

# **1999 ACCOMPLISHMENT REPORT**

**GULFPORT PLANT PROTECTION STATION**  
**CENTER FOR PLANT HEALTH SCIENCE AND TECHNOLOGY**  
**PLANT PROTECTION AND QUARANTINE**  
**U.S. DEPARTMENT OF AGRICULTURE**

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These reports were prepared for the information of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service personnel, and others interested in imported fire ant control 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:

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## **FY 1999 IMPORTED FIRE ANT OBJECTIVES**

GULFPORT PLANT PROTECTION STATION  
GULFPORT, MS

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

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

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

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

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

OBJECTIVE 4:

Determine impact of IFA on biodiversity of various ecosystems.

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

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PROJECT NO: FA01G037

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

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Lee McAnally

### INTRODUCTION:

Fipronil 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, U.S. registrations for the product include mole cricket control in sod, termite control in structures and fleas on dogs. 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 dispersible 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 and aged at the Gulfport site.

### MATERIALS AND METHODS:

Fipronil was blended into nursery potting soil from Windmill Nursery, Folsom, LA (bulk density of media was 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 standard 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. An additional 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:*

Through 9 months (35 ppm and below) and 5 months (40 and 50 ppm) these trials were evaluated on the 7-day exposure period. Seven days is the traditional exposure period called for in the standard protocol used by this lab. Due to the slower acting nature of some of the newer compounds, it was decided to change the exposure period to 14 days. It was also decided to start



checking the bioassays daily to determine the length of exposure required by each rate to reach 100% mortality. Bioassays were checked daily for a 14-day exposure period beginning at 10 and 6 months post-treatment.

Treatment rates below 35 ppm were either poor or erratic at the 7 days exposure period (Table 1). The 7 and 10.5 ppm rates were dropped from the trial and discarded after 4 months. The higher rates maintained 95-100% efficacy through 15 months for the 35 ppm rate, and 100% efficacy through 11 months for the 40 ppm rate and 27 months for the 50 ppm rate in 7 days or less. All rates retained in the trial, 14-50 ppm, have reached 100% within 14 days exposure through 31 months (Table 2).

*MAFES media:*

The 10 ppm rate maintained 95-100% through 14 months when the ants were exposed for 7 days (Table 3). The 25 ppm rate maintained 100% efficacy through 17 months, the 40 ppm rate through 26 months, and the 50 and 75 ppm rates through 29 months. Through 7 months these trials were evaluated on the 7-day exposure period. Bioassays conducted at the 14-day exposure period began at 8 months post-treatment. Bioassays were also checked daily beginning at 8 months. All rates have maintained 100% efficacy through 29 months at the 14 day exposure except the 10 and 25 ppm rates which were 100% effective through 27 months (Table 4).

*Flowerwood media:*

Treatment rates of 10-40 ppm maintained 100% efficacy through 26 months at exposures of 7 days or less and the 50 and 75 ppm rates through 29 months (Table 5). Through 7 months these trials were evaluated on the 7-day exposure period. Bioassays conducted at the 14-day exposure period began at 8 months post-treatment. Bioassays were also checked daily beginning at 8 months. All rates have maintained 100% efficacy through 29 months at the 14 day exposure (Table 6).

Table 1. Residual Activity of Fipronil 0.1G in Windmill Media After 7 Days Exposure to Treated Media

Rate of application (ppm)	% mortality of alate females at indicated months post-treatment (7 days exposure)																		
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-15	-16	-17	-18	-19
7	30	60	10	30	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
10.5	30	30	5	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
14	65	55	15	25	10	0	20	--	--	--	60	30	50	25	20	*	10	10	40
17.5	95	75	75	20	10	55	55	60	100	35	95	75	60	40	60	*	35	35	70
28	100	100	90	100	50	100	100	90	100	95	100	100	100	100	100	*	40	80	100
35	100	100	100	100	95	100	100	100	100	100	100	100	100	100	100	*	75	80	85
Check	0	25	15	15	0	5	5	15	10	5	10	10	40	15	15	*	5	5	10
40	100	100	100	100	100	100	100	100	100	100	100	*	80	95	100	100	100	**	100
50	100	100	100	100	100	100	100	100	100	100	100	*	100	100	100	100	100	**	100
Check	0	5	5	15	10	5	10	10	40	15	15	*	5	5	10	0	5	**	15

\* not evaluated at 7 days due to Hurricane Georges

\*\*not evaluated due to lack of alate queens

Table 1. ( Cont.)Residual Activity of Fipronil 0.1G in Windmill Media After 7 Days Exposure to Treated Media

Rate of application (ppm)	% mortality of alate females at indicated months post-treatment (7 days exposure)																		
	-20	-21	-22	-23	-24	-25	-26	-27	-28	-29	-30	-31	-32	-33	-34	-35	-36	-37	-38
7	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
10.5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
14	65	55	**	45	85	**	85	75	30	65	50	85							
17.5	35	85	**	95	90	**	60	85	10	50	95	60							
28	90	100	**	100	100	**	100	80	85	100	100	100							
35	100	100	**	100	100	**	100	100	95	100	100	100							
Check	0	10	**	15	10	**	10	5	5	10	0	5							
40	100	**	80	100	100	100	100	100											
50	100	**	100	100	100	100	100	100											
Check	10	**	10	5	5	10	0	5											

\*\*not evaluated due lack of alate queens

Table 2. Residual Activity of Fipronil 0.1G in Windmill Media During 14 Day Exposure Periods.

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (# of days to reach 100% mortality or % mortality at 14 days exposure)																		
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-15	-16	-17	-18	-19
7	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
10.5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
14	--	--	--	--	--	--	--	--	--	10d*	12d	9d	13d	11d	**	12d	10d	13d	
17.5	--	--	--	--	--	--	--	--	9d*	10d	12d	9d	10d	8d	**	12d	10d	11d	
28	--	--	--	--	--	--	--	--	8d*	5d	12d	7d	7d	7d	**	12d	10d	7d	
35	--	--	--	--	--	--	--	--	7d*	5d	6d	4d	7d	7d	**	8d	10d	11d	
Check	--	--	--	--	--	--	--	--	5%*	10%	10%	40%	15%	15%	45%	10%	15%	10%	
40	--	--	--	--	--	7d	4d	5d	4d	6d	5d	**	8d	10d	7d	6d	6d	***	4d
50	--	--	--	--	--	7d	4d	5d	4d	6d	5d	**	7d	7d	7d	6d	6d	***	4d
Check	--	--	--	--	--	5%	10%	10%	40%	15%	15%	45%	10%	15%	10%	0%	10%	***	15%

- daily readings of bioassays not performed prior to this evaluation period
- \*\* was not read 4-9 days exposure due to Hurricane Georges all were 100% at 10 days
- \*\*\*not evaluated due to lack of alate queens

Table 2. (Cont.) Residual Activity of Fipronil 0.1G in Windmill Media During 14 Day Exposure Periods.

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (# of days to reach 100% mortality or % mortality at 14 days exposure)																		
	-20	-21	-22	-23	-24	-25	-26	-27	-28	-29	-30	-31	-32	-33	-34	-35	-36	-37	-38
7	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
10.5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
14	12d	10d	***	12d	10d	***	10d	10d	10d	11d	9d	8d							
17.5	12d	8d	***	8d	10d	***	10d	10d	10d	8d	8d	8d							
28	8d	7d	***	5d	6d	***	7d	10d	10d	5d	7d	7d							
35	7d	7d	***	4	5d	***	5d	7d	10d	6d	7d	6d							
Check	5%	10%	***	15%	10%	***	20%	10%	10%	15%	10%	5%							
40	5d	***	10d	7d	5d	5d	7d	6d											
50	5d	***	7d	6d	6d	6d	7d	5d											
Check	10%	***	20%	10%	10%	15%	10%	5%											

\*\*\*not evaluated due to lack of alate queens

Table 3. Residual Activity of Fipronil 0.1G in MAFES Media After 7 Days Exposure to Treated Media

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (7 days exposure)																	
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	(13)*	-14	-15	-16	-17	-18
10	100	100	100	100	100	100	100	100	100	100	100	100	95	100	60	85	80	35
15	95	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	95	65
20	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	90	85
25	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	85
40	100	100	100	100	100	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	100	100	100	100	100
75	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Check	0	10	10	5	5	15	0	10	15	15	15	15	15	10	15	10	0	5

\* readings taken at 6 days exposure due to Hurricane Georges

Table 3. (cont.)Residual Activity of Fipronil 0.1G in MAFES Media After 7 Days Exposure to Treated Media

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (7 days exposure)*																	
	-20	-21	-23	-24	-25	-26	-27	-28	-29	-30	-31	-32	-33	-34	-35	-36	-37	-38
10	95	100	80	50	90	100	0	5	100									
15	90	100	85	70	100	100	10	25	85									
20	100	100	100	100	100	100	30	50	100									
25	95	100	100	100	100	100	30	40	100									
40	100	100	100	100	100	100	75	100	100									
50	100	100	100	100	100	100	100	100	100									
75	100	100	100	100	100	100	100	100	100									
Check	5	10	25	15	15	10	0	0	5									

\* months 19 & 22 were not evaluated due to lack of alate queens

Table 4. Residual Activity of Fipronil 0.1G in MAFES Media During 14 Day Exposure Periods.

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (# of days to reach 100% mortality or % mortality at 14 days exposure)																	
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-15	-16	-17	-18
10	--	--	--	--	--	--	--	5d*	6d	7d	7d	7d	13d**	7d	9d	11d	9d	9d
15	--	--	--	--	--	--	--	4d*	6d	7d	7d	6d	6d	7d	7d	7d	8d	9d
20	--	--	--	--	--	--	--	5d*	6d	4d	5d	5d	5d	6d	7d	7d	12d	9d
25	--	--	--	--	--	--	--	4d*	6d	4d	6d	5d	5d	6d	7d	7d	7d	8d
40	--	--	--	--	--	--	--	4d*	6d	3d	4d	5d	4d	6d	7d	7d	6d	7d
50	--	--	--	--	--	--	--	3d*	3d	3d	5d	5d	4d	6d	4d	4d	6d	6d
75	--	--	--	--	--	--	--	3d*	3d	3d	4d	4d	3d	3d	4d	4d	6d	6d
Check	--	--	--	--	--	--	--	15%*	15%	15%	15%	15%	55%	10%	15%	15%	10%	15%

\* daily readings of bioassays not performed prior to this evaluation period

\*\* was not read 7-12 days exposure due to Hurricane Georges



Table 4. (cont.) Residual Activity of Fipronil 0.1G in MAFES Media During 14 Day Exposure Periods.

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (# of days to reach 100% mortality or % mortality at 14 days exposure) *																	
	-20	-21	-23	-24	-25	-26	-27	-28	-29	-30	-31	-32	-33	-34	-35	-36	-37	-38
10	8d	7d	10d	9d	8d	7d	11d	85%	7d									
15	8d	7d	10d	8d	6d	6d	11d	10d	10d									
20	7d	6d	7d	7d	6d	6d	11d	10d	7d									
25	8d	5d	7d	7d	6d	4d	11d	85%	7d									
40	7d	6d	6d	6d	6d	4d	9d	6d	6d									
50	6d	5d	5d	6d	6d	4d	7d	7d	5d									
75	5d	5d	5d	5d	3d	4d	7d	5d	4d									
Check	5%	10%	25%	15%	15%	10%	25%	10%	5%									

\* months 19 & 22 were not evaluated due to lack of alate queens

Table 5. Residual Activity of Fipronil 0.1G in Flowerwood Media

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (7 days exposure)																	
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-15	-16	-17	-18
10	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
15	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
20	100	100	100	100	100	100	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	100	100	100	100	100	100
40	100	100	100	100	100	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	100	100	100	100	100
75	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Check	0	5	0	30	20	0	10	0	25	25	20	5	5	5	0	15	0	25

Table 5. (cont.) Residual Activity of Fipronil 0.1G in Flowerwood Media

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (7 days exposure) *																	
	-20	-21	-23	-24	-25	-26	-27	-28	-29	-30	-31	-32	-33	-34	-35	-36		
10	100	100	100	90	100	100	5	5	100									
15	100	100	100	100	100	100	75	100	100									
20	100	100	100	100	100	100	85	100	100									
25	100	100	100	100	100	100	20	100	100									
40	100	100	100	100	100	100	15	100	100									
50	100	100	100	100	100	100	100	100	100									
75	100	100	100	100	100	100	100	100	100									
Check	0	5	50	5	5	0	10	5	15									

\*months 19 & 22 not evaluated due to lack of alate queens

Table 6. Residual Activity of Fipronil 0.1G in Flowerwood Media During 14 Day Exposure Periods.

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (# days to reach 100% mortality or % mortality at 14 days exposure)																	
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-15	-16	-17	-18
10	--	--	--	--	--	--	--	5d*	7d	7d	7d	7d	6d	7d	7d	7d	6d	7d
15	--	--	--	--	--	--	--	4d*	6d	7d	5d	5d	6d	6d	7d	4d	6d	3d
20	--	--	--	--	--	--	--	4d*	6d	4d	5d	7d	6d	6d	7d	4d	6d	3d
25	--	--	--	--	--	--	--	3d*	6d	4d	5d	5d	5d	6d	7d	4d	6d	3d
40	--	--	--	--	--	--	--	3d*	3d	3d	4d	4d	5d	6d	4d	4d	6d	3d
50	--	--	--	--	--	--	--	4d*	3d	3d	4d	4d	5d	6d	4d	4d	6d	3d
75	--	--	--	--	--	--	--	3d*	3d	2d	3d	4d	5d	3d	3d	4d	6d	2d
Check	--	--	--	--	--	--	--	0%*	25%	25%	20%	5%	5%	5%	0%	15%	0%	25%

\* daily readings of bioassays not performed prior to this evaluation period

Table 6. (cont.) Residual Activity of Fipronil 0.1G in Flowerwood Media During 14 Day Exposure Periods.

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (# days to reach 100% mortality or % mortality at 14 days exposure)																	
	-20	-21	-23	-24	-25	-26	-27	-28	-29	-30	-31	-32	-33	-34	-35	-36	-37	-38
10	7d	7d	6d	10d	7d	6d	14d	10d	7d									
15	6d	6d	6d	6d	7d	5d	9d	7d	5d									
20	4d	5d	5d	5d	6d	5d	8d	6d	5d									
25	7d	5d	5d	5d	6d	4d	8d	6d	5d									
40	4d	5d	5d	4d	6d	4d	8d	6d	5d									
50	4d	5d	4d	4d	6d	4d	5d	5d	4d									
75	4d	4d	4d	4d	3d	4d	4d	4d	4d									
Check	0%	5%	50%	5%	5%	0%	10%	5%	15%	25%	20%	5%	5%	5%	0%	15%		

PROJECT NO: FA01G067

PROJECT TITLE: Residual Activity of Fipronil 0.05G Incorporated into Potting Media and Applied "Over-the-Top"

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Lee McAnally

### INTRODUCTION:

Fipronil 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, U.S. registrations for the product include mole cricket control on golf courses, termites in structures and fleas on dogs. 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:*

Granular 0.05% fipronil was blended into nursery potting soil (MAFES mix, 650 pounds per cubic yard) on 2 October 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. Treatments rates used were 5, 10, 15, 25, and 50 ppm. Treated media was then poured into one-gallon capacity plastic nursery 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 moisture 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. Initially queen mortality was assessed after seven days of continuous confinement to the treated soil. At 6 months post-treatment, bioassays were checked daily for 14 days or until 100% mortality was attained. On 23 June 1998 Windmill potting media (Windmill Nursery, Folsom, LA, 200 pounds per cubic yard) at rates of 5, 10, 15, 20, 25, 40, and 50 ppm was added to the trial. On 1 July 1998 Flowerwood potting media (Flowerwood Nursery, Mobile, AL, 390 pounds per cubic yard) was also added at the same rates as the Windmill media. Both of these later trials were bioassayed in the same manner described above and were read daily for 14 days or until 100% mortality.

### *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:*

#### MAFES Media:

When using the 7 day exposure period, the 50 ppm rates provided 95-100% efficacy for 25 months, the 25 ppm rate through 21 months and 10 ppm rate through 12 months (Table 1). The 5 ppm rate was somewhat erratic through 27 months. The 15 ppm rate showed some unexplained drops in efficacy at 1 and 4 months post-treatment, but otherwise has maintained 100% efficacy through 21 months. However, all rates were still attaining 100% mortality using the 14 days exposure period with the exception of the 15 ppm rate attaining only 75% efficacy at 14 days exposure at the 23 month time period (Table 2).

#### Windmill Media:

At six months post-treatment, the 5, 10, 15 and 20 ppm rates evaluated at the 7 day exposure period showed poor results and became erratic after that (Table 3). The 25 ppm rate showed a decline at 6, 11, and 13 months, the 40 ppm rate showed a decline at 6 and 11 months but otherwise maintained 100% through 18 months. The 50 ppm rate has provided 100%. All rates are attaining 100% mortality at 14 days or less (Table 4).

#### Flowerwood Media:

At 18 months post-treatment, all rates except the 5 and 10 ppm rate showed 100% mortality at less than 7 days with the exception of the 20 ppm rate dropping to 75% at 17 months (Table 5). The 10 ppm rate provided 90-100% mortality through 7 months and became erratic after that, while the 5 ppm rate was effective through 4 months and became erratic after that at 7 days exposure. All rates were at 100% at 14 days exposure or less (Table 6).

### *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. This is probably due to the low mobility of fipronil in soil, and supports the necessity of incorporation into potting media.

References Cited:

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



Table 1. Residual Activity of Fipronil 0.05G in MAFES Media (7 days exposure)

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment *																
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-15	-16	-18
5	100	30	100	100	75	95	60	60	25	20	60	100	100	50	90	100	15
10	100	100	100	95	100	100	100	100	100	100	100	100	80	100	95	100	100
15	55	100	100	50	100	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	100	100	100	100	100
50	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Check	5	0	0	10	0	20	10	15	15	0	15	35	15	10	15	10	0

\* not evaluated at 17 months due to lack of alate queens

Table 1. (cont.) Residual Activity of Fipronil 0.05G in MAFES Media (7 days exposure)

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment *																
	-19	-21	-22	-23	-24	-25	-26	-27	-28	-29	-30	-31	-32	-33	-34	-35	-36
5	100	95	20	85	5	55	55	45									
10	100	100	5	100	20	95	95	100									
15	100	100	50	15	80	100	35	80									
25	100	100	85	95	100	60	100	100									
50	100	100	100	100	100	100	35	100									
Check	10	0	10	10	0	0	5	5									

\* not evaluated at 20 months due to lack of alate queens

Table 2. Residual Activity of Fipronil 0.05G in MAFES Media During 14 Day Exposure Periods.

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (# of days to reach 100% mortality or % mortality at 14 days exposure) **																
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-15	-16	-18
5	--	--	--	--	--	8d*	10d	10d	10d	11d	8d	5d	7d	10d	11d	6d	13d
10	--	--	--	--	--	7d*	5d	7d	7d	6d	5d	5d	9d	6d	11d	7d	6d
15	--	--	--	--	--	5d*	5d	5d	7d	7d	5d	4d	6d	6d	6d	7d	6d
25	--	--	--	--	--	5d*	4d	3d	5d	5d	5d	3d	6d	6d	5d	4d	3d
50	--	--	--	--	--	5d*	4d	3d	4d	4d	5d	5d	6d	6d	4d	3d	3d
Check	--	--	--	--	--	20%*	10%	20%	20%	5%	15%	35%	25%	25%	15%	10%	5%

\* daily readings of bioassays and exposure beyond 7 days began

\*\* 17 months not evaluated due to lack of alate queens

Table 2. (cont.) Residual Activity of Fipronil 0.05G in MAFES Media During 14 Day Exposure Periods.

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (# of days to reach 100% mortality or % mortality at 14 days exposure) *																
	-19	-21	-22	-23	-24	-25	-26	-27	-28	-29	-30	-31	-32	-33	-34	-35	-36
5	6d	8d	10d	8d	10d	12d	11d	11d									
10	6d	7d	10d	7d	10d	9d	10d	7d									
15	6d	6d	10d	75%	10d	7d	10d	11d									
25	5d	6d	10d	8d	7d	12d	10d	5d									
50	4d	4d	5d	6d	4d	5d	10d	4d									
Check	10%	10%	15%	15%	10%	20%	10%	5%									

\* 20 months not evaluated due to lack of alate queens

Table 3. Residual Activity of Fipronil 0.05G in Windmill Media (7 days exposure)

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment *															
	-1	-2	-3	-4	-5	-6	-7	-9	-10	-11	-13	-14	-15	-16	-17	-18
5	70	20	40	35	0	5	60	35	25	5	45	10	55	0	60	25
10	95	80	65	55	20	10	100	70	75	15	100	70	90	10	100	80
15	100	100	95	90	75	45	100	100	100	20	100	55	65	30	100	95
20	100	100	100	100	85	95	100	100	100	45	100	75	100	80	100	100
25	100	100	100	100	100	75	100	100	100	45	80	100	100	100	100	100
40	100	100	100	100	100	75	100	100	100	75	100	100	100	100	100	100
50	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Check	60	20	50	35	20	25	5	5	10	5	20	5	5	0	5	0

\* 8 & 12 months not evaluated due lack of alate queens

Table 4. Residual Activity of Fipronil 0.05G in Windmill Media During 14 Day Exposure Periods.

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (# of days to reach 100% mortality or % mortality at 14 days exposure) *															
	-1	-2	-3	-4	-5	-6	-7	-9	-10	-11	-13	-14	-15	-16	-17	-18
5	10d	11d	13d	10d	14d	14d	10d	9d	11d	11d	12d	10d	11d	11d	12d	12d
10	8d	8d	9d	10d	11d	14d	7d	9d	10d	10d	7d	10	10d	10d	8d	8d
15	7d	6d	8d	10d	9d	9d	6d	7d	6d	9d	7d	10d	10d	9d	8d	8d
20	6d	6d	7d	5d	8d	9d	6d	6d	6d	8d	7d	10d	7d	8d	7d	5d
25	6d	5d	7d	5d	7d	9d	6d	6d	5d	8d	8d	7d	7d	7d	7d	5d
40	6d	5d	7d	4d	7d	9d	5d	5d	5d	8d	5d	4d	5d	7d	6d	5d
50	6d	5d	7d	4d	4d	7d	5d	5d	5d	4d	4d	5d	5d	7d	6d	5d
Check	60%	20%	50%	40%	30%	25%	10%	15%	20%	15%	25%	10%	5%	5%	10%	0%

\* 8 & 12 months not evaluated due lack of alate queens

Table 5. Residual Activity of Fipronil 0.05G in Flowerwood media (7 days exposure)

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment *															
	-1	-2	-3	-4	-5	-6	-7	-9	-10	-12	-13	-14	-15	-16	-17	-18
5	75	100	100	100	50	55	100	80	90	70	30	75	30	5	15	10
10	100	100	100	100	90	95	100	80	100	100	95	100	45	65	50	100
15	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
20	100	100	100	100	100	100	100	100	100	100	100	100	100	100	75	100
25	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
40	100	100	100	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	100	100	100
Check	0	0	5	5	20	20	0	15	20	15	0	15	10	5	0	5

\* 8 & 11 months not evaluated due to lack of alate queens

Table 6. Residual Activity of Fipronil 0.05G in Flowerwood media During 14 Day Exposure Periods.

Rate of Application (ppm)	% mortality of alate females at indicated months post-treatment (# of days to reach 100% mortality or % mortality at 14 days exposure) *															
	-1	-2	-3	-4	-5	-6	-7	-9	-10	-12	-13	-14	-15	-16	-17	-18
5	8d	7d	5d	6d	10d	11d	7d	9d	8d	8d	11d	8d	10d	14d	13d	11d
10	6d	5d	5d	6d	8d	11d	5d	8d	7d	7d	10d	7d	10d	12d	10d	7d
15	6d	5d	5d	6d	6d	6d	4d	6d	6d	6d	6d	6d	7d	7d	7d	7d
20	4d	5d	3d	6d	6d	7d	4d	6d	6d	5d	6d	5d	7d	7d	10d	6d
25	4d	5d	5d	6d	6d	5d	3d	6d	6d	5d	6d	5d	7d	6d	5d	6d
40	4d	5d	3d	6d	6d	6d	3d	3d	5d	5d	4d	5d	5d	6d	5d	5d
50	4d	5d	3d	3d	6d	4d	3d	3d	4d	5d	4d	5d	4d	5d	4d	4d
Check	0%	0%	5%	5%	25%	20%	0%	15%	20%	20%	5%	15%	20%	10%	10%	5%

\* 8 & 11 months not evaluated due to lack of alate queens

PROJECT NO: FA01G097

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

PROJECT TYPE: Final

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 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 were 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 inverted over the tops of the pots prevent escape from the top of the test chambers. Prior to placing queens in the test chamber, 50 cc of treated potting media was placed in the bottom of each pot. Each treatment to be evaluated was subdivided into 4 replicates, with one test chamber per replicate. Five alate queens were 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 specified intervals, 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* - Granular chlorfenapyr 0.5% 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. 1-1/2" 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* - Traditionally, we have utilized a 7-day exposure period for our bioassays. However at 8 months after treatment, we began determining mortality daily for a 14-day exposure period. 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 >85% efficacy through 26 months, and the 200 ppm rate has been 100% effective through 26 months. When the exposure period is extended to 14 days all rates except the 25 ppm rate have consistently provided 100% mortality through 26 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 where 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 (based on 7-day exposure results). 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 (7 day exposure).

Rate of Application	Percent mortality at indicated months post-treatment											
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
25 PPM	20	25	40	15	10	20	10	75	65	75	20	35
50 PPM	85	40	50	100	100	95	95	95	100	95	50	50
75 PPM	95	95	100	100	100	95	100	100	100	100	85	100
100 PPM	100	90	100	100	100	100	100	100	100	100	100	100
200 PPM	100	100	100	100	100	100	100	100	100	100	100	100
CHECK	5	20	5	10	0	10	0	20	15	15	15	0

Table 4. Cont.

Rate of Application	Percent mortality at indicated months post-treatment											
	-13	-14	-15	-16	-17	-18	-20	-21	-23	-24	-25	-26
25 PPM	20	40	50	25	15	100	65	10	15	40	50	20
50 PPM	100	95	100	90	75	100	100	70	50	95	95	60
75 PPM	100	100	100	85	100	100	100	100	80	100	100	95
100 PPM	100	100	100	100	100	100	100	100	100	100	95	100
200 PPM	100	100	100	100	100	100	100	100	100	100	100	100
CHECK	15	35	35	10	15	10	0	10	15	10	10	0

Table 5. Efficacy of chlorfenapyr as an incorporated treatment against RIFA alate females (14 day exposure).

Rate of Application	Percent mortality at indicated months post-treatment at 14 days or # days to reach 100% mortality											
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
25 PPM	**	**	**	**	**	**	**	12d	11d	11d	13d	70%
50 PPM	**	**	**	**	**	**	**	8d	6d	10d	10d	11d
75 PPM	**	**	**	**	**	**	**	6d	7d	7d	10d	7d
100 PPM	**	**	**	**	**	**	**	6d	4d	5d	7d	5d
200 PPM	**	**	**	**	**	**	**	5d	4d	4d	5d	4d
CHECK	**	**	**	**	**	**	**	25	15	20	20	0

\*\* First seven months were only given 7 day exposure

Table 5. Cont.

Rate of Application	Percent mortality at indicated months post-treatment at 14 days or # days to reach 100% mortality											
	-13	-14	-15	-16	-17	-18	-20	-21	-23	-24	-25	-26
25 PPM	12d	13d	10d	13d	12d	7d	14d	95%	80%	3d	13d	70%
50 PPM	7d	9d	7d	8d	11d	7d	7d	11d	12d	5d	8d	10d
75 PPM	7d	7d	7d	8d	7d	5d	7d	7d	8d	7d	7d	10d
100 PPM	5d	7d	6d	6d	5d	4d	6d	7d	6d	10d	8d	6d
200 PPM	5d	7d	6d	6d	5d	3d	3d	5d	4d	12d	6d	4d
CHECK	15	45	35	25	15	10	5	20	20	10	15	10

At 19 and 22 months bioassays were not done due to scarcity of alate queens

PROJECT NO: FA01G019

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

REPORT TYPE: Interim

PROJECT LEADER/PARTICIPANT(s): Lee McAnally

### INTRODUCTION:

The Federal Imported Fire Ant Quarantine Program (7CFR §301.81) states that all regulated products (nursery stock) leaving the quarantined area must be treated in a prescribed manner. Currently, treatments for containerized nursery include the use of granular insecticides incorporated into potting media or liquid drenches applied prior to shipping. Nursery stock treated with incorporated insecticides (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 (FA01G097).

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

### MATERIALS AND METHODS:

#### *Drench Treatments:*

A 2SC liquid formulation of chlorfenapyr was tested as a drench application for containerized nursery stock. Trade gallon nursery pots were filled with three different media, our standard (MAFES) potting media (3:1:1 pine bark: sphagnum peat moss sand - bulk density = 720 lb/cu yd), Flowerwood media (Flowerwood Nursery, Mobile, AL, bulk density = 470 lb/cu yd), and Windmill media (Windmill Nursery, Folsom, LA, bulk density = 235 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 on 28 June 1999 at rates of 25, 50, 75, 100, and 200 ppm. Each pot was drenched with a volume of solution equal to 1/5 the volume of the pot (i.e. 400 ml solution). Standard alate queen bioassays were then performed at 24 hrs., 1 week, 2 weeks, and monthly through 6 months after treatment.

*Incorporated Treatments:*

Granular treatments included 1%, 1.5% and 2% products formulated either on clay or corn cob grit carriers. Each of the granular formulations were blended into each of the three media described above at rates of 10, 25, 50, 75 and 100 ppm. 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 nursery pots and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week. At monthly intervals, subsamples were taken from 3 pots of each treatment and composited and subjected to standard alate queen bioassay. The 1.0G formulations were mixed from 30 June to 2 July 1999, the 1.5G formulations were mixed from 17 through 20 August 1999, and the 2.0G formulations were mixed from 15 through 17 September 1999.

RESULTS:

*Drench Treatments:*

Results to date indicate that media type has an impact on chlorfenapyr efficacy. Rates of 100 ppm and greater have provided 100% mortality in both Flowerwood and MAFES media in 7 days or less up to 1 month after treatment and in 12 days or less up to 5 months after treatment (Table 1). Lower rates were slower at achieving 100% mortality. In the Windmill media only the 200 ppm was 100% effective against IFA.

*Incorporated Treatments:*

Results of the granular treatments also indicate that media type effects the efficacy of chlorfenapyr. Rates of 25 ppm and higher in the Flowerwood media have been 100% effective through three months, regardless of chlorfenapyr formulation (Tables 2, 3, and 4). The MAFES media has been less consistent with all grit formulations providing excellent control at rates of 50 ppm and higher. Windmill media treated with the test formulations of chlorfenapyr has not provided consistent control of IFA at the rates applied.



Table 1. Residual activity of chlorfenapyr 2SC.

Media Treated	Rate of App (ppm)	Mean % mortality to alate females at indicated period post-treatment after 14 days exposure (days required to reach 100% mortality)								
		24 Hours	1 wk	2 wks	1 mth	2 mths	3 mths	4 mths	5 mths	6 months
Flower-wood	25	90	70	100 (8)	100 (8)	55	90	70	35	60
	50	100 (7)	100 (9)	100 (8)	100 (8)	100 (7)	90	95	100 (13)	100 (13)
	75	100 (7)	100 (8)	100 (6)	100 (6)	100 (7)	100 (8)	100 (10)	100 (9)	100 (12)
	100	100 (7)	100 (6)	100 (5)	100 (5)	100 (5)	100 (6)	100 (6)	100 (7)	100 (6)
	200	100 (3)	100 (6)	100 (5)	100 (5)	100 (5)	100 (6)	100 (6)	100 (5)	100 (4)
	Check	10	40	70	15	25	15	5	5	10
MAFES	25	85	65	55	100 (13)	60	60	70	25	60
	50	100 (7)	100 (14)	100 (12)	100 (11)	85	100 (9)	75	75	95
	75	100 (11)	100 (7)	100 (9)	100 (6)	100 (9)	100 (8)	100 (8)	90	100 (12)
	100	100 (7)	100 (7)	100 (7)	100 (5)	100 (12)	100 (6)	100 (8)	100 (9)	100 (13)
	200	100 (7)	100 (6)	100 (7)	100 (4)	100 (6)	100 (6)	100 (6)	100 (7)	100 (8)
	Check	15	15	70	5	10	15	20	5	5
Windmill	25	15	5	75	60	85	30	0	0	0
	50	30	5	65	45	55	30	10	10	0
	75	70	60	65	55	30	35	5	10	0
	100	90	35	85	95	45	40	25	10	0
	200	100 (9)	100 (14)	100 (13)	100 (13)	95	65	50	30	80
	Check	10	25	95	20	30	20	0	5	5

Table 2. Residual activity of chlorfenapyr 1.0 G.

Media Treated	Rate of Application	Mean % mortality to alate females at indicated months post-treatment after 14 days exposure (days required to reach 100% mortality)								
		1	3	4	6	7	8	9	10	11
Flowerwood	Clay									
	10	100 (11)	65	0	25					
	25	100 (7)	100 (10)	70	15					
	50	100 (4)	100 (8)	100 (9)	100 (8)					
	75	100 (3)	100 (7)	100 (6)	100 (7)					
	100	100 (3)	100 (7)	100 (5)	100 (5)					
	Grit									
	10	100 (11)	80	25	5					
	25	100 (8)	100 (7)	100 (12)	80					
	50	100 (4)	100 (7)	100 (7)	100 (8)					
	75	100 (2)	100 (7)	100 (6)	100 (7)					
100	100 (2)	100 (7)	100 (5)	100 (5)						
	Check	5	20	20	5					
MAFES	Clay									
	10	55	75	20	20					
	25	80	90	10	100 (14)					
	50	100 (10)	100 (10)	95	100 (11)					
	75	100 (7)	100 (7)	95	100 (6)					
	100	100 (7)	100 (7)	100 (8)	100 (6)					
	Grit									
	10	35	60	0	10					
	25	50	100 (14)	10	45					
	50	100 (13)	100 (9)	100 (13)	100 (11)					
	75	100 (7)	100 (7)	100 (10)	100 (7)					
100	100 (7)	100 (7)	100 (8)	100 (6)						
	Check	10	10	0	5					
Windmill	Clay									
	10	85	75	40	15					
	25	55	70	35	20					
	50	80	90	0	65					
	75	100 (12)	95	45	10					
	100	100 (12)	100 (13)	95	75					
	Grit									
	10	40	60	0	25					
	25	40	35	10	5					
	50	70	95	40	10					
	75	85	90	40	70					
100	100 (12)	100 (13)	75	95						
	Check	10	10	5	15					

Table 3. Residual activity of chlorfenapyr 1.5G.

Media Treated	Rate of Application	Mean % mortality to alate females at indicated months post-treatment after 14 days exposure (days required to reach 100% mortality)									
		1	2	3	5						
Flowerwood	Clay										
	10	100 (5)	85	100 (12)							
	25	100 (6)	100 (7)	100 (9)							
	50	100 (5)	100 (7)	100 (5)							
	75	100 (4)	100 (7)	100 (5)							
	100	100 (4)	100 (7)	100 (5)							
	Grit										
	10	100 (6)	85	100 (12)							
	25	100 (5)	100 (5)	100 (7)							
	50	100 (4)	100 (7)	100 (5)							
	75	100 (4)	100 (5)	100 (5)							
	100	100 (4)	100 (5)	100 (5)							
	Check	5	15	10							
	MAFES	Clay									
		10	5	10	5						
25		20	15	20							
50		85	50	85							
75		100 (11)	100 (12)	100 (11)							
100		100 (8)	100 (12)	100 (8)							
Grit											
10		20	15	20							
25		100 (14)	60	100 (14)							
50		100 (8)	100 (12)	100 (8)							
75		100 (7)	100 (8)	100 (6)							
100		100 (6)	100 (7)	100 (5)							
Check		15	5	15							
Windmill		Clay									
		10	0	5	5						
	25	5	5	15							
	50	10	0	50							
	75	30	20	5							
	100	65	65	50							
	Grit										
	10	30	5	5							
	25	5	10	0							
	50	25	55	25							
	75	80	50	40							
	100	100 (11)	75	90							
	Check	10	0	0							

Table 4. Residual activity of chlorfenapyr 2.0G.

Media Treated	Rate of Application	Mean % mortality to alate females at indicated months post-treatment after 14 days exposure (days required to reach 100% mortality)								
		1	2	3	4	7	8	9	10	11
Flowerwood	Clay									
	10	100 (8)	100 (10)	100 (11)	100 (7)					
	25	100 (8)	100 (6)	100 (11)	100 (5)					
	50	100 (11)	100 (7)	100 (6)	100 (4)					
	75	100 (7)	100 (6)	100 (4)	100 (4)					
	100	100 (6)	100 (6)	100 (4)	100 (4)					
	Grit									
	10	100 (8)	100 (8)	100 (4)	100 (6)					
	25	100 (8)	100 (6)	100 (7)	100 (4)					
	50	100 (5)	100 (6)	100 (4)	100 (4)					
	75	100 (6)	100 (3)	100 (4)	100 (4)					
	100	100 (5)	100 (3)	100 (4)	100 (4)					
	Check	10	25	33*	5					
MAFES	Clay									
	10	45	5	0	0					
	25	25	15	0	5					
	50	70	25	30	50					
	75	90	65	70	95					
	100	100 (12)	85	100 (11)	100 (11)					
	Grit									
	10	5	10	0	30					
	25	65	30	0	100 (11)					
	50	100 (9)	100 (10)	100 (13)	100 (7)					
	75	100 (8)	100 (8)	100 (11)	100 (6)					
	100	100 (7)	100 (8)	100 (11)	100 (6)					
	Check	10	20	10	10					
Windmill	Clay									
	10	15	0	15	0					
	25	30	5	30	5					
	50	10	0	10	10					
	75	55	5	55	5					
	100	40	20	40	60					
	Grit									
	10	20	0	20	0					
	25	20	20	20	10					
	50	80	25	80	65					
	75	85	80	85	90					
	100	100 (11)	100 (14)	100 (13)	95					
	Check	5	15	5	0					

\* Queens escaped from 1 replicate

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: Final

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

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 tefluthrin (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 or tefluthrin prevented fire ant colonies from invading the test pots for at least 24 months (see 1997 Annual Report). These preliminary results warranted 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 as cooperators 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	<i>Buxus microphylla</i> 'Winter Gem'	<i>Buxus microphylla</i> 'Winter Gem'
Grass Roots	Leesburg, FL	60% pine bark 40% local peat	<i>Juniperus parsonnii</i>	<i>Ilex crenata</i>
Flowerwood	Loxley, AL	65% new bark 35% aged bark	<i>Ilex crenata</i>	<i>Ilex crenata</i>
Windmill	Folsom, LA	100% pine bark	<i>Ilex crenata</i>	<i>Ilex crenata</i>
Wight	Cairo, GA	80% bark 20% sand	<i>Juniperus horizontalis</i>	<i>Juniperus horizontalis</i>

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 and 3 months after the pots were filled with media, then quarterly after that. 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 or Fluon® 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. A pot was considered infested if +25 workers were inside the pot.

*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 9 months showed some movement of whole colonies (workers and brood) into pots treated with 0.25 and 0.5% bifenthrin (Fig. 2). A few workers (<100) had moved into some of the 3 gallon pots treated at 1.0%, but no whole colonies had moved into these pots. No workers moved into any of the 1.0% 1 gallon pots through 9 months. 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 1/3 the amount applied to the pots we began testing in 1995. This may account for the differences in efficacy seen between the two trials.

After a meeting with Lerio and Griffin to discuss results and "next steps", it was decided to drop the lower two rates from the trial, and only continue evaluating the 1.0% bifenthrin rate from all sites except Turkey Creek (dropped due to delivery logistics). Efficacy continued to be erratic. Two and one 1 gallon pots were infested with workers only at 12 and 15 months, respectively, while only one 3 gallon pot was infested at 12 months (none infested at 15 months). The 3 gallon pot was infested by a whole colony that entered the pot by climbing a trailing branch of the juniper plant in the pot and avoided having to enter through a "treated" drain hole. This problem was eliminated in subsequent tests by trimming the trailing limbs prior to testing. At 18 months, two more nurseries were dropped due to logistics, leaving two nurseries with the test pots to be evaluated (Flowerwood and Windmill). At 27 months, the Flowerwood trial was inadvertently destroyed by nursery workers, leaving only the Windmill trial. By 30 months, the Windmill trial had no remaining 1 gallon pots to be evaluated, only the 3 gallon 1.0% pots were

left. Therefore, the results in Figure 2 are skewed due to the decrease in number of replicates per treatment (15 replicates at the beginning down to 3 replicates by the 30 month evaluation). The 1.0% rates continued erratic through 30 months.

*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 were performed at 1 and 3 months after potting up. No polygynous queens or workers survived in the treated pots (all rates), while queens and workers survived in the untreated check pots.

#### CONCLUSIONS:

It was evident that with the Spin Out 300 formulation the thinner coating of latex and therefore less insecticide applied to the pots had affected the efficacy of the treatment. It also appeared that the larger pots (3-gallon) with proportionally larger drain holes may require more insecticide per surface area to adequately protect this vulnerable spot. The 0.25 and 0.50% rates were dropped from the trial 9 months after treatment due to unacceptable efficacy. Additional trials were initiated in the spring of 1998 utilizing a greater amount of insecticide in the latex coating (see FA01G018).



Figure 1. Diagram of test arena.

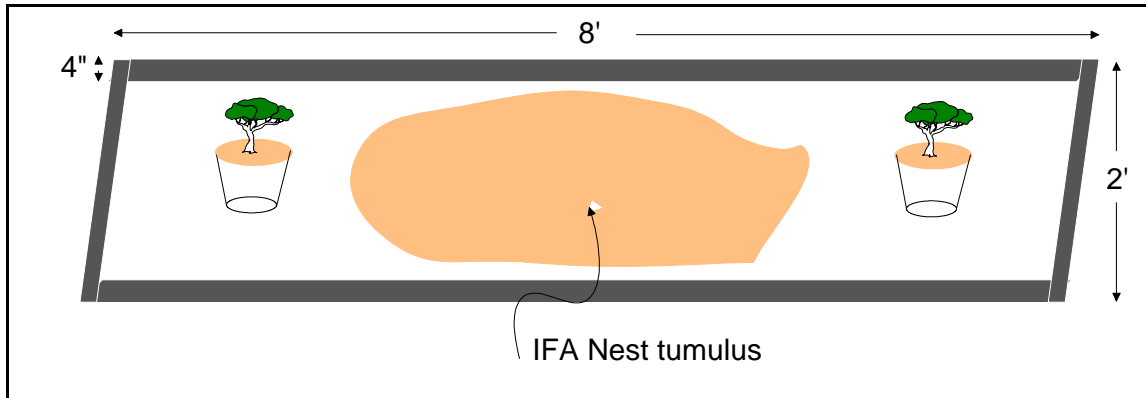
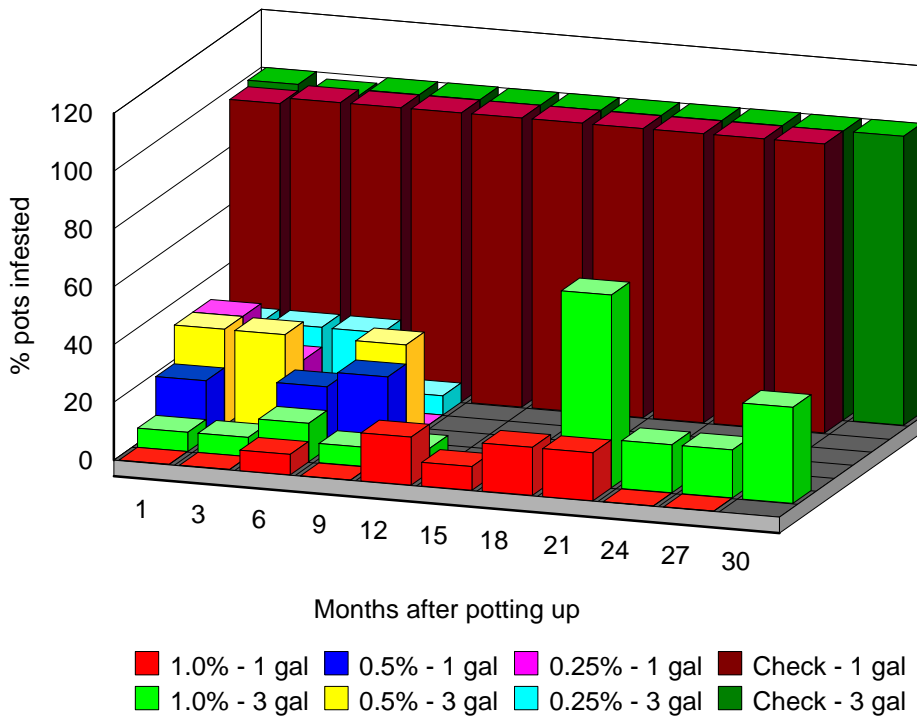


Figure 2. Percent of Spin Out 300 + Bifenthrin Treated pots Infested with Imported Fire Ants. Multi-State Trial - initiated March 1997.



PROJECT NO: FA01G018

PROJECT TITLE: 1998 Expanded Trials with Spin Out® Technology Used in Combination with Bifenthrin Insecticide to Prevent Imported Fire Ant Infestations in Containerized Nursery Stock

TYPE REPORT: Interim

PROJECT LEADER/PARTICIPANTS: Homer Collins, Anne-Marie Callcott, Lee McAnally, Avel Ladner and Shannon Wade

COOPERATORS: Lerio Corporation, Mobile, AL (Mark Christian and Andy Zimlich)  
Griffin Corporation, Valdosta, GA (Mark Crawford)  
Windmill Nursery, Folsom, LA (Dennis McCloskey)  
Flowerwood Nursery, Loxley, AL (Jim Berry)  
Rocky Creek Nursery, Lucedale, MS (Lee Howell)

#### INTRODUCTION:

Spin Out root growth regulator technology was developed by Griffin Corporation to enhance the development of dense, compact root growth. The active ingredient in the current formulation is 7% 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, 0.5, and 1.0% AI). 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 had prevented fire ant colonies from invading the test pots for 24 months (report FA01G155). These results warranted an expanded study to include actual rather than simulated nursery conditions, various size containers, different potting medias, and varying climatic conditions. A trial initiated in 1997 (report FA01G017) at 5 nurseries in the Southeast used Spin Out 300 combined with various concentrations of bifenthrin (0.25, 0.5 and 1.0% AI). The Spin Out 300 was applied to 1 and 3 gallon nursery pots, however the rate of application of bulk material was 3X less than in the original 1995 trial (this was the result of Griffin perfecting the Spin Out product to deal with economic concerns). Therefore 3X less bifenthrin was available in each pot as an insecticide. Results from this trial were less effective, with 7-33% of the 0.25 and 0.5% treated pots becoming infested with fire ants at 1-6 months after treatment. The 1-gallon pots treated at 1.0% provided protection from infestation for 3 months, while the 3-gallon pots treated at 1.0% failed 7-13% of the time at 1, 3, and 6 months after treatment.

Due to the less than acceptable results from the 1997 trial, Lerio, Griffin, and GPPS jointly decided to initiate another trial in 1998 using higher rates of bifenthrin (1.5 and 2.0% AI) combined with the Spin Out 300 to accommodate the lower amount of material applied to the

pots. We also evaluated pots with the Spin Out/bifenthrin applied in a band to the inside or outside of the pot.

**MATERIALS AND METHODS:**

Griffin Corporation supplied Spin Out 300 containing bifenthrin (1.5% and 2.0% 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. Treatments included:

- 1.5% AI combined with Spin Out 300 sprayed on entire inside of pot; both 1-gallon and 3-gallon sizes
- 2.0% AI combined with Spin Out 300 sprayed on entire inside of pot; both 1-gallon and 3-gallon sizes
- 2.0% AI combined with Spin Out 300 sprayed in a 1-2” band on the inside bottom side of pot; band will overlap side drainage holes; there will be no bottom drainage hole; both 1-gallon and 3-gallon sizes
- 2.0% AI combined with Spin Out 300 sprayed in a 1-2” band on the outside bottom side of pot; band will overlap side drainage holes; there will be no bottom drainage hole; both 1-gallon and 3-gallon sizes

Lerio color coded the pots for identification of treatment type. After treatment, Lerio delivered treated pots to the nurseries and assisted with potting up, and at each sampling period, they transported test pots from each nursery to this laboratory. Griffin and Lerio purchased all plants for the test. Cooperating nurseries provided space, irrigation, fertilization, and weed control for the test pots. Gulfport personnel 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	Soil Type	Plants (1-gal and 3-gal)
Flowerwood	Loxley, AL	65% new bark 35% aged bark	<i>Berberis thunbergii atropurpurea</i> Redleaf Japanese Barberry
Windmill	Folsom, LA	100% bark	<i>Ilex vomitoria nana</i> Dwarf Yaupon Holly
Rocky Creek	Lucedale, MS	unknown	<i>Cleyera japonica</i> Japanese Cleyara

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 and 3 months after the pots are filled with media, and then quarterly thereafter until activity

ceased. 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. Sides of the test arena were coated with talcum powder or Fluon® 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 (see FA01G017 for diagram of 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 an 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. A pot was considered infested if +25 workers were inside the pot.

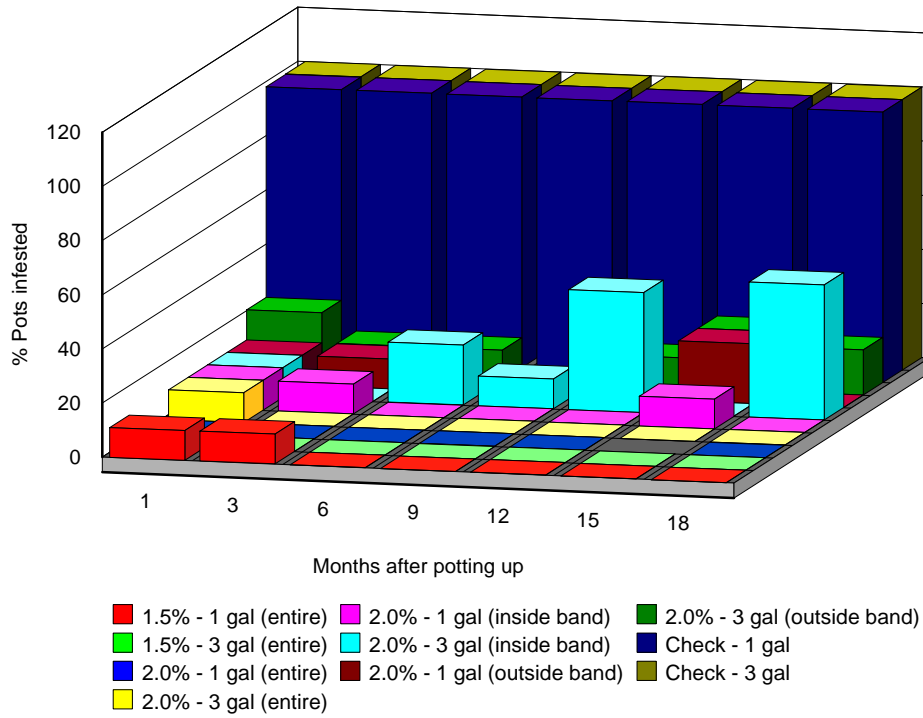
### RESULTS:

The higher rates of insecticide in latex coating the entire inside of the pot appear to be providing better protection against IFA infestation than the previous year's lower rates of insecticide (Figure 1). The 1.5% 1-gallon rate, and the 2.0% 1-gallon and 3-gallon rates have had one pot each infested within the first 3 months of testing, although these infestations involved less than 100 workers and no brood. There was 100% exclusion of IFA from all the pots with the entire inside coating from 6 to 18 months after treatment. The band treatments are not working as well as the entire inside coating, and many of these pots were infested with 1000+ ants with brood. Unfortunately, the Flowerwood trial was inadvertently discarded prior to the 18 month evaluation, therefore data from 18 months on includes 6 replicates instead of the original 9.

### CONCLUSIONS:

The 1.5% and 2.0% rates of bifenthrin applied in Spin Out to the entire inside of 1 and 3 gallon nursery pots have successfully prevented infestation of nursery pots for 15 months after a 1 to 3 month exposure period. This trial, thus far, supports our original data (FA01G115) as discussed above. This treatment, if granted approval for use in the Federal Imported Fire Ant Quarantine would give nurserymen the advantage of the Spin Out technology for better root growth and control, as well as, protection against transporting IFA in their containerized nursery stock. We will recommend that another trial duplicating this data be initiated in the spring of 2000 to provide confidence in this treatment for quarantine purposes.

Figure 1. Percent of Spin Out 300 + Bifenthrin Treated Pots Infested with IFA.  
Multi-State Trial - Initiated March 1998.



PROJECT NO: FA01G038

PROJECT TITLE: Effectiveness of Permethrin Impregnated Nursery Pots in Preventing Imported Fire Ant Invasion of Containerized Nursery Stock: A Preliminary Appraisal

TYPE REPORT: Interim

PROJECT LEADERS: Homer Collins, Anne-Marie Callcott and Shannon Wade

### INTRODUCTION:

Nursery stock and other regulated articles cannot be shipped outside the imported fire ant (IFA) quarantined area unless treated with an approved insecticide (7CFR §301.81) to prevent inadvertent spread of IFA. Several treatment options are approved and registered for this use pattern. Both liquid drenches (chlorpyrifos, diazinon, and bifenthrin), and granular insecticides (tefluthrin and bifenthrin) are approved for use. The most frequently used treatment is incorporation of either granular tefluthrin or bifenthrin into the potting media prior to "potting up". The residual activity of the insecticide prevents IFA invasion of containerized nursery stock for up to 24 months, depending upon dose rate employed.

A totally new and novel approach currently under investigation by us involves the addition of the insecticide bifenthrin to the SpinOut® technology. SpinOut (Griffin Corp., Valdosta, GA) is a coating of copper hydroxide applied to the interior surface of pots, grow bags, etc. to control root development by chemical root pruning. We have hypothesized that IFA colonies invade containerized nursery stock through the drain holes in the bottom of the pots, rather than climbing up the sides of the pot and entering from the top. Preliminary studies with SpinOut have demonstrated potential for use of this technology to prevent IFA invasion of nursery containers (FA01G017, FA01G018).

### MATERIALS AND METHODS:

Following several telephone conversations with Dr. Earl Tryon, representing Brandywine Compounding, Chadds Ford, PA, we received 12 one pint (4.75" x 4.75") nursery containers on October 7, 1997. No information other than the pots contained permethrin was provided. The pots were filled with potting media (MAFES mix: 3:1:1 mix of pine bark, sphagnum peat and sand), and placed on raised benches in a simulated can yard. Irrigation water (ca. 1½" per week) was provided through overhead pulsating sprinklers in addition to natural rainfall. The pots did not contain plants because the extremely low number of pots provided required that we conduct periodic resampling of the same 12 pots rather than destructive sampling normally done in similar trials. Bioassays were conducted in the laboratory in 2' x 4' test arenas (Figure 1). Sides of the test arena were talced to prevent ants from climbing out and escaping. An impregnated pot was placed at one end of the arena, and an untreated check container filled with potting media was placed at the distal end of the arena. A field collected IFA colony complete with associated

soil and nest tumulus was then placed in the center of the arena. Overhead incandescent light bulbs (60 watts, placed 14" above the test arena) slowly desiccated the nest so that the ants were encouraged to migrate to the more moist containers. The IFA colony had an equal opportunity to move into either a permethrin pot or the untreated check pot. Pots were observed at 24 hour intervals for 7 days after introduction, and the estimated number of worker ants successfully invading each pot was recorded. There were 3 replicates per sampling interval.

At 4, 6 and 9 months after potting up, we subjected four permethrin pots to a test in which the ants had no choice of pots. Only a permethrin treated pot was placed in the test arena with a field collected colony. Pots were observed for 7 days after introduction.

### RESULTS:

Permethrin treated pots prevented IFA invasion for 11 months (Figure 2) when ants were given a choice of pots. There has been one pot infested with 25-100 workers, but no brood, at several evaluation periods since the 12 month evaluation. Otherwise, IFA have been excluded from these small containers impregnated with permethrin for 26 months. When ants had no choice, they did try to move into the permethrin treated pots. At 4 mths, all ants exposed to the permethrin pots died. At 6 mths, some ants successfully infested the pots, but the majority of the workers preferred to live in the arena. By 9 mths, ants successfully infested all the replicates when there was no other choice. Based on these promising results, we recommended that a larger trial with various dose rates and different container sizes be initiated (FA01G069).

Figure 1. Diagram of test arena.

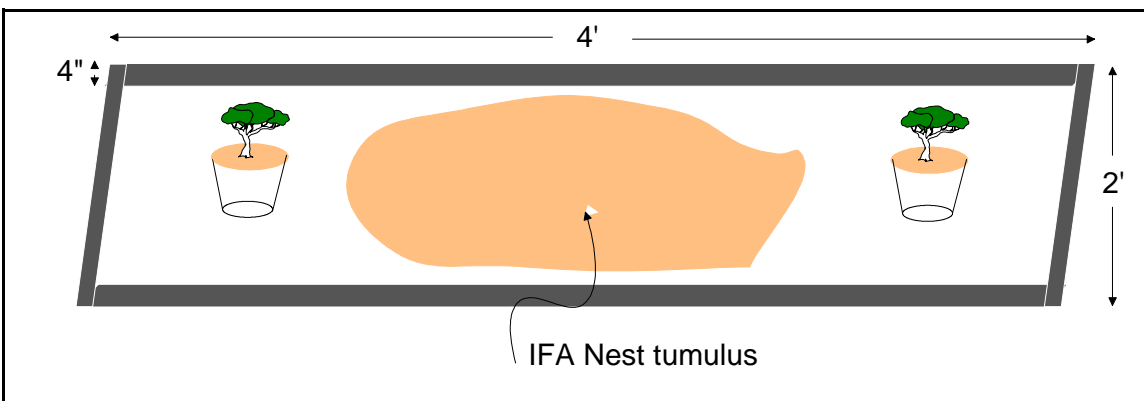
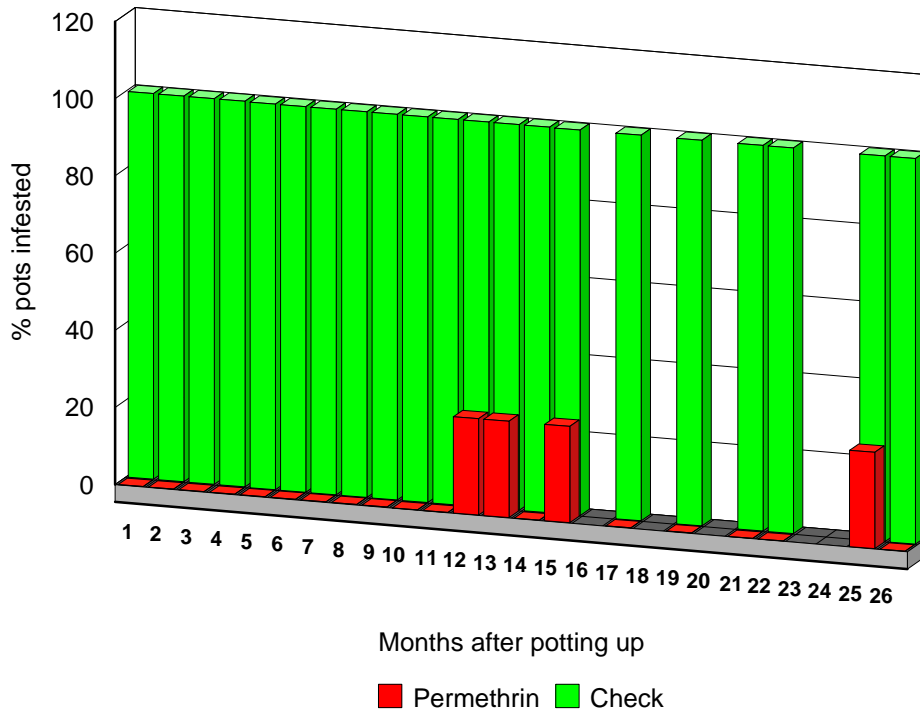


Figure 2. Percent of Original Permethrin Pots Infested with IFA - Preliminary Trail at Gulfport, MS - initiated 1997.





PROJECT NO: FA01G069

PROJECT TITLE: Effectiveness of Permethrin Impregnated Nursery Pots in Preventing Imported Fire Ant Invasion of Containerized Nursery Stock, 1999

TYPE REPORT: Interim

PROJECT LEADERS: Homer Collins, Anne-Marie Callcott and Shannon Wade

COOPERATORS: Premium Compounded Products, LLC (Corinne Brothers)  
Nursery Supplies, Inc. (Henry Guarriello, Jr.)  
AgrEvo Environmental Health (John Lucas)  
Windmill Nursery (Tom Cooper)

### INTRODUCTION:

Nursery stock and other regulated articles cannot be shipped outside the imported fire ant (IFA) quarantined area unless treated with an approved insecticide (7CFR §301.81) to prevent inadvertent spread of IFA. Several treatment options are approved and registered for this use pattern. Both liquid drenches (chlorpyrifos, diazinon, and bifenthrin), and granular insecticides (tefluthrin and bifenthrin) are approved for use. The most frequently used treatment is incorporation of either granular tefluthrin or bifenthrin into the potting media prior to "potting up". The residual activity of the insecticide prevents IFA invasion of containerized nursery stock for up to 24 months, depending upon dose rate employed.

New technologies utilizing insecticides applied to the nursery pot or insecticides impregnated into the plastic of the nursery pot to prevent IFA invasion have been investigated by our laboratory over the past several years. Preliminary work with permethrin impregnated nursery pots has shown the potential for preventing IFA infestation of small nursery containers (report FA01G038). This trial was initiated to expand on our preliminary observations and test the impregnated containers in actual nursery conditions with plants added.

### MATERIALS AND METHODS:

Three sizes of nursery containers (1, 3, and 10 gallon) impregnated with permethrin or deltamethrin were produced the week of December 14, 1998 by Premium Compounded Products. Concentrations of permethrin in the plastic were 0.25, 0.50, 0.75 and 1.0%, and of deltamethrin were 0.025, 0.050, 0.075 and 0.10%. Containers were potted up at Windmill Nursery on May 1, 1999. Due to logistics and resources, only three treatments were subjected to bioassay at our laboratory: 0.5 and 1.0% permethrin, and 0.10% deltamethrin. Pots were transported to the Gulfport laboratory quarterly for bioassay testing. The 0.05% deltamethrin concentration was tested at another laboratory at 6 month intervals. The other rates will be held for testing as needed. Other trials, not reported here, were initiated in other nurseries and bioassays performed by other laboratories.

Bioassays were conducted in the laboratory in 2' x 8' test arenas (Figure 1). Sides of the test arena were talced to prevent ants from climbing out and escaping. An impregnated pot was placed at one end of the arena, and an untreated check container filled with potting media was placed at the distal end of the arena. A field collected IFA colony complete with associated soil and nest tumulus was then placed in the center of the arena. Overhead incandescent light bulbs (60 watts, placed 14" above the test arena) slowly desiccated the nest so that the ants were encouraged to migrate to the more moist containers. Therefore, the IFA colony had an equal opportunity to move into either a permethrin pot or the untreated check pot. Pots were observed at 24 hour intervals for 7 days after introduction, and the estimated number of worker ants successfully invading each pot was recorded. There were 3 replicates per sampling interval.

### RESULTS:

At 3 and 6 months after potting up, the 1 and 10 gallon containers impregnated with 0.5 and 1.0% permethrin and 0.1% deltamethrin have excluded IFA, as have the 3 gallon containers impregnated with 1.0% permethrin (Figs. 2, 3 and 4). One container in each of the other treatment rates in the 3 gallon containers have become infested with 50-500 workers.

Figure 1. Diagram of test arena.

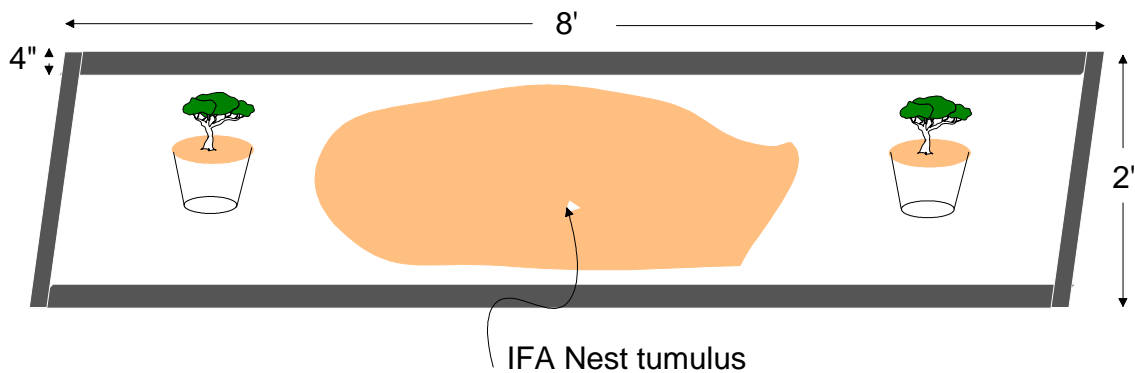


Figure 2. Percent of insecticide impregnated 1 gallon pots infested with IFA - Trial at Windmill Nursery - initiated 1999.

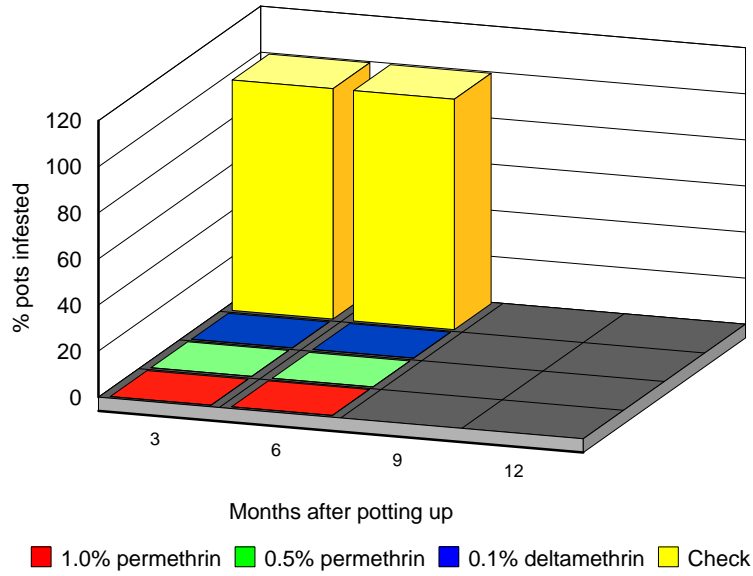


Figure 3. Percent of insecticide impregnated 3 gallon pots infested with IFA - Trial at Windmill Nursery - initiated 1999.

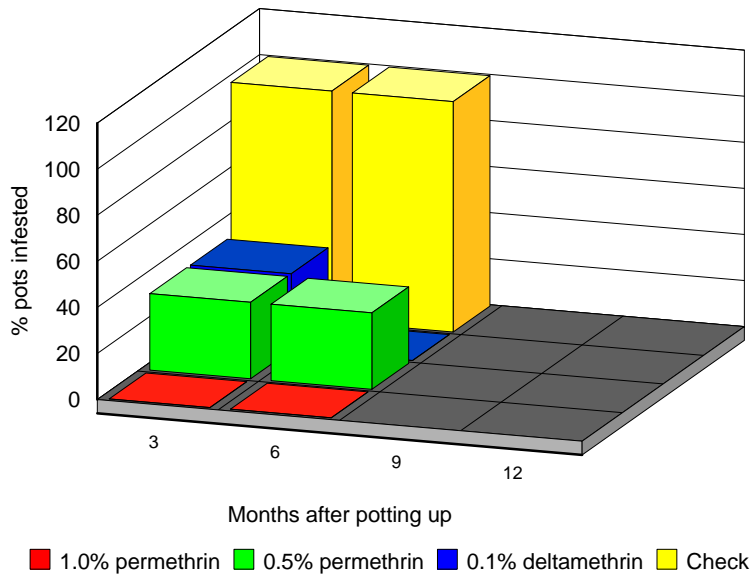
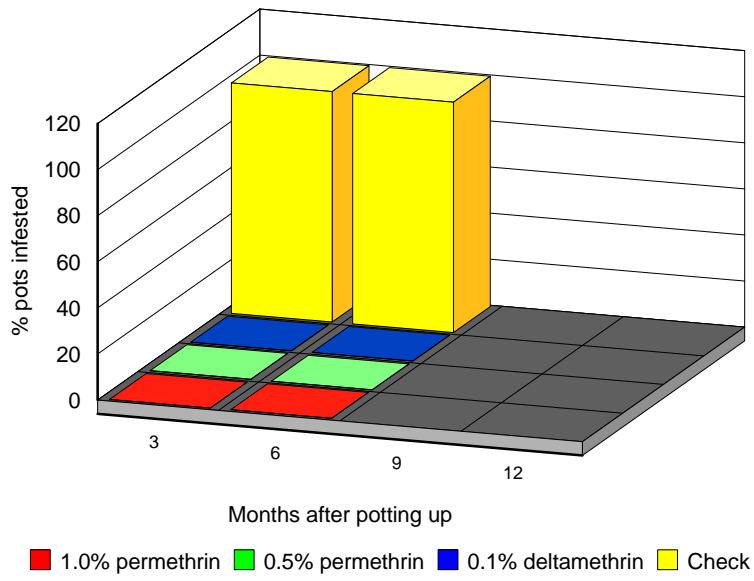


Figure 4. Percent of insecticide impregnated 10 gallon pots infested with IFA - Trial at Windmill Nursery - initiated 1999.



PROJECT TITLE: Development of IFA Quarantine Treatments for Balled & Burlapped Nursery Stock

COOPERATORS: USDA, APHIS, PPQ, Gulfport Plant Protection Station  
USDA, APHIS, PPQ, Nashville, TN  
USDA, ARS, CMAVE, Gainesville, FL  
Tennessee Dept. Agric., Nashville, TN  
University of Tennessee, Knoxville, TN  
Tennessee State Univ. Nursery Crop Res. Stn, McMinnville, TN

### Introduction:

Practical and cost-effective quarantine treatments for certification of 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§301.81 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 Association of Nurserymen, Tennessee Nurserymen's Association, and the Southern Nurserymen's Association.

The Gulfport Plant Protection Station will be focusing research efforts into the following areas:

### SHORT TERM EFFORTS:

- I. Spot insecticide treatments applied in-field prior to harvest will be investigated. Such treatments will not necessarily be lethal to ant colonies that may be nesting adjacent to B&B stock that is being harvested for shipment. Induced movement of the ant colony away from the harvest zone will be sufficient. In preliminary studies (see 1996 IFA Station Annual Report), acephate and other insecticides provoked colony movement (relocation) within 7 days of application. Acephate is currently labelled for spot treatment of IFA colonies. Therefore, registration or drastic label changes would be unnecessary. Additional testing under various soil and climatic conditions are needed to confirm the efficacy of this and other insecticides applied as spot treatments to the base of B&B nursery stock 1-2 weeks prior to harvest.

- II. In-field broadcast treatments with baits such as Amdro® or Award® used in combination with granular chlorpyrifos are approved for certification of B&B stock. However granular formulations of chlorpyrifos are no longer marketed for this use pattern. It is highly probable that other granular formulations of alternate insecticides such as fipronil (Chipco Choice®) or tefluthrin (Force 1.5G®) would be equally or more effective than the chlorpyrifos. However, the research to show this has not been done.

#### LONG TERM EFFORTS:

- I. Novel approaches such as encasing B&B stock in plastic sheeting that has been impregnated with a volatile insecticide has not been investigated. Several groups are exploring this technology for use as termiticides at the present time. However, it has not been evaluated against IFA.
- II. In the southern part of the IFA range mating flights have been recorded in every month of the year. Since newly mated queens and small incipient colonies are impossible to visibly detect with 100% assurance, negative surveys have not been used to certify movement of nursery stock. However, IFA may differ biologically along the northern extremities of the range in areas such as Oklahoma and Tennessee. If it can be determined that mating flights do not occur in certain parts of the year, i.e., December through February, certification based on extremely careful negative survey may be feasible. Additional biological studies along the northern extremities of the range are needed to confirm this hypothesis. We will cooperate very closely with ARS, CMAVE, Gainesville, FL to develop the biological data to support this type of certification. The Tennessee Department of Agriculture, University of Tennessee at Knoxville, and Tennessee State University at McMinnville will also be involved in these studies.
- III. Fumigants such as DDVP (Vapona®) and metam-sodium (Vapam®) and possibly others, will be evaluated as post-harvest treatments for individual plants. DDVP will be tested by placing various sized "kill strips" beneath the burlap liner to determine if ant colonies are killed or induced to move out of the ball. Metam-sodium will be applied post harvest as a "pour-on" treatment at various rates of application. If either treatment is effective in eliminating ant colonies, extensive phytotoxicity trials will be required to assure that treated plants will not be affected by the treatment. Hopefully the potential phytotoxic effects will be mitigated since the plants will be in a dormant state when treated. A distinct disadvantage of metam-sodium is that it is a restricted use pesticide, and requires use of extensive personal protective equipment.

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)  
Nancy King, USDA, APHIS, PPQ (Memphis, 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 §301.81 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 bait 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.

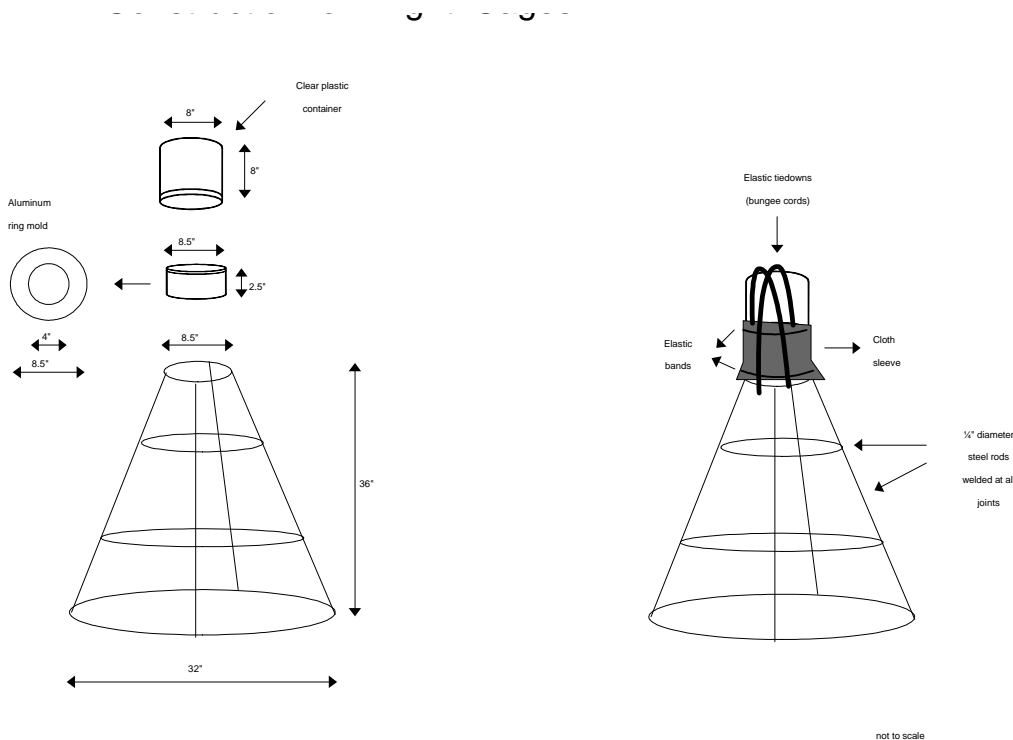
Since imported fire ant (IFA) mating flights occur year-round in most of the infested area, we have historically relied entirely upon contact action insecticides to certify movement of nursery stock outside the quarantine area. However, it seems likely that as the IFA extends its range to more northerly areas, differences in the biology of the ant may begin to occur. Mating flight cages were 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 initiated in cooperation with ARS, PPQ, Tennessee Department Agriculture, and Tennessee State University. Flight cages were deployed at three sites (east, central, and west Tennessee), and were monitored to determine mating flight patterns. This study was 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 1/4" diameter steel rods (Figure 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. 1/2 full with preservative (1:1 mixture of antifreeze and 70% alcohol). A 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 coordinates with collectors, assists in maintenance of traps and transmits collected data to ARS, CMAVE. ARS, CMAVE will correlate flight trap data with satellite information.

## RESULTS:

### *East Tennessee (Ooltewah):*

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 (Figure 2). No alates were captured between October 13, 1997 and March 13, 1998 when 2 males were captured in one trap. In 1998, major alate flights in this area began in mid June and continued through the end of July. There were small flights of alates in September and October, and flights had ceased by mid-November, 1998. Sporadic alate flights (1-3 alates captured) occurred between March and early April, 1999. Major flights in 1999 occurred between late May and early July. Small flights continued through December 6, 1999. Data is current through December 17, 1999, at which time no alates were captured.

### *Central Tennessee (Pulaski):*

Only males were captured at the central Tennessee site in Pulaski between September 9 and October 13, 1997; one male was captured between October 13 and October 28, 1997 (Figure 3). No alates were captured between October 28, 1997 and February 10, 1998 when 2 males were captured in two traps. In 1998, there was one surge of alate flight in this area between March 27 and April 13, with the major flights beginning in late May and continuing through the end of July. A reduced rate of alate flight continued through November 11, 1998. No flights occurred from mid-November, 1998 through late March, 1999. Only one alate was captured per capture date between late March and early May, 1999. Once flights began in late May, they continued until December 2, 1999, with peaks occurring in June and September. Data is current through January 5, 2000, at which time no alates were captured.

### *West Tennessee (La Grange):*

Both male and female alates were captured at the western site in La Grange between September 10 and September 29, 1997 (Figure 4). Between late September and October 17, only females were captured. No alates were captured between late October, 1997 and mid May, 1998. In 1998, major alate flights at this site began in late May and continued through the end of June. A reduced rate of alate flight continued through the first of December, 1998. With the exception of two males captured between late January and late February, 1999, no alate flights occurred from early December, 1998 to early May, 1999. Flights began in early May, 1999 and continued through October 27, 1999, with peaks in early June and September. Data is current through

December 9, 1999, at which time no alates were captured.

Overall, in the fall/winter of 1997, the last alates attempting flights and thus capable of mating were captured at all sites within a two week period in mid to late October. During 1998, the last alates were captured in a three week period from mid-November to early December. And in 1999, captures of the last alates at all sites occurred over a much longer period of time from late October to early December. These results parallel temperatures during those years. In 1997, average high temperatures at the three test sites in November were in the mid-50's, while in 1998, average high temperatures in November were in the low to mid-60's. Data for fall/winter 1999 has not yet been received.

We will continue this study through the spring of 2000 to complete data for the onset of mating flights in the spring. This data may be useful in future protocols to control IFA.

### CONCLUSIONS:

Our original premise was that if the last fall flights occurred at such time that a bait could be applied approximately 30 days later (when the first mimums from the newly mated queens appear to forage), field grown nursery stock could be certified to move out of the quarantined area without further treatment prior to spring mating flights. However, from the data collected in this trial, it appears that IFA alates are flying and thus capable of mating as late as the first week in December. This would push back the bait application until late December or early January. IFA forage when soil temperatures at 2 cm are 59 to 109°F (15-43°C), with optimum foraging occurring when temperatures are between 71 and 96°F (22-36°C) (Porter and Tschinkel 1987), supporting the general guideline that bait applications should be made when air temperatures are at or above 65°F. Therefore, with mating flights occurring so late in the year, the possibility of a day in late December or early January having temperatures conducive to bait application would be remote in Tennessee. Even if bait applications could be made with confidence of efficacy, the length of time between the last mating flights of the year and control of the subsequent small incipient colonies, which could be as late as the end of January, would probably be unacceptable to the nurserymen. In general, field grown nursery stock is harvested and sold from December through March or April in Tennessee.

In conclusion, mating flights are occurring much later than predicted in Tennessee, rendering the use of baits 30 days after the last mating flights unacceptable as a quarantine treatment for field grown/B&B nursery stock in Tennessee.

### REFERENCES CITED:

Porter, S.D. and W.R. Tschinkel. 1987. Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae): effects of weather and season. *Environ. Entomol.* 16: 802-808.

Figure 2. Alates collected at the eastern Tennessee site: Ooltewah (Hamilton Co.)

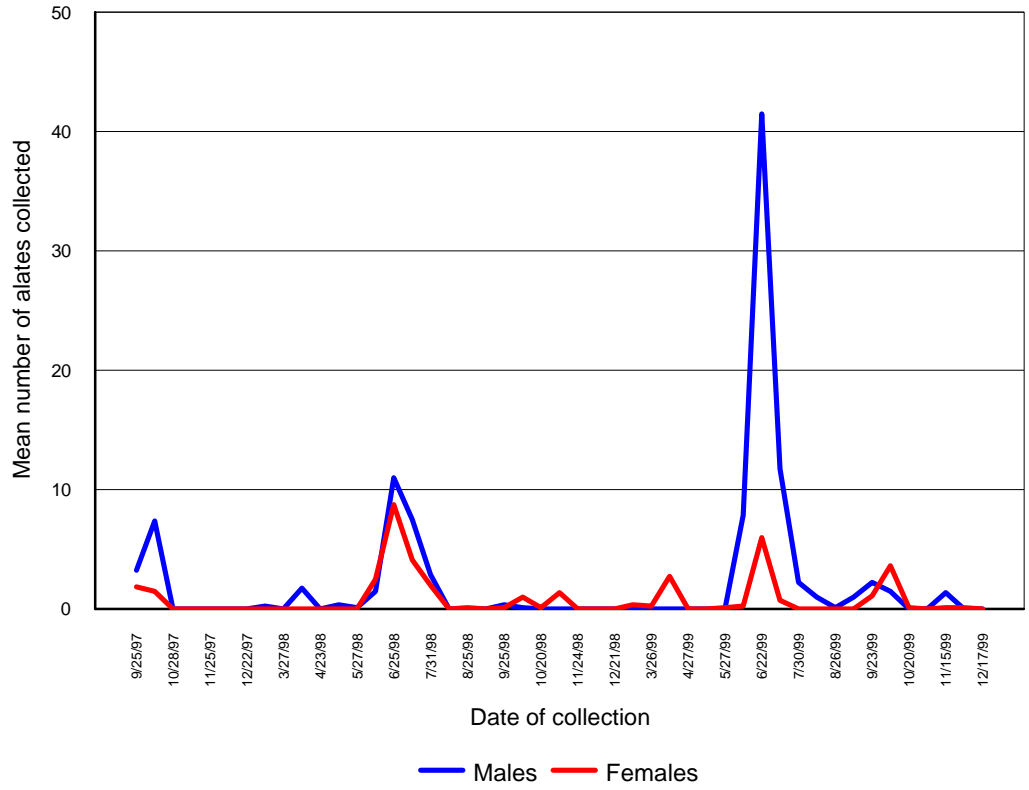


Figure 3. Alates collected at the central Tennessee site: Pulaski (Giles Co.)

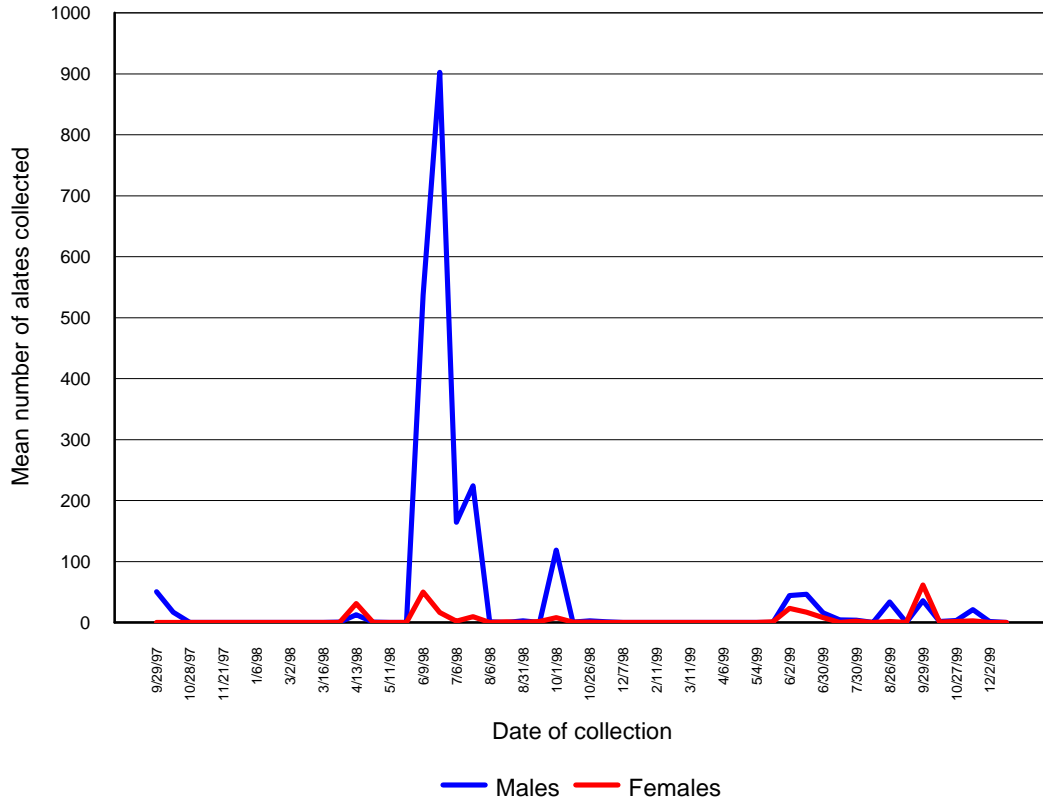
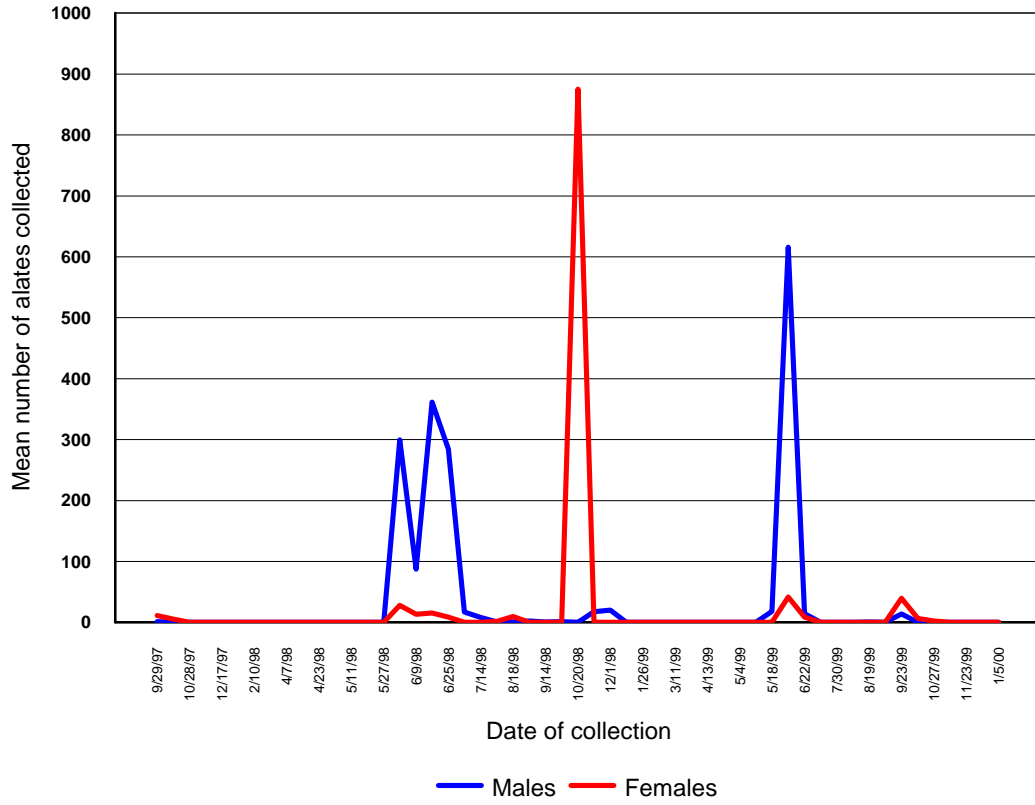


Figure 4. Alates collected at the western Tennessee site: La Grange (Fayette Co.)



PROJECT NO: FA05G055

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

TYPE REPORT: Final

LEADER/PARTICIPANTS: Timothy C. Lockley/Barbara J. Smith<sup>1</sup>

### INTRODUCTION:

Phytophthora Root Rot (*Phytophthora 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 phytophthora 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 phytophthora 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 (FA05G045). Tests were undertaken at the USDA-ARS-Small Fruits Research Laboratory in Poplarville, MS to determine the effects Biobarrier would have on the phytophthora 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 phytophthora root rot symptoms every 3 months and were assayed for the presence of *Phytophthora cinammoni* every 6 months. Fire ant populations were determined every 6 months.

### RESULTS:

After 54 months, RIFA colonies had established themselves throughout the untreated plots. No activity was noted within any of the treated plots. However, a significant reduction in plant vigor was observed within the Biobarrier mulched plots. This was most notable on the Misty variety with the Tifblue cultivar showing the least effect. A statistical analysis made by BJS

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<sup>1</sup> USDA, ARS, Poplarville, MS

showed significant reductions in plant growth, vigor; though fruit production did not seem to be significantly impacted.

REFERENCES CITED:

Caruso, F.L. and D.C. Ramsdell. 1995. *In: Compendium of Blueberry and Cranberry Diseases.* Amer. Pathol. Soc. Press, St. Paul, MN. pp. 7-8.

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

No.	Treatments Chemical	Bed Height	Cultivars					TOTAL
			Tifblue	Gulfcoast	Misty	Reville	Other*	
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. Phytophthora 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: 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: Final

LEADER/PARTICIPANTS: Timothy C. Lockley/Barbara Smith<sup>2</sup>

### INTRODUCTION:

The Grape Root Borer (*Vitacea polistiformis*) is a pest of muscadine grapes *Vitis rotundifolia 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, burrow into the soil and 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 were maintained according to Mississippi Agriculture and Forestry Experiment Station recommendations. Irrigation was supplied as needed via drip lines placed under mulch. Plants were monitored three times a year for potential phytotoxicity and surveyed quarterly for the presence of fireants and weeds. Plants were assayed for the presence of crown gall bacterium and, at the end of the study (24 months), grape root borer damage assessed.

Mulches consisted 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 muscadine cultivars were used in the trials: Carlos, Doreen, Jumbo, Magnolia, Noble, Sterling, Summit, and Tarheel.

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<sup>2</sup> USDA, ARS, Poplarville, MS



## RESULTS:

Examination of the various replicates throughout 1996, 1997, 1998 and 1999 showed no indication of fire ant activity among any of the Biobarrier treatments (Table 1).

## REFERENCES CITED:

Olien, W.C., B.J. Smith & C.P. Hegwood, Jr. 1993. Grape root borer: a review of the life cycle and strategies for integrated control. Hortscience. 28: 1154-1156.

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

PLOT	CULTIVAR	MULCH TREATMENT	NUMBER OF RIFA MOUNDS			
			1996	1997	1998	1999
1	Tarheel	Biobarrier	0	0	0	0
2	Carlos	Fabric	5	4	1	3
3	Summit	Fabric	4	4	0	1
4	Carlos	Bark	4	3	1	1
5	Sterling	Bark	3	4	2	3
6	Carlos	Biobarrier	0	0	0	0
7	Noble	Biobarrier	0	0	0	0
8	Carlos	Fabric	2	5	1	0
9	Magnolia	Fabric	3	2	0	1
10	Carlos	Bark	8	4	1	1
11	Jumbo	Biobarrier	0	0	0	0
12	Carlos	Bark	2	4	0	2
13	Doreen	Bark	1	2	0	2

PROJECT NO: FA01G028

PROJECT TITLE: Evaluation of Various Granular Insecticides for Control of Imported Fire Ants in Turfgrass, 1998

TYPE REPORT: Interim

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

INTRODUCTION:

Several companies are developing new chemistries with potential as granular products for control of imported fire ants. These products have the potential to be used in both the homeowner market and the Federal IFA Quarantine as lawn or sod treatments. In 1998, several companies supplied us with new granular products for evaluation.

MATERIALS AND METHODS:

The test site was located at the Slidell Municipal Airport in Slidell, Louisiana. Mowed areas between taxiways and runways simulated conditions that occur in commercial turfgrass. Granular products were applied broadcast to test plots using a Herd® GT-77 spreader (Herd Seeder Co., Logansport, IN) on a farm tractor on June 3-4, 1998. There were 3 replicates per treatment. Products, producers, and rates of application are listed below:

Product	% active ingredient	Producer	Rate of application (lbs/acre)
Fipronil	0.05%	Rhone-Poulenc Ag. Co., Raleigh, NC	25.0, 37.5, 50.0
Force	1.5% tefluthrin	Zeneca Ag Products, Wilmington, DE	46.7
Lambda-cyhalothrin	0.1%	Zeneca Ag Products, Wilmington, DE	120
Talstar	0.2% bifenthrin	FMC Corp., Philadelphia, PA	100

A ¼ acre circular efficacy plot was established in the center of each 1.0 acre test plot. Prior to and at 2, 4, and 6 weeks after treatment, 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 pre-treatment population index were calculated. Data were statistically analyzed

using analysis of variance and means separated using Tukey's test (P=0.05) for each post-treatment interval.

## RESULTS:

Treatments were applied on June 3-4, 1998 with air temperatures between 87-94°F and soil temperatures between 80-82°F. At 2 weeks after treatment, Talstar and the two high rates of fipronil provided the greatest control of IFA (Tables 1 & 2). By 4 weeks, all treatments, except lambda-cyhalothrin reduced pretreatment population indices by >85%, and all except lambda-cyhalothrin and the lowest fipronil rate, reduced colony numbers by >70%. The 50.0 lb/acre rate of fipronil provided 100% control at 4 weeks. At 6 weeks after treatment, all fipronil rates and the Force treatment were still significantly better than the check; the low fipronil rate and Force providing >80% reduction in population indices, and the two higher fipronil rates providing >95% reduction in population indices.

By 12 weeks after treatment, all fipronil rates provided 100% control of IFA. All other products provided 60-75% control of IFA. At 18 weeks after treatment, the 2 lower fipronil rates were still providing 100% control of IFA; one plot in the high fipronil rate had one large reproductive colony, which probably moved into the area from the untreated surrounding area. All other treatments had reproductively viable colonies. The Talstar and lambda-cyhalothrin plots were not evaluated after this time.

At 24 weeks after treatment, the two higher fipronil rates were at 100% mortality; the one mound found on a high rate plot at 18 weeks had either moved out of the area or succumbed to the treatment. Two of the plots of the lowest fipronil rate (25 lb/acre) had reproductively viable colonies on them, showing possible reinfestation. The Force plots were all reinfested with reproductively viable colonies, and were not evaluated after this time.

Fipronil rates of 37.5 and 50 lb/acre continue to provide excellent control of IFA through 71 weeks after treatment. Plots treated with the low rate continue to have a few reproductively viable colonies present.

All fipronil treatments will continue to be evaluated until general reinfestation is noted; although the time interval between evaluations was lengthened to 8 weeks during the winter months.

## DISCUSSION:

Talstar 0.2G, at 100 lbs/acre (0.2 lb AI/acre), provided extremely fast knockdown of IFA (>88% mortality at 2 weeks after treatment), but did not provide adequate residual control, as evidenced by the rapid reinfestation by either movement into the treated area, or possibly rejuvenation of treated colonies. Lambda-cyhalothrin at the rate used (120 lbs/acre or 0.12 lb AI/acre) never provided more than 75% control of IFA, and at all evaluation periods there were reproductively viable colonies present. Force at 46.7 lbs/acre (0.7 lb AI/acre) performed a little better numerically, providing 70-80% control of IFA through 18 weeks.

In general, the higher the rate of application with fipronil, the faster the control of IFA in grass sod. The 2 higher rates provided >84% control of IFA by 4 weeks after treatment, >91% after 6 weeks, and by 12 weeks after treatment, all rates provided 100% control. The lower rate achieved the same control (100%) at 12 weeks, but in the preceding weeks was numerically inferior to the higher rates of application. The lowest rate has shown good control of IFA through 18 weeks, while the 2 higher rates have provided excellent control through 71 weeks.

References Cited:

- Harlan, D.P., W.A. Banks, H.L. Collins & C.E. Stringer. 1981. Large are test of AC217,300 bait for control of imported fire ant in Alabama, Louisiana, and Texas. Southwest. Entomol. 8: 42-45.
- Lofgren, C.S. & D.F. Williams. 1982. Avermetin B<sub>1a</sub>, a highly potent inhibitor of reproduction by queens of the red imported fire ant. J. Econ. Entomol. 75: 798-803.

Table 1. Efficacy of various granular insecticides applied broadcast to grass sod: change in pretreatment population indices. Slidell Airport, LA; June 3-4, 1998.

Treatment	Rate of Applic. (lb/acre)	Mean pretreat population index/acre*	% change in pretreatment population indices at indicated wks PT**							
			2 wks	4 wks	6 wks	12 wks	18 wks***	24 wks	32 wks	41 wks
Fipronil	25.0	526.8	-43.0abc	-85.1a	-85.9a	-100.0a	-100.0a	-85.1ab	-86.3a	-87.5a
Fipronil	37.5	313.2	-91.1a	-89.7a	-99.3a	-100.0a	-100.0a	-100.0a	-100.0a	-100.0a
Fipronil	50.0	404.0	-76.9a	-100.0a	-95.2a	-100.0a	-90.5a	-100.0a	-100.0a	-96.4a
Force	46.7	440.0	-70.5ab	-89.6a	-80.2a	-59.2ab	-75.7a	-23.9c	--	--
Lambda-cyhalothrin	120.0	366.8	-16.2c	-64.9ab	-74.3ab	-61.6ab	-66.9a	--	--	--
Talstar	100.0	312.0	-94.7a	-88.7a	-63.9ab	-75.6a	-20.3a	--	--	--
Check	--	620.0	-19.4bc	-34.0b	-30.1b	-28.8b	-48.1a	-36.8bc	-37.3b	-18.3b

\* Mean of 3 replicates.

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

\*\*\* 18 wk count made on 10/9/98 after Hurricane Earl (9/1-9/3), T.S. Frances (9/10-9/13), T.S. Hermine (9/17-9/22), and Hurricane Georges (9/26-9/28) impacted the count area with tremendous rainfall amounts. Total rainfall for the 5 weeks preceding this count was approximately 32 inches.

Table 1. Cont.

Treatment	Rate of Applic. (lb/acre)	Mean pretreat population index/acre*	% change in pretreatment population indices at indicated wks PT**							
			47 wks	53 wks	59 wks	65 wks	71 wks	79 wks	89 wks	
Fipronil	25.0	526.8	-87.5a	-93.8a	-91.3a	-100.0a	-97.0a	-97.0a	-89.4a	
Fipronil	37.5	313.2	-100.0a	-100.0a	-91.7a	-100.0a	-100.0a	-88.3a	-85.0a	
Fipronil	50.0	404.0	-96.0a	-100.0a	-100.0a	-100.0a	-92.9a	-93.0a	-84.8a	
Force	46.7	440.0	--	--	--	--	--	--		
Lambda-cyhalothrin	120.0	366.8	--	--	--	--	--	--		
Talstar	100.0	312.0	--	--	--	--	--	--		
Check	--	620.0	-3.0b	-49.0b	-20.6b	-47.3b	-38.6b	6.6b	8.2b	

\* Mean of 3 replicates.

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

Table 2. Efficacy of various granular insecticides applied broadcast to grass sod: decrease in pretreatment colony numbers. Slidell Airport, LA; June 3-4, 1998.

Treatment	Rate of Applic (lb/acre)	Mean no. pretreat colonies/ acre*	% decrease in no. of pretreatment colonies at indicated wks PT*							
			2 wks	4 wks	6 wks	12 wks	18 wks***	24 wks	32 wks	41 wks
Fipronil	25.0	30.8	41.1bc	56.1ab	72.3ab	100.0a	100.0a	83.9ab	81.1a	86.7a
Fipronil	37.5	24.0	88.6a	83.8ab	95.2a	100.0a	100.0a	100.0a	100.0a	100.0a
Fipronil	50.0	25.2	68.3ab	100.0a	91.7a	100.0a	93.3ab	100.0a	100.0a	95.8a
Force	46.7	29.2	63.9ab	79.3ab	74.5ab	63.4ab	76.3ab	34.8c	--	--
Lambda-cyhalothrin	120.0	24.0	22.9c	35.7b	73.0ab	56.8ab	67.1ab	--	--	--
Talstar	100.0	21.2	88.6a	70.0ab	53.8ab	75.2ab	28.6b	--	--	--
Check	--	40.0	19.7c	35.6b	31.4b	27.8b	40.3ab	41.7bc	30.6b	25.3b

\* Mean of 3 replicates.

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

\*\*\* 18 wk count made on 10/9/98 after Hurricane Earl (9/1-9/3), T.S. Frances (9/10-9/13), T.S. Hermine (9/17-9/22), and Hurricane Georges (9/26-9/28) impacted the count area with tremendous rainfall amounts. Total rainfall for the 5 weeks preceding this count was approximately 32 inches.

Table 2. Cont.

Treatment	Rate of Applic (lb/acre)	Mean no. pretreat colonies/ acre*	% decrease in no. of pretreatment colonies at indicated wks PT*							
			47 wks	53 wks	59 wks	65 wks	71 wks	79 wks	89 wks	
Fipronil	25.0	30.8	86.7a	93.3a	90.6a	100.0a	94.4a	94.4a	83.3a	
Fipronil	37.5	24.0	100.0a	100.0a	93.3a	100.0a	100.0a	90.5a	85.7a	
Fipronil	50.0	25.2	90.3a	100.0a	100.0a	100.0a	93.3a	94.4a	82.2ab	
Force	46.7	29.2	--	--	--	--	--	--		
Lambda-cyhalothrin	120.0	24.0	--	--	--	--	--	--		
Talstar	100.0	21.2	--	--	--	--	--	--		
Check	--	40.0	10.0b	44.4b	21.1b	55.3b	41.1b	13.3b	16.7b	

\* Mean of 3 replicates.

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



PROJECT NO: FA01G059

PROJECT TITLE: Evaluation of Fipronil Insecticide For Control of Imported Fire Ants in Turf Grass, 1999

TYPE REPORT: Interim

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

COOPERATORS: Rhone-Poulenc Ag Company

### INTRODUCTION:

Fipronil is a relatively new broad spectrum pyrazole insecticide currently under development by Rhone-Poulenc Ag Company, Research Triangle Park, NC. Fipronil has demonstrated potent insecticide and acaricide properties on a large number of pests including fleas, ticks, boll weevils, thrips, flies, fire ants, and others (Colliot et al. 1992, Burris et al. 1994, Postal et al. 1995, Searle et al. 1995, Collins and Callcott 1998). Trials initiated in June 1998 are still in progress, but 100% control was obtained through 65 weeks at rates of 37.5 and 50.0 lbs formulated product per acre. Additional studies were needed to confirm the results obtained in prior studies. Therefore a trial was conducted at an abandoned grass sod farm near Gulfport, Mississippi in June 1999.

### MATERIALS AND METHODS:

Test plots were one acre in size, with a ¼ acre efficacy subplot located in the center of the test plots. Granular fipronil (0.05 G) was applied with a Herd® granular applicator mounted on a farm tractor on July 7, 1999. Rates of application were 25, 37.5, and 50 lbs. formulated product per acre. Prior to treatment and at 6 week intervals thereafter, evaluations of IFA populations were made in each ¼ acre efficacy subplot using the procedures described by Lofgren and Williams (1982) and Collins and Callcott (1995). Differences in treatment means will be separated by a LSD test (P=0.05).

It is anticipated that this trial will continue approximately one year, with test plot evaluations conducted at 6-8 week intervals.

### RESULTS:

At 6 weeks after treatment, fipronil showed 100% control of IFA in the plots treated at the two high rates and greater than 95% control in plots treated at the lowest rate of application (Tables 1 & 2). Results, thus far in this trial, have been very different from numerous previous trials with this product. One or more colonies have appeared on the treated plots 12 and 18 weeks after treatment, signs of normal reinfestation. However, in all other trials with this product if a mound appeared on a plot within 6 months of the treatment, it generally was not present at the next

evaluation period. It should also be noted that the mean population index of the check plots had increased 100% from the pretreatment index at the 18 week count, and the mean number of colonies present on the check plots increase 73% during the same period. There may have been uncommon pressure to inhabit the treated areas at this site during the fall and winter due to the unseasonably warm weather. By 27 weeks after treatment, the 50 lb/acre plots had only one small colony class 7 mound present, indicating that the other mounds that had inhabited those plots in the previous count had either succumbed to the treatment or moved outside the evaluation area. We will continue to evaluate these plots.

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Table 1. Efficacy of granular fipronil applied broadcast to grass sod: change in pretreatment population indices. Harrison Co., MS; July 7, 1999.

Treatment	Rate of Applic. (lb/acre)	Mean pretreat population index/acre*	% change in pretreatment population indices at indicated wks PT**						
			6 wks	12 wks	18 wks	27 wks	33 wks		
Fipronil	25.0	720.0	-99.0a	-90.0a	-71.2a	-83.8a	-82.0a		
Fipronil	37.5	640.0	-100.0a	-94.2a	-83.2a	-86.3a	-77.7a		
Fipronil	50.0	733.3	-100.0a	-100.0a	-88.4a	-97.5a	-93.0a		
Check	--	593.3	7.1b	2.1b	100.4b	59.1b	107.5b		

Table 2. Efficacy of granular fipronil applied broadcast to grass sod: change in pretreatment colony numbers. Harrison Co., MS; July 7, 1999.

Treatment	Rate of Applic. (lb/acre)	Mean pretreat no. colonies/acre*	% change in pretreatment population indices at indicated wks PT**						
			6 wks	12 wks	18 wks	27 wks	33 wks		
Fipronil	25.0	50.7	94.2a	85.1a	66.5a	83.1a	78.1ab		
Fipronil	37.5	48.0	100.0b	91.2a	83.8a	84.5a	71.8a		
Fipronil	50.0	53.2	100.0b	100.0a	86.2a	96.7a	94.3b		
Check	--	40.0	0.0c	15.2b	0.0b	0.0b	0.0c		

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

PROJECT NUMBER: FA02G028

PROJECT TITLE: Field Evaluation of Extinguish™ (methoprene) Fire Ant Bait, Fall 1998 and Spring 1999

TYPE REPORT: Final

PROJECT LEADERS/PARTICIPANTS: Homer Collins, Anne-Marie Callcott, Lee McAnally, Avel Ladner, and Shannon Wade

## INTRODUCTION:

Juvenile hormone analogues or insect growth regulators (IGR's) were investigated by several researchers for control of imported fire ants in the 1970's. Laboratory studies by Cupp and O'Neal (1973) found that methoprene (ENT-70460) and another compound (hydroprene), caused deformities in immatures and increased mortality. Troisi and Riddiford (1974) reported that the same two compounds interfered with egg production, embryonic development, and metamorphosis in *Solenopsis invicta*. Vinson et al. (1974) reported that topical application of several insect growth regulators including methoprene to pharate reproductive pupae reduced the number of adults eclosing and prevented the development of normal pigmentation in those adults that did eclose. Vinson et al. (1974) found that insect growth regulators administered to small colonies (300-400 workers) by feeding or through contact or fumigation caused delayed cessation of egg laying by the queen, initiation of sexual brood production, and ultimate death of the colony.

Research with numerous other IGR's continued throughout the 1980's (Banks et. al. 1978, Banks and Schwarz 1980, Banks and Harlan 1982), but additional studies with methoprene were not conducted until several trials were conducted between 1992-1996 (Drees and Barr 1998). Results of those tests suggested that methoprene baits provide control equivalent to a fenoxycarb standard. In the summer of 1998, Extinguish™ Professional Fire Ant Bait containing 0.5% (S)-methoprene was registered by Wellmark International (Bensenville, IL 60106). Extinguish may be applied to, but not limited to, the following areas: residential turf and landscape, parks, zoos, golf courses, roadsides, airports, cemeteries, perimeter areas of buildings, homes, sheds, electrical and phone boxes, pump houses, and other associated areas, forestry sites, commercial nurseries including field grown and container stock, school grounds, sports fields, pastures, rangeland, citrus groves, sod farms, and cropland. We initiated field studies in October 1998 and April 1999 to examine effects of methoprene on colonies of IFA and to compare efficacy and rate of control of fall versus spring applications of Extinguish.

## MATERIALS AND METHODS:

### *1998 Fall Field Trial:*

The test site was located in a pasture located in Harrison County, MS. All baits were applied to test plots using a shop-built granular applicator mounted on a farm tractor on October 19, 1998.

Soil temperature at the time of application was 73°F, and air temperature was 78°F. Extinguish was applied at a rate of 1.5 lbs formulated bait per acre. Logic® was also applied at 1.5 lbs per acre as a standard, and there was also an untreated control. There were 3 replicates per treatment, and all test plots were 1.0 acre in size. A ¼-acre circular efficacy plot was established in the center of each 1.0 acre test plot. Prior to bait application and at 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 pretreatment population index were calculated. Experimental data were statistically analyzed using analysis of variance and means were separated using the LSD test (P=0.05) for each posttreatment rating interval.

#### *1999 Spring Field Trial:*

The test site was located in a pasture located in Jackson County, MS. All baits were applied to test plots as described above on April 27, 1999. Extinguish was applied at a rate of 1.5 lbs formulated bait per acre. Logic was also applied at 1.5 lbs per acre as a standard, and there was also an untreated control. Replicates, test plot size, and evaluation intervals were as above.

### RESULTS:

#### *1998 Fall Field Trial:*

Fall is not the optimum time to treat IFA colonies with baits. With fall bait applications, we usually do not see optimal control until the following spring (Collins et al. 1992). As seen in the data from this trial (Table 1), neither Extinguish or Logic reduced the number of colonies present at 12-20 weeks after treatment. However, since we did not find worker brood present in many of the treated colonies, the population indices were significantly reduced (Table 2). As anticipated, the level of control increased significantly at the 27 - 39 week counts (April, June, July 1999), with Extinguish providing 100% control 39 weeks after application. Reinfestation with healthy reproducing colonies followed after that time.

#### *Reproductive Status of Sexual Females:*

On January 19, 1999, we collected alate and dealate females from field treated colonies and control colonies, as well as alate females from a series of laboratory treated colonies. These females were sent to USDA, ARS, CMAVE (Gainesville, FL) for determination of reproductive status. Dissection and microscopic examination found no ovaries in any females collected from methoprene treated colonies. Therefore, even if methoprene-treated alates fly and mate, they would not be capable of producing viable eggs and beginning new colonies. Thus, the life cycle appeared to have been broken in methoprene treated colonies.

#### *1999 Spring Field Trial:*

At 6 weeks after treatment, the number of colonies present in either bait treated area had not decreased significantly as compared to the untreated check (Table 3), however population indices had decreased significantly (Table 4). At 12 and 18 weeks after treatment, both Extinguish and Logic provided significant reduction in both number of colonies present and population index. By 26 weeks, reinfestation was evident in both bait treatment areas.

CONCLUSION:

Methoprene bait provides effective IFA population control. These results again demonstrate the desirability of a spring bait application as opposed to a fall application, particularly if only one application per year is performed.

Table 1. Effect of Extinguish fire ant bait on number of IFA colonies present - 1998 fall trial.

Treatment	Mean no. colonies/acre - pretreat	% decrease in no. colonies at indicated wks posttreatment							
		-5-	-12-	-20-	-27-	-33-	-39-	-47-	-52-
Extinguish	78.7	0.0a	0.0a	0.0a	9.3a	79.9a	100.0a	97.1a	86.1a
Logic	38.7	0.0a	5.6a	16.7a	44.4a	68.7a	97.0a	91.2ab	64.9ab
Untreated	60.0	3.0a	0.0a	0.0a	3.0a	3.0b	29.8b	84.2b	34.4b

Table 2. Effect of Extinguish fire ant bait on IFA population indices - 1998 fall trial.

Treatment	Mean pop. index/acre - pretreat	% change in population indices at indicated wks posttreatment							
		-5-	-12-	-20-	-27-	-33-	-39-	-47-	-52-
Extinguish	1000.0	-29.4a	-53.3a	-56.6a	-80.4a	-96.2a	-100.0a	-97.9a	-84.8a
Logic	513.3	-37.6a	-67.6a	-70.0a	-88.8a	-95.0a	-96.8a	-96.3a	-68.7ab
Untreated	1013.3	17.8b	21.7b	27.7b	14.7b	3.5b	-33.3b	-86.1b	-36.3b

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

Table 3. Effect of Extinguish fire ant bait on number of IFA colonies present - 1999 spring trial.

Bait	Mean no. colonies/acre - pretreat	% decrease in no. colonies at indicated wks. posttreatment			
		-6-	-12-	-18-	-26-
Logic	49.2	31.8a	100.0a	100.0a	71.1ab
Extinguish	52.0	2.8a	81.8b	92.2a	83.9a
Check	33.2	19.5a	29.3c	35.9b	21.2b

Table 4. Effect of Extinguish fire ant bait on IFA population indices - 1999 spring trial.

Bait	Mean pop. index/acre - pretreat	% change in population indices at indicated wks. PT			
		-6-	-12-	-18-	-26-
Logic	900.0	-90.4a	-100.0a	-100.0a	-84.5a
Extinguish	1026.8	-83.0a	-98.2a	-99.0a	-89.9a
Check	680.0	-32.7b	-33.9b	-56.4b	-38.2b

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



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- Banks, W. A., C. S. Lofgren, and J. K. Plumley. 1978. Red imported fire ants: effects of insect growth regulators on caste formation, and colony growth and survival. *J. Econ. Entomol.* 71: 75-78.
- Banks, W.A. and D.P. Harlan. 1982. Tests with the insect growth regulator Ciba-Geigy CGA-38531 against laboratory and field colonies of red imported fire ants. *J. Georgia Entomol. Soc.* 17: 460-466.
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- Collins, H. L., A.-M. A. Callcott, T. C. Lockley and A. L. Ladner. 1992. Seasonal trends in effectiveness of hydramethylnon and fenoxycarb for control of red imported fire ants. *J. Econ. Entomol.* 85: 2131-2137.
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- Lofgren, C. S. and D. F. Williams. 1982. Avermectin B<sub>1a</sub>, a highly potent inhibitor of reproduction by queens of the red imported fire ant. *J. Econ. Entomol.* 75: 798-803.
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PROJECT NUMBER: FA02G019

PROJECT TITLE: Field Evaluation of Various Fire Ant Baits, Spring 1999

TYPE REPORT: Final

PROJECT LEADERS/PARTICIPANTS: Homer Collins, Anne-Marie Callcott, Lee  
McAnally, Avel Ladner, and Shannon Wade

## INTRODUCTION:

A variety of baits are currently on the market for control of imported fire ants. In the summer of 1998, Extinguish™ Professional Fire Ant Bait containing 0.5% (S)-methoprene was registered by Wellmark International (Bensenville, IL 60106). Extinguish may be applied to, but not limited to, the following areas: residential turf and landscape, parks, zoos, golf courses, roadsides, airports, cemeteries, perimeter areas of buildings, homes, sheds, electrical and phone boxes, pump houses, and other associated areas, forestry sites, commercial nurseries including field grown and container stock, school grounds, sports fields, pastures, rangeland, citrus groves, sod farms, and cropland. For a detailed report on methoprene applied as a bait to control IFA see FA02G028. Novartis produces Clinch™ Ant Bait (0.011% abamectin), and Logic® IGR Bait Formulation (1.0% fenoxycarb).

## MATERIALS AND METHODS:

The test site was located in a pasture located in Jackson County, MS. All baits were applied to test plots using a shop-built granular applicator mounted on a farm tractor on April 27, 1999. All baits were applied at a rate of 1.0 to 1.5 lbs formulated bait per acre. There were 3 replicates per treatment, including an untreated control, and all test plots were 1.0 acre in size. A ¼-acre circular efficacy plot was established in the center of each 1.0 acre test plot. Prior to bait application and at 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 pretreatment population index were calculated. Experimental data were statistically analyzed using analysis of variance and means were separated using the LSD test (P=0.05) for each posttreatment rating interval.

Baits used in this trial included Extinguish, Logic from a newly opened bag, old/aged Logic (bag had been opened 5/13/98 and stored under normal storage conditions), and two formulations of Clinch (FL-990324 and FL-990373).

## RESULTS:

All baits significantly reduced population indices 6 weeks after treatment (Table 2), but decreases in colony numbers were not significantly different from the check in any treatment (Table 1).

This indicated that while most treated colonies were still active, they were no longer producing worker brood, an effect of the IGR activity of all of these baits. By 18 weeks all baits, except Clinch FL-990373, provided >92% decrease in colony numbers (Table 1) and population indices (Table 2). By 26 weeks after treatment, all plots were being reinfested with small healthy colonies.

All baits decreased population indices at similar rates, but the speed at which decreases in colony numbers occurred varied: at 12 weeks after treatment (when decreases were significantly different from the check), fresh Logic was the fastest numerically, followed by aged Logic, Extinguish, Clinch FL-990324, Clinch FL-990373.

Table 1. Efficacy of various bait formulations - decrease in colony number.

Bait	Mean no. colonies/acre - pretreat	% decrease in no. colonies at indicated wks. posttreatment			
		-6-	-12-	-18-	-26-
Aged Logic	52.0	0.0a	84.5ab	100.0a	68.4ab
Fresh Logic	49.2	31.8b	100.0a	100.0a	71.0ab
Clinch FL990324	46.8	35.3b	72.4b	96.3a	12.8b
Clinch FL990373	53.2	26.2ab	65.7b	86.7a	23.1ab
Extinguish	52.0	2.8a	81.8ab	92.2a	83.9a
Check	33.2	19.5ab	29.3c	35.9b	21.2b

Table 2. Efficacy of various bait formulations - change in population indices.

Bait	Mean pop. index/acre - pretreat	% change in population index at indicated wks. PT			
		-6-	-12-	-18-	-26-
Aged Logic	1000.0	-80.6a	-98.0a	-100.0a	-81.8a
Fresh Logic	900.0	-90.4a	-100.0a	-100.0a	-84.5a
Clinch FL990324	813.2	-90.7a	-94.1a	-96.8a	-20.2b
Clinch FL990373	1033.2	-84.8a	-95.5a	-97.5a	-15.8b
Extinguish	1026.8	-83.8a	-98.2a	-99.0a	-89.9a
Check	680.0	-32.7b	-33.9b	-56.4b	-38.2ab

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

PROJECT NO: FA02G029

PROJECT TITLE: Dual applications of Amdro® for late season control of RIFA

TYPE REPORT: Interim

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

### 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. Currently we have a study underway in Tennessee (see report number FA01G077) to determine when mating flights cease. Once that has been determined, it may be possible to use a fast-acting metabolic inhibitor bait such as Amdro to certify movement of B&B nursery stock in Tennessee. The bait would be applied after mating flights have stopped. Rapid rate of kill would increase the window of opportunity for certification purposes. We hypothesized that dual applications of Amdro, spaced about one week apart might increase the rate of kill obtained from a single Amdro application.

### MATERIALS AND METHODS:

The test site was located in a pasture in Jackson County, MS. Amdro was applied to test plots using a shop-built granular applicator mounted on a farm tractor on Nov. 23, 1999. The equipment provided a 21' swath and was operated at 4 mph. Soil temperature at the time of the first application was 70°F and air temperature was 76°F. The second application was made on Nov. 29, 1999, at which time the soil and air temperature were 60°F and 65°F, respectively. Treatments were a single Amdro application, two Amdro applications spaced 1 week apart, and an untreated control. There were 4 replicates per treatment, and all test plots were 1.0 acre in size. A ¼-acre circular efficacy plot was established in the center of each 1.0 test plot. Prior to bait application and at 3 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 pretreatment population index were calculated. Experimental data were statistically analyzed using analysis of variance, and means were separated using the LSD test (P=0.05) for each posttreatment rating interval.

### RESULTS:

In the first 13 weeks after treatment, the dual Amdro application was numerically superior to the single treatment, but this superiority was not significant (Tables 1 and 2). At the 10 week evaluation (Feb. 4, 2000), the check plots experienced large decreases in colonies and populations, probably due to a week of cold weather just prior to the evaluation and tall grass in

the check plots. By 13 weeks, we were able to detect many colonies on the check plots that we apparently could not detect 3 weeks earlier. Also at 13 weeks, there were several small reproductively viable colonies on both the single and double treated Amdro plots, indicating reinfestation.

Table 1. Efficacy of single versus double applications of Amdro for control of imported fire ants in the fall - decrease in colony numbers.

Treatment	% decrease in no. pretreat colonies present at indicated weeks after treatment				
	3	6	10	13	
Amdro - 1X	73.0a	83.1a	85.5a	76.7a	
Amdro - 2X	77.9a	91.9a	90.6a	87.2a	
Check	18.2b	22.9b	55.8a	18.2b	

Table 2. Efficacy of single versus double applications of Amdro for control of imported fire ants in the fall - change in population indices.

Treatment	% change in pretreat population index at indicated weeks after treatment				
	3	6	10	13	
Amdro - 1X	-77.5a	-91.0a	-88.1ab	-77.5a	
Amdro - 2X	-83.8a	-97.2a	-93.0a	-88.9a	
Check	-15.8b	-17.3b	-57.1b	-8.2b	

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

References Cited:

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

Lofgren, C.S. & D.F. Williams. 1982. Avermetin B<sub>1a</sub>, a highly potent inhibitor of reproduction by queens of the red imported fire ant. J. Econ. Entomol. 75: 798-803.

PROJECT NO: FA01G039

PROJECT TITLE: Preliminary Trial using Chlorfenapyr 2SC as an Individual Mound Treatment

TYPE REPORT: Final

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

### INTRODUCTION:

Chlorfenapyr 2SC (AC 303,630) is a new pyrrole chemical from American Cyanamid under development for a variety of insect and mite pests. This product was evaluated in a non-replicated trial for efficacy against mounds of imported fire ants in the field.

### MATERIALS AND METHODS:

Chlorfenapyr 2SC was supplied by the company and rates of application were determined by the company. The rate of application was 3.6 ml. 2SC per mound, applied as a drench treatment in either one gallon or one quart of water. One acre test plots were set out in Harrison County, MS, and all the mounds within the test plot flagged to facilitate treatment. A ¼ acre circular efficacy plot was established in the center of each 1.0 acre test plot. Prior to and at 1, 2, 4, 8 and 13 weeks after treatment, IFA populations in each efficacy plot were evaluated using the population index system developed by Harlan et al. (1981) and revised by Lofgren and Williams (1982).

Due to the preliminary nature of this trial, and the logistics involved in individual mound treatments, only one acre (replicate) was treated with each treatment.

### RESULTS:

Treatments were made on July 13, 1999. The one gallon treatment reduced the number of IFA colonies in the treated area by 70% at 1 and 2 weeks after treatment, while the one quart treatment reduced colony number by 50% during the same time period. Those remaining colonies appeared to contain a reproducing queen, as all colonies detected throughout the trial contained worker brood. At 4 to 8 weeks after treatment, both application rates provided 85-90% decrease in number of colonies present, however, the untreated check also experienced a 50-65% decrease in colonies during the same time. This was probably due to the hot, dry conditions prevalent during late August and September. By 13 weeks after treatment, the one gallon treatment plot had several healthy colonies on it and the one quart treatment plot had more colonies on it than prior to treatment, as did the untreated plot.

Thus, while this trial was preliminary and not conclusive, it does indicate that chlorfenapyr 2SC may provide some short term control of IFA when used as an individual mound drench. However, as with all individual mound treatments it is hard to determine whether "new" colonies

which appear after a treatment are truly new colonies which were too small to detect at treatment time and thus not treated, or whether they are colonies that were severely depopulated by the treatment, but the queen survived to replenish the colony.

References Cited:

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

Lofgren, C.S. & D.F. Williams. 1982. Avermetin B<sub>1a</sub>, a highly potent inhibitor of reproduction by queens of the red imported fire ant. *J. Econ. Entomol.* 75: 798-803.

PROJECT NO: FA02G048

PROJECT TITLE: Evaluation of Field Releases of *Thelohania solenopsae*

TYPE REPORT: Final

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

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

### INTRODUCTION:

The microsporidium *Thelohania solenopsae* (Microsporidia: Thelohaniidae) was discovered in Brazil in the red imported fire ant (Knell et al. 1977). Since that time, USDA, ARS, CMAVE personnel in Argentina have also discovered the pathogen in the black imported fire ant in that country and have determined that the pathogen does decrease colonies and colony vigor and therefore may be a good candidate for use as a biological control agent in the United States (Briano et al. 1995a, 1995b, 1996). *T. solenopsae*, which previously was found only in South America, was discovered in limited locations in the U.S. (Williams et al. 1998).

### MATERIALS AND METHODS:

In June, 1998 we assisted ARS with the initiation of a trial to evaluate field releases of the pathogen *Thelohania solenopsae*. On June 10, five mounds at two sites (polygyne site in Hancock Co. and monogyne site in Harrison Co.) were inoculated with brood infected with *T. solenopsae*. Every two months we monitored the inoculated plots and corresponding non-inoculated control plots by evaluating mounds with the mound index system, geo-referencing each mound within the plots, and collecting worker samples from each mound within the plots. We also assisted by microscopically examining collected workers for pathogen spores.

### RESULTS:

#### *Colony mortality*

##### Polygyne Site

At 8 weeks after inoculation, the inoculated and the control sites had large decreases in number of colonies and in population indices (Tables 1 & 2). By late November, 1998 (25 weeks after inoculation), the number of colonies in the inoculated site was almost back to the pretreatment level, but the population index of the site was 34% less than the pretreatment level. The control site had the opposite; colony numbers were down 33% compared to the pretreatment level, but the population index of the site was a little above pretreatment levels. Unfortunately, this site was lost due to construction.



### Monogyne Site

At 8 weeks after inoculation, the control plot had an increase in number of colonies and in population index, while the inoculated site had a decrease in number of colonies and in population index (Tables 1 & 2). At this evaluation period, we tried to collect worker brood from the inoculated colonies. This resulted in a lot of mound destruction, and was therefore not attempted at future evaluation periods. After the 8 week evaluation, we experienced difficulty in finding mounds close to the original inoculated mounds. In general, the inoculated monogyne site has shown a decrease in colony numbers and population indices throughout the 34 week evaluation period, while the control site has shown an increase in colony and ant numbers.

### *Presence of pathogen*

Pretreatment samples were examined and no spores were detected at either site.

### Polygyne Site

In the polygyne site, spores were not detected in any of the inoculated mounds at the 8 week evaluation. At 18 weeks after inoculation, a few spores were detected in two of the originally inoculated mounds, and numerous spores were detected in one mound not originally inoculated. By 25 weeks after inoculation, we were not able to find a mound associated with one of the original inoculated sites. Three of the remaining four inoculated mounds were no longer reproductively viable (class 2s). These plots were lost due to construction after the 25 week evaluation.

### Monogyne Site

In the monogyne site, at 8 weeks after inoculation, numerous spores were detected in one of the inoculated mounds. Two of the inoculated mounds were deserted and no new mounds were detected within an 8-10' radius of the original inoculated mounds. At 18 weeks after inoculation, we were not able to find any monogyne inoculated mounds (no mound within 8-10 ft of the placard), and no workers collected from any mounds contained *Thelohania* spores. By 25 weeks after there were 2 reproductively active mounds very near the sites of 2 of the original inoculated mounds, and one non-reproductively active (class 4) within about 10-12 feet of another original site. However, again at 34 weeks after inoculation, no mounds were found within 10 feet of the original inoculation sites. No spores were found in any sample at the 25 or 34 week count. This trial has been terminated due to poor results and the loss of the polygyne site.

References Cited:

- Briano, J., R. Patterson and H. Cordo. 1995a. Relationship between colony size of *Solenopsis richteri* (Hymenoptera: Formicidae) and infection with *Thelohania solenopsae* (Microsporidae: Thelohaniidae) in Argentina. *J. Econ. Entomol.* 88: 1233-1237.
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- Briano, J.A., R.S. Patterson, J.J. Becnel and H.A. Cordo. The black imported fire ant, *Solenopsis richteri*, infected with *Thelohania solenopsae*: intracolony prevalence of infection and evidence for transovarial transmission. *J. Invertebr. Pathol.* 67: 178-179.
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- Williams, D.F., G.J. Knue, and J.J. Becnel. 1998. Discovery of *Thelohania solenopsae* from the red imported fire ant, *Solenopsea invicta* in the United States. *J. Invert. Pathol.* 71:175-176.

Table 1. Change in colony numbers of *Thelohania solenopsae* inoculated sites.

Type of Site	Treatment	No. colonies/ acre - pretreat	% change in number of colonies at indicated weeks after inoculation				
			-8-	-18-	-25-	-34-	
Monogyne	Inoculated	52	-7.7	-30.8	-23.1	-46.1	
	Control	40	30.0	-20.0	40.0	20.0	
Polygyne	Inoculated	384	-39.3	-39.3	-7.1	*	
	Control	896	-37.5	-41.7	-33.3	*	

\* lost due to construction

Table 2. Change in population indices of *Thelohania solenopsae* inoculated sites.

Type of Site	Treatment	No. colonies/ acre - pretreat	% change in population indices at indicated weeks after inoculation				
			-8-	-18-	-25-	-34-	
Monogyne	Inoculated	880	-23.6	-25.0	-52.7	-38.6	
	Control	680	23.5	-14.7	52.9	11.8	
Polygyne	Inoculated	10,784	-44.5	-27.3	-34.4	*	
	Control	4,336	-29.9	-16.9	9.6	*	

\* lost due to construction

PROJECT NO: FA02G049

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

TYPE REPORT: Interim

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

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

### INTRODUCTION:

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

### MATERIALS AND METHODS:

In October, 1999 we assisted ARS with the initiation of a trial to evaluate field releases of the pathogen *Thelohania solenopsae*. Two sites, one polygyne in Hancock Co. and one monogyne in Harrison Co., were selected for the inoculation and four plots set up at each site. At the polygyne site, circular test plot evaluation areas were 1/16 an acre in size due to the large number of mounds in the area. Two plots were used as inoculation plots and two were maintained as non-inoculated control plots. On October 19, 1999 nine mounds in each of the inoculation plots were inoculated with 3.5g of brood infected with *T. solenopsae* (field collected by ARS prior to study). At the monogyne site, circular test plot evaluation areas were the standard ¼ acre size. Inoculations were also made on October 19 to nine mounds in each of two test plots. Every two months we monitor the inoculated plots and corresponding non-inoculated control plots by evaluating mounds with the mound index system, geo-referencing each mound within the plots, and collecting worker samples from each mound within the plots. We also assist by microscopically examining collected workers for pathogen spores.

### RESULTS:

#### *Colony mortality*

Polygyne Site/Monogyne Site: Due to the holidays, our first evaluation was done at 12 weeks after inoculation. At the 12 week evaluation, small decreases in number of colonies present in

both the monogyne and polygyne site were seen (Table 1). Since decreases also occurred in the control plots, these decreases probably cannot, at this time, be attributed to the pathogen. Decreases in population indices were more significant, particularly in the monogyne site. However, these decreases were mainly due to many colonies not having worker pupae present. This may be a seasonal trend (brood production slows down in the winter months).

*Presence of pathogen*

Pretreatment samples were examined and no spores were detected at either site. The 12 week evaluation samples have not yet been examined.

Table 1. Change in colony numbers of *Thelohania solenopsae* inoculated sites.

Type of Site	Treatment	Mean no. colonies/ acre - pretreat	% change in number of colonies at indicated weeks after inoculation				
			-12-	-18-	-25-	-34-	
Monogyne	Inoculated	66	-6.1				
	Control	34	+40.0				
Polygyne	Inoculated	304	-13.2				
	Control	336	-19.0				

Table 2. Change in population indices of *Thelohania solenopsae* inoculated sites.

Type of Site	Treatment	Mean no. colonies/ acre - pretreat	% change in population indices at indicated weeks after inoculation				
			-12-	-18-	-25-	-34-	
Monogyne	Inoculated	1,160	-53.1				
	Control	590	-11.5				
Polygyne	Inoculated	4,440	-29.9				
	Control	4,800	-20.8				

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PROJECT NO: FA02G039

PROJECT TITLE: Field and Laboratory Efficacy of *Beauveria bassiana* Alone or in Combination with Imidacloprid Against RIFA

TYPE REPORT: Final

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

COOPERATORS: Dr. Harlan Thorvilson<sup>3</sup> and Dr. Jim Cink<sup>4</sup>

### INTRODUCTION:

*Beauveria bassiana* (Hyphomycetes: Moniliales) is a general fungal pathogen of many insects, including imported fire ants. Broome (1974) and Broom et al. (1976) observed a mortality rate of 90% when *Solenopsis richteri* was exposed to *B. bassiana*. Stimac et al. (1990) noted that the pathogen could be passed from ant to ant. Sanchez-Pena (1992) found an effective method of infecting RIFA with subsequent mortality. Encapsulation of mycelia in alginate pellets improved the mortality rate of RIFA (White 1995). Alginate pellets coated with peanut oil greatly reduced RIFA population in field trials, both as broadcast and individual mound applications (Bextine and Thorvilson 1998).

Termites exposed to the insecticide imidacloprid cease grooming, have paralyzed mandibles, and become vulnerable to infections by soil fungi. RIFA exposed to imidacloprid become comotose or moribund, cease colony maintenance, and are non-aggressive for a temporary period but eventually recover (HLC, unpublished data). We hypothesized that pathogenicity of *B. bassiana* might be enhanced by imidacloprid induced behavior in RIFA.

### MATERIALS AND METHODS:

Alginate pellets were furnished by Dr. Harlan Thorvilson. The *B. bassiana* used to produce these pellets (ARSEF#2484, BB2484) was originally isolated from workers of the Mexican leaf-cutting ant, *Atta mexicana* collected in Sinaloa, Mexico in 1986 (Sanchez-Pena 1992). The BB2484 was re-isolated several times from infected RIFA and grown and stored on Sabrouad's dextrose agar medium with 1% yeast extract in the fire ant laboratory at Texas Tech University (Bextine and Thorvilson 1998).

#### *Laboratory trials*

Field colony trial: Field colonies of RIFA were collected in two gallon pails on July 28, 1999 and allowed to acclimate until August 2, 1999, at which time the trial was initiated. There were 3 replicates per treatment. One treatment consisted of 1 ml imidacloprid in 500 ml water

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<sup>3</sup> Texas Tech University

<sup>4</sup> Bayer Corporation

and applied to the nest surface as a liquid drench. Another treatment was 1 ml imidacloprid + 0.5 g alginate pellets in 500 ml water. Alginate pellets (0.5 g in 500 cc water) were also applied, and there was an untreated control. Colonies were visually observed daily for evidence of infection (size of "bone piles") for four weeks. Colonies were watered as needed and fed crickets weekly.

Laboratory colony trial: Laboratory colonies maintained in artificial nests on a diet of crickets and sugar water were also challenged with alginate pellets coated with 20% peanut oil. On June 16, 1999, 0.5 g of *Beauveria* bait were fed to three lab colonies. Three control colonies were also included in the trial. Again colonies were visually observed daily for evidence of infection. The three treated colonies were re-inoculated on August 20, 1999 with 1.0 gram of *Beauveria* bait per colony.

#### *Field trial*

A preliminary field trial comparing efficacy of peanut oil coated pellets, imidacloprid applied as a liquid drench, and a mixture of imidacloprid plus alginate pellets, and an untreated control was initiated on August 25, 1999. Alginate pellets containing 20% peanut oil (w/w) were applied at a rate of 1.0 g formulated product per RIFA nest. The bait was scattered over about 2 sq ft around each nest in a one acre test plot. One ml imidacloprid (Premise® 2) in one gallon of water was used to treat individual RIFA nests. One g of alginate pellets along with one ml imidacloprid in one gallon of water was applied as an additional treatment, and there was an untreated control. Test plots were 210' x 210' (ca. one acre), with a ¼ acre circular subplot located in the center of each test plot. Each test plot was closely searched by a team of six highly experienced IFA researchers, and all active colonies were flagged. Treatments were then applied to all flagged RIFA colonies. Population estimates were made prior to and at 2 and 4 weeks after treatment by the population indexing system described by Harlan et al. (1981) and modified by Lofgren and Williams (1982).

## RESULTS AND DISCUSSION:

#### *Laboratory trials*

Field colony trial: All imidacloprid treated colonies were comotose within 3 hrs of treatment. At 48 hrs posttreatment imidacloprid treated colonies were beginning to return to normal activity. No evidence of *Beauveria* infection was noted at any time. All colonies remained active and normal and bone piles in the treated colonies were not different from the untreated controls. The trial was terminated after 4 weeks.

Laboratory colony trial: No evidence of infection of any colony was noted. The treated colonies were re-inoculated approximately 60 days after the first inoculation but no infection was noted.

#### *Field trial*

Test plots were evaluated at 2 and 4 weeks posttreatment after which they were ploughed up, prematurely terminating the trial. The *Beauveria* baits provided a 68% reduction in the pretreatment index 4 weeks after application (Tables 1 and 2).



Table 1. Efficacy of *Beauveria bassiana* as an individual mound treatment for control of imported fire ants - decrease in colony numbers.

Treatment	No. colonies/plot pretreat	% decrease in no. colonies at indicated wks. posttreatment			
		-2-	-4-	-8-	-12-
Imidacloprid	7.0	71.4	-28.6		
<i>Beauveria</i>	10.0	-30.0	-40.0		
Imid + <i>Beauveria</i>	12.0	-8.3	-25.0		
Check	8.0	-12.5	0.0		

Table 2. Efficacy of *Beauveria bassiana* as an individual mound treatment for control of imported fire ants - change in population indices.

Treatment	Population index/plot - pretreat	% change in population index at indicated wks. posttreatment			
		-2-	-4-	-8-	-12-
Imidacloprid	135.0	33.3	-53.3		
<i>Beauveria</i>	210.0	-35.7	-68.0		
Imid + <i>Beauveria</i>	225.0	-15.6	-52.4		
Check	160.0	-18.8	-25.0		

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PROJECT NO: FA05G038

PROJECT TITLE: Effects of Fipronil Formulations on Non-target Species of Ants

TYPE REPORT: Final

LEADER/PARTICIPANTS: T.C. Lockley & H. L. Collins

### INTRODUCTION:

Both bait and granular formulations of fipronil are proposed for use against imported fire ants. To ascertain effects on non-target ant species and to quantify any deleterious results, the following project was undertaken.

### MATERIALS AND METHODS:

Sixteen acres of grass dominated field (Bobby Chain Airport, Hattiesburg, MS) were separated into 6 roughly equal parts. The site was surveyed and delineated in April 1998. Six pitfall traps consisting of a permanent 1 inch PVC sleeve and a 75 ml glass test tube were placed in a T pattern from the middle of the plot to the closest peripheral margin. Traps were set on approximately 6 week intervals and retrieved 72 hours after placement. Bait traps were also set adjacent to each pitfall trap and left in place for 1 hour. All traps were returned to the Gulfport Plant Protection Station where ants were separated, identified and counted under magnification. Trapping began immediately prior to fipronil application.

Application of fipronil in bait and granular formulations was made on 11 May, 1998. Air temperature was 74°F and soil temperature was 80°F (one inch depth) at the time of application. The bait formulation was applied using a Herd Seed Spreader (model GT-77) mounted on a farm tractor operated at 4 mph with a 21 foot swath. Bait was applied at a rate of 15.0 lbs per acre. The granular formulation was applied at 15.0 lbs per acre using a shop built granular applicator (double throat) mounted on a farm tractor operated at 4 mph using a 21 foot swath.

### RESULTS:

Twenty-eight species of ants were collected within all sites (Table 1). Within these, six nontarget species or species complexes were commonly collected in the untreated sites by pitfall trapping. In the bait treated plots, only three species/complexes were collected with any regularity; and in the granular treated plots, one nontarget species and one species complex were found through most of the trapping periods (Table 2). The red imported fire ant was continuously taken at all sites and during all sampling periods.

Greater species diversity occurred in the untreated sites. Ants capable of directly competing with imported fire ants (RIFA) such as *Forelius* spp. and species that aggressively defend their nests such as *Dorymyrmex* spp. and *Pheidole* spp. were more commonly found within these plots. All

three of these species complexes are omnivorous and, with the possible exception of *Pheidole* spp. because of their relatively small size, would have been directly impacted by bait applications. *Forelius* spp. generally have large colony sizes and can recruit to food sources in numbers readily comparable to those produced by RIFA (TLC pers. obs.). *Dorymyrmex* spp. have only modest sized colonies even when polydomous and can only recruit to food sources in relatively small numbers. The impact of bait would have had a disproportional impact on this complex because of the small size of the colonies. *Brachymyrmex depilis* is primarily a sugar feeder and would not have been affected by bait application. Their small size (generally 1.0 mm) would have precluded their collecting any bait granules and returning them to the colony even if they had been so disposed. Why they were found in so few numbers within the bait plots cannot be ascertained. *Monomorium minimum* is another omnivorous feeder with a relatively large colony size. However, like *B. depilis*, their small size would have made collection of the bait granules difficult. Their numbers in the bait treated plots were higher and more consistent than in the untreated plot. *Cyphomyrmex rimosus* is a fungus feeder. It collects organic material (eg. dead insects, leaves) to use as a substrate on which it grows its fungi. It would not have been affected by bait treatment nor would it have been a competitor of any of the other species collected in any of the plots. It is so noncompetitive that colonies of this species have been excavated directly from RIFA colonies (pers. obs.).

The granular treatment plots showed the least number of dominant species. However, *Pheidole* spp. complex was the most consistent and numerous in these plots; as were the numbers for *B. depilis*.

Insufficient diversity was observed among bait trap sites and dates to draw any conclusions on the impact of fipronil on nontarget species.

Table 1. Species of Ants Collected By Bait Sampling and Pitfall Trapping At the Bobby Chain Airport, Hattiesburg, MS.

PONERINAE

1. *Hypoponera opacipes*
2. *Proceratium croceum*

MYRMECINAE

3. *Aphaenogaster carolinse*
4. *Crematogaster ashmeadi*
5. *C. clara*
6. *C. missouriensis*
7. *Cyphomyrmex rimosus*
8. *Leptothorax pergandei*
9. *Monomorium minimum*
10. *Myrmecina americana*
11. *Pheidole dentata*
12. *P. dentigula*
13. *P. moerens*
14. *P. tysoni*
15. *Pseudomyrma pallida*
16. *Solenopsis invicta*
17. *S. molesta*

DOLICHODERINAE

18. *Dorymyrmex bureni*
19. *D. medeis*
20. *Forelius mccooki*
21. *F. pruinosus*

FORMICINAE

22. *Brachymyrmex depilis*
23. *Camponotus pennsylvanicus*
24. *Paratrechina faisonensis*
25. *P. longicarnis*
26. *P. parvula*
27. *P. phantasma*
28. *P. terricola*

Table 2. Numerically Dominant Species of Ants Collected By Pitfall Trapping By Date and Treatment.

TAXON	UNTREATED SITES									
	V	VI	VII	IX	X	XI	XII	I	II	III
<i>Solenopsis invicta</i>	8	72	70	19	22	4	9	18	5	24
<i>Brachymyrmex depilis</i>		2	1		2			3		6
<i>Cyphomyrmex rimosus</i>	1	3			1	1				8
<i>Forelius</i> spp.	3	2	21	2		2				5
<i>Monomorium minimum</i>		4	6	1	1					1
<i>Paratrechina</i> spp.	2	4	8	5	1					11
<i>Pheidole</i> spp.	5	4	4	4					2	3
	<b>BAIT TREATMENT</b>									
<i>Solenopsis invicta</i>	24	4	40	65	27	18	6		31	36
<i>Cyphomyrmex rimosus</i>		1		4	1	1			2	2
<i>Monomorium minimum</i>	8	6	3	11	2	2			8	8
<i>Pheidole</i> spp.	1		1	4		1	1		1	1
	<b>GRANULAR TREATMENT</b>									
<i>Solenopsis invicta</i>	11	6	1	7	6	4	1	6	3	25
<i>Brachymyrmex depilis</i>		1		2	5	1				12
<i>Pheidole</i> spp.		2	6	6	15	4	1	1	1	2

PROJECT NO: FA05G028

PROJECT TITLE: Impact of Fipronil on Non-target Ant Species

TYPE REPORT: Final

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

COOPERATORS: Mississippi Dept. of Wildlife, Fisheries, and Parks (Deborah Epperson)  
Camp Shelby Environmental (Cindy King)  
Rhone-Poulenc Ag Company (Ken Lewis)  
USDA, ARS (Dan Wojcik)

### INTRODUCTION:

Fipronil is a relatively new insecticide under development by Rhone-Poulenc, Research Triangle Park, NC. Fipronil has been shown to be effective for control of imported fire ants when applied as a 15 ppm bait (Collins & Callcott 1998). Non-bait granular formulations are also highly effective for control of imported fire ants (1997 Annual Report, Gulfport Plant Protection Station). Impact of fipronil on native ant species such as *Solenopsis molesta*, *Paratrechina sp.*, *Brachymyrmex sp.*, and others has not been determined, although a pilot study is currently underway at the Hattiesburg, MS airport by T. C. Lockley and H. L. Collins (FA05G038). Native ant species compete with, and to some degree assist with control of imported fire ants by preying upon newly mated fire ant queens. Therefore information on the impact of fipronil on native ant populations could be very useful.

### MATERIALS AND METHODS:

Test plots were located in an ecologically diverse habitat in order to maximize the number of native ant species present. Non-diverse habitats such as grazed pastures, athletic fields, airports, etc. do not support large numbers of native ant species. The test site chosen for this study was a power line right of way at Camp Shelby National Guard Base (Mississippi). Test plots were one acre in size, with three replicates for each treatment. Treatments were fipronil 0.00015% bait applied at 10 lbs bait per acre, fipronil 0.05% granular applied at 25 lbs formulated product per acre, and an untreated control. Insecticide treatments were applied with a shop-build granular applicator mounted on a farm tractor. Ants were collected prior to application of fipronil using bait and pitfall transects placed diagonally across each test plot. Ten bait stations spaced ca. 45 feet apart were used in each transect. Stations were baited with the multiple ant bait (MAB) developed by Williams, Oi, and Vail (ARS, patent pending). Pitfall traps, consisting of 4" diameter plastic cups (16 oz) containing ethyl alcohol, were established adjacent to each bait station. Pitfalls and bait stations were employed prior to application and at 5, 13, 20, 28, 36, 44, and 53 weeks after application. Pitfalls were left in place for 48 hour intervals, and bait stations were retrieved after being in place for approximately one hour. Tim Lockley from this laboratory

provided taxonomic identification and enumeration of all ants collected. Verification of taxonomic accuracy was provided by Dr. Dan Wojcik, ARS, Gainesville, FL. Voucher specimens of all species were retained at the Gulfport Plant Protection Station.

## RESULTS:

Statistical analyses have not been performed on any data at this time. Therefore all observations are preliminary and based on numerical trends.

*Granular Treatment:* The granular treatment reduced the number of IFA collected in bait and pitfall traps by 70% 5 weeks after treatment and 92% after 13 weeks (Fig. 1). Numbers of IFA fluctuated somewhat through 53 weeks after treatment, however numbers were always dramatically lower than on the check plots.

Although IFA made up approximately 83% of the ants collected before treatment, collections included *Dorymyrmex bureni* (10%) and *Brachymyrmex depilis* (4%) (Figs. 2 and 3). IFA remained greater than 70% of the ants collected in the granular treated plots through 28 weeks after treatment. In July 1999, 36 weeks after treatment, less than 6 IFA were collected per acre (in both trap types), constituting less than 14% of the total ants collected. At that time, we collected very few total ants (<50/acre), but the *Dorymyrmex* sp. (92% *D. medei*, 8% *D. bureni*), *B. depilis* and *Forelius pruinosus*, all outnumbered the IFA. While IFA numbers increased at the 44 and 53 week collections, *B. depilis* maintained fairly large numbers, constituting 23 and 34% of the ants collected, respectively. *Dorymyrmex* sp. were 38 and 30% of the ants collected at 36 and 44 weeks after treatment, respectively, but dropped to pretreatment levels at 53 weeks. *Cyphomyrmex rimosus* was not present on these plots prior to treatment and were only collected at two collection periods in very small numbers during the study.

*Bait Treatment:* The bait treatment reduced the number of IFA collected in bait and pitfall traps by 96% 5 weeks after treatment and maintained greater than 92% reduction in pretreatment numbers through the 36 week collection (Fig. 1). There were small fluctuations in numbers of IFA collected, but numbers were dramatically lower than on the check plots.

Although IFA made up approximately 88% of the ants collected prior to treatment, we also collected *D. bureni* (9%) and *B. depilis* (2%) (Figs. 4 and 5). At 5 to 20 weeks after treatment, collections consisted of less than 22 ants per acre, but IFA remained greater than 60% of those ants collected. At 28 weeks after treatment, the number of ants collected per acre increased to greater than 100. At this time, IFA constituted 26% of the collection while *Dorymyrmex* sp., *B. depilis*, and *F. pruinosus* were 30, 19 and 22% of the collection, respectively. IFA dropped to less than 15% of the collection at 36 weeks after treatment, but increased over the next two collections to 65% (and more than 100 ants/acre) by 53 weeks after treatment. *D. medei* constituted 59% of the ants collected at 36 weeks after treatment, and *D. bureni* were 39% of the ants collected at 44 weeks. By 53 weeks after treatment, *D. bureni* and *D. medei* were 13 and 4% of the collection, respectively, approaching pretreatment levels. While *B. depilis* was approximately 20% of the collections at 5 and 28 weeks after treatment, the actual number of



ants collected per acre in all collections was 20 or fewer ants per acre. *C. rimosus* were collected at all collections times, but always in very small numbers (<10 ants/acre).

The large percentage of other ants noted in Figure 5 at 13 weeks after treatment indicate a few *Prenolepis imparis* and *Paratrechina phantasma*. At 36 weeks, the others noted in Figure 5 are mostly *Monomorium minimum*, all collected in one bait trap at the edge of one plot.

*Untreated Checks:* IFA were 86% of the ants collected prior to treatment of the other plots (Figs. 6 and 7). *D. bureni* and *F. pruinosis* were 5.5 and 5% of the original collection, respectively. In these plots, *B. depilis* was only 1% of the pretreatment collection. IFA remained at 86 to 97% of the ants collected in the check plots over the course of the study, except in July 1999 at 36 weeks after treatment. At this time, IFA were 69% of the ants collected while *D. bureni* and *F. pruinosis* were 11 and 9%, respectively. *B. depilis* was collected at all collection periods, but were never found in large numbers (generally <5 ants/acre) and always constituted <5% of the total collection. *C. rimosus* were collected in all collections, except 13 weeks after treatment, in very small numbers (<10 ants/acre) similar to the bait treated plots.

*Number of species collected:* In the fall of 1998, prior to treatments, bait and pitfall collections combined consisted of an average of 5.3, 4.7, and 5.0 species of ants per acre (including *S. invicta*) in check, granular and bait plots, respectively (Fig. 8). In January 1999, 13 weeks after treatments, all plots, including the checks showed reduced numbers of species present, probably due to the winter weather. By 28 to 36 weeks after treatments, when IFA were at their lowest percentages in the treated plots, numbers of species in the treated plots were not numerically much different from the untreated check.

## DISCUSSION:

Mortality of IFA in the fipronil granular treated plots was not as complete and consistent as we have seen in numerous other granular treated sites. This may have been due to the terrain of this particular site. This site was a typical power line right of way with tall grass and weeds and rough terrain. The granular treatment may not have been applied in a true broadcast application due to deflection by some tall vegetation. Unlike the bait treatment which the ants retrieve and take to the nest, the granular application depends on accurate and consistent application. However, despite the less than expected performance of the granular treatment, both granular and bait treatments provided dramatically less numbers of IFA present on their respective plots compared to the check plot. It is hard to determine the true effect of the treatments on other ant species since the check plot had very few other ant species present during the winter months (5-20 weeks after treatment - November through March). However, it does appear that while *D. medei*, *D. bureni* and *B. depilis* are able to co-exist with IFA in small numbers, they, in particular, are able to produce larger numbers of ants when IFA populations are reduced. At no time did the dramatic decrease in numbers of IFA present in the treated plots translate into large increases in numbers of other ant species present.

Figure 1. Mean number of *Solenopsis invicta* collected in all traps - initiated 10/28/98.

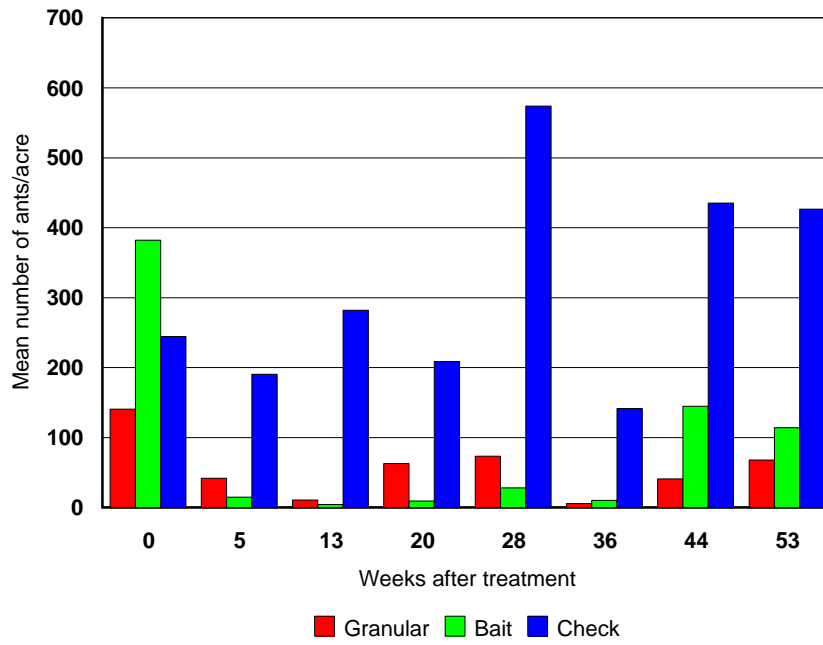


Figure 2. Numbers of ants collected in all trap types in the granular treated plot.

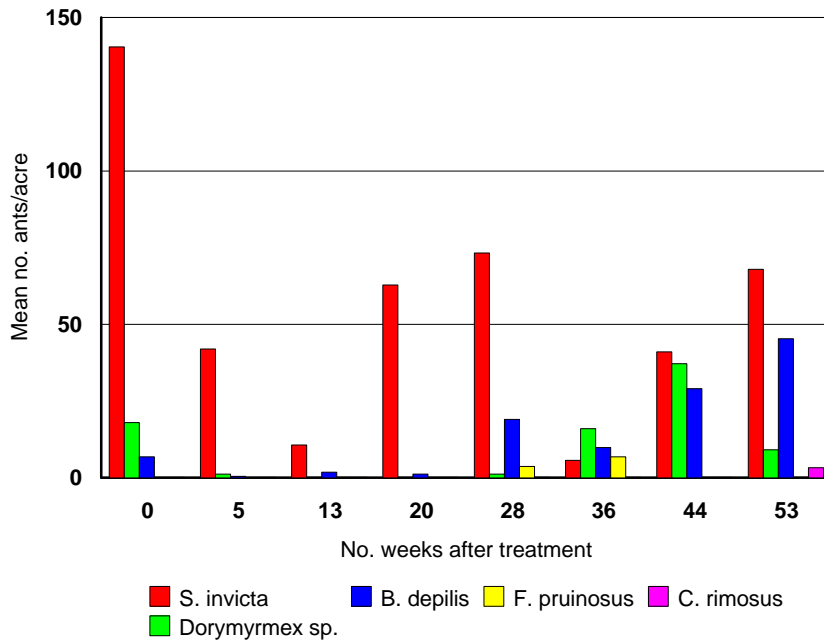


Figure 3. Percentage of ants collected in all trap types in the granular treated plots.

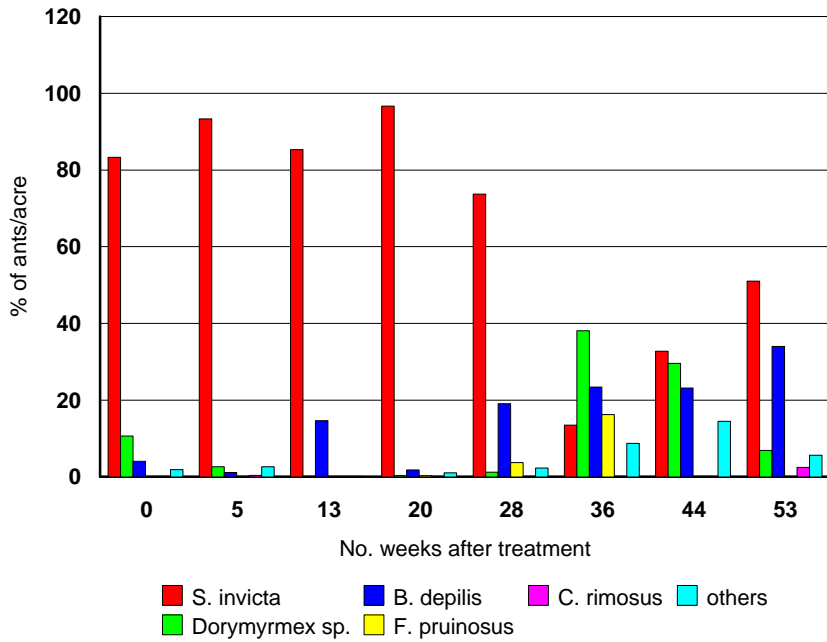


Figure 4. Numbers of ants collected in all trap types in the bait treated plot.

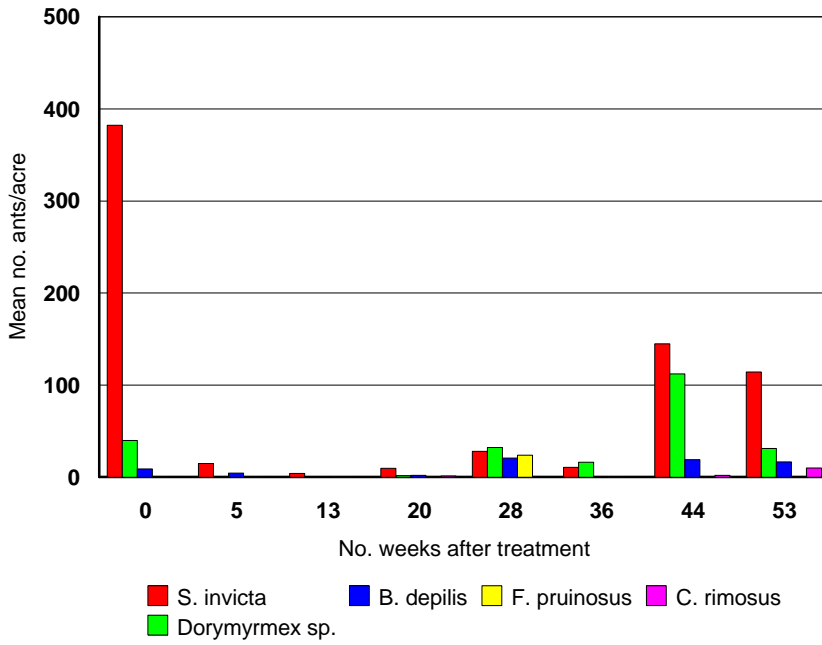


Figure 5. Percentage of ants collected in all trap types in the bait treated plots.

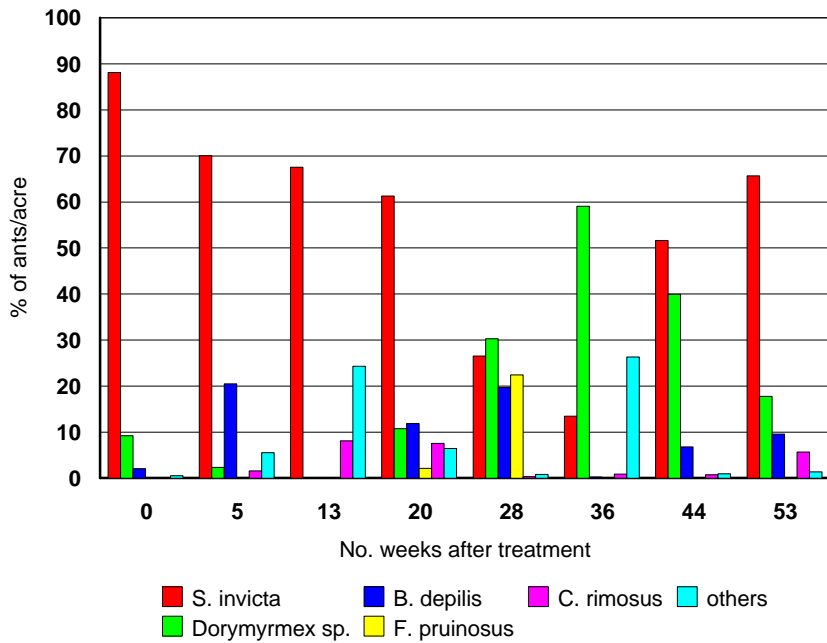


Figure 6. Numbers of ants collected in all trap types in the check plot.

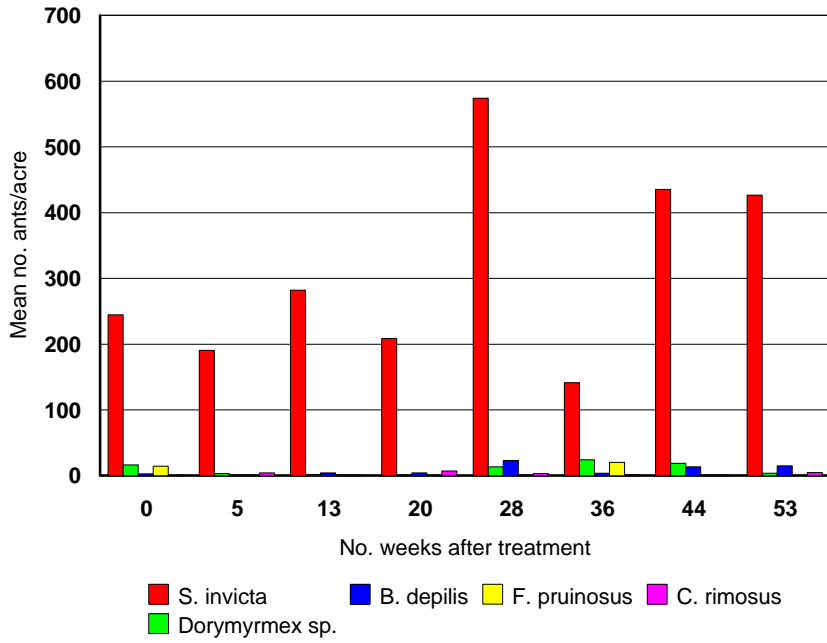


Figure 7. Percentage of ants collected in all trap types in the check plots.

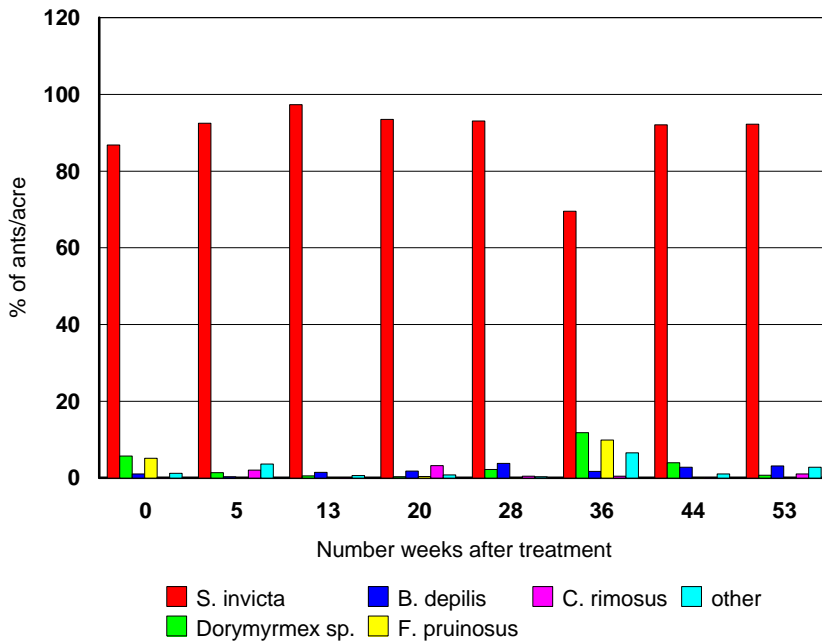
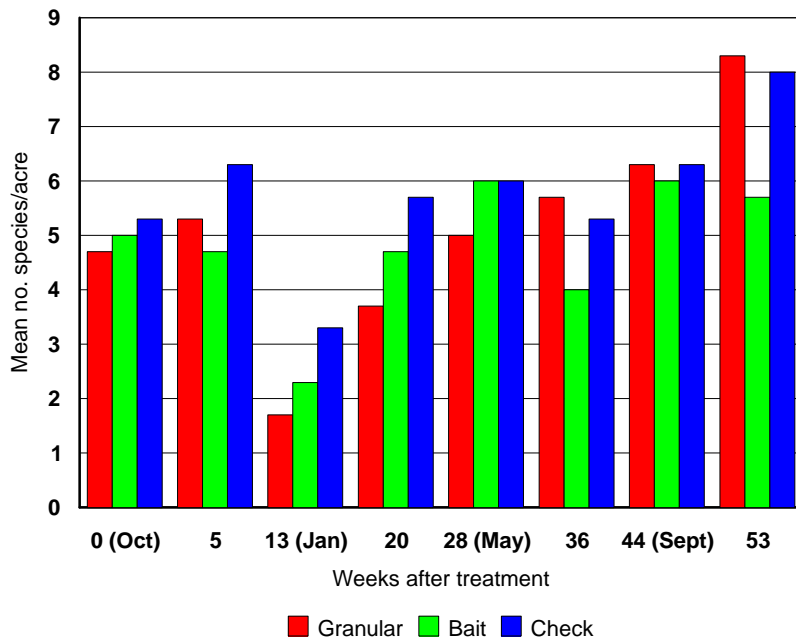


Figure 8. Mean number of genera/species collected in both trap types (includes *S. invicta*).



PROJECT NO: FA04G019

PROJECT TITLE: The Terrestrial Arthropods of Horn Island, MS: A Comparison with a Survey Conducted Between 1943 and 1961

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Tim Lockley

### INTRODUCTION:

In 1962, and in a supplement published in 1967, E. A. Richmond described the flora and fauna of Horn Island, Mississippi. The data presented in these publications was a composite of over 20 years of research. Within these publications, Richmond listed sixteen orders, 160 families, 478 genera and 712 species of insects as well as three orders, 15 families, 24 genera and 27 species of arachnids. Among the insects was the first imported fire ant (IFA) collected in the state of Mississippi (1943). An initial survey of the island revealed a light population of IFA. To our knowledge, Horn Island has never been treated for fire ant control. Questions this study will ask include: why are populations of IFA low when compared with adjoining islands and the mainland and what are the dominant competitive species of ants on the island?

It has been over fifty years since the first arthropod collections were carried out on Horn Island and over 35 years since these data were published. Collections from the Horn Island arthropod population were undertaken in 1999 to compare populations of selected groups to see if any major shifts in species abundance/richness has occurred over those years.

### MATERIALS AND METHODS:

Collections were begun in April 1999. Samples were taken using an ultraviolet light trap, pitfall traps, bait traps and hand sampling. UV lights and pitfall traps were set for ca. 24 hours; at which point they were removed from the island. Twelve pitfall traps were set from the dunes adjacent to the pier area on the north side of the island and running the width of the island along a path to the southern end of the island. Pitfall traps consisted of 10 oz. capacity cups placed in the ground with 70% ethanol used as the killing/ preserving agent. Bait traps (for collection of ant species) were set within two meters of the pitfall traps and allowed to remain in place for ca. 1.0 hour. Traps were located with the multiple ant bait (MAB) developed by Williams, Oi, and Vail (ARS, patent pending). Bait traps were collected and returned to the lab the first day of each sampling period.

### RESULTS:

From collections made from April through October 1999, three previously undescribed species of Scarabaeidae were found as well as a new species of ant in the genus *Brachymyrmex* (Table 1). Conversations with other taxonomists working on other species groups suggest that more new

species will be forthcoming. With these new species, new records for both Horn Island and the state of Mississippi were collected.

Among the scarabs, three new island and one new state record were recorded. Among the carabids, 11 names were added to the list of the island's fauna. With the cicindellid, this brings the total of new records for beetles to 19.

Among the ants, seven species were found not previously recorded from Horn Island. One of these, *Pogonomyrmex badius*, may only occur in Mississippi on Horn Island. Never numerous, this ant has been heavily impacted directly by IFA and man's attempts to control fire ants in the past. Some remnant populations occur in the central Florida area and in isolated pockets in the panhandle (Wojcik, pers. comm.).

Along with the insects, a number of spiders have also been added to the record for Horn Island. Although not specifically designed for the capture of spiders (except ground dwelling species), 18 new names were added to the island list with one as a new record for the state.

Collections will begin again in the spring of 2000 and proceed through out the summer and early fall. It is anticipated that collecting on the island will continue over the next two years.

#### REFERENCES CITED:

Richmond, E.A. 1962. The Fauna and Flora of Horn Island, Mississippi. Gulf Research Reports 1(1): 59-106.

Richmond, E.A. 1967. A Supplement to the Fauna and Flora of Horn Island, Mississippi. Gulf Research Reports 7(1): 213-254.



Table 1. HORN ISLAND ARTHROPODS

## INSECTA

### Coleoptera

#### Scarabaeidae

\**Anomala undulata* Melsch.

#### *Aphodius* n. sp. A

#### *Ataenius* n. sp. A

#### *Ataenius* n. sp. B

*A. alternatus* (Melsch.)

*A. wenzeli* Horn

\**Cyclocephala lurida* Bland

*Diplotaxis bidentata* Lec.

*Dyscinetus morator* (F.)

#### \*\**Gronocarus autumnalis* Schaeffer

\**Lygyrus gibbosus* (DeGeer)

*Phyllophaga latifrons* (Lec.)

*P. prununculina* (Burm.)

#### Carabidae

\**Agonum punctiforme* (Say)

\**Bembidion viridicolle* (LeFerte-Senectere)

\**Dyschiriodes abbreviatus* (Putzeys)

\**Notiobia purpurescens* (Bates)

\**N. terminata* (Say)

\**Oodes amaroides* DeJean

\**Platynus cincticollis* (Say)

\**Pterostichus ophryoderus* (Chaudoir)

\**Scarites subterraneus* Fab.

*Selenophorus* sp.

\**Stenolophus ochropezus* (Say)

\**Semiardistomis puncticollis* (DeJean)

#### Cicindellidae

\**Cicindela trifasciata ascendens* LeConte

#### Staphylinidae

### Hymenoptera

#### Formicidae

\**Aphaenogaster treatae* Forel

#### \*\**Brachymyrmex* n. sp.

\**B. depilis* Emery

*Camponotus abdominalis floridanus* (Buckley)

*Crematogaster clara* Emery

*Dorymyrmex bureni* Trager

\**D. medeis* Trager

*Forelius* sp.

Table 1. cont'd

## Hymenoptera

### Formicidae

- \**F. maccooki* Forel
- F. pruinosus* (Roger)
- \**Paratrechina phantasma* Trager
- Pheidole* sp.
- \**P. moerens* Emery
- Pogonomyrmex badius* Latr.
- Solenopsis invicta* Buren
- \**S. molesta* Say

## ARACHNIDA

### ARANEAE

#### Araneidae

- \**Argiope aurantia* Lucas
- \**Nephila clavipes* (L.)

#### Oxyopidae

- \**Oxyopes aglossus* Hentz
- \* *O. salticus* Hentz

#### Gnaphosidae

- \**Drassylus dixinus* Chamb.
- \**Zelotes pullus* Bryant

#### Agelenidae

- \**Agelenopsis barrowsi* (Gertsch)

#### Lycosidae

- \*\**Arctosa sanctaerosae* Gertsch
- \**A. littoralis* (Hentz)
- \**Gladicosa gulosa* (Walck.)
- \**G. pulchra* (Keys.)
- \**G. huberti* (Chamb.)
- \**Lycosa lenta* Hentz
- \**L. rabida* Walck.
- \**L. timuqua* Wallace
- \**Pardosa delicatula* Gertsch & Wallace
- \**Schizocosa crassipes* (Walck.)
- \**Sossipus mimus* Chamb.

\* New record Horn Island; \*\* New record Mississippi

# APPENDIX I - LABORATORY BIOASSAY PROCEDURE

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

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

Collection of test insects: Field collected alate imported fire ant queens are used as the test insect. IFA colonies are opened with a spade and given a cursory examination for the presence of this life stage. Alate queens are seldom, if ever, present in all IFA colonies in a given area. Some colonies will contain only males, others may have few or no reproductive forms present, others may contain both males and queens, while some will contain only alate queens. Seasonal differences in the abundance of queens is quite evident; in the warmer months of the year 50% or more of the colonies in a given area may contain queens. However, in the cooler months, it is not uncommon to find that less than 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

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  
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
- 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)

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.