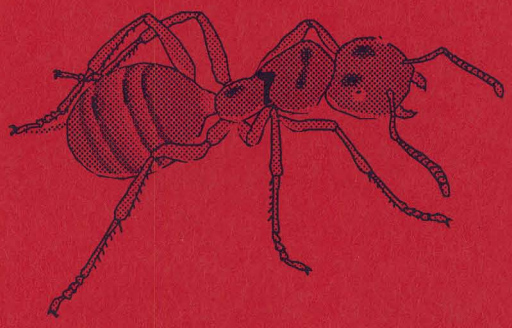
1997 ACCOMPLISHMENT REPORT

GULFPORT PLANT PROTECTION STATION

U.S. DEPARTMENT OF AGRICULTURE

ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE
CENTER FOR PLANT HEALTH SCIENCE AND TECHNOLOGY



3505 25th Ave., Bldg. 16
Gulfport, MS 39501

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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 or sweet potato weevil 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 Callcott and Homer L. Collins

February 1998

GENERAL TABLE OF CONTENTS

TITLE	PAGE
FY 1996 Imported Fire Ant Objectives	1
IFA Contents	2
IFA Projects	5
FY 1996 Sweet Potato Weevil Objectives	177
SPW Contents	178
SPW Projects	179

IMPORTED FIRE ANT

FY 1996 IMPORTED FIRE ANT OBJECTIVES

GULFPORT PLANT PROTECTION STATION
GULFPORT, MS

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

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

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

- o New product/formulation testing and evaluation.
- o Conduct label expansion studies
- o Evaluation of non-chemical biocides 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:

- o Provide training to state regulatory agencies and nursery associations.
- o Publish and distribute a directory of research, regulatory. and Extension Services involved in IFA activities.

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

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

IFA CONTENTS

SECTION I

DEVELOPMENT OF QUARANTINE TREATMENTS FOR CONTAINERIZED AND BALLED & BURLAPPED NURSERY STOCK

PROJECT:	PROJECT TITLE:	PAGE
FA01G025	Evaluation of Fipronil as a Potting Media Toxicant under Actual Nursery Conditions	5
FA01G076	Expanded Trials with Fipronil as a Quarantine Treatment for Containerized Nursery Stock - Incorporated and Drench Treatments	10
FA01G037	Residual Activity of Fipronil 0.1G in Nursery Potting Media	14
FA01G067	Residual Activity of Fipronil 0.05G Incorporated into Potting Media and Applied "Over-the-Top"	17
FA01G145	Evaluation of Bifenthrin 0.2G on a new Inert Carrier	20
FA01G035	Influence of Sand Content of Commercial Nursery Potting Media on Residual Activity of Bifenthrin and Tefluthrin, 1995	23
FA01G086	Evaluation of Deltamethrin as a Potting Media Toxicant, 1996	28
FA01G085	Residual Activity of suSCon® Green in Various Types of Nursery Potting Media	30
FA01G016	Preliminary Testing of Organic Solutions™ Multipurpose Fireant Killer for Use as a Containerized Nursery Stock Treatment	36
FA01G056	Preliminary Testing of Organic Plus Multipurpose Fireant Killer for Use as a Containerized Nursery Stock Treatment	40
FA01G057	Expanded Trials with Organic Plus Multipurpose Fireant Killer for Use as a Quarantine Treatment for Containerized Nursery Stock	44
FA01G097	Efficacy of Chlorfenapyr as an Imported Fire Ant Quarantine Treatment	51
FA01G026	Residual Activity of Silafluofen Drench in Nursery Potting Media	58

PROJECT NO:	PROJECT TITLE:	PAGE
FA01G155	Eval'uation of Spin Out® Techno1ogy in Combination with Insecticides as a Quarantine Procedure for Containerized Nursery Stock	60
FA01G017	Expanded Trials with Spin Out® Technology Used in Combination with Bifenthrin Insecticide to Prevent Imported Fire Ant Infestations in Containerized Nursery Stock, 1997	66
FA01G116	Evaluation of Spot Treatments with Granular Insecticides for Quarantine Treatment of Red Imported Fire Ants in Field Grown Nursery Stock	72
FA01G047	Quarantine Treatment of Balled-and-Burlapped Nursery Stock with Fumigants	80
FA05G055	Biobarrier as a Prophylactic Treatment for Control of Phytophera Root Rot in Blueberry and its Effects on Populations of Imported Fire Ants in Commercially Grown Blueberry Orchards	89
FA05G075	Biobarrier as a Prophylactic Against Infestation of Containerized and Ball & Burlap Nursery Stock	92
FA05G065	Effect of Mulches on Grape Root Borer and Establishment of Colonies of the Imported Fire Ant in Orchards of Commercially Grown Muscadines	94
FA01G077	Tennessee Mating Flight Study: Can the Biology of the Imported Fire Ant be Used to Aid in Certification of Field Grown Nursery Stock ?	96
FA01G087	Prevention of IFA Colony Establishment in Hay Bales Stored Outdoors on the Ground	100

SECTION II

DEVELOPMENT OF QUARANTINE TREATMENTS FOR GRASS SOD

FA01G066	Evaluation of Candidate Chemicals for Control of IFA in Commercial Grass Sod, 1996	102
FA01G027	Multi-State Field Trials with Fipronil Bait and Chipco® Exclude™ for Control of Imported Fire Ants (IFA), Cutworms (Noctuidae), and Ground Pearls (Margarodes spp.)	106

PROJECT:	PROJECT TITLE:	PAGE

SECTION III

POPULATIONS SUPPRESSION TRIALS

FA02G017 Efficacy of Various Fenoxycarb Formulations. 1997.....117

FA02G026 Evaluation of New Bait Products for Control of Imported Fire Ants.....119

FA02G036 Efficacy of AC 303,630 as an Imported Fire Ant Bait.....123

SECTION IV

BIODIVERSITY

Impact of Imported Fire Ants on Biodiversity.....131

FA05G017 The Ants of East Texas: A Twenty-Five Year Comparative Survey.....132

FA05G013 Ecological Studies of an Isolated Imported Fire Ant Population in Eastern Tennessee.....,137

FA04G017 Seasonal Biology of Polygynous RIFA Colonies Along the Mississippi Gulf Coast.....158

REFERENCES CITED.....161

APPENDIX I Publications/Presentations.....163

APPENDIX II Protocol for Bioassay of Insecticide Treated Potting Media with Alate IFA Queens.....164

APPENDIX III Protocol for Rearing of Imported Fire Ants.....167

APPENDIX IV Technology Transfer.....173

APPENDIX V IFA Interceptions. 1997.....174

SECTION I

DEVELOPMENT
QUARA
TREAT
CONT
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PROJECT NO: FA01G025

PROJECT TITLE: Evaluation of Fipronil as a Potting Media Toxicant Under Actual Nursery Conditions

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Homer Collins, Anne-Marie Callcott, Avel Ladner, Charlene Russell, and Lee McAnally

COOPERATORS: Flowerwood Nursery (Loxley, AL), Wight Nursery (Cairo, GA), Georgia Dept. of Agric., Windmill Nursery (Folsom, LA) and Louisiana Dept. of Agric.

INTRODUCTION:

Fipronil is a broad spectrum pyrazole insecticide under development by Rhone-Poulenc Ag. Co. The insecticide acts on the central nervous system of the insect and enters by ingestion or contact. Thus, fipronil has shown excellent control of a variety of foliar and soil insects (Colliot et al. 1992).

In 1993, we initiated a trial using fipronil as a preplant incorporated treatment against IFA (FA01G123). In this trial fipronil provided 24 months of excellent control at rates of 100 and 300 ppm. The 25 ppm rate provided 23 months of residual activity and rates of 5 and 10 ppm provided 8 and 13 months of residual activity, respectively.

Products which show promise as potting media treatments for control of IFA are further evaluated by subjecting them to actual nursery conditions. However, due to cost restrictions, plants are not included in the pots with treated media. Colliot et al. (1992) reported no phytotoxicity on any crops tested and our lab in a limited trial underway (FA01G015), also found no apparent phytoxicity.

MATERIALS AND METHODS:

Three nurseries cooperated in this trial: Wight Nursery (Cairo, GA), Windmill Nursery (Folsom, LA), and Flowerwood Nursery (Mobile, AL). IFA lab personnel blended fipronil granular insecticide into media provided by each nursery on site. The media contained all fertilizer and nutrients normally used by the nursery. After blending, treated media was potted up in trade gallon nursery

pots and aged on site under normal conditions of irrigation and other cultural practices. Fipronil 0.1G (EXP 60818A) was incorporated at rates of 5, 10, 25 and 50 ppm.

The Flowerwood trial was initiated on April 25, 1995, the Wight trial on August 9, 1995, and the Windmill trial on October 24, 1995.

At monthly intervals, three pots from each treatment rate were collected, composited, and sent to the IFA lab. IFA personnel subjected the treated media to a standard alate female bioassay as described in Appendix II (Annual Report).

RESULTS:

Media was treated at Flowerwood Nursery, Loxley, AL on April 25, 1995. The 10 ppm rate was effective for 4 mths and rates of 25 and 50 ppm provided excellent control through 15 mths PT (Table 1). However, all test pots were accidently destroyed by nursery workers after 15 mths.

Media at Wight Nursery in Cairo, GA was treated on August 9, 1995. The 5 ppm rate was effective for 9 mths, and the 10 and 25 rates for 13 mths. Both the 10 and 25 ppm rates showed decreased efficacy at 14 mths after treatment, however, both recovered and maintained ≥90% mortality through 23 and 28 mths, respectively. The 50 ppm rate has been effective for 28 mths.

Media was treated at Windmill Nursery, Folsom, LA on October 24, 1995. The 10 ppm rate was effective for 2 mths, the 25 ppm rate for 18 mths, and the 50 ppm rate has been effective for 25 mths.

At this time, there appears to be some variability at the 5 and 10 ppm rates of application, probably due to non-uniform mixing, but the 25 and 50 ppm rates are providing excellent control.

Table 1. Efficacy of Fipronil Incorporated into Nursery Potting Media and Aged Under Actual Nursery Conditions.

Nursery	Rate (ppm)	% Mortality to Alate Females at Indicated Mths PT												
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Flowerwood	5	95	45	50	30	15	20	0	5	100	45	55	--	--
	10	100	100	100	95	65	10	100	100	100	100	100	--	--
	25	100	100	100	100	100	100	100	100	100	100	100	--	--
	50	100	100	100	100	100	100	100	100	100	100	100	--	--
	Check	80	20	5	0	0	5	10	0	0	10	5	--	--
Wight	5	100	100	100	100	100	100	100	100	100	---	85	75	100
	10	100	100	100	100	100	100	100	100	100	--	100	100	100
	25	100	100	100	100	100	100	100	100	100	--	100	100	100
	50	100	100	100	100	100	100	100	100	100	--	100	100	100
	Check	10	10	15	25	0	100*	100*	10	55	--	0	25	40
Windmill	5	0	65	5	55	5	30	20	35	10	60	35	15	10
	10	95	100	75	100	100	100	90	65	15	100	25	90	25
	25	100	100	100	100	100	100	100	100	100	100	100	100	100
	50	100	100	100	100	100	100	100	100	100	100	100	100	100
	Check	5	0	5	10	0	15	5	15	5	30	20	20	5

* high check mortality unexplained, possibly human error in collection

Table 1. Cont.

Nursery	Rate (ppm)	% Mortality to Alate Females at Indicated Mths PT												
		(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)
Flowerwood	5	50	45	*										
	10	55	80	*										
	25	100	100	*										
	50	100	100	*										
	Check	55	10	*										
Wight	5	0	65	35	100	--	100	95	--	50	20	20	--	5
	10	20	100	100	100	--	100	90	--	100	100	30	--	5
	25	40	100	100	100	--	100	100	--	100	90	100	--	100
	50	100	100	100	100	--	100	100	--	100	100	100	--	100
	Check	0	0	0	15	--	60	10	--	0	10	15	--	0
Windmill	5	85	35	5	65	40	15	25	20	20	20	10	20	
	10	100	100	60	100	10	10	30	60	10	0	95	40	
	25	100	100	100	100	100	85	95	100	100	80	100	100	
	50	100	100	100	100	100	100	100	100	100	100	100	100	
	Check	0	15	10	70	5	5	15	0	5	0	0	10	

* trial terminated prematurely due to destruction by nursery workers

Table 1. Cont.

Nursery	Rate (ppm)	% Mortality to Alate Females at Indicated Mths PT						
		(27)	(28)	(29)	(30)	(31)	(32)	(33)
Flowerwood	5							
	10							
	25							
	50							
	Check							
Wight	5	30	5					
	10	100	95					
	25	100	100					
	50	100	100					
	Check	5	5					
Windmill	5							
	10							
	25							
	50							
	Check							

PROJECT NO: FA01G076

PROJECT TITLE: Expanded Trials with Fipronil as a Quarantine Treatment for Containerized Nursery Stock - Incorporated and Drench Treatments

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Homer Collins, Anne-Marie Callcott and Lee McAnally

COOPERATORS: Turkey Creek Farms, Houston, TX
Tavo Garza, Texas Dept. of Agriculture

INTRODUCTION:

Fipronil (common name) is produced by Rhone-Poulenc Ag. Co. (Research Triangle Park, NC) and is currently marketed in numerous countries for control of many insect pests in a variety of crops. Currently, the only U.S. registration for the product is for mole cricket control in sod. Our laboratory has achieved excellent results with a granular formulation of the product when used as a preplant incorporated treatment (FA01G123, FA01G025). In 1996, we expanded our evaluation of the 0.1% granular formulation of fipronil, and also began preliminary testing of a water dispersable formulation as a drench treatment.

MATERIALS AND METHODS:

Incorporated trial:
Treatments were applied at Turkey Creek Farms. Houston, TX on July 9, 1996. Media and space was provided by the nursery. We traveled to Turkey Creek Farms and incorporated granular fipronil (EXP 60818A) into the nursery media at rates of 10, 15, 20 and 25 ppm. Incorporation was achieved by using a portable cement mixer to blend the toxicant into the media. Media was aged on site, under normal agronomic practices of irrigation, weeding. etc. On a monthly basis, three pots from each treatment group were collected and composited into one sample, and sent to the IFA laboratory. At the IFA lab. the samples were subjected to standard alate female bioassay.

Drench trial:
Treatments were applied at the IFA laboratory on June 25, 1996. Media consisted of the standard IFA media mix (MAFES mix - 3:1:1 pine bark:sphagnum

peat moss: sand). One gallon nursery pots were filled with the untreated potting media. Fipronil 80WD (EXP 60720A) was applied at rates of 10 and 20 ppm. The drench solution was applied at a rate of 400 ml of finished solution per container. Containers were placed outdoors under simulated nursery conditions, including a pulsating overhead irrigation system which supplied ca 1-1½" water per week. At monthly intervals, three pots from each treatment were composited and subjected to standard alate female bioassay.

RESULTS:

Incorporated trial:
The 10 ppm rate provided excellent control of IFA through 9 months (Table 1). There have been two dates of significant decreased efficacy for all rates, once at 12 months after treatment and again at 16 months after treatment. Because of these decreases we have only achieved 11 months of residual activity with rates of 15, 20 and 25 ppm.

Drench trial:
The 20 ppm rate provided >95% control for 5 months (Table 2). The lower rate of 10 ppm, did not provide consistent control.

Table 1. Residual Activity of 0.1% Granular Fipronil Incorporated into Nursery Potting Media at Turkey Creek Farms.

Treatment	Rate of Application (ppm)	% mortality of alate females at indicated mths PT												
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Fipronil	10	100	100	100	90	100	100	100	100	100	25	70	15	80
	15	100	100	100	100	100	100	100	100	100	90	100	30	100
	20	100	100	100	100	100	100	100	100	100	100	100	60	100
	25	100	100	100	100	100	100	100	100	100	100	100	40	100
Check	--	40	0	10	5	30	0	5	5	15	0	0	5	20

Table 1. Cont.

Treatment	Rate of Application (ppm)	% mortality of alate females at indicated mths PT										
		(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)
Fipronil	10	100	100	25	--	100						
	15	100	100	40	--	100						
	20	100	100	50	--	100						
	25	100	100	55	--	100						
Check	--	15	40	10	--	0						

Table 2. Residual Activity of Fipronil 80WD Applied as a Drench Treatment to Containerized Nursery Stock.

Treatment	Rate of Application (ppm)	% mortality of alate females at indicated mths PT											
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
Fipronil	10	80	90	100	100	80	45	100	100	100	100	100	100
	20	100	100	100	100	95	75	100	95	100	100	100	95
Check	--	20	15	10	0	5	0	0	0	0	5	5	10

PROJECT NO: FA01G037

PROJECT TITLE: Residual Activity of Fipronil 0.1G in Nursery Potting Media

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Lee McAnally

INTRODUCTION:

Fipronil (common name) is produced by Rhone-Poulenc Ag. Co. (Research Triangle Park, NC) and is currently marketed in numerous countries for control of many insect pests in a variety of crops. Currently, the only U.S. registration for the product is for mole cricket control in sod. Our laboratory has achieved excellent results with a granular formulation of the product when used as a preplant incorporated treatment (FA01G123, FA01G025). In 1996, we expanded our evaluation of the 0.1% granular formulation of fipronil, and also began preliminary testing of a water dispersable formulation as a drench treatment (FA01G076). In 1997, we again expanded our evaluation to include the incorporation of the granular formulation into several different media here at the Gulfport site.

MATERIALS AND METHODS:

The product was blended into nursery potting soil from Windmill Nursery, Folsom, LA (360 pounds per cubic yard) on May 14, 1997. A portable cement mixer (2 cu ft capacity) was used 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 pots and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½" water per week. At monthly intervals, 3 pots from each treatment were composited and subjected to alate queen bioassay.

Theoretical treatment rates used were 10, 15, 20, 25, 40 and 50 ppm. Due to an error in calculating bulk density, the actual initial treatment rates were 7, 10.5, 14, 17.5, 28, and 35 ppm. Rates of 40 and 50 ppm were mixed and added to the trial in September 1997. A further trial was set up on August 7, 1997 using Flowerwood media (Flowerwood Nursery, Mobile, AL, 550 pounds per

cubic yard), and on August 11, 1997 with our standard potting mix (MAFES mix, 650 pounds per cubic yard).

RESULTS:

Windmill media:
Treatment rates below 35 ppm were either poor or erratic (Table 1). The higher rates however, maintained 95-100% efficacy through 7 months for the 35 ppm rate, and 100% efficacy through 3 months for the 40 and 50 ppm rates.

MAFES media:
All treatment rates maintained 95-100% efficacy through 4 months (Table 2).

Flowerwood media:
All treatment rates maintained 100% efficacy through 4 months (Table 3).

Table 1. Residual Activity of Fipronil 0.1G in Windmill Media

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment						
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
7	30	60	10	30	--	--	--
10.5	30	30	5	10	--	--	--
14	65	55	15	25	10	0	20
17.5	95	75	75	20	10	55	55
28	100	100	90	100	50	100	100
35	100	100	100	100	95	100	100
Check	0	25	15	15	0	5	5
40	100	100	100				
50	100	100	100				
Check	0	5	5				

Table 2. Residual Activity of Fipronil 0.1G in MAFES Media

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment			
	(1)	(2)	(3)	(4)
10	100	100	100	100
15	95	100	100	100
20	100	100	100	100
25	100	100	100	100
40	100	100	100	100
50	100	100	100	100
75	100	100	100	100
Check	0	10	10	5

Table 3. Residual Activity of Fipronil 0.1G in Flowerwood Media

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment			
	(1)	(2)	(3)	(4)
10	100	100	100	100
15	100	100	100	100
20	100	100	100	100
25	100	100	100	100
40	100	100	100	100
50	100	100	100	100
75	100	100	100	100
Check	0	5	0	30

PROJECT NO: FA01G067

PROJECT TITLE: Residual Activity of Fipronil 0.056 Incorporated into Potting Media and Applied "Over-the-Top"

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Lee McAnally

INTRODUCTION:

Fipronil (common name) is produced by Rhone-Poulenc Ag. Co. (Research Triangle Park, NC) and is currently marketed in numerous countries for control of many insect pests in a variety of crops. Currently, the only U.S. registration for the product is for mole cricket control on golf courses. Our laboratory has achieved excellent results with a 0.1% granular formulation of the product when used as a preplant incorporated treatment for containerized nursery stock (FA01G123, FA01G025). In 1996, we expanded our evaluation of the 0.1% granular formulation of fipronil, and also began preliminary testing of a water dispersable formulation as a drench treatment (FA01G076). In 1997, we again expanded our evaluation of the 0.05% granular formulation to include incorporation and an over-the top application.

MATERIALS AND METHODS:

Incorporated Treatment:

Test procedures used were as follows: The product was blended into nursery potting soil (MAFES mix, 650 pounds per cubic yard). A portable cement mixer (2 cu ft capacity) was used to blend the toxicant into the potting media, and was operated for 15 minutes per batch to insure thorough blending. Treatments rates used were 5, 10, 15, 25, and 50 ppm. Treated media was then poured into one-gallon capacity plastic pots and weathered outdoors under simulated nursery conditions for one month prior to the first bioassay. Subsequent bioassays were conducted at monthly intervals. A pulsating overhead irrigation system supplied ca. 1-1½" water per week. Bioassays were conducted in the laboratory by confining alate queens to treated soil placed in 2" x 2" plastic flower pots equipped with a Labstone® bottom. The labstone absorbs mixture from an underlying bed of damp peat moss. There were four replicates per treatment in each bioassay. Each pot (replicate) contained 50 cc of

treated soil and five alate queens. Queen mortality was assessed after seven days of continuous confinement to the treated soil.

Over-the-top Treatment

One gal. nursery pots were filled with media and placed on a masonry brick in a 12" x 18" x 5" plastic pan. The sides of the pan were talced and ca. 1" of water was added to prevent escape. Five replicates per treatment rate were set up. Field collected colonies were separated from their nest tumulus by the floatation method (Banks et al. 1981) and 50 cc of workers and brood were added to each media-filled pot. The fragmented colonies were allowed to acclimate 3-5 days before treatment. Fipronil 0.05G was applied by sprinkling over the surface of the soil. Each container was then watered in with approximately 400 ml of water. Rates of 0.012, 0.12, and 1.2 grams per pot were used in the first trial. (approximately 0.01 ppm, 0.1 ppm, and 1 ppm). A second trial was initiated using rates of 12, 18, and 25 grams per pot (10 ppm, 15 ppm, and 25 ppm). Containers were watered as needed for the duration of the 7 day trial. Ants were inspected daily for mortality and colonies were considered dead when less than 20 workers were present.

RESULTS:

Incorporated Treatment:

At three months post-treatment the 10, 25, and 50 ppm rates provided 100% efficacy with the 5 and 15 ppm rates showing unexplained drops in efficacy at 2 and 1 months respectively (Table 1).

Over-the-top Treatment:

In the first trial, no treatment rate provided more than 30% efficacy. In the second trial, no treatment rate provided more than 75% efficacy.

Table 1. Residual Activity of Fipronil 0.05G

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment		
	(1)	(2)	(3)
5	100	30	100
10	100	100	100
15	55	100	100
25	100	100	100
50	100	100	100
Check	5	0	0

PROJECT NO: FA01G145

PROJECT TITLE: Evaluation of Bifenthrin 0.2G on a New Inert Carrier

TYPE REPORT: Final

LEADER/PARTICIPANTS: Lee McAnally and Kirk Irby

INTRODUCTION:

Talstar® T&O Granular (FMC Corp.; currently marketed by The Scotts Co.) is registered and approved for use in the Imported Fire Ant Federal Quarantine as a preplant incorporation treatment. Rates and certification periods range from 10 ppm for a 6 mth certification to 25 ppm for continuous certification (7CFR §301.81). The product currently on the market is formulated on an inert sand carrier. Formulators frequently evaluate new carriers for a variety of reasons, including better mix-ability, easier handling, etc. In 1995, FMC formulated bifenthrin on an organic type carrier at 0.2% AI and requested our evaluation of this formulation.

MATERIALS AND METHODS:

Standard commercial Talstar T&O Granular (0.2G on a sand carrier) and bifenthrin 0.2G on an experimental organic carrier (SPG 95-07) were incorporated into standard nursery potting media at rates of 10, 12. 15 and 25 ppm. The standard MAFES media used by the IFA Laboratory (3:1:1 pine bark:peat moss:sand) was used. Treated media was placed into trade gallon pots and weathered under simulated nursery conditions, including irrigation (ca. 1½ inches/week) plus rainfall. Three pots from each treatment were collected monthly and subjected to a standard alate female bioassay (Appendix II).

RESULTS:

Results show 95-100% efficacy, regardless of carrier or rate of application, through 25 months posttreatment (Table 1).

Table 1. Efficacy of Bifenthrin 0.2% on an Organic Carrier (SPG95-07) vs. the Standard Sand Carrier (Talstar T&O).

Formulation	Rate of Application (ppm)	Mean % mortality to alate females at indicated months PT										
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Talstar T&O Granular	10	100	100	100	100	100	100	100	100	100	100	100
	12	100	100	100	100	100	100	100	100	100	100	100
	15	100	100	100	100	100	100	100	100	100	100	100
	25	100	100	100	100	100	100	100	100	100	100	100
SPG95-07	10	100	100	100	100	100	100	100	100	100	100	100
	12	100	100	100	100	100	100	100	100	100	100	100
	15	100	100	100	100	100	100	100	100	100	100	100
	25	100	100	100	100	100	100	100	100	100	100	100
Untreated Check	---	5	20	5	15	5	5	5	0	10	15	10

Table 1. Cont.

Formulation	Rate of Application (ppm)	Mean % mortality to alate females at indicated months PT												
		(12)	(13)	(14)	(16)	(18)	(20)	(21)	(22)	(23)	(24)	(25)	(26)	(27)
Talstar T&O Granular	10	100	100	100	100	100	100	100	100	100	100	100	100	100
	12	100	100	100	100	100	100	100	100	100	100	100	100	100
	15	100	100	100	100	100	100	100	100	100	100	95	100	100
	25	100	100	100	100	100	100	100	100	100	100	100	100	100
SPG95-07	10	100	100	100	100	100	100	100	100	95	100	100	100	95
	12	100	100	100	100	95	100	100	100	100	100	100	15	100
	15	100	100	100	100	100	100	100	100	100	100	100	85	100
	25	100	100	100	100	100	100	100	100	100	100	100	100	100
Untreated Check	---	5	60	5	10	0	5	5	10	10	5	0	0	0

PROJECT NO: FA01G035

PROJECT TITLE: Influence of Sand Content of Commercial Nursery Potting Media on Residual Activity of Bifenthrin and Tefluthrin, 1995

TYPE REPORT: Final

LEADER/PARTICIPANTS: Homer Collins, Anne-Marie Callcott, Jim Berry (Flowerwood Nursery. Mobile, AL) and Lee McAnally

INTRODUCTION:

Historically, insecticidal treatments for nursery potting media were administered on a weight to volume basis, i.e. 1 lb of Dursban® 2.5G per cubic yard of media. However, this resulted in tremendous variations in the effective dose rates, with "lighter" media receiving far more actual toxicant than "heavier" media. In order to compensate for these differences, Talstar T&O Granular@, and tefluthrin 1.5G are applied at a rates of 10 to 25 ppm. based on the dry weight bulk density of the potting media. Potting media is a tremendously variable entity containing highly organic components such as milled pine bark, hardwood sawdust, rice hulls, sphagnum peat, etc. Undoubtedly, the most common component of all southern nursery media is pine bark. Some media contain sand in varying amounts, while others do not. The ratio of sand to bark may vary from 1 part sand to 2 parts. bark, to 1 part sand to 8 parts bark. Sand is an inert component that contributes little other than weight to the media. Pesticide binding to sand particles is not known to occur. However, the weight of the sand influences the amount of pesticide required because dose rates are bulk density dependent.

Due to the high costs of the insecticides approved for IFA containerized quarantine treatments, nurserymen would prefer to disregard sand and base their application rates on the bulk density of the other components in their media mix. In other words, incorporate 25 ppm of bifenthrin or tefluthrin into the potting media based on the bulk density of all the components of the media except the sand. However, in a trial initiated in 1993 (FA01G053), we found that large quantities of sand, and the bulk density that it adds, appeared to affect the efficacy of both bifenthrin and tefluthrin. In that trial, insecticides were incorporated into media of various pine bark to sand ratios at 3.915 g AI/cu (equivalent to 25 ppm in pure pine bark).

The current study also investigated the impact of sand content of potting media on the residual activity of bifenthrin (Talstar®) and tefluthrin (Fireban®) in commercial nursery potting media blended and aged on site. In this trial, insecticides were incorporated at a rate of 25 ppm based on the bulk density of all media components except the sand.

MATERIALS AND METHODS:

Two media types were used in this trial: Flowerwood Nursery media (Mobile, AL) and MAFES media. Flowerwood Nursery provided the bark component of their 19:3 (pine bark:sand) basic potting mix. The MAFES mix is a 3:1:1 (pine bark:peatmoss:sand) potting media used by the IFA Station. The IFA lab provided masonry sand for the sand component. At the IFA Station, lab personnel blended the candidate toxicants into the potting media at 25 ppm, based on the bulk density of the non-sand components. The ratios of other media components to sand used are indicated in Table 1. The two products currently registered for IFA quarantine use, bifenthrin (Talstar T&O® Granular 0.2%) and tefluthrin (Fireban® 1.5%) were used as test toxicants. After blending, treated media was potted up in trade gallon nursery pots and aged under simulated nursery conditions of irrigation (ca 1-1½ inches per week).

At monthly intervals, three pots from each treatment rate were collected and composited. IFA personnel subjected the treated media to a standard alate female bioassay as described in Appendix II.

RESULTS:

Results are summarized in Table 1. Talstar in the Flowerwood media with no sand (ratio 1:0; 25 ppm) has provided excellent control for 20 mths. Talstar in the MAFES media with no sand (ratio 1:0; 25 ppm) has provided excellent control for 25 mths. Talstar in all other media ratios provided excellent control through 14 mths. At 15 mths through the end of the trial, there has been a decrease in efficacy in MAFES media, and a more definite decrease in efficacy in Flowerwood media.

Fireban in both media types with no sand showed excellent control for 12 mths with variable control through 18 mths. All other media ratios began to show

variable control after 12 mths.

Results of this trial are in general agreement with a prior study (FA01G053) that indicates that sand, i.e. bulk density, definitely affects efficacy of bifenthrin and tefluthrin. Therefore, at this time, it would not be prudent to disregard sand in bulk density determinations and subsequent blending techniques.

Table 1. Effect of Sand Content in Nursery Media on Residual Activity of Bifenthrin and Tefluthrin.

TREATMENT			PERCENT MORTALITY AT INDICATED MONTHS POST-TREATMENT											
INSECT-ICIDE	MEDIA TYPE	RATIO*	1	2	3	4	5	6	7	8	9	10	11	12
TALSTAR	MAFES	1:0	100	100	100	100	100	100	100	100	100	100	100	100
		1:1	100	100	100	100	100	100	100	100	100	100	100	95
		2:1	100	100	100	100	100	100	100	100	100	100	100	100
		3:1	100	100	100	100	100	100	100	100	100	100	100	100
		6:1	100	100	100	100	100	100	100	100	100	100	100	100
		8:1	100	100	100	100	100	100	100	100	100	100	100	100
	FLOWER-WOOD	1:0	100	100	100	100	100	100	100	100	100	100	100	100
		1:1	100	100	100	100	100	100	100	100	100	100	75	90
		2:1	100	100	100	100	100	100	100	100	100	100	100	100
		3:1	100	100	100	100	100	100	100	100	100	100	95	100
		6:1	100	100	100	100	100	100	100	100	100	100	80	80
		8:1	100	100	100	100	100	100	100	100	100	100	100	95
	SAND	0:1	100	100	100	100	100	100	100	90	100	100	100	65
FIREBAN	MAFES	1:0	100	100	100	100	100	100	100	100	100	100	100	100
		1:1	100	100	100	100	100	100	100	100	100	100	100	90
		2:1	100	100	100	100	100	100	100	100	100	100	100	95
		3:1	100	100	100	100	100	100	100	100	100	100	100	85
		6:1	100	100	100	100	100	100	100	100	100	100	100	100
		8:1	100	100	100	100	100	100	100	100	100	100	100	85
	FLOWER-WOOD	1:0	100	100	100	100	100	100	100	100	100	100	100	100
		1:1	100	100	100	100	100	100	100	100	100	100	100	100
		2:1	100	100	100	100	100	100	100	100	100	100	100	85
		3:1	100	100	100	100	100	100	100	100	100	100	100	100
		6:1	100	100	100	100	100	100	100	100	100	100	100	100
		8:1	100	100	100	100	100	100	100	100	100	100	100	100
	SAND	0:1	100	100	100	100	100	100	100	100	100	100	100	100
CHECK	SAND		0	20	0	0	5	5	5	0	0	0	25	10
	FLOWER-WOOD		55	35	5	20	5	15	5	15	0	15	30	0
	MAFES		10	0	5	15	25	10	10	0	0	5	45	0

* non-sand components:sand

Table 1. Cont.

TREATMENT			PERCENT MORTALITY AT INDICATED MONTHS POST-TREATMENT											
INSECT-ICIDE	MEDIA TYPE	RATIO*	13	14	15	16	17	18	19	20	22	23	24	25
TALSTAR	MAFES	1:0	100	100	100	100	100	100	100	100	100	100	100	100
		1:1	100	100	85	100	90	95	100	100	100	15	40	50
		2:1	100	100	90	100	100	100	100	100	70	35	0	25
		3:1	100	100	100	100	100	100	100	100	100	60	100	100
		6:1	100	100	95	100	100	100	100	100	95	65	100	80
		8:1	100	100	95	100	100	100	100	100	100	55	55	50
	FLOWER-WOOD	1:0	100	100	100	100	90	100	100	100	80	90	15	45
		1:1	100	100	85	95	95	100	100	100	90	65	80	50
		2:1	100	100	100	95	85	100	100	100	100	40	20	65
		3:1	100	100	65	95	100	100	100	100	90	55	55	70
		6:1	100	100	100	100	100	100	100	100	100	50	45	65
		8:1	100	100	95	100	100	100	100	100	100	75	55	90
	SAND	0:1	55	95	5	70	85	70	30	10	0	0	25	15
FIREBAN	MAFES	1:0	55	100	100	100	75	70	85	45	100	25	60	65
		1:1	90	90	75	100	55	100	65	15	95	65	40	60
		2:1	95	100	100	75	85	75	70	75	60	55	45	45
		3:1	85	65	40	50	30	100	95	100	65	65	25	35
		6:1	95	50	90	90	75	100	100	100	95	40	5	25
		8:1	95	65	95	95	70	100	100	90	25	35	15	5
	FLOWER-WOOD	1:0	100	50	100	85	75	100	100	90	40	65	20	25
		1:1	100	100	100	95	80	60	100	85	15	30	100	40
		2:1	100	40	100	15	95	100	90	25	40	90	75	25
		3:1	100	85	100	100	40	100	100	75	15	40	25	30
		6:1	100	70	100	95	85	100	100	100	10	70	35	15
		8:1	100	100	95	100	95	100	100	100	35	50	25	20
	SAND	0:1	100	100	100	100	100	100	100	100	100	100	100	100
CHECK	SAND		0	5	0	0	0	20	0	0	10	0	5	30
	FLOWER-WOOD		10	10	5	20	5	30	10	0	20	20	5	40
	MAFES		10	5	0	30	0	0	5	0	50	5	10	15

* non-sand component:sand

PROJECT NO: FA01G086

PROJECT TITLE: Evaluation of Deltamethrin as a Potting Media Toxicant, 1996

TYPE REPORT: Interim

LEADER/PARTICIPANT(s): Lee McAnally

INTRODUCTION:

An ongoing screening program to evaluate insecticides for use as quarantine treatments for nursery potting media has been conducted by the IFA Station since 1974. This trial tested a 0.1% granular formulation of deltamethrin produced by AgrEvo USA.

MATERIALS AND METHODS:

The product was blended into nursery potting soil (MAFES mix, 750 pounds per cubic yard). A portable cement mixer (2 cu ft capacity) was used 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 pots and weathered outdoors under simulated nursery conditions. Treatment rates used were 10, 20, 30, and 40 ppm.

A pulsating overhead irrigation system supplied ca. 1-1½" water per week. At monthly intervals, 3 pots from each treatment were composited and subjected to alate queen bioassay.

RESULTS:

The 10 ppm rate showed some variability through 6 months post-treatment but maintained 80% or better mortality through 11 months (Table 1). All other rates maintained 100% efficacy through 11 months post-treatment (20 & 30 ppm) and 13 months post-treatment (40 ppm).

Table 1. Residual Activity of Deltamethrin Granule.

Rate of Application (ppm)	Mean % mortality to alate females at indicated months PT									
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
10	95	100	95	80	95	85	100	100	100	90
20	100	100	100	100	100	100	100	100	100	100
30	100	100	100	100	100	100	95	100	100	100
40	100	100	100	100	100	100	100	100	100	100
Check	10	15	10	0	5	0	0	0	0	5

Rate of Application (ppm)	Mean % mortality to alate females at indicated months PT								
	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)
10	85	25	40	35	20	--	--		
20	100	85	95	45	80	75	85		
30	100	85	100	60	35	70	95		
40	100	100	100	90	45	50	100		
Check	5	10	15	10	5	0	0		

PROJECT NO: FA01G085

PROJECT TITLE: Residual Activity of suSCon® Green in Various Types of Nursery Potting Media

TYPE REPORT: Final

LEADER/PARTICIPANTS: Lee McAnally

INTRODUCTION:

Numerous prior studies conducted by this laboratory have conclusively shown that the residual activity of chlorpyrifos, regardless of formulation, is media dependent (Callcott et al. 1995). Similar effects have been observed when suSCon Green is incorporated into a limited number of media (McAnally 1995). In that study, suSCon green incorporated into the media at 400 ppm was active against alate IFA queens for 24 months in MAFES potting media versus only 3 months in Grace Sierra media. Theoretically, it may be possible to compile a list of media in which suSCon Green can be expected to provide the long residual activity required of an effective IFA quarantine treatment. Some growers might then opt to use a given media treated with suSCon green in lieu of other treatment options.

MATERIALS AND METHODS:

Potting media from 10 different sources (commercial nurseries that custom blend their own media, bulk media from firms that sell media, and commercially bagged media) were acquired. Exact media composition was not available for all mixes, especially the commercially pre-bagged mixes (Table 1). suSCon Green was incorporated into each media at a rate of 400 ppm based on the dry weight bulk density of the media. Certain characteristics of each media including pH, % organic matter, bulk density, ion exchange capacity, etc. was determined by the Mississippi State University Soil Testing Laboratory (Mississippi State, MS). After treatment, media was placed in one-gallon plastic nursery containers and weathered outdoors under simulated nursery conditions (black groundcloth culture, overhead pulsating irrigation system). At monthly intervals, three pots from each treatment were destructively sampled and subjected to a standard alate queen bioassay (Appendix II).

RESULTS:

Results show 4 months of residual activity in Wight potting media (Table 2). The Mobile and MAFES media have produced 95-100% efficacy through 14 and 24 months respectively. All other media gave at least one month of activity.

Soil analyses indicate that high hydrogen ion content in the soil, and thus low pH, is very important in the prolonged activity of chlorpyrifos (Table 3). Past trials investigated the role of the pH of the irrigation water on chlorpyrifos degradation (1990 IFA Annual Report - FA01G079), but not the pH of the media itself. The technical data sheet for Dursban® insecticides (Form No. 134-289-R585, Dow Chemical U.S.A., Agric. Prod. Dept., Midland, MI) shows a negative relationship between pH of water and half-life of chlorpyrifos in water (pH 7, half-life 35 days; pH 5, half-life 63 days). However, it does not address the effect of soil pH on the half-life of the insecticide in soil. In this trial, when soil pH exceeded 7.0, chlorpyrifos was no longer effective against IFA. Apparently soil pH should be below 6 for chlorpyrifos to be effective as a long term residual treatment. However, if low soil pH is necessary for IFA control with chlorpyrifos in containerized nursery stock, this will not be an acceptable option for nursery growers since most amend their media to a pH of ca. 7 for plant health.

Table 1. Composition and Producer of Various Media Mixes.

Media Name	Abbreviation for Future Tables	Composition	Producer/ Address
Wight Nursery	Wight	4:1 bark:sand	Wight Nursery P.O. Box 390 Cairo, GA 31728
Flowerwood Nursery	Flowerwood	ca. 19:3 bark:sand	Flowerwood Nursery P.O. Box 7 Loxley, AL 36551
Reliable Peat Montage Blend	Montage Blend	50% Florida Peat 20% Hardwood Bark 20% Cypress Chips 10% Hardwood Dust 10% sand	Reliable Peat Co. Hwy. 50 W Wintergreen, FL
Reliable Peat Florida Peat	Florida Peat	100% Reede Sedge Florida Peat	Reliable Peat Co. Hwy. 50 W Wintergreen, FL
Reliable Peat Potting Soil	Reliable Potting	50% Florida Peat 20% Cypress Chips 20% Hardwood Dust 10% Hardwood Bark 10% sand	Reliable Peat Co. Hwy. 50 W Wintergreen, FL
Fafard Mix No. 2-S	Fafard	contains sphagnum peat moss, hort. vermiculite and polybeads	Conrad Fafard Inc. P.O. Box 790 Agawam, MA 01001
Mobile Media	Mobile	3:1 pinebark:sand	Alabama Agric. Exper. Station Mobile, AL
Grace Metro Mix 360	Metro Mix		Grace Sierra Hort. Products Co. 1001 Yosemite Dr. Milpitas, CA 95035
Premier Pro-Mix BX	Pro-Mix		manufactured for: Premier Brands Inc Red Hill, PA 18076
MAFES Media	MAFES	3:1:1 bark:peat: sand	USDA, APHIS, PPQ IFA Station Gulfport, MS 39501

Table 2. Residual Activity of suSCon Green 10CR in Various Potting Media.

TREATED SOIL

Media Type	% Mortality to Alate Females at Indicated Mths PT*																			
	1	2	3	4	5	6	8	10	12	14	15	16	17	18	19	20	21	22	23	24
MAFES	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	95
Mobile	100	100	100	100	100	100	100	100	100	100	80	60	80	85	80	40	25	55	30	25
Wight	100	100	100	100	65	35	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Flowerwood	100	100	55	50	45	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Reliable Potting	100	45	25	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Montage Blend	100	25	40	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Florida Peat	100	55	50	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Fafard	100	15	25	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
ProMix	100	95	75	0	5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
MetroMix	100	25	100	0	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

* Some Data columns omitted due to space limitations. Data for these months does not effect final outcome.

Table 2. Cont.

CHECK SOIL

Media Type	% Mortality to Alate Females at Indicated Mths PT*																			
	1	2	3	4	5	6	8	10	12	14	15	16	17	18	19	20	21	22	23	24
MAFES	25	10	10	0	5	10	0	5	5	10	0	5	15	0	10	0	5	5	15	5
Mobile	30	0	20	5	10	10	20	15	0	15	40	10	20	5	15	25	10	10	10	20
Wight	90	45	0	50	0	0	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Flowerwood	35	0	10	5	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Reliable Potting	0	15	35	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Montage Blend	20	15	20	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Florida Peat	0	10	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Fafard	0	5	5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
ProMix	0	10	5	0	0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
MetroMix	5	10	5	5	0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

* Some Data columns omitted due to space limitations. Data for these months does not effect final outcome.

Table 3. Presence of Hydrogen in the Media As Indicated by Various Soil Analyses.

Media Type	Presence of Hydrogen at Indicated Months Post treatment								
	pH			CEC (meq/100 g)			% Base Saturation		
	0	3	6	0	3	6	0	3	6
MAFES	4.0	5.3	5.4	10.4	7.0	5.2	79.0	65.6	82.93
Mobile	4.3	5.0	5.8	5.9	4.8	2.9	56.9	58.2	45.96
Wight	6.0	6.9	7.8	1.5	1.6	0.0	19.0	14.7	0.0
Flowerwood	5.8	6.7	7.5	2.4	2.1	0.0	14.9	14.9	0.0
Reliable Potting	5.8	7.7	7.8	1.2	0.0	0.0	6.2	0.0	0.0
Montage Blend	6.5	7.7	8.1	0.3	0.0	0.0	1.5	0.0	0.0
Florida Peat	6.8	8.1	8.0	0.4	0.0	0.0	2.9	0.0	0.0
Fafard	5.7	8.1	8.2	0.6	0.0	0.0	7.2	0.0	0.0
ProMix	6.1	7.3	8.3	1.5	0.0	0.0	11.7	0.0	0.0
MetroMix	6.6	7.3	8.2	0.0	0.0	0.0	6.5	0.0	0.0

PROJECT NO: FA01G016

PROJECT TITLE: Preliminary Testing of Organic Solutions™ Multipurpose Fireant Killer for Use as a Containerized Nursery Stock Treatment

TYPE REPORT: Final

LEADER/PARTICIPANTS: Lee McAnally and Kirk Irby

INTRODUCTION:

Organic Solutions™ (San Antonio, Texas) produces a dust formulation that contains pyrethrins (0.1%), piperonyl butoxide (1.0%), and silicon dioxide (83.3%) as the active ingredients. At this time the product is sold for homeowner control of IFA as a dust or drench treatment. The label directs the user to "dust the perimeter of the mound first. With a stick, disturb the mound, then dust the mound in a circular motion working toward the center until the entire mound is thoroughly dusted." The drench treatment is applied using 6 oz. of product per gal. of water (1 gal. of drench per 12-14" dia. mound). The company was interested in the possible use of this product as a containerized stock treatment in the Federal IFA quarantine program.

MATERIALS AND METHODS:

Prep7ant incorporation treatment:
Organic Solutions Fireant Killer™ was incorporated into the standard laboratory MAFES potting media at rates of 1, 2, 6, 10, 100 and 200 ppm based on the 0.1% pyrethrins as the active ingredient. Pots filled with treated media were weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½" water per week. On a monthly basis, three pots from each treatment group were composited, brought to the laboratory and subjected to standard IFA alate female bioassay.

Containerized drench treatment:
The product was used to drench 1 gal. nursery pots containing the standard laboratory MAFES potting media at a rate of 17.0 g material per pot (equivalent to 0.6 oz per "mound" as stated on label). The drench solution was applied at a rate of 400 mls of finished solution per container. Pots

were weathered as above. Bioassays were performed at 1, 2, 4, and 8 weeks after treatment.

Elimination of established colonies:
One gal. nursery pots were filled with MAFES media and placed on a brick in a 12" x 18" x 5" plastic pan. The sides of the pan were talced and ca. 1" of water was added to prevent escape. A hole 1½" from the bottom of the pan allowed excess water to drain. Five replicates were utilized. Field collected colonies were separated from their nest tumulus by the floatation method (Banks et al. 1981) and 50 cc of workers and brood were added to each media-filled pot. The fragmented colonies were allowed to acclimate 3-5 days before treatment. Organic Solutions Fireant Killer at a rate of 17.0 g per pot was used to drench each container (400 ml finished solution per container). Containers were watered twice a week, or as needed for the duration of the trial. Pots were inspected daily for mortality and were considered dead when less than 20 workers were present.

Repellency determination:
At monthly intervals (beginning at four months post-treatment), 1 pot each from the 6, 10, 100, & 200 ppm incorporated treatments was placed in 2' X 8' test arenas along with untreated check pots so that each arena contained one treated and one untreated pot at opposite ends. A freshly collected field colony with nest tumulus was evenly spread over the bottom of the test arena. Sides of the arenas were talced to prevent escape. Colonies were allowed 7 days to move into either the treated or check pots. Estimations of the percentage of the colony that moved into each pot were made on a daily basis.

RESULTS:

Preplant incorporation treatment:
The 200 ppm rate produced 100% mortality through three months post-treatment and began to decline thereafter (Table 1). The 100 ppm rate provided 100% mortality through two months. All other rates failed to provide more than 65% mortality at any time and were dropped from the test after two months.

Containerized drench treatment:
Drench treatment provided 65% mortality at one week post-treatment, 30%

mortality at 2 and 4 weeks post-treatment and 5% at 8 weeks post-treatment.

Elimination of established colonies:
Drench of existing colonies eliminated 75-80% at 24 hours post-treatment and 100% within 5 days post-treatment.

Repellency determination:
Results shown in Table 2 indicate estimated percentage of ants which moved into each pot at 7 days post-exposure. Rates of 6 and 10 ppm had no apparent effect on colony movement. Rates of 100 and 200 ppm effectively prevented movement into treated pots at 4 and 5 months post-treatment and 200 ppm prevented movement at 6 months. However, by month 7 approximately 50% of the test colony had invaded the 200 ppm pot. By months 10 and 11, most of the ants from the test colonies had invaded the treated pots.

Table 1. Residual Activity of Organic Solutions Incorporated into Potting Media.

Rate of Application	Percent mortality at indicated months post-treatment									
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1 ppm	0	5	---	---	---	---	---	---	---	---
2 ppm	5	0	---	---	---	---	---	---	---	---
6 ppm	15	10	---	---	---	---	---	---	---	---
10 ppm	65	15	---	---	---	---	---	---	---	---
100 ppm	100	100	80	25	35	10	0	15	10	0
200 ppm	100	100	100	95	85	75	80	10	25	10
CHECK	0	0	5	10	15	15	10	5	0	0

Table 2. Efficacy of Organic Solutions as a Repellant when Incorporated into Potting Media.

Treatment Rate	Estimated percentage of Ants in Pots @ Indicated Post-Treatment Interval (months)															
	(4)		(5)		(6)		(7)		(8)		(9)		(10)		(11)	
	TR	CK	TR	CK	TR	CK	TR	CK	TR	CK	TR	CK	TR	CK	TR	CK
6 ppm	50	50	0	100	100	0	5	95	100	0	---	---	---	---		
10 ppm	0	100	100	0	100	0	10	90	100	0	---	---	---	---		
100 ppm	0	100	0	100	90	10	100	0	100	0	10	90	75	25	90	10
200 ppm	0	100	0	100	0	100	50	50	75	25	0	100	25	75	80	20

PROJECT NO: FA01G056

PROJECT TITLE: Preliminary Testing of Organic Plus Multipurpose Fireant Killer for Use as a Containerized Nursery Stock Treatment

TYPE REPORT: Final

LEADER/PARTICIPANTS: Lee McAnally

INTRODUCTION:

Organic Plus Inc. (Albuquerque, NM) produces a dust formulation that contains pyrethrins (0.2%), piperonyl butoxide (1.1%), and silicon dioxide (97.9%) as the active ingredients. This product is labelled for homeowner control of IFA as a drench treatment. The label directs the user to "drench both the mound and the area within six inches of the mound" using 1 pound of product per 25 gal. of water or 4 tablespoons per gal. of water (1 gal. of drench per mound). The company is interested in the possible use of this product as a containerized stock treatment in the Federal IFA Quarantine Program.

MATERIALS AND METHODS:

Prep7ant incorporation treatment:
Organic Plus" Fireant Killer was incorporated into the standard laboratory MAFES potting media at rates of 1, 2, 6, 10, 100 and 200 ppm. Pots filled with treated media were weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca 1-1½" water per week. On a monthly basis, three pots from each treatment group were composited, brought to the laboratory and subjected to standard IFA alate female bioassay.

Containerized drench treatment:
The product was used to drench 1 gal. nursery pots containing the standard laboratory MAFES potting media at a rate of 1.97 grams per container (approximately 3 ppm). The drench solution was applied at a rate of 400 mls of finished solution per container. Pots were weathered as above. Bioassays were performed at 1, 2, 4, and 8 weeks after treatment.

Elimination of established colonies:
One gal. nursery pots were filled with MAFES media and placed on a brick in a

12" x 18" x 5" plastic pan. The sides of the pan were talced and ca. 1" of water was added to prevent escape. A hole 1½" from the bottom of the pan allowed excess water to drain. Five replicates were set up. Field collected colonies were separated from their nest tumulus by the floatation method (Banks et al. 1981) and 50 cc of workers and brood were added to each media-filled pot. The fragmented colonies were allowed to acclimate 3-5 days before treatment. Organic Plus Fireant Killer at a rate of 1 pound per 25 gallons of water (18.2 grams per gallon) was used to drench each container (400 ml finished solution per container). Containers were watered twice a week, or as needed for the duration of the trial. Pots were inspected daily for mortality and were considered dead when less than 20 workers were present.

A second trial was initiated in March 1997, duplicating the above procedures. The above treatment rate was used as well as a 2X and 3X rate.

Repellency determination:
At monthly intervals (beginning at four months post-treatment), 1 pot each from the 6, 10, 100, & 200 ppm incorporated treatments was placed in 2' X 8' test arenas along with untreated check pots so that each arena contained one treated and one untreated pot at opposite ends. A freshly collected field colony with nest tumulus was evenly spread over the bottom of the test area. Sides of the arenas were talced to prevent escape. Colonies were allowed 7 days to move into either the treated or check pots. Estimations of the percentage of the colony that moved into each pot were made on a daily basis.

RESULTS:

Preplant incorporation treatment:
The 200 ppm rate produced 100% mortality through three months post-treatment and began to decline thereafter (Table 1). The 100 ppm rate provided 100% mortality through two months. All other rates failed to provide more than 45% mortality at any time and were dropped from the test after two months.

Containerized drench treatment:
The drench treatment provided 0% mortality at one week post-treatment, 5% mortality at 2 weeks post-treatment, 0% mortality at 4 weeks post-treatment, and 5% mortality at 8 weeks post-treatment.

Elimination of established colonies (1st trial):
The drenching of existing colonies provided 75% mortality at 24 hours post-treatment and 90% at 48 hours through 1 week post-treatment.

(2nd trial):
1X rate- 50-75% mortality at 24 hours post-treatment, 75-100% at one week, and 100% at 11 days.
2X rate- 90-100% mortality at 24 hours post-treatment, and 100% at 3 days.
3X rate- 90-100% mortality at 24 hours post-treatment, and 100% at 4 days.

Repellency determination:
Results shown in Table 2 indicate estimated percentage of ants that moved into each pot at 7 days post-exposure. Rates of 100 and 200 ppm effectively prevented movement into treated pots at 4 and 5 months post-treatment, however, by month 6 approximately 5% of the test colonies had invaded the bottom portion of the pots. By 7-9 months 25-50% of the colonies were moving into the treated pots. By 10-11 months 50-95% were moving into the treated pots, and at 12 months, 100% of the colonies had moved into the treated pots.

Table 1. Residual Activity of Organic Plus Incorporated into Potting Media.

Rate of Application	Percent mortality at indicated months post-treatment								
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1 ppm	5	5	---	---	---	---	---	---	---
2 ppm	5	5	---	---	---	---	---	---	---
6 ppm	25	15	---	---	---	---	---	---	---
10 ppm	45	20	---	---	---	---	---	---	---
100 ppm	100	100	45	30	25	65	20	30	20
200 ppm	100	100	100	80	85	70	55	45	55
CHECK	5	10	5	10	10	15	0	5	10

Table 2. Efficacy of Organic Plus as a Repellant when Incorporated into Potting Media.

Treatment Rate	Estimated percentage of Ants in Pots @ Indicated Post-Treatment Interval (months)																	
	(4)		(5)		(6)		(7)		(8)		(9)		(10)		(11)		(12)	
	TR	CK	TR	CK	TR	CK	TR	CK	TR	CK	TR	CK	TR	CK	TR	CK	TR	CK
6 ppm	50	50	0	100	95	5	100	0	100	0	---	---	---	---	---	---	---	---
10 ppm	100	0	0	100	0	100	100	0	100	0	---	---	---	---	---	---	---	---
100 ppm	0	100	0	100	5	95	50	50	25	75	50	50	95	5	95	5	100	0
200 ppm	0	100	0	100	5	95	25	75	25	75	25	75	50	50	90	10	100	0

PROJECT NO: FA01G057

PROJECT TITLE: Expanded Trials with Organic Plus Multipurpose Fireant Killer for Use as a Quarantine Treatment for Containerized Nursery Stock

TYPE REPORT: Final

LEADER/PARTICIPANTS: Lee McAnally

INTRODUCTION:

Organic Plus Inc. (2525 N. Austin St., Seguin, TX 78155) produces a dust formulation that contains pyrethrins (0.2%), piperonyl butoxide (1.1%) and silicon dioxide (97.9%) as the active ingredients. This product is labelled for homeowner control of imported fire ants as a drench treatment. The label directs the user to "drench both the mound and the area within six inches of the mound" using 1 pound of product per 25 gal. of water or 4 tablespoons per gal. of water (1 gal. of drench per mound). Organic Plus Inc. is interested in the possible use of this product in the Federal IFA Quarantine Program, and requested that we evaluate their product. In a preliminary trial (FA01G056), we tested Organic Plus™ in several different use patterns including a drench treatment for containerized nursery stock. Our results indicated that Organic Plus™ might be suitable as a "treat and ship" procedure for certification of containerized nursery stock. In the expanded trial reported here we tested Organic Plus™ at three rates of application in 4 different types of potting media.

MATERIALS AND METHODS:

One gal. nursery pots were filled with media and placed on a masonry brick in a 12" x 18" x 5" plastic pan. The sides of the pan were talced and ca. 1" of water was added to prevent escape. A hole 1½" from the bottom of the pan allowed excess water to drain. Five replicates were set up. Field collected colonies were separated from their nest tumulus by the floatation method (Banks et al. 1981) and 50 cc of workers and brood were added to each media-filled pot. The fragmented colonies were allowed to acclimate 3-5 days before treatment. Organic Plus™ Fireant Killer at a rate of 1 pound per 25 gallons of water (1X rate) was used to drench each container (400 ml finished solution

per container). Rates of 2 pounds per 25 gal. (2X), and 3 pounds per 25 gal. (3X) of water were also used. Containers were watered twice a week, or as needed for the duration of the 14 day trial. Ants were inspected daily for mortality and colonies were considered dead when less than 20 workers were present.

Four different types of media were tested: Mississippi Agriculture & Forestry Experiment Station (MAFES) Media; Flowerwood Nursery (Mobile AL); Windmill Nursery (Folsom, LA) and Grassroots Nursery (Leesburg, FL). Media compositions are shown below:

Media Type	Components	Bulk Density
MAFES	3:1:1 Pine Bark, Peat Moss, Sand	650 Lbs./Cu. Yd.
Flowerwood	19:3 Pine Bark, Sand	550 Lbs./Cu. Yd.
Windmill	Pine Bark	230 Lbs./Cu. Yd.
Grassroots	60% Pine Bark, 40% Florida Peat	N/A

This study was initiated on February 27, 1997 and terminated on September 22, 1997.

RESULTS and DISCUSSION:

Results of this trial are shown in Tables 1-4, and are summarized in Table 5. The 1X rate of application was less than 100% effective in both Windmill and Grassroots media. However 100% mortality was achieved in the MAFES and Flowerwood media after an exposure interval of 11-14 days. The 2X rate was about equal to the 1X rate. The 3X rate did achieve 100% mortality in all 4 types of media, but required exposure intervals of 3-11 days to reach this level of control.

Table 1. Results with Organic Plus applied at 3 rates to MAFES potting Media

TREATMENT RATE	REP No.	Percent Mortality at Indicated Days Post-treatment									
		1	2	3	4	7	8	9	10	11	14
1#/25 gal H_2O	1	75	90	90	90	100	--	--	--	--	--
	2	75	75	75	90	90	100	--	--	--	--
	3	50	50	50	75	75	90	90	90	100	
	4	75	90	90	90	90	90	100	--	--	--
	5	75	90	90	90	90	100	--	--	--	--
2#/25 gal H_2O	1	90	100	--	--	--	--	--	--	--	--
	2	100	--	--	--	--	--	--	--	--	--
	3	90	100	--	--	--	--	--	--	--	--
	4	90	90	100	--	--	--	--	--	--	--
	5	90	100	--	--	--	--	--	--	--	--
3#/25 gal H_2O	1	90	90	90	100	--	--	--	--	--	--
	2	100	--	--	--	--	--	--	--	--	--
	3	90	100	--	--	--	--	--	--	--	--
	4	90	100	--	--	--	--	--	--	--	--
	5	90	100	--	--	--	--	--	--	--	--
Untreated CK	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0

Table 2. Results with Organic Plus applied at 3 rates to Windmill Media

TREATMENT RATE	REP No.	Percent Mortality at Indicated Days Post-treatment									
		1	2	3	4	7	8	9	10	11	14
1#/25 gal H_2O	1	10	25	25	25	50	50	50	50	50	50
	2	10	10	25	50	75	75	75	75	75	75
	3	25	25	50	75	100	--	--	--	--	--
	4	10	10	25	25	25	50	50	50	75	75
	5	10	25	25	50	75	75	75	75	75	75
2#/25 gal H_2O	1	50	50	50	50	75	75	75	75	75	100
	2	50	50	50	50	50	50	50	50	50	50
	3	50	75	75	75	75	90	100	--	--	--
	4	75	75	75	75	75	75	75	75	75	75
	5	50	50	50	75	75	75	75	75	75	75
3#/25 gal H_2O	1	50	75	75	75	75	75	75	75	75	75
	2	75	75	75	75	75	75	75	75	75	75
	3	75	90	90	90	90	90	90	90	100	--
	4	50	75	90	90	90	90	90	90	90	100
	5	50	50	75	75	90	90	90	90	90	90
Untreated CK	1	0	0	0	0	10	25	25	25	25	25
	2	0	0	0	10	10	10	10	10	10	10
	3	0	0	0	10	10	25	25	25	25	25
	4	0	0	0	0	10	10	10	10	10	10
	5	0	0	0	0	10	10	10	10	10	10

Table 3. Results with Organic Plus applied at 3 rates to Flowerwood Media

TREATMENT RATE	REP No.	Percent Mortality at Indicated Days Post-treatment									
		1	2	3	4	7	8	9	10	11	14
1#/25 gal H_2O	1	25	50	75	90	90	90	90	100	--	--
	2	0	25	75	90	90	90	90	100	--	--
	3	0	50	50	75	90	90	90	90	90	100
	4	0	50	50	90	90	90	90	90	90	100
	5	0	25	50	75	90	90	90	90	90	100
2#/25 gal H_2O	1	10	50	90	90	100	--	--	--	--	--
	2	25	50	75	90	90	90	90	90	90	100
	3	25	75	90	90	90	90	90	90	90	100
	4	50	90	90	90	100	--	--	--	--	--
	5	25	75	100	--	--	--	--	--	--	--
3#/25 gal H_2O	1	50	75	100	--	--	--	--	--	--	--
	2	50	90	100	--	--	--	--	--	--	--
	3	50	75	100	--	--	--	--	--	--	--
	4	75	90	100	--	--	--	--	--	--	--
	5	50	90	100	--	--	--	--	--	--	--
Untreated CK	1	0	0	0	0	10	10	10	10	10	10
	2	0	0	0	0	10	10	10	10	10	10
	3	0	0	0	0	0	10	10	10	10	10
	4	0	0	0	0	10	10	10	10	10	10
	5	0	0	10	10	20	20	20	20	20	20

Table 4. Results with Organic Plus applied at 3 rates to Grassroots Media

TREATMENT RATE	REP No.	Percent Mortality at Indicated Days Post-treatment									
		1	2	3	4	7	8	9	10	11	14
1#/25 gal H_2O	1	10	25	25	50	75	75	90	100	--	--
	2	10	25	50	50	75	75	75	90	100	--
	3	10	25	50	50	50	50	50	75	75	75
	4	10	50	50	50	50	50	50	50	75	75
	5	10	25	50	50	50	50	50	75	75	75
2#/25 gal H_2O	1	10	25	50	75	75	75	75	90	90	90
	2	25	50	50	90	90	90	90	90	90	90
	3	25	50	75	75	75	75	75	90	90	90
	4	10	25	50	50	75	75	75	75	75	90
	5	25	50	75	90	90	90	90	90	90	90
3#/25 gal H_2O	1	75	90	90	90	100	--	--	--	--	--
	2	75	90	100	--	--	--	--	--	--	--
	3	50	90	90	100	--	--	--	--	--	--
	4	75	90	100	--	--	--	--	--	--	--
	5	90	90	100	--	--	--	--	--	--	--
Untreated CK	1	0	0	0	0	10	10	10	10	10	10
	2	0	0	0	0	10	10	10	10	10	10
	3	0	0	0	0	10	10	10	25	25	25
	4	0	0	0	0	10	10	25	25	25	25
	5	0	0	0	0	0	0	0	10	10	10

Table 5. Summary of results with Organic Plus applied to 4 different nursery medias at 3 rates of application.

TREATMENT RATE	MEDIA USED	Average Percent Mortality at Indicated Days Post-treatment									
		1	2	3	4	7	8	9	10	11	14
1#/25gal H_2O	MAFES	70	79	79	87	89	96	98	98	100	--
	Windmill	13	19	30	45	65	70	70	70	75	75
	Flowerwood	7	40	60	84	90	90	90	94	94	100
	Grassroots	10	30	45	50	60	60	63	83	86	85
2#/25gal H_2O	MAFES	92	98	100	--	--	--	--	--	--	--
	Windmill	55	60	60	65	70	73	75	75	75	80
	Flowerwood	27	68	89	92	96	96	96	96	96	100
	Grassroots	19	40	60	76	81	81	81	87	87	90
3#/25gal H_2O	MAFES	92	98	98	100	--	--	--	--	--	--
	Windmill	60	78	81	81	84	84	84	84	86	88
	Flowerwood	55	84	100	--	--	--	--	--	--	--
	Grassroots	73	90	96	98	100	--	--	--	--	--
UNTREATED CHECK	MAFES	0	0	0	0	0	0	0	0	0	0
	Windmill	0	0	0	4	10	16	16	16	16	16
	Flowerwood	0	0	2	2	10	12	12	12	12	12
	Grassroots	0	0	0	0	8	8	11	16	16	16

PROJECT NO: FA01G097

PROJECT TITLE: Efficacy of Chlorfenapyr as an Imported Fire Ant Quarantine Treatment

PROJECT TYPE: Interim

PROJECT LEADER/PARTICIPANT(s): Anne-Marie Callcott, Lee McAnally, Charlene Russell

INTRODUCTION:

The Federal Imported Fire Ant Quarantine Program (7CFR §301.81) states that all regulated products (nursery stock) leaving the quarantined area (all or part of 11 southern states and Puerto Rico; 308,000,000 acres) 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 (bifenthrin or tefluthrin) 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.

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. 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.

MATERIALS AND METHODS:

Drench Trials

A 2SC liquid formulation of chlorfenapyr was tested as a drench application to

containerized nursery pots. Trade gallon nursery pots were filled with standard potting media (3:1:1 sphagnum peatmoss: pine bark: sand - bulk density = 650 lb/cu yd). The filled pots were left for 3-5 days under simulated nursery conditions (ca. 1-1½" irrigation per week) to allow the media to become fully saturated before treating. Individual pots were then drenched at rates of 25, 50, 100 and 200 ppm. Each pot (ca. 900 g media) was drenched with a volume of solution equal to 1/5 the volume of the pot (i.e. 400 ml solution). Two types of bioassays were performed at 72 hours after treatment, and then monthly thereafter.

Whole colony bioassay - The first bioassay evaluated chlorfenapyr in its ability to prevent infestation or eliminate whole colonies in nursery pots using field collected RIFA colonies. One container from each treatment series was placed in the center of a 2' x 8' test arena. Sides of the test arena were talced to prevent ants from climbing out and escaping. A field collected colony complete with associated soil and nest tumulus was then placed in the arena. Overhead incandescent bulbs slowly desiccated the nest so that the ants were encouraged to migrate to the more moist container. Pots were observed at 24 hour intervals for at least 21 days after introduction, and rate of infestation and percent mortality recorded.

Standard alate female bioassay - The second bioassay performed on the drench treated media was a standard alate female bioassay. Test chambers are 2.5" x 2.5" plastic flower pots which have been equipped with a labstone bottom (Patterson Dental Co., Metairie, LA). 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. 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. 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. All evaluations were based on at least 14 days continuous exposure period. i.e., introduced queens remain in the test chambers for at least 14 days. At this time the contents of each chamber were expelled into a shallow laboratory pan and closely searched for the presence of live IFA alate queens. Data was recorded as percent mortality.

Incorporated treatment - The granular 0.5% formulation was blended into our standard potting mix (MAFES mix, 650 pounds per cubic yard). A portable cement mixer (2 cu ft capacity) was used 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 pots and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½" water per week. At monthly intervals, 3 pots from each treatment were composited and subjected to alate queen bioassay described above for a 7 day exposure period.

RESULTS:

Drench trials

Whole colony bioassay - RIFA did move into pots containing potting media treated with chlorfenapyr (drench), but the ants did not behave normally; they tended to move into the edges of the pot (between the media and the pot) and under the pot, or in the bottom 1" layer of media, and did not actively "work up" the media. The 200 ppm rate provided 100% colony mortality within 7-14 days of moving into the treated media through the 3 month assessment, and within 14-21 days through the 5 month assessment (Tables 1 & 2). The 100 ppm rate provided 100% mortality within 14-21 days through the 3 month assessment. The 50 ppm rate provided 100% mortality through 2 months, but required a longer period of time to cause mortality (up to 30 days). The 25 ppm rate was very slow to kill whole colonies (always >21 days). At 6 months after treatment, all rates required >21 days to achieve 100% mortality. At 7-12 months after treatment the 25 ppm rate still required >21 days to kill the whole colony, while the other rates became somewhat variable in the length of time required to kill the whole colony.

Standard alate female bioassay - We have achieved similar results with this bioassay. No bioassay was performed at 3 months after treatment, and the 4 month test was inadvertently destroyed after 7 days. Through 7 months after treatment, the 200 ppm rate provided 100% alate female mortality within 7-12 days, while the 50 and 100 ppm rates required up to 14 days to achieve 100% mortality (Table 3). The 25 ppm rate was not consistent. The alate female bioassays were terminated at 7 months since drench treatments do not need to be long term.

Incorporated treatment - Rates of 25 and 50 ppm have not provided adequate control of IFA under the 7-day exposure testing standard (Table 4). The 75 and 100 ppm rates have provided >90% efficacy through 4 months, and the 200 ppm rate has been 100% effective through 4 months.

CONCLUSIONS:

Drench Trials

The high rates of 100 and 200 ppm, showed fairly fast and consistent results through 3 months, which is acceptable for quarantine drench treatments. Drench treatments have a distinct disadvantage over granular incorporated treatments in that thorough saturation is not always achieved. This was particularly evident in the whole colony bioassays were colonies survived in the bottom layer of media in the pot, indicating that the active ingredient remained in the top layers of the media, not penetrating the entire pot. This is a definite problem with products whose active ingredient is fairly immobile in potting media. A granular formulation of chlorfenapyr may alleviate this problem since granular products are blended into the media prior to potting up, ensuring a fairly even distribution of active ingredient.

Incorporated trials

Incorporation at rates of 75-200 ppm look promising thus far. This trial will continue for another 20 months and will give a better indication of effectiveness as time goes on. These rates are much higher than those of insecticides currently approved for use in the IFA quarantine. However, actual cost of material, not the dose rate required, will be the deciding factor for the end-user (nurserymen).

Table 1. Efficacy of chlorfenapyr as a drench against whole RIFA colonies.

Rate	% population mortality at indicated time after treatment and days after colony introduction																			
	3 days						1 mth					3 mth					6 mth			
	1d	3	7	14	21	30	1	3	7	14	21	1	3	7	14	21	1	3	7	14	21
25	0	0	10	10	50	100	0	0	75	75	95	0	0	0	50	75	0	0	0	5	5
50	0	0	20	95	99	100	0	0	75	95	100	0	25	50	75	95	0	0	0	25	25
100	0	50	75	99	100	--	0	10	99	100	--	0	25	75	100	--	0	0	5	75	90
200	25	95	100	--	--	--	0	20	99	100	--	0	50	75	100	--	0	0	50	95	95

Table 1. Cont.

Rate	% population mortality at indicated time after treatment and days after colony introduction									
	9 mth					12 mth				
	1d	3	7	14	21	1	3	7	14	21
25	0	0	10	95	95	--	0	52	75	90
50	0	10	50	99	100	--	25	90	100	--
100	0	50	75	99	100	--	50	99	100	--
200	0	50	90	99	100	--	100	--	--	--

Table 2. Efficacy of chlorfenapyr as a drench against whole RIFA colonies - summary.

Rate of Application	Days required to obtain 100% mortality at indicated months PT												
	(3 days)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
25 ppm	30	+28	22	+21	+21	+21	+21	+21	+21	+21	+21	21	+21
50 ppm	30	22	14	+21	+21	+21	+21	14	21	21	14	14	14
100 ppm	21	14	14	14	+21	14	+21	14	21	21	14	7	14
200 ppm	7	14	7	14	21	14	+21	+21	10	21	14	7	7

Table 3. Efficacy of chlorfenapyr as a drench against RIFA alate females.

Rate of Application	Days required to obtain 100% mortality at indicated months PT							
	(3 days)	(1)	(2)	(3)	(4)	(5)	(6)	(7)
25 ppm	9	+21	14	--	+7	14	10	12
50 ppm	7	7	7	--	+7	7	14	7
100 ppm	7	7	14	--	7	7	7	7
200 ppm	7	12	7	--	7	7	7	7

Table 4. Efficacy of chlorfenapyr as an incorporated treatment against RIFA alate females.

Rate of Application	Percent mortality at indicated months post-treatment						
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
25 PPM	20	25	40	15			
50 PPM	85	40	50	100			
75 PPM	95	95	100	100			
100 PPM	100	90	100	100			
200 PPM	100	100	100	100			
CHECK	5	20	5	10			

PROJECT NO: FA01G026

PROJECT TITLE: Residual Activity of Silafluofen Drench in Nursery Potting Media

TYPE REPORT: Final

LEADER/PARTICIPANTS: Lee McAnally

INTRODUCTION:

Drench trials are a part of the ongoing search for new and/or better toxicants for use in the IFA quarantine program. While granular incorporation treatments are commonly used by large nurseries which routinely ship outside the quarantine area, drench treatments are used by smaller nurseries which ship limited quantities of plant material outside the quarantine area. We routinely test new compounds and products not currently registered for IFA use. Silafluofen is a relatively new product by AgrEvo Environmental Health (Montvale, NJ). We tested silafluofen earlier as a drench and obtained 100% efficacy for 6 mths at rates of 1.300, 6,500 and 13,000 ppm (FA01G074). A later test at rates of 5, 10, 15 & 25 ppm produced 30% efficacy or less (FA01G115). This trial was set up to test rates between the first and second trials.

MATERIALS AND METHODS:

Trade gallon nursery pots were filled with MAFES mix and set in a simulated can yard to allow the media to become fully saturated before the drench was added. Drench solutions were applied to each container at a rate of 400 ml drench solution per container. All containers were then aged under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½" water per week. At monthly intervals, three pots from each treatment were cornposited and subjected to standard IFA alate female bioassay (Appendix II).

The formulation evaluated in this trial was an 80% EC formulation (800 g AI/L). Rates of 50, 100, 200, 300 and 500 ppm were tested.

RESULTS:

As indicated in Table 1, the 50 ppm rate was effective through 3 months. The 100 ppm rate obtained 100% efficacy through 4 months and at 6 & 7 months posttreatment. Rates of 200 ppm and above were still at 100% efficacy through 12 months, at which the trial was terminated as originally scheduled.

Table 1. Efficacy of Silafluofen as a Containerized Drench Treatment.

Rate of Application (ppm)	Mean % mortality to alate females at indicated months Post-Treatment											
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
50	100	100	100	75	40	95	35	70	50	45	90	25
100	100	100	100	100	85	100	100	45	55	90	70	80
200	100	100	100	100	100	100	100	100	100	100	100	100
300	100	100	100	100	100	100	100	100	100	100	100	100
500	100	100	100	100	100	100	100	100	100	100	100	100
check	0	15	5	10	20	5	5	0	5	5	25	0

PROJECT NO: FA01G155

PROJECT TITLE: Evaluation of Spin Out® Technology in Combination with Insecticides as a Quarantine Procedure for Containerized Nursery Stock

TYPE REPORT: Final

PROJECT LEADER: Anne-Marie Callcott, Lee McAnally and Charlene Russell

INTRODUCTION

Spin Out root growth regulator technology was developed by Griffin Corporation. Valdosta, GA to enhance the development of dense, compact root growth. When applied to the inner surface of a nursery container, Spin Out inhibits the root tips when they reach the container sides. As the plant continues to produce new lateral roots, Spin Out serves as a growth regulator to the new roots, thus helping to produce a very fibrous root system. Spin Out is a liquid flowable that can be applied with a variety of airless sprayers. The active ingredient is 7.1% copper hydroxide. Spin Out treated containers have been tested one year after treatment with out any loss of activity. It has been hypothesized that fire ant colonies enter nursery containers through the drain holes in the bottom of pots (H. L. Collins, personal communication). Use of the Spin Out technology in which an insecticide such as bifenthrin or tefluthrin replaces or is used in conjunction with copper hydroxide might prevent IFA invasion of nursery containers by creating a toxic barrier at or around the drain holes. The current study was designed to evaluate the potential of Spin Out technology used in concert with bifenthrin and tefluthrin to prevent invasion of treated nursery containers by RIFA colonies.

MATERIALS AND METHODS:

Treatment of Containers – Standard trade one gallon nursery containers (Lerio Corp.. Mobile, AL) were treated by Griffin Corporation with various concentrations of bifenthrin in Spin Out (0.25% AI, 0.5% AI, and 1.0% AI). The pots were shipped to this laboratory and received on September 27. 1995. On October 2, 1995. 25 pots of each bifenthrin concentration were filled with a standard growing medium comprised of pine bark. sphagnum peat and sand

(3:1:1)[1]. After the pots were filled with media, they were placed in a simulated can yard and aged under simulated nursery conditions. Irrigation was provided through an automatic overhead pulsating irrigation system programmed to deliver 1.0-1.5 inches of water per week. In addition to the supplemental irrigation, all pots received natural rainfall.

In January, 1996, Griffin Corporation supplied us with the Spin Out product containing 0.1% tefluthrin. This mixture was applied with a paint brush to the interior of 25 pots. Pots were filled with media, aged and periodically bioassayed as described above.

Bioassay - prevention of whole colony infestation: Treatments were bioassayed in the laboratory with field collected RIFA colonies. One container from each treatment series was placed at one end of a 2' x 8' test arena. Sides of the test arena were talced to prevent ants from climbing out and escaping. An untreated check container filled with media was placed at the distal end of the arena. A field collected colony complete with associated soil and nest tumulus was then placed in the center of the arena. Overhead incandescent bulbs slowly desiccated the nest so that the ants were encouraged to migrate to the more moist containers. Therefore the colony had an equal opportunity to move into either a Spin Out treated pot or the untreated check pot. Pots were observed at 24 hour intervals for 7 days after introduction, and the estimated number of workers successfully invading each pot recorded.

Bioassay - partitioning of bifenthrin between Spin Out coating and potting media: To determine whether partitioning of bifenthrin between the Spin Out coating and the potting media was occurring, a second bioassay was performed beginning at 15 months after treatment. Media from each bifenthrin treated pot was placed in a new untreated pot. Untreated MAFES media was placed in the now empty Spin Out/bifenthrin treated pot. Each newly matched pair was bioassayed as described above.

Bioassay - invasion by "newly mated" queens: To determine whether Spin Out/bifenthrin treated pots could prevent a newly mated queen from founding an

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[1] This is the standard media used by Miss. Agr. Forestry Expt. Stn., Poplarville, MS, and adopted by us for many such trials.

incipient colony, a trial simulating a newly mated queen flying into the top of a pot was initiated. Nursery pots from the original set of bifenthrin treated pots described above (0.25% AI, 0.5% AI, and 1.0% AI) were filled with potting media on December 4, 1996, and placed in a simulated can yard with irrigation for 4-6 days. Due to the time of year, and the difficulty in collecting newly mated females, we collected fertile queens from polygynous colonies in Hancock County, MS, along with workers from the same colonies. Since mature, mated queens do not have a reserve of wing muscle for a nutrient source, workers to tend her in our trial were necessary. One fertile female, along with approximately 100 workers, was introduced into each test nursery pot in the first trial initiated in Dec., 1996. In a second trial, initiated in Feb., 1997, two fertile queens from a polygynous colony along with ca. 100 workers were introduced into each test pot. Treatments included three bifenthrin rates, one Spin Out treatment, and one untreated check. There were four replicates per treatment. Pots were watered as needed. Meal worm larvae or peanut butter was placed near the drain holes of each pot twice a week. Treatments were observed daily for the first week and then twice weekly thereafter for a total of 3 months. Observations included noting feeding habits, worker activity, mortality, movement out of the pot, etc.

RESULTS:

Bioassay - prevention of whole colony infestation: All rates of bifenthrin in the Spin Out technology have successfully prevented RIFA infestation in nursery pots for 24 months (Fig. 1). The Spin Out technology without an insecticide does not exclude RIFA. Tefluthrin also has successfully prevented IFA infestation for 24 months, although a few workers (<30) were found under the tefluthrin treated pot at 11 months. These preliminary results demonstrate the potential for Spin Out technology as a quarantine treatment for containerized plants. Additional studies on a larger scale were initiated in the spring of 1997 (see FA01G017).

Bioassay - partitioning of bifenthrin between Spin Out coating and potting media: At 15 months after treatment, ants did not readily move into the Spin Out pot or the media from a Spin Out pot, and 50-75% of the ants died. However, after 7 days, some ants had moved into the center of the "clean" pots filled with "treated" media (media originally in treated Spin Out pot) from

the 0.25 and 1.0% rates (1000 and 500 ants, respectively). These ants did not inhabit the entire pot as is normal, nor did they respond to outside stimuli such as watering, or tapping on the pot. As previously stated, they had moved into the center of the media from the Spin Out treated pots. No ants invaded the Spin Out treated pots which had been refilled with fresh media.

This test was preformed twice more in February and March of 1997 (16 and 17 months after initiation of original trial). Over a 7 day period, no ants moved into either of the offered pots in the February trial, preferring to bunch up in the tray or die. In the March trial, at the end of the 7 day observation period, >50% of the ants moved under, around, and into the clean pot filled with "treated" media regardless of rate. However, in all rates some of the ants were under and in the "treated" pot with clean media as well as bunched up in the tray.

The position of the ants in the media suggests that some bifenthrin leached into the outer edges of the media, but did not penetrate into the center of the media. Media from the Spin Out treated pots was inverted into the clean pots for this trial, meaning that the original top of the media was now on the bottom of the new pot. Therefore, ants were able to move into the media through the bottom of the pot through the media that had not been in direct contact with the bifenthrin treated Spin Out coating. Ants exposed to all rates avoided the original Spin Out treated pots in the first trial and second trials, but did infest the lower rates in the third trial.

Bioassay - invasion by "newly mated" queens: This trial was initiated on December 10, 1996, 14 months after the pots had been treated with the Spin Out/bifenthrin coating. One day after introduction, there was no visible activity in any of the bifenthrin treated pots. There was activity in all checks and Spin Out only pots. Three months after introduction, pots were destructively sampled. All bifenthrin treated pots contained no live IFA. Three of the four Spin Out only treated pots contained a live queen and worker pupae and/or brood; one had no live IFA. All the untreated pots contained workers (+20 workers) and/or brood.

The second trial was initiated on February 18, 1997, 16 months after the pots had been treated with the Spin Out/bifenthrin coating. One day after

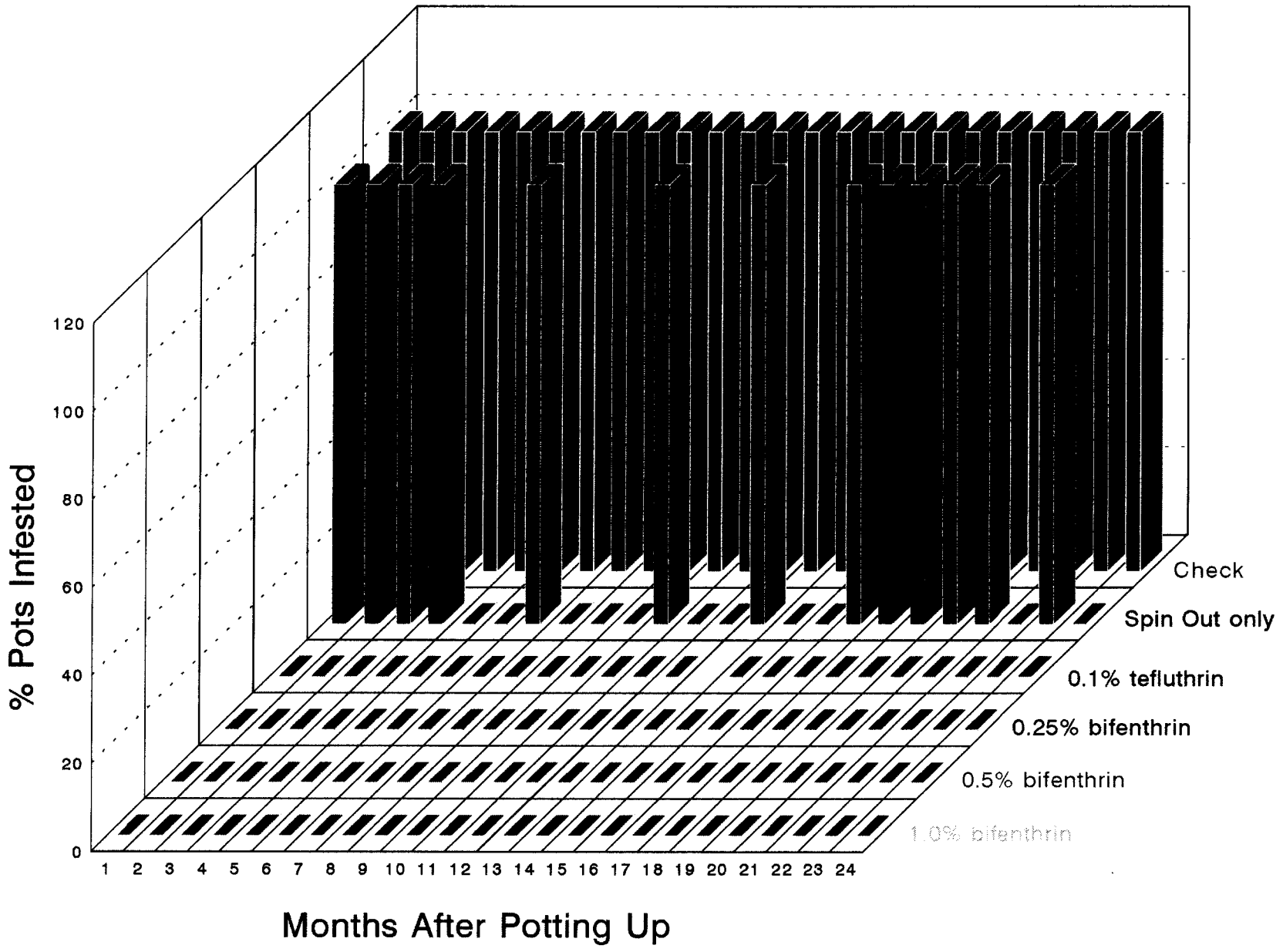
introduction, very few ants were visible in the bifenthrin treated pots and these were moribund. By 2 days after introduction, no ants were visible in the bifenthrin treated pots. Three months after introduction, none of the bifenthrin treated pots or the Spin Out only pots contained any live IFA. The untreated check pots all contained +50 workers and brood.

DISCUSSION:

Nursery pots with an interior coating of latex containing either bifenthrin or tefluthrin is a deterrent to IFA colonies when give a choice of treated or untreated pots to invade. An interior coating of Spin Out alone is not a deterrent to IFA colonies. There appears to be enough insecticide leached into the media to eliminate newly mated IFA queens from a treated pot at least 16 months after a pot was treated and filled with media. This preliminary work (partitioning work) also indicates that some of the insecticide is leaching into the surrounding media as shown by IFA not infesting either pot when the "treated" media was switched into an untreated pot. However, this data is not conclusive, and does indicate that the best results are the combination of the treated pot and the media that has been in contact with the latex coating over time.

As mentioned earlier, results of this trial promoted the initiation of a larger scale trial at commercial nurseries in the spring of 1997. See report FA01G017 for the results of that ongoing trial.

Figure 1. Percent of Spin Out 100 + Insecticide Treated Pots Infested with Imported Fire Ants. Preliminary Trial at Gulfport, MS - initiated 1995-1996.



Infested = >20 worker ants in pot

PROJECT NO: FA01G017

PROJECT TITLE: Expanded Trials with Spin Out® Technology Used in Combination with Bifenthrin Insecticide to Prevent Imported Fire Ant Infestations in Containerized Nursery Stock, 1997

TYPE REPORT: Interim

PROJECT LEADER/PARTICIPANTS: Anne-Marie Callcott, Homer Collins, Charlene Russell, and Lee McAnally

COOPERATORS: Lerio Corporation, Mobile, AL (Mark Christian)
Griffin Corporation, Valdosta, GA (Mark Crawford)
Turkey Creek Nursery, Houston, TX (Tom Henry)
Windmill Nursery, Folsom, LA (Dennis McCloskey)
Flowerwood Nursery, Loxley, AL (Jim Berry)
Wight Nursery, Cairo, GA (Jerry Lee)
Grass Roots Nursery, Leesburg, FL

INTRODUCTION:

Spin Out root growth regulator technology was developed by Griffin Corporation to enhance the development of dense, compact root growth. The active ingredient is 26% copper hydroxide contained in a latex matrix which is sprayed onto the interior of plastic nursery pots. In 1995 Griffin supplied this laboratory with Spin Out treated pots that also contained various concentrations of bifenthrin (0.25% AI, 0.5% AI, and 1.0% AI or teflutrin 0.1%). Pots were filled with potting media and then placed in a simulated can yard. Laboratory bioassays were then conducted on a monthly basis by allowing red imported fire ant (RIFA) colonies free choice to move into either a Spin Out treated pot or an untreated control pot. The Spin Out treated pots containing bifenthrin prevented fire ant colonies from invading the test pots for at least 24 months, (24 for tefluthrin). These results warrant an expanded study to include actual rather than simulated nursery conditions, various size containers, different potting medias, and varying climatic conditions.

MATERIALS AND METHODS:

Griffin Corporation supplied Spin Out 300 (a different formulation than used in the preliminary trial mentioned above) containing bifenthrin (0.25%. 0.5%, and 1.0% AI by weight) for treatment of a sufficient number of pots to conduct

a three year study. Lerio Corporation provided the containers (one and three gallon sizes) used in the expanded study and applied the Spin Out 300/bifenthrin to the pots using automated equipment. After treatment, Lerio delivered treated pots to the Gulfport Plant Protection Station for distribution to the nurseries. Lerio also transported test pots from each nursery to this laboratory at each sampling interval. Griffin and Lerio purchased all plants for the test. Nurseries listed above provided space, irrigation, fertilization, and weed control for the test pots. The Gulfport Plant Protection Station color coded the pots with paint on the exterior of the pots to designate the different bifenthrin concentrations. Gulfport personnel delivered treated pots to the participating nurseries, purchased potting media from each nursery, filled the pots and placed the test pots in a designated test area (or bed). All pots were planted with slow growing, relatively pest free woody ornamentals such as Japanese boxwood. Each individual nursery determined the best species of plants to use at that particular location.

Nursery	Location	Media Type	1-gal Plants	3-gal Plants
Turkey Creek	Houston, TX	70% bark 8% sand 8% hadite 14% peat	*Buxus microphylla* 'Winter Gem'	*Buxus microphylla* 'Winter Gem'
Grass Roots	Leesburg, FL	60% pine bark 40% local peat	*Juniperus parsonnii*	*Ilex crenata*
Flowerwood	Loxley, AL	65% new bark 35% aged bark	*Ilex crenata*	*Ilex crenata*
Windmill	Folsom, LA	100% pine bark	*Ilex crenata*	*Ilex crenata*
Wight	Cairo, GA	80% bark 20% sand	*Juniperus horizontalis*	*Juniperus horizontalis*

The Gulfport Plant Protection Station was responsible for determining the efficacy of the Spin Out technology by conducting bioassays in the laboratory.

Bioassay - prevention of whole colony infestation: All treatments were tested against field collected RIFA colonies. Three containers (replicates) from each treatment were bioassayed at 1, 3, 6, and 9 months after the pots were filled with media, plant tops were removed prior to bioassaying as needed. Each replicate was bioassayed by placing the treated container at one end of a

2' x 8' test arena (Fig. 1). Sides of the test arena were coated with talcum powder to prevent the ants from climbing out and escaping. An untreated check container filled with media was placed at the distal end of the test arena. A field collected RIFA colony complete with associated soil and nest tumulus was then placed in the center of the arena. Overhead incandescent light bulbs slowly desiccated the nest tumulus so the ants were encouraged to migrate to the more moist and hospitable containers. Therefore, the RIFA colony was provided a free choice to invade either a Spin Out treated pot or the untreated check pot. Pots were observed at 24 hour intervals for 7 days after introduction, and the estimated number of workers successfully invading each pot recorded.

Bioassay - partitioning of bifenthrin between Spin Out coating and potting media: A second bioassay was conducted at 6 months after treatment to determine partitioning of bifenthrin between the Spin Out/bifenthrin coating and the potting media. Media from each nursery was transported back to the Gulfport lab, placed in untreated pots (1 and 3 gallon), and placed in our simulated nursery for aging. At the prescribed interval, media from each treated pot was swapped with media from an untreated but aged pot of the corresponding size; i.e. the treated pot now contained "clean" media and the untreated pot now contained "treated" media. Bioassays were performed as described above using the newly paired pots.

Bioassay - invasion by "newly mated" queens: To determine whether Spin Out/bifenthrin treated pots could prevent a newly mated queen from founding an incipient colony, a trial simulating a newly mated queen flying into the top of a pot was initiated at 1, 3, and 9 months. The one-gallon treated nursery pots from Wight Nursery only were used in this trial. Due to the difficulty in collecting newly mated females, we collected fertile queens from polygynous colonies in Hancock County, MS, along with workers from the same colonies. Since mature, mated queens do not have a reserve of wing muscle for a nutrient source, workers to tend her in our trial were necessary. Two fertile females, along with approximately 100 workers, were introduced into each test nursery pot. Untreated check pots filled with MAFES media were used as controls. There were three replicates per treatment. Pots were watered as needed. Meal worm larvae or peanut butter was placed near the drain holes of each pot twice a week. Treatments were observed daily for the first week and then twice

weekly thereafter for a total of 3 months. Observations included noting feeding habits, worker activity, mortality, movement out of the pot, etc.

RESULTS:

Bioassay - prevention of whole colony infestation: Results through 6 months show some movement of whole colonies (workers and brood) into pots treated with 0.25% and 0.5% bifenthrin (Fig. 2). A few workers (<100) have moved into some of the 3-gallon pots treated at 1.0% during all evaluation periods, and <50 ants moved into one of the 1.0% 1-gallon pots at 6 months after potting up. The pots used in this trial were treated by the companies (Griffin and Lerio) and the amount of latex coating applied to the pots was about ⅓ the amount applied to the pots we began testing in 1995. This may account for the differences in efficacy seen between the two trials.

Bioassay - partitioning of bifenthrin between Spin Out coating and potting media: Because ants readily moved into pots treated with the two lower rates of bifenthrin, only the 1.0% bifenthrin treated pots were used in this trial. At 6 months after potting up, results were very inconsistent. While the ants did not immediately move into either pot and many died (probably due to contact with the insecticide), eventually (after 7 days) the ants moved into either pot at about the same rate, showing no preference for either the treated pot with "clean" media or the untreated pot with "treated" media. This indicates that some of the bifenthrin is moving into the media from the latex coating.

Bioassay - invasion by "newly mated" queens: Trials have been completed at 1 and 3 months after potting up. No polygynous queens or workers have survived in the treated pots (all rates), while queens and workers in the untreated check pots have survived.

CONCLUSIONS:

At this time, it is evident that with the Spin Out 300 formulation the thinner coating of latex and therefore less insecticide applied to the pots has affected the efficacy of the treatment. It also appears that the larger pots (3-gallon) with proportionally larger drain holes may require more insecticide

per surface area to adequately protect this vulnerable spot. We propose that the 0.25 and 0.50% rates be dropped from the trial at this time, and additional trials be initiated in the spring of 1998. These new trials would require either a thicker coating of the Spin Out/insecticide equivalent to our original trial (FA01G155) or 3X the amount of insecticide that was present in this trial to bring the amount of insecticide present per pot (surface area) up to that which was present in the original trial.

Figure 1. Diagram of test arena.

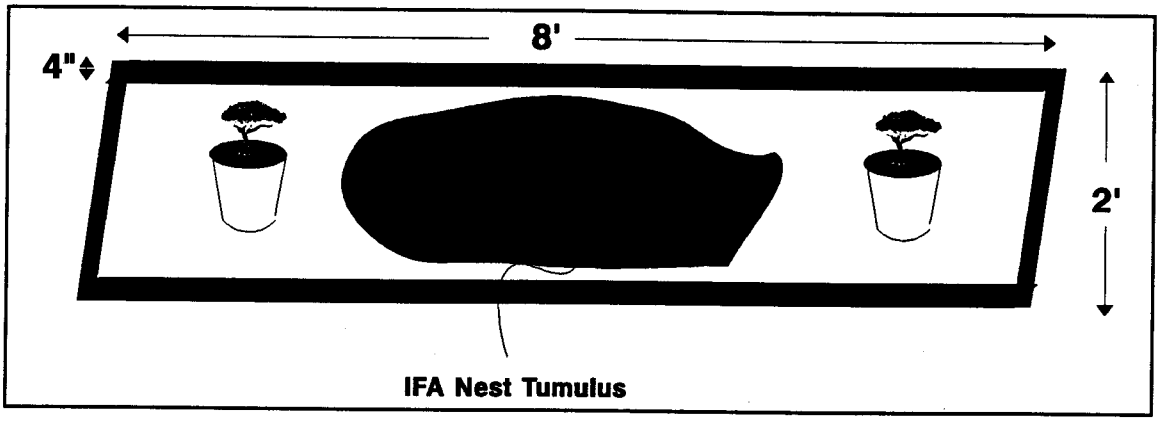
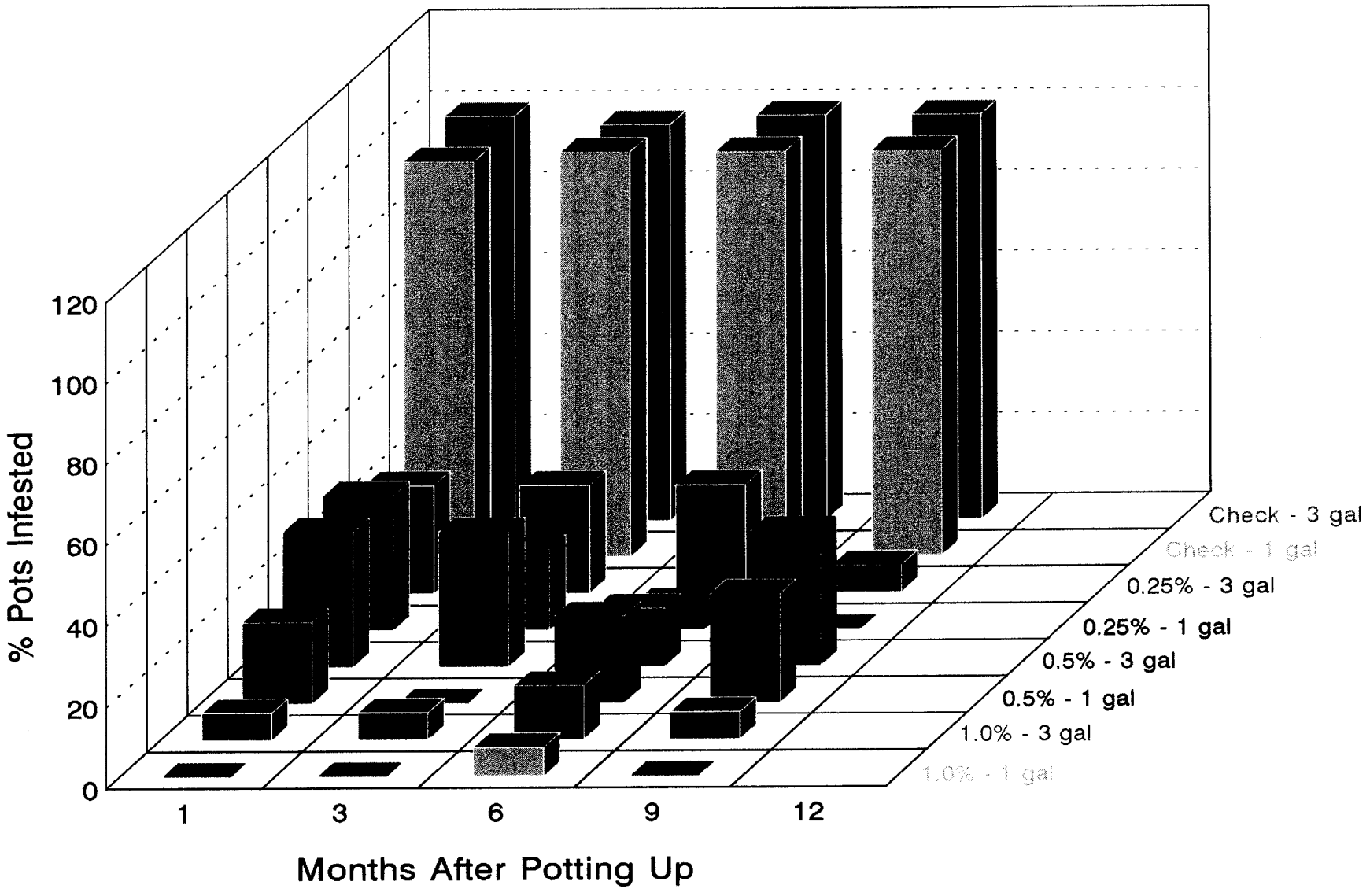


Figure 2. Percent of Spin Out 300 + Bifenthrin Treated Pots Infested with Imported Fire Ants. Multi-State Trial - initiated March 1997



Infested = >20 worker ants in pot

PROJECT NO: FA01G116

PROJECT TITLE: Evaluation of Spot Treatments with Granular Insecticides for Quarantine Treatment of Red Imported Fire Ants in Field Grown Nursery Stock

TYPE REPORT: Interim

LEADERS/PARTICIPANTS: Homer Collins, Charlene Russell, Lee McAnally, and Avel Ladner

COOPERATORS: Windmill Nurseries, Inc., Folsom, LA, Harrison County Farm, Gulfport, MS

INTRODUCTION:

Nursery stock and other regulated articles cannot be shipped outside the quarantined area unless treated with an approved insecticide (7CFR § 301.81) Balled and burlapped plants can be immersed in a chlorpyrifos solution or subjected to a twice daily spray-on with chlorpyrifos for three consecutive days. The immersion process is labor intensive and disruptive to the root ball. The spray-on treatment is also labor intensive and somewhat impractical. A simple, cost effective, and practical treatment is needed to enable growers to ship non-infested plants outside the quarantined area.

Numerous granular formulations of common insecticides such as diazinon, chlorpyrifos, acephate, and others are labelled for spot treatment of imported fire ant colonies. Talstar PL Granular (0.2% bifenthrin) is labelled for broadcast application to lawns and landscape areas for control of imported fire ants. Rate of application is 200-400 lbs formulated product per acre. Talstar T & O Granular is in general use in many nurseries because it is also labelled for incorporation into nursery potting media as a fire ant quarantine treatment for containerized nursery stock. We evaluated 1% granular Dursban® (Hi-Yield@Kill-a-Bug Lawn Granules), Orthene® 75S, Fireban® 1.5G, and Talstar T&O Granular at various rates of application for quarantine treatment of B & B nursery stock.

MATERIALS AND METHODS:

Test I - Windmill Nursery, Folsom, LA (Nov. 5, 1996)

A twenty acre planting of field grown nursery stock (Crepe Myrtles,

approximately 2' in height) was closely inspected for presence of live fire ant colonies. Ants typically nest adjacent to the trunks of woody ornamentals which are grown on raised beds. Nests are less commonly located away from the trunks in areas such as the "middles", or between plants on the row. When a nest in direct contact with the trunk or roots was detected, the plant was marked with an engineering flag, and the nest was rated on the population index scale described by Lofgren and Williams (1982). All marked plants were then treated. Treatments evaluated included three rates of Talstar T&O, plus an untreated check. Each treatment was replicated 10 times. Rates tested were 200, 100, and 50 lb formulated material per acre. Treatments were applied with a shaker can in a circular pattern beginning at the trunk of each infested plant and extending outward to the drip line (ca. 12.5 ft^2).

Treatments were evaluated at 2, 7, 14, and 21 days after application. Evaluation consisted of a re-examination of each previously marked and treated fire ant nest (replicate) to determine if the nest remained active. Again, all active nests were rated on the population index scale at each posttreatment rating interval. Inactive nests, whether the colony had succumbed to the treatment or simply relocated to a new site, were rated as "0". Data for each posttreatment rating interval were analyzed by ANOVA and treatment means separated by LSD test.

Test II - Harrison County Farm, Gulfport, MS (Nov. 5, 1996)
Procedures described above were essentially repeated except the test site was an open, mowed field located at the Harrison County Farm. Individual IFA nests were marked with a stake prior to treatment. Talstar Granular was applied at rates of 200, 100, and 50 lbs of formulated material per acre to a two foot diameter circular area around each replicate (nest). There were 24 replicates per treatment.

Test III - Windmill Nursery, Folsom, LA (Nov. 19, 1996)
Procedures described above were used to evaluate labelled rates of application of Orthene and Dursban. Rates of application were two teaspoons of orthene and 2 ounces (56.7g) Dursban per mound, with 10 replicates per treatment.

Test IV - Harrison County Farm, Gulfport, MS (Nov. 20, 1996)
Procedures described above were used to evaluate labelled rates of application

of Orthene and Dursban. Rates of application were two teaspoons of Orthene and 2 ounces (56.7g) Dursban per mound, with 24 replicates per treatment.

Test V - Harrison County Farm, Gulfport, MS (May 27, 1997 and Oct. 27, 1997)
Procedures described above were used to evaluate acephate (Orthene 75S) and tefluthrin (Fireban® 1.5G). Rates of application were two teaspoons of Orthene 75S per mound (labelled rate) and 1.5 g of Fireban 1.5G per mound (2 ft diameter circle - extrapolated from maximum labelled rate of granular tefluthrin for corn rootworm). There were 15 to 24 replicates per treatment, including untreated checks.

RESULTS AND DISCUSSION:

Test I - Windmill Nursery, Folsom, LA
Treatments were applied on November 5, 1996. Air and soil temperature were 71°F and 70°F, respectively at the time of application. Talstar T&O Granular applied at a rate of 200 lb/acre (2g/ft^2) resulted in an 94.8% reduction in the pretreatment population index 2 d after application (Table 1). By 21 d after application a 100% reduction in the pretreatment population was achieved with all applications.

Table 1. Efficacy of Talstar T&O Granular as a quarantine treatment for field grown nursery stock

Treatment	Pretreat Pop. Index†	% Change in Pretreat Pop. Index at indicated days posttreatment			
		(2)	(7)	(14)	(21)
200 lb/acre	17.0	-94.8a	-99.2a	-100.0a	-100.0a
100 lb/acre	15.0	-98.0a	-90.0a	-93.3a	-100.0a
50 lb/acre	14.5	-73.3a	-83.3a	-94.3a	-100.0a
Untreated Check	16.0	0.0b	-30.0b	-34.0b	-54.0b

† mean based on 10 replicates

Test II - Harrison County Farm, Gulfport, MS

Air and soil temperatures were 82°F and 72°F, respectively at the time of application. All rates of application provided 100% control or colony relocation by 21 d after treatment, but in general, lower rates of application required more time to reach this level of control (Table 2).

Table 2. Efficacy of Talstar T&O Granular as a quarantine treatment for field grown nursery stock

Treatment	Pretreat Pop. Indext	% Change in Pretreat Pop. Index at indicated days posttreatment			
		(2)	(7)	(14)	(21)
200 lb/acre	13.5	-51.5a	-58.5a	-100.0a	-100.0a
100 lb/acre	17.5	-43.1ab	-53.2a	-97.5a	-100.0a
50 lb/acre	13.3	-18.4bc	-25.3ab	-99.9a	-100.0a
Untreated Check	15.6	-5.3c	-5.3b	-30.1b	-27.8b

† Mean based on 24 replicates

Test III - Windmill Nursery, Folsom, LA

Air and soil temperatures were 75°F and 70°F, respectively at the time of application. Both Orthene 75S and 1% Dursban granules were highly effective in eliminating treated IFA colonies. Both treatments were 100% effective within 7 days of application (Table 3).

Table 3. Efficacy of Orthene 75S and Dursban 1% Granular in causing colony mortality or relocation.

Treatment	Pretreat Pop. Indext	% Change in Pretreat Pop. Index at indicated days posttreatment			
		(2)	(7)	(14)	(21)
Orthene 75S	13.5	-93.3a	-100.0a	-100.0a	-100.0a
Dursban 1G	15.5	-51.8b	-100.0a	-100.0a	-100.0a
Untreated Check	14.5	0.0c	8.0b	-3.7b	1.3b

† Mean based on 10 replicates

Test IV - Harrison County Farm, Gulfport, MS

As shown in Table 4, Orthene was very effective in eliminating treated IFA colonies. Dursban 1G did not perform as well in this trial as it did in the previous trial.

Table 4. Efficacy of Orthene 75S and Dursban 1% Granular in causing colony mortality or relocation.

Treatment	Pretreat Pop. Index†	% Change in Pretreat Pop. Index at indicated days posttreatment			
		(2)	(7)	(14)	(21)
Orthene 75S	15.0	-100.0a	-100.0a	-100.0a	-100.0a
Dursban 1G	15.0	-46.5b	-83.3b	-83.3b	-91.7a
Untreated Check	14.0	4.2c	2.8c	2.8c	5.9b

† Mean based on 24 replicates

Test V - Harrison County Farm, Gulfport, MS (May 27, 1997 and Oct. 27, 1997)
Air and soil temperatures were 84°F and 78°F, respectively, on the May 27 treatment date. Between the 7 and 14 day counts, the area had been mowed, inducing many of the check mounds to relocate and also removing flags which marked treated mounds. Therefore, those lost due to flag removal were eliminated from the entire trial (2 check mounds, 2 Orthene mounds, and 5 Fireban mounds). We believe that this is what caused the higher than normal check mortality. Within 7 days after treatment the acephate had provided 100% mortality or movement >5 ft. away from the original mound (Table 5). The Fireban provided 100% mortality or movement within 14 days.

Table 5. Efficacy of Orthene 75S and Fireban 1.5% Granular in causing colony mortality or relocation.

Treatment	Pretreat Pop. Index†	% Change in Pretreat Pop. Index at indicated days posttreatment			
		(2)	(7)	(14)	(21)
Orthene 75S	16.82	-96.9a	-100.0a	-100.0a	-100.0a
Fireban 0.1G	17.82	-71.8b	-94.7a	-100.0a	-100.0a
Untreated Check	19.32	-4.5c	-9.1b	-36.4b	-53.0b

† Mean based on 19-22 replicates

Air and soil temperatures for the Oct. 27 treatment date were 56°F and 60°F, respectively. Due to a shortage of mounds in an area that we were certain would not be disturbed for the duration of the trial only 15 replicates per treatment were used. In this trial, acephate required 14 days to achieve 100% mortality or movement >5 ft. away from the original mound (Table 6). The Fireban did not provide good control in this trial with >30% of the mounds surviving during the rating period.

Table 6. Efficacy of Orthene 75S and Fireban 1.5% Granular in causing colony mortality or relocation.

Treatment	Pretreat Pop. Index†	% Change in Pretreat Pop. Index at indicated days posttreatment			
		(2)	(7)	(14)	(21)
Orthene 75S	16.82	-35.8a	-97.9a	-100.0a	-100.0a
Fireban 0.1G	17.82	-18.3a	-47.7b	-66.0b	-67.7b
Untreated Check	19.32	5.5b	3.3c	3.3c	-3.9c

† Mean based on 15 replicates

DISCUSSION:

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 (personal observation). The 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 root ball of plants intended for harvest. The results of these preliminary trials indicate that Orthene 75S and Talstar T&O are effective in eliminating active IFA colonies from treated nests and could possibly be used as a quarantine treatment for field grown nursery stock, if registered for this use pattern.

ACKNOWLEDGEMENTS:

We thank Anne-Marie Callcott for providing statistical analysis of raw data.

PROJECT NUMBER: FA01G047

PROJECT TITLE: Quarantine Treatment of Balled-and-Burlapped Nursery Stock with Fumigants

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Anne-Marie Callcott, Charlene Russell, Lee McAnally

INTRODUCTION:

Practical and cost-effective quarantine treatments for balled-and-burlapped nursery stock are not currently available. Current treatment options include (1) dipping the root ball; (2) twice daily irrigation of root ball for three consecutive days; (3) in field treatment with a combination of baits and granular chlorpyrifos. These treatments are labor-intensive, costly, and cause worker and environmental exposure problems. Therefore the Gulfport Plant Protection Station is investigating several practical and cost-effective treatments for B&B stock including the use of fumigants as additives inside the burlap wrapping or as field treatments prior to digging and balling.

MATERIALS AND METHODS:

A variety of fumigants were used in several different trials reported herein:

Fumigant	% Active Ingredient1 description	Manufacturer
Naphtha1ene (Moth Balls)	1.0% naphtha1ene/ moth balls (2.95g/tablet)	Enoz® Old Fashioned Moth Balls Willert Home Products St. Louis, MO 63110
2,2-Dichlorovinyl dimethyl phosphate (DDVP)	10% DDVP/ 1" x 4" strips	Hercon® Vaportape" II Hercon Environmental Company Emigsville, PA 17318-0467
Aluminum phosphide	55% aluminum phosphide/tablets (3 g/tablet = 1 g hydrogen phosphide gas)	Phostoxin® Tablets Degesch America, Inc. Weyers Cave, VA 24486

Bioassay with Alate Females

Field collected IFA alate females were exposed to moth ball vapor to determine mortality. Standard desiccators with and without covers were used to contain ants and moth balls. Square nursery pots (2" x 2") with Labstone® bottoms (see Appendix II, Standard alate female bioassay), IFA alate females, standard GPPS potting media, and moth balls were used. Each desiccator contained 5 alate females in a nursery pot with potting media or 5 females in a nursery pot without potting media. One moth ball was introduced into each desiccator. There was also an untreated check (no moth ball) of females in a nursery pot with or without media. In one trial, the glass cover was placed on the desiccator and in another trial the glass cover was replaced with a screen to allow constant air flow. Mortality was recorded periodically over a one week period.

Induced Movement out of B&B Stock

Trial I: IFA field colonies (colony class 7-8; Lofgren and Williams 1982) were dug to simulate 12" root balls and placed in 36" square sheets of burlap, tied at the top to resemble B&B nursery stock and placed in talced pans to transport them back to the laboratory. Ants were allowed to acclimate to the burlap sack in the laboratory for 3-5 days. Each IFA-infested burlap sack was then placed outside on a grassy lawn at least 10 feet from an existing IFA mound and 20 feet from each other. Each sack was then treated with either moth balls or DDVP strips. Balls or strips were distributed evenly around the "root ball" (between the burlap and the soil) by making small incisions in the burlap and inserting the ball or strip. Treatments included 1, 3, or 6 units (balls or strips) of each fumigant, and each treatment was replicated three times. Untreated checks were also placed on the lawn. Burlap sacks were watered as needed to simulate a nursery environment. Colony activity and location (in ball, new mound present within 5' to 8' of sack, or ants not found) were recorded every other day for 7 days. On the final day, the sacks were opened and the "mound" destructively sampled for live IFA.

Trial II: The protocol above was followed: field colonies placed in burlap sacks, treated with 1, 3, or 6 moth balls, Phostoxin tablets or DDVP strips, replicated three times. However in this trial, sacks were placed at one end of a 2' x 5' tray while artificial plaster nests were placed at the other end. The sides of the tray were coated with Fluon® to prevent escape from the tray.

In this environment, we were able to accurately determine whether treatments caused mortality of the IFA colony or caused movement out of the burlap sack.

Enhancement of Fumigant Activity by Use of Plastic Enclosure
Since we were not able to induce movement out of the burlap balls with the introduction of fumigants, and only phostoxin was providing 100% mortality in the root balls, we explored the possibility of enclosing the burlap covered root ball in a plastic bag for a period of time after introduction of the fumigant to enhance/concentrate the effects of the fumigant.

Placement of field collected IFA colonies into burlap sacks was performed as described above. Burlap covered root balls (IFA colonies) were placed in a 17 x 22" plastic pan with 3-5" sides. The sides of the pan had been treated with Fluon® to prevent ants from escaping. Tablets or strips of fumigants were introduced to the root ball in two ways: (1) placing the fumigant inside the burlap, usually on top of the root ball, securing the top of the burlap, and then placing the burlap ball into a plastic bag which was closed by twisting the top of the bag and securing with a twist tie; or (2) placing the fumigant in the bottom of the plastic bag before placing the burlap ball into the plastic bag, closing as above. The plastic bags were left on the burlap ball for a 24 or 48 hour exposure period. After the exposure period, the plastic was removed and the ball examined for presence of live ants. Balls were observed for 7 days and activity noted. Un-fumigated check balls were also observed, both those that had been enclosed in plastic bags and those that had not been enclosed in plastic. There were three replicates per fumigant.

After several trials, we discovered that placing the fumigant in the bottom of the plastic bag was not the best method to use since the tablets could get caught in the folds of the plastic and thus not be able to actively fumigate the entire root ball. Therefore those trials were eliminated from this discussion.

RESULTS:

Bioassay with Alate Females
In the glass covered desiccators, 100% mortality of IFA alate females was achieved in less than 24 hours. In the screen covered desiccators, less than

20% mortality was achieved 2 days after treatment. By 5 days after treatment, 100% mortality was achieved in the desiccator without potting media, and 40% in the desiccator with potting media.

Induced Movement out of B&B Stock

Trial I: This trial was initiated on June 2, 1997. One day after application, no treatment had induced IFA movement from the burlap sacks (Table 1). By 3 days after treatment, the sacks treated with 6 moth balls had either caused 100% mortality of the colonies or had induced them to move greater than 8' from the sack. Over time, the lower moth ball treatments also either caused mortality or induced movement in two of the three replicates of each treatment. The single DDVP strip treatment only caused mortality or induced movement in one of the replicates, while the two higher rates of DDVP caused mortality or induced movement in two of the three replicates. The checks also, over the 7 day test period, lost all IFA activity.

Due to the uncertainty of whether IFA colonies were being killed by the fumigants or being induced to move out of the burlap sacks, a second trial was initiated as described above to better control and track movement of the colonies.

Trial II:

DDVP was not included in this trial because of the poor results obtained in the first trial. Acephate, at the labelled rate of 2 tsp/mound was included in this trial as a standard, and provided 100% mortality in two of three replicates (Table 2). No colonies attempted to relocate to the cast nest, but the surviving colony did move into the center of the ball of dirt. Naphthalene applied at 1, 3 or 6 tablets per root ball did not produce significant mortality nor did it induce movement out of the burlap into the cast nest. Phostoxin at a rate of 1 tablet per root ball produced 50-99% mortality, while the 3 tablet per root ball provided 100% mortality in two of the replicates. The highest Phostoxin rate of 6 tablets per root ball provided 100% mortality in all replicates within 24 hours. These were not re-examined on subsequent days, and we discovered in future trials that the ants did occasionally recover (see below). However, 6 tablets may be enough to ensure that the ants are all killed and not just temporarily incapacitated.

Enhancement of Fumigant Activity by Use of Plastic Enclosure

Enclosing the burlap ball in a plastic bag did not enhance the activity of DDVP or naphthalene against IFA (Tables 3 & 4). After several trials these products were dropped from the study and efforts were concentrated on Phostoxin.

Three Phostoxin tablets provided greater than 99% mortality regardless of the length of time the plastic bag remained on the burlap ball (Tables 3 & 4). In most cases, immediately after removing the plastic bag, ants appeared dead or moribund (slowly moving legs or bodies, very few actually walking around). However, some of the ants did recover from the fumigant over the next few days, but were never as active as the check ants. In the trial in which the plastic bag remained in place for 48 hrs, temperatures during treatment (while plastic bag was in place) were 60-69°F, however during the following 5 days, including evaluations at day 4 and 7, the temperature was 37-48°F. In this trial (Table 3), 2 of the 3 replicates treated with the 3 phostoxin tablets appeared 100% dead after the plastic was removed (48 hrs). Some ants in one of the replicates recovered by day 4, and by day 7 all the replicates were dead. This indicates that the cold weather may have "finished off" the weakened survivor ants in these root balls.

DISCUSSION:

These results suggest that Phostoxin tablets placed on the root ball and under the burlaps of B & B nursery stock post harvest may eliminate IFA colonies. Additional trials with Phostoxin will be conducted. Variables such as temperature at the time of treatment, dose rate, size of root balls, soil type, etc. will be investigated. Current costs estimates for Phostoxin tablets (2/98) from Degesch America, Inc. are about $0.065 when purchased in bulk. This would make the cost of treating one ball with 6 tablets $0.40.

Table 1. Observations of Induced Movement Trial Conducted on the Lawn.

Treatment	Observation at indicated days after treatment			
	(1)	(3)	(5)	(7)
1 Moth Ball	ants in sacks	ants in sacks	2 in sacks 1 not found	1 in sack 1 mound adjacent to sack 1 not found
3 Moth Balls	ants in sacks	no ants found	1 in sack 2 not found	1 in sack 2 not found
6 Moth Balls	ants in sacks	no ants found	no ants found	no ants found
1 DDVP Strip	ants in sacks	2 in sacks 1 not found	2 in sacks 1 not found	2 in sacks 1 not found
3 DDVPs	ants in sacks	ants in sacks	ants in sacks	1 in sack 1 mound under sack 1 not found
6 DDVPs	ants in sacks	1 in sack 2 not found	2 in sacks 1 mound adjacent to sack	1 in sack 1 mound adjacent to sack 1 not found
Check	ants in sacks	2 in sacks 1 not found	1 in sack 2 not found	no ants found

Table 2. Results of Tray Test with Fumigants.

Fumigant	Number Used	% mortality at indicated days after treatment								
		1 day			3 days			7 days		
		Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Phostoxin	1	50	50	99	50	50	99	50	50	99.9
	3	90	100	100*	90	100	100*	90	100	100*
	6	100	100	100	--	--	--	--	--	--
Naphthalene	1	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0
Acephate	2 tsp/ mound	50	90	0	50	90	0	100	100	0
Check	1	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0

Table 3. Results of Fumigants in Burlap Balls Enclosed in Plastic Bags for 24 Hours.

Fumigant	Temp. Range while Plastic on Ball (°F)	Number Used	% mortality at indicated days after treatment								
			1 day			3 days			7 days		
			Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Phostoxin	50-70	1	100	100	99.9	99.9	99.9	50	100	99	50
	41-57	3	100	100	99.9	100	99.9	99.9	100	100	99.9
		6									
Naphthalene	50-70	1	0	0	0	0	0	0	0	0	0
	41-57	6	0	0	0	5	0	0	5	0	0
DDVP	50-70	1	0	0	0	0	0	0	0	0	0
	41-57	6	0	0	0	0	0	0	0	0	0
Check	50-70	1	0	0	0	0	0	0	0	0	0
	41-57	3	0	0	0	0	0	0	0	0	0
	41-57	6	0	0	0	0	0	0	0	0	0
Check	no plastic	1	0	0	0	0	0	0	0	0	0
	no plastic	3	0	0	0	0	0	0	0	0	0
	no plastic	6	0	0	0	0	0	0	0	0	0

Table 4. Results of Fumigants in Burlap Balls Enclosed in Plastic Bags for 48 Hours.

Fumigant	Temp. Range while Plastic on Ball (°F)	Number Used	% mortality at indicated days after treatment								
			2 day			4 days			7 days		
			Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Phostoxin	60-69	1	99	99	50	90	90	50	90	100	90
	60-69	3	99	100	100	99	100	99	100	100	100
Naphthalene	60-69	3	0	0	0	0	0	0	0	50	0
DDVP	60-69	3	0	0	0	0	10	25	0	10	25
Check	60-69	0	0	0	0	0	0	0	0	0	0
Check	no plastic	0	0	0	0	0	0	0	0	0	0

PROJECT NO: FA05G055

PROJECT TITLE: Biobarrier as a Prophylactic Treatment for Control of Phytophera Root Rot in Blueberry and Its Effects on Populations of Imported Fire Ants in Commercially Grown Blueberry Orchards

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Timothy C. Lockley/Barbara J. Smith

INTRODUCTION:

Phytophera Root Rot (Phytophera *cinammoni*) is a major pest of blueberries in commercial orchards in the southeastern United States and causes considerable economic losses every year (Caruso & Ramsdell 1995). The mode of transmission of phytophera is from wild host plants to the blueberries. Standard practice is to spray various biocides to either kill the pathogen or the host plant. By restricting the development of the wild hosts. the effects of the phytophera on commercial blueberries can be limited to acceptable economic levels. Biobarrier (Reemay Corp., Nashville. TN) is designed to eliminate weedy species from landscaping by emitting small amounts of Treflan herbicide over extended periods. Imported fire ants, although not a direct pest of blueberries, also play a major deleterious role in commercial blueberry fields by clogging drip irrigation systems and stinging field workers and customers in "pick-your-own" fields. Biobarrier has shown significant potential to repel active fire ant colonies (1996 Annual Report FA05G045). Tests were undertaken at the USDA-ARS-Small Fruits Research Laboratory in Poplarville, MS to determine the effects Biobarrier would have on the phytophera pathogen. In conjunction with this trial, Biobarrier was also examined for its effects on imported fire ants and the movement of colonies of this pest ant.

MATERIALS AND METHODS:

Blueberries/treatments were established in April 1995 as shown in Table 1. Plot design for the Biobarrier treatment is shown in Table 2. Plants were spaced ca. 1.0 m apart. Peatmoss was incorporated into the plantings with the exception of the Tung Press Cake treatment. Post planting treatments of Ridomil and Subdue were applied in the spring of 1996. Plants were visually rated for phytophera root rot symptoms every 3 months and were assayed for the

presence of *Phytophera cinammoni* every 6 months. Fire ant populations were determined every 6 months.

RESULTS:

At 30 months into the evaluation, RIFA colonies have established themselves throughout the untreated plots. To date, no activity has been noted within any of the treated plots. However, a significant reduction in vigor has been observed within the Biobarrier mulched plots. This was most notable on the Misty variety with the Tifblue cultivar showing the least effect. Currently, a statistical analysis is being made by BJS to determine if significant reductions in plant growth, vigor or fruit production did occur.

Table 1. Phytophera Root Rot/Fire Ant Experimental Protocol.

Treatments		Bed	Cultivars					
No.	Chemical	Height	Tifblue	Gulfcoast	Misty	Reville	Other*	TOTAL
1	None	Raised	4	4	4	4	27	43
2	None	Flat	4	4	3	3	0	14
3	Ridomil Prep	Raised	4	4	4	3	0	15
4	Ridomil Prep	Flat	4	4	4	3	0	15
5	Ridomil Post	Raised	4	4	4	3	0	15
6	Ridomil Post	Flat	4	4	4	3	0	15
7	Subdue Post	Raised	4	4	4	3	0	15
8	Biobarrier	Raised	4	4	4	3	0	15
9	Tung Press Cake	Raised	4	4	4	3	0	15

* Other cultivars: Georgia Gem; Pearl River; Magnolia; Marimba; Cooper; Premier.

Table 2. Phytophera Root Rot Study Biobarrier Plot Protocol.

CULTIVAR	REP	NO.	BED HEIGHT	ROW	PLOT
Gulfcoast	1	8	Raised	4	31
Misty	1	8	Raised	4	33
Reville	1	8	Raised	4	32
Tifblue	1	8	Raised	4	34
Gulfcoast	2	8	Raised	3	23
Misty	2	8	Raised	3	24
Reville	2	8	Raised	3	22
Tifblue	2	8	Raised	3	25
Gulfcoast	3	8	Raised	3	9
Misty	3	8	Raised	3	8
Reville	3	8	Raised	3	6
Tifblue	3	8	Raised	3	7
Gulfcoast	4	8	Raised	1	29
Misty	4	8	Raised	1	30
Tifblue	4	8	Raised	1	31

PROJECT NO: FA05G075

PROJECT TITLE: Biobarrier as a Prophylactic Against Infestation of Containerized and Ball & Burlap Nursery Stock

TYPE REPORT: Final.

LEADER/PARTICIPANTS: Timothy C. Lockley and Lee McAnally

INTRODUCTION:

Over the years, the spread of imported fire ants has been primarily associated with the movement of infested containerized and ball & burlap nursery stock. Various treatment regimens have been developed to prevent these infestations. Although the majority of infestations occur when a newly-mated queen establishes her brood cell at the base of the plant or in the container, some nursery stock is infested by the movement of established colonies. Biobarrier (Reemay Corp., Nashville. TN) was developed as a method of controlling invasive roots in pipes. Lately, it has found numerous uses in the nursery industry for control of weeds in landscaping projects and as root control in field-grown nursery stock. Fire ants are chemically sensitive and respond negatively to even small quantities of various biocides.

A project was established at the Harrison County Work Farm to evaluate Biobarrier as a potential tool in restricting movement of imported fire ants.

MATERIALS AND METHODS:

On 8 May 1995, twenty Biobarrier Root Control Bags were placed in the ground at the Harrison County Work Farm and back filled with soil. Each bag then had a woody ornamental plant transplanted into it. Each bag was positioned in such a way that the inert plastic strip remained above ground and the remainder (the "active" portion of the bag) was below the soil surface. Plants were watered through an underground drip irrigation system and examined monthly for imported fire ant colony activity.

RESULTS:

At 24 months post planting, no fire ant activity had been observed at any of

the plants contained in the Biobarrier bags. Examinations were discontinued in May 1997.

PROJECT NO: FA05G065

PROJECT TITLE: Effect of Mulches on Grape Root Borer and Establishment of Colonies of the Imported Fire Ant in Orchards of Commercially Grown Muscadines

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Timothy C. Lockley/Barbara Smith

INTRODUCTION:

The Grape Root Borer (Vitacea *polistiformis*) of muscadine grapes Vitis *rotundifo7ia* muscadina in the southeastern United States (Olien et al. 1993). The presence of this pest often goes unnoticed by commercial growers until the vines begin to decline in vigor. Grape Root Borer (GRB) is difficult to detect and control because it spends most of its life on the roots and in the crown of muscadine vines. Vines are infected over several seasons and decline may not become evident for several years. Adult GRB emerge in mid-summer and oviposit on the shoots and foliage of muscadines and other plants. The larvae emerge ca. 2 weeks later, fall to the ground and burrow into the soil; then into the roots.

Imported fire ants (IFA), while not a major pest of muscadines, do present unique problems when they infest these plants. The mounds' tend to be built at the base of the plant. When the grapes are harvested mechanically, the ants respond aggressively to the vibrations of the machine and can swarm over the picker and sting the operator. In pick-your-own fields, people harvesting the grapes can unwittingly step into mounds and be subjected to numerous stings. In fields irrigated by constant drip. IFA mounds are often built over the emitters clogging or restricting the flow of the system. Fire ant workers nurse numerous homopteran species including hard scales, soft scales and aphids, all of which can cause significant primary damage to muscadine vines.

MATERIALS AND METHODS:

Muscadine plants were set at 20 foot intervals and trained on wire trellis. Plants are maintained according to MAFES recommendations. Irrigation is as needed via drip lines placed under mulch. Plants are monitored three times a

year for potential phytotoxicity and will be surveyed quarterly for the presence of fireants and weeds. Plants will be assayed for the presence of crown gall bacterium and, at the end of the study (24 months), grape root borer damage will be assessed.

Mulches consists of Pine bark (2-4" depth); black fabric with pine bark and Biobarrier with pine bark. Mulches were applied on raised beds after plants were transplanted. Eight muscadene cultivars were used in the trials: Carlos; Doreen; Jumbo; Magnolia; Noble; Sterling; Summit; and Tarheel.

RESULTS:

Examination of the various replicates throughout 1996 and 1997 showed no indication of fire ant activity among any of the Biobarrier treatments (Table 1).

Table 1. Efficacy of Biobarrier as a Deterrent to the Red Imported Fire Ant.

PLOT	CULTIVAR	MULCH TREATMENT	NUMBER OF RIFA MOUNDS 1996	1997
1	Tarheel	Biobarrier	0	0
2	Carlos	Fabric	5	4
3	Summit	Fabric	4	4
4	Carlos	Bark	4	3
5	Sterling	Bark	3	4
6	Carlos	Biobarrier	0	0
7	Noble	Biobarrier	0	0
8	Carlos	Fabric	2	5
9	Magnolia	Fabric	3	2
10	Carlos	Bark	8	4
11	Jumbo	Biobarrier	0	0
12	Carlos	Bark	2	4
13	Doreen	Bark	1	2

PROJECT NO: FA01G077

PROJECT TITLE: Tennessee Mating Flight Study: Can the Biology of the Imported Fire Ant be used to Aid in Certification of Field Grown Nursery Stock?

TYPE REPORT: Interim

LEADERS/PARTICIPANTS: Homer Collins and Anne-Marie Callcott

COOPERATORS: Drs. Rick Brenner and David Williams, USDA, ARS, CMAVE (Gainesville, FL)
Randy Dodd. Frank Heery and Rick Joyce. Tennessee Dept. of Agriculture (Jackson and Chattanooga. TN)
Dr. Catharine Mannion, Tennessee State University (McMinnville, TN)

INTRODUCTION:

Practical and cost-effective quarantine treatments for certification of field grown/balled and burlapped (B&B) nursery stock are not currently available. Growers wanting to ship their nursery stock outside the IFA quarantined area have several treatment options available, but none are user-friendly or practical. Treatment options that are currently listed in 7CFR 5301.81 include the following:

1. Total immersion (dipping) the root ball in a chlorpyrifos solution
2. Twice daily irrigation of root balls for three consecutive days with a chlorpyrifos solution
3. In-field treatments with a combination of baits plus granular chlorpyrifos

Total immersion of the root balls in insecticidal solutions disrupts the root ball, is logistically unfeasible, and causes worker exposure problems. Other options such as twice daily irrigation of root balls with insecticidal solutions causes problems with run-off and is highly labor intensive. Research into development of practical and cost-effective treatments is a high priority with the American Nursery & Landscape Association, Tennessee Nurserymen's Association, and the Southern Nurserymen's Association.

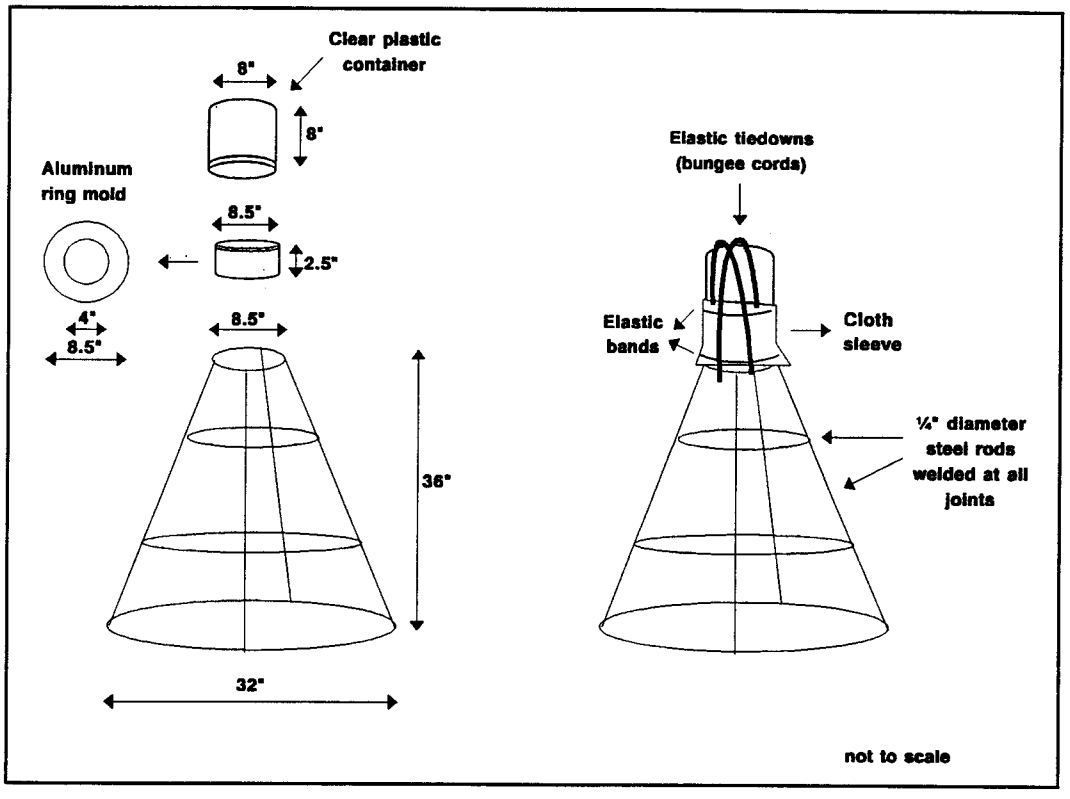
Since imported fire ant (IFA) mating flights occur year-round in most of the infested area, we have historically relied entirely upon contact action

insecticides to certify movement of nursery stock outside the quarantine area. However, it seems likely that as the IFA extends its range to more northerly areas, differences in the biology of the ant may begin to occur. Mating flight cages will be used in a pilot study in Tennessee to determine if a window of opportunity exists in which we can use the biology of the pest in combination with easily applied and highly efficacious baits to certify movement of nursery stock, specifically field grown nursery stock. The Tennessee pilot study was recently initiated in cooperation with ARS, PPQ, Tennessee Department Agriculture, and Tennessee State University. Flight cages were deployed at three sites (East, Central, and Western Tennessee), and will be monitored to determine mating flight patterns. This study is designed to last two or more years, and will be used to correlate weather with emergence, and thereby, use satellite information to develop probability contour maps showing the likelihood of alate emergence at any part of the infested area.

MATERIALS AND METHODS:

Flight traps were supplied by USDA, ARS, CMAVE in Gainesville, FL to the Gulfport Plant Protection Station (GPPS). GPPS repaired and slightly modified traps for use in clay soils of Tennessee. The cages are conical in shape with the frames made of ¼" diameter steel rods (Fig. 1). Fiberglass window screen was inserted in the frame and secured in place with 50 lb. test monofilament fishing line. The cages were placed over active IFA mounds and held in place with 8" aluminum tent pegs. An elastic cotton cloth sleeve (8" diameter) was placed over the top of the cage and the bottom of the sleeve held in place with an elastic band or bungee cord. An aluminum ring mold (cake pan) was placed on top of the cage and filled ca. ½ full with preservative (1:1 mixture of antifreeze and 70% alcohol). The plastic dome covered the ring mold. The cloth sleeve was pulled up over the ring pan onto the plastic dome and held in place with another elastic band. Elastic tiedowns (bungee cords) were criss-crossed over the dome, securing the entire catching apparatus to the wire cage.

Figure 1. Construction of Flight Cages.



Flight traps were placed over 8 mounds in three locations in Tennessee the week of September 8, 1997; Ooltewah (Hamilton Co.), Pulaski (Giles Co.) and La Grange (Fayette Co.), TN. Every two weeks, cooperators count and record the number of male and female alates caught in the preservative in the ring mold of each trap. Alates are discarded after counting. If a mound has moved the trap is also moved. Recorded data is transmitted to the Gulfport Plant Protection Station and ARS, CMAVE.

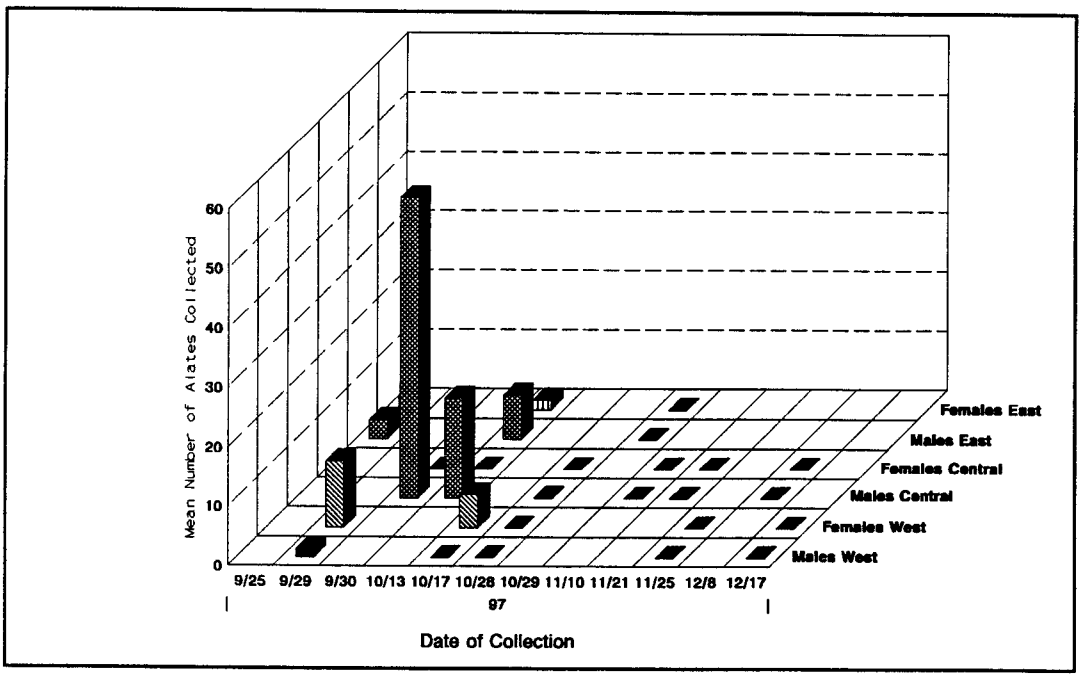
The Gulfport Plant Protection Station will coordinate with collectors, assist in maintenance of traps and transmit collected data to ARS, CMAVE. ARS, CMAVE will correlate flight trap data with satellite information.

RESULTS:

Results show that all sites were producing alates through the middle of October, 1997 (Fig. 2). Both male and female alates were captured at the

eastern Tennessee site in Ooltewah between September 9 (when the traps were set up) and October 13, 1997. No alates have been captured since October 13, 1997. Both male and female alates were also captured at the western site in La Grange also captured between September 10 and September 29, 1997. Between late September and October 17, only females were captured. No alates have been captured since October 17, 1997. Only males were captured at the central Tennessee site in Pulaski between September 9 and October 13; one male was captured between October 13 and October 28, 1997. This was the only site with any alates captured after mid-October. None of the sites captured alates during November and December, 1997.

Figure 2. Average Number of Alates Captured in Flight Traps at Three Sites in Tennessee.



PROJECT NO: FA01G087

PROJECT TITLE: Prevention of IFA Colony Establishment in Hay Bales Stored Outdoors on the Ground

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Homer Collins, Anne-Marie Callcott, Lee McAnally, Charlene Russell

INTRODUCTION:

Baled hay or straw stored in direct contact with the ground is ineligible for movement outside the imported fire ant quarantine area (7CFR Ch. III §301.81-16). Fire ant colonies will nest in baled hay that is left in place for relatively long periods of time. Usually the colonies will seek refuge and invade the bales when the soil moisture is at or above field capacity. We conducted a small field trial to determine if one or more simple, cost effective procedures would prevent IFA nesting in baled hay stored on the ground. We evaluated baiting the storage site prior to moving the hay, and also placing the hay on polyethylene sheeting rather than in direct contact with the ground.

MATERIALS AND METHODS:

The test site was a well drained bahia grass field in Harrison County, Mississippi. The site was part of the Harrison County Prison Farm, and was kept mowed for appearance. Immediately prior to initiating the study, the IFA population was determined by visually counting all colonies within three g-acre plots. Test plots were 16' x 16' with 10' borders on all sides. Round hay bales (4' x 4') were placed in the center of each test plot. There were three treatments, and each treatment was replicated four times. Treatments included Amdro® bait applied with a hand seeder at labelled rate of application (1.5 lb bait/acre), 6-mil black polyethylene sheeting, and an untreated check. The polyethylene sheeting was secured on all four sides with nails driven into the ground. By placing the 4' x 4' bale in the center of 16' x 16' plots, the bales were 6' from the nearest uncovered ground. Bales were visually inspected quarterly for the presence of IFA colonies.

RESULTS:

The IFA population at the test site averaged 56 colonies per acre prior to initiating the test. Hay bales were put in place on August 18, 1997. Polyethylene sheets were positioned before the hay bales were placed. Amdro was applied, as stated above, on August 20, 1997. Three months after treatment (November 17, 1997), no hay bale within either the Amdro or polyethylene were infected with IFA colonies. Two of the four check bales did contain IFA colonies. This study will continue in 1998, and possibly be expanded to include actual rather then simulated conditions.

SECTION II

DEVELOPMENT
QUARA
TREAT

PROJECT NO: FA01G066

PROJECT TITLE: Evaluation of Candidate Chemicals for Control of IFA in Commercial Grass Sod, 1996

TYPE REPORT: Final

LEADER/PARTICIPANTS: Homer Collins, Anne-Marie Callcott, Avel Ladner, Lee McAnally, Tim Lockley and Charlene Russell

COOPERATOR(S): Jack Allegri, Woerner Turf Farm, Elberta. AL

INTRODUCTION:

Currently, the only product available for certification of grass sod under the Federal IFA Quarantine is Dursban® 50-WP, which is applied at a rate of 8 1bs AI/acre. Therefore, our laboratory is continuously looking for other products to add to this use pattern. In 1996, we tested three candidate insecticides for use on grass sod. Fipronil is a relatively new product produced by Rhone-Poulenc Ag. Co. (Research Triangle Park, NC), and is presently marketed in the U.S. for control of mole crickets in sod when applied with a granular slit applicator. Last year, in an unreplicated trial, suSCon® on a starch carrier (10% chlorpyrifos - Crop Care, Brisbane, Australia) showed promising results. Talstar@(FMC Corp., Princeton, NJ) granular and flowable formulations have also shown excellent results in previous trials conducted by this laboratory (FA01G063, FA01G065). Fipronil 0.1G, suSCon 10G, and Talstar granular and flowable were evaluated in 1996.

MATERIALS AND METHODS:

Woerner Turf Farms in Elberta, AL was the site for this trial. All treatments were applied to one-acre test plots on July 2, 1996. Air temperature ranged from 82-92°F and the soil temperature ranged form 75-80°F. Treatments and rates of application are listed below:

Treatment	Formulation	Rate of Application (lb AI/acre)	Rate of Application (lb material/acre
starch suSCon	10% chlorpyrifos	4.0	40
Fipronil-EXP60818A	0.1% fipronil	0.0125	12.5
Talstar granular	0.2% bifenthrin	0.40	200
Talstar flowable*	7.9% bifenthrin	0.20 0.20	1135 ml/acre 1135 ml/acre
Untreated check	----	----	----

* split application applied one week apart per FMC instructions

Applications of fipronil (EXP60818A) were made using a shop-built spreader mounted on a farm tractor. The suSCon and Talstar granular applications were made with a Herd® 96 granular applicator mounted on a farm tractor. The Talstar flowable application was made using a 16-ft roller pump boom sprayer calibrated to deliver 40 gallons of finished spray per acre.

Prior to treatment and at 6 week intervals thereafter, evaluations of IFA populations were made in ¼-acre circular plots in the center of each one-acre test plot, using the procedures described by Lofgren and Williams (1982) and Collins and Callcott (1995). Differences in treatment means were separated by a LSD test (P=0.05).

RESULTS:

Results indicate that all treatments provide significant reduction in population indices and number of colonies at 6 weeks after treatment (Tables 1 & 2). Split applications with Talstar Flowable provided greater than 95% reduction in pretreatment population indices as well as colony mortality at 6 weeks PT, and 100% control at 12, 20 and 28 weeks. The fipronil EXP60818A provided 100% control at 6 and 12 weeks posttreatment (PT), but reinfestation had begun at 20 weeks PT. However, in January, 28 weeks PT, after several bouts of freezing weather, the fipronil plots were again ant free. The suSCon starch granule provided greater than 85% reduction in population and number of colonies at 12 weeks PT. Also at 12 weeks PT, reinfestation was noted on two of the Talstar granular plots, while the other plot remained IFA free. By 20

weeks PT, reinfestation was evident on both the suSCon starch granule and Talstar granule plots.

At the time of the second Talstar Flowable application, 1 week after the initial application, no phytotoxic effects on the sod were noted. At the 6, 12 and 18 week PT counts, no phytotoxic effects were noted on the Talstar Flowable plots, or any other treated plots.

Overall, the Talstar Flowable treatment has provided excellent control for at least 6.5 months (28 weeks), and the Fipronil granule for at least 3 months (12 weeks). Three small mounds were detected on the fipronil plots at 20 weeks PT, but these had disappeared by the 28 week count. This may have been a result of the weather (several periods of successive freezing nights), or a combination of the weather and the chemical. As a comparison, the number of mounds present on the check plots decreased during this time also. The suSCon starch granule did not preform as well as the previously extruded plastic formulation. The Talstar granular formulation provided good control at 6 weeks, but did not provide any residual activity after that time.

The entire field was harvested prior to the 36 week count. Therefore the trial was terminated with the fipronil and Talstar Flowable still providing excellent control. Additional trials with granular fipronil were conducted in 1997. See report FA016027 for details of these trials.

Table 1 . Efficacy of Candidate Grass Sod Treatments - reduction in pretreatment population index.

Treatment	Rate of Application (lb material/ acre)	Mean % change in population index at indicated wks PT			
		(6)	(12)	(20)	(28)
Fipronil EXP60818A	12.0	-100a	-100a	-82.3a	-100a
Talstar flowable	2270 ml/acre	-99.2a	-100a	-100a	-100a
Talstar granular	200.0	-95.1a	-39.5a	78.2ab	--
starch suSCon	40.0	-77.4a	-87.7a	-74.9a	--
Untreated check	----	-39.3b	85.3b	207.6b	-37.5b

Means within a column followed by the same letter are not significantly different according to a LSD test (P=0.05).

Field harvested prior to 36 week count.

Table 2 . Efficacy of Candidate Grass Sod Treatments - colony kill.

Treatment	Rate of Application (lb material/ acre)	Mean % decrease in number of colonies at indicated wks PT			
		(6)	(12)	(20)	(28)
Fipronil EXP60818A	12.0	100a	100a	83.3ab	100a
Talstar flowable	2270 ml/acre	95.2a	100a	100a	100a
Talstar granular	200.0	90.5a	52.4bc	0.0c	--
starch suSCon	40.0	77.3a	85.9ab	66.4b	--
Untreated check	----	32.1b	12.1c	0.0c	21.2b

Means within a column followed by the same letter are not significantly different according to a LSD test (P=0.05).

Field harvested prior to 36 week count.

PROJECT NO: FA01G027

PROJECT TITLE: Multi-State Field Trials with Fipronil Bait (EXP 61443A) and Chipco® (EXP 60818A) for Control of Imported Fire Ants (IFA), Cutworms (Noctuidae), and Ground Pearls (Margarodes spp.)

TYPE REPORT: Interim

LEADERS/PARTICIPANTS: Homer Collins, Anne-Marie Callcott, Avel Ladner, Lee McAnally, and Charlene Russell

COOPERATORS: Rhone-Poulenc AG Company; Hattiesburg, MS Airport; Camden, AR Airport; La Porte, TX Airport; University of Arkansas; Texas Extension Service; Texas Dept. Agriculture

INTRODUCTION:

Fipronil is a relatively new broad spectrum pyrazole insecticide currently under development by Rhone-Poulenc Ag Company, Research Triangle Park, NC. Fipronil is registered in the U.S. as Chipco® Choice™ (0.1% AI granule) for control of mole crickets on golf courses. Fipronil has demonstrated potent insecticide and acaricide properties on a large number of pests including fleas, ticks, boll weevils, thrips, flies, and others (Colliot et al. 1992. Burris et al, 1994, Postal et al. 1995, Searle et al. 1995). We tested fipronil as a bait toxicant in 1996 and found it to be effective for control of IFA (Collins and Callcott, in press). Chipco® Choice™ applied at 12.5 lbs formulated material per acre provided 100% control of IFA at 6 and 12 weeks after application, but plots became reinfested by the 20th week (Collins et al., unpublished data). These trials demonstrated the potential for both fipronil bait (EXP 61443A-15 ppm ai on defatted corn grit + 20% vegetable oil) and Chipco® Choice™ for control of IFA. Larger, and more extensive trials with both fipronil bait and Chipco® EXP 60818A (0.1% AI granule) were needed to confirm our preliminary results.

MATERIALS AND METHODS:

Test sites were located on airports in Camden, AR, Hattiesburg, MS, and La Porte, TX. All test plots were 0.8 acre in size, with a ¼ acre efficacy subplot located in the center of the test plots. Bait applications were made with a shop-built granular applicator mounted on a farm tractor. Chipco® EXP 60443A was applied with a Herd@Granular Applicator mounted on a farm tractor.

Liquid treatments (Dursban® 50WP) were applied with a roller pump boom sprayer. The spray rig was equipped with three TKSS tips which provided a 10 ft. swath. The system was operated at 50 psi providing 37.5 gallons of finished spray per acre. Each treatment was replicated three times. Rates of application, and formulations tested are as follows:

TREATMENT	RATE OF APPLICATION
EXP 61443A bait (15 ppm ai)	1.5 lbs formulated material/acre
Commercial standard bait (Amdro®)	1.5 lbs formulated material/acre
Chipco® EXP 60818A (0.1G)	12.5 lbs formulated material/acre
Chipco® EXP 60818A (0.1G)	18.75 lbs formulated material/acre
Chipco® EXP 60818A (0.1G)	25.0 lbs formulated material/acre
Commercial std. (Dursban® 50WP)	16.0 lbs formulated material/acre
Untreated check	-

Treatment dates were May 21, 1997 in Hattiesburg, MS with the air temperature at 75-85°F, the soil temperature at 72-80°F, and the soil moist; June 11, 1997 in Camden, AR with the air temperature at 72-82°F, the soil temperature at 72-76°F, and the soil moist; and June 18, 1997 in La Porte, TX with the air temperature at 80-89°F, the soil temperature at 80°F, and the soil moist.

Temperature and rainfall data for all treatment sites was acquired from the National Climatic Data Center, Asheville, NC.

Imported Fire Ant Populations - Imported fire ant population were evaluated primarily at the Arkansas site, but also at the other sites as they occurred. Population counts were made before application and at 6 week intervals thereafter using the population indexing system described by Lofgren and Williams (1982). Colony mortality and changes in pretreatment population indices were calculated. Data was statistically analyzed by ANOVA and treatment means separated by LSD test ($P \leq 0.05$) for each posttreatment rating interval.

Cutworm Populations - Cutworm populations were evaluated at the Mississippi site by excavating 6 in^3 areas within the ¼ acre circular subplot and examining the dirt for presence of cutworms. There were 5-15 examinations per

plot.

Ground Pearl Populations - Ground pearl populations were evaluated at the Texas site by excavating 6 in^3 areas within the ¼ acre circular subplot and examining the dirt for presence of cutworms. There were 5-15 examinations per plot.

RESULTS AND DISCUSSION:

Imported Fire Ant Populations

Arkansas site: The Camden, AR site was composed of Cohoba fine sandy loam soil and was heavily infested with imported fire ants. Initial colony counts at this site, averaging 204 colonies/acre, indicated that this may be a polygynous site. Exhumation and examination indicated that over half of the colonies examined had multiple de-alated females with worker ants congregating around said females. Thus this site was considered a polygynous IFA site.

Granular formulations of fipronil have provided excellent control of IFA 24 weeks after treatment, providing >92% reduction in colony numbers throughout the period and >95% reduction in population indices (Tables 1 & 2). There were no statistical differences in the various rates of fipronil. Control with fipronil was not different from the Dursban standard until 24 weeks after treatment, at which time all fipronil rates were maintaining significantly better reduction of IFA colonies and populations indices.

The fipronil bait formulation numerically outperformed the Amdro standard throughout the trial. Both baits provided >90% reduction in colony numbers and population indices at 6 and 12 weeks after treatment (Tables 3 & 4). Reinfestation of plots treated with both baits began at the 18 week evaluation, however, reinfestation of the Amdro plots was much faster than that of the fipronil plots.

The untreated control (check) plots experienced heavy losses in number of colonies and population indices (Tables 1, 2, 3 & 4). This is a fairly normal cycle for the deep south (FA05G013), but usually not seen this prominently in more temperate areas of the IFA infestation.

Mississippi site: No cutworms were observed in any of the plots before or after insecticide applications.

However, the Hattiesburg, MS site, comprised of Trebloc silt loam soil, was lightly infested with IFA. Initial colony counts averaged 29.3 colonies/acre, indicating a monogynous IFA site.

All rates of the granular fipronil provided >91% reduction of colony numbers and >94% reduction in population indices through 24 weeks after treatment (Tables 5 & 6). There were no statistical differences between the various rates of fipronil and the Dursban standard.

The fipronil bait formulation decreased colony numbers by more than 83% over an 18 week period and reduced population indices by more than 94% over the same time period (Table 7 & 8). By the 24 week evaluation, reinfestation with small incipient colonies had occurred.

Texas site: No ground pearls were observed in any of the plots before or after insecticide applications.

However, the La Porte, TX site, comprised of Bernard clay loam soil, was moderately infested with IFA. Initial colony counts averaged 39.2 colonies/acre, indicating that this site was monogynous.

Again, excellent control was obtained with the fipronil granular formulations. The 12.5 lb/acre rate provided 93-100% reduction of IFA colonies and population indices through 18 weeks (Tables 9 & 10). However, by the 24 week evaluation, reinfestation was noted for this rate as well as the Dursban standard. Rates of 18.75 and 25.0 lb/acre provided 100% control throughout the 24 week evaluation period. While these two rates were not significantly better than the lower fipronil rate or the Dursban standard due to large variations in plot counts, numerically they provided greater control of IFA.

The fipronil bait formulation decreased colony numbers by more than 93% over an 18 week period and also reduced population indices by more than 93% over the same time period (Table 11 & 12). By the 24 week evaluation, reinfestation with small incipient colonies had occurred.

CONCLUSIONS:

These results clearly indicate that 0.1% granular fipronil (EXP 60818A) applied at rates of 12.5, 18.75 and 25.0 lbs formulated material per acre effectively controls IFA in turfgrass. Test plots will continue to be monitored to determine length of residual activity provided by the higher rates of application. Fipronil formulated as a 15 ppm bait on defatted corn grit carrier (EXP 61443A) provided control equivalent to the Amdro standard.

Table 1. Control of Imported Fire Ants in Turfgrass with Fipronil 0.1% Granular - Decrease in Number of Colonies; Camden, AR. Airport

Treatment	Rate of Application (lb/acre)	Mean No. Colonies Pretreat (per acre)	% decrease in number of colonies at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP60818A 0.1G	12.5	232.0	98.9a	99.5a	98.2a	100.0a
EXP60818A 0.1G	18.75	132.0	96.1a	100.0a	92.4a	100.0a
EXP60818A 0.1G	25.0	181.3	99.2a	100.0a	97.0a	98.4a
Dursban	16.0	222.7	100.0a	100.0a	91.5a	88.5b
Check	--	142.7	53.5b	85.6b	61.8b	20.5c

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

Table 2. Control of Imported Fire Ants in Turfgrass with Fipronil 0.1% Granular - Change in Population Index; Camden, AR. Airport

Treatment	Rate of Application (lb/acre)	Mean Pop. Index Pretreat (per acre)	% change in population index at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP 60818A 0.1G	12.5	3257.3	-99.7a	-99.9a	-99.3a	-100.0a
EXP 60818A 0.1G	18.75	1638.7	-98.2a	-100.0a	-95.4a	-100.0a
EXP 60818A 0.1G	25.0	2297.3	-99.9a	-100.0a	-98.3a	-99.6a
Dursban	16.0	3100.0	-100.0a	-100.0a	-92.9a	-92.3b
Check	--	1873.3	-58.1b	-88.2b	-66.3b	-32.0c

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

Table 3. Control of Imported Fire Ants in Turfgrass with Fipronil 0.0015% Bait - Decrease in Number of Colonies; Camden, AR. Airport

Treatment	Rate of Application (lb/acre)	Mean No. Colonies Pretreat (per acre)	% decrease in number of colonies at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP61443A bait	1.5	314.7	97.3a	99.3a	96.7a	92.4a
Amdro	1.5	202.7	90.3a	94.7a	77.2b	74.2a
Check	--	142.7	53.5b	85.6a	61.8b	20.5b

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

Table 4. Control of Imported Fire Ants in Turfgrass with Fipronil 0.0015% Bait - Change in Population Index; Camden, AR. Airport

Treatment	Rate of Application (lb/acre)	Mean Pop. Index Pretreat (per acre)	% change in population index at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP61443A bait	1.5	4142.7	-98.8a	-99.9a	-96.9a	-94.9a
Amdro	1.5	2900.0	-90.6a	-96.3a	-80.1b	-79.4a
Check	--	1873.3	-58.1b	-88.2a	-66.3b	-32.0b

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

Table 5. Control of Imported Fire Ants in Turfgrass with Fipronil 0.1% Granular - Decrease in Number of Colonies; Hattiesburg, MS. Airport

Treatment	Rate of Application (lb/acre)	Mean No. Colonies Pretreat (per acre)	% decrease in number of colonies at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP60818A 0.1G	12.5	26.7	95.8a	95.2a	100.0a	95.8a
EXP60818A 0.1G	18.75	30.7	100.0a	100.0a	100.0a	100.0a
EXP60818A 0.1G	25.0	32.0	100.0a	95.8a	95.8a	91.7a
Dursban	16.0	29.3	100.0a	100.0a	100.0a	100.0a
Check	--	29.3	3.3b	33.3b	25.6b	31.1b

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

Table 6. Control of Imported Fire Ants in Turfgrass with Fipronil 0.1% Granular - Change in Population Index; Hattiesburg, MS. Airport

Treatment	Rate of Application (lb/acre)	Mean Pop. Index Pretreat (per acre)	% change in population index at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP60818A 0.1G	12.5	360.0	-99.4a	-99.3a	-100.0a	-97.0a
EXP60818A 0.1G	18.75	413.3	-100.0a	-100.0a	-100.0a	-100.0a
EXP60818A 0.1G	25.0	426.7	-100.0a	-99.4a	-99.0a	-94.2a
Dursban	16.0	413.3	-100.0a	-100.0a	-100.0a	-100.0a
Check	--	380.0	43.7b	-46.1b	-34.8b	-15.8b

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

Table 7. Control of Imported Fire Ants in Turfgrass with Fipronil 0.0015% Bait - Decrease in Number of Colonies; Hattiesburg, MS. Airport

Treatment	Rate of Application (lb/acre)	Mean No. Colonies Pretreat (per acre)	% decrease in number of colonies at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP61443A bait	1.5	24.0	88.9a	83.3a	94.4a	50.0a
Amdro	1.5	33.3	100.0a	100.0a	100.0a	92.6a
Check	--	29.3	3.3b	33.3b	25.6b	31.1a

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

Table 8. Control of Imported Fire Ants in Turfgrass with Fipronil 0.0015% Bait - Change in Population Index; Hattiesburg, MS. Airport

Treatment	Rate of Application (lb/acre)	Mean Pop. Index Pretreat (per acre)	% change in population index at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP61443A bait	1.5	340.0	-97.5a	-94.2a	-98.8a	-25.8a
Amdro	1.5	453.3	-100.0a	-100.0a	-100.0a	-94.7a
Check	--	380.0	43.7b	-46.1b	-34.8b	-15.8a

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

Table 9. Control of Imported Fire Ants in Turfgrass with Fipronil 0.1% Granular - Decrease in Number of Colonies; La Porte, TX. Airport

Treatment	Rate of Application (lb/acre)	Mean No. Colonies Pretreat (per acre)	% decrease in number of colonies at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP60818A 0.1G	12.5	34.7	93.3a	100.0a	100.0a	73.3a
EXP60818A 0.1G	18.75	34.7	100.0a	100.0a	100.0a	100.0a
EXP60818A 0.1G	25.0	37.3	100.0a	100.0a	100.0a	100.0a
Dursban	16.0	48.0	100.0a	97.0a	89.8a	62.1a
Check	--	37.3	53.3b	37.1b	4.8b	0.0b

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

Table 10. Control of Imported Fire Ants in Turfgrass with Fipronil 0.1% Granular - Change in Population Index; La Porte, TX. Airport

Treatment	Rate of Application (lb/acre)	Mean Pop. Index Pretreat (per acre)	% change in population index at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP60818A 0.1G	12.5	586.7	-99.1a	-100.0a	-100.0a	-75.6a
EXP60818A 0.1G	18.75	522.7	-100.0a	-100.0a	-100.0a	-100.0a
EXP60818A 0.1G	25.0	452.0	-100.0a	-100.0a	-100.0a	-100.0a
Dursban	16.0	889.3	-100.0a	-99.5a	-91.6b	-73.9a
Check	--	513.3	-51.0b	-42.3b	+9.3c	+189.6b

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

Table 11. Control of Imported Fire Ants in Turfgrass with Fipronil 0.0015% Bait - Decrease in Number of Colonies; La Porte, TX. Airport

Treatment	Rate of Application (lb/acre)	Mean No. Colonies Pretreat (per acre)	% decrease in number of colonies at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP61443A bait	1.5	42.7	86.9a	90.5a	93.3a	68.5a
Amdro	1.5	40.0	90.8a	100.0a	97.9a	58.8ab
Check	--	37.3	53.3b	37.1b	4.8b	0.0b

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

Table 12. Control of Imported Fire Ants in Turfgrass with Fipronil 0.0015% Bait - Change in Population Index; La Porte, TX. Airport

Treatment	Rate of Application (lb/acre)	Mean Pop. Index Pretreat (per acre)	% change in population index at indicated weeks PT*			
			(6)	(12)	(18)	(24)
EXP61443A bait	1.5	702.7	-96.5a	-97.7a	-93.3a	-70.1a
Amdro	1.5	693.3	-98.4a	-100.0a	-98.7a	+9.7ab
Check	--	513.3	-51.0b	-42.3b	+9.3b	+189.6b

* means within a column followed by the same letter are not significantly different, LSD test, P=0.05.

PROJECT NO: FA02G017

PROJECT TITLE: Efficacy of Various Fenoxycarb Formulations, 1997

TYPE REPORT: Interim

LEADERS/PARTICIPANTS: Homer Collins, Anne-Marie Callcott, Avel Ladner. Lee McAnally, Charlene Russell

INTRODUCTION:

Logic® is a commercially available fire ant bait containing the insect growth regulator fenoxycarb (1%) as the active ingredient, and is produced by Novartis Crop Protection, Inc. (Greensboro, NC). In 1997, Novartis supplied us with several different formulations of the fenoxycarb-based bait for field testing. All baits were comprised of corn grit, soybean oil and 1% fenoxycarb.

MATERIALS AND METHODS:

Four baits were sent to us as numbered formulations. One was the standard commercially available formulation, the other three were proprietary formulations. All baits were applied to 1-acre test plots at a rate of 1.5 1b/acre on June 24, 1997 using a shop-built spreader mounted on a farm tractor. Prior to treatment and at 6 week intervals thereafter, evaluations of IFA populations were made in ¼-acre circular plots in the center of each one-acre test plot, using the procedures described by Lofgren and Williams (1982) and Collins and Callcott (1995). Differences in treatment means were separated by a LSD test (P=0.05).

RESULTS:

All treatments showed a significant decrease in populations at 6 weeks after treatment, but reduction in number of colonies was not significantly different from the check (Tables 1 & 2). By 12 weeks after treatment, there was >91% reduction in populations and >97% reduction in colony numbers in all treatments. Control has remained high (>80%) in all treatments through 24

weeks after treatment. Further evaluations will be made in the spring of 1998.

Table 1. Efficacy of Various Fenoxycarb Formulation on Field Populations of Imported Fire Ants, 1997 - decrease in number of colonies.

Treatment	Mean No. Colonies - Pretreat (per acre)	% decrease in number of colonies (wks posttreat)			
		(6)	(12)	(18)	(24)
FL971038	61.2	68.5a	94.4a	100.0a	100.0a
FL971039	52.0	67.8ab	91.0a	88.7ab	100.0a
FL971040	57.2	66.8ab	95.1a	81.2b	90.4b
FL971041	80.0	62.9ab	95.8a	95.8ab	89.4b
CHECK	56.0	48.2b	45.0b	39.2c	0.0c

LSD test, P=0.05

Table 2. Efficacy of Various Fenoxycarb Formulation on Field Populations of Imported Fire Ants, 1997 - change in pretreat population index.

Treatment	Mean Pop. Index - Pretreat (per acre)	% change in population index (wks posttreat)			
		(6)	(12)	(18)	(24)
FL971038	993.2	-94.7a	-97.9a	-100.0a	-100.0a
FL971039	996.8	-95.6a	-99.1a	-97.6a	-100.0a
FL971040	1244.0	-95.8a	-99.3a	-95.1a	-98.9a
FL971041	1413.2	-95.0a	-99.6a	-97.4a	-96.7a
CHECK	913.2	-50.5b	-57.6b	-44.9b	-0.2b

LSD test, P=0.05

SECTION III

POPULATION SUPPRESSION

PROJECT NO: FA02G026

PROJECT TITLE: Evaluation of New Bait Products for Control of Imported Fire Ants

REPORT TYPE: Final

LEADER/PARTICIPANTS: Homer Collins, Anne-Marie Callcott, Lee McAnally, Avel Ladner and Tim Lockley

INTRODUCTION:

In 1996, we evaluated two relatively new bait products. Fipronil (common name) is produced by Rhone-Poulenc Ag. Co. (Research Triangle Park, NC) and is currently marketed in numerous countries for control of many insect pests in a variety of crops. It is also labeled in Germany as a bait against cockroaches and ants for use by homeowners. Currently, the only U.S. registration for the product is for mole cricket control on golf courses. Several laboratory trials had previously shown that fipronil has excellent potential as a fire ant bait (see internal report on fipronil dated December 1995). This season Rhone-Poulenc produced a 15 ppm fipronil bait on the traditional pregelled corn grit carrier, using corn oil as the attractant.

Valent USA (Walnut Creek, CA) provided a 0.5% pyriproxyfen bait for these trials.

MATERIALS AND METHODS:

The test site was located in Harrison Co., MS. All baits were applied to test plots using a shop-built granular applicator on a farm tractor on June 18, 1996. There were 3 replicates per treatment. The fipronil bait was applied at rates of 1.5 and 3.0 lb/acre. The pyriproxyfen bait was applied at 1.0 and 1.5 lb/acre. Logic® and Amdro® were each applied as standards at 1.5 lb bait per acre. All test plots were 1.0 acre in size except for the fipronil plots which were 0.5 acres each.

A ¼-acre circular efficacy plot was established in the center of each 0.5 or 1.0 acre test plot. Prior to application and at 6 week intervals thereafter, IFA populations in each efficacy plot were evaluated using the population

index system developed by Harlen et al. (1981) and revised by Lofgren and Williams (1982). Using this data, both colony mortality and decrease in population index were calculated. Experimental data were statistically analyzed using analysis of variance and means separated using Tukey's test (P=0.05) for each posttreatment rating interval.

RESULTS:

At 6 and 12 weeks posttreatment (PT), all treatments provided excellent control, 88 - 98% decrease in population indices (Table 1 & 2). Prior to the 18 week count, the land owner lightly disked many of the treated areas, and as a result, one Amdro and one check replicate were not included in the 18 week count, but were included in the following counts. Therefore, means for the Amdro and check evaluations at 18 weeks include 2 replicates, rather than 3. All the Logic and pyriproxyfen plots were also disked, but all were included in the counts. However, the results are probably biased due to the disturbance. At 18 weeks PT, both pyriproxyfen rates and the Logic treatment provided 100% control of IFA. As stated above, this is probably a result of the disturbance caused by the owner disking the plots as well as the action of the treatments. Also at 18 weeks PT, the 1.5 lb fipronil rate provided 94.2% reduction in pretreatment population indices and 87.5% reduction in pretreatment colony numbers. The 3.0 lb rate of fipronil and the Amdro standard were both reinfested with healthy colonies 18 weeks after treatment.

By 24 weeks PT, the fipronil 1.5 lb rate, the Logic and the pyriproxyfen plots were reinfested with small class 7 incipient colonies.

We have previously reported the importance of using RIFA test plots at least 1.0 acre in size. Plots this large (1.0 acre) provide a treated buffer area of 14.2 m (46.6 ft), which minimizes colony relocation into the ¼ acre efficacy plots from adjacent untreated areas (Collins & Callcott 1995). However, treated buffers as large as 75 m do not necessarily prevent all movement from outside the treated area since Callcott & Collins (1992) noted the appearance of large, mature colonies in treated test plots with a 75 m treated buffer approximately 16 weeks PT. As stated in the materials and methods above, plot size for the fipronil plots was 0.5 acres. This was due

to limited availability of the experimental bait, and therefore, these plots had treated buffers of only 4.8 m (15.7 ft). At 6 and 12 weeks PT, the few remaining colonies in fipronil plots did not contain worker brood, indicating abnormal colonies and the absence of a functioning queen. However, at 18 weeks PT, all of the plots treated at the 3.0 lb rate contained 1-4 large, normal colonies (colony class 8 according to the population index scale of Lofgren & Williams 1982). While it is possible for these colonies to have developed from newly mated queens entering the treated area just after treatment, it is more likely that the entire colonies simply migrated into test plots from the adjacent untreated area. Adoption of newly mated queens by surviving broodless colonies could also account for the sudden appearance of category 8 nests within the test plots.

Ultimately, fipronil and pyriproxyfen candidate baits controlled IFA in the field as well as the standard commercial baits.

Table 1. 1996 Bait Trials - population index change.

Treatment	Rate of Applic.- lb/acre	% change in population indices at indicated wks PT*					
		(6)	(12)	(18)	(24)	(33)	(41)
Fipronil	1.5	-96.6a	-96.9a	-94.2ab	-67.1a	--	--
Fipronil	3.0	-97.3a	-96.2a	-81.7b	-62.8a	--	--
Pyriproxyfen	1.0	-92.9a	-97.6a	-100a	-95.5a	-98.2a	-84.3a
Pyriproxyfen	1.5	-90.8a	-97.7a	-100a	-98.8a	-97.5a	-76.8a
Logic	1.5	-88.5a	-98.8a	-100a	-97.0a	-96.6a	-80.8a
Amdro	1.5	-90.1a	-88.3a	-82.4b	-76.0a	--	--
Untreated Check	---	-36.3b	-28.2b	12.0c	54.4b	63.6b	150.7b

* means within a column followed by the same letter are not significantly different (Tukey's test, P=0.05)

Table 2. 1996 Bait Trials - decrease in number of colonies.

Treatment	Rate of Applic.- lb/acre	% decrease in no. of colonies at indicated wks PT*					
		(6)	(12)	(18)	(24)	(33)	(41)
Fipronil	1.5	81.9a	87.5a	87.4a	61.6ab	--	--
Fipronil	3.0	84.1a	84.6a	68.6b	56.9b	--	--
Pyriproxyfen	1.0	65.2a	83.3a	100a	91.8ab	94.1a	74.1a
Pyriproxyfen	1.5	63.5a	84.9a	100a	98.4a	86.1a	66.2a
Logic	1.5	65.3a	92.4a	100a	96.5a	93.1a	79.6a
Amdro	1.5	77.4a	79.3a	79.6ab	76.1ab	--	--
Untreated Check	---	47.5a	36.3b	3.6c	0.0c	0.0b	0.0b

* means within a column followed by the same letter are not significantly different (Tukey's test, P=0.05)

PROJECT NO: FA02G036

PROJECT TITLE: Efficacy of AC 303,630 as an Imported Fire Ant Bait

PROJECT TYPE: Final

PROJECT LEADER/PARTICIPANT(s): Anne-Marie Callcott, Lee McAnally, Charlene Hebert

INTRODUCTION:

AC 303,630 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. American Cyanamid conducted some preliminary trials against imported fire ants with the product formulated into a bait and showed promising results. Therefore, in 1996, our laboratory began screening the product for use as a bait against IFA. Stringer et al. (1964) noted that an effective bait toxicant must: (1) exhibit delayed kill over at least a ten-fold dosage range and preferably above a 100-fold dosage range, (2) be rapidly transferred from one ant to another via trophallaxis and kill the recipient, and (3) not be repellent to foraging ants.

MATERIALS AND METHODS:

Bait' Tria7s

Laboratory testing with field collected RIFA workers - AC 303,630 was tested as a bait toxicant for control of red imported fire ants (RIFA) in the laboratory using techniques and procedures described by Lofgren et al. (1967). A 0.1% stock solution was prepared by adding 0.1 g technical material to 99.1 g Crisco® vegetable oil. The mixture was vigorously shaken and slowly heated to approximately 43°C. This process appeared to form saturated solution. Concentrations of 25, 50. 75, 100 and 150 ppm were then prepared from the stock solution by serial dilutions with pure Crisco vegetable oil. All dilutions appeared to form true solutions.

Each concentration, plus an untreated check (0 ppm), was tested against field collected RIFA workers in the laboratory. Four replicates of each treatment were tested. Each replicate consisted of 20 RIFA workers (mix of minors and

majors), confined in test chambers consisting of 5 cm x 5 cm plastic flower pots that had been furnished with a Labstone® (Bayer Corp. Dental Products, South Bend, IN) bottom, which wicked moisture from an underlying bed of damp peat moss. This arrangement provided a confined area with high humidity, which is necessary for survival of the ants. The vegetable oil baits were offered to the ants by soaking 2 mm cotton balls with each concentration prior to placing in the test chambers. The cotton balls were removed from the test chambers after a 24 hr feeding period. The ants were thereby provided a free choice to either feed or not feed upon each bait concentration. Mortality was assessed at 0, 1, 3, 5, 7 and 14 days after treatment.

Laboratory trials with whole RIFA colonies - Baits formulated by American Cyanamid for laboratory testing included rates of 5, 10, 25, 50 and 75 ppm in the first trial initiated on July 15, 1996, and rates of 100 and 150 ppm in a second trial initiated on August 28, 1996. An Amdro standard and a non-toxic bait standard (30:70 volume to volume ratio of soybean oil to corn grit carrier) were included in both trials. Field collected RIFA colonies were confined in 12-liter plastic pails and allowed to acclimate in the laboratory for 5 days prior to testing. Water was provided as needed, but no food was offered prior to testing. Queen status was not determined but each colony contained several thousand workers, immatures, and alates. Five grams of formulated bait contained in a petri dish was placed on the surface of each of 3 colonies (replicates) for each bait concentration tested. The ants were allowed to feed *ad. lib.* on the baits for 24 hours at which time the petri dishes were removed and the amount of bait removed by the ants was recorded. Colonies were maintained in the laboratory under ambient temperature, watered as needed, and provided peanut butter and live mealworms as food. Quantitative data was not recorded, but colonies were observed weekly for 12 weeks. Behavioral changes and mortality were noted.

Laboratory bait acceptance trial - Using the bait formulated by American Cyanamid, a bait acceptance trial was initiated with the 50, 75, 100 and 150 ppm AC 303,630 baits. This type of trial compares a candidate (toxic) bait to a non-toxic standard bait or to a toxic standard bait such as Amdro. In this trial we compared the AC 303,630 baits to a non-toxic standard and to an Amdro standard. Field collected RIFA colonies were allowed to acclimate in the laboratory approximately 5-7 days. A control bait was prepared by mixing

fresh soybean oil and pregelled defatted corn grits in a 30:70% weight to weight ratio. Four grams of a candidate bait contained in a petri dish were placed on the surface of each of five test colonies. Simultaneously, four grams of the standard bait (either non-toxic standard or Amdro standard) 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 removed by the ants was determined by weighing. Differences between the amount of bait removed was compared using a t-test (P=0.05).

Over the years, we have found that the laboratory bait acceptance test tends to give varied and inconsistent results. Therefore, while the test provides a good indication whether a bait is acceptable to RIFA, field testing must occur to ensure that the bait is truly attractive to ants in a situation where there are many available food sources.

Field testing - Based on laboratory trials, a small field trial was initiated in the fall of 1996. The test site was located in Harrison Co., MS. All baits were applied on October 9, 1996 to 1.0 acre test plots using a shop-built granular applicator mounted on a farm tractor. There were 3 replicates per treatment. Treatments included AC 303,630 at rates of 50, 75 and 100 ppm applied at a rate 1.5 lb/acre, an Amdro® standard applied at 1.5 lb/acre, and an untreated check.

A 0.25 acre circular efficacy plot was established in the center of each 1.0 acre plot. Prior to application and at 4 week intervals thereafter, RIFA populations in each efficacy plot were evaluated using the population indexing system described by Lofgren and Williams (1982). Both colony mortality and changes in population indices were calculated. Experimental data were statistically analyzed using analysis of variance and means were separated using Tukey's test (P=0.05) for each posttreatment rating interval.

RESULTS:

Laboratory testing with field collected RIFA workers - As shown in Figure 1, rates of 25-75 ppm produced <10% mortality in RIFA workers during the 14 day test period. The 100 ppm rate produced 68% mortality at 14 days after exposure, while the 150 ppm rate gave 90% mortality at 14 days. These two rates showed the necessary delayed toxicity, but did not achieve 100% mortality within 14 days.

Laboratory trials with whole RIFA colonies - Colonies removed all AC 303,630 bait at rates of 5 to 75 ppm and the non-toxic bait in the first trial within 24 hours (Table 1). The higher rates (100-150 ppm) appeared to be progressively less attractive to the ants. One replicate removed all the Amdro standard, while the other two replicates removed 62-76% of the available bait in the 24 hour access period. Remaining bait was removed and discarded.

Visual results over the 12 week trial period indicated that rates of 50 - 150 ppm adversely affected whole colonies of RIFA, however, results were not consistent nor were they as definitive as Amdro.

Laboratory bait acceptance trial - As shown in Table 2, there was no significant difference in the amount of AC 303,630 bait removed compared to a standard in any of the comparisons. Numerically, the ants did appear to accept the 50 and 75 ppm rates of the AC 303,630 bait as well as or better than either the non-toxic standard or the Amdro standard. The higher rates, 100 and 150 ppm, were accepted less than the non-toxic standard but more than the Amdro standard.

Field testing - Fall applications of RIFA baits are not always as effective as those made in the spring due to the biology of the insect. At 4 weeks after treatment, none of the AC 303,630 bait formulations had significantly reduced populations or colony numbers (Tables 3 & 4). By 8 weeks after treatment, all of the AC 303,630 treatment plots had increases in population indices and colony numbers. We did evaluate the plots in late winter (Feb., 1997), 17 weeks after treatment. While population indices in all treatments and the check had drastically decreased (winter kill), there was no significant

difference between the AC 303,630 treatments and the check. Colony numbers had not decreased significantly either. The Amdro standard was significantly different from the check in both population indices and colony numbers.

CONCLUSIONS:

While laboratory trials were encouraging, providing 100% mortality of colonies or greatly diminished colonies with rates of 50-100 ppm, field trials with these rates when applied at 1.5 lb material/acre were unsuccessful.

Figure 1. Toxicity of AC 303,630/Crisco oil baits to RIFA workers.

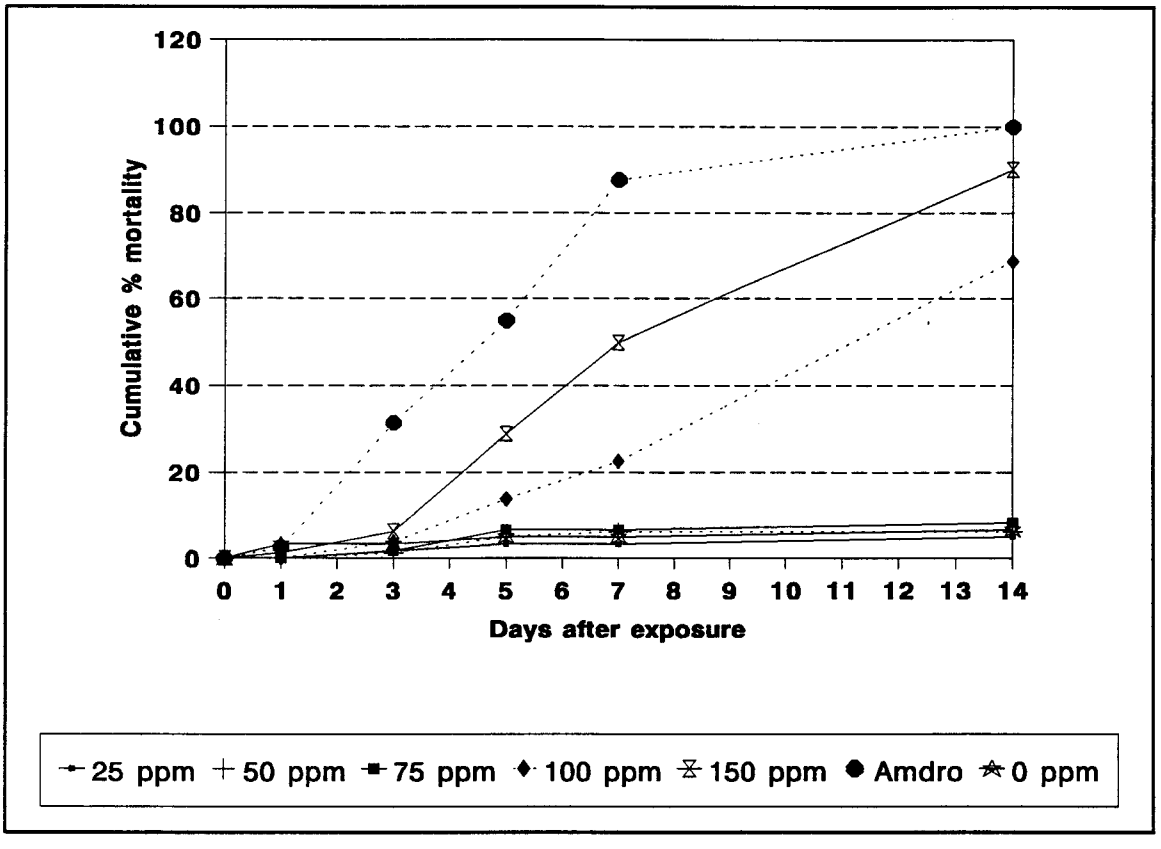


Table 1. Subjective results with various concentrations of AC 303,630 baits against whole RIFA colonies in the laboratory.

Subjective Criteria	AI Concentration (ppm)								
	5.0	10.0	25.0	50.0	75.0	100 ppm*	150 ppm	Amdro	0.0 (Check)
Mean amt. bait removed in X no. hours	5 g in 24 hr	5 g in 24 hr	5 g in 24 hr	5 g in 24 hr	5 g in 24 hr	4.6 g in 24 hr	3.4 g in 24 hr	4.0 g in 24 hr	5 g in 24 hr
Feeding habits	normal	normal thru 2 mth; progressively fewer and less responsive thru 3 mth	normal thru 2 mth; progressively fewer and less responsive thru 3 mth	normal thru 1½ mth; progressively fewer and less responsive thru 3 mth	survivors less actively feeding over time	minimal within 2 wks	minimal within 2 wks	minimal within 3 days	normal
Colony maintenance	normal	same as feeding habits	same as feeding habits	same as feeding habits	decreased over time	minimal within 2 wks	minimal within 2 wks	minimal within 3 days	normal
Dead workers	typical small numbers	typical thru 2 mth; then increased thru 3 mth	typical thru 2 mth; then increased thru 3 mth	larger than checks and increased over time	much larger than checks and increased over time	increased over time	increased over time	minimal (ants probably too sick to remove dead)	typical small numbers
No. of weeks until mortality	survived test period	+500 survived test period	+500 survived test period	2 reps dead within 7 wks; one with <50 workers survived test	2 reps dead within 7 wks; one with +100 workers survived test	1 rep dead within 4 wks; one with +200 workers survived test	2 reps dead within 6 wks; one with +200 workers survived test	2 wks	all survive d test period

* 1 replicate did not remove any bait; colony was not as active as others and was eliminated from the trial; therefore only 2 replicates evaluated for the 100 ppm rate

Table 2. Bait acceptance comparison of AC 303,630 and a non-toxic standard bait or an Amdro standard.

Bait Comparison (ppm AC 303,630/standard)	Mean g. bait removed ± SEM*	
	AC 303,630	Standard
50 ppm AC vs. 0 ppm	2.52 ± 0.91a	2.52 ± 0.91a
50 ppm AC vs. Amdro	2.62 ± 0.67a	1.60 ± 0.51a
75 ppm AC vs. 0 ppm	3.20 ± 0.49a	1.94 ± 0.73a
75 ppm AC vs. Amdro	3.74 ± 0.26a	3.34 ± 0.59a
100 ppm AC vs. 0 ppm	2.08 ± 0.71a	2.28 ± 0.43a
100 ppm AC vs. Amdro	1.70 ± 0.62a	1.48 ± 0.52a
150 ppm AC vs. 0 ppm	1.36 ± 0.73a	1.84 ± 0.87a
150 ppm AC vs. Amdro	1.64 ± 0.53a	1.26 ± 0.29a

Means within a row followed by the same letter are not significantly different (t-test)

Table 3. Efficacy of AC 303,630 baits - change in population indices.

Treatment	Formulation (ppm)	% change in population index at indicated wks PT*		
		(4)	(8)	(17)
AC 303,630	50	-30.3a	8.6ab	-74.5ab
AC 303,630	75	-14.1a	24.9ab	-68.0b
AC 303,630	100	-32.2a	24.7ab	-76.0ab
Amdro	0.7%	-57.4b	-65.5a	-91.3a
Untreated Check	---	-25.8a	106.3b	-62.1b

* Means within a column followed by the same letter are not significantly different (Tukey's test, P=0.05)

Table 4. Efficacy of AC 303,630 baits - reduction in number of colonies.

Treatment	Formulation (ppm)	% decrease in number of colonies at indicated wks PT*		
		(4)	(8)	(17)
AC 303,630	50	28.5abc	3.0a	12.1a
AC 303,630	75	3.0a	0.0a	3.0a
AC 303,630	100	33.6bc	0.0a	3.3a
Amdro	0.7%	46.3c	6.7a	67.6b
Untreated Check	---	15.8ab	46.3b	0.0a

* Means within a column followed by the same letter are not significantly different (Tukey's test, P=0.05)

SECTION IV

BIODIV

PROJECT TITLE: Impact of Imported Fire Ants on Biodiversity

Personnel from the Gulfport Plant Protection Station provided technical support and assistance to several biodiversity studies that are being concocted by various research organizations. A brief summary of each project follows:

- Determination of impact of IFA on the Florida tree snail

  Coo~erators ARS, CMAVE, University of Florida, Eckerd College

  In March 1997 we provided technical support to this project by treating test plots at Key Largo, FL with Amdro.

- Effect of the red imported fire ant on the Florida Grasshopper Sparrow, an endangered species

  Coo~erators ARS, CMAVE, University of Florida, Eckerd College

  The impact of IFA on Grasshopper Sparrow food sources, especially Orthopeterans, is being determined by UV-light trap sampling at 12 study sites near Avon Park, FL. Sampling will occur quarterly until the study is completed.

- Impact of IFA on natural populations of the Gopher Tortoise

  Coo~erators Mississippi Wildlife Heritage Foundation, ARS, CMAVE, University of Florida

  We are collaborating on a project to determine if fire ants impact hatchlings of the Gopher Tortoise, a T & E species. Light trap sampling of arthropod populations will begin in May 1998, and bait application to test plots will occur in May/June 1998.

PROJECT NO: FA05G017

PROJECT TITLE: The Ants of East Texas: A Twenty-Five Year Comparative Survey

TYPE REPORT: Interim

LEADER/PARTICIPANT: Tim Lockley

INTRODUCTION:

In 1973, a survey was conducted to determine the ant species currently extant in eastern Texas. Four counties (Bexar. Cameron, Galveston, Nueces) were selected for the survey based upon their history with regards to the imported fire ant, *Solenopsis invicta.* Although separated from the contiguously infested area of Texas until the mid 1980's, Bexar county has been under federal quarantine the longest of the four (1959) for red imported fire ants (RIFA). Galveston County was next to be declared officially infested by RIFA in 1969. Nueces County came under Federal quarantine in 1973 (the first year of the survey). Cameron County was the last to come under the quarantine in 1996.

In 1997, the first of two follow-up surveys was made to determine the change in RIFA and native ant status in east Texas.

MATERIALS AND METHODS:

In September, October, and November, 1997 bait transects were run along major roadways within each county at 0.5 mile intervals. Care was made to attempt, where possible, to repeat the same lines of transect as used in the Glancey et al. (1973) unpublished study. A liquid bait supplied by Dr. David Williams (USDA, ARS. CMAVE, Gainesville, FL) was used as the attractant. Bait traps were placed along the roadsides and left for ca. one hour before retrieval. Within two hours of collection, specimens were removed from the bait traps: separated into distinct groupings and counted. Ants were placed in 70% ETOH and returned to the USDA facilities at Gulfport MS where they were examined under magnification and identified to the lowest level possible. Dr. Daniel Wojcik (USDA. ARS, CMAVE, Gainesville, FL) confirmed the identifications. Specimens are maintained at the Gulfport Plant Protection, Station.

RESULTS:

In all four counties, increases in RIFA abundance (as a percentage of ants caught) were observed (Table 1). Both Bexar and Nueces counties had exceptionally large increases in RIFA numbers. These counties also reflected the largest decreases in ant species diversity (Table 2).

The congenera of RIFA (*Solenopsis geminata* and *S. xyloni*) were completely eliminated in three of the four counties surveyed (Bexar, Galveston, and Nueces). In 1973, *S. geminata* was found at 4.2% of the sites in Galveston county, 18.0% of the sites in Bexar County, and 23.6% of the sites in Nueces county. Some decrease in *S. geminata* numbers was also noted in Cameron county. With *S. xyloni*, similar results were observed with decreases occurring in the three counties where it had been previously collected (Table 3).

Precipitous decreases in numbers of other ant species was noted in the 1997 survey. In Galveston county (1973), *Monomorium minimum* had been collected at 50.0% of the sites. By 1997, it could only be found at 4.8% of the collection points. *Tetramorium bicarinatum* dropped from 8.3% to 0.0%.

In Bexar county, *Pheidole dentata* had been collected at 32.5% of the sites in the first survey. In 1997, it had declined to only 1.8%. During that same period, RIFA increased from zero collections to being found at 36.9% of the sites. *Forelius pruinosus*, a species of ant that seems to compete well with RIFA (pers. obs.) also increased significantly between samplings; going from 0.0% (1973) to 15.8% of the locations (1997).

Data from Nueces County reflected much the same changes as seen in Bexar county. RIFA numbers rose from 3.9% of the sites to 31.3%. Ten of the native ant species found in 1973 were not taken in the 1997 collections. One of these, *Pheidole floridana*, had previously been found at 7.9% of the sites. In Cameron County, some decline in numbers of *S. geminata* was seen. A big headed ant, *Ph. floridana*, was not taken in 1997. Another of the big headed ants, *Ph. dentata*, had also decreased; falling from a high of 18.1% in 1973 to a low of 3.5% in the 1997 sampling. *Monomorium minimum* likewise declined sharply from 15.8% to 1.2%. Some increases had also occurred. The harvester ant,

Pogonomyrmex barbatus, went from being found at 7.9% of the sampling sites to 13.8%. A *Paratrechina* species also increased going from 0.8% to 6.9% during the same period. Six species of ants found in 1973 were not found in 1997. While seven new species were collected in 1997 as compared with 1973. An unidentified *Forelius* species not found in 1973 was taken from 9.8% of the sites and a yellow form of *Dorymyrmex* was found at 4.6% of the sites.

Another set of samples will be taken in the early to late spring of 1998 to eliminate any possible seasonal bias.

Table 1. Red Imported Fire Ants as a Percentage of Ants Collected

LOCATION	1973	1997
Galveston Co.	35.0	74.7
Nueces Co.	8.9	90.4
Bexar Co.	0.0	71.7
Cameron Co.	0.0	6.4

Table 2. Changes in Diversity of Ant Species Captured In Four Texas Counties From 1973 to 1997.

	1973		1997	
LOCATION	NO. GENERA	NO. SPECIES	NO. GENERA	NO. SPECIES
Galveston Co.	8	10	8	8
Nueces Co.	7	13	5	5
Bexar Co.	6	9	5	5
Cameron Co.	10	13	10	13

Table 3. Species List of Ants Captured By Bait Trap in Four Texas Counties in 1973 and 1997.

GALVESTON CO.	NUMBER COLLECTED		MEAN NUMBER PER SITE		PERCENT OF SITES	
TAXON	1973	1997	1973	1997	1973	1997
Solenopsis invicta	452	700	37.6	38.9	**50.0**	**28.6**
Monomorium minimum	367	72	30.2	24.0	**50.0**	**4.8**
Paratrechina terricola	27	143	5.4	11.0	20.8	20.6
Tetramorium bicarinatum	151		75.5		**8.3**	**0.0**
Crematogaster sp.	161		161.0		**4.2**	**0.0**
S. geminata	120		120.0		**4.2**	**0.0**
Pheidole dentata	10	8	10.0	2.7	4.2	4.8
Forelius pruinosus	3	6	3.0	3.0	4.2	3.2
S. xyloni	1		1.0		**4.2**	**0.0**
Cardiocondyla nuda	1		1.0		**4.2**	**0.0**
Aphaenogaster texana		5		5.0	0.0	1.6
Crematogaster pilosus		2		2.0	0.0	1.6
Pseudomyrmex pallidus		1		1.0	0.0	1.6

BEXAR CO.	NUMBER COLLECTED		MEAN NUMBER PER SITE		PERCENT OF SITES	
TAXON	1973	1997	1973	1997	1973	1997
Ph. dentata	455	76	12.0	76.0	**32.5**	**1.8**
S. geminata	471		22.4		**18.0**	**0.0**
M. minimum	140		28.0		**4.3**	**0.0**
Pheidole tepicana	39		9.8		**3.4**	**0.0**
S. xyloni	80		40.0		**1.7**	**0.0**
Pheidole floridana	12		12.0		0.9	0.0
P. terricola	9		9.0		0.9	0.0
Crematogaster laeviuscula	2		2.0		0.9	0.0
Dorymyrmex sp.	1		1.0		0.9	0.0
S. invicta		1046		49.8	**0.0**	**36.9**
F. pruinosus		250		27.8	**0.0**	**15.8**
Pogonomyrmex barbatus		31		15.5	**0.0**	**3.5**
Leptothorax nr schmitti		56		56.0	0.0	1.8

Table 3. Cont.

NUECES CO. TAXON	NUMBER COLLECTED 1973	NUMBER COLLECTED 1997	MEAN NUMBER PER SITE 1973	MEAN NUMBER PER SITE 1997	PERCENT OF SITES 1973	PERCENT OF SITES 1997
S. geminata	1658		55.3		**23.6**	**0.0**
S. xyloni	1769		63.2		**22.0**	**0.0**
Ph. dentata	621	3	51.8	3.0	**9.5**	**1.3**
Ph. floridana	416		41.6		**7.9**	**0.0**
S. invicta	449	1106	99.8	44.2	**3.9**	**31.3**
F. pruinosus	141	64	28.2	21.3	3.9	3.8
M. minimum	75		15.0		**3.9**	**0.0**
Ph. tepicana	356		89.0		**3.1**	**0.0**
S. (Diplorhoptrum) sp.	40		10.0		**3.1**	**0.0**
C. laeviuscula	27		13.5		1.6	0.0
C. nuda	2		1.0		1.6	0.0
Paratrechina sp.	2		2.0		0.8	0.0
P. terricola	3	46	3.0	4.6	**0.8**	**12.5**
Dorymyrmex sp.		4		1.3	**0.0**	**3.8**

CAMERON CO. TAXON	NUMBER COLLECTED 1973	NUMBER COLLECTED 1997	MEAN NUMBER PER SITE 1973	MEAN NUMBER PER SITE 1997	PERCENT OF SITES 1973	PERCENT OF SITES 1997
S. geminata	3817	1333	61.6	49.4	**48.8**	**31.0**
Ph. floridna	953		31.8		**23.6**	**0.0**
F. pruinosus	635	554	23.5	36.9	21.3	17.2
Ph. dentata	390	92	17.0	30.7	**18.1**	**3.5**
M. minimum	665	3	33.3	3.0	**15.8**	**1.2**
P. barbatus	57	85	5.7	7.1	**7.9**	**13.8**
C. laeviuscula	148	1	124.0	1.0	1.6	1.2
S. (Diplorhoptrum) sp.	2	40	2.0	40.0	0.8	1.2
Paratrechina sp.	1	64	1.0	10.7	**0.8**	**6.9**
P. terricola	1		1.0		0.8	0.0
Tetramorium similimum	1		1.0		0.8	0.0
Cardiocondyla emeryi	1		1.0		0.8	0.0
Macromischa subditiva	1		1.0		0.8	0.0
S. invicta		163		163.0	**0.0**	**1.2**
Forelius sp.		40		5.0	**0.0**	**9.8**
Dorymyrmex sp.		127		31.8	**0.0**	**4.6**
Pheidole sp.		2		2.0	0.0	1.2
Leptothorax nr. schmitti		64		64.0	0.0	1.2
A. texana		1		1.0	0.0	1.2

PROJECT NO: FA05G013

PROJECT TITLE: Ecological Studies of an Isolated Imported Fire Ant Population in Eastern Tennessee

TYPE REPORT: Final

LEADERS/PARTICIPANTS: Anne-Marie Callcott[2], David F. Williams[3], Homer Collins[2], Tim Lockley[2], David Oi[3], R.G. Milam[4] and others

INTRODUCTION:

Red imported fire ants (RIFA), *Solenopsis invicta*, currently infest over 308,000,000 acres in 11 states and Puerto Rico. A congener species S. *richteri*, inhabits a relatively small area in northwestern Alabama, northeastern Mississippi, and southern Tennessee. Interbreeding between S. *invicta* and *S. richteri* occurs and results in the production of a fertile hybrid form. Range expansion primarily by S. *invicta* and the hybrid form, by both natural and artificial means, continues. Recent isolated infestation in Phoenix, AZ (Collins unpublished), several sites in Virginia (Waller 1993), and numerous sites in Tennessee (Milam unpublished), are suggestive that acclimation to harsh environments may result in continued range expansion by this serious pest. Many scientists have speculated that hybrid vigor may enable the hybrid to survive in colder areas than either parental form.

In December 1992 an isolated RIFA infestation approximately 3000 acres in size was detected approximately 45 miles northeast of Chattanooga, TN near the town of Calhoun, TN. A wood pulp processing plant (Bowater Corporation) appears to be the source of the infestation. The original infestation may have been introduced onto the plant site on construction equipment or material; however this cannot be proven. This population appeared to be well adapted and thriving. A site visit by HLC and RGM in April 1993 revealed numerous large nests in open areas, presence of alate forms, and generally healthy colonies.

Various ecological studies of this isolated population were initiated in 1993

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in an effort to learn more about this highly adaptable insect. Results of these studies could lead to better estimates of the ultimate range of imported fire ants.

MATERIALS AND METHODS:

Four different, but interrelated, studies were conducted. Winter kill, seasonal life cycle, survivability of incipient colonies/newly mated queens under sub-optimal temperatures, and impact of RIFA on local myrmecofauna were determined.

WINTER KILL: Winter kill (survival) of colonies in the Calhoun, TN infestation were compared to a control site at Gulfport, MS. Population estimates in eight ¼-acre test plots were made in October 1993 at each site using the population indexing system described by Harlan et al. (1981) and modified by Lofgren and Williams (1982). Test plots were re-evaluated in April, July and October 1994 and 1995, May and October 1996, and June 1997. Maximum and minimum air temperatures and rainfall were collected at both sites. Mean number of colonies present at each site was compared with a *t*-test, as were mean population indices.

SEASONAL LIFE CYCLE: Markin and Dillier (1971) reported on the seasonal life cycle of RIFA along the Gulf Coast of Mississippi. Techniques and procedures used in that study were utilized to compare life cycle of RIFA in Calhoun, TN with Gulfport, MS. Briefly, those procedures consisted of monthly collections of field colonies of RIFA (n=4) and using the desiccation technique described by Markin (1968) to separate all life stages of the ant from the associated nest tumulus. Separated colonies were then preserved in 500 ml high density polyethylene bottles filled with isopropyl alcohol. The preserved colonies were thoroughly mixed using a magnetic stirrer, and while the solution was mixing, a 5 ml subsample was removed by dipping a ¼ teaspoon into the mixture 3-6 times. Each "spoonful" or aliquot was dumped into a calibrated beaker with a screen bottom. This allowed the alcohol to drain off and to accurately obtain a 5 ml ant subsample. The 5 ml subsample was then placed on filter paper and the excess alcohol drawn off through a Buchner funnel. Finally, each subsample was dried under a hood for 1 hr prior to evaluation. Each life stage present was then enumerated. From this data, the percentage of all life

stages present on each sample date was determined for each location, and compared with a t-test ($P<0.10$).

SURVIVABILITY OF QUEENS & INCIPIENT UNDER SUBOPTIMAL TEMPERATURES IN THE LABORATORY: Bioclimatic chambers programmed for sub-optimal developmental temperatures (Williams 1990) were used to compare nesting success of ants collected near Calhoun, TN with those collected in Gainesville, FL. Newly mated queens were collected serendipitously following mating flights. Also, small incipient colonies were field collected by shovelling the entire nest tumulus into plastic pails. Ants were then transported to Gainesville, FL for laboratory rearing in bioclimatic chambers. Colonies were subjected to temperature regimes that mimic average monthly conditions in several U.S. cities including Calhoun, TN (negative control), Gainesville, FL (positive control), Nashville, TN, Washington D.C., and St. Louis, MO. Nesting success was based on colony development rates (colony weight per time). An analysis of variance was used to compare development rates among the various temperature regimes and collection locations.

IMPACT OF RIFA ON LOCAL MYRMECOFAUNA: Fire ants readily compete with different arthropod species including other ants (Porter & Savignano 1990). Ant species diversity in the RIFA infested area of Calhoun, TN (McMinn Co.) were compared with a non-infested control area of similar or identical habitat approximately 10-20 miles from the RIFA infested area (Rhea Co.). Collections were made at monthly intervals for the length of the study. Two collection procedures were utilized.

Bait transect: Transects, 200 meters in length (n=4), were placed in similar habitats at both infested and non-infested sites, i.e. open fields, old field successional habitats, etc. These transects were used for both bait and pitfall traps. Bait and pitfall stations were alternated along each transect at 5 m intervals. Each bait transect was comprised of 10 bait stations, five baited with canned sausage and five baited with maple syrup. Baits were placed in snap-top vials, and were left in place for 1 hr prior to collecting. Collections were immediately frozen and returned to the Gulfport Plant Protection Station for sorting and identification. Bait transects provided a measure of the abundance and diversity of ants that might compete for food resources with RIFA.

Pitfall traps: Pitfall traps were also employed to collect ants and other arthropods in both the RIFA infested and the nearby non-infested site. These traps indicated the abundance and diversity of ants and other arthropods that may not be attracted to the diurnally placed baits used in the bait transect. A total of 10 pitfall traps located along the 200 meter transect were utilized at each site. Each transect traversed similar habitats and efforts were made to include as much habitat diversity as possible. "Permanent" type pitfalls were utilized. Pitfall traps at each site consisted of test tubes containing 70% ethanol placed in preset PVC sleeves spaced 10 meters apart along the transect. Traps were collected ca. 24 hrs after placement. All samples from the site were composited, placed in a 50 cc polyethylene bottles, and mailed to the IFA laboratory in Gulfport, MS for sorting and identification.

RESULTS:

WINTER KILL: In October 1993, both the Tennessee and control (Mississippi) site had similar population indices and numbers of colonies present (Figures 1 & 2). By April 1994, the Tennessee site had significantly lower IFA populations and colony numbers compared to the Mississippi site, indicating notable winter kill. Temperatures at the Tennessee site between December 20, 1993 and January 19, 1994 averaged a high of 39.8°F (range 16°-58°F) and a low of 23.7°F (range 0°-42°F) (Figure 3). There were 5 days that winter where high temperatures did not exceed 32°F. During mid-January there were 7 days in a row in which the high temperature did not exceed 34°F (avg. high = 27.7°F, range 16°-34°F; avg. low = 8.1°F, range 0°-16°F). On February 8, 1994, Greg Adyelotte (USDA, APHIS, PPQ Officer, Knoxville, TN) observed considerable mound mortality, and stated that it was very difficult to find active mounds (temperature range on 2/8 was 58°-67°F). This dramatic decline, indicating winter kill, was quantitatively noted in our April 1994 population assessment which showed 87% colony mortality (92% decrease in mean population index) between October 1993 and April 1994.

The Tennessee population did not rebound as expected over the summer months of 1994, but remained at very low levels, with only small increases in population indices and colony number between April and October. Average high temperatures in the summer months of June, July, and August 1994, were 87.4°F (range 75°-95°F), 86.6°F (range 73°-94°F), and 87.4°F (range 73°-90°F),

respectively. Rainfall during those months was 3.38, 8.13, and 6.17 inches, respectively.

In April 1995, there was a drop in both population indices and colony numbers in the Tennessee plots compared to the previous fall count (October 1994), again indicating some over winter kill that season. There was ca. 60% colony mortality (58% decrease in mean population index) between October 1994 and April 1995. Temperatures were not as extreme over this winter season. Lowest temperatures were recorded between January 20, 1995 and February 20, 1995 when the average high was 45.1°F (range 26°-67°F) and the average low was 31.2°F (range 10°-52°F). With only 1 exception (26°F on February 8, 1995), daily high temperatures were at least 32°F that winter season.

RIFA populations finally began to rebound over the summer of 1995. By October 1995, populations had risen substantially compared to the previous year, and both population indices and colony numbers were not significantly different from that of the Mississippi population. At the Tennessee site, the average high temperatures in the summer months of June, July, and August 1995, were 84.8°F (range 70°-93°F), 89.4°F (range 82°-96°F), and 92.5°F (range 83°-100°F), respectively. Rainfall during these months was 2.70, 2.46, and 4.68 inches, respectively.

There was, again, a substantial decrease in both mean population indices and colony numbers over the winter of 1995-1996 in the Tennessee plots. There was a 90% decrease in the mean population index and a 79% decrease in mean colony number between October 1995 and May 1996. The 1995-1996 winter was colder than the 1994-1995 winter, but not quite as severe as the 1993-1994 winter. The coldest temperatures were between December 20, 1995 and January 19, 1996, and between January 20 and February 19, 1996, with an average high of 43.4°F (range 23-67°F) and 43.9°F (range 22-63°F), respectively, and an average low of 28.6°F (range 12-48°F) and 28.7°F (range 0-45°F), respectively. Records show 8 days that winter where the high temperature did not exceed 32°F; 4 of these were consecutive days in early February (avg. high = 23.5°F, range 22-25°F; avg. low = 8.5°F, range 0-19°F). This cold weather impacted the RIFA populations as indicated by the substantial winter kill.

By October 1996, mean number of colonies present at the Tennessee site had not

increased significantly (16% increase), however, those colonies that had survived the previous winter were increasing in size, as indicated by an increase in mean population index between May 1996 and October 1996 (166% increase). Summer temperatures were similar to the two previous summers; June average high temperature was 84.8°F (range 74-92°F), July average high temperature was 89.9°F (range 83-95°F), and August average high temperature was 89.2°F (range 79-96°F). Rainfall during these months was 7.89, 1.16, and 5.94 inches, respectively.

The final evaluation was made in early June 1997. At that time, little or no winter kill was noted in the Tennessee plots (9% increase in mean number of colonies present, and 8% decrease in mean population index). The late count (June vs. April/May) may have allowed surviving colonies to grow prior to our count and new colonies to develop into visible colonies. However, most of the colonies detected were fairly large, indicating that they had overwintered. While the site had some very low temperatures during the 1996-1997 winter, average high temperatures were warmer than in previous winters. The lowest average high temperature for the winter was 50.2°F (range 26-69°F) and occurred between December 20, 1996 and January 19, 1997. While there were numerous nights when the low temperature was below 32°F, as evidenced by an average low of 20.2°F (range 0-53°F) between January 20 and February 19, 1997, there were only 2 days when the high temperature did not exceed 32°F. Therefore, there was no extended period of constant freezing temperatures that particular winter.

The Mississippi site showed a large reduction in population indices and number of colonies at the July 1994 assessment (Figures 1 & 2). This mid-summer drop in RIFA populations appears to be a natural phenomenon in the hot, dry summer climate of south Mississippi (pers. obs.). Populations had rebounded well by the October 1994 counts. There was another dramatic decrease in populations at the July 1995 assessment reinforcing the observation of this summer decline phenomenon. However, populations did not rebound in October 1995 as they had the previous year. 1995 was an exceptionally long, hot summer in south Mississippi which probably attributed to the slow recovery (Figure. 4). There were three months this summer (July, August, September) where the average high temperature exceeded 90°F (92.6°F, 92.3°F, 92.0°F, respectively). Again, the Mississippi RIFA populations showed an increase between October 1995 and May

1996, and between October 1996 and June 1997, and a decrease over the hot summer months into fall. The final count in June 1997 showed both the mean number of colonies and the mean population index almost identical to those when the trial was initiated 3½ years previously.

In general, there was little numerical difference in average summer temperatures, both highs and lows, between the Mississippi and Tennessee sites (Figures 3 & 4), although the Mississippi site sustained the high summer temperatures over a longer period of time. The sustained high temperatures (2-3 months) in the summer appeared to contribute to the decrease in RIFA colonies and populations in Mississippi, whereas the brief high temperatures in Tennessee (1 month) did not appear to inhibit RIFA growth. There were, however, large and significant differences in both the mean high temperatures and the mean low temperatures between the two sites during the winter months. For example, in January 1995, the Mississippi mean high temperature was 62.4°F, and the Tennessee mean high was 51.1°F (difference = 11.3°F, $P<0.0001$). During the same period the Mississippi mean low was 43.5°F, and the Tennessee mean low was 33.9°F (difference = 9.6°F, $P<0.0001$). These lower winter temperatures at the Tennessee site, coupled with RIFA mortality during the winter months, strongly indicate that Tennessee winter temperatures have a severe adverse impact on RIFA colonies. Our data indicates that winter survival of RIFA may be more dependent on the mean high temperatures in an area rather than the mean low temperatures. The lowest average low temperature at the Tennessee site was over the 1996-1997 winter (20.2°F). However, the average high temperature during this period was 50.2°F, and there were only 2 days when the high temperature did not get above 32°F. During that winter, the mean number of RIFA colonies at the Tennessee site actually increased by 9% (population index decreased by only 8%). The 1993-1994 winter had the lowest average high temperature (39.8°F), 5 days where the high temperature did not exceed 32°F, and the greatest decrease in mean number of RIFA colonies (87%) and in mean population index (92%). At the other extreme, prolonged high summer temperatures appeared to adversely affect RIFA populations in Mississippi. Summer and fall evaluations of colony numbers and population indices in Mississippi were consistently lower during these seasons than during the corresponding spring months.

SEASONAL LIFE CYCLE: Life stage data was collected from November 1993 through

November 1996. The dramatic reduction in RIFA colonies in the Tennessee plots over the winter of 1993-1994, and subsequent winter kills, made colony collection for this portion of the study somewhat difficult. Five times during the study we were unable to collect colonies at the Tennessee site: February and June 1994, May 1995, and February and October 1996. On five occasions, only three colonies were collected (instead of 4), and on three occasions, only two were collected. Gravid females and eggs were rarely collected at either site. The biology of the insect precludes easy collection of gravid females and our extraction technique may be the cause of the low egg numbers. The mesh screens used in the process may not have been fine enough to retain all the eggs in the samples.

Small larvae were collected in every collection at the Mississippi site, with a drop in production in the winter months (December - February) (Figure 5). In the Tennessee site, small larvae were collected at least once in every calendar month, with similar reductions or cessation in production during the winter months (none collected January, March, and April, 1996). There were more significant differences in presence of this life form between the two sites than any other. Out of 32 monthly comparisons (t-test, $P<0.10$), the Mississippi site produced a greater percentage of small larvae than the Tennessee site did in 6 comparisons (November 1993, March, August, and December 1994, March and April 1995). The Tennessee site produced significantly more small larvae in 2 comparisons (August 1995, August 1996).

A cessation in worker pupae production occurred at the Mississippi site in January 1994, and a substantial drop occurred in February 1995 and again in December - March 1996. The Tennessee site ceased or substantially dropped worker pupae production January - March 1994, December 1994 - March 1995, and January - April 1996. Significant differences in worker pupae were only noted twice; Mississippi produced a greater percentage of worker pupae than Tennessee in December 1994 and April 1996.

Sexual larvae presence fluctuated at both sites. In general, sexual larvae were present in the Mississippi colonies in the spring to early summer (April - July 1994, March - June 1995, and April - July 1996) and again late summer through fall (September - October 1994, and August - October 1995). In the Tennessee colonies, sexual larvae presence occurred slightly later in the

spring (May - July 1994, April - June 1995, and May - July 1996), and about the same time in the fall (September - October, 1994 and October 1995). The Mississippi colonies contained significantly more sexual larvae and pupae than the Tennessee colonies in April 1995, significantly more sexual larvae in April 1994, and more sexual pupae in April 1996 (t-test, $P<0.10$). The Tennessee colonies contained significantly more sexual brood than the Mississippi colonies in May 1994, more sexual larvae in June 1996, and more sexual pupae in October 1995 (t-test, $P<0.10$). This trend indicates that major springtime production of sexual brood in southern Mississippi begins in April, a full month before the major production in Tennessee.

Minor workers were the most abundant life stage year round in both sites, and in general, minor production was similar at both sites (Figure 6). In January 1994, December 1994 and August 1996, the Mississippi colonies had significantly more minors than the Tennessee colonies, and only in March 1994 did the Tennessee colonies have significantly more present (t-test, $P<0.10$). Of the adult life forms, the most differences appeared in the major worker ant. In 7 of 32 comparisons (22%), the Tennessee colonies contained a significantly greater percentage of major workers than the Mississippi colonies (December 1994, June, October, and November 1995, March, May, and November 1996). Four of the dates are during the winter-early spring months (November - March), indicating the obvious advantages of worker size on over winter survival.

The most interesting trend at this time is that of the alate females. At the Tennessee site alate females were present continuously from November 1993 through March 1995. Only at three collection dates were alate females not collected in Tennessee (April, August and September 1995). At the Mississippi site, where climatic conditions seem more suitable for continuous alate female production, alate females were collected at least once in each calendar month over the three year collection period, but no alate females were collected in 10 of the collection dates (December 1993, January and February 1994, January, April and November 1995, and March, April, September and November 1996). However, only in November 1994 were there significant differences between the sites when more alate females were produced in the Tennessee colonies (t-test, $P<0.10$).

Male production in Tennessee was evident at least once in every calendar month except April. In the Mississippi site, male production was not evident in January, February or March of any year. There were no significant differences between sites during any monthly comparison over the 3 year collection period (t-test, $P<0.10$).

Due to the difficulty in adequate and constant monthly sampling because of low colony numbers from winter kill at the Tennessee site, our assessment of fluctuations in life cycles may not be accurate. However, our data does demonstrate the fluctuations in various life stages present in a new and environmentally susceptible RIFA population. Major workers appeared to comprise a greater percentage of the worker population in the Tennessee colonies during the colder months, probably due to better winter survivability. Minor workers, small larvae and worker pupae were present in every collection in Mississippi (except no worker pupae in January 1994). Minor workers were present in all Tennessee collections, and small larvae and worker pupae were collected at least once in every calendar month during the course of the study, indicating that production can occur year round in Tennessee.

Sexual life stages were less consistently produced. At the Tennessee site, sexual brood was not collected in any year during December or March. Sexual larvae were not collected in any January, and sexual pupae were not collected during any April. On the other hand, alate females were collected at least once in every calendar month and males were collected at least once in every calendar month except April. This indicates that while sexual brood production may cease during the winter months, both alate forms may over winter in the mound, possibly waiting for the warmth of spring to commence their mating flights. Mississippi colonies had similar trends, though total lack of sexual brood production was generally a month earlier. Sexual brood was not collected in Mississippi during the months of November, December or February. Sexual larvae were not collected in any January, and sexual pupae were not collected during any March. Alate females were collected at least once in every calendar month of the year in Mississippi, but males were not collected in any January, February or March. Again, this indicates the possibility of over wintering alate forms, particularly the females.

SURVIVABILITY OF QUEENS & INCIPIENT UNDER SUBOPTIMAL TEMPERATURES IN THE LABORATORY: This portion of the study was conducted at USDA, ARS, CMAVE, in Gainesville, FL. Eight dealate queens collected in Chattanooga, TN were sent by overnight mail to Gainesville, FL in June 1994. Alate queens were collected in Gainesville within a week of receiving the Tennessee queens. When eggs were observed, queens from each location were placed in temperature chambers that followed the average monthly temperatures of either Orlando, FL, or Chattanooga, TN. After 3 months (October), the Florida queens had produced small colonies under both temperature regimes, but all the eggs from the Tennessee queens did not hatch or were eaten. It was assumed that the Tennessee queens had not mated and the study was terminated.

IMPACT OF RIFA ON LOCAL MYRMECOFAUNA: A total of 16,918 ants (including RIFA) were collected in the RIFA infested area (Table 1) compared to 7,104 in the non-RIFA infested area (Table 2), through October 1996. Excluding RIFA in the infested site, 14,229 other ants were collected. Total number of species collected from October 1993 through October 1996, excluding RIFA, was 27 in the infested plots, and 32 in the non-infested plots, of which 25 species were common to both sites.

We arranged the data seasonally for presentation: spring = March, April, May; summer = June, July, August; fall = September, October, November; winter = December, January, February. Over the winter months very few ants were collected in any of the traps, so this data has been omitted from this discussion. Numerically, RIFA comprised 97% of the ants collected in the fall of 1993. By the spring of 1994, RIFA comprised only 7.7% of the total number of ants collected in the infested site. By the fall of 1995, they comprised 32.8% of the sample. This high level of RIFA activity corresponds with the time frame (October 1995) in which we found the greatest number of active RIFA colonies in the site since the original fall 1993 count.

The number of non-RIFA species collected in both infested and non-infested plots varied seasonally. Significantly more species of non-RIFA ants were collected in the non-infested plots in the spring of 1994, the fall of 1994 and the summer of 1995, than in the RIFA-infested plots (Figure. 7). However, when RIFA are added to the equation, there is no significant difference in any season or year between mean number of ant species captured in RIFA-infested

vs. non-infested sites.

When trap types are evaluated separately, we find more species of ants were collected in the pitfall traps than in the bait traps in both the infested and non-infested sites, regardless of whether RIFA are included in the data. On a seasonal basis, more species of ants were regularly collected in the pitfall traps. The difference was significant in the infested site (including RIFA data) in fall 1995 (bait = 4.7, pitfall = 11.0, P=0.076) and summer 1996 (bait = 5.7, pitfall = 11.7, P=0.027). In the non-infested site, significant differences were found in summer 1994 (bait = 6.0, pitfall = 7.3, P=0.057), fall 1994 (bait = 2.0, pitfall = 5.7, P=0.093), and fall 1995 (bait = 4.0, pitfall = 10.0, P=0.074). There were no significant differences in mean number of ant species collected within trap types between the infested and non-infested sites on a seasonal basis or over the course of the study.

The most numerous ants collected in the infested area were *Forelius pruinosus*, *Forelius* sp. A, and *Solenopsis invicta*, with 36.6%, 26.4%, and 15.9%, respectively, of the total number of ants collected through October 1996 (Table 1). *Forelius* sp. A was most prevalent in the spring of 1994, after the extremely cold winter of 1993-1994 had severely depleted the RIFA populations (Figure 8), indicating a negative, though insignificant correlation between the two species (df=27, r=-0.1407, P=0.475). On the other hand, *F. pruinosus* was most numerous in the summer of 1995 when RIFA populations had increased dramatically, and again in the summer of 1996 when RIFA populations were moderate. Initially, it appeared that there may be a positive correlation between RIFA and *F. pruinosus*, however data from the third year of sampling negated that possibility. There is a slight positive, though very insignificant, correlation between these two species after 3 years of collecting (df=27, r=0.069, P=0.727). However, at the beginning of this trial, fall 1993, when the RIFA population was at its strongest, no *F. pruinosus* were present and only a few *Forelius* sp. A were present (Table 1, Figure 8). There was also no significant correlation between RIFA density (no. of RIFA collected in traps) and number of non-RIFA ant species present (df=27, r=0.0584, P=0.768).

S. invicta were collected in the infested site during all 10 seasonal collections, as were *Paratrechina terricola* and *Pheidole vinelandica* (Table

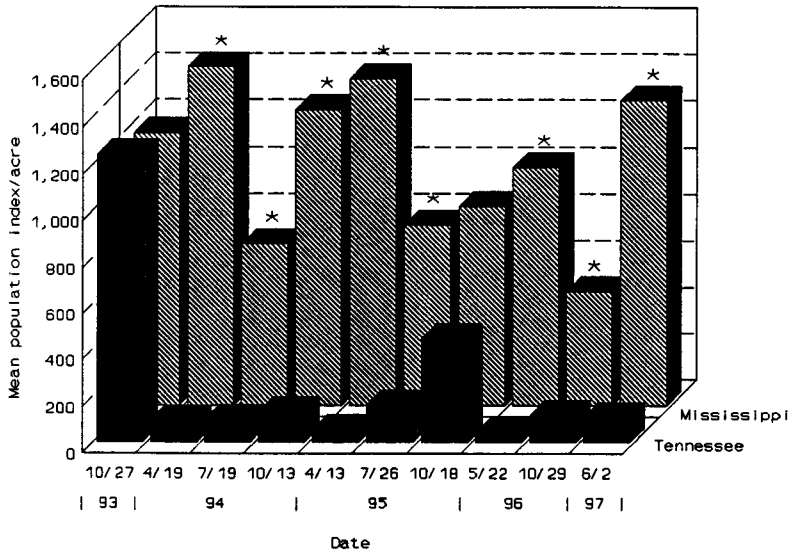
1). *Forelius* sp. A was collected in 9 of the 10 seasonal collections (not collected in fall 1996). Six other ant species were collected in at least 7 of the 10 seasonal collection periods: *Crematogaster clara*, *C. missouriensis*, *Monomorium minimum*, *Paratrechina faisonensis*, *Pheidole dentata*, and *Ph. tysoni*.

In the non-RIFA infested area, the most numerous ants were *Forelius* sp. A, *Monomorium minimum*, and *Crematogaster clara* with 21.1%, 19.9%, and 17.4%, respectively, of the total number of ants collected between October 1993 and October 1996 (Table 2). *Leptothorax pergandei* was collected during all 10 seasonal collections, and *Monomorium minimum* was collected during 9 seasons. Six other species were collected in at least 7 of the 10 collections: *Aphaenogaster treatae*, *Crematogaster clara*, *Forelius* sp. A, *F. dolosa*, *Pheidole dentata*, and *Ph. vinelandica*.

Five species of ants were collected in at least 7 of the 10 seasonal collecting periods in both the RIFA-infested and non-infested sites: *Crematogaster clara*, *Forelius* sp. A, *Monomorium minimum*, *Pheidole dentata* and *Ph. vinelandica*. In the first seasonal collection in fall 1993, when RIFA colony numbers and activity, noted by high numbers of RIFA collected in traps, were high, very small numbers of these five species were collected (Table 1), and no *C. clara* or *M. minimum* were collected. Subsequently, all of these species were collected in the infested site, even during seasons of fairly high RIFA activity as indicated by colony numbers and numbers of RIFA collected in traps. This indicates that these species may be able to compete with RIFA on a limited basis when RIFA numbers are low, but as RIFA populations increase and surpass some threshold, these species can no longer effectively compete.

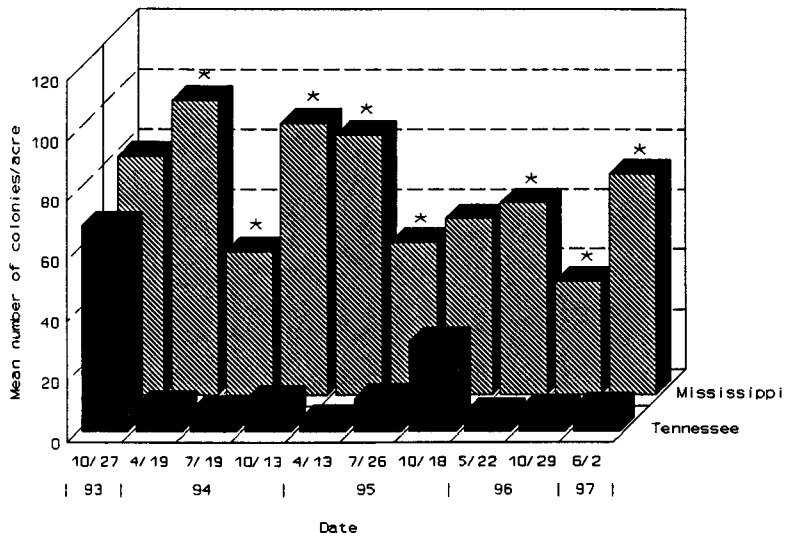
All data generated by this study will be analyzed further and published in a referred journal in late 1998 or early 1999.

Figure 1. RIFA Population Indices Over Time at Two Climatically Different Sites.



* denotes significant difference between sites (t-test, P=0.05)
TN site - one plot lost to construction prior to 10/13/94, one lost prior to 10/29/96
MS site - one plot lost prior to 10/29/96

Figure 2. Number of Colonies Present Over time at Two Climatically Different Sites.



* denotes significant difference between sites (t-test, P=0.05)
TN site - one plot lost to construction prior to 10/13/94, one lost prior to 10/29/96
MS site - one plot lost prior to 10/29/96

Figure 3. Trends of Temperature vs. Number of RIFA Colonies Present in Tennessee

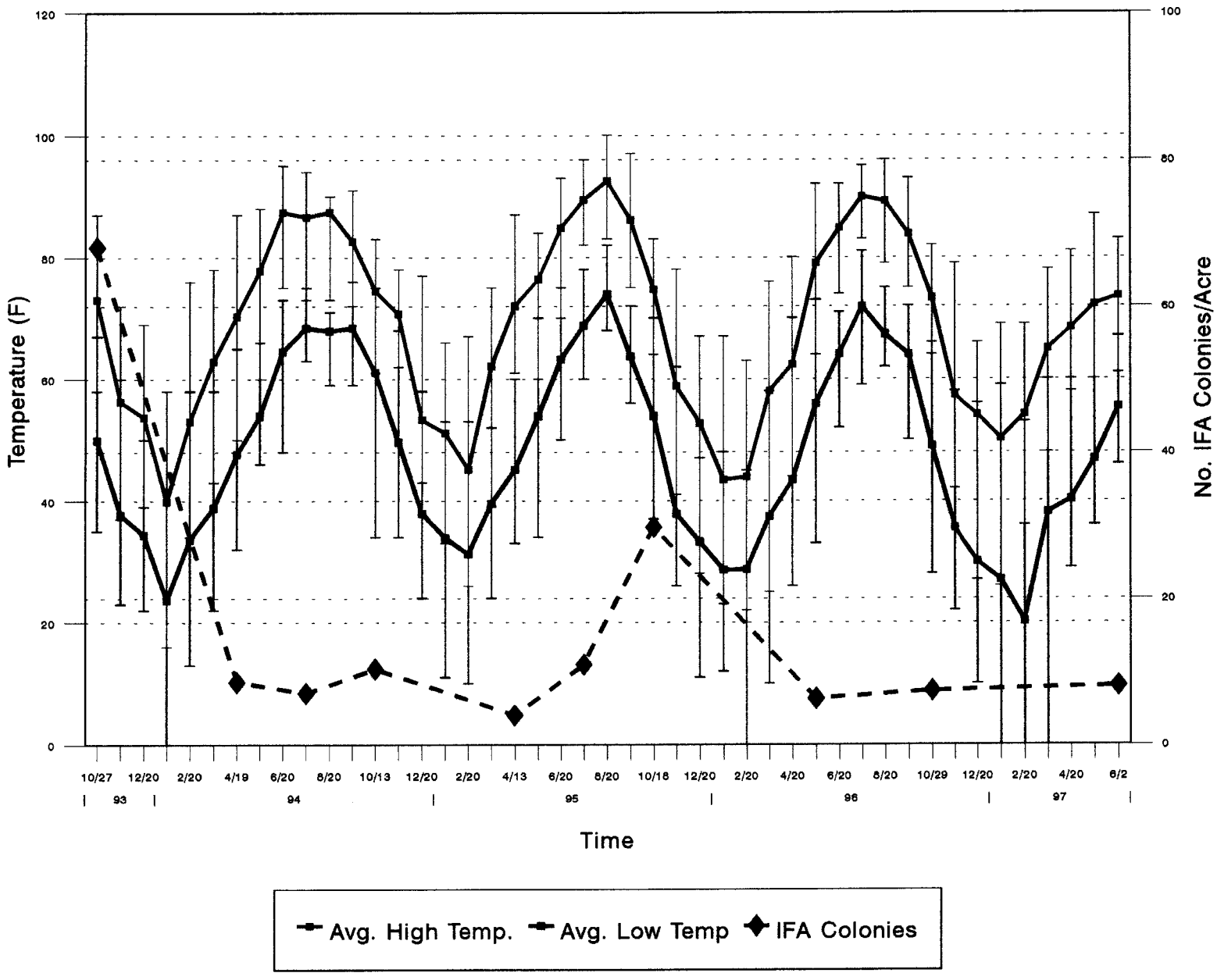


Figure 4. Trends of Temperature vs. Number of RIFA Colonies Present in Mississippi

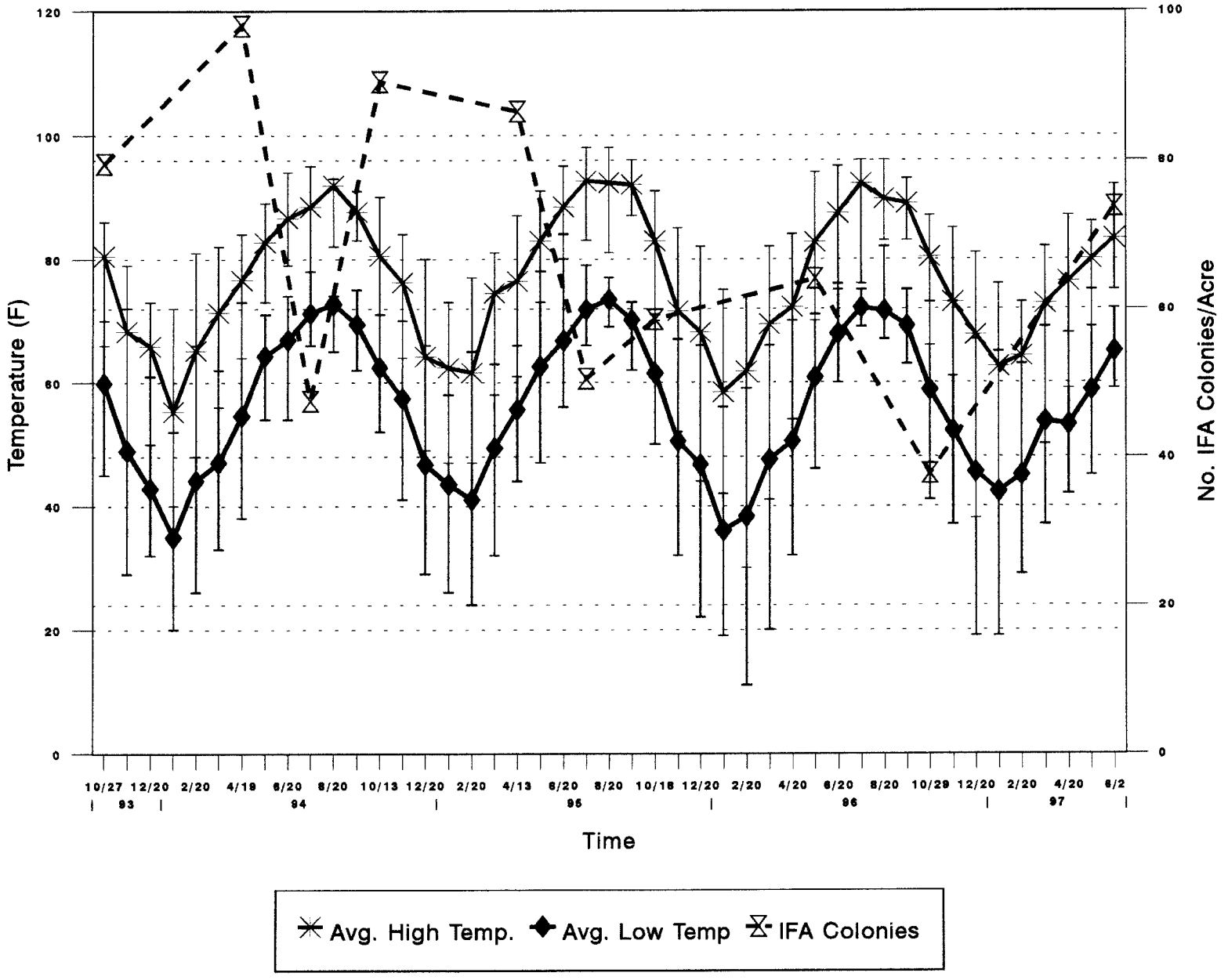
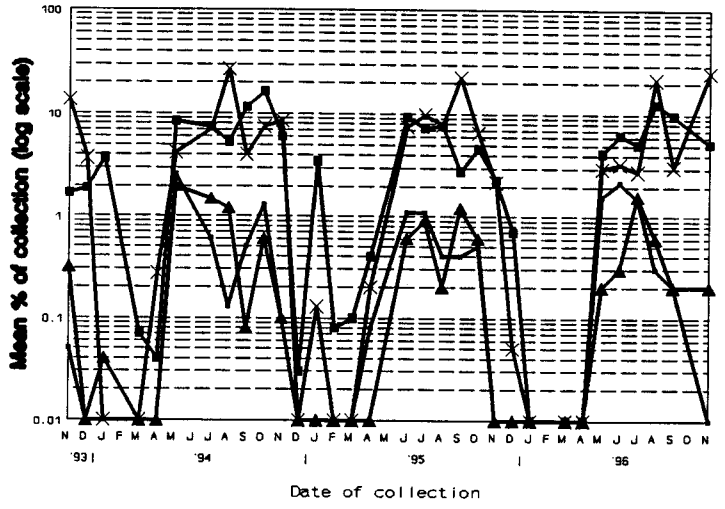


Figure 5. Life Stage Data for Immature Forms Collected at Mississippi and Tennessee Sites; a) Tennessee, b) Mississippi.

a) Percentage of immature forms present in Tennessee plots over time



No TN collections were made in Feb 94, June 94, May 95, Feb 96, or Oct 96.

b) Percentage of immature forms present in Mississippi plots over time

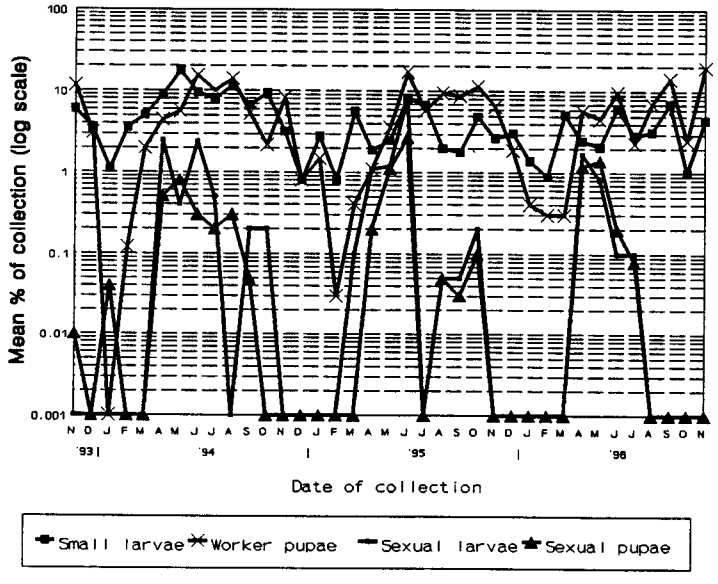
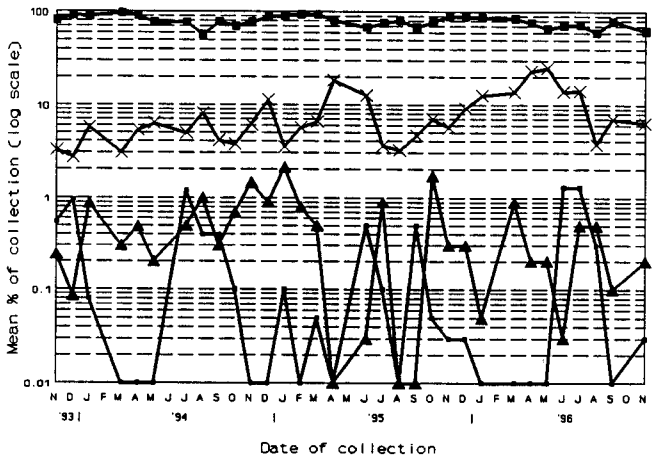


Figure 6. Life Stage Data for Adult Forms Collected at Mississippi and Tennessee Sites; a) Tennessee, b) Mississippi.

a) Percentage of adult forms present in Tennessee plots over time



No Tn collections were made in Feb 94, June 94, May 95, Feb 96, or Oct 96.

b) Percentage of adult forms present in Mississippi plots over time

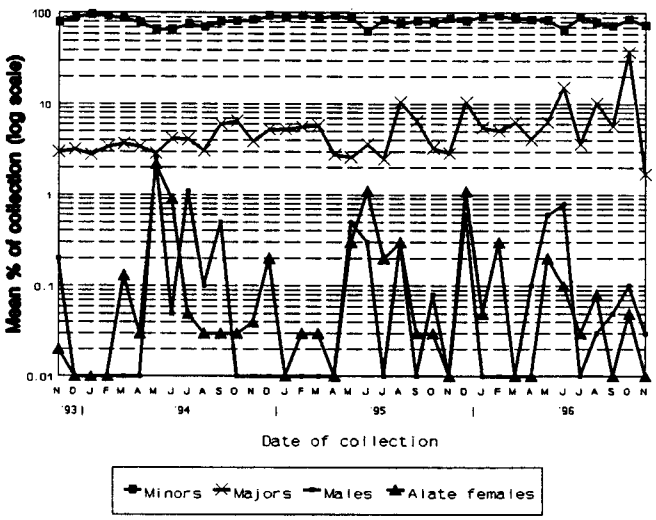
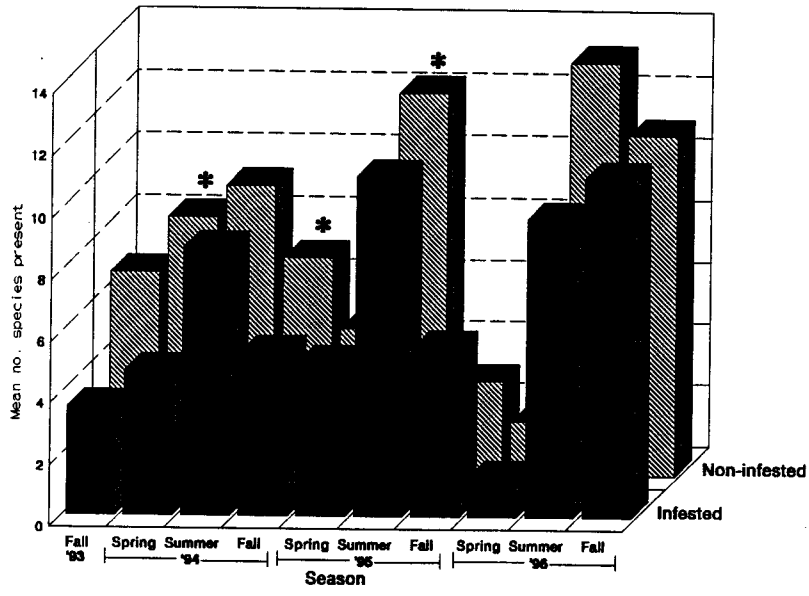


Figure 7. Number of Non-RIFA Ant Species Present in RIFA Infested vs. Non-infested Plots.



* indicates significant difference (t-test, P<0.06)

Figure 8. Number of Most Abundant Ant Species Present in RIFA-infested Plots.

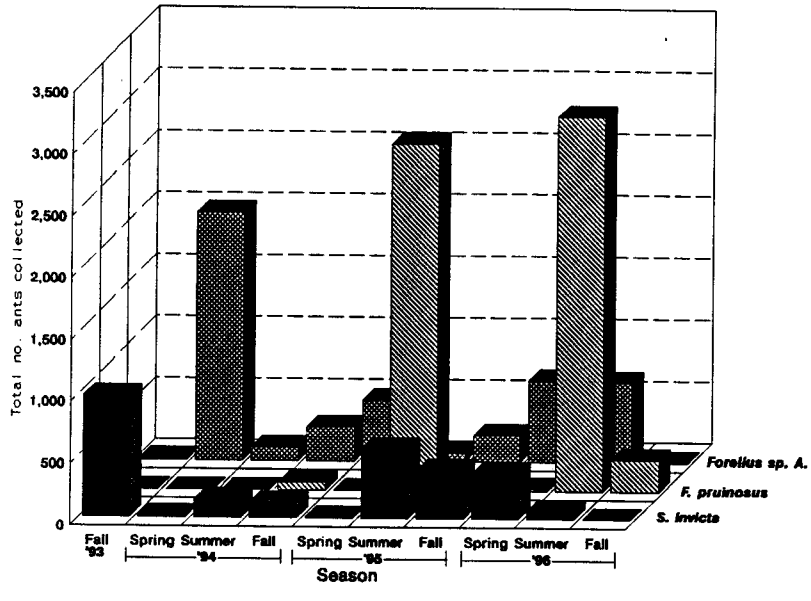


Table 1. Mean no. of ants trapped in bait and pitfall traps seasonally in INFESTED plots.

Species name	1993	1994			1995			1996			TOTAL	PERCENT OF TOTAL
	Fall	Spr	Summ	Fall	Spr	Summ	Fall	Spr	Summ	Fall		
Aphaenogaster sp. A.			1			2					3	0.02
A. lamellidens											0	0.00
A. treatae											0	0.00
Crematogaster sp. A.											0	0.00
C. ashmeadi		7			45						52	0.31
C. clara		6	1			430	66	9	212	3	727	4.30
C. lineolata		1	49								50	0.30
C. missouriensis	2	1	7			3	2		10	1	26	0.15
Forelius sp. A.	8	2006	105	268	496	74	206	663	646		4472	26.43
F. sp. B.							1	1	1		3	0.02
F. sp. C.							58				58	0.34
F. pruinosus				63		2800	38	16	3027	251	6195	36.62
Formica sp. A.											0	0.00
F. sp. B.											0	0.00
F. dolosa		4									4	0.02
Labidus sp. A.				36			137		2		175	1.03
Lasuis sp. A.							1				1	0.01
L. neoniger											0	0.00
Leptothorax sp. A.		13							2		15	0.09
L. pergandei			14			1	5		1		21	0.12
Monomorium sp. A.		2									2	0.01
M. minimum		257	1			58	35	2	5	10	368	2.18
Paratrechina sp. A.			1	2					1		4	0.02
P. faisonensis			51	9		8	9	1	23	3	104	0.61
P. parvula			1								1	0.01
P. terricola	5	1	66	1	1	28	13	1	76	121	313	1.85
Pheidole sp. A.	4						1	1			6	0.04
P. dentata	3			1		8	129	39	85	104	369	2.18
P. tysoni		16	6	14		4	18		2	7	67	0.40
P. vinelandica	5	3	36	12	2	96	63	128	116	194	655	3.87
Proceratium silaceum				1		2		2			5	0.03
Tapinoma sessile									1		1	0.01
Trachyomyrmex sp. A.											0	0.00
S. molesta	3		185	13		237	29		65		532	3.14
S. invicta	984	18	152	128	10	558	395	374	68	2	2689	15.89
S. richteri											0	0.00
TOTAL	1014	2335	676	548	554	4309	1206	1237	4343	696	16918	

Unidentified species (i.e. identified to sp. A, B or C) will be identified by experts in each genus as available.

Table 2. Mean no. of ants trapped in bait and pitfall traps seasonally in NON-INFESTED plots.

Species name	1993	1994			1995			1996			TOTAL	PERCENT OF TOTAL
	Fall	Spr	Summ	Fall	Spr	Summ	Fall	Spr	Summ	Fall		
Aphaenogaster sp. A.		1						1	1		3	0.04
A. lamellidens		1	27								28	0.39
A. treatae	11		6	14	1	38	9		14	15	108	1.52
Crematogaster sp. A.		27		1			2		1		31	0.44
C. ashmeadi				2	85				3		90	1.27
C. clara	3	235	7			616	12	39	321	6	1239	17.44
C. lineolata		265	99								364	5.12
C. missouriensis	1	2	3	1			6		38		51	0.72
Forelius sp. A.		518	836	5	4	47	86	1	4		1501	21.13
F. sp. B.						43		1	2		46	0.65
F. sp. C.								1			1	0.01
F. pruinosus	2					299	6	12	518		837	11.78
Formica sp. A.		14				1			1		16	0.23
F. sp. B.		4									4	0.06
F. dolosa	2	22	23			19	8		12	1	87	1.22
Labidus sp. A.											0	0.00
Lasuis sp. A.			9								9	0.13
L. neoniger		6	19								25	0.35
Leptothorax sp. A.		15	2				4		45		66	0.93
L. pergandei	8	39	24	4	1	53	12	13	24	6	184	2.59
Monomorium sp. A.		1									1	0.01
M. minimum	1	3	502	7	3	504	9	4	383		1416	19.93
Paratrechina sp. A.					2	3					5	0.07
P. faisonensis	4			1		2	2		6		15	0.21
P. parvula											0	0.00
P. terricola				1					3		4	0.06
Pheidole sp. A.	10	2				2	1				15	0.21
P. dentata	53	5	7	5		112	44	2	16		244	3.43
P. tysoni	10		70	3		57	35		78		253	3.56
P. vinelandica	15		144	3		54	13	4	203		436	6.14
Proceratium silaceum								1			1	0.01
Tapinoma sessile				1							1	0.01
Trachyomyrmex sp. A.						1					1	0.01
S. molesta			1			16	3		2		22	0.31
S. invicta											0	0.00
S. richteri											0	0.00
TOTAL	120	1160	1779	48	94	1866	255	79	1675	28	7104	

PROJECT NO: FA04G017

PROJECT TITLE: Seasonal Biology of Polygynous RIFA Colonies Along the Mississippi Gulf Coast

TYPE REPORT: Interim

LEADERS/PARTICIPANTS: Tim Lockley and Charlene Russell

INTRODUCTION:

Polygyny was not known to occur in RIFA colonies until Glancey et al. (1973) found 2 small nests of *Solenopsis invicta* Buren (estimated to be 6-8 months old) that contained multiple fertile queens. These colonies were near Jackson, MS. Later, Glancey et al. (1975) reported that a group of 10 nests of S. *invicta* in Southeastern Mississippi contained from 7 to 677 fertile queens. This site was 3 miles east of Hurley, MS (Jackson County), adjacent to an abandoned garbage dump on State Highway 614. Lockley (1995) surveyed this site over a three year period and found that multiple queen colonies were still present. Additionally he found one site in Harrison County and one site in Hancock County that contained polygynous colonies. Except for these three polygynous sites, all other RIFA colonies along the Mississippi Gulf Coast are thought to be monogynous.

Markin and Dillier (1971) reported on the seasonal life cycle of monogynous RIFA colonies along the Mississippi Gulf Coast. They found that production of worker brood was diminished in the cooler months and alate production peaked in late spring and early fall. The seasonal life cycle of polygynous colonies along the Mississippi Gulf Coast has not been studied. We compared the seasonal biology of monogynous and polygynous colonies in the study described herein.

MATERIALS AND METHODS:

Seasonal life stages present: Five RIFA colonies were collected from the Hancock County site each month (Bordages Brothers Marina and Shipyard property). Collections were made by shovelling the nest tumulus and as many ants as possible into 12 liter plastic pails. Five monogynous colonies were

collected from the Harrison County Farm in a like manner. Colonies were transported into the laboratory where the ants were separated from the nest tumulus by the desiccation technique described by Markin (1968). Separated colonies were preserved in 500 ml high density polyethylene bottles filled with isopropyl alcohol. Preserved colonies were thoroughly mixed with a magnetic stirrer, and while the ant/alcohol suspension was mixing, a 5 cc subsample was removed. This 5 cc subsample was then placed on filter paper and the alcohol allowed to drain off through a Buchner funnel. After allowing to air dry under a hood for 1 hr, each life stage present in each subsample was enumerated. From this data the percentage of all life stages present from each sample on each sampling date was determined. Differences in mean percentages of life stages present in each population type at each sampling date was determined by t-test (P=0.05).

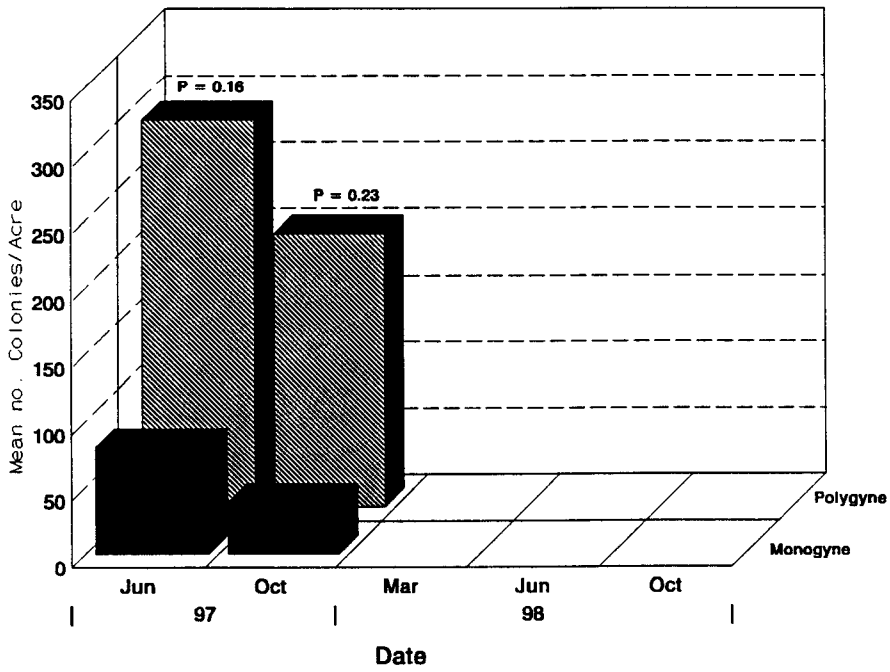
Seasonal variations in population densities: Population densities were measured by the standard method utilized by the Gulfport Plant Protection Station. Evaluations of IFA populations were made in three ¼-acre circular plots, using the procedures described by Lofgren and Williams (1982) and Collins and Callcott (1995). Differences in treatment means were separated by a t-test (P=0.05).

RESULTS:

Seasonal life stages present: Colonies are being collected at the end of every month. Therefore reference to a month, i.e. January, refers to samples collected on or about the 29th of that month. Between January and November, 1997, very few statistical differences in life stages were detected. As expected, there were significantly more minors in the polygyne colonies than in the monogyne colonies in January, March and September, 1997 (during other months there were no significant differences). While there were significantly more majors in the monogyne colonies than in the polygyne colonies in January and November, 1997, there were significantly more majors in the polygyne colonies in July, 1997. Other life stages where there were significant differences between the population types were gravid queens, significantly more in polygyne colonies in January, February and March, 1997, and small larvae, significantly more in monogyne colonies in January, March and September, 1997.

Seasonal variations in population densities: Only two counts have been made to date; June and October 1997 (Fig. 1). Statistically, colony numbers in the polygyne and monogyne sites are not significantly different, however there are enormous numerical differences. The poor statistics are due to the range in number of polygyne colonies per acre (June 1997 mean ± SEM = 290.7 ± 94.4; October 1997 mean ± SEM = 205.3 ± 98.4), compared with the range in number of monogyne colonies per acre (June 1997 mean ± SEM = 80.0 ± 6.1; October 1997 mean ± SEM = 38.7 ± 8.1). There is one polygyne plot which is causing the large variation in colony numbers, however we hesitate to replace the plot because it does contain polygynous colonies. This plot is separated from the main body of the polygynous site by a two-lane asphalt drive (i.e. across the street).

Figure 1. Number of colonies present in monogyne vs. polygyne areas in South Mississippi.



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APPENDIX I

1997 Publications/Presentations

PUBLICATIONS:

Collins, H. L., and A.-M. A. Callcott. 1998. Fipronil: An ultra-low-dose bait toxicant for control of red imported fire ants. Florida Entomologist (In Press).

PRESENTATIONS:

Callcott, A.-M. A. Range expansion of the imported fire ant. Oklahoma Farmer's Union Workshop on Imported Fire Ants in Oklahoma. Durant, OK. April 25, 1997.

Callcott, A.-M. A. and H. L. Collins. Update on new IFA quarantine treatment. 1997 Imported Fire Ant Research Conference. Gainesville, FL. April 15-16, 1997.

Collins, H. L. Sweetpotato Weevil: Evaluation of alternate hosts and results of mark and release studies in southern Mississippi. Southern Plant Board Meeting, Charleston, South Carolina. April 8, 1997.

Collins, H. L. Imported fire ant update. Southern Plant Board Meeting, Charleston, South Carolina. April 9, 1997.

Collins. H. L. and Anne-Marie Callcott. Fipronil: An ultra-low-dose bait toxicant for control of red imported fire ants. 1997 Imported Fire Ant Research Conference. Gainesville, FL. April 15-16, 1997. (Poster).

Collins, H. L. and A.-M. A. Callcott. Control of red imported fire ants with fipronil. National Plant Board Meeting. St. Petersburg, FL. August 9-13, 1997.

Lockley, T. C. Imported fire ants: a threat to native flora and fauna. Mississippi Academy of Sciences. Biloxi, MS. February, 1997.

Lockley, T. C. Control of the red imported fire ant. Southeastern Regional Public Health and Vector Management Conference. Panama City. FL. February, 1997.

Lockley, T. C. Population fluctuations of the sweetpotato weevil on wild host plants in southeast Mississippi. Entomological Society of America Annual Meeting. Nashville, TN. December 14-18, 1997.

T. C. Lockley presented general information to numerous local schools and civic organizations.

APPENDIX II - 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 (Jour. Econ. Ent. 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 minimizes 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 III

PROTOCOL FOR REARING OF IMPORTED FIRE ANTS

INTRODUCTION

Research and development studies for control of the red imported fire ant, *Solenopsis invicta,* have been in progress since late 1957 when a federal control program was initiated. Investigations on the biology, ecology, behavior, biocontrol, and pheromones of imported fire ants, as well as chemicals and formulations needed for control, require techniques for collecting, handling, rearing and maintaining ant colonies in the laboratory. Many techniques developed in earlier studies are being used at the Gulfport Plant Protection Station. However, some techniques have been revised or replaced by newer methods.

NEWLY MATED QUEENS (NMQ)

Colonies of known age and condition can be obtained only by rearing newly mated queens collected after mating flights. Mating or nuptial flights of ants serve as a means of dispersal of inseminated females and a way to increase genetic mixing.

FLIGHTS

Flights are found after rains when temperatures rise above 24°C (Rhoades and Davis 1967, Markin et al. 1971). Activity in the mound increases prior to flight. Internal tunnels are opened to the outside with exit holes 1/4" to 1/2" in diameter for alates to emerge (Markin et al. 1971). Mating flights occur any month of the year, but weather may prohibit regular flights. Flights may occur as early as 11:30 am and as late as 4:00 pm, but the majority occur between 1:00 and 3:00 pm (Roe 1973). Favorable conditions include temperature ranging between 23.8° and 33.3°C, light winds up to 5 mph, and rain 1-3 days before flight.

Ninety percent of flights from individual colonies are composed of predominately (>75%) one sex of individuals (Morrill 1974). Males normally emerge before females. Males form a layer over the flight area up to a height of 1000 ft. Females fly up to 800 ft. Most queens land less than 1/4 mile from the flight's point of origin. However, evidence has shown in extreme

cases queens can fly or be carried by wind 7 to 10 miles. Within 1/2 hour after taking flight, mated females descend to the ground and shed wings within minutes (Markin et al. 1971).

COLLECTION

Newly mated queens can be collected using light featherweight forceps. Queens are placed in a vial with moist toweling for transport to the laboratory. Queens are then transferred to "rearing tubes" (see SUPPLIES section) immediately for colony production. Rearing tubes are capped and placed lengthwise in a 9" X 3" X 2" Rubbermaid® pan.

GROWTH

Studies by O'Neal and Markin (1975) and Markin et al. (1972) indicate an average of 26 eggs laid by newly mated queens the day after isolation. The ideal temperature for brood development is 32°C. However, laboratory personnel find this uncomfortable. Between 27°C and 32°C is acceptable. An increase in egg production occurs through the 5th day and decreases until pupation occurs. After pupation on the 12th day, egg production again increases. Minim workers enclose on the 18th day and begin tending queen and brood. Minor workers and major workers appear on the 24th and 28th days, respectively. Sexual brood produce alates in 34 days; developmental times are identical for males and females (O'Neal and Markin 1975). Complete life cycle occurs in 30-40 days.

Rearing tubes should be uncapped when 10-12 minims have eclosed. A 16x125mm test tube of water and a 16x125mm tube of 10% sugar water should be added to the box, as well as a small amount of food (see SMALL COLONIES/FOOD). When the population becomes too much for the rearing tube, ants can be moved to a small rearing nest (see SUPPLIES) in a 9" x 6" x 2" Rubbermaid® pan. Small test tubes can be replaced with 20x150mm tubes and a larger amount of food. As colonies increase in size, nests can be upgraded as needed (see SMALL COLONIES/GROWTH).

SMALL COLONIES

Some research must be done in a timely manner with no regard to the age of the colonies used. Collecting newly mated queens and beginning a new colony in the laboratory will take much longer than collecting established colonies or fragments of colonies from the field.

COLLECTION

Colony fragments containing workers, brood, sexuals, and infrequently, the mated queen can be obtained by shoveling a portion of the nest tumulus into a bucket or similar container. The inner walls of the container must be carefully coated with Fluon (ICI Flouropolymers, Downington, PA) or dusted with inert talc to prevent escape. This method can be used if the queen is not required. Odds of capturing the queen increase during the early morning hours of bright, sunny days after cool nights from late fall to early spring. Brood and queen can usually be found near top of mound on side exposed to sunlight (Banks et al. 1981).

Colonies can be transported to the laboratory, fed, maintained, and used directly from the container for various studies. However, the ants also may be separated from the soil by spreading the soil in large trays. As the soil dries, the ants are forced to move into moist plaster of paris nests (Markin 1968).

Colonies collected from the field also may be separated using the "drip" method described by Jouvenaz et al. (1977). After transporting ants to the laboratory, buckets are left undisturbed overnight to allow the ants to reestablish tunnels and to collect buried brood. Water is then slowly dripped from a weighted drip emitter connected to standard drip irrigation tubing. The rising water level forces the ants to the surface. When the soil is completely submerged, the ants and the brood they have rescued float or cling in masses to the sides of the bucket. A cup or ladle can be used to transfer the masses to a new container minus the soil. If queen is present, colony can be maintained in laboratory.

GROWTH

After separating ants from soil, place ants, brood, and queen in 9" x 6" x 2" Rubbermaid® pan coated with fluon to prevent escape. A small 25x100mm rearing nest (see SUPPLIES), 20x150mm test tubes of water and sugar water, and a small weighing boat for food should be placed in pan with ants. A 250- or 500-ml cup or beaker can be used for holding colony debris (see SUPPLIES/CLEANING).

When colony becomes too large for nest, another small nest can be added. A large 25x150mm nest may be necessary depending on colony size. As colony grows, larger amounts of water and food will be required. Water tubes (25x150mm) and sugar tubes (25x200mm) for large colonies can be used, as well

as transferring colonies to larger rearing pans (22"l x 17 1/2"w x 3"d).

FOOD

Fire ants, like most ants, are omnivorous and feed primarily on sugar solutions and arthropods. Both are important components in an ant's diet. Sugars serve as a fuel source to power worker activities; and the protein from arthropods is used for brood production. The absence of protein causes colonies to cease brood production within a week or so (Porter 1989).

Arthropods of any kind can be used. Crickets are a favorite source. However, crickets for colonies being fed weekly can become quite costly. Substitutions may be made--such as mealy bug larvae. Additional items which can be added to the diet include peanut butter and boiled egg yolks.

Food may be place directly in pan, but this increases mortality of workers and queens due to fungal growth on unused food (Banks et al. 1981). Therefore, weighing boats of varying sizes are recommended for holding food in colony rearing pan. Food source can be placed in weighing boats on a weekly basis while disposing of "leftovers" in debris cup mentioned above. Amount of food source used depends on size of colony, availability of food source, and frequency of feedings.

SUPPLIES

NEWLY MATED QUEENS

light featherweight forceps for collection
small vial with moist toweling for transport to lab
9"x3"x2" Rubbermaid® pan (painted with fluon)
"rearing tube"
*16x150mm test tube filled halfway with water
*cotton is placed directly above water
*dental labstone (Patterson Dental, Metairie, LA) is poured on top of cotton using a modified squirt bottle
*when labstone dries, surface provides moisture for queen while laying eggs
*place 16mm plastic cap on tube to prevent escape of moisture--and queen when present
16x125mm tubes for water and sugar water--when 10-12 minims have appeared (remove cap)
small rubberbands (for sugar tubes to distinguish from water)
small weighing boat for food as colony grows
fluon (may be colored with dye)
small foam paintbrush for fluon

talc powder
large cotton balls for test tubes
powdered labstone

SMALL COLONIES

9"x6"x2" Rubbermaid® pan (painted with fluon)
fluon (may be colored with dye)
foam paintbrush for fluon
buckets and shovel
talc powder
drip system with tubing and emitters
weighing boats
20x150mm test tubes for water and sugar water for small colonies
small rubberbands (placed on sugar water to distinguish from H_2O)
large cotton balls
25x150mm test tubes for large colony water
25x200mm test tubes for large colony sugar water
250- or 500-ml cup or beaker for debris pail
plastic rearing pan (22"x17 1/2"x3")stored in grocery bread racks
full grown crickets (frozen)
mealy bug larvae (reared in laboratory)
peanut butter
small "rearing nest"
*15x60mm plastic petri dish with thin sponge in bottom
*make 2 holes 1/2" apart in lid for piece of 1/4" tubing
*place wax paper (with center cut out) in bottom of large plastic petri dish 25x100mm
*place small dish inside middle of large dish
*pour labstone in large dish until only tubing is exposed
*make small holes in sides of large dish for nest access
*paint lid of large dish with black shoe polish
large "rearing nest"
*15x100mm plastic petri dish with thin sponge in bottom
*make 2 holes 1/2" apart in lid for piece of 3/8" tubing
*place wax paper (with center cut out) in bottom of larger plastic petri dish 25x150mm
*place smaller dish inside middle of larger dish
*pour labstone in larger dish until only tubing is exposed
*make small holes in sides of larger dish for nest access
*paint lid of larger dish with black shoe polish

CLEANING

Periodically, rearing nests become dirty and must be replaced. A new nest is placed in pan and the top of the old nest is removed. Ants will move into the new nest. The old nest is removed when vacated. Pans also require changing when dirty. A clean tray is prepared and painted with fluon. Nests are transferred to clean tray. Water tubes are removed; ants are shaken out of tubes into clean tray. Tubes are placed in hot soapy water to be cleaned (long forceps will be needed to remove cotton). Ants and debris remaining in bottom of dirty tray should be scraped into a pile and placed in the debris pail. Debris pail is then transferred to clean tray where live ants can crawl out of pail. Debris left in pail can be thrown out when vacated by ants.

Rearing nest should be monitored for excess or lack of moisture. Too much moisture may promote fungal growth. If this occurs, replace nest as mentioned above. If nest begins to become dry, use a large hypodermic needle to insert water in the tubing of the nest. Add water until sponge is moist. If nests become too dry, ants may eat through tubing and move into sponge area.

CONCLUSION

Since the red imported fire ant, *Solenopsis invicta* Buren, was first collected in 1945 in Mississippi, it has spread across the southeast United States at an average yearly expansion of 3-5 miles (Morrill 1974).

Laboratory rearing of the imported fire ant is essential to research. It provides a means for controlled investigations on the biology of this ant. Large numbers of colonies of different sizes and ages can easily be reared and maintained in the laboratory for experimental use if guidelines are followed.

APPENDIX IV

TECHNOLOGY TRANSFER

Training and techno1ogy transfer were provided at the request of several stakeholders in 1997:

o Anne-Marie Callcott presented a seminar on IFA control around poultry production houses to pesticide distributors. The seminar was given at Birmingham, AL on February 6, 1997 at the request of Novartis Crop Protection, Inc.

o Homer Collins discussed biology and control of IFA at a workshop on impact of IFA on quail populations. The workshop was held at the request of Mossy Dell Plantation. Albany, GA on February 25, 1997.

o Training on all aspects of IFA biology and control was provided to Rhone-Poulenc personnel on February 19, 1997. This workshop was held in Gulfport, MS. Tim Lockley covered biology, Homer Collins addressed control, and Anne-Marie Callcott discussed test plot techniques, data collection, and statistical analyses.

o Training on biology of the pest and the quarantine program was presented to Georgia Department of Agriculture personnel by Anne-Marie Callcott and Homer Collins. This seminar was held on September 23, 1997 in Macon, GA.

o Tim Lockley addressed the Forrest/Lamar County Cattlemen's Association on biology and control of IFA at Hattiesburg. MS on September 22, 1997.

o Tim Lockley provided an update on IFA control to the Mississippi Aerial Applicators Association in Jackson, MS on November 14, 1997.

o A 51 page booklet entitled "Imported Fire Ant Quarantine Treatment for Nursery Stock and Other Regulated Articles" was revised, updated, and distributed to SPROs and SPHDs in 13 states and Puerto Rico. A total of 150 copies were distributed. In addition to listing treatments for all regulated articles, the booklet contains copies of pesticide labels, and names and addresses of affected SPROs and SPHDs.

APPENDIX V

IFA INTERCEPTIONS. 1997

Nursery stock shipped out of the imported fire ant quarantine area is routinely inspected for presence of live IFA upon arrival at its destination, or at some check point in route. The state of Arizona is especially diligent in this endeavor. An informal system has evolved whereby, if live IFA are found in a non-quarantined state on incoming nursery stock from a quarantined area, the ants are usually sent to the state laboratory for taxonomic verification. Media from the suspect nursery stock is collected and sent to the USDA, APHIS, PPQ, National Monitoring and Residue Analysis Laboratory (NMRAL) in Gulfport. MS for chemical analysis. NMRAL routinely analyzes these samples for certain program chemicals; chlorpyrifos (Dursban), diazinon, and bifenthrin. A portion of the collected media sent to NMRAL is shared with the Gulfport Plant Protection Station where IFA worker bioassays are performed (Appendix II; modified to use 20 major workers in lieu of 5 alate females per replicate). Results from both testing procedures are forwarded to Joe Vorgetts (USDA, APHIS, PPQ, TSS, Riverdale, MD; formerly BBEP, TSS) for reporting to the collecting agency.

Trials conducted in our laboratory have shown that residual activity of chlorpyrifos (Dursban) is affected by media type; some media requires more chlorpyrifos than others to be effective against IFA (Callcott et al. 1995. Proc. of Southern Nurserymen's Assoc. pp. 158-177). In a prior trial, we tested three types of media treated with chlorpyrifos against IFA alate females and found that a minimum of 10 ppm of chlorpyrifos is necessary to insure adequate activity against IFA (FA01G024; 1995 Annual Report). This same report indicates that a minimum of 5.0 ppm of bifenthrin is necessary for adequate control. We have no data at this time on rates of tefluthrin or diazinon needed to obtain 100% kill in most medias.

In 1997, 19 media samples from nursery stock originating within the IFA quarantined area were submitted to NMRAL for testing. Fifteen of these interceptions were detected in Arizona, the remaining 4 interceptions were made in California. Plant material from all of the 19 interceptions were from nurseries in Florida. There may have been additional interceptions in other states that we have no record of. Results of chemical analyses and bioassays that were conducted in 1997 are shown in Table 1.

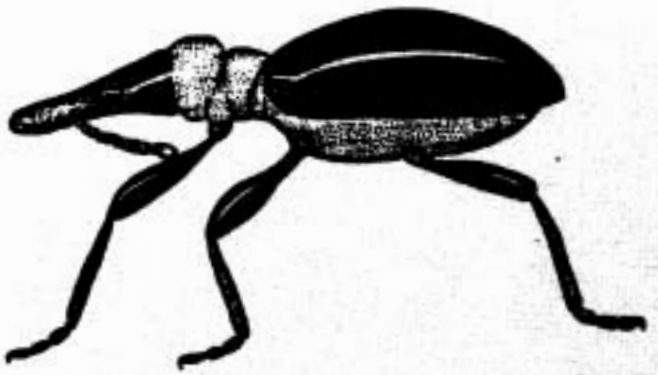
Table 1. Results of all IFA Quarantine Violations Which Have Passed Through the IFA Laboratory in 1997.

NMRAL Lab Number	Plant Material Origin	Plant Material Sampled At . . . (Destination)	Collect-ion Date	IFA Lab Bioassay Results (% mortality)	NMRAL Results (ppm)				Comments
					Dursban	Diazinon	Bifenthrin	Tefluthrin	
AB61819	Homestead, FL	San Simon border, AZ	2/22/97	100	27.2	<0.024	--	--	live IFA found
AB63606 AB63607 AB63608	Botek Botanicals Homestead, FL	L.A. Tropicals Whittier, CA	5/29/97	2.5 NA 12.5	<0.1 <0.1 <0.1	<0.1 <0.1 <0.1	<0.9 <0.9 <0.9	<0.12 <0.12 <0.12	live IFA found; also analyzed for malathion - 1.6, 1.9, 1.2 ppm, respectively; carbaryl - ND
AB63952	Botek Botanicals Homestead, FL	Nurserymen's Exch. Half Moon Bay, CA	6/11/97	100	<0.64	<0.60	ND	<0.12	non-infested plants
AB63489 AB63495	Costa Nursery, Goulds, FL	The Plant Stand Phoenix, AZ	6/3/97	52.5	0.896	2.38	ND	--	
AB66769 AB66775	Campbell's Foliage Homestead, FL	Altman Specialty Phoenix, AZ	8/12/97	100	31.0	<0.01	ND	--	
AB67215 AB67216	Brynteson's Nursery Lake Worth, FL	Family Tree Farms Phoenix, AZ	8/27/97	17.5	<0.015	<0.007	<0.9	--	
AB67190 AB67196	Stewart Nursery Boynton Beach, FL	Green Things Tuscon, AZ	8/25/97	100	37.0	<0.007	<0.9	--	live IFA found
AB67640 AB67646	Lake Worth, FL	Sanders border, AZ	8/24/97	100	59.0	<0.007	<0.9	--	
AB68055 AB68061	Lake Worth, FL	San Simon border, AZ	9/8/97	100	9.9	<0.007	<0.9	--	live IFA found
AB68415	Pine Island Nursery Miami, FL	Temple Gardens El Monte, CA	9/17/97	10.4	--	ND	--	--	live IFA colonies found
AB69415 AB69421 AB69427 AB69428	two growers in FL	Home Depot, Milpitas, CA	10/28/97	100	7.7	<0.007	<0.9	<0.12	live IFA found

NMRAL Lab Number	Plant Material Origin	Plant Material Sampled At . . . (Destination)	Collect-ion Date	IFA Lab Bioassay Results (% mortality)	NMRAL Results (ppm)				Comments
					Dursban	Diazinon	Bifenthrin	Tefluthrin	
AB69849 AB69855	Carol City, FL	San Simon border, AZ	10/29/97	100	ND	--	<0.9	--	live IFA found
AB62514 AB62520	Bokeelia, FL	San Simon border, AZ	4/6/97	NA	<0.007	<0.005	<0.16	--	live IFA found
AB62812 AB62818	Deerfield Beach, FL	San Simon border, AZ	4/24/97	NA	ND	<0.022	ND	--	live IFA found
AB62850	Plant World Nursery Clearwater, FL	Sanders border, AZ	4/28/97	NA	<0.032	<0.022	--	--	live IFA found
AB64252 AB64258	B & L Farms, Lake Worth, FL	The Plant Stand, Phoenix, AZ	6/17/97	NA	13.2	0.14	ND	--	
AB65117 AB65123	Alva, FL	San Simon border, AZ	7/12/97	NA	ND	<0.01	<0.9	--	live IFA found
AB66775	Campbell's Foliage Homestead, FL	Altman Specialty Phoenix, AZ	8/12/97	NA	--	--	ND	--	
AB66911	Goulds, FL	San Simon border, AZ	8/12/97	NA	62.0	<0.007	<0.9	--	live IFA found

ND - not detected; detection limits vary
NA - not enough sample media for both bioassay and chemical analysis, therefore only chemical analysis performed
-- no results; probably not analyzed for particular chemical in that sample

SWEETPOTATO WEEVIL



FY 1996 SWEET POTATO WEEVIL OBJECTIVES

GULFPORT PLANT PROTECTION STATION
GULFPORT. MS

The Sweet Potato Weevil (SPW), *Cylas formicarius elegantulus* (Summers) is one of the most devastating pests of sweet potatoes worldwide. It occurs in southern parts of Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Texas. Recent advances in the development of pheromone traps and other issues have spawned renewed interest in this old program pest.

At the request of several members of the Souther Plant Board we initiated a small project to test and develop improved trapping techniques for use in storage facilities and growing fields. Short term goals for 1996 were as follows:

- Establish a reprint file of selected scientific papers and become familiarized with the biology, rearing, and trapping of SPW.
- Establish a laboratory culture of SPW using standardized rearing techniques.[5]
- Establish a field planting of sweet potatoes on USDA farm property in Harrison County, MS[6]; artificially infest this field in early season with SPW in anticipation of conducting late season trapping studies.
- Conduct trapping studies on alternate hosts in Harrison County, MS.
- Establish working relationships with Louisiana State University research and extension personnel so that expanded trapping studies can be expedited.
- Establish working relationships with State Regulatory Agencies in Louisiana and Mississippi in order to foster future cooperative efforts on SPW.

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[5] Physical Location: Gulfport Plant Protection Station. USDA. APHIS, PPQ, 3505 25th Ave., Gulfport, MS 39501

[6] Physical Location: Harrison County Farm, County Farm Road, Gulfport. MS.

SPW CONTENTS

PROJECT NO:	PROJECT TITLE:	PAGE
97SPW001	Sweetpotato Weevil Trap Efficacy	179
97SPW006	Attractiveness of the Sweetpotato Weevil to Various Light Sources	183
96SPW001	Population Fluctuations of the Sweetpotato Weevil on Wild Host Plants in South Mississippi	185
96SPW003	Feeding of Sweetpotato Weevils on Non-Convolvulaceae Hosts	191
96SPW004	Laboratory Evaluations of *Ipomoea* Species as Host Plants for the Sweetpotato Weevil	193
97SPW002	Relative Attractiveness of Wild and Cultivated Alternate Host Plants (Convolvulaceae) to the Sweet Potato Weevil	197
97SPW004	Attraction of Leaf and Root Volatiles of Wild Host Plants to the Sweetpotato Weevil, *Cylas formicarius elegantulus* (Summers)	200
	REFERENCES CITED	204
APPENDIX I	Sweetpotato Weevil Alternate Host Plants	206

PROJECT NO: 97SPW001

PROJECT TITLE: Sweet Potato Weevil Trap Efficacy

TYPE REPORT: Final

LEADER/PARTICIPANTS: Tim Lockley & Charlene Russell

INTRODUCTION:

As a sampling tool for the sweet potato weevil (SPW) in storage facilities, the Dickerson Type trap is somewhat inefficient. Its current popularity is due to its being relatively inexpensive when compared to other traps available. Weevil behavior has not been taken into consideration. Data suggests that the weevil does not fly directly to the trap but lands at a distance and approaches the trap on the ground. The distance a weevil flies to reach the trap has not been determined nor the distance traveled on the substrate. As an initial evaluation of the Dickerson Type trap efficacy in storage facilities, the following experiment was devised.

MATERIALS AND METHODS:

A 1/4 inch thick clear plexiglas sheet (4' x 4') was gridded in 3 inch squares Fig. 1). Tanglefoot® was applied to the surface of the plexiglas sheet at a thickness of 1/16 inch. A Dickerson Type trap with a fresh SPW pheromone septa was placed in the middle of the sheet. In Trial I. fifty male SPW were released on 6 January 1997 at a distance of 25 feet from the trap. Release was made in an enclosed area ca. 500 sq ft. The grid was examined at 24 hour intervals for 14 days for the presence of entrapped SPW. Trial II was initiated on 21 January 1997 with the release of 50 marked SPW males within the same facility.

RESULTS:

Trial I:

At 24 hours post release, no SPW were observed in the pheromone trap nor were any found on the grid. Of the 50 SPW released, 4 remained in the container; 3 alive and one dead. A cursory examination of the room revealed no sign of the released SPW. Temperature within the room was ca. 70°F.

At 192 hours into the trial, one SPW male was captured on the outside edge of grid L-9 (Fig. 1). No other SPW were captured during the two week monitoring period (Table 1).

Trial II.

At 24 hours, no dead weevils were observed at the point of release. One male SPW was found within grid K-11. Two males were found <u>under</u> the plexiglas sheet. They were removed and placed at the original point of release. No SPW were caught after 48 hours. At 72 hours, 8 males were captured; two from quadrant II, five from quadrant III and one from quadrant IV. All eight SPW males captured were found along the outside edge of each quadrant (Table 2). No SPW were captured from that point until termination of the trial at 360 hours.

Explanations for the lack of captures range from too cold temperatures during Trial I to rapid inundation of the air within the test area by the pheromone making detection of the trap highly unlikely. In Trial II, space heaters were set up within the test area to maintain temperatures at ca. 18 - 20°C. Other less likely scenarios include photophobia by SPW. The room in which the weevils were released was not completely light free. The eastern end of the room held a double door with glass panes. This allowed a significant amount of light into the room during the day thus retarding the activity of this nocturnally active insect.

Insufficient numbers of SPW were captured to draw any real conclusions from these tests as regards the efficiency of the Dickerson Type trap. The pattern of capture would seem to indicate that approach to the trap is indirect with the weevil traveling along the substrate to the trap rather than flying directly to it.

Figure 1. Grid/Quadrant Pattern for Trap Efficacy Trials.

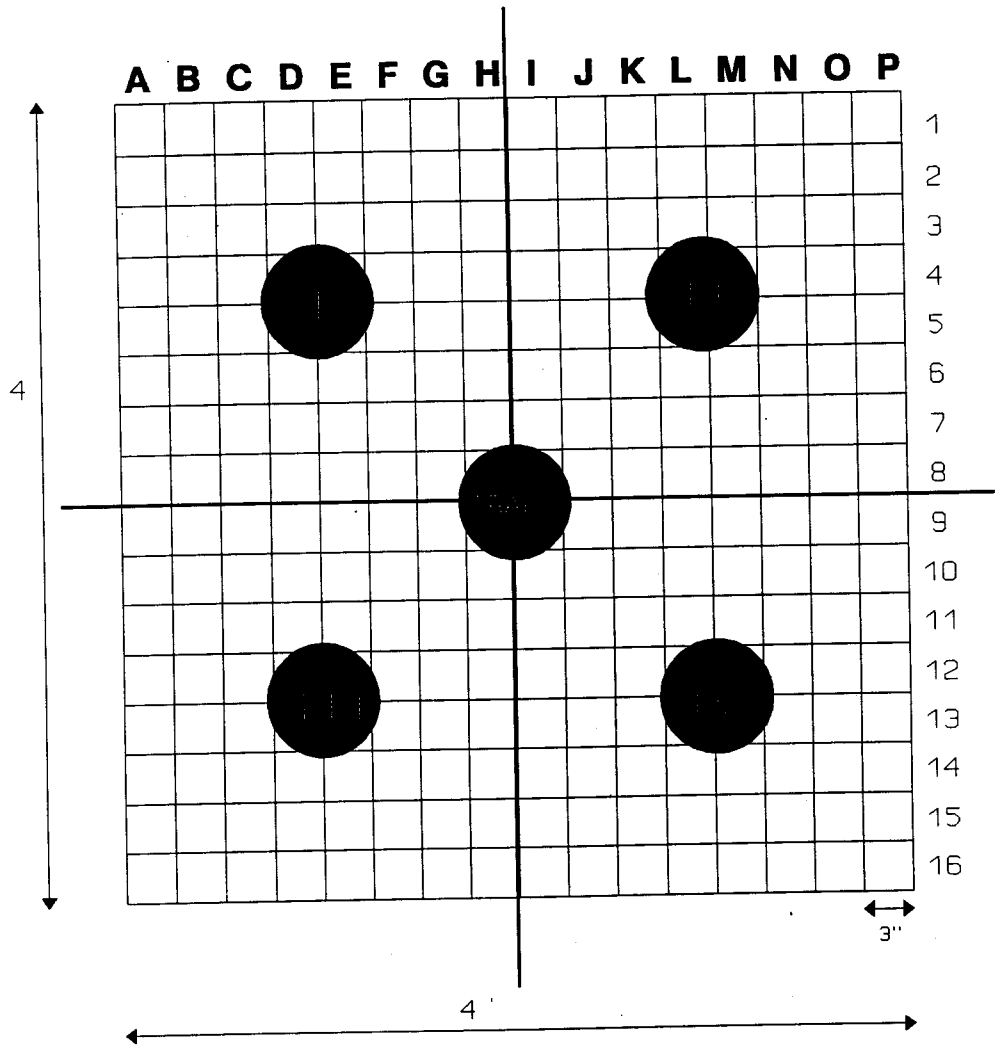


Table 1. Relative Number and Placement of Male SPW Captured by the Dickerson Type Trap in a Simulated Storage Environment in Trial I.

HOURS POST RELEASE	NUMBER MALE SPW RELEASED	NUMBER MALE SPW CAPTURED	NUMBER MALE SPW IN TRAP	NUMBER FOUND IN GRID QUADRANTS QUADRANT			
				I	II	III	IV
24	46*	0	0	0	0	0	0
48	47*	0	0	0	0	0	0
72	47*	0	0	0	0	0	0
96	47	0	0	0	0	0	0
120	47	0	0	0	0	0	0
144	47	0	0	0	0	0	0
168	47	0	0	0	0	0	0
192	47	1	0	0	0	0	1#
216	47	0	0	0	0	0	0
240	47	0	0	0	0	0	0
264	47	0	0	0	0	0	0
288	47	0	0	0	0	0	0
312	47	0	0	0	0	0	0
336	47	0	0	0	0	0	0
360	47	0	0	0	0	0	0

* Fifty male SPW released: 24 hrs - 3 remained in container (1 dead)
48 hrs - 2 remained in container (1 dead)
72 hrs - 3 dead in container

Table 2. Relative Number and Placement of Male SPW Captured by the Dickerson Type Trap in a Simulated Storage Environment in Trial II.

HOURS POST RELEASE	NUMBER MALE SPW RELEASED	NUMBER MALE SPW CAPTURED	NUMBER MALE SPW IN TRAP	NUMBER FOUND IN GRID QUADRANTS QUADRANT			
				I	II	III	IV
24	50	1	0	0	0	1	0
48	50	0	0	0	0	0	0
72	50	8	0	0	2	5	1
96	50	0	0	0	0	0	0
120	50	0	0	0	0	0	0
144	50	0	0	0	0	0	0
168	50	0	0	0	0	0	0
192	50	0	0	0	0	0	0
216	50	0	0	0	0	0	0
264	50	0	0	0	0	0	0
288	50	0	0	0	0	0	0
312	50	0	0	0	0	0	0
336	50	0	0	0	0	0	0
360	50	0	0	0	0	0	0

PROJECT NUMBER: 97SPW006

PROJECT TITLE: Attractiveness of the Sweetpotato Weevil to Various Light Sources

TYPE REPORT: Final

LEADER/PARTICIPANTS: Timothy C. Lockley & Homer Collins

INTRODUCTION:

Although the artificial pheromone is an effective attractant for SPW males in the field, it is of little use within storage facilities. Many insects are photophilic and can be readily captured by various light sources. Cockerham (1954) indicated in his study of the biology of the sweetpotato weevil that they were attracted to light, and were easily captured in light traps. Though no other corroborative evidence could be found in the SPW literature, an experiment was devised to determine if SPW were, indeed attracted to light, and, if so, the specific type of light to which they were attracted.

MATERIALS AND METHODS:

Locally purchased lights were mounted in or suspended above a modified bucket light trap and placed along side a field of sweet potatoes that had been artificially infested with sweet potato weevils. Traps were run from ca. 2-3 hours before dark until 2-3 hours after dawn (ca. 14 hours). Kill strips were placed in the bottom of the bucket to preclude escape. Captured arthropods were collected in a cloth bag and transported to the laboratory where they were examined for the presence of SPW. Two traps were run each night.

RESULTS:

Fifty types of light sources were used in this study. Lights sources (both incandescent and fluorescent) ranged throughout the visible spectrum from infrared to ultraviolet. A small number of males were found in some of the traps but were probably not captured due to any attractiveness of the light sources. Attempts to repeat the successful captures using the same light source failed. No female SPW were ever collected. Table 1 shows the various types of light sources used and their relative success in capturing SPW.

Table 1. Number of sweetpotato weevils captured by light trap using various light sources.

LIGHT SOURCE COLOUR/NAME	WATTAGE	TYPE	MANUFACTURER	NO. SPW CAUGHT FEMALES	MALES
Yellow, opaque	60	Incandescent	Commercial Service	0	5
Infra-red (heat)	125	Incandescent	Phillips	0	5
Grow light	65	Incandescent	GE	0	4
Clear (chrome)	50	Incandescent	Phillips	0	4
Quadlite	15	Fluorescent	Lights of America	0	4
Pink, opaque	60	Incandescent	Hand-dipped	0	3
Clear	60	Halogen	Phillips	0	3
Arctic Brite	15	Fluorescent	Lights of America	0	3
Display Pink	15	Fluorescent	Service	0	2
Actinic, 17DAS	15	Fluorescent	Voltarc	0	1
Hearth Glo	15	Fluorescent	Service	0	1
Soft Pink	60	Incandescent	GE	0	1
Teal, clear	25	Incandescent	GE	0	1
Blue, clear	25	Incandescent	GE	0	1
Hot Pink, clear	25	Incandescent	GE	0	1
Yellow, clear	25	Incandescent	Sylvania	0	1
Red, clear	25	Incandescent	ABCO	0	1
Actinic 18DAS	15	Fluorescent	Voltarc	0	0
Agro Lite	60	Incandescent	Phillips	0	0
Brite Lite	13	Fluorescent	Lights of America	0	0
Blue, clear	25	Incandescent	Feit Electric	0	0
Blue, clear	25	Incandescent	ABCO	0	0
Daylight	32	Fluorescent	Sylvania Circline	0	0
Frosted	50	Halogen	GE	0	0
Germicidal	15	Fluorescent	Phillips	0	0
Green, clear	25	Incandescent	ABCO	0	0
Green, clear	25	Incandescent	GE	0	0
Grow Light	15	Fluorescent	Market Lite	0	0
Grow Light	50	Incandescent	Bulbrite	0	0
Mercury Vapor	160	Incandescent	Iwasaki	0	0
Natural	100	Incandescent	Neo Dyne	0	0
Orange, clear	25	Incandescent	ABCO	0	0
Orange, clear	25	Incandescent	Fiet Electric	0	0
Purple, clear	25	Incandescent	GE	0	0
Red	15	Fluorescent	Phillips	0	0
Red, clear	25	Incandescent	Feit Electric	0	0
Red, clear	25	Incandescent	GE	0	0
Ultraviolet	15	Fluorescent	GE	0	0
Ultraviolet	15	Fluorescent	Sylvania Circline	0	0
Ultraviolet	75	Incandescent	ABCO	0	0
White, cool	22	Fluorescent	Sylvania Cicline	0	0
White, soft	25	Fluorescent	GE	0	0
White, flame tip	25	Incandescent	GE	0	0
White, earthlite	20	Fluorescent	Phillips	0	0
Yellow, opaque	60	Incandescent	Commercial Service	0	0
Yellow, opaque	40	Incandescent	Feit Electric	0	0
Yellow, clear	25	Incandescent	Feit Electric	0	0
75 PAR 30	75	Fluorescent	Neo Dyne	0	0
5900K White	15	Fluorescent	Service	0	0
Kerosene Lantern	NA	Natural Flame		0	0

PROJECT NUMBER: 96SPW001

PROJECT TITLE: Population Fluctuations of the Sweetpotato Weevil on Wild Host Plants in South Mississippi

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Timothy C. Lockley and Homer Collins

INTRODUCTION:

The sweet potato weevil, *Cylas* formicarius *elegantulus* (Summers) (Coleoptera: Curculionidae), is one of the most destructive pests of sweet potato, Ipomoea *batatas* (L.) Lam., world wide (Sutherland 1986). Because of the production of terpenoids caused by feeding, both yield and quality of sweet potatoes can be markedly reduced by even low level infestations of the sweetpotato weevil (SPW) (Uritani et al. 1975).

Infestations of SPW in commercial sweet potato fields can come from three sources: 1) nearby infested sweet potato fields (Jannson et al. 1989); 2) infested planting material (Rolston et al. 1983) and; 3) wild host plants (Cockerham et al. 1954).

The sweetpotato weevil has been known for decades to breed on a number of wild host plants in the family Convolvulaceae. Over 35 species of Convolvulaceae among seven genera in six tribes have been reported as serving as hosts for SPW (Austin 1991). In the three coastal counties of southeast Mississippi, at least 23 wild host plants (most of which are in the genus *Ipomoea*) are present (Lockley, pers. obs.) Information on these wild host plants (eg. local species and their abundance, host preference, etc.) is lacking from the literature.

To increase our knowledge of sweetpotato weevil and the role wild host plants play in population levels of this pest, a survey was begun in Hancock and Harrison Counties, Mississippi in June 1996.

MATERIALS AND METHODS:

Beginning in June 1996, screen-cone boll weevil traps (Dickerson et al. 1981) containing SPW pheromone (Agrisense®, Colombia, MD) were deployed throughout Hancock and Harrison counties Mississippi. Traps were placed at two week intervals among stands of nine different species of the Convolvulaceae (*Calystegia sepium, Ipomoea coccinea, I. cordato-triloba, I. imperati, I. indica, I. x leucantha, I. pandurata, I. purpurea, Merremia dissecta*). Traps remained in place for 24 hours and were then retrieved. Counts were made in the field and, in order to avoid "trapping out" the resident population, weevils were released at the point of capture. Monitoring of these sites is continuing and will proceed throughout CY 1998.

RESULTS:

Ipomoea cordato-triloba is the most commonly occurring of the morning glories in southeast Mississippi (Lockley, pers. obs.) and comprised 34.5% of the 29 sites investigated. A perennial along the Gulf Coast, *I. cordato-triloba* (sharp-pod morning glory) is one of the first of the hosts to bloom in the spring. It has an extensive tuberous root system and produces numerous vines from a single root source. Flowering begins in mid May and continues until early November. Hedge bind weed (*Calystegia sepium*) is next in overall abundance among the convolvulaceae in the study area. It is the first to be observed blooming in the spring (mid April). Flowering continued at most sites until late July. Some sporadic flowering was observed in late October. The roots of hedge bind weed are fleshy and extensive with numerous vines emerging along the length of the roots. An annual throughout most of its range, it acts as a perennial along the Gulf Coast.

Ipomoea pandurata and *Merremia dissecta* were each sampled at three sites. Both have thick, woody roots and are perennials in the area. *Ipomoea coccinea* and *Ipomoea indica* were found in sufficient abundance at two sites. *I. coccinea* is an annual with a fibrous root system. *I. indica* has an extensive fibrous root system that occurs along the length of the vines emerging at each node in contact with the soil surface. In southeastern Mississippi, *I. indica* is a perennial and, in protected areas, continues to bloom until the first heavy frost. In 1997, blooms could be seen until the end of December.

Ipomoea imperati, *I. purpurea* and *I. x leucantha* were examined at only one site each and were the least abundant morning glories studied.

Two other morning glories, *Ipomoea quamoclit* and *I. sagittata*, were found to occur extensively throughout the coastal area surveyed; but they were not in sufficient concentration to be added to the study. Twelve other species of morning glory (excluding the dodders within the genus *Cuscuta*) were found to occur sporadically in the study area (Table 1).

The sharp-pod morning glory (*I. cordato-triloba*) had the highest number of SPW captured averaging ca. 29.8 adult male weevils per trap night. Numbers were averaged from ten sites over 209 trap nights. Sharp-pod morning glories also recorded the highest single night capture with 484 SPW taken. Second in mean capture rate was *Ipomoea purpurea* (tall morning glory) with 20.4 SPW taken per trap night. Tall morning glory was found at only one site and, as such, may not reflect a "typical" population of the weevil on this host.

Three other wild hosts had modest to large numbers of SPW taken: *Ipomoea imperati* (beach morning glory); *I. coccinea* (red morning glory) and *Calystegia sepium*. Smaller numbers were found in *Ipomoea indica*, *I. pandurata* (man root morning glory) and *Merremia dissecta*. The hybrid *Ipomoea x leucantha* had the lowest capture rate averaging 0.5 SPW per trap night (Table 2).

Initial sampling was limited to only five sites representing three species of alternate hosts. Numbers of SPW from these sites were low with an average catch of only 7.9 per trap night. By mid July of 1996, trapping sites had increased to 15 and numbers of SPW taken had also increased dramatically. Whether these numbers reflected a normal population trend or were an artifact of the sampling can not be answered at this time. By early August (1996), numbers had stabilized (24 sampling sites) and remained steady until mid September when a precipitous decline in numbers began. This decline in overall populations of SPW continued until mid-December; at which point, a steady rise in numbers began. This increase in SPW population continued until the latter part of August (1997) and remained at roughly the same level until mid October (Figures 1 and 2).

These initial data would seem to indicate that any of the convolvulaceae

investigated can act as a significant reservoir of sweetpotato weevil populations and have the potential to be sources of infestation in adjacent or nearby plantings of commercial sweet potatoes. Sanitation procedures, such as removal or destruction of the alternate host plants should be seriously considered in any integrated pest management system of sweetpotato weevils. Monitoring of these sites and future sites will continue. It is anticipated that three years of monitoring will be needed to determine the "normal" fluctuations of SPW populations in southeast Mississippi.

Table 1. List of Known Alternate Host Plants (Convolvulaceae) of the Sweetpotato Weevil Found In Southeastern Mississippi.

Calystegia sepium (L.) R. Brown
Ipomoea alba L.
I. coccinea L.
I. cordato-triloba Dennst.
I. cordato-triloba var. *torreyana* (A. Gray) D.F. Austin
I. hederaceae var. *hederaceae* (L.) Jacq.
I. hederaceae var. *integriuscula* (L.) Jacq.
I. imperati (Vahl) Griseb.
I. indica (Burm.) Merr.
I. lacunosa L.
I. macrorhiza Michx.
I. nil (L.) Roth
I. pandurata (L.) G. Mey
I. pes-caprae (L.) R. Brown
I. purpurea (L.) Roth
I. quamoclit L.
I. sagittata Poir.
I. tricolor Cav.
I. turbinata Lag.
I. wrightii A. Gray
I. x leucantha Jacq.
Jacquemontia tamnifolia Choisy
Merremia dissecta (Jacq.) Hall

Table 2. Relative Average Numbers of Sweetpotato Weevil Captured Per Host Plant.

HOST	TOTAL NO. OF SITES	TOTAL NO. SPW CAPTURED	TOTAL TRAP NIGHTS	AVERAGE NO. SPW CAPTURED
Calystegia sepium	6	2082	174	12.0
Ipomoea coccinea	2	855	63	13.6
I. cordato-triloba	10	6226	209	29.8
I. imperati	1	390	27	14.4
I. indica	2	286	36	7.9
I. pandurata	3	437	103	4.2
I. purpurea	1	735	36	20.4
I. x leucantha	1	16	35	0.5
Merremia dissecta	3	390	80	4.9

Figure 1. Sweet Potato Weevil Populations on Wild Hosts along the Mississippi Gulf Coast.

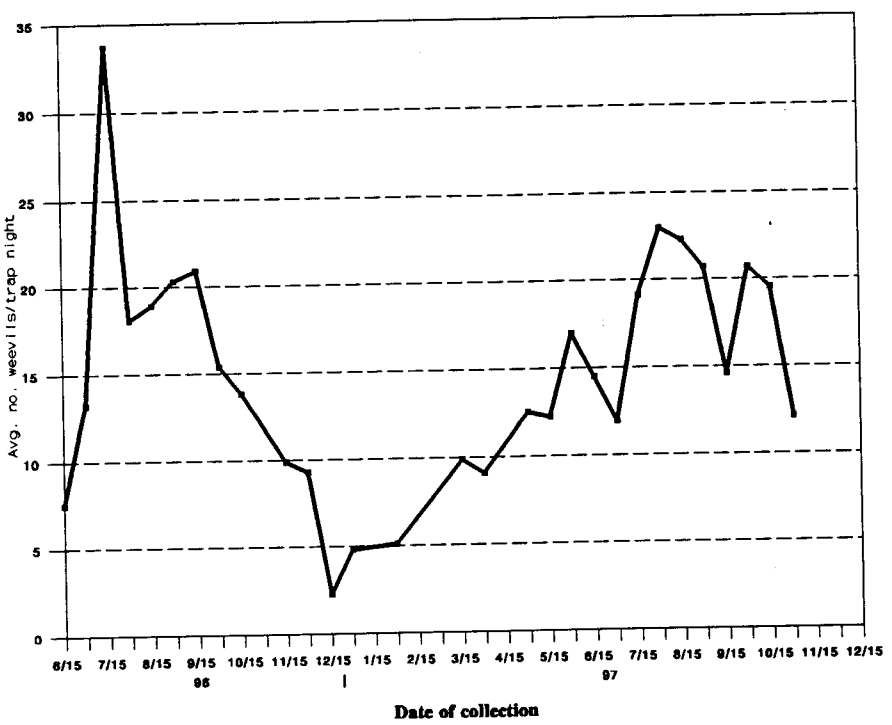
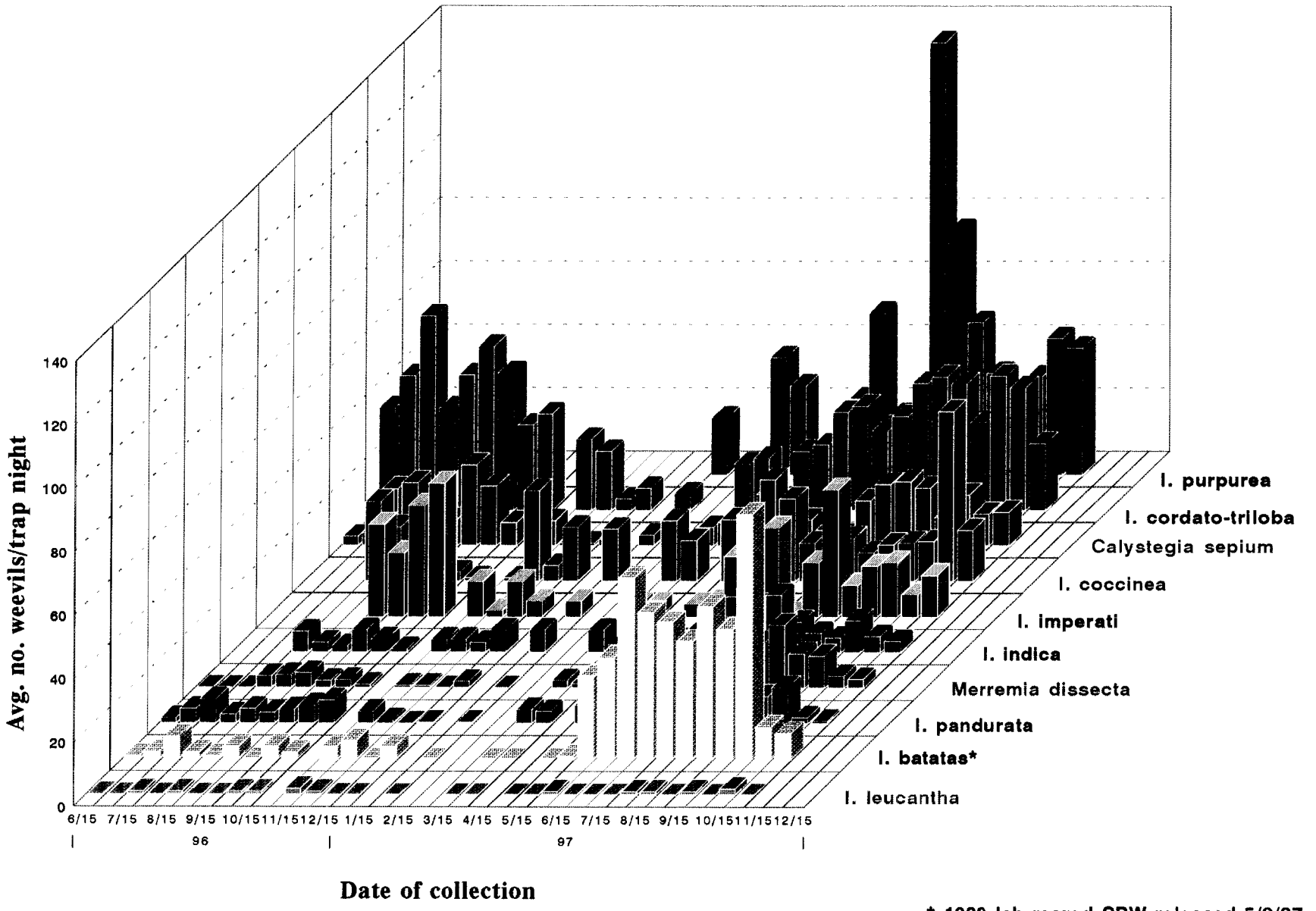


Figure 2. Sweetpotato Weevil Populations on All Hosts along the Mississippi Gulf Coast.



* 1000 lab reared SPW released 5/9/97

PROJECT NUMBER: 96SPW003

PROJECT TITLE: Feeding of Sweetpotato Weevils on Non-convolvulaceae Hosts

TYPE REPORT: Final

LEADER/PARTICIPANTS: Timothy C. Lockley and Charlene Russell

INTRODUCTION:

Although it has been shown that the preferred host plant of the sweetpotato weevil is *Ipomoea batatas* (Cockerham 1943), several other species in the family Convolvulaceae also serve as alternate hosts. Some attempts to use these alternate hosts as trap crops have been attempted in tropical areas with mixed results (Kemner 1924). Although few details are given, Jayaramaiah (1976) under laboratory conditions, succeeded in breeding SPW on carrot, *Dacus carota* (Umbelliferae); cassava. *Manihot esculenta (ultissima)* (Euphorbiaceae); and *I. campanulata* (Convolvulaceae). Das (1992) listed fourteen plants from nine families as alterative hosts for SPW. The author indicated that SPW could survive on these alternate hosts when Convolvulaceae (specifically the sweet potato) was unavailable. Many authors consider the presence of alternate hosts as potentials for major infestations and recommend their removal as a cultural control measure.

MATERIALS AND METHODS:

Investigations on the root preferences of SPW were conducted in the laboratory. Whole and sectioned roots of ten species of plants in 8 families were placed in 1-pint capacity jars. Tops were covered with insect screen. Five male and five female SPW were introduced into each jar (three replicates per species) where they remained throughout the study. Observations were made under magnification at one hour intervals for eight continuous hours each day over a three day period. One replicate per species of root stock was dissected every seven days under a binocular microscope to determine the presence of immature stages of SPW. Root stocks were maintained in the laboratory. Temperatures ranged from 20 - 25°C. Weevils used in the test were reared from sweet potato cultivars.

RESULTS:

Observations during the first day indicated feeding activity on Ginger roots (*Zingiber officinale*). During the second day of observations, feeding continued on ginger. Some signs of feeding were also noted in two of the three replicates of Chinese yam (*Discorea batatas*) and in one of the replicates of Taro (*Colocasia esculenta*). On the third day of the study, feeding continued on ginger. All three replicates of Taro and Chinese yam showed feeding activity by SPW; as did the three replicates of carrot (*Dacus carota*). In one replicate, SPW were seen feeding on the roots of collards (*Brassica oleraceae* var. *acephala*).

Although previously reported as alternate hosts (Muruvanda et al. 1986.), radishes showed no sign of feeding damage and neither radishes nor carrots housed any immature stages of SPW when dissected and examined under magnification. None of the other root stocks tested contained larval stages.

PROJECT NUMBER: 96SPW004

PROJECT TITLE: Laboratory Evaluations of *Ipomoea* Species as Host Plants for the Sweetpotato Weevil

TYPE REPORT: Final

LEADER/PARTICIPANTS: Timothy C. Lockley

INTRODUCTION:

Although it has been known for many years that the sweetpotato weevil, *Cylas formicarius elegantulus*, breeds on a number of wild host plants within the family Convolvulaceae, no study has been undertaken to determine the efficacy of these alternate hosts in contributing to overall weevil populations.

Tests were initiated to determine (1) if wild and cultivated morningglories can support SPW and (2) how many weevils can be generated by one plant.

MATERIALS AND METHODS:

With notable exceptions, all experimental plants were grown from seed in standard industry two-gallon containers. At maturity, three plants from each species were discarded and 15 cm of stem and 15 cm of root were collected. Each severed section of plant was placed on a moistened filter paper in a 100mm petri dish with one female and one male SPW. After 72 hours, stems and roots were examined under a binocular microscope for signs of feeding damage.

In a second study, plants were placed in screened cages with two male and two female SPW to determine the potential number of SPW each species of morning glory is capable of producing as a seedling plant. Plants were watered through a drip irrigation system on an as-needed basis. Plants were removed at 40 days post introduction and the top 33 cm of stem and major root trunks were excised, dissected and examined under magnification for the presence of SPW immatures.

RESULTS:

In the first trial, all species of morning glories showed signs of feeding activity. Feeding punctures for both stems and roots seemed to be

concentrated near the crown line (soil level) in most of the tests (Table 1).

The greatest feeding activity was in *Ipomoea cordato-triloba*, sharppod morning glory, with an average of 7.3 feeding punctures along the root and 4.3 punctures along the stem. *I. batatas*, sweet potato, ranked second with an average of 5.7 feeding punctures along the root and 3.0 along the stem. The commercially grown morning glory, *I. aquatica* (water spinach), had all of its stem feeding punctures located within the hollow portion. No punctures were found on the exterior surface of the stem.

In the second study, all morning glory species, with the exception of *Ipomoea quamoclit* and *Jacquemontia tamnifolia*, had latter instar SPW immatures within stems and roots (Table 2). Both the cypressvine morning glory and the smallflower morning glory had been previously indicated by other researchers as acting as wild hosts for SPW.

Table 1. Feeding activity of Sweet Potato Weevils on Alternate Host Plants.

Species	ROOTS		STEMS	
	Average number of punctures	Primary location of punctures	Average number of punctures	Primary location of punctures
Convolvulus tricolor	2.3	Crown	1.3	Crown
Ipomoea tricolor "Pearly Gates"	3.0	Throughout	1.3	Crown
I. alba	3.0	Crown	2.0	Crown
I. aquatica	3.3	Throughout	7.3	Interior
I. batatas	5.7	Throughout	3.0	Crown
I. coccinea	2.0	Crown	1.7	Crown
I. cordato-triloba	7.3	Throughout	4.3	Throughout
I. hederaceae	3.7	Crown	1.7	Crown
I. hederaceae var. *intergriuscula*	3.0	Crown	2.3	Crown
I. indica	3.7	Throughout	2.7	Crown
I. lacunosa	1.7	Crown	1.0	Crown
I. nil	3.7	Throughout	2.0	Crown
I. pandurata	4.3	Throughout	1.7	Crown
I. purpurea	2.0	Crown	2.0	Crown
I. quamoclit	2.0	Crown	1.7	Crown
I. wrightii	4.3	Throughout	2.3	Crown
I. x leucantha	1.3	Crown	1.7	Crown
I. x multifida	1.0	Crown	0.7	Crown
Jacquemontia tamnifolia	1.7	Crown	1.0	Crown
Merremia dissecta	3.7	Throughout	2.0	Crown

Table 2. Relative Efficacy of Differing Species of Alternate Host Plants in the Production of the Sweetpotato Weevil.

SPECIES	Date SPW introduced	Date examined	Mean no. larvae	Mean number larvae present	
				stem	root
Ipomoea tricolor "Pearly Gates"	25 IX 96	5 XI 96	0.7	0.3	0.3
I. alba Moon Flower	6 XI 96	18 XII 96	0.7	0.3	0.3
I. aquatica Water Spinach	6 XI 96	18 XII 96	1.7	1.3	0.3
I. batatas Sweet Potato	25 IX 96	5 XI 96	21.3	0.3	21.0
I. coccinea Scarlet M.G.	19 II 97	2 IV 97	0.3	0.0	0.3
I. hederaceae Blue M.G.	3 IV 97	14 V 97	1.0	0.7	0.3
I. hederaceae var. *integriuscula* Ivyleaf M.G.	19 II 97	2 IV 97	0.7	0.3	0.3
I. imperati Beach M.G.	7 I 97	18 II 97	2.7	0.3	2.3
I. lacunosa Pitted M.G.	19 II 97	2 IV 97	0.3	0.0	0.3
I. x multifida "Cardinal Climber"	3 IV 97	14 V 97	0.7	0.7	0.0
I. nil "Scarlet O'Hara"	25 IX 96	5 XI 96	0.7	0.7	0.0
I. pandurata Bigroot M.G.	19 II 97	2 IV 97	1.7	0.3	1.3
I. purpurea Tall M.G.	7 I 97	18 II 97	0.3	0.3	0.0
I. quamoclit Cypressvine M.G.	6 XI 96	18 XII 96	0.0	0.0	0.0
I. tricolor "Heavenly Blue"	25 IX 96	5 XI 96	1.7	1.0	0.7
I. cordato-triloba Sharppod M.G.	19 II 97	2 IV 97	3.7	0.7	3.0
I. turbinata Purple Moonflower	6 XI 96	18 XII 96	0.7	0.7	0.0
I. wrightii Palmleaf M.G.	7 I 97	18 II 97	0.7	0.7	0.0
I. x leucantha	3 IV 97	14 V 97	0.3	0.3	0.0
Jacquemontia tamnifolia Smallflower M.G.	6 XI 96	18 XII 96	0.0	0.0	0.0
Merremia dissecta	7 I 97	18 II 97	0.3	0.3	0.0

PROJECT NO: 97SPW002

PROJECT TITLE: Relative Attractiveness of Wild and Cultivated Alternate Host Plants (Convolvulaceae) to the Sweet Potato Weevil

TYPE REPORT: Final

LEADER/PARTICIPANTS: Tim Lockley

INTRODUCTION:

There are at least 23 species of wild host plants found in southeastern Mississippi as well as a number of commercially grown species (pers. obs.). Much study has been devoted to the effect of the sweetpotato weevil on commercial sweet potatoes; but little is known about the relative attractiveness of alternate hosts when compared to the sweet potato.

MATERIALS AND METHODS:

Fifteen species (17 cultivars) in four genera of Convolvulaceae grown from seed were transplanted into standard industry two-gallon containers. Fifteen of each species/cultivar were divided into three replicates of five plants each and set, still in the container, into the ground in a random block design on 29 April, 1997. On 9 May, 1660 SPW (638 male/1014 female) were released along the entire length of the row in which the hosts were arranged. One plant per replicate was removed each month and returned to the laboratory where stems and roots were examined for evidence of SPW larvae, larval damage or emergence.

RESULTS:

At one month into the study, dissections of sampled plants showed larval infestations in only three of the cultivars: *Ipomoea grandifolia* (with the highest infestation of 5), *I. wrightii* and *Mina lobata citronella*. By the second month, ten of the seventeen cultivars examined revealed the presence of either SPW larvae or larval damage. The highest infestation occurred among *Calystegia sepium*, *Ipomoea imperati*, *I. ni7* and I. *tricolor* (3 each). *Ipomoea alba*, *I. x leucantha* and *I. setosa* each had 2 and *I. batatas* "Blacky", *I. grandifolia* and *I. hederaceae* each had 1. Two of the cultivars were dead at

month three: *Ipomoea tricolor* and *Mina lobata citronella*. Insufficient plant remains were available to determine if SPW larval damage was the cause of their demise. Of the remaining 15 cultivars, twelve were infested. At 120 days into the study, three more cultivars were dead (a 7 week drought had begun in the latter part of month two and this was the probable culprit in their death). Eight of the remaining cultivars were infested. Before the end of month five, the site had been destroyed by mowing.

Of all the cultivars evaluated, only two failed to ever be infested by SPW: *Convolvulus tricolor* and *Merremia dissecta*. *Convolvulus tricolor* is a commercially grown ornamental and has not been indicated as an alternate host of SPW. It has a shallow, extensive fibrous root system and the stems are numerous and woody. *Merremia dissecta* is a known host of SPW (see 96SPW001 and 96SPW004). Only *Ipomoea grandifolia* (a close relative of the sweet potato) was infested throughout the trial. *Ipomoea alba* and I. *batatas* "Blacky" were infested through three months (Table 1).

Table 1. Comparative Number of Sweet Potato Weevil Larvae Found in Alternate Convolvulaceae.

	NUMBER OF LARVAE MONTHS POST-PLANTING			
TAXON	I	II	III	IV
Calystegia sepium	0	3	1	*
Convolvulus tricolor	0	0	0	0
Ipomoea alba	0	2	1	1
I. aquatica	0	0	3	3
I. batatas "Blacky"	0	1	1	2
I. batatas "Margarita"	0	0	1	4
I. batatas "Tricolor"	0	0	4	2
I. grandifolia	5	1	1	2
I. hederaceae	0	1	2	*
I. imperati	0	3	2	0
I. x leucantha	0	2	1	3
I. nil	0	3	0	*
I. setosa	0	2	2	*
I. tricolor "Heavenly Blue"	0	3	*	
I. wrightii	1	0	4	10
Merremia dissecta	0	0	0	0
Mina lobata citronella	1	0	*	

* all plants dead

PROJECT NO: 97SPW004

PROJECT TITLE: Attraction of Leaf and Root Volatiles of Wild Host Plants to the Sweet Potato Weevil, *Cylas forrnicarius elegantulus* (Summers)

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Tim Lockley, Mike Legendre[7], Flowers White[7], & Charlene Russell

INTRODUCTION:

Cylas species weevils are serious pests of sweet potato, *Ipomoea batatas* (L.) Lam. throughout the world. They infest plants and storage roots both in the field and during postharvest storage (Nottingham et al. 1989). Plant volatiles are considered to be the main attractants to phytophagous species of insects (Visser 1986). Over 300 insect species within five orders were listed in Metcalf's review (1987) as selectively responding to 64 different plant odorants. Although some data has been collected about volatiles of sweet potatoes (Nottingham et al. 1989), little is understood concerning alternate wild hosts within the convolvulaceae.

MATERIALS AND METHODS:

Sweetpotato weevils (SPW) were reared in the laboratory on sweet potato storage roots in containers held at ca. 28°C and 70% relative humidity. Emerging weevils were removed weekly and transferred to containers with fresh sweet potato storage roots. SPW had no previous contact with sweet potato leaves or to any wild host plants.

WHOLE LEAF/ROOT STUDY - Leaves and roots were collected ca. 1 hour prior to use. Leaves sufficient to equal an area of ca. 50 cm. sq. were used. Root periderm was shaved from the root surface in amounts equal to ca. 50 cm. sq. Experimental protocol for both trials is described below.

LEAF AND ROOT VOLATILE STUDY - Leaves were collected from 7:00 to 10:00 am on the 25th and 26th of August, 1997. Leaves were placed in plastic bags and transported to the laboratory where they were cooled to 10° C and held for 24

[7]National Monitoring and Residue Analysis Laboratory

hours. Leaves, sufficient to make 100g, were removed from the terminal 1.0 meter of healthy vines.

Leaf Material. All leaf material was collected in the field from healthy, growing plants. Leaf surface extracts were made by placing 100g of leaf material in 300 ml of methylene chloride for 24 hours in a 1 liter teflon jar. The jar was agitated for two hours on a shaker and the liquid was filtered into a 500ml roundbottom flask and rotovapped. Hexane (50 ml) was added and the extract was rotovapped almost to complete dryness. The material was transferred with a Pasteur pipet into a 15 ml centrifuge tube and reduced to 1.0 ml on N-Vap. The material was then transferred into a glass vial, sealed with a Teflon-lined cap and kept at 0°C until required.

Root Material. Roots were collected from perennial wild host plants at the same time as the leaves were collected. Roots were severed at the root crown, transported in separate plastic bags and stored in the same manner as the leaves. One hundred grams of root from wild host plants and 100 g of sweet potato root periderm were used in the extractions. Procedures for extraction were accomplished as described for leaf material.

Bioassays. A dual-choice olfactometer, as described by Nottingham et al. (1989) was used to test the response of both male and female SPW to volatiles of various wild hosts.

Experimental procedures. SPW were starved for 2 hours prior to exposure to whole leaf/root or plant volatiles. Twenty-five male or female SPW were placed in the main arena. Air was drawn through the experimental chamber by vacuum pump. Plant volatile extracts were dried on filter paper and placed in test tubes. Root periderm or whole leaves were placed on a moistened 100mm filter paper and placed in the bottom of the chamber. Weevils were exposed for a period of 2 hours. After that time, numbers of weevils in each chamber were counted. Three replicates per host plant were carried out.

RESULTS:

In those trials comparing relative attractiveness of whole leaves to root periderm, females showed a decided preference for roots over leaves. Male SPW displayed the opposite preference (Table 1). The sweet potato (45%) and its closest relatives (*Ipomoea cordato-triloba* [35%], *I. cyanchifolia* [50%], *I. trifida* [35%], *I. tiliaceae* [20%]) along with two closely related species (*I. imperati* [30%], *I. pes-caprae* [35%]) and *Turbina corymbosa* [30%] showed the greatest attractiveness for male SPW. Among female SPW, the greatest attractiveness were for, in descending order of preference, *I. pes-caprae* (45%), *I. imperati* (40%), *I. aquatica* (20%), *I. batatas* (20%) and *I. triloba* (20%).

The second phase of the study, the leaf/root volatile choice test, will be undertaken in the spring of 1998 or such time as sufficient numbers of laboratory reared SPW are available.

Table 1. Relative Attractiveness of Various Whole Leaves and Root Surfaces to Male and Female Sweetpotato Weevils.

	MEAN NUMBER OF SPW			
	LEAVES		ROOTS	
TAXON	MALES	FEMALES	MALES	FEMALES
I. alba	2	1	NA	NA
I. aquatica	0	5	NA	NA
I. batatas	9	5	1	8
I. coccinea	5	2	NA	NA
I. cordato-triloba	7	4	0	9
I. cordato-triloba x lacunosa	4	3	1	6
I. cyanchifolia	10	4	NA	NA
I. grandifolia	0	2	NA	NA
I. hederaceae	1	0	0	3
I. imperati	6	8	NA	NA
I. indica	4	3	NA	NA
I. lacunosa	2	1	NA	NA
I. x leucantha	1	0	NA	NA
I. pandurata	0	1	1	3
I. pes-caprae	7	9	NA	NA
I. quamoclit	1	0	NA	NA
I. tiliacea	5	2	NA	NA
I. trifida	7	4	NA	NA
I. triloba	2	5	NA	NA
I. umbraticola	1	4	NA	NA
I. wrightii	0	2	NA	NA
M. dissecta	2	2	0	3
Turbina corymbosa	6	4	NA	NA

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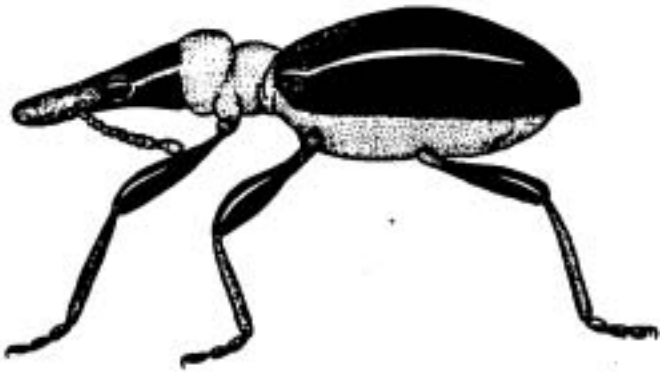
APPENDIX I

PROTOCOL FOR SWEETPOTATO WEEVIL REARING PROCEDURES



ADULT SWEETPOTATO WEEVIL, *Cylas formicarius elegantulus*

INTRODUCTION

The sweetpotato weevil, *Cylas formicarius elegantulus* (Summers), is the most devastating insect pest of the sweet potato. *Ipomoea batatas* (L.) Lam, worldwide. The sweetpotato weevil was introduced into the United States in the early 1900s from the West Indies and was first reported in New Orleans. LA. Populations have spread throughout the southern United States from Texas to South Carolina (Edmond and Ammerman 1971). The weevils feed almost exclusively on the leaves, vines, and storage roots of members of the *Ipomoea* genus. Storage roots of the sweetpotato are attacked in the field and during storage (Kays et al. 1993).

The negative impact of sweetpotato weevils on previous potato crops has increased the need for additional research. The process of rearing sweetpotato weevils in the laboratory has become necessary to conduct field and laboratory experiments efficiently. The information and protocol listed below has been compiled from rearing studies practiced in the Gulfport Plant Protection Station.

COLLECTION

Collection of wild sweetpotato weevils takes place periodically to increase numbers and genetic variation. Screen-cone boll weevil type traps are baited with sweetpotato weevil pheromone septa (AgriSense. Palo Alto. CA 94303) to attract male sweetpotato weevils. Females are collected using

coffee cans baited with sweetpotato roots and placed in patches of *Ipomoea trichocarpa*, sharppod morningglory, a natural sweetpotato weevil host plant.

CAGES

Upon capture weevils are transferred to rearing cages inside the lab. Cages are standard 10-gallon glass aquariums. Wooden lids 9 1/2"w x 19"l x 3 1/2" d are cut to snugly fit inside the top of the aquarium. A 3/8"wide x 1/4"deep lip is routered on the underside of the lid to ensure a tighter fit. The lid's center is removed and covered with standard household nylon screen. Sweet potato roots are placed in a pile inside the cage.

CONDITIONS

Nottingham et al. (1989) and Kays et al. (1993) reared sweetpotato weevils for research on feeding habits. Results from those studies indicate the optimum conditions for sweetpotato weevil development as 27°C and 65% relative humidity. Adult weevils should be transferred to fresh potatoes every 7 days, with old infested potatoes set aside until emergence of new adults occurs.

FOOD

Little is known about the sweetpotato weevils' ability to distinguish members of the *Ipomoea* genus from other species (Kays et al. 1993). Nottingham et al. (1988) has shown both male and female weevils are attracted by volatile compounds emitted from aerial plant parts; whereas, only female weevils are attracted to volatiles emitted from storage roots, the primary site of oviposition.

The primary sugars present in sweet potato roots are sucrose, fructose, and glucose. (Sugar concentrations are cultivar dependent.) Sucrose appears to act as a feeding stimulant for the sweetpotato weevil (Kays and Howard 1984). Weevils usually move beneath the root, where they feed in groups. The weevil feeds until its head and snout are in the root then moves to a new feeding site (Sherman and Tamashiro 1954).

GROWTH

The complete life cycle for the sweetpotato weevil takes approximately 33 days. Once weevils reach the adult stage, mean longevity at 27°C and relative humidity of 65% is approximately 93 days (Mullen 1981). Mating is

required for normal oviposition (Cockerham et al. 1954) and more than one mating is required for a female weevil to achieve her full reproductive capacity (Mullen 1981).

Adult females excavate cavities (1.4mm deep and 0.4mm in diameter) in which to lay single cream-colored eggs (Jayaramaiah 1975). The female then seals the cavity with a gray fecal plug to preserve moisture, protect the egg, and disguise the location of oviposition. The rate of oviposition varies from 1-17 eggs per female per day until shortly prior to death (Sherman and Tamashiro 1954).

The developing larvae feed within the root and usually bore down into the potato. The winding feeding tunnel is narrow at first but enlarges with growth of the insect. The tunnel ends in the pupation chamber.

After emerging from the pupal stage, the new adult remains within the pupal chamber or larval tunnel for 6-9 days. It emerges with full coloration, but does not develop normal cuticular hardness until after feeding begins (Sherman and Tamashiro 1954).

Cylas formicarius elegantulus is a smooth shiny weevil. The head is black, the thorax is orange to reddish brown, and the posterior portion, including the elytra, is dark metallic blue. The sexes can be determined by view of the antennae. Distal segments are longer in males (figure 1) and shorter in females (figure 2). Weevils vary in length from 5.5 to 7.0mm (Sherman and Tamashiro 1954).

CONCLUSION

Sweetpotato weevils, *Cylas formicarius*, may be reared in the laboratory for research purposes. Ideal conditions include 27°C and 65% relative humidity. Cages need to be secure with good airflow. Potatoes for feeding and oviposition should be changed out every 7-10 days. Weevils can be counted every few days. They may be identified and separated for certain experiments bases on antennae differences. Sweetpotato weevils, upon being disturbed, will feign death. They fall on their backs, keep legs extended in a relaxed position and remain motionless. If left alone, the weevil will resume activity within several minutes. Breathing on them or holding them close to a hot surface may provoke them into "awaking" earlier. Weevils may be identified, even when feigning death, as long as antennae are visible.

Several studies including Mullen (1981), Reinhard (1923), and Subramanian (1959) have reported a 1:1 sex ratio in adult weevil production.

This seems to hold true, except in cold winter months when numbers decrease. However, population increases soon after. Laboratory rearing of sweetpotato weevils can be efficient, beneficial, and entirely successful.

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ADULT SWEETPOTATO WEEVIL, *Cylas formicarius elegantulus*

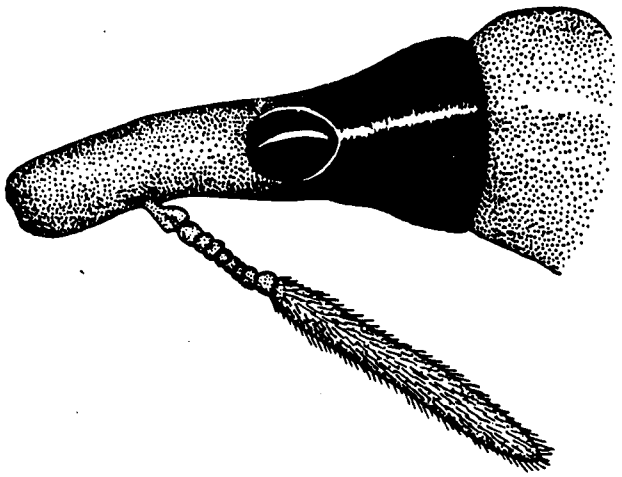

Figure 1. Head and antenna of male sweetpotato weevil.

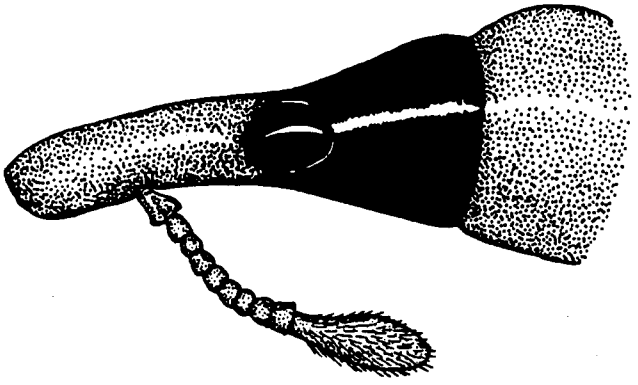

Figure 2. Head and antenna of female sweetpotato weevil.