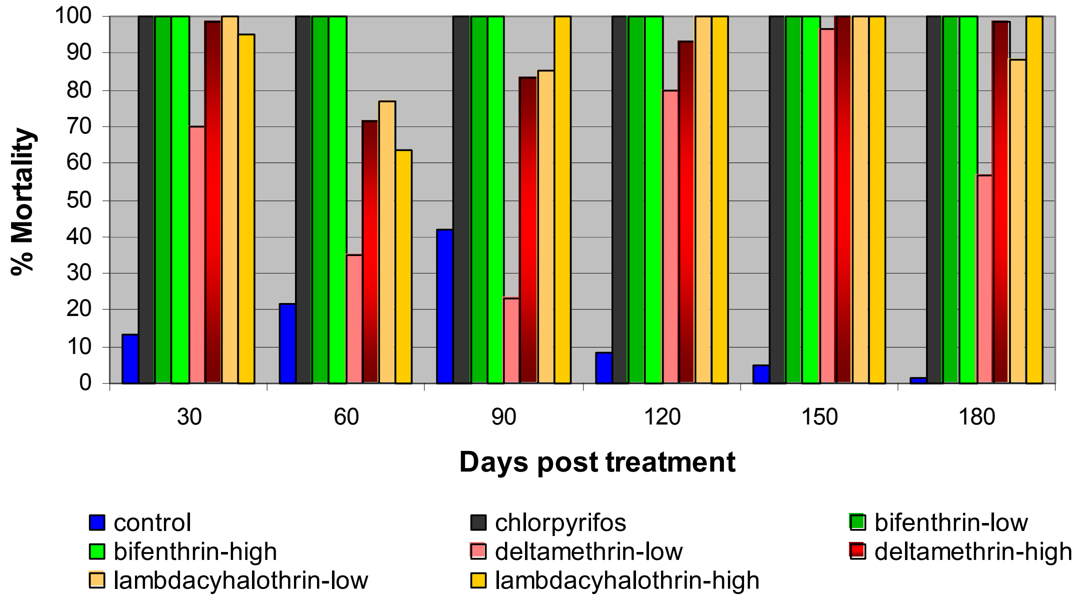


Figure 2. Penetration and evenness of treatment efficacy of various immersion treatments for B&B nursery stock applied in Mississippi 2003.

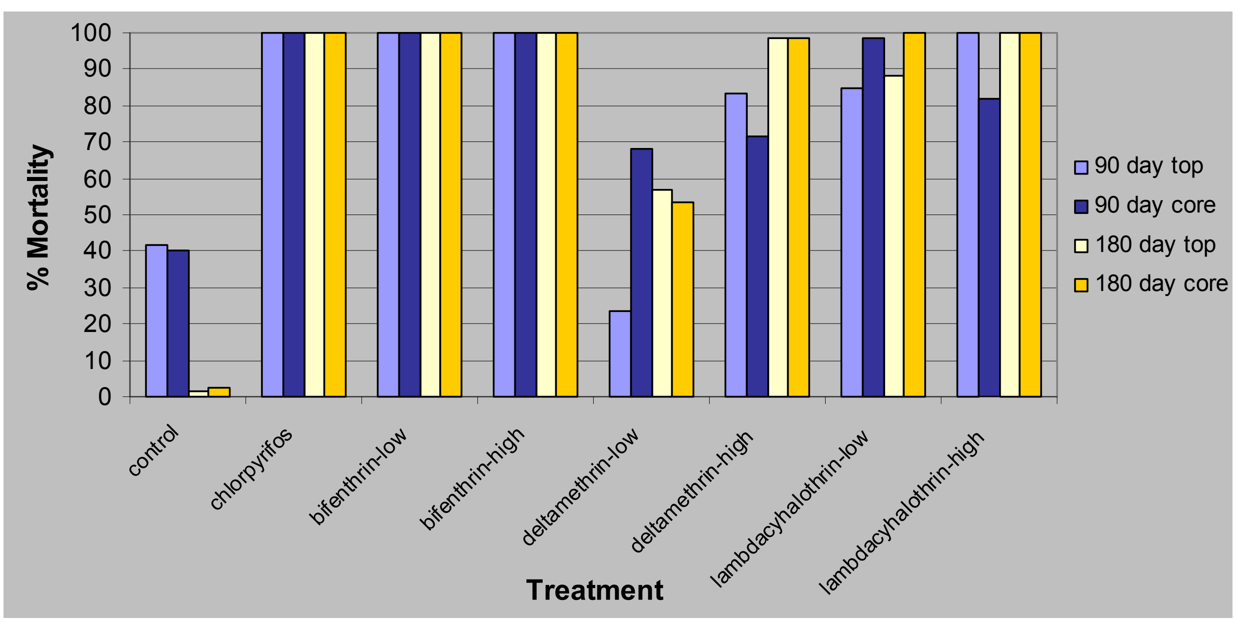


Figure 3. Efficacy of B&B drenches (2X/day on 3 consecutive days) testing media collected from various parts of the root ball – top of ball; Mississippi 2003.

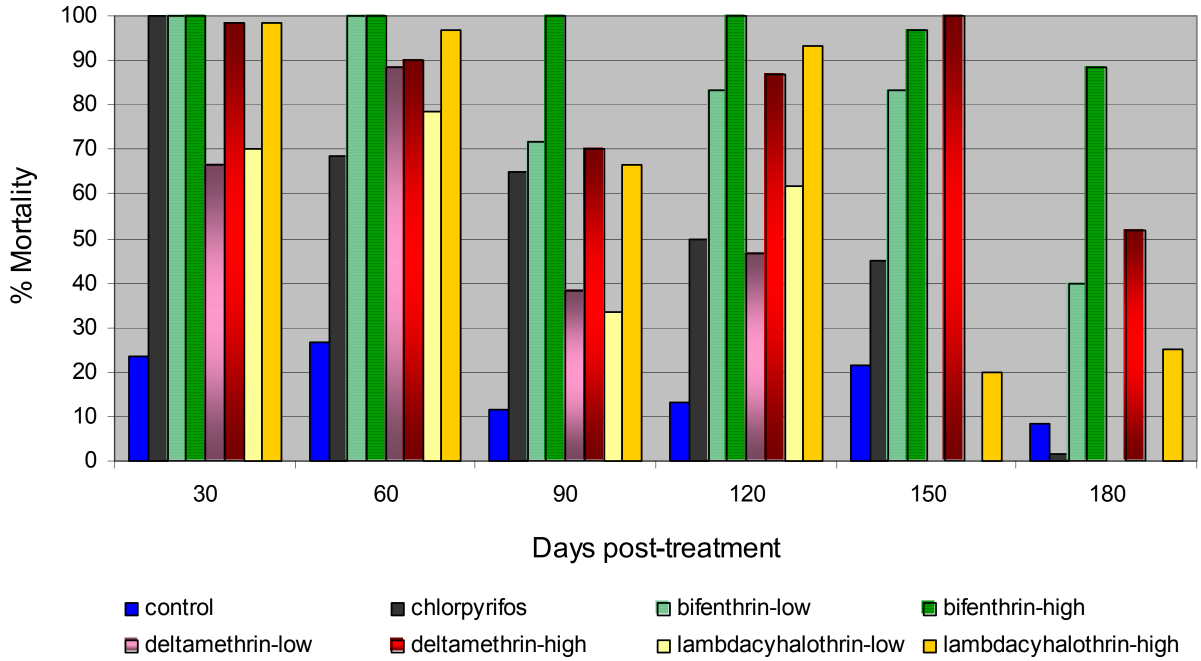


Figure 4. Efficacy of B&B drenches (2X/day on 3 consecutive days) testing media collected from various parts of the root ball – middle of ball; Mississippi 2003.

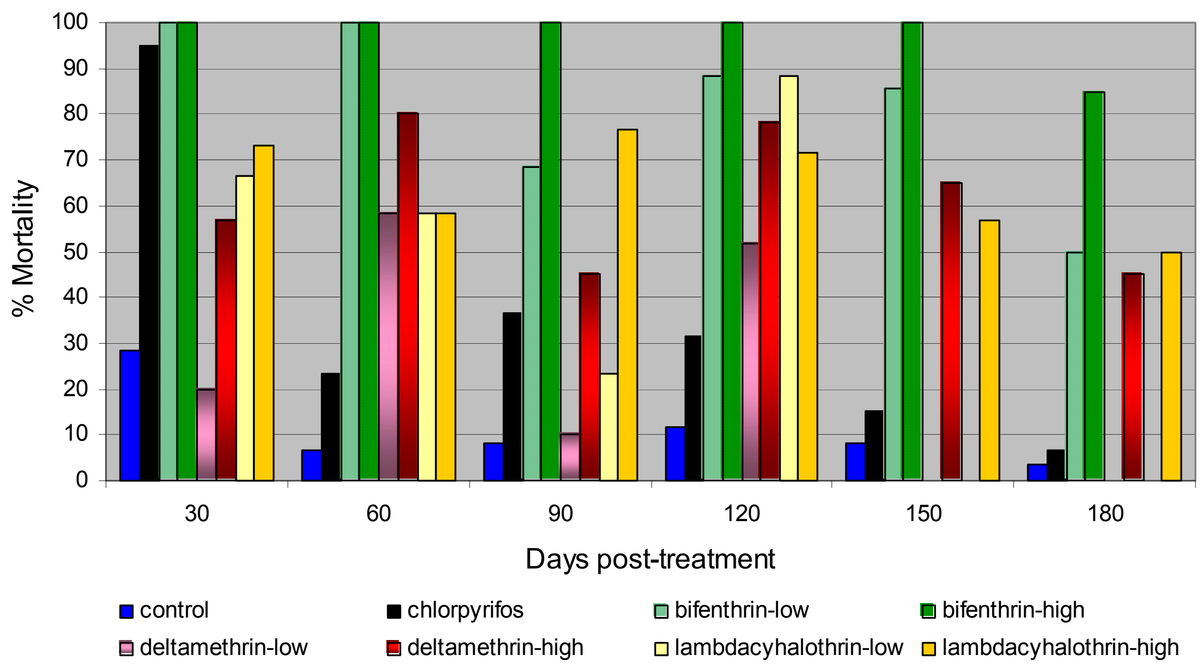


Figure 5. Efficacy of B&B drenches (2X/day on 3 consecutive days) testing media collected from various parts of the root ball – bottom of ball; Mississippi 2003.

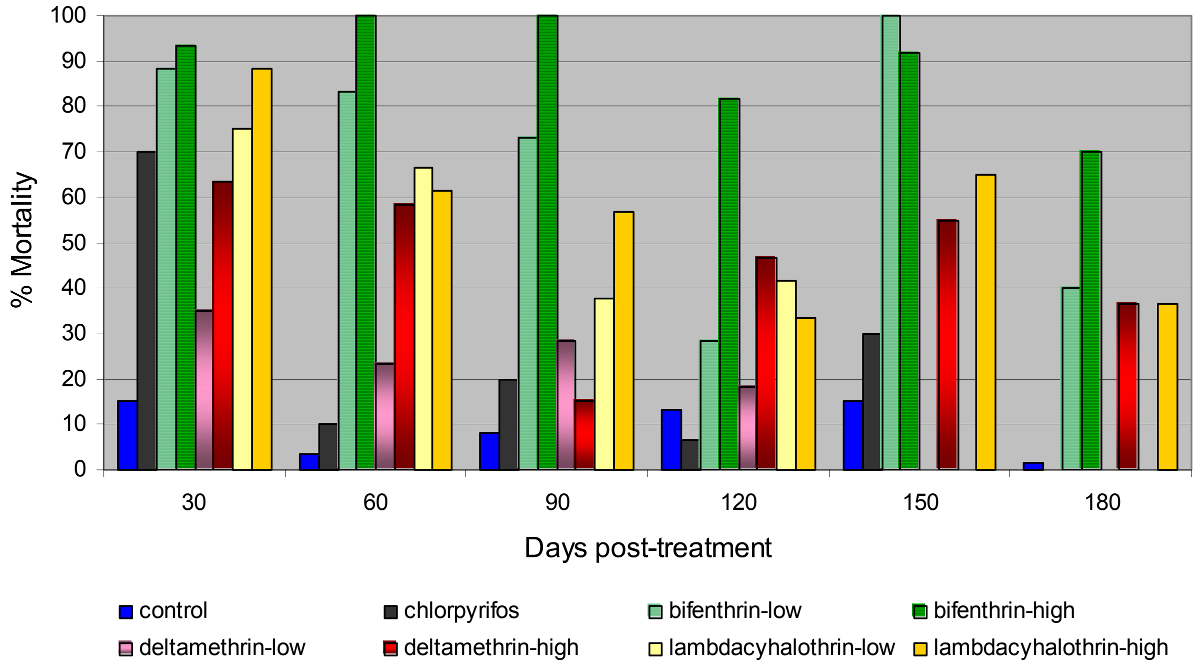


Figure 6. Penetration and evenness of treatment of various drench treatments for B&B nursery stock sampled at 90 days after final drench application; Mississippi 2003.

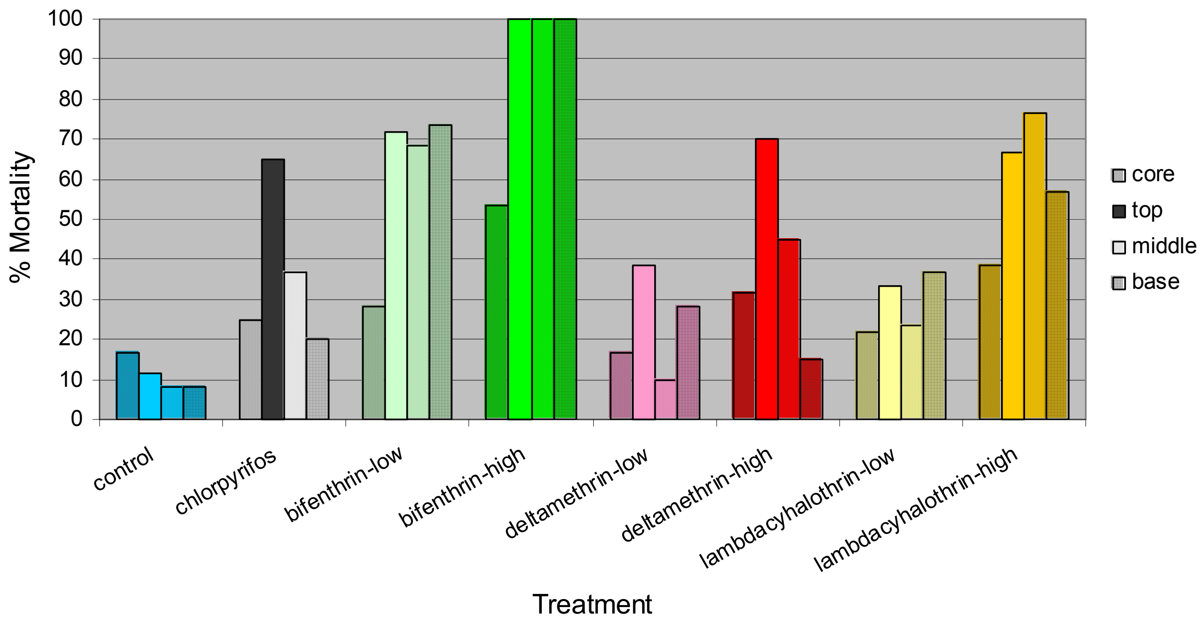
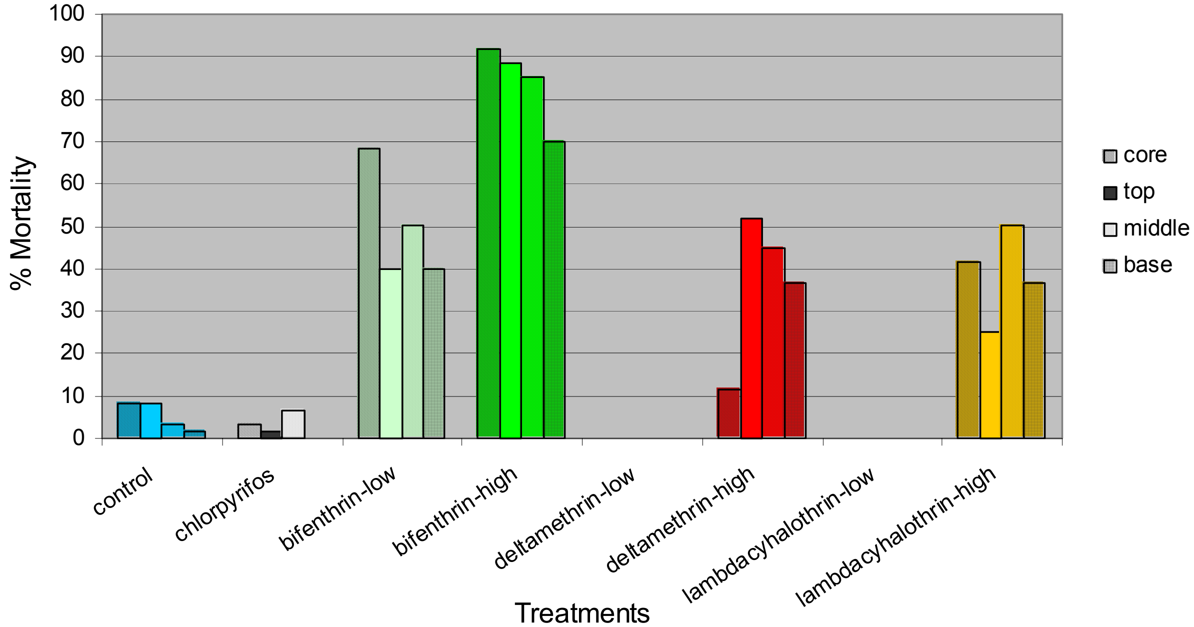


Figure 7. Penetration and evenness of treatment of various drench treatments for B&B nursery stock sampled at 180 days after final drench application; Mississippi 2003.



Due to consistently low results the deltamethrin and lambdacyhalothrin low rate treatments were discarded prior to day 180.

Figure 8. Efficacy of various immersion/dip treatments for B&B nursery stock as tested by surface location or "top" sample bioassays – Tennessee 2003.

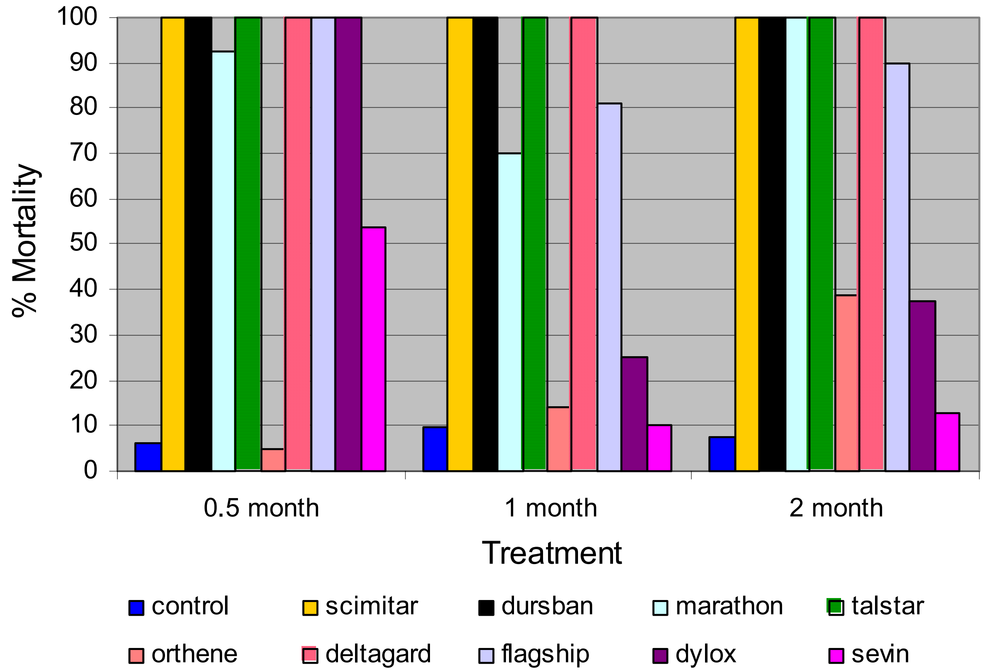


Figure 9. Efficacy of various immersion/dip treatments for B&B nursery stock as tested by center sample bioassays – Tennessee 2003.

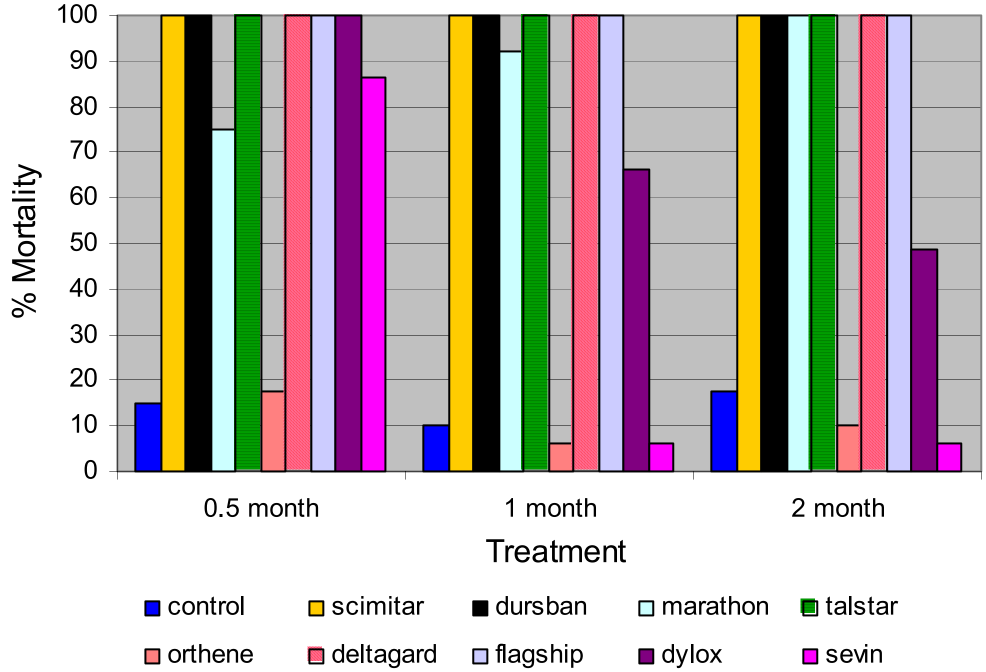


Figure 10. Efficacy of treatment and evenness of application of B&B drench (2X/day on 3 consecutive days) as tested through soil samples collected from various parts of the root ball Tennessee 2003 – **Top-surface**.

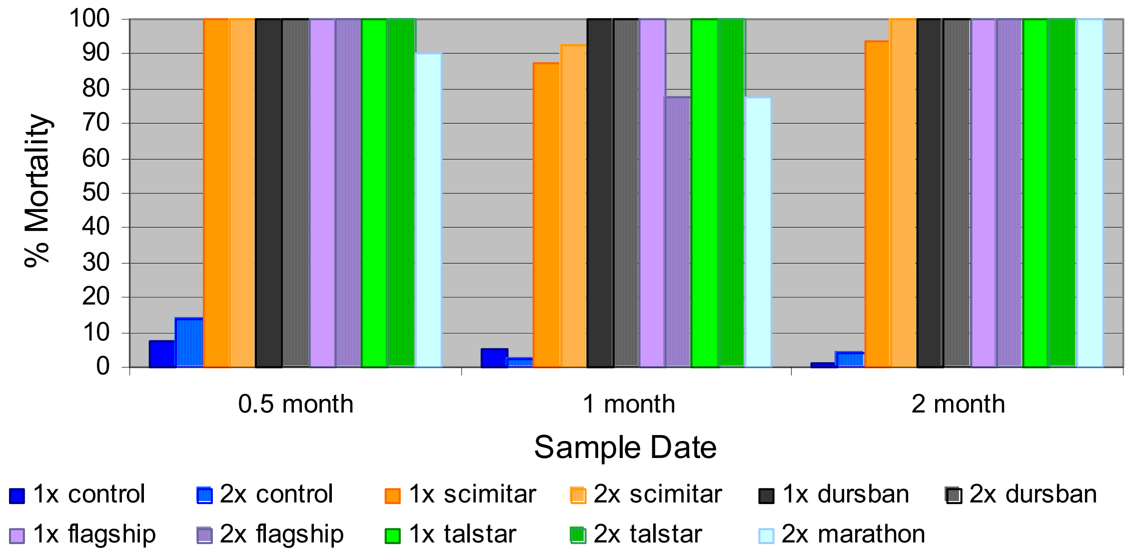


Figure 11. Efficacy of treatment and evenness of application of B&B drench (2X/day on 3 consecutive days) as tested through soil samples collected from various parts of the root ball Tennessee 2003 –**Center**.

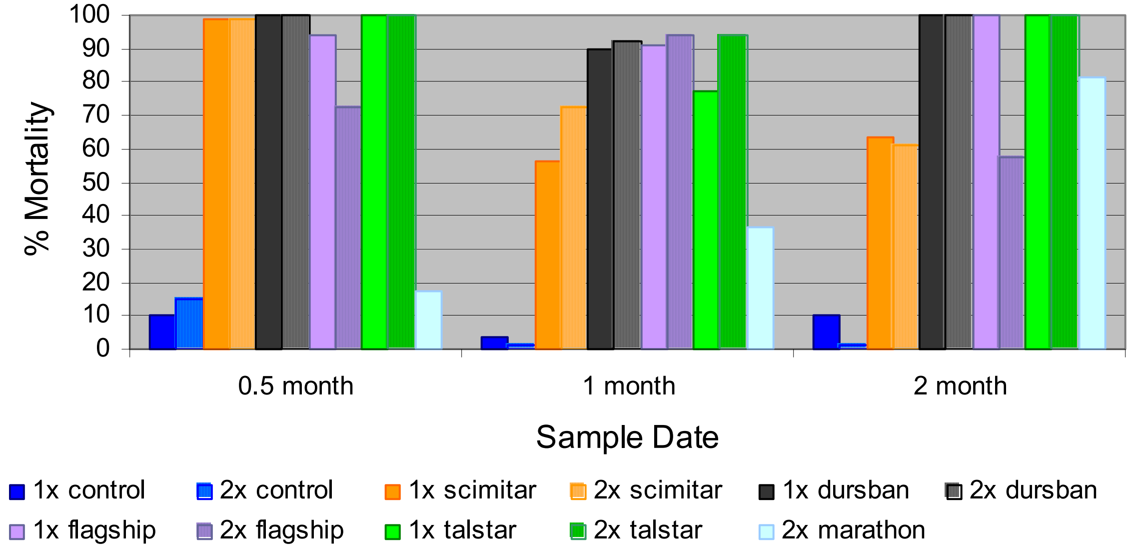
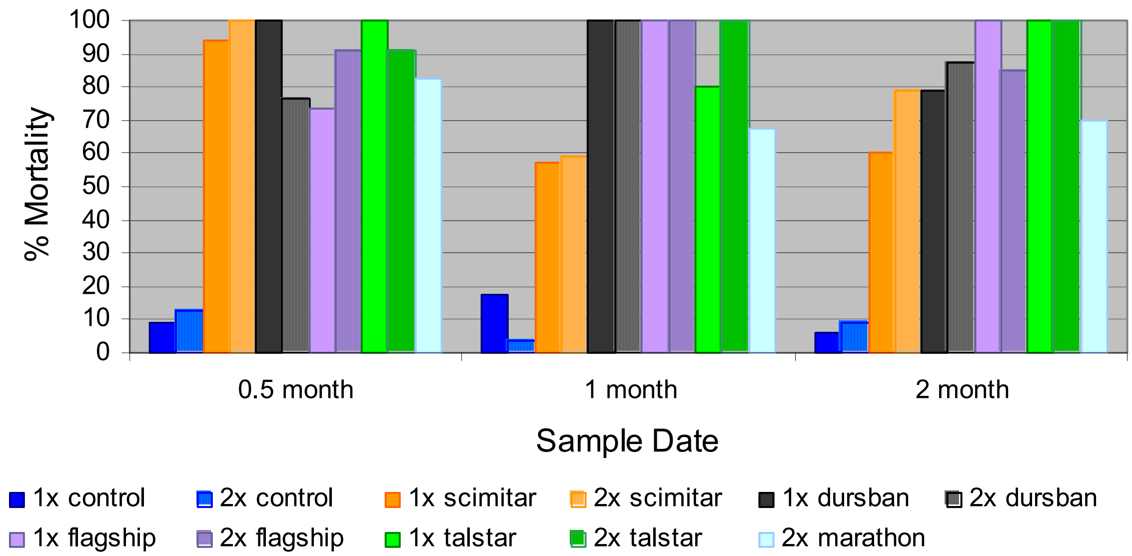


Figure 12. Efficacy of treatment and evenness of application of B&B drench (2X/day on 3 consecutive days) as tested through soil samples collected from various parts of the root ball Tennessee 2003 – **Bottom-surface**.



PROJECT NO: A3Q01

PROJECT TITLE: Evaluation of Methods to Prevent Imported Fire Ants from Infesting Commercial Honey Bee Pollination Operations, 2003 Trials

TYPE REPORT: Final

LEADER/PARTICIPANTS: Ronald D. Weeks

INTRODUCTION:

Commercial pollination with honey bees is a highly mobile business, and bee colonies are frequently moved on pallets among holding yards and over-wintering and pollination sites. These activities increase the probability that imported fire ants (IFA) *Solenopsis spp.* will be inadvertently transported from fire ant-infested areas to non-infested areas with beehives or in soil adhering to apiary equipment. Currently, bees and bee equipment are not listed as regulated items within the Federal IFA Quarantine, however many states vigorously inspect and regulate these items coming from IFA infested states. Beehives and associated equipment are under consideration for addition to the Federal Imported Fire Ant Quarantine (Federal Code of Regulations, Title 7, Part 301.81). No quarantine treatments have been approved for assuring that transported hives are IFA free. The objectives of this project are to evaluate the efficacy, longevity, and effective rates and formulations of several candidate chemicals applied to support pallets or soil area as barrier treatments around apiculture equipment in preventing IFA activity, such as foraging or nesting, on apiary equipment. In 2003, two treatment methods (chemical soil and pallet applications) and a broadcast bait application were evaluated.

MATERIALS AND METHODS:

In 2003, several IFA exclusion methods were evaluated in two fields in south Mississippi. Evaluations were conducted in fields infested with monogyne red imported fire ants, *Solenopsis invicta*. Routine ant sampling and visual examinations of pallets and soil areas for IFA activity were made 3-4 days post chemical applications and at weekly intervals. A 2.5 x 2.5 cm. card soaked in corn oil was placed on support pallets or the soil area and used as an attractant to determine the presence of foraging workers. Sampling was conducted for 45-60 minutes in each treatment. Visual inspections were made under and around the base of pallets for evidence of reproducing colonies.

Evaluation 1

This study evaluated the efficacy of chemical barrier applications on support pallets in excluding IFA foraging or nesting on pallets. Four liquid chemicals from a range of manufactures with a variety of active ingredients effective against fire ants were selected for treatment evaluations; lambda-cyhalothrin (Scimitar® GC, Syngenta), deltamethrin (Deltagard® T&O 5SC, Bayer/Chipco), chlorpyrifos (Dursban™, Dow AgriSciences), and permethrin (Gardstar® 40% EC, Y-Tex). Support pallets were treated with ≈ 0.5 gal each chemical formulation using a 2.5 gal. hand pump sprayer until saturation and run-off (Table 1). Four replicates of each liquid chemical treatment and a set of untreated control pallets were evaluated. Four bait cards were placed on untreated soil to confirm ant foraging activity during all pallet sampling periods. Treatments and controls were placed in a randomized block design in an IFA-infested field. Barrier treatments were intended to provide short-term protection (2 – 6 weeks) against fire ant foraging and infestation. A bait-card, soaked in vegetable oil, was placed directly on treated and untreated support pallets to detect IFA foraging as previously described. This experiment began on January 28, 2003 and ended in March 2003.

Table 1. Chemical formulations and application rates for both pallet and soil applications – Eval. 1 and 2

Chemical	Active ingredient	Formulation	Rate
Scimitar GC	Lambda-cyhalothrin (9.7%)	1.48 ml/gal	0.5 gal
DeltaGard T&O 5 SC	Deltamethrin (4.75%)	44.4 ml/gal	0.5 gal
GardStar 40% EC	Permethrin (40.0%)	10 ml/gal	0.5 gal
Dursban 4E	Chlorpyrifos (47%)	16.4 ml/gal	0.5 gal

Evaluation 2

This study evaluated the efficacy of chemicals applied to 3.05 x 3.05 m soil areas as barrier applications to exclude IFA foraging in the area, under apiary equipment. Each liquid chemical was applied at 0.5 gal rate using a hand-held sprayer. Three liquid chemicals were selected for evaluations; lambda-cyhalothrin, deltamethrin, and permethrin (Table 1). Four replicates of each chemical treatment and a set of untreated controls were evaluated. Treatments and controls were placed in a randomized block design in the field. Two bait-cards, soaked in vegetable oil, were placed in the center of the treated areas to detect IFA foraging as previously described. This experiment began on May 9, 2003 and ended on June 16, 2003.

Evaluation 3

This study evaluated both soil and pallet chemical barrier treatments. An additional factor included the impact of a single broadcast bait application of Amdro® (hydramethylnon, AmBrands) on the time, measured in days, for treated areas or pallets to become infested with IFA. August 15, 2003, Amdro bait was applied to the southern half (ca. 2.0 ha) of the trial area. The northern half of the field remained untreated. Pre-treatment surveys of the entire area by visual transect count showed the population level of active imported fire ant mounds to be ca. 45/acre.

Four liquid chemicals were selected for evaluations; lambda-cyhalothrin (Scimitar® GC, Syngenta), deltamethrin (Deltagard ® T&O 5SC, Bayer/Chipco), chlorpyrifos (Dursban™, Dow AgriSciences), and permethrin (Gardstar® 40% EC, Y-Tex) (Table 2). Pallets were treated with ≈ 0.5 gal each chemical formulation using an electric sprayer set to low pressure and force until saturation and run-off (Table 1). Soil areas (3.05 × 3.05 m) were treated by spraying 6.0 gal formulations in each plot using an electric sprayer (≈ 20 p.s.i with a 30 – 40 hole spray nozzle). Four replicates of each chemical treatment and a set of untreated soil and pallet controls were evaluated in both bait treated and untreated areas. Treatments and controls were placed in a randomized block design in the field. Two bait-cards, soaked in vegetable oil, were placed on pallets or in the center of the 3.05 x 3.05 m treated areas to detect IFA foraging described. This experiment began on Sept. 19, 2003 and ended on Nov. 7, 2003.

Table 2. Chemical formulations and application rates for both pallet and soil applications – Eval 3			
Chemical	**Active ingredient**	**Formulation**	**Rate**
Scimitar GC	Lambda-cyhalothrin (9.7%)	1.48 ml/gal	6.0 gal
DeltaGard T&O 5 SC	Deltamethrin (4.75%)	44.4 ml/gal	6.0 gal
GardStar 40% EC	Permethrin (40.0%)	10 ml/gal	6.0 gal
Dursban 4e	Chlorpyrifos (47%)	16.4ml/gal	6.0 gal

RESULTS:

Evaluation 1

Despite active ant foraging on control bait cards placed on the soil, there was insufficient ant activity on both treated and untreated pallets during this study for any meaningful interpretations to be made. A similar evaluation using untreated pallets was conducted during April 4 – 28, 2003. Results again were unsatisfactory due to the lack measurable ant activity on both untreated and treated pallets.

Evaluation 2

This study compared the overall mean rank infestation levels on bait cards placed in treated and untreated soil areas. Results show that all three chemical treatments reduced IFA foraging on bait cards compared to controls (Figure 1). However, most chemical barrier treatments showed ant foraging on bait cards after four days post application. Both permethrin and deltamethrin treatments reduced ant foraging on bait cards to lower levels compared to lambda-cyhalothrin and control treatments. These results indicate that the 0.5 finished solution rate of application was too low for our strict project objectives.

Evaluation 3

This study compared the number of occurrences of IFA infestations on soil and/or pallet treatments. Both permethrin and chlorpyrifos provided protection from IFA infestation compared to deltamethrin and control treatments (Figure 2A). Protection was on going at the conclusion of sampling, which ended after 45 days. Unlike the pallet studies conducted in January and April, there was enough foraging on pallets in this study for comparisons to be made among infestation occurrences between treated and untreated pallets.

Results from the broadcast bait applications show that the number of times IFA was detected on bait cards was half as much compared to bait cards in the untreated part of the field (Figure 2B).

DISCUSSION:

Variation in seasonal activity of IFA foraging on pallets was observed during the 2003 trials. During the winter (January) and early spring (April) trials no IFA foraging was observed on untreated pallets. This may be related to the reduced demand for protein and foraging motivation during non-reproductive periods in the ant's life cycle. If this is the case, this behavior fits well into migratory bee pollination operations where most bees are moved to pollination sites during winter and early spring. However, this needs further evaluation and variation in local conditions may not provide similar results in other areas. Therefore, the assurance of chemical or mechanical protection is still needed.

Both permethrin (Gardstar 40% EC) and chlorpyrifos (Dursban 4E) applications used in evaluation 3 on support pallets and soil areas provided excellent protection from IFA infestation. Permethrin is currently used by beekeepers to control hive beetle around apiary equipment. Studies in 2004 will focus on effective rates and formulations of permethrin in preventing IFA infestation of apiary equipment. Also, studies are being conducted in the ARS bee laboratory in Arizona to determine the impact of liquid chemical and broadcast granular chemical applications, aimed at IFA, on honey bee mortality, foraging, and colony growth.

Figure 1. Overall mean rank infestation levels of foraging ants on bait cards in treated and untreated areas. Ranks are defined as 0 = 0, 1 = 1 – 25, 2 = 26 – 50, 3 = 51 – 75, 4 = 76 - 100, and 5 = > 100 ants. Four replicates of each treatment were compared, n = 16.

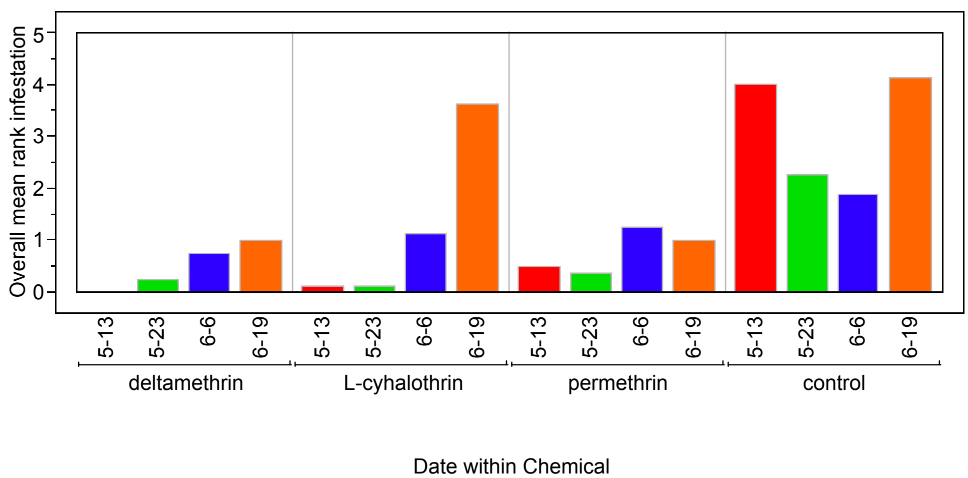
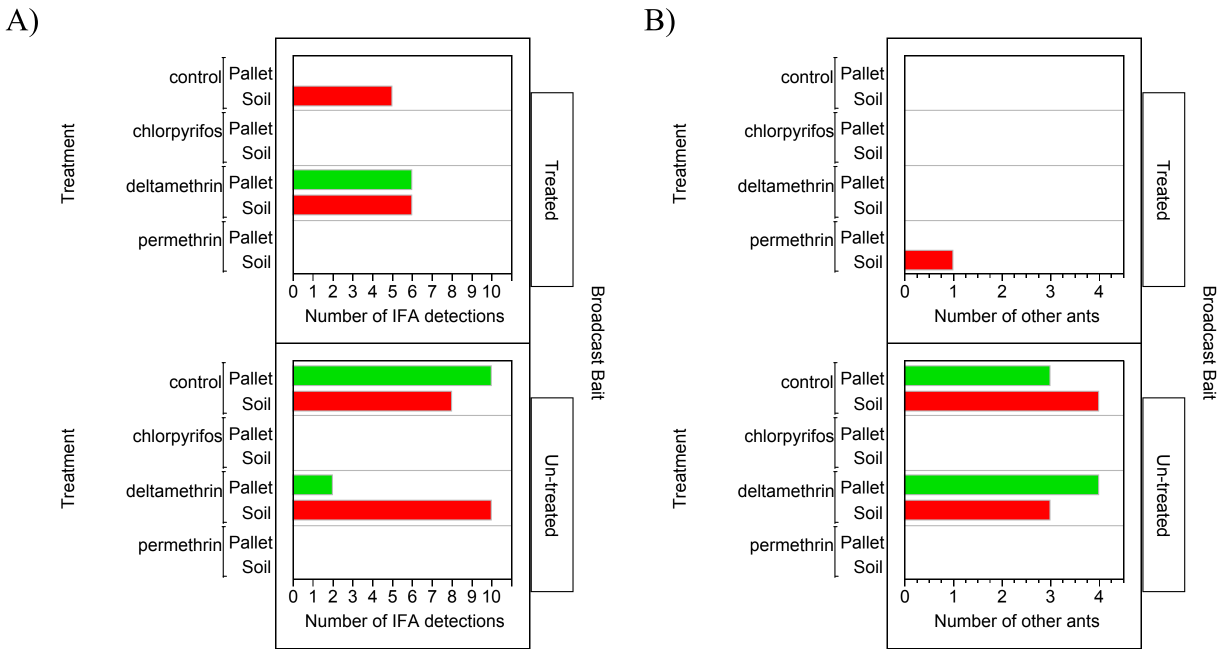


Figure 2. A) Total number of IFA detections over the course of the six-week study. B) Total number of other ant species detected on pallets over the course of the six-week study.



PROJECT NO: A1P03

PROJECT TITLE: Evaluation of Methods to Exclude Imported Fire Ants (IFA) From Infesting Baled Hay, 2003 Trials

TYPE REPORT: Final

LEADER/PARTICIPANTS: Ron Weeks, Timothy Lockley

INTRODUCTION:

Commercial hay operations are highly mobile and imported fire ant (*Solenopsis invicta* Buren) colonies can be moved in the commodity. As a federally regulated item under the Federal Imported Fire Ant Quarantine (7CFR 301.81), hay stored in direct contact with the ground cannot be moved outside the quarantine. No quarantine treatments have been approved for assuring that transported hay bales are *S. invicta*-free. The objectives of this project are to evaluate the efficacy, longevity, rates and formulations of several candidate chemicals applied to support pallets or the soil area as barrier treatments around hay bales in preventing *S. invicta* activity, such as foraging or nesting, on baled hay. In 2003, three treatment/storage methods and a broadcast bait application were evaluated.

MATERIALS AND METHODS:

In 2003 all hay evaluations were conducted at the White Sands Mississippi Agriculture and Forestry Experiment Station in Pearl River County. Evaluations were conducted in a 2.25 ha field infested with monogyne red imported fire ants *S. invicta*. Infestation is defined as either individual foraging ants or reproducing colonies. Routine ant sampling and visual examinations of each bale were made 3-4 days post hay placement and at weekly intervals. A 2.5 x 2.5 cm. card soaked in corn oil and placed on the south side of each bale was used as an attractant to determine the presence of foraging workers. Cards were attached to the bale with survey flags for 30 minutes. Visual inspections were made under and around the base of each bale/pallet for evidence of reproducing colonies. Colonies were ranked by size using a ranking system developed by Harlan et al. (1981) and revised by Lofgren and Williams (1982).

Evaluation 1

This study evaluated the efficacy of chemical barrier applications on support pallets and soil area around round hay bales. Four treatments were evaluated. Round bales of hay were placed on 1) support pallets on top of chemically treated pallets, 2) untreated double pallets, 3) the ground area with a 1 meter strip of chemical applied to soil surrounding bales, and 4) the ground with no barrier treatment. Four replicates of each treatment were set up (n = 16). The chemically active ingredient for this method was deltamethrin 4.75%, a pyrethroid product for insect control on sod farms, commercial and residential turf, and landscape and nursery ornamental plants. Hay for this evaluation was cut and baled in an adjacent field and moved within 24 hours to an IFA designated field. Treatments were arranged approximately 7m apart in a randomized block in the field. This experiment began on 6 June 2003 and ended on 23 June.

Evaluation 2

This method evaluated the timing of ant foraging and colony movement on hay bales that were left either "in-situ" in the field after baling or moved after baling and stored on the perimeter of the field. An additional factor included the impact of a single broadcast bait application of Amdro® (hydramethylnon) on the time, measured in days, for hay bales to become infested with IFA. July 2003, Amdro (hydramethylnon) bait was applied to the western half (ca. 1.12 ha) of the trial area. The eastern half of the field remained untreated. Pre-treatment surveys of the entire area by visual transect count showed the population level of active imported fire ant mounds to be ca. 70/hectare.

Twelve square bales were left "in-situ" after baling on the bait treated side of the field and 10 bales were left on the untreated side. Four stacks of four square bales each were moved onto support pallets on the perimeter of the field; two stacks on the treated side and two on the untreated side. Bait cards and visual counts were made 3 days post baling and at weekly intervals. Hay was baled August 22, 2003. Ant sampling occurred August 26 – September 2, 2003.

Evaluation 3

This method evaluated the impact of broadcast bait applications and pallet or soil applications of chemical barriers in preventing ant infestation of square baled hay. As in evaluation 2, this study compared infestation rates among treatments in bait treated [Amdro (hydramethylnon)] and untreated areas (bait applied July 2003). Hay was baled August 22, 2003 and stored off of the ground in a covered area until field placement September 11, 2003. Ant sampling occurred Sept. 12 – Dec. 1, 2003.

Forty-eight replicates were used for this study. Eight pallets were treated with either deltamethrin (Deltagard® GC 5 SC; Bayer/Chipco) at 0.44 ml/gal or permethrin (Gardstar® 40% EC Livestock and Premise Insecticide; Y-Tex) at 10.0 ml/gal. A drench application was made to each pallet. All exposed surfaces of the pallet were sprayed until thoroughly wet and then allowed to dry. Hay was not stored directly on treated soil or pallet areas, but was stored on an untreated support pallet. Pallet treatments were comprised of an untreated pallet set on a treated pallet or on the treated soil area and three hay bales were stacked on top of the untreated pallet. Also eight 3.048 x 3.048 m plots were treated with either deltamethrin or permethrin at 6.0 gal. of finished material as formulated for the pallet treatments. An untreated pallet was then placed in the center of the treated area and two square bales were set on top. Controls consisted of three bales placed atop an untreated pallet (four per treatment area) and three bales set directly on untreated ground. Hay bales were inspected at varying intervals using oil-cards placed on the shaded side of the bales and by visual inspection of the base of the pallet or ground bales for active colonies.

RESULTS:

Evaluation 1
After three days in the field, checks (pallets and ground) and the deltamethrin soil treatment had active foraging workers on the bales. Pallets treated with deltamethrin had no activity. At 10 days post-treatment, all treatments and checks had foraging activity; although only one of the deltamethrin treated pallets showed worker ants. At 17 days, all treatments and checks had foraging workers (Table 1). Once again, only one deltamethrin pallet (though not the same one seen on day 10) had foraging workers.

Evaluation 2
A single broadcast bait application of Amdro® did not eliminate infestation of hay bales in either storage method, "in-situ" or moved. In the "in-situ" evaluation, most hay bales stored in both bait treated and untreated areas showed ant activity, after four days in the field. Four days post-harvest sampling showed hay bales stored in the treated side of the field had fewer bales infested (6 out of 10) compared to bales stored in the untreated side (11/12). All bales tested positive for fire ant activity 11 days post-harvest.

Although individual ants were detected on all hay bales, the number of active colonies detected on bales was significantly less in the treated area compared to the untreated area; n = 3 and 16 colonies respectively. Broadcast baits can be expected to reduce the number fire ant colonies in an area up to 90%. Yet, despite a marked reduction in active colonies in the treated area, colonies were still able to "cover the area" with foragers and infest bales with active workers and use hay bales for foraging.

Comparisons among bales from treated and untreated areas that were moved and stored at the perimeter of the field were somewhat more successful. Although moving hay bales did not totally eliminate fire ant infestations, one of two hay stacks in the treated side of the field did not show any evidence of fire ant infestation for 11 days post- harvest. However, evaluations using more replications may have shown different results.

Evaluation 3
There were significant differences among chemicals and soil and/or pallet applications in this study. Permethrin soil and pallet chemical applications provided excellent protection to bales from all ant foraging and nesting behavior (Fig. 1). The number of days in the field until first ant detection was greater than 45 days for both permethrin treatments. Both soil and pallet applications of deltamethrin failed to provide satisfactory IFA protection (Fig. 1). Untreated soil and pallet treatments became infested within a few days.

For the permethrin trials, the single broadcast bait application of Amdro® did have a marked impact on the rate or intensity of worker ant activity on hay bales between treated and untreated areas (Fig. 1). Bales stored in the untreated area were infested with reproducing colonies much sooner than in the treated area. Also, there was a significant difference in the total number of reproducing colonies detected on bales (both pallet and soil treatments) during the six week study between the bait treated and untreated areas; n = 6 and 26 reproducing colonies respectively.

DISCUSSION:

Round hay bales do not fit well on support pallets due to their size and weight. Soil barrier treatments of a permethrin product may be a more reasonable treatment approach. Deltamethrin used at the labeled rate for turf grass did not provide acceptable results for our trials. Permethrin soil and pallet applications worked well and provide ca. 6 weeks of protection from IFA infestation. Although the single broadcast bait application to the field didn't reduce the occurrence of foragers on hay bales, it did reduce the number active colonies in hay bales. Also, there were more occurrences of native ants foraging on hay bales in treated areas (personal observations). These results suggest some benefits to using broadcast bait applications in a systems approach to hay production. Broadcast baits reduce active colony presence in bales and increases the number and diversity of other ant species foraging on hay bales. Although foraging ants were able to find sampling cards on all of the bales, a repeated application of broadcast bait may eliminate remaining colonies, and demonstrate a positive impact on the presence of individual foragers.

Permethrins are relatively safe chemicals that provided long-term protection of stored hay. Future studies need to evaluate several rates and formulations of permethrin to maximize protection from IFA and to eliminate chemical overuse.

References Cited:

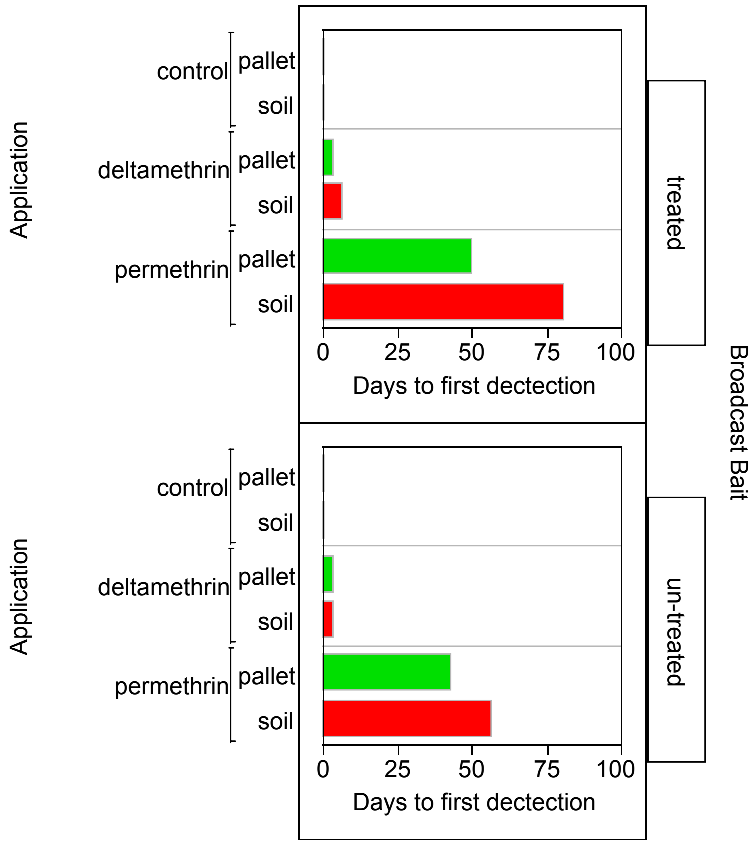
Harlan, D. P., W. A. Banks, H. L. Collins, and C. E. Stringer. 1981. Large area tests of AC217,300 bait for control of imported fire ants in Alabama, Louisiana, and Texas. Southwest. Entomol. 8: 42-45.

Lofgren, C. S. and D. F. Williams. 1982. Avermectin B1a, a highly potent inhibitor of reproduction by queens of the red imported fire ant. J. Econ. Entomol. 75: 798-803.

Table 1. Number of infested hay bales in each barrier treatment by days post-treatment for Evaluation 1.

Treatment	**# bales/treatment**	**Number of days post-treatment**		
		3	**10**	**17**
Ground Check	4	3	4	4
Pallet Check	4	3	4	3
Ground deltamethrin	4	3	2	3
Pallet deltamethrin	4	0	1	1

Figure 1. Number of days in the field until first day of infestation (n = 48 hay stacks, 24/side) for Evaluation 3.



PROJECT NO: A2P01

PROJECT TITLE: Development and Evaluation of Universally Acceptable Fire Ant Bait Attractant for Survey Traps – Starkville, MS Report

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Robert G. Jones, Ph.D, Timothy Lockley

INTRODUCTION:

Numerous baits are used to attract the imported fire ant (IFA) for both survey and research studies. There is no consistency in what is being used among the states. The use of many are laborious, messy or both. While attractants have been evaluated over the years, most researchers have not determined that one bait attractant is superior to others for use in survey traps (Lofgren et al. 1961, Brinkman et al. 2001). There are some bait attractants currently in use that have not been compared for effectiveness. None have been tested for attractiveness to all three types of imported fire ants; the red, black and hybrid. The Laboratory testing was done to screen out the most promising products from the less promising. The more promising "off the shelf" products were then tested in the field. This study has developed into three parts: 1.Laboratory Testing, 2.Bait Attractant Development and 3.Field Studies. The work reported on was done at Starkville, MS with the hybrid IFA.

1. LABORATORY TESTING:

INTRODUCTION: Five commercial products were selected from the 2002 laboratory testing. These were Original Lay's Potato Chips®, Mini Ritz Crackers®, Fritos® (original corn chips), Kebbler's Pecan Sandies® and SPAM® (classic). In 2003 there had been some changes in the commercial marketplace. Mini Ritz Crackers were not available so the original Ritz Crackers® were used. Original Lay's Potato Chips® apparently got a packaging facelift and are now Lay's Classic Potato Chips®. There is enough confusion in the variety of favors and dietary types of these brand name products. Now, further confusion in these commercial products is beginning to develop (www.bantransfats.com). Most of these products have trans or hydrogenated fats which maybe eliminated because of human health concerns in the near future. This could change the products' ingredients, flavor and possibly their attraction for IFA. The 2002 laboratory tests showed that corn oil was better than both peanut and soybean oils for IFA response. This created some questions that retesting can answer since these results differed from those of Lofgren et al (1964). The attraction of other individual ingredients also needed testing for work in developing universally acceptable IFA bait attractant.

METHODS AND MATERIALS: The laboratory test method was a standardized Gulfport IFA Laboratory protocol based on Lofgren et al. (1961). Field collected hybrid IFA worker ants (adult and immature) with mound soil were collected with a small bladed shovel and placed in a plastic 11.4 quart dishpan or 12 qt. sweater box. Each treatment was composed of 5 replications of collected ants in plastic boxes. The insides of the plastic boxes where dusted with talcum powder to prevent escape. The ants were held for 3 to 5 days before testing. On the day of the test the soil was watered and a board (1"x2"x12") was placed in the box on the soil. At each end of the board a petri dish (100mm x 15mm square or round) was placed. Each dish bottom contained 4 grams of the test product. After a period of 24 hours the petri dish bottoms were collected and weighed. The dishes had been numbered, weighed and recorded per replication per treatment before being tested. The finished weights were subtracted from the beginning weights. These weights were then recorded and an acceptance ratio (grams candidate bait removed/grams standard bait removed) was calculated for each replication with a mean acceptance ratio calculated per treatment (Table 1).

The baits reported on were mixed and divided into two portions one for each of two test dates and vacuumed sealed. The test baits were Pregelled Corn (70% by weight) mixed with 30% by weight of each of three vegetable cooking oils: peanut, corn and soybean oil (Table 1). These were compared to each other, corn x peanut, corn x soybean and peanut x soybean on April 7 and again in May 1. Several other ingredients were tested to find candidates for creating a survey bait attractant. These are not discussed here in case it is decided to patent the bait.

<u>RESULTS AND DISCUSSION</u>: The results of the laboratory tests are presented in Table 1. In 2002 both corn and soybean oil with pregelled corn carrier were more attractive compared to the peanut oil standard for the hybrid IFA. Acceptance ratios of 1 indicate no difference (Lofgren et al.1961). All of the extensive studies by Lofgren et al (1961, 1964) were done to develop toxic baits. None of these studies compared cooking quality vegetable oil from corn, peanuts and soybeans to each other. This study indicates that all three are equal in attraction to IFA.

Table 1. Laboratory Tested Products (Vegetable Oil, 30%) on Pregelled Corn Carrier (70%) with Results Shown as Acceptance Ratios (1 = no difference).

VEGETABLE OILS	2002 RATIOS	4/2003 RATIOS	5/2003 RATIOS
CORN/PEANUT	2.49	1.03	1.00
SOYBEAN/PEANUT	1.34	1.08	1.00
CORN/SOYBEAN	--	1.00	1.00

<u>2. BAIT ATTRACTANT DEVELOPMENT:</u>

<u>INTRODUCTION:</u> Developing a bait or attractant for IFA (imported fire ant) survey needs to consider both the IFA and the surveyor. The IFA like all biological organisms have specific nutritional needs. While IFA appears to eat almost anything that grows, crawls, walks or flies, it has certain basic requirements. These are oils, carbohydrates and proteins (Vinson 1968). These needs vary with season. Stein et al. (1990) found that carbohydrate baits are more attractive in lower temperatures (mean = 17°C) while proteinaceous bait is best at higher temperatures (mean = 25°C). Oils are important in any attractant (Lofgren et al.1964, Vinson 1968). With oils a carrier is necessary. Lofgren, et al (1963) found that pregelled corn worked well. Size of the particle was critical as discussed by Lofgren et al (1963) and Hooper-Bùi et al (2002). A good artificial diet media would give the IFA all its nutritional requirements throughout the year and

thus be attractive to foraging IFA. This is the approach that was taken based on Jones and Brindley (1969).

The surveyor needs a bait attractant that is easy to use or handle. This means not greasy, sticky or crumbly. It also means no cutting to size or weighing. Thus, it should be a prepared bait or attractant made specific for this survey. Stability under the handling stress of most field surveys and resistance to spoilage in storage or after exposure to the elements are critical.

METHODS AND MATERIALS: A variety of methods were attempted and numerous individual or basic ingredients were compared for acceptance ratios. Most of these will not be discussed until a decision can be made about a patent. Numerous cookie, granola and candy recipes were examined for ingredients and mixing procedures through the courtesy of the internet.

For economic reasons the emphasis was placed on using ingredients that could be purchased in grocery stores. Pregelled corn is the exception since it is a byproduct of processing other corn products. Cost is not a factor with it and it is a proven IFA bait carrier of the correct particle size. Of the other ingredients, corn oil was chosen because of its availability and being labeled as corn oil. Peanut oil may not be available every where and soybean oil is available only when the market price of soybeans is lower than some of the other vegetable oils. Carbohydrates were chosen for both furnishing essential elements of an insect diet, their ability to hold other materials together and their properties of preservation. Both vegetable and animal protein sources were used in the final bait. The final bait is mixed and formed in equal sized units. It is stored in plastic bags under refrigeration until used (Fig. 1).

RESULTS AND DISCUSSIONS: Several developmental versions of the survey bait were compared to the standard of pregelled corn and peanut oil. In all tests the developmental versions and the final bait had acceptance ratios equal to or slightly better than the standard. As these versions were made, 12 unit samples were selected and set out on the laboratory bench for 2 months. When exposed as such there was no molding or spoilage. They all dried hard on their surfaces. When pressed in filter paper the oil came out. No odor of rancid oil was noted. The quality of the bait has not changed after several months refrigerated in sealed plastic bags.

Figure 1. Laboratory produced bait attractant.

3. FIELD STUDIES:

INTRODUCTION: Comparing these baits in the field is the true test. The 2002 field tests worked fine in southern Mississippi on the red IFA. In northeastern Mississippi on the hybrid IFA the opposite was the result. The side by side comparisons would have IFA with one bait and native ants with the comparison. This nullified the tests and attempts to modify the test protocol and retain the simple comparisons failed. A totally new protocol was developed in 2003 for the hybrid IFA. Since IFA foraging can change with the season or temperature it is necessary to test any new bait or attractant for year round use. Due to the nature of field research testing for 1 year is usually not enough. Two years of testing would be more appropriate and then only if the same trends or results occurred both years.

METHODS AND MATERIALS

1. Hybrid IFA Test Site: The South Farm at Mississippi State University was selected as the test site. This farm is composed of numerous fenced pastures totaling several hundred acres. The pastures are well managed for livestock grazing and other research experiments. All pastures are accessible on at least one side by gravel roads. In the past the vegetation had been removed by herbicide treatment under the pasture fencing. This has left long narrow strips of bare ground. Bare ground is preferred for the placement of bait holders or traps. Placement on grass or other vegetation creates too many variables to consider. The IFA population or numbers of mounds varies from moderate (ca. 15/acre) to none observed in the different pastures. The tests were set out on the peripheral fence lines.

2. Preparation of the Standard Bait: The control or standard bait known to be attractive to ants was prepared by mixing peanut oil (cooking quality) and pregelled defatted corn grits 30%:70% w/w. The standard bait was prepared mixed for each monthly series of tests and stored in sealed containers and refrigeration during this period.

3. Candidate Baits: The candidate baits are attractive commercial products, the standard bait and the survey bait formulation. These are 1.original corn chips, 2. classic potato chips, 3. pecan shortbread sandies, 4. Ritz Crackers, 5. Spam Classic, 6. the standard bait and this laboratory's formulation of animal and plant proteins, carbohydrates and oils made in the form of a cookie. The commercial products are known to have extended shelf lives but after their packages are opened each was resealed, stored in an additional sealed plastic bag and refrigerated until used. This laboratory's survey bait formulation was prepared before each monthly series of tests and also sealed and refrigerated until used.

4. Hybrid IFA Bioassay: A new procedure was developed for the hybrid IFA. The 7 baits and the control compose the 8 treatments in each replication. Each bait is in a 9x50mm petri dish bottom on bare soil as a bait station. The stations were spaced at 6 foot (±2 feet) intervals along a line or in this case a fence row that had vegetation removed in past years with herbicide. The first station of each replication was marked with a surveyor flag and the baits are placed at random among the replication's stations. The spacing between replications was the same as between treatments except for skips

where changes in ground type occurred such as driveways, low wet areas etc. The number of replications was determined by time taken to place them and return to start collecting 60 minutes after placement. This developed to 25 replications for each test date. Soil and air temperatures were recorded both after placing the bait stations and collecting them. Collecting was done by putting the petri dish lids on the bottoms and placing the 7 treatments of a replication together in a plastic jar. Prior to going to the field all baits are weighed (1.5 ±.25 grams) and placed in petri dishes. These 7 dishes each with a different bait or treatment were stacked in a plastic jar and were randomly placed in the field.

5. Analyses of data will be done in the future using ANOVA AND LSD.

RESULTS AND DISCUSSION: The results of the 2003 tests are summarized in Table 2. No attempt was made to statistically treat this data since a full year of testing has not been completed. The first test of the seven treatments was on June 4. There were 4 tests in June, 5 in July, 1 in August, 4 in September, 4 in October 1 in November and 1 in December. The first 2 tests were composed of 20 replications of the 7 treatments. The remaining tests were replicated 25 times. This made a total of 20 tests and 490 replications.

The SPAM appears to be the best in all categories or at least provided the highest capture rate. The key figure here for confirming presence and delineating infestations is the % replications with IFA captures. There are three baits or treatments with the same %: Fritos, potato chips and the survey bait. Since there are numerous replications in all treatments with no captures of IFA there probably is no significant difference between or among any of them. The solution to this quandary is more testing and increase the data base. This has been planned since in this type of field testing there are numerous uncontrollable variables from climatic events, ecological factors and native ants competing for the same baits. The average IFA trap capture per treatment is a factor of the total treatment capture divided by number of replications. This data is presented for interest but may not have any validity for determining the most attractive bait. All of these treatments attract IFA to forage. Kidd et al (1985) discusses the variations of this phenomenon on the recruitment of IFA to various baits. The larger the surface area of the bait is, the more ants will be there. With the treatments in this test the largest recruitment of ants was to the three treatments with the largest surface area: Fritos, SPAM and potato chips. The standard and the sandies were in small pieces of foraging size and the survey bait was a single piece designed so the ants can remove forage size pieces easily. The Ritz was a single piece or ½ of a standard cracker but only the upper surface was available to the ants. However, it did, like the standard and the sandies, have the lowest % replication capture. The SPAM differed from all of the other baits in that liquid spread out around the bait. Ants were observed taking in this liquid which meant each ant spent more time at that bait station than at one where they picked up and carried off particles. Again, this is not a completed test so no conclusions should be made.

TABLE 2. Summary of Hybrid Imported Fire Ant (IFA) and Little Black Ant (LBA, *Monomorium minimum*) Bait Captures for the Months of June through December 2003.

	Ritz	Fritos	Standard	SPAM	SANDIES	Chips	Survey Bait
% Reps with IFA Captures	51.6	56.7	48.98	64.0	52.9	56.7	56.7
Total IFA Captures: All Reps	15506	36154	20498	35479	15701	31103	16297
Avg IFA Capture per Rep	31.6	73.8	41.8	72.4	32.0	63.5	33.3
Total LBA Capture: All Reps	16074	11594	17005	18831	18586	12573	10391

<u>LITERATURE CITED:</u>

Brinkman, M. A., W. A. Gardner, R. M. Ipser, and S. K. Diffie. 2001. Ground-Dwelling Ant Species Attracted to Four Baits in Georgia. J. Entomol. Scien. 36: 461-463.

Hooper-Bùi, L. M., A. G. Appel and M. K. Rust. Preference of Food Particle Size Among Several Urban Ant Species. J. Econ. Entomol. 95:1222-1228.

Jones, R. G. and W. A. Brindley. 1970. Test of Eight Rearing Media for the Mountain Pine Beetle, *Dendroctonus ponderosae* Hopkins, from Lodgepole Pine. Ann. Entomol. Soc. Amer. 63:313-316.

Lofgren, C. S., F. J. Bartlett and C. E. Stringer. 1964. The Acceptability of Some Fats and Oils as Food to Imported Fire Ants. J. Econ. Entomol. 57:602-602.

Lofgren, C. S., F. J. Bartlett and C. E. Stringer. 1963. Imported Fire Ant Toxic Bait Studies: Evaluation of Carriers for Oil Baits. J. Econ. Entomol. 56:62-66.

Lofgren, C. S., F. J. Bartlett and C. E. Stringer. 1961. Imported Fire Ant Toxic Bait Studies: The Evaluation of Various Food Materials. J. Econ. Entomol. 54: 1096-1100.

Stein, M. B. H. G. Thorvilson, and J. W. Johnson. 1990. Seasonal Changes in Bait Preference by Red Imported Fire Ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Florida Entomol. 73117-123.

Vinson, S. B. 1968. Distribution of an Oil, Carbohydrate and Protein Food Source to Members of the Imported Fire Ant Colony. J. Econ. Entomol. 61:712-714.

PROJECT NO: A2P01

PROJECT TITLE: Comparative Seasonal Evaluation of Commercially Available Products and An Artificial Bait for Use in Surveying Populations of Imported Fire Ants – Gulfport, MS Report

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Tim Lockley, Robert Jones

INTRODUCTION:

Among the various products used to survey imported fire ants (IFA), no real consistency exists. Many require wasteful effort to use and /or are messy. And, while researchers have evaluated numerous attractants (Lofgren et al. 1961; Brinkman et al. 2001), no one bait attractant has found to be superior over another. Studies undertaken in 2001 and 2002 found a number of commercially available products that showed excellent results in the laboratory.

Field evaluations were done to determine if any differences could be seen when these products were made available to IFA under normal, seasonal conditions.

MATERIALS AND METHODS:

Field tests were conducted in March, June, September and December of 2003. Products tested were: Fritos Corn Chips; Lay's Original Potato Chips; Pecan Sandies; Ritz Crackers; and Spam. The control consisted of pregelled corn and corn oil (70/30 by weight). A laboratory survey bait, created by RJ, was tested in the fall and winter. Materials tested were weighed out at 1.0 gram, placed in a bait station (55mm Petri dish, Gelmar Sciences) and set along a transect spaced ca. 3 m apart. Five replicates of each product were evaluated on each sampling date. Samples were kept in the field for a minimum of 30 minutes at which point they were retrieved from the field, sealed and returned to the laboratory where they were frozen for a minimum of 12 hours. Each sample was then opened and IFA workers were counted.

RESULTS:

The results of the four seasonal evaluations are shown in Tables 1 – 4.

In the spring, fall and winter seasonal trials, Pecan Sandies had the highest mean consistency of the baits tested. The summer evaluations indicated a slightly higher preference for Spam followed closely by Pecan Sandies. In the fall, the laboratory developed survey bait compared well with the commercial products. In the winter trials, the survey bait was very close to the Pecan Sandies in mean number of ants collected.

<u>References Cited</u>:

Brinkman, M.A., W.A. Gardner, R.M. Ipser & S.K. Diffie. 2001. Ground-dwelling ant species attracted to four baits in Georgia. J. Entomol. Sci. 36: 461-463.

Lofgren, C.S., F.J. Bartlett & C.E. Stringer. 1961. Imported fire ant toxic bait studies: the evaluation of various food materials. J. Econ. Entomol. 54: 1096-1100.

Table 1. 2003 Seasonal Field Evaluation of Five Commercially Available Products as Possible Survey Bait Attractants for Imported Fire Ants.

	Mean no. workers collected						
	Control	Survey Bait	Chips	Fritos	Ritz	Sandy	Spam
Spring	16.7	na	26.0	19.0	33.2	38.8	34.8
Summer	63.6	na	67.2	71.0	66.4	92.8	97.6
Fall	128.8	177.0	184.0	0.8	199.0	250.4	139.4
Winter	17.4	48.4	3.4	4.0	2.6	50.0	15.6
Mean							

Note: Spring - Air Temperature 63°F; Ground Temperature 55°F
Summer - Air Temp. 78°F; Ground Temp. 71°F
Fall - Air Temp. 81°F; Ground Temp. 76°F
Winter - Air temp 59°F; Ground temp. 54°F

PROJECT NO: A2P06

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

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Robert G. Jones, Ph.D, Timothy Lockley

INTRODUCTION:

Currently, the Federal Imported Fire Ant Quarantine (7CFR301.81) has no recommended survey trap. States and researchers have used a variety of traps with no known consistency of trapping or survey results. Some traps are handmade increasing survey planning and logistic problems. Others are difficult to handle under field conditions. This means increased time needed to set out and collect traps. The collecting, preserving and recording of specimens must be done in the field when it can be done better and faster in a laboratory situation.

The traps being used or have been used include vials and jars of various sizes. The most convenient and economical is a plastic centrifuge tube or vial with an attached lid. Index cards have been used as a substrate or holder for sticky baits, such as peanut butter. The state of California uses a plastic cage or basket to hold the bait (1/4 inch cube of SPAM®). In essence these “traps” are containers or substrates for bait attractants. The reason for this is that the foraging ants will carry some of the bait back to the nest leaving a pheromone trail for others to follow. The survey needs to demonstrate that there are established nests present. To do this, catching both major and minor worker IFA or numerous workers produces proof of an established colony. Thus a trap that catches or entraps the foraging ant will not work for this quarantine survey.

This is a report of an effort to develop an Imported Fire Ant (IFA) survey trap that is effective, simple and easy to use. Methods used included literature surveys, questioning experienced workers and looking at a variety of containers. Both purchasing and altering a variety of containers was done. These products were then tested and observed in both the laboratory and in the field with IFA. In differing ways, a good survey trap is user friendly to both the subject insect and the surveyor. There has never been a reported test to compare the efficiency of different types of potential IFA survey traps. This work is to compare examples of these containers and holders. It is also to compare a candidate trap or bait container developed to improve this survey need and improve on survey efficiency and cost.

METHODS:

Candidate Traps: Five candidate “traps” (containers and holders) are compared. This includes: 1. clear, polystyrene, 11 ml, snap lid vial; 2. opaque, polyethylene, 10 ml, hinged cap vial; 3. plastic, open mesh basket or California IFA bait station; 4. clear, polystyrene, 9X50mm petri dish bottom and 5. clear polystyrene, 9X50mm petri dish (Pall Gelman Brand), modified with 12 ($1/8^{th}$ inch) holes evenly spaced around sides and the top (or the Petri dishes’ wide bottom) covered with aluminum foil. All containers except the basket were selected for being close to the same volume size.

Trap Bait Attractant: The same bait or food attractant will be used throughout this series of tests. The attractant will be this lab's formulated survey bait that has performed well in laboratory bait acceptance tests. It will be formulated for this study in equal size pieces so that cutting and weighing baits for tests is not necessary. The bait will be prepared prior to use then packaged in small lots and refrigerated until use.

Collection of Traps: Four of the trap containers can be easily sealed to prevent loss of ants or data. To make collecting efficient and painless the basket trap is dropped into a 110 ml hinged capped, polyethylene container. All traps are then placed into a single 16 ounce plastic jar with a screw type lid. This allows for a complete test replication of all treatments to be kept together and data is recorded by replication and treatments.

Bioassay: The traps will be set out individually in a line with 6 foot (± 2 feet allowing for setting on bare ground) spacing between each treatment. The next replication of the five treatments will continue on this line at the same spacing between replications and treatments. The first treatment placing of each replication is marked by a surveyor's flag and the 16 oz jar is placed there for use in collection. Traps or treatments are placed at random within each replication. In the case of poor collecting sites along the line such as wet areas, heavy grass covering or driveways, the spacing between replications will vary to maintain similarity and close proximity among treatments in the same replications. No trap location should be within 3 feet of a mound. If more than one line or replication is possible in the same pasture or location the lines should be established no closer than 10 feet apart. In the hybrid area or northern half of Mississippi the trap lines are setup along fence lines in order to place traps on bare ground. Lines or replications can be parallel, perpendicular or as a continuation of another line, but can not cross. The number of replications will be determined by setup time since collecting begins at 60 minutes after the first is set out. Soil and air temperature readings are recorded after setup and after collection. These test replications will be done at least four times during each month with favorable foraging weather.

Analyses: The numbers of IFA caught by each treatment and the numbers of traps in each treatment catching IFA will be analyzed and compared using ANOVA and LSD. There is possibility of seasonal occurrence and location providing further data. The analyses used will be determined if this occurs.

RESULTS AND DISCUSSION:

Preliminary: The need to consider all available candidate or potential traps was important. In searching the literature and catalogues for candidates it became obvious that they all fit into three categories. These are 1. substrates or open bait holders such as index cards and the California bait station, 2. vials and jars of various sizes and 3. lids of small containers. This simplifies the need to limit treatment numbers for field testing but consider all basic possibilities. Being able to see ants in the containers or traps simplifies handling. There are two types of plastic containers that make this possible. These are the clear and opaque or "milky" plastics. These types are common in the vial and jar category. It is in this category that this physical difference could be a factor by the effect on light reflection or heat accumulation in the vials. A series of observational studies was initiated on numerous containers in the laboratory with some carry over into the field. Category 1: the California Basket or IFA bait station is an accepted trap making it the one to test. Category 2: numerous candidates have potential but observations showed that the ants had no difficulty entering the straight sided vials. The opening was flat on the ground and had no obstacles to entry. Category 3: the petri dish lid has been used in numerous bait studies on IFA. While it may not be the most economical it can be cleaned and reused numerous times. It is easy for the ants to enter and exit. The tight fitting dish bottom can easily be replaced into the lid and these petri dishes are stackable for ease in transportation. Many of the other lids examined which locked onto their container presented some obstacles for entry. The lids were also flimsy and needed care in setting out. For this test the petri dish was determined to be an excellent representative for this category. The IFA Survey Trap developed by this laboratory as a candidate was altered slightly from that reported last year. The number of entrance holes or openings was increased from eight to twelve.

Field Study: This study began on June 4, 2003 with 20 replications of the five treatments. On subsequent test days this was increased to 30 replications. There were a total of 530 replications of each treatment tested. This included 4 test days in June, 3 in July, 1 in August, 3 in September, 5 in October, 1 in November and 1 in December. Since this is a year round test there are some months left as well as a second year of testing. Since a complete annual data set has not been obtained no statistical treatment has been attempted. A summary of the data is found in Table 1. There were numerous variables encountered during these tests. These are too numerous to discuss and since they can not be eliminated in field testing only increasing numbers of samples will diminish their effect. These relate to microclimatic, micro ecological and micro geologic variables as well as competition from other ant species. The Little Black Ant (LBA, *Monomorium minimum*) is the most common of these.

The data in Table 1 shows that the laboratory produced survey trap is a very viable candidate. It is the only trap that has a reflective cover on it which may be a factor its higher capture rates for IFA. The California Basket is at a disadvantage in capture numbers since many ants fall off or out through the large mesh openings during collection. The numbers of replicates with IFA or the % is the more important number in Table 1. This shows the presence of IFA which is the purpose of the IFA Survey. The survey trap had a 70.8% rate of IFA captures versus the next highest, California IFA Bait Station, with 63.4%. From this preliminary data it can be concluded that the California IFA Eradication Program made a good choice in their selection of a survey tool but maybe we can improve on it (Fig. 1).

TABLE 1. Summary of Hybrid Imported Fire Ants (IFA) and Little Black Ant (LBA, *Monomorium minimum*) Trap Captures for the Months of June through December 2003.

	California Basket	Opaque Plastic Vial	Clear Plastic Vial	Plastic Petri Dish	Survey Trap Candidate
% Replications with IFA Captures	63.4	58.1	57.8	61.1	70.8
Total IFA Captures For All Replications	15818	11767	10861	17133	36067
Average IFA Capture Per Replication	29.9	22.2	20.5	32.4	68.2
Total LBA Capture For All replications	13245	10505	8660	11563	10469

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

PROJECT NO: A2P02

PROJECT TITLE: Cooperative Project with ARS – Area-Wide Suppression of Fire Ant Populations in Pastures

TYPE REPORT: Interim

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

INTRODUCTION:

The USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE – Gainesville, FL) received a grant for a 5-year area-wide pest management demonstration project for control of imported fire ants (IFA). The CPHST Soil Inhabiting Pests Laboratory (aka Imported Fire Ant Lab) was asked to participate in the program as a Core member and Co-Principal Investigator. The Core team is responsible for oversight and review of the project and includes all external collaborators. Not only will the CPHST lab be participating in the project, but PPQ, AEO has agreed to aerially treat as many of the sites as possible (see note at end of template). In APHIS's role of safeguarding American agriculture, expanding our fire ant work from its traditional focus on quarantine methods development to including work on controlling fire ants and their impact on the environment through an integrated pest management approach, is a logical step. The Gulfport lab routinely cooperates with ARS on projects, and the expertise we bring to the program will contribute to the success of the project. For detailed information on the ARS project see http://fireant.ifas.ufl.edu.

This ARS project includes USDA-ARS, USDA-APHIS, and university and state personnel. The project is investigating the effectiveness of utilizing bait treatments combined with biological control agents to control IFA with demonstration projects in five states; FL, SC, MS, TX and OK. PPQ, AEO is providing a pilot and plane to apply bait treatments approximately twice a year, and CPHST, ANPCL, SIPL is providing coordination of pilot and plane, expertise and ground support for the aerial treatments. Due to complicated state regulations we are not aerially treating in FL.

MATERIALS AND METHODS:

There will be 2 sites per state. One will receive aerial bait applications only, and the other (referred to as IPM plot) will receive an initial bait application as well as inoculations of mounds with phorid flies and the microsporidia, *Thelohania solenopsae*. IFA mound counts within the bait treatment area of the IPM plot will trigger future bait applications. Numerous assessments will occur within each set of paired plots, including IFA populations, insect biodiversity (bait and pitfall traps), biological control agents population assessments, etc.

ARS is the lead agency on this project. State cooperators will select sites, and conduct pre-treatment site evaluations. CPHST Gulfport obtained assistance of PPQ, AEO personnel to provide aerial application of bait treatments over the course of the study.

RESULTS:

Initial aerial applications by AEO occurred in 2002 in 3 states, with a second application done in one of the original states. CPHST Gulfport personnel assisted with initial aerial applications at all sites treated by AEO, and assisted with follow up applications when needed. Due to complicated state regulations we are not aerially treating in FL. Aerial treatments were completed in 2002 in the spring in MS and TX; and in the fall in TX and SC. In 2003, aerial applications were done in the spring in MS and OK; and in the fall in OK and MS only. Weather precluded a treatment in TX in the fall of 2003. We anticipate continued treatments in the spring of 2004.

Data for the project is being collected by state cooperators and compiled by ARS. No specific results have been communicated to the CORE group at this time.

PROJECT NO: A1P02

PROJECT TITLE: Bait Acceptance of Various Formulations Provided by Cerexagri, Inc.

PROJECT TYPE: Final

LEADER/PARTICIPANTS: Anne-Marie Callcott, Shannon Wade

INTRODUCTION/METHODS AND MATERIALS:

Samples of various bait formulations on Tast-e-bait were provided by Cerexagri in Dec. 2002. The laboratory bioassay for feeding acceptance is a standard test used to determine the relative attractancy of various IFA baits or components of baits. Field-collected captive ant colonies are given a free choice to select and feed on either a candidate bait (the bait under evaluation) or a freshly prepared standard bait. It is assumed that the ants will indicate their preference by consuming greater quantities of the bait of their choice. Fragments of colonies containing all life forms (workers, immature, winged sexuals and occasionally, the mated queen) were collected from infested fields by shoveling a portion of the nest tumulus into a plastic dishpan. The colonies were then transported into the laboratory and allowed to acclimate and rebuild the nest structure for 5-7 days prior to testing. A standard bait known to be attractive to ants was prepared by mixing fresh soybean oil and pregelled defatted corn grits at 30%:70% w/w. The standard bait was prepared one day prior to the test. The candidate bait can be any potentially attractive oil, experimental bait formulation, or formulated bait which may have deteriorated due to storage, etc. Each candidate bait was tested on five different colonies, and the results reported as an average response of all colonies.

Four grams of the candidate bait contained in a plastic petri dish were placed on the surface of each of the 5 test colonies. Simultaneously, 4 grams of the freshly prepared standard bait in an identical dish were placed approximately 4-5 inches from the candidate bait. Foraging workers were then provided a free choice to feed on the bait of their preference. After a 24 hour feeding period, the dishes were removed and the amount of each bait consumed determined by weighing.

An acceptance ratio for each candidate bait is computed in the following manner:

$$\frac{\text{No. grams candidate consumed}}{\text{No. grams standard consumed}} = \text{acceptance ratio}$$

An acceptance ratio with a value of less than 1.0 indicated that a given candidate was less attractive than the standard. Values equal to or greater than 1.0 indicated that a candidate was equally or more attractive than the standard.

RESULTS:

Test I: On February 3, 2003 the bait acceptance trial was initiated with the 8 candidates provided by the company. Results are in Table 1.

Table 1. Bait acceptance of various Ceraxagri candidate formulations, Feb. 3, 2003.

Formulation Code	Series	% AI	Bait Acceptance Ratio ± SD	Mean amt. of bait removed (g)	
				Candidate	Standard
TD#2462-01	A	1.0	0.16 ± 0.16	0.66	3.74
TD#2462-02	A	9.7	0.03 ± 0.04	0.15	4.00
TD#2462-03	A	19.3	0.01 ± 0.01	0.03	3.24
TD#2462-04	B	9.9	0.11 ± 0.09	0.43	4.00
TD#2462-05	B	19.6	0.02 ± 0.01	0.07	4.00
TD#2462-06	C	1.0	0.17 ± 0.18	0.68	4.00
TD#2462-07	C	9.4	0.01 ± 0.01	0.04	3.24
TD#2462-08	C	18.6	0.01 ± 0.02	0.05	3.20

None of the candidate baits were acceptable to the IFA as a food source as indicated by bait acceptance ratios of less than 0.2. In all comparisons, the ants removed 80% or more of the standard bait compared to less than 20% of the candidate bait. In the lowest ai of each sample series the ants removed 10-20% of the candidate bait, while in all other rates the ants removed only 1-4% of the candidate.

The lowest ai rates did allow some feeding by IFA, but due to the large amount of standard bait consumed mortality rates were not determined. Since the bait acceptance ratio is far below acceptable rates, additional studies on these samples will not be performed. All the samples were on Tast-e-bait, however they were on a larger size particle than is normally considered acceptable by IFA standards. This may have had an impact on the ability of the ants to remove the particles. Removal of particles is the basis on which this particular protocol is based. It is possible that the ai would give better results on a carrier size more appropriate for IFA use.

The size of the carrier particle may have made it difficult for the ants to remove the particles from the dish as they like to do. Therefore, with the 3 formulations that showed some promise (01, 04 and 06), the carrier was crushed into smaller particles and 1g of vegetable oil added to 20 g of the crushed bait. These new formulations were then subjected to bait acceptance testing against the standard bait. In this trial, none of the candidate baits did as well as they had in the original trial (0.04, 0.05 and 0.09 acceptance ratios).

Test II: In mid-July, 2003 we received 3 additional samples from Cerexagri, Inc.

TD-2462-09: AI + Tast-E-Bait + REAX88B
TD-2468-01: AI in soybean oil + REAX88B
TD-2469-01: AI in soybean oil + Morwet

On July 25, IFA colonies were collected in the field and transported back to the lab in plastic pans. Colonies were watered as necessary, but not fed prior to testing. On July 30, testing was initiated. TD-2462-09 (TEB) was crushed into smaller sized pieces to facilitate ant removal. Four grams of this bait was presented to each of 5 colonies. The other 2 samples were in liquid soybean oil formulations, and 1.5 grams of each of these oil baits was presented to each of 5 colonies. The liquid was well mixed prior to measuring and placing in a shallow dish.

After 24 hours, each of the colonies presented the TD-2462-09 (TEB) bait had removed a good portion of the solid bait. The liquid baits had separated and much of the oil had been removed leaving a moist to dry patch of material in the bottom of the dish. After 1 week, an average of 2.60 grams of the solid bait had been removed by the colonies, and the liquid baits had become powdery material. All baits were removed at this time and the ants fed and watered normally.


TD-2468-01


TD-2469-01

At the 1 week evaluation, only the solid bait fed colonies showed significant mortality when compared to the untreated control colonies. IFA bring their dead to the surface and place them in "bone piles". The bone piles for 4 of the 5 TEB colonies were about twice the size as those of the control colonies (the other colony was similar to controls). However, the surviving ants were very active, responded well to stimuli and actively fed when presented the traditional lab diet of crickets. All colonies for both liquid baits had bone piles similar to the controls and also responded well to stimuli and actively fed.

Colonies were fed, watered and visually monitored weekly through September 23, 2003 (8 weeks after treatment). There was no significant change in the colonies during the remainder of the trial that could not be attributed to normal attrition. At the time the trial was terminated, 2 TEB colonies were smaller than the controls by about 50%, 2 were smaller by about 20%, and the remaining colony was similar in population to the controls. All the liquid treated colonies were similar to the controls.

PROJECT NO: A1P02

PROJECT TITLE: Tast-e-Bait Acceptance – Various Formulations, March 2003

TYPE REPORT: Final

LEADER/PARTICIPANTS: Anne-Marie Callcott, Shannon Wade

INTRODUCTION:

Tast-e-bait is a carrier product formulated from the by-products of bakery products by Advanced Organics. This carrier has been tested by this laboratory for several years and has proven to be an acceptable carrier. The company is continuing to refine the carrier to provide even better acceptability to imported fire ant (IFA) as well as better flowability through application devices. Samples of various Tast-e-bait formulations were provided by Advanced Organics in March 2003 and additional samples of new and aged -10+30 Tast-e-bait granule were provided by Advanced Organics in August 2003. Both sets of samples were tested for acceptability to IFA.

METHODS AND MATERIALS:

The laboratory bioassay for feeding acceptance is a standard test used to determine the relative attractancy of various IFA baits or components of baits. Field-collected captive ant colonies are given a free choice to select and feed on either a candidate bait (the bait under evaluation) or a standard bait. It is assumed that the ants will indicate their preference by consuming greater quantities of the bait of their choice. Fragments of colonies containing all life forms (workers, immature, winged sexuals and occasionally, the mated queen) were collected from infested fields by shoveling a portion of the nest tumulus into a plastic dishpan. The colonies were then transported into the laboratory and allowed to acclimate and rebuild the nest structure for 5-7 days prior to testing. A standard pregelled corn bait known to be attractive to ants was prepared by mixing fresh soybean oil and pregelled defatted corn grits at 30%:70% w/w. The standard bait was prepared one day prior to the test. The candidate bait can be any potentially attractive oil, experimental bait formulation, or formulated bait which may have deteriorated due to storage, etc. Each candidate bait was tested on five different colonies, and the results reported as an average response of all colonies.

Four grams of the candidate bait contained in a plastic petri dish were placed on the surface of each of the 5 test colonies. Simultaneously, 4 grams of the standard bait in an identical dish were placed approximately 4-5 inches from the candidate bait. Foraging workers were then provided a free choice to feed on the bait of their preference. After a 24 hour feeding period, the dishes were removed and the amount of each bait consumed determined by weighing.

An acceptance ratio for each candidate bait is computed in the following manner:

$$\frac{\text{No. grams candidate consumed}}{\text{No. grams standard consumed}} = \text{acceptance ratio}$$

An acceptance ratio with a value of less than 1.0 indicated that a given candidate was less attractive than the standard. Values equal to or greater than 1.0 indicated that a candidate was equally or more attractive than the standard.

RESULTS:

March samples: Only the ground formulation showed any problems with acceptability, and this was probably due to the particle size not the attractiveness. The extremely fine particle size appeared harder for the ants to pick up and carry off. All other formulations were attractive and acceptable to the ants (Table 1).

Table 1. Acceptability of various Tast-e-bait formulations, March 2003.

Candidate	Standard	Bait Acceptance Ratio ± SD	Mean amt. of bait removed (g)	
			Candidate	Standard
Ground w/ calcium	Ground w/o calcium	0.97 ± 0.18	3.34	3.33
Weather res w/ 3% oil	Weather res w/o 3% oil	1.00 ± 0.00	4.00	4.00
Weather res w/ 3%	Pregel	1.00 ± 0.00	4.00	4.00
April 2002 sample	Mar 2003 sample	1.00 ± 0.00	4.00	4.00

August samples: No difference was noted in acceptability of any of the baits (Table 2). One colony from the TEB vs. pregel test did not remove a lot of either bait (1.7g TEB vs. 0.5g pregel), whereas all other colonies in that comparison and all other comparisons removed all bait regardless of age or carrier.

Table 2. Acceptability of various Tast-e-bait formulations, March 2003.

Candidate	Standard	Bait Acceptance Ratio ± SD	Mean amt. of bait removed (g)	
			Candidate	Standard
TEB - 7/22/01	TEB – 7/30/03	1.00 ± 0.00	4.00	4.00
TEB – 5/9/03	TEB – 7/30/03	1.00 ± 0.00	4.00	4.00
TEB – 7/22/03	TEB – 7/30/03	1.00 ± 0.00	4.00	4.00
TEB – 7/30/03+4% soybean oil	Pregel corn grit standard	1.48 ± 1.07	3.54	3.33

PROJECT NO: A9P03

PROJECT TITLE: Mississippi Phorid Fly Release Project, 2000

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Tim Lockley, Sanford Porter (USDA, ARS), Shannon James

INTRODUCTION:

Pseudacteon species are endoparasites of *Solenopsis* species and are widely distributed throughout the fire ant range in their natural habitats. These phorid flies have the potential to suppress imported fire ant populations if they can be established in North America. To determine their ability to acclimatize, phorids were released in the spring of 2000 in Harrison County, MS and in August 2002 in Forrest Co., MS.

MATERIALS & METHODS:

Release I: A release site near Saucier and a paired control at the Harrison County Work Farm were selected for the study. The sites were ca. 20 km apart. Each site was similar in habitat at the time of the release; consisting of grasslands with deciduous woods and a large pond adjacent. Both the release site and the control site had ca. 100 active mounds per hectare. Emerged adult flies of *Pseudacteon tricuspis*, supplied by S. Porter, were released daily, per the protocol supplied by S. Porter, at the Saucier site on 11 April, 2000 with the final release occurring on 20 April. A total of 2612 phorids were released on 45 separate imported fire ant colonies.

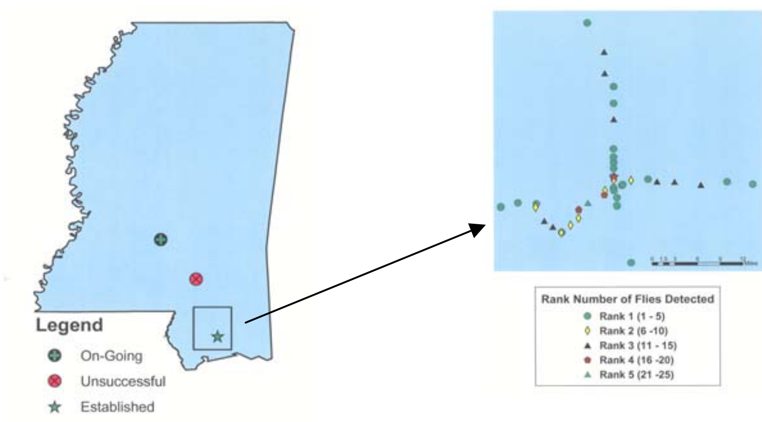
Release II: In August 2002, over 2000 phorids were released at the Hattiesburg Airport (Bobby Chain Air Field) as a part of the APHIS phorid fly rearing and release project. 42 IFA colonies were treated.

RESULTS:

Release I: One and one-half years after release, fall 2001, flies were found 3.5 km south of the release site. In fall 2002, 2½ years after release, flies were detected north, south and east of the release site at distances of ca. 7.5 km. A survey carried out in October 2003 along the cardinal points from the release site showed a significant increase in the area occupied by *P. tricuspis* (Fig. 1). The greatest distance flies were detected from the release site was ca. 39.1 km east of the release site. The spread of phorids was ca. 19.3 km south of the original point of release. To the north, *P. tricuspis* was detected 34.5 km, and to the west, phorids were observed at 24.9 km from the original site. In 3½ years, *P. tricuspis* has spread over an area of ca. 3443 square kilometers.

Release II: Surveys conducted through out the spring summer and early fall of 2003 failed to reveal the presence of any phorids at the release site.

Figure 1. Distribution of phorid flies at one site in Mississippi
Left: Mississippi phorid fly release sites
Right: Distribution of flies 3½ years after release at established site in south Mississippi



PROJECT NO: A1P01

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

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Anne-Marie Callcott, Debbie Roberts, Tim Lockley, FL DPI, ARS CMAVE, state departments of agriculture and their designees

INTRODUCTION:

In a recent USDA-APHIS survey, seven southern states ranked IFA as a top priority target organism for biological control. To date, most research on phorid flies has been under the direction of ARS in Gainesville, FL. USDA, APHIS, PPQ began funding a cooperative project in 2001 to rear and release this potential biological control agent for imported fire ants. 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.

MATERIALS AND METHODS:

Preliminary research and rearing techniques have been developed by USDA, ARS for two species, with other types under development. ARS will continue to evaluate other phorid fly species for potential use in the U.S., and transfer rearing techniques to the rearing facility as the new species are ready for mass rearing. Mass rearing of flies is being conducted by the Florida Department of Agriculture, Dept. of Plant Industries (DPI), in Gainesville, FL. The CPHST biological technician assigned to the rearing facility will continue to conduct small methods development projects aimed at improving efficiency of fly production and shipping. In 2003, a second species of fly was transferred to the FL-DPI rearing facility, but the rearing of the first species will continue for another few years for complete distribution. Currently (winter 2003) ca. 10 attack (rearing) boxes are online producing one species of fly, *P. tricuspis*, and 2 boxes are online producing a second species, *P. curvatus*. The second species of fly will be ready for limited release in spring 2004. Funding supplied in FY03 through all sources, particularly first time funding by CPHST and PPQ-WR, enabled an increase from 8 boxes to 12 boxes. A total of 16 boxes are available for rearing and FL-DPI may be ready to increase production up to that number by late FY04 depending on funding.

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. An excellent pictorial and text description of the rearing technique is available online from the FL DPI at:
http://www.doacs.state.fl.us/pi/methods/images/biocontrolfacilitybooklet.pdf.

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 production. Adult flies live only a few days and are very fragile, therefore it is impractical to ship and release adult flies.

Release techniques for the first fly species, *P. tricuspis*, are also labor intensive. Approximately 5000-6000 parasitized worker ant head capsules are shipped to the cooperator. The cooperator must then place the head capsules in an enclosed emergence box and allow the adult flies to emerge daily over 10-14 days. Adult flies are then aspirated into vials, carried to the field and released over IFA mounds. The mounds are disturbed frequently for 2 hours to insure worker ants are available on the soil surface for the flies to attack. One "release" encompasses 10-14 days of daily fly collection and release over mounds. Detailed instructions are available on: http://www.cphst.org/pages/FlyRearing/.

<u>RESULTS</u>:

While flies have been and will continue to be released by various research agencies, including ARS, in various 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. There were 10 releases in 2002 with at least 5 of these successfully overwintering. To date (December 31, 2003) there have been 1-3 releases in each of 12 states and Puerto Rico (a total of 26 releases from 2002-2003). Overwinter success of flies released in 2003 can not be determined until spring or summer of 2004. Included in these release counts are flies shipped to New Mexico for research purposes, and one shipment of flies to Louisiana to "seed" their own rearing project. These uses of flies have been approved by the states. California is the only state within the federal IFA quarantine not receiving flies. Since California is under an eradication program, they have elected not to participate at this time. Multiple releases of each fly species in each state are anticipated, depending on total acreage quarantined or generally infested within each state. A new CPHST project initiated in FY2003, utilizing spatial technology to assist in monitoring and evaluating the success of these fly releases (A3Q02/A3M02), will hopefully allow us to more efficiently target sites and states where each fly species would be most successful in establishment.

In addition to the flies being released by state collaborators, the rearing facility is providing flies, as available, for educational purposes. For example, when state regulatory or extension personnel conduct field days or have booths at state fairs, etc., ca. 100 flies are provided as an educational live exhibit.

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.

PROJECT NO: A1P01

PROJECT TITLE: Comparative Study of Shimmed and Non-shimmed Cups in Phorid Fly Rearing Attack Box

TYPE REPORT: Final

LEADER/PARTICIPANTS: Deborah Roberts

INTRODUCTION:

The lifter cup system in the phorid fly attack boxes in the present configuration appears to lead to a high rate of mortality or injury to the ants and the squishing of brood. Dr. Sanford Porter suggested that a cup shim be tried in order to increase production. Several different types of shims in varying numbers per cup were tried during 2002 and reported here in 2003.

MATERIALS AND METHODS:

Many factors influence the type of shim and the material it should be made of. High humidity must be maintained in the attack box at all times, thus a material that would not rust was imperative. The main housing of the attack cups is made of a black spray can lid. Assembly of the housing starts with the nipping of three semi-holes at the base of the lid, allowing the ants access to the interior during phorid attacks. Within the cap is another flange in which several holes are drilled or punched out. This is so the plaster will form a stronger bond within the cap and not fall out or come loose. A gap of a quarter of an inch is left between the bottom of the cap and the plaster.

The first shims that were tried were crude pieces of wire in an L-shape and glued to the outside of the cup. These were unsatisfactory for several reasons. The wire was difficult to hold in place while the glue was being applied. The wire was not stiffly held in place by the glue, and the point of the L was very sharp. This configuration was disregarded. Next a small hole was drilled through the plastic: one, two, or three per cup. A small piece of brass wire was run through the hole and then twisted to form a loop that held the wire in place while the other end formed a “foot”. This was much stronger, and did not move, but still presented the problem of the sharp end point. This would be less of a problem for the ants, but more so for the technicians when it was time to remove the ants.   Some were covered in a spot of hot glue to prevent the ants from using the new hole as pathway into the protective area and to protect the technician.

A third configuration which appeared to work had the brass wire running through the hole and pinched into a loop with no jutting points. This allowed only the width of the wire as the spacer for the cup. Several of these types of cups were constructed. Hot glue was also placed on three spots and allowed to drip down in order to form a leg. It was believed that the ants may have a propensity for chewing the glue and failure of this shim seemed likely.

Finally, a type of shim that had to be placed in the cup prior to plastering was constructed. Paperclips were hot glued into place on the inside of the cap, and plaster was then poured in as usual. Brass wire was looped and glued in the same way the paperclips were.

A wide variety of shimmed cups and non-shimmed cups were placed into Attack Box E, in a somewhat random pattern. Visual observations were made to see if the ants would still trail, if flies would try and get under the gap and if any adverse effects would come of this raised cup due to a possible change in humidity. Additionally, during the time when the ants were being transferred out after a period of attacks by the phorid flies (this was done on Tuesdays and Fridays), the dead ants and other debris that had been piled up and lying about the pan were vacuumed up on a pan by pan basis. This material was then placed under the dissecting scope and the individual ants were counted. The count was based on the number of ant heads, whether they were still attached to a body or not. Headless bodies were not counted.

A second experiment was run, when all the shimmed cups were removed and replaced with the final configuration of brass wire held in place by the plaster. Fourteen cups (seven pans worth) of shimmed cups and fourteen non-shimmed cups were tried. Pans 1 through 7 contained the shim cups and 8-14 the non-shimmed. All cups were the same with the exception of the cups in pan 12, where the mylar strips were replaced with a heavy duck cloth and fray check on the edges.

<u>RESULTS AND DISCUSSION:</u>

Eleven evaluations over time were conducted with the cups in the initial trial containing the wide variety of shimmed cups. The arrangement within the attack box was as follows:

Pan 1 Glue Shims on both cups
2 No shim on the front cup / Two brass legs on the back cups
3 No shims
4 Tripod of brass legs on both cups
5 Single brass leg on both cups
6 Glue Shims on both cups
7 Tripod of brass loops covered with glue on both cups
8 Tripod of brass loops in plaster on both cups
9 Single brass leg on front cup / Tripod of brass legs on back cup
10 Tripod of paperclips in plaster on both cups
11 No shims
12 No shims
13 No shims
14 No shims

The treatment cups were put into the pans with no particular attention being given to the order. The arrangement of the cups and wide variability of the shims types may have adversely affected the observable differences between shimmed and non-shimmed cups. The second experiment eliminated this variability. The data gathered from the first experiment still has credence though and appears in Table 1. Non-shimmed cups appeared to have a higher rate of mortality over the shimmed cups. Tripod cups had a lower rate of mortality over all the other treatments. The pans that had two entirely different treatments such as Pan 2 and 9 should be discounted from the study. The cups that exhibited the lowest rate of mortality were those in which the shim had been implanted into the plaster. The first evaluation of the brass wire in plaster shims (7 Jun) was high due to a large amount of dead ants being introduced into the pan initially (Table 1). Fluctuation from time point to time point cannot be adequately rationalized. It does appear that on days when there were higher numbers of dead in the shimmed cups, those same numbers can be reflected in the non-shimmed cups.

The data in Table 2 represents the second time the experiment was run, comparing tripod shimmed cups to non-shimmed cups. All cups on each side of the attack box were the same with the exception of cups in tray #12. Instead of mylar strips, this cup had black duck cloth with fray check on the edges. The lower mortality numbers seen in this tray may be contributed to the fact that the black cloth acted somewhat like a shim.

Suzanne Fraser ran statistics on the numbers with the following Tukey Comparisons:

Treatment	Mean	Homogenous Groups
No Shims	72.929	A
Tripod Shim	28.310	·· B

There was significantly less mortality with the shimmed cups than with the non-shimmed cups. Based on this data, it is recommended that shimmed cups be utilized in all the attack boxes.

Table 1. Comparative counts of dead ants within pans of Attack Box E.

SHIMMED AND NON-SHIMMED CUP COMPARISON												
PAN	7-Jun	14-Jun	18-Jun	7-Jun	25-Jun	2-Jul	9-Jul	12-Jul	16-Jul	18-Jul	23-Jul	TOTAL
1	28	12	45	9	30	59	21	55	38	23	36	356
2	79	32	77	39	62	35	56	75	39	38	62	594
3	62	68	75	22	44	52	58	81	74	37	100	673
4	38	34	53	13	21	35	7	36	13	19	17	286
5	78	45	60	20	68	53	91	120	46	36	70	687
6	37	23	37	36	15	20	61	44	29	12	18	332
7	35	22	32	12	3	17	35	no ants	55	38	20	269
8	50	21	20	8	10	11	15	50	21	17	14	237
9	39	18	31	6	13	16	24	32	57	25	83	344
10	38	5	22	8	10	33	18	34	20	66	30	284
11	108	35	76	73	69	76	83	58	96	40	75	789
12	80	31	48	34	38	61	52	38	71	38	50	541
13	139	69	70	61	99	139	64	65	100	48	79	933
14	138	59	139	57	85	110	111	65	101	60	105	1030

Table. 2 Brass shims vs. non-shimmed cups within Attack Box E.

EXPERIMENT 2 SHIMMED AND NON-SHIMMED CUP COMPARISON								
CUP TREATMENT	PAN	30-Jul	2-Aug	6-Aug	9-Aug	13-Aug	16-Aug	TOTAL
Tripod of Brass Loops	1	7	28	54	29	25	21	164
Tripod of Brass Loops	2	25	39	75	39	28	10	216
Tripod of Brass Loops	3	10	58	40	33	23	5	169
Tripod of Brass Loops	4	24	39	30	20	16	2	131
Tripod of Brass Loops	5	22	44	40	25	20	31	182
Tripod of Brass Loops	6	22	42	33	17	37	11	168
Tripod of Brass Loops	7	23	31	29	18	30	34	165
No Shims	8	87	89	71	49	96	59	451
No Shims	9	86	90	127	76	73	65	517
No Shims	10	69	117	73	88	59	74	480
No Shims	11	71	70	80	83	56	35	395
No Shims (Black Strips)	12	22	57	42	48	76	25	270
No Shims	13	76	83	85	75	92	53	464
No Shims	14	77	64	108	86	106	45	486

PROJECT NO: A9P03

PROJECT TITLE: Evaluation of Field Releases of *Thelohania solenopsae*, 1999-2003

TYPE REPORT: Final

LEADER/PARTICIPANTS: Shannon James, Anne-Marie Callcott, Shannon Wade, Lee McAnally, Tim Lockley, Ron Weeks, Homer Collins, and Avel Ladner

COOPERATORS: Drs. David Williams and David Oi, USDA, ARS, CMAVE, Gainesville, FL

INTRODUCTION:

The microsporidium *Thelohania solenopsae* (Microsporidia: Thelohaniidae) was discovered in Brazil in the red imported fire ant (Knell et al. 1977). Since that time, USDA, ARS, CMAVE personnel in Argentina also discovered the pathogen in the black imported fire ant in that country and determined that the pathogen does decrease colonies and colony vigor and therefore may be a good candidate for use as a biological control agent in the United States (Briano et al. 1995a, 1995b, 1996). In 1998, we initiated a trial releasing the microsporidium in Harrison and Hancock counties, MS (see 1999 annual report for FA02G048). These initial inoculation sites were lost or had poor results. Therefore, we repeated the trial in the fall of 1999.

MATERIALS AND METHODS:

In October 1999 we assisted ARS with the initiation of a trial to evaluate field releases of *T. solenopsae*. Two sites in southern Mississippi, one polygyne in Hancock Co. and one monogyne in Harrison Co. were selected for inoculation trials. Four plots at each site were divided into two inoculation plots and two non-inoculated control plots. The monogyne site was lost early in the study due to pasture improvements. Standard plot sizes are generally 0.25 acre (1012 m^2), however, due to the high density of mounds at the polygyne site, the circular test plots were 0.0625 acre (253 m^2) in size. Brood infected with *T. solenopsae* (field collected in Florida by ARS prior to study) was introduced in 3.5-g amounts to nine mounds within each of the inoculation plots. As weather and circumstance permitted, plot evaluations consisting of number, mound index, and within-plot location of mounds were conducted every few months. Evaluations occurred at weeks 0 (pre-inoculation), 12, 20, 28, 36, 49, 76, 84, 92, 100, 109, 118, 127, 141, 155, 161, 173, 191, and 198. Repeated measures ANOVA, with treatment, year, and season nested within year were performed on both colony number and population index. Population indices with brood were scaled for a heavier weighting than those without. Flooding from tropical storm Isadore and hurricane Lili in weeks 152 and 153 significantly disturbed the site, so only data from weeks 0 – 141 are used in statistical analysis.

Worker samples were collected from each mound during these evaluations and frozen until they could be examined. Ants from each sample were ground in a tissue grinder and wet mount slides were made of the resulting slurry. The slides were studied under 400x magnification on a phase contrast microscope for presence of *T. solenopsae* spores (Briano et al. 1995a). Valles et al. (2002) have recently determined a highly sensitive technique of *T. solenopsae* detection using PCR. Samples from weeks 191 and 198 are being held pending authorization for PCR examination to verify accuracy of microscopic examination.

Sampling on weeks 161, 191, and 198 was altered due to concern that flooding may have moved infected colonies out of the original plots. The sampling area of the plots was increased for these dates to 506 m^2. Additionally, on weeks 191 and 198, a grid divided into 10 x 10 meter squares was used to determine 32 random samples collected over the remainder of the pasture outside of the extended plots.

RESULTS:

Colony mortality:
The numbers of colonies and the population indices in plots were significantly influenced by treatment, year of the trial, and season within the year (df = 55, F = 4.481, P < 0.001; df = 55, F = 5.958, P < 0.0001 respectively). Colony mortality, measured in numbers of colonies and total population index per plot, displayed fluctuation within each year based on season (effects test, df = 8, F = 2.863, P = 0.0118; df = 8, F = 3.737, P = 0.0021) (Figure 1). Often ants travel deeper in the soil to avoid desiccation in summer and thus are harder to adequately sample causing an apparent if not actual drop in populations. Peak populations, likewise, annually coincide with sampling in optimal weather when new colonies become evident and ants are most active.

Inoculated and control plots displayed similar colony numbers and population indices for the first year of the trial and to some extent in the second year. However, by the third year exposure to *T. solenopsae* infection caused inoculated plots to deviate from the seasonal cycling demonstrated by the control plots (year effects test, df = 2, F = 9.224, P = 0.0005; df = 2, F = 10.441, P =0.0021) (treatment effects test, df = 1, F = 8.843, P < 0.005; df = 1, F = 14.235, P = 0.0005). Very few colonies were located in the site after flooding that occurred a few weeks prior to the week 155 sampling. As of week 191 the number of control plot colonies had not returned to the level expected for that time of year. Plot evaluation at week 198 (Oct. 20, 2003), has shown indication of recolonization.

Presence of pathogen:
No spores were detected in pretreatment samples (Table 1). At 12 weeks after inoculation, two mounds in one of the inoculated plots tested positive for spores. By week 20, both of the inoculated plots had three positive colonies, and spores were detected from 2 mounds in one of the control plots. No other spore positive results were found in control plot samples through the last evaluation prior to the storms. The number of colonies decreased in both inoculated polygyne plots as the percent of infected colonies increased (Figure 2). Highest numbers of spore positive colonies occurred in the third year while the inoculated plots deviated from the seasonal population increase seen in the controls.

One of the properties of an ideal biological control agent is the ability to sustain itself in the field. Since inoculation, only one sample date has not shown continued spore presence in the plots. Fluctuation of infection does appear in the data, as colonies recorded in the same location have shown spores on one date, none on another, and then spores again later on. Potential contributing factors to this phenomenon include ability to detect other stages of the disease, inadequate sample collection due to seasonal influence, and colony movement. Evidence of the propagation of *T. solenopsae* among colonies was supported by the increase in spore-positive colonies within the plots over time.

After the hurricane disturbance, the sample regime was expanded to better determine occurrence of movement of infection. The extended sampling areas around plots in samples during weeks 161 and 191 revealed additional spore positive colonies. The week 161 sampling had no positives within the original 253 m^2 of one inoculated plot but did have one positive colony at 0.3 m into the extended zone. The other inoculated plot at this date doubled its number of positive colonies with the addition of the extended sample area. Samples at week 191 displayed a similar trend with one inoculated plot having two positive colonies and adding two more with the extended zone, and one control plot showing a single infected colony in the original area and two more in the extension. Since this extended area was not sampled prior to week 161, it is unknown if positive colonies found there were displaced by the flooding or were present in this area earlier.

Spore positive samples were detected in the same control plot both on week 173 and week 191 indicating sustained infection within this control. The week 191 random samples produced spore positive colonies in samples only near the infected control plot. The infection of this control plot shows movement of the infection within the pasture, but whether the movement is a result of movement of old colonies, establishment of new infected colonies through mating flights, or brood transferal during the flooding is unknown.

DISCUSSION:

Results from this trial support the assessment of *T. solenopsae* as a useful biological control agent. South American plots infected with *T. solenopsae* demonstrated significant decrease in mound density over a four-year period of observation (Briano et al. 1995b). Similarly, significant reduction of imported fire ant populations and ability to sustain infection in the area were demonstrated in the three-year period prior to major site disturbance here in Mississippi. Furthermore, while the number of spore positive colonies observed in a single sample date never exceeded the initial inoculation total of 18, the low numbers of infected colonies in the first two years indicate that increase in year three was due to spread of the infection.

Transfer of this technology to state agencies is pending improved availability of the microsporidum and method of inoculation. A second trial of *T. solenopsae* will be established further inland in 2004 with method modifications based on experiences with this first trial. Information from this established infected site coupled with that from new sites should in time produce valuable clues to questions about environmental factors influencing successful inoculation, infection, and *T. solenopsae* spread.

REFERENCES CITED:

Briano, J., R. Patterson and H. Cordo. 1995a. Relationship between colony size of *Solenopsis richteri* (Hymenoptera: Formicidae) in Argentina. J. Econ. Entomol. 88: 1233-1237.

Briano, J.A., R.S. Patterson and H.A. Cordo. 1995b. Long-term studies of the black imported fire ant (Hymenoptera: Formicidae) infected with a microsporidium. Environ. Entomol. 24: 1328-1332

Briano, J.A., R.S. Patterson, J.J. Becnel and H.A. Cordo. 1996. The black imported fire ant, *Solenopsis richteri*, infected with *Thelohania solenopsae*: intracolonial prevalence of infection and evidence for transovarial transmission. J. Invertebr. Pathol. 67:178-179.

Knell, J.D., G.E. Allen and E.I. Hazard. 1977. Light and electron microscope study of *Thelohania solenopsae* n. Sp. (Microsporidae: Protozoa) in the red imported fire ant, *Solenopsis invicta*. J. INvertebr. Pathol. 29: 192-200.

Valles, S.M, D.H. Oi, O.P. Perera and D.F. Williams. 2002. Detection of *Thelohania solenopsae* (Mircosporidia: Thelohaniidae) in *Solenopsis invicta* (Hymenoptera: Formicidae) by multiplex PCR. J. Invertebr. Pathol. 81: 196-201.

Figure 1. Fire ant colony abundance over three years of exposure to *T. solenopsae*

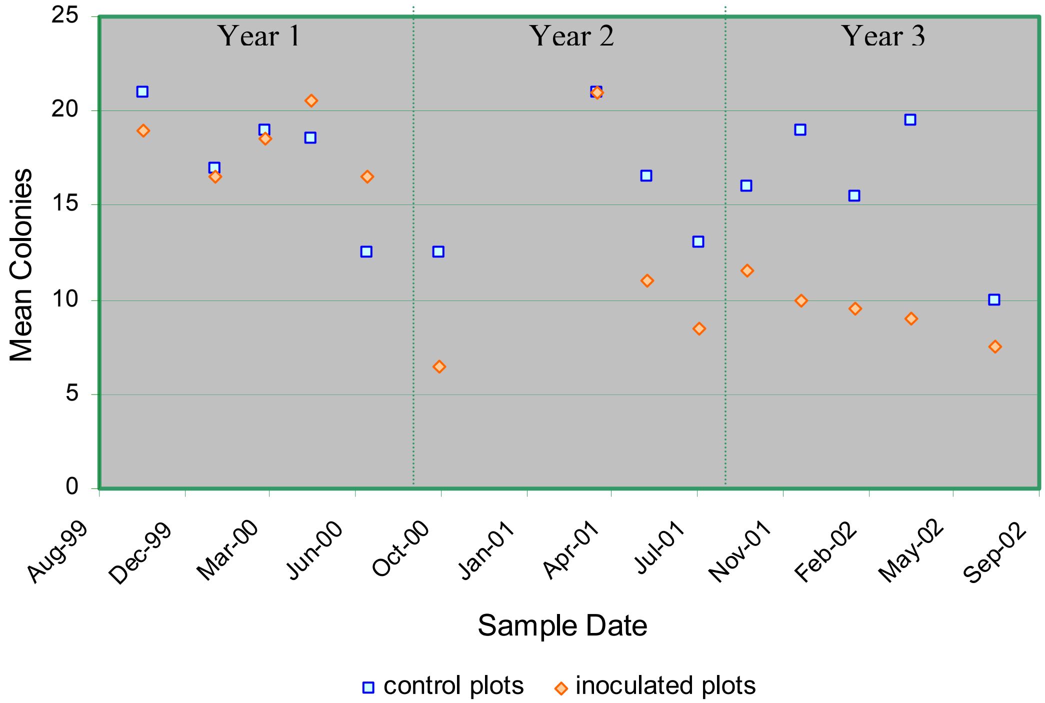


Figure 2. Effect of increased proportion of colonies infected with *T. solenopsae* on colony abundance.

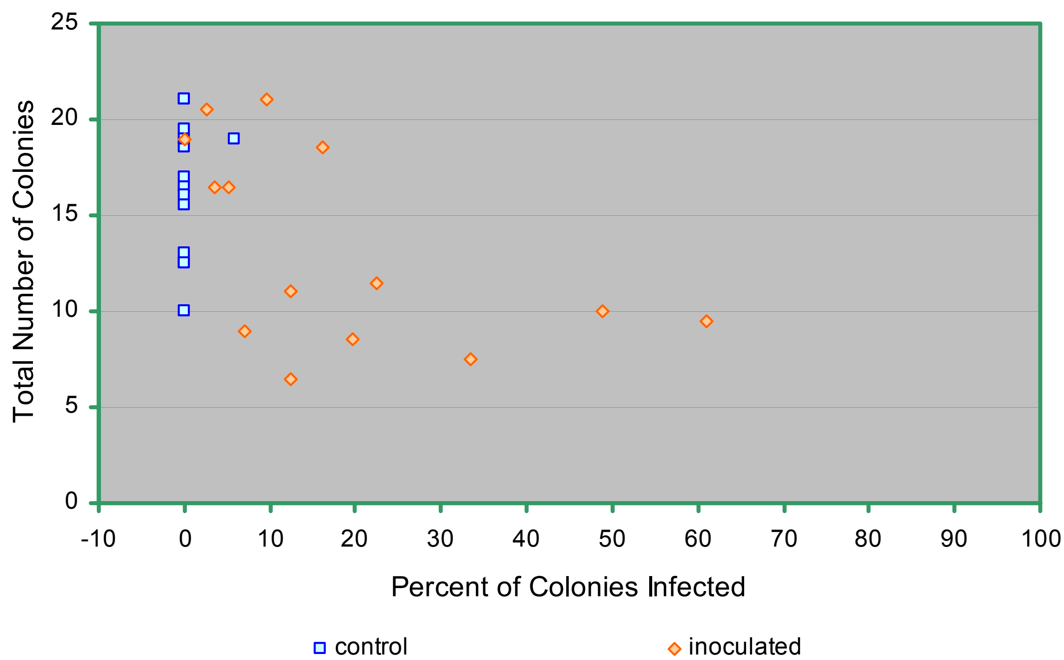


Table 1. Number of colonies in each plot determined positive for *T. solenopsae* spores for all sample dates. †

Location	Sample date in weeks after inoculation																	
	0	12	20	28	36	49	76	84	92	100	109	118	127	141	155	161	173	191
Plot Five*	0	0	3	1	0	0	1	0	2	1	1	2	2	0	2	0	2	2
Plot Six	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
Plot Seven*	0	2	3	0	1	2	3	0	0	4	4	8	8	1	0	5	11	0
Plot Eight	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

† Week 198 samples are being held for PCR examination
* Denotes inoculated plots

PROJECT N0: A3SO2/A3M02

PROJECT TITLE: Geographic Information Systems (GIS) Decision Support and Management Program for Monitoring and Evaluation of Beneficial Exotic Arthropod (i.e. phorid fly – *Pseudacteon* spp.) Releases, Establishment, and Spread in Imported Fire Ant *Solenopsis* spp. Populations

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Ronald D. Weeks

INTRODUCTION:

APHIS is allocating significant funding to the rearing and distribution of phorid flies to State collaborators for releases in numerous imported fire ant (IFA) infested states and varying habitats (see Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing Project in Integrated Pest Management section of report). Spatially explicit factors that affect successful phorid fly releases and establishment include; habitat type, eco-region, land use, previous treatment strategies (poison bait applications), and IFA population densities and social form (mongyne vs. polygyne). GIS (geographic information systems) is a dynamic tool that CPHST can use to organize and compile these factors into an integrated program. This approach can be of immense value in targeting areas for efficient and effective phorid fly releases.

This CPHST program will be delivered as a web-based application to State collaborators as a decision and management system. Currently, only one phorid fly species has been released in the APHIS release program, *Pseudacteon tricuspus*. However, another species, *Pseudacteon curvatus,* is planned for release in 2004. As more phorid species are released, this program will provide regulatory officials a tool to monitor multiple phorid species releases, establishments, and spread. As a clearly defined and well focused project, this GIS program will serve as a model for future CPHST survey and monitoring programs. Along this line of reasoning, the GIS-Phorid program would be structured to enable future linkage with other IFA control strategies or biological control agents, which would allow for estimation of their impact on IFA populations under different management scenarios. There are two related components to the GIS project; 1) a phorid fly tracking program, and 2) a predictive decision and management support program. Each component of the project is structured as a stand alone program with supporting data for the predictive model relying on data from the tracking component. The tracking component of the project is being initiated in the first two years of the multi-year project. The tracking program uses GIS technology to display and organize information on phorid fly releases within states.

Spatial data are collected using a GPS (Teletype WorldNavigator® Global Positioning System) attached to an iPAQ® Pocket PC H3955. Additional iPAQ® hardware includes an iPAQ® Expansion Pack Plus® and 256MB SecureDigital® Card. Data are entered into GPS/Pocket PC units via customized application forms running in ARCPAD 6 (ESRI®). Application forms were designed using ARCPAD Application Builder 6 (ESRI®). Data are maintained using

ARCGIS 8.3 (ESRI®) software on a Dell® Precision 650 Workstation computer in Gulfport, MS at the Soil Inhabiting Pests Laboratory **http://www.cphst.org/pages/IFASIPL**.

Map of phorid fly releases in southeastern United States. Includes all APHIS releases from 2002-2003, and additional releases made by Sanford Porter (USDA, ARS, CMAVE, Florida) and Larry Gilbert (University of Texas).

PROJECT HIGHLIGHTS:

- There are two components to this GIS project; 1) a phorid fly tracking program, and 2) a predictive decision and management support program
- This program will provide regulatory officials a tool to monitor multiple phorid species releases, establishments, and spread
- Completed electronic data entry forms and GPS integration of handheld units
- Completed "in-house" beta testing of all software and hardware
- Near completion of data dictionary and user manual for handheld devices and software
- Completed general map of phorid fly releases in southeastern United States (above) and detailed map of releases and establishments in Mississippi (see IPM report; Biological Control of IFA)

PROJECT NO: A3S01

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

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Robert G. Jones, Ph.D

INTRODUCTION:

The Boll Weevil Eradication Programs were operated for many years by USDA APHIS PPQ. The boll weevil is an introduced pest of cotton from southern Mexico. The present eradication effort started with the 1978 Trial Program in North Carolina and Virginia. This was then expanded into a North and South Carolina Program in 1983.

With the expansion into parts of Alabama, Georgia and Florida this became the Southeastern Boll Weevil Eradication Program (SEBWEP) in 1987. The Southeastern Boll Weevil Eradication Foundation was created during this expansion and was composed of representatives from each of the individual state foundations. By 1997 the Foundation had taken over the management of program operations with technical advice and funding from USDA APHIS PPQ. The SEBWEP now includes all of the formerly mentioned states with Mississippi, Tennessee and Missouri. The other boll weevil eradication programs are run by their individual state foundations in Arkansas, Louisiana, Texas, Oklahoma, Kansas, New Mexico, Arizona and California.

Identification of boll weevils is critical to eradication programs. Control decisions are based on adult boll weevils caught in survey traps. These traps are baited with an aggregation pheromone lure. While this lure is species distinct it has components found in pheromones of other weevil species. This odor as well as the trap's color and reflective attraction cause numerous assortments of weevil and other insect species to be captured. These trapped insects must be sorted with the boll weevils identified and counted. These boll weevil counts become the data used for program operational decision making. Insecticide treatments resulting from misidentified non boll weevils are environmentally disruptive, expensive and can lead to legal problems. If boll weevils go unrecognized as such the program can have numerous costly problems. This can mean crop damage and area wide insecticide applications instead of a single field being treated.

Forensic examinations of trap caught boll weevils have four areas where it can help in program operational decision making or justification for decisions. (1) Sexing the boll weevils. Normally the male boll weevil comes to the trap in a seasonal pattern. This is early and late in the cotton plant growth cycle. To catch a male in traps during flower bud formation means one of two things, the presence of a large population or the only boll weevil present. The male boll weevil produces pheromone as he feeds to attract others to him. If others do not come he moves to pheromone sources searching for others. (2) The aging of adult boll weevils indicates different occurrences dependent on season or human interferences. For example the presence of teneral adults in traps means there has been reproduction in the immediate area or field. Of course teneral adults in traps before cotton fruiting has started means someone is tampering with the traps. This has been done by both individuals who lost their jobs with the program and growers who needed their fields sprayed for plant bugs. (3) Determining the diapause condition and (4) the reproductive condition of adult boll weevils is done by dissecting specimens. This is difficult with specimens from program traps since they are generally dead and desiccated. On occasion and when large numbers are sent in it has been possible. Boll weevils coming out of and going into the physiological diapause condition indicates problems ahead for programs. The same is true with the presence of both mated and reproductively active females.

METHODS AND MATERIALS:

Identification, sexing and aging adult boll weevils is based on Jones, Robert G. and Michael Williams. 2001. A Field Guide to Boll Weevil Identification. Mississippi Agriculture & Forestry Experiment Station. Technical Bulletin 228.10 pages.

Dissections to determine the physiological diapause and reproductive conditions are based on Brazzel, J. R. and L. D. Newsom.1959. Diapause in *Anthonomus grandis* Boh. Journal of Economic Entomology. 52:603-611 and Burke, H. R. 1959. Morphology of the Reproductive Systems of the Cotton Boll Weevil (Coleoptera, Curculionidae). Annals Entomological Society of America 52:287-294.

Materials include microscope and dissecting tools that were purchased several years ago for this project that was started in 1983. No further materials are necessary in the foreseeable future. SEBWEP pays for the overnight or two day mailing expenses of specimens.

RESULTS AND DISCUSSION:

The numbers of weevils and boll weevils submitted in 2002 and 2003 has been greatly reduced from previous years. The numbers of samples for both years was about 20. This included samples with multiple specimens and individual boll weevils and non boll weevils. The progress of the Program was evident between the two years. In 2002 samples came from several locations in northeast Mississippi, Alabama and Georgia, Tennessee. There was only one boll weevil caught in Alabama in 2003. This was the only boll weevil found in any of the cotton states east of the state of Mississippi during 2003. This was a female boll weevil that had been an adult for over three days. The trap location was at a cotton field west of Mobile, Alabama near the Mississippi state line. The field or area is near Interstate Highway 10 and on the road to a major tourist attraction. The area has a history of reinfestations since it was eradicated in the early 1990's. More information on this is found in "Robert G. Jones and James A. Wilson. 2002. Boll Weevil: Post Eradication Outbreaks in Cotton in the Southeastern United States. Proceedings of the National Cotton Council". Since it was trapped July 22, 2003 and the last previous boll weevils trapped in the area was in June 2002 this was determined to be a "hitchhiker". Progress in the Texas Program should continue to eliminate boll weevil introduction along Interstate 10. That Program has eradication efforts in the Upper Coastal Bend and the Southern Blacklands well under way. This greatly increases the distance between boll weevil populations and southeastern cotton along this transportation route. The situation along Interstate 20 is progressing with growers in the Northern Blacklands voting for an eradication program last November. The cotton in Louisiana acts as a buffer or protection area for Mississippi and eastward on this transportation route. The Louisiana Boll Weevil Eradication Program has been operating for a number of years.

In 2003 the remaining 19 boll weevil samples came from north central and north western Mississippi, western Tennessee and Missouri. Four of these were for validation as non boll weevil specimens. The other samples were for forensic evaluation. These included early, emerged over wintered adult boll weevils which probably could not survive until the late plant cotton sprouted; adult boll weevils emerging from fruit after defoliation and harvest of cotton; and sexing and aging individual specimen samples to determine possibility of local infestations. Indications are that many of these boll weevil samples were an effect of the two Arkansas counties just south of Missouri on the Mississippi River. These two counties were legally forced into the Arkansas Boll Weevil Eradication Program in 2002. The first year that no boll weevils were trapped in middle Tennessee, northeast Mississippi and the Tennessee River Valley of Alabama was 2003. After one more full year of eradication actions in the two Arkansas counties, good program actions in Tennessee, Mississippi and Missouri and a cold wet winter, boll weevils will be scarce or even eradicated in the SEBWEP area.

This CPHST Project A3S01 was approved for no more than 10% of the lead scientist's time. The best calculation made for 2003 was that it took 5% or less to handle the samples received and as stated fewer samples are anticipated in the 2004 calendar year. While the work of this project may be greatly reduced, the need to do it becomes greater. To verify the eradication of the boll weevil in the Southeastern United States every questionable weevil trapped will need to be examined by someone with recognized expertise.

APPENDIX I - LABORATORY BIOASSAY PROCEDURE

PROTOCOL FOR BIOASSAY OF INSECTICIDE TREATED POTTING MEDIA WITH ALATE IFA QUEENS

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

Collection of test insects: Field collected alate imported fire ant queens are used as the test insect. IFA colonies are opened with a spade and given a cursory examination for the presence of this life stage. Alate queens are seldom, if ever, present in all IFA colonies in a given area. Some colonies will contain only males, others may have few or no reproductive forms present, others may contain both males and queens, while some will contain only alate queens. Seasonal differences in the abundance of queens is quite evident; in the warmer months of the year 50% or more of the colonies in a given area may contain queens. However, in the cooler months, it is not uncommon to find that less that 10% of the colonies checked will contain an abundance of alate queens. Therefore, it is necessary to examine numerous colonies, selecting only those which contain large numbers of alate queens for collection. During winter, ants will often cluster near the surface of the mound facing the sun. Collection during midday on bright, sunny days is highly recommended for winter; whereas the cooler time of day is recommended for hot, dry days of summer. Once a colony (or colonies) has been selected for collection, the entire nest tumulus is shovelled into a 3-5 gallon pail. Pails should be given a liberal dusting with talcum powder on the interior sides to prevent the ants from climbing up the sides of the pail and escaping. Approximately 3-6" head room should be left to prevent escape. An effort should be made to collect as many ants as possible while minimizing the collection of adjacent soil which will contain few ants. Collected colonies are then transported to the laboratory for a 3-5 day acclimation period. The addition of food or water during this short acclimation period is not necessary. Alate queens are collected with forceps after placing a 1-2 liter aliquot of the nest tumulus in a shallow laboratory pan. 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 2-300 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 Patterson Dental Co., 2323 Edenborn Ave., Metairie, Louisiana. 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 (see Figure 1). 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. Plastic petri dishes are inverted over the tops of the pots to prevent escape from the top of the test chambers. Prior to placing queens in the test chamber, 50 cc of treated potting media is placed in the bottom of each pot. Due to possible pesticide contamination, test chambers are discarded after use.

Replicates: 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.

Test interval: All evaluations are based on a 7 day continuous exposure period. i.e., introduced queens remain in the test chambers for 7 days. At this time the contents of each chamber are expelled into a shallow laboratory pan and closely searched for the presence of live IFA alate queens.

Recording of data: Results of each bioassay are entered on the attached 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 18 months.

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

B&B Wire basket dimensions

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

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

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