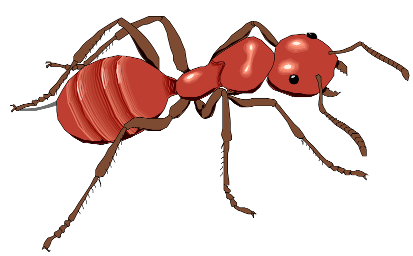
# 2006 ACCOMPLISHMENT REPORT

# SOIL INHABITING PESTS SECTION

U.S. DEPARTMENT OF AGRICULTURE

ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE
CENTER FOR PLANT HEALTH SCIENCE AND TECHNOLOGY
ANALYTICAL AND NATURAL PRODUCTS CHEMISTRY LABORATORY



3505 25TH Ave.
Gulfport, MS 39501

# 2006 ACCOMPLISHMENT REPORT

**SOIL INHABITING PESTS SECTION**

ANALYTICAL AND NATURAL PRODUCTS CHEMISTRY LABORATORY

CENTER FOR PLANT HEALTH SCIENCE AND TECHNOLOGY

PLANT PROTECTION AND QUARANTINE

ANIMAL AND PLANT HEALTH INSPECTION STATION

U.S. DEPARTMENT OF AGRICULTURE

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

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


Compiled and Edited by:

Anne-Marie A. Callcott



February 2007

Available online at the CPHST-SIPS website:
http://cphst.aphis.usda.gov/sections/SIPS/

# 2006 IMPORTED FIRE ANT OBJECTIVES

SOIL INHABITING PESTS SECTION
GULFPORT, MS

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

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

OBJECTIVE 2: Development and refinement of quarantine treatments for certification of non-traditional or non-specified articles.

- Emphasis development of treatments for baled hay and straw and bee equipment.
- Evaluate candidate toxicants, formulation, and dose rates for various use patterns.
- Assist in registration of all treatments shown to be effective.

OBJECTIVE 3: Advancement of technology for population suppression and control.

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

OBJECTIVE 4: Development of survey and detection tools and technologies.

- Evaluate efficacy of survey traps
- Evaluate attractants for use in traps determining differences in seasonal preference and efficacy across species/hybrids
- Standardize trapping and survey techniques for regulatory use

OBJECTIVE 5: Technology transfer of all methods developed by laboratory.

- Provide training in quarantine treatments to stakeholders as requested
- Transfer all methods and technologies developed in lab to stakeholders through training, user's guides, web pages, etc.

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## QUARANTINE TREATMENTS FOR CONTAINERIZED NURSERY STOCK



## QUARANTINE TREATMENTS FOR FIELD GROWN NURSERY STOCK

## TREATMENT OPTIONS FOR BALED HAY/PINE STRAW



## POPULATION SUPPRESSION/INTEGRATED PEST MANAGEMENT



## BIOLOGICAL CONTROL AND BIODIVERSITY

## MISCELLANEOUS

CPHST PIC NO: A9F01/A9M01

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

TYPE REPORT: Final

LEADER/PARTICIPANTS: Lee McAnally and Shannon James

## INTRODUCTION:

The Federal Imported Fire Ant Quarantine Program (7CFR §301.81) states that all regulated products (nursery stock) leaving the quarantined area must be treated in a prescribed manner. Currently, treatments for containerized nursery stock include the use of granular insecticides incorporated into potting media or liquid drenches applied prior to shipping. Nursery stock treated with incorporated insecticides may be certified for 6 months to 2 years, depending on the rate incorporated into the media (10-25 ppm based on bulk density of media). This allows the grower to use less insecticide on nursery stock that will be held on site for a short period of time, and more on those that need a longer growing period prior to selling. Drench treatments 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.

Original efficacy trials evaluating bifenthrin for inclusion in the IFA quarantine as both an incorporation and a drench container treatment utilized FMC formulations of bifenthrin. TopPro Specialties, in conjunction with Micro Flo Company began the manufacture of bifenthrin in both granular (0.2%) and liquid flowable (7.9%) formulations around 2002. The granular formulation was produced on two different carriers, sand and DG lite. In August 2002 a study was initiated to determine the efficacy of TopPro bifenthrin. Each formulation was set up in treatment rates equivalent to those specified in the quarantine treatment manual for durations corresponding to the certification periods for each treatment rate.

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

## MATERIALS AND METHODS:

### Granular Incorporation Treatment:

On July 31 and August 1, 2002 both formulations (carriers) of TopPro granular bifenthrin were blended into the MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 850 lb/cu yd) at rates of 10, 12, 15, and 25 ppm. A portable cement mixer (2 cu ft capacity) was use to blend the toxicant into the potting media, and was operated for 15 minutes per batch to insure thorough blending. Treated media was then poured into one-gallon capacity plastic nursery pots and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week. At monthly intervals, sub samples were taken from 2 pots of each treatment and composited and subjected to standard alate queen bioassay (Appendix I).

On December 1 and 2, 2003 further testing of the sand carrier granular formulation was initiated in media obtained from Windmill Nursery, Folsom, LA (bulk density 310 pounds per cubic yard) and Flowerwood Nursery, Mobile, AL (bulk density 500 pounds per cubic yard). Methods and materials for mixing, aging, and testing were the same as described above.

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

On December 2, 2003 Windmill and Flowerwood media were drench treated in the manner described above.

RESULTS:

MAFES Media:
The drench and the 10 ppm incorporation rates provided 100% mortality in 3 days or less through 6 months (Table 1). This was the planned duration for these treatment rates based on the IFA quarantine certification period, and thus the drench and 10 ppm incorporation rates were terminated after the 6 month evaluation. The incorporated 12 ppm rates with both carrier types were effective through 12 months per the quarantine certification period and were terminated at 12 months. The 15 ppm rates were also 100% effective through the 24 month certification period and were terminated at 24 months. The 25 ppm rate, used in conjunction with the Fire Ant Free Nursery Program, for continuous certification has remained 100% effective through 35 months. The final 36 month result (data sheet) was lost due to hurricane Katrina.

Flowerwood and Windmill Media:
The Flowerwood drench provided 100% efficacy through 6 months, but the Windmill drench maintained 100% mortality through 4 months and dropped to 95% and 85% in months 5 & 6 respectively (Table 2).

Through 6 months post-treatment the 10 ppm incorporation rate in both media types maintained 100% efficacy and were terminated at that time (Table 2). The 12 ppm rate was also 100% effective in both media types for 12 months per the quarantine certification period. Bioassays were not set up in months 20 through 23 due to cleanup activities associated with hurricane Katrina. The 24 month bioassay indicated an unexplained drop in efficacy in both the 15 ppm and 25 ppm rates of incorporation, especially in the Flowerwood media. The 15 ppm rates were terminated after 24 months (scheduled). The 25 ppm rates continued to provide 100% efficacy through 31 months. The test was terminated at this time because numerous check and treated pots were intermingled while being moved.

DISCUSSION:

The TopPro Specialties/BASF flowable and granular bifenthrin formulations in the MAFES media are as effective as the FMC formulation indicating acceptability as a product to be used in the IFA quarantine. Unfortunately, hurricane Katrina interrupted this trial at a crucial point for the testing in the Flowerwood and Windmill nursery medias. Since the 25 ppm rate in the Flowerwood media at 24 months performed so poorly, but then returned to 100% efficacy for the remaining 7 months, we can speculate that in our cleanup of the can yard after the hurricane we inadvertently mixed up pots. Knowing that in past trials, slight occasional decreases in efficacy in the Windmill media has been noted, we are not overly concerned with the slight decrease in efficacy of the drench at 6 months or of the 15 ppm incorporation treatment at 24 months.

Table 1. Residual activity of TopPro bifenthrin in MAFES media.

| Formulation Tested | Rate of Application (ppm) | Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| DG lite Carrier | 10 | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) | *** | *** | *** | *** | *** | *** |
| | 12 | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) |
| | 15 | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) |
| | 25 | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) |
| Sand Carrier | 10 | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) | *** | *** | *** | *** | *** | *** |
| | 12 | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) |
| | 15 | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) |
| | 25 | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) |
| Drench | 25 | 100(1) | 100(3) | 100(1) | 100(3) | 100(3) | 100(1) | *** | *** | *** | *** | *** | *** |
| | Check | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 10 | 5 |

| Formulation Tested | Rate of Application (ppm) | Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| DG lite Carrier | 10 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 12 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 15 | 100(3) | 100(3) | 100(1) | 100(3) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(2) | 100(1) |
| | 25 | 100(3) | 100(3) | 100(1) | 100(3) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) |
| Sand Carrier | 10 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 12 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 15 | 100(3) | 100(3) | 100(1) | 100(3) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) |
| | 25 | 100(3) | 100(3) | 100(1) | 100(3) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) |
| Drench | 25 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | Check | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |

*** terminated based on IFA quarantine certification period

Table 1. cont.

| Formulation Tested | Rate of Application (ppm) | Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 |
| DG lite Carrier | 10 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 12 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 15 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 25 | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(3) | 100(4) | 100(7) | 100(4) | 100(4) | *** |
| Sand Carrier | 10 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 12 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 15 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 25 | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(3) | 100(1) | 100(5) | 100(4) | 100(3) | *** |
| Drench | 25 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | Check | | | | | | | | | | | | |

*** terminated based on IFA quarantine certification period
*** Data sheet lost due to hurricane Katrina

Table 2. Residual activity of TopPro bifenthrin in Flowerwood and Windmill media.

| Media Tested | Rate of Application (ppm) | Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Flowerwood | 10 | 100(1) | 100(1) | 100(1) | 100(1) | 100(3) | 100(1) | *** | *** | *** | *** | *** | *** |
| | 12 | 100(1) | 100(1) | 100(1) | 100(1) | 100(3) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) |
| | 15 | 100(1) | 100(1) | 100(1) | 100(1) | 100(3) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) |
| | 25 | 100(1) | 100(1) | 100(1) | 100(1) | 100(3) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) |
| | Drench | 100(1) | 100(1) | 100(1) | 100(1) | 100(3) | 100(1) | *** | *** | *** | *** | *** | *** |
| | Check | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 5 | 0 | 10 | 0 |
| Windmill | 10 | 100(1) | 100(1) | 100(14) | 100(7) | 100(4) | 100(1) | *** | *** | *** | *** | *** | *** |
| | 12 | 100(1) | 100(1) | 100(7) | 100(8) | 100(3) | 100(2) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) |
| | 15 | 100(1) | 100(1) | 100(1) | 100(1) | 100(3) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) |
| | 25 | 100(1) | 100(1) | 100(1) | 100(1) | 100(3) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) | 100(1) |
| | Drench | 100(1) | 100(1) | 100(1) | 100(14) | 95 | 85 | *** | *** | *** | *** | *** | *** |
| | Check | 0 | 0 | 0 | 5 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 2. (cont.) Residual activity of TopPro bifenthrin in Flowerwood and Windmill media.

| Media Tested | Rate of Application (ppm) | Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| Flowerwood | 10 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 12 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 15 | 100(1) | 100(1) | 100(3) | 100(4) | 100(7) | 100(7) | 100(10) | *** | *** | *** | *** | 10 |
| | 25 | 100(1) | 100(1) | 100(3) | 100(1) | 100(5) | 100(4) | 100(7) | *** | *** | *** | *** | 20 |
| | Check | 0 | 0 | 0 | 0 | 0 | 5 | 15 | | | | | 15 |
| Windmill | 10 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 12 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| | 15 | 100(1) | 100(1) | 100(4) | 100(4) | 100(5) | 100(7) | 100(7) | *** | *** | *** | *** | 75 |
| | 25 | 100(1) | 100(1) | 100(3) | 100(4) | 100(7) | 100(7) | 100(6) | *** | *** | *** | *** | 100(9) |
| | Check | 0 | 0 | 0 | 5 | 0 | 10 | 5 | | | | | 5 |

*** Data sheet lost due to hurricane Katrina

*** Bioassays not run due to post-Katrina clean-up

Table 2. (cont.) Residual activity of TopPro bifenthrin in Flowerwood and Windmill media.

| Media Tested | Rate of Application (ppm) | Mean % mortality to alate females at indicated months post-treatment (days required to reach 100% mortality) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
| Flowerwood | 10 | *** | *** | *** | *** | *** | *** | *** |
| | 12 | *** | *** | *** | *** | *** | *** | *** |
| | 15 | *** | *** | *** | *** | *** | *** | *** |
| | 25 | 100(8) | 100(10) | 100(11) | 100(9) | 100(8) | 100(11) | 100(11) |
| | Check | 10 | 15 | 5 | 20 | 10 | 5 | 10 |
| Windmill | 10 | *** | *** | *** | *** | *** | *** | *** |
| | 12 | *** | *** | *** | *** | *** | *** | *** |
| | 15 | *** | *** | *** | *** | *** | *** | *** |
| | 25 | 100(6) | 100(9) | 100(7) | 100(9) | 100(9) | 100(8) | 100(11) |
| | Check | 5 | 5 | 10 | 15 | 10 | 10 | 15 |

CPHST PIC NO: A9F01/A9M01

PROJECT TITLE: Chemical Degradation of IFA Quarantine Program Insecticides Used for Incorporation into Containerized Nursery Stock Potting Media, 2006

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Anne-Marie Callcott, Lee McAnally, Jennifer Lamont

## INTRODUCTION:

For certification in the Federal Imported Fire Ant Quarantine (7CFR 301.81), containerized nursery stock can be treated by incorporating granular insecticide into the potting media prior to potting. Various initial treatment dose rates result in various certification periods (e.g., 12 ppm dose rate of bifenthrin provides 12 months certification). For quality assurance, i.e. to determine whether the nursery properly applied the insecticide to the potting media, PPQ and state inspectors routinely collect media samples which are submitted to laboratories for chemical analysis to determine amount of insecticide present in the media (usually reported in parts per million – ppm). These media samples can be collected from nurseries using this quarantine treatment, as well as from nursery container shipments with suspect or confirmed IFA infestations.

Original trials to determine effective dose rates and certification periods of incorporated insecticides focused on the efficacy of the insecticide on the target insect, and no studies were conducted to determine the chemical degradation of the insecticide in potting media. In late 2004, a series of trials were initiated to determine levels of program chemicals detected by chemical analysis over the certification/aging period of the treated media. The first chemical evaluated was granular bifenthrin incorporated into different potting media. This testing was done in cooperation with the ANPCL Chemical Analysis section who conducted the chemical residue analyses. Data collected from these trials will allow the quarantine program to better evaluate results from chemical analyses of samples collected by inspectors.

The initial test was prematurely terminated due to hurricane Katrina. The data generated by the limited sampling was inconsistent and highly variable, and no significant conclusions could be formed with this data. As a result, a new trial was initiated in 2006 incorporating lessons learned about the sampling and mixing procedures.

## MATERIALS AND METHODS:

Potting media used in this test were: MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 875 lb/cu yd); Windmill media (Windmill Nursery, Folsom, LA - bulk density = 310 pounds per cubic yard).

The MAFES media is being tested in two ways; bulk mixing where a large quantity of untreated media was mixed, measured out into 1.5 cu. ft. loads then chemical treatments applied, and batch mixing where each individual batch (mixer load) of ingredients and chemical treatments was mixed. Windmill media is obtained in bulk and required no difference in handling.

Each media/mixture type was then mixed either wet or dry at 10 and 25 ppm. Dry mixing meant that no additional moisture other than what was already in the media was added. The wet mixes were done by adding approximately 1 liter of water per mixer load (1.5 cu. ft.). The wet loads were allowed to mix for approximately 5 minutes to ensure a uniform moisture content before the chemical treatments were added. 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 load to insure thorough blending. Treated media was then placed 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.

Immediately after potting, samples were taken for chemical analysis. Each sample consisted of one full pot and three such samples per treatment type were submitted for analysis. Samples will be taken at 0, 3, and 6 months post-treatment for the 10 ppm treatments and at 0, 6, 12, 18, and 24 months post-treatment for the 25 ppm treatments.

RESULTS:

Results to date are summarized in Table 1. At this time, only the samples taken immediately after mixing and the 3 month samples of the 10 ppm mixtures have been collected and chemically analyzed. There is not enough data to draw any conclusions at this time.

Table 1. Chemical Analysis for Bifenthrin Incorporated into Various Potting Media and Aged

| Soil Type | Mixing Method | Soil Moisture | Treatment Rate | PPM at indicated months post-treatment (Avg. of 3 samples) | |
|---|---|---|---|---|---|
| | | | | 0 mth | 3 mth |
| MAFES | Bulk | Dry | 10 ppm | 7.4 | 5.6 |
| | | | 25 ppm | 25.033 | N/A |
| | | Wet | 10 ppm | 14.4 | 5.267 |
| | | | 25 ppm | 18.767 | N/A |
| | Batch | Dry | 10 ppm | 9.8 | 6.467 |
| | | | 25 ppm | 46.733 | N/A |
| | | Wet | 10 ppm | 11.6 | 7.233 |
| | | | 25 ppm | 26.567 | N/A |
| Windmill | Bulk only | Dry | 10 ppm | 15.167 | 11.5 |
| | | | 25 ppm | 26.733 | N/A |
| | | Wet | 10 ppm | 13.167 | 9.033 |
| | | | 25 ppm | 24.2 | N/A |

CPHST PIC NO:  A9F01/A9M01

PROJECT TITLE:  Residual Activity of BASF BAS 320 001 as a Containerized Drench Treatment

REPORT TYPE:  Final

LEADER/PARTICIPANTS:  Shannon Wade, Lee McAnally

## INTRODUCTION:

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

BASF BAS 320 001 is a candidate pesticide for use as a drench treatment.

## MATERIALS AND METHODS:

On March 15, 2006 untreated MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 700 lb/cu yd) was placed in 1-gallon nursery pots and drenched with BAS 320 001 (240SC) using a 400ml finished solution at a rates of 25, 50, and 100 ppm.  The pots were weathered outdoors under simulated nursery conditions.  A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week.  At 2 weeks and thereafter at monthly intervals, sub samples were taken from 3 pots of each treatment and composited and subjected to standard alate queen bioassay (Appendix I) evaluated at 7 and 14 days after exposure.

## RESULTS:

The trial was scheduled to run for six months.  However, it was terminated after 2 months due to a lack of sufficient efficacy.  At no time were the treatments significantly effective against IFA (Table 1).  At the one month evaluation, all treatments and the untreated check had higher mortality than at any other testing period, indicating a possible problem with test insects.  At 2 weeks and at 2 months, activity was not sufficient to warrant continued testing.

Table 1. Residual Activity of BASF BAS 320 001 as a Containerized Drench Treatment.

| Rate of Application (ppm) | Mean % mortality to alate females at indicated post-treatment interval | | |
|---|---|---|---|
| | 2wks | 1 month | 2 months |
| 25 | 15 | 55 | 25 |
| 50 | 0 | 55 | 15 |
| 100 | 25 | 45 | 15 |
| Check | 0 | 65 | 15 |

CPHST PIC NO: A9F01/A9M01

PROJECT TITLE: Residual Activity of Nature's Care (BioGreen Soft) as a Containerized Drench Treatment

REPORT TYPE: Final

LEADER/PARTICIPANTS: Shannon Wade, Anne-Marie Callcott

## INTRODUCTION:

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

Nature's Care/BioGreen Soft is a candidate pesticide for use as a drench treatment.

## MATERIALS AND METHODS:

On August 1, 2006 untreated MAFES media (3:1:1 pine bark: sphagnum peat moss: sand - bulk density = 700 lb/cu yd) was placed in 1-gallon nursery pots and drenched with Nature's Care/BioGreen Soft using a 400ml finished solution at a rates of 2ml/400 ml and 6 ml/400 ml. The pots were weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1½ inches water per week. At 2 weeks and thereafter at monthly intervals, sub samples were taken from 3 pots of each treatment and composited and subjected to standard alate queen bioassay (Appendix I) evaluated at 7 and 14 days after exposure.

## RESULTS:

The trial was scheduled to run for six months. However, it was terminated after 5 weeks due to a lack of sufficient efficacy. At no time were the treatments significantly effective against IFA (Table 1). Due to time issues, testing was conducted at 3 and 5 weeks after application. At no time, was more than 25% mortality noted, thus testing was terminated due to lack of efficacy against IFA.

CPHST PIC NO: A9F01/A9M01

PROJECT TITLE: Effectiveness of Permethrin Treated Nursery Pots in Preventing Imported Fire Ant Invasion of Containerized Nursery Stock, 2004

TYPE REPORT: Final

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

## 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 and granular insecticides (preplant incorporation treatments) are approved for use. The most frequently used treatment is incorporation of granular 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 showed potential for preventing IFA infestation of small nursery containers (report FA01G038 – 2000 Accomplishment Report), and a large scale trial confirmed the potential of this type of treatment for containerized nursery stock (A9M01/FA01G069 – 2002 Accomplishment Report).

In 2004, Premium Compounded Products changed the way in which it produced the insecticide impregnated containers. Instead of the insecticide being distributed throughout the plastic, there is now a 3-layer system in place for the plastic whereby 3 layers of plastic (inside, middle and outside layers) are molded together to make the container. Therefore the insecticide can be placed in any or all of the layers. Testing to insure the efficacy of the permethrin impregnated into the plastic of the container in this manner was initiated in 2004. The company is pursuing EPA registration of the impregnated containers.

## MATERIALS AND METHODS:

One gallon nursery containers were provided by Premium Compounded Products. These containers were impregnated with 1% permethrin within each treated layer of the 3-layer plastic system. Three different types of sample pots were provided. One with the outside layer only treated, one with the inside layer only treated, and one with the inside and outside layers treated. Therefore, the inside and outside treated containers contain twice as much active ingredient than the single layer treated containers. Untreated check pots in the same size were also provided by the company.

Containers were potted up at the Gulfport, MS lab with the MAFES media (3:1:1 pine bark: sphagnum peat moss: sand) on August 2, 3 and 6, 2004. A portable cement mixer was used to blend the media, and operated for 15 minutes per batch to insure thorough blending. The MAFES media was then poured into the one gallon pots provided and weathered outdoors under simulated nursery conditions. A pulsating overhead irrigation system supplied ca. 1-1 ½ inches of water per week. Samples were taken at 2 week, 1 month, 2 month and every other month after that.

Bioassays were conducted in the laboratory in 2' x 8' test arenas (Figure 1 to right). Sides of the test arena were talced to prevent ants from climbing out and escaping. A permethrin 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 tumulus 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. A pot was considered infested if there were +25 workers inside the pot. There were 3 replicates per sampling interval.

RESULTS:

At 2 weeks the inside only treated pots had 500 or more ants living in each of the pots at the end of the 7 days (Figures 1 & 2). The inside only treatment continued to be ineffective at preventing IFA from infesting containers throughout the duration of the trial.

At 2 months, two of the three outside only treated pots had >500 ants living in them at the end of the 7 days evaluation period (Figures 1 & 3). These containers continued to provide erratic results throughout the trial, with most failures having more than 200 workers infesting a treated container.

The inside/outside treated containers were the most effective and consistent within this trial (Figures 1 & 4). At 2 months, one pot contained ca. 100 workers at the end of the evaluation period. At 15 months, two of the three pots had ca.100 ants living in them by the end of the 7 days. At 18 through 22 months no ants were present in the containers at the end of the evaluation period. At 24 months there was a significant failure with one container, where the majority of the colony (4000+ workers and brood) moved into the treated container.

Outside only treated | Inside only treated | Untreated

DISCUSSION:

These results indicate that treating the inside layer only of containers with 1% permethrin does not exclude IFA, probably due to the ants being able to avoid or limit contact with the inside of the container. While ants did infest the outside only treated containers early in the trial, we speculate they were able to infest the containers by pulling “untreated” media from the container around the drain holes (similar to the inside-only treated picture above), allowing the ants to enter and exit the container while limiting contact with the treated plastic. However, over time the media in the drain hole area may take on some chemical through leaching, thus providing the extra barrier needed to exclude IFA from the container. Unfortunately, this extended period of time required to achieve exclusion is not acceptable under the IFA Quarantine and the number of ants infesting the treated containers was large (>200 workers). The inside/outside treated containers showed good results through 22 months, although the break in efficacy at 2 and 15 months is of concern. The containers that were infested at these times were infested with less than 100 workers in all cases, while the failure at 24 months consisted of 4000 workers (basically the whole colony), and truly indicates breakdown in efficacy of the treatment.

Previous testing showed that one-gallon containers treated with 1% permethrin impregnated throughout the plastic were effective in excluding IFA for 16 months. However, through 24 months, any failures consisted of only 1 container out of 3 (per evaluation period) with less than100 workers present, similar to the data collected here.

The previous trial also tested 3-gallon and 10-gallon containers, with the larger treated containers not losing efficacy until 36 months or greater. In the original trial we speculated that the one-gallon containers may not have been of sufficient size to contain the entire IFA colony resulting in a few workers living in or around the treated container. Since less than 100 workers infested containers in the failures at less than 24 months in both the original trial and the inside/outside treatment in this current trial, this hypothesis may be correct. However, in all testing in all container sizes, no treatment truly repelled IFA. All trials and all treatments had worker ants at some time crawling in, on or under a treated container, which is not different from other acceptable IFA containerized treatments.

Figure 1. Percentage of containers infested with IFA (>25 workers) at indicated time after potting up.

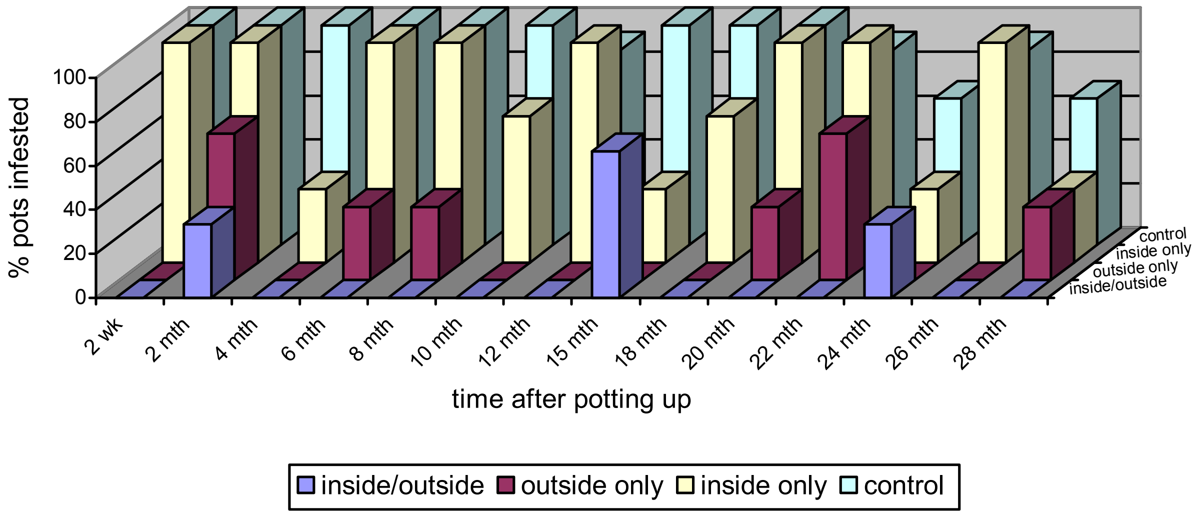



Figure 2. No. workers infested each replicate at indicated time after potting up for inside treated only containers.

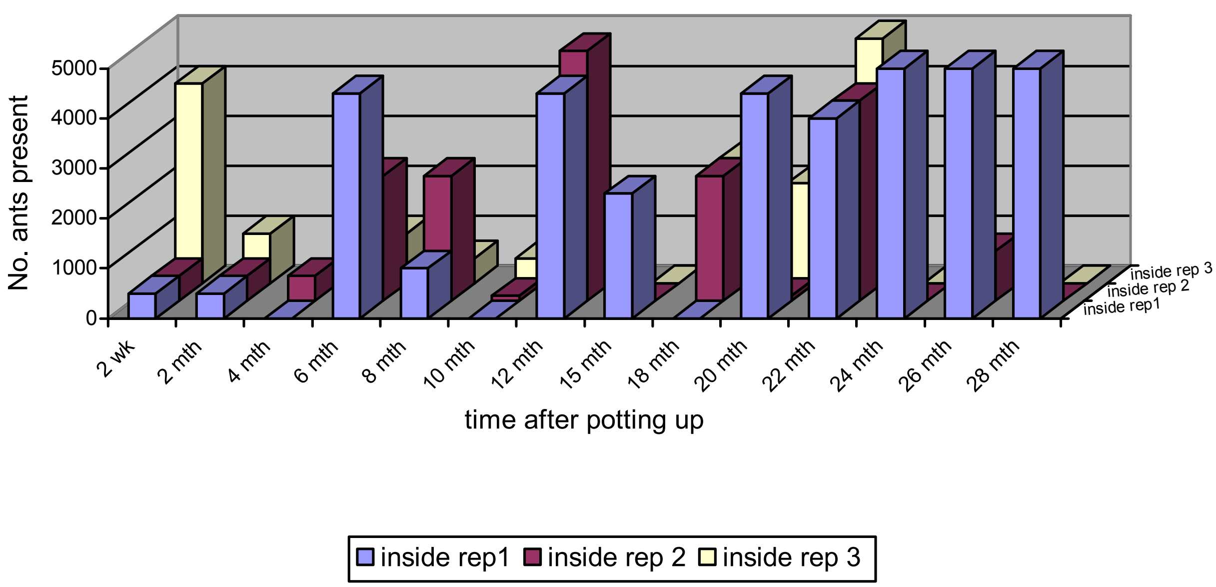


Figure 3. No. workers infested each replicate at indicated time after potting up for outside treated only containers.

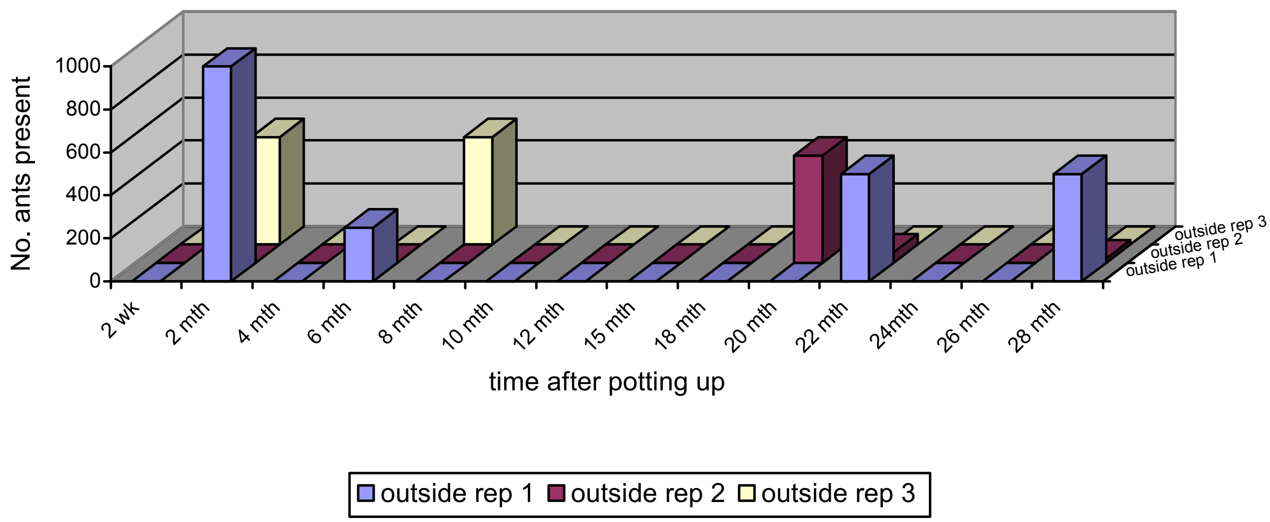
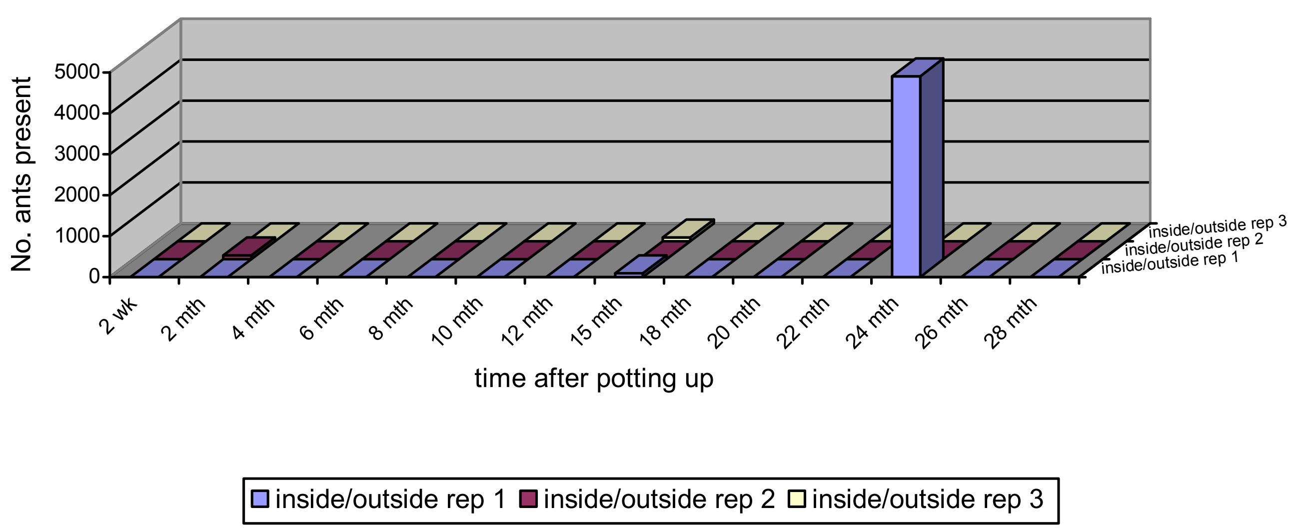



Figure 4. No. workers infested each replicate at indicated time after potting up for inside and outside treated containers.



CPHST PIC NO: A1F04/A1M04

PROJECT TITLE: Alternative Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine, Spring 2005

REPORT TYPE: Final

LEADER/PARTICIPANTS: Shannon James, Lee McAnally, Anne-Marie Callcott Jennifer Lamont; Jason Oliver, Sam Dennis and Nadeer Youssef of Tennessee State University; Michael Reding and Jim Moyseenko of USDA-ARS

## INTRODUCTION:

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

Current certification options for harvested B&B stock are immersion in a chlorpyrifos solution (dipping) or watering twice daily with a chlorpyrifos solution for three consecutive days (drenching). Likewise, the current treatment for Japanese beetle (*Poppillia japonica* Newman) in B&B requires dipping in chlorpyrifos. Since both imported fire ants (IFA) and Japanese beetle (JB) are a concern for the Tennessee field-grown nursery industry, the trials detailed in this report were conducted in cooperation with the Tennessee State University Nursery Research Center (TSU-NRC) with the goal of determining treatments useful against both pests. The JB testing portion of this trial was planned and conducted by TSU-NRC and the USDA-ARS Horticultural Insects Research Laboratory in Wooster, OH, and they report the details and results for that portion of these trials.

Standard IFA testing of chemical treatments for both dip and drench applications has been conducted through female alate bioassays on soil core samples from the treated root balls (Appendix A). Soil core bioassays for drenches conducted in 2002 and spring 2003 yielded erratic results over time and among replicates within treatments. The same chemicals at equal or lower rates, when applied by immersion, were consistent, thus indicating insufficiency in either application or the mode of testing for the drench applied treatments. Drench trials conducted in fall 2003 and spring 2004 determined that doubling the volume of solution applied failed to eliminate inconsistent results.

During drenching, B&B normally rests on a single side of the root ball throughout the three-day drench process. This possibly restricts treatment coverage on the resting side, while giving the surface of direct application a higher concentration of chemical and deeper penetration. The 2004 fall drench strongly suggested that rotating root balls during treatment, regardless of frequency, improved the consistency of bioassay results and could potentially cut the number of days spent applying drenches from three down to one. Trials were repeated in spring 2005 to examine whether changes in plant handling during application improve penetration and coverage and possibly allow reduction in the number of days required to complete a drench.

MATERIALS and METHODS:

In March 2005 TSU-NRC and USDA-ARS personnel completed drench applications on B&B plants with 25-inch diameter root balls at the TSU-NRC in Warren Co., TN. Drench treatments consisted of one of three chemical solutions or a water control in each of four application methods. In order to focus on the effect of application variation, the variety of chemicals applied was reduced from previous trials to three of the more promising insecticides that demonstrated control with both IFA and JB. Solutions, final rates, and handling which composed the treatments are listed in the table below.

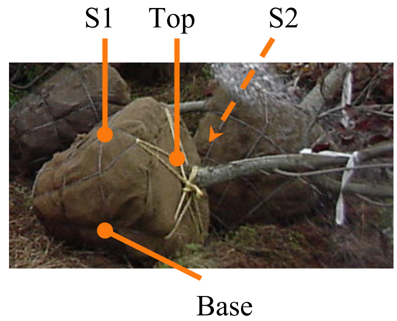
| Product | Active Ingredient | Rate (lb a.i./ 100 gal $H_2O$) | Handling | | | |
|---|---|---|---|---|---|---|
| | | | F2 | F4 | F6 | NF |
| Flagship 25WG | Thiamethoxam | 0.260 | X | X | X | X |
| Dursban TNP | Chlorpyrifos | 2.000* | X | X | X | X |
| Talstar Lawn & Tree Flowable | Bifenthrin | 0.230 | X | X | X | X |
| Control | ---- | 0.000 | | | | X |

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

Insecticidal solutions were prepared in 30-gal drums with polypropylene liners and pumped through a hose attached to a shower-headed nozzle using a Shur-Dri battery-powered pump (Figure 1). Solutions were applied twice daily (once in the morning and again in the afternoon) and between these applications in the flip-handled regimes the root balls were rotated or flipped to expose a different side to the direct application. The three regimes with flip-handling were two drench applications in one day (F2), drench applications twice daily for two consecutive days (F4), and drenches twice daily for three consecutive days (F6). Each root ball received approximately 0.67 gallons of drench solution at each drenching totaling 1.354 gallons a day. The amount used per drench application was based on the amount needed to achieve, "the point of runoff," required in the IFA quarantine. Although the volume of solution applied increased as the number of days drenched increased, the amount of chemical in the solution was adjusted so that within a single chemical group, regardless of the number of drench days, each plant was exposed to the same total amount of pesticide by the conclusion of its final drench. Each pesticide and the water control also had a no-flips (NF) treatment group that remained stationary, while receiving two applications a day for three consecutive days as is the current regulatory treatment.

Figure 1. Drench application

Figure 2. Soil core sample collection sites. The root ball is resting on an edge, so the base is visible and the lateral side two (S2) is opposite the camera.




After final treatment, the plants were maintained outside to weather naturally. Four replicate plants were selected out of the twenty root balls in each treatment group at 0.5, 1, 2, 4, and 6 months after final treatment for soil core sample collection. Four locations corresponding to the four sides of a root ball, top-as-planted (top), lateral side 1 (S1), lateral side 2 (S2), and the lateral side the plant rested on at the first drench application (base), were sampled on each plant to explore evenness of coverage (Figure 2). Samples were collected from within the first four inches of soil depth for testing against red, black, and red x black hybrid IFA as well as analytical chemical analysis. TSU-NRC is responsible for conducting and reporting testing on locally available black and hybrid IFA and chemical analysis. The samples for testing against red IFA were frozen and sent to the CPHST-ANPCL Soil Inhabiting Pests Section in Gulfport, MS where they were utilized in modified alate queen bioassays. Each soil sample was split into two sub-samples and ten alates were tested in each (Figures 3 & 4). Results were analyzed by ANOVA using JMP 5.1 (SAS Institute Inc., Cary, NC). Means were separated using Tukey's HSD at $\alpha = 0.05$.

Figure 3. A tray of alate mortality bioassay cups.

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

Through the course of testing samples from the fall 2004 trial, several control samples had rapid onset of high mortality, which may be indicative of chemical contamination. The chemical analysis from the same plants revealed that indeed a portion of the control group received some accidental chlorpyrifos application. Since no other treatment groups had contamination and within each round of applications chlorpyrifos was always the final chemical in the drench apparatus and the control was always first, it is expected contamination occurred due to a failure to clean before the next round of drench application began. The drench apparatus was normally washed between chemical treatments by running the pump for 20 seconds in a soap solution followed by 20 to 30 seconds of clean water. After the incident with high mortality in the controls, a second group of control samples using Mississippi soils was added to the groups tested in the spring 2005 drench as a backup verification of the health of the alates used.

RESULTS AND DISCUSSION:

The results of the fall 2004 trial were reported in the 2005 IFA Annual Accomplishment Report.

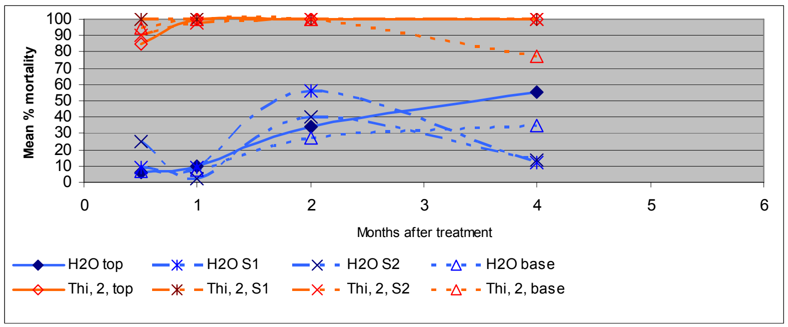
*Spring 2005 trial*:
Samples were not collected at the 6 month sampling period, therefore results are only through 4 months. Soil from the thiamethoxam treated balls provided inconsistent results regardless of application technique throughout the 4 months of the trial (Figure 5). The bifenthrin treated balls were fairly consistent with only the base soil samples giving less than 100% mortality regardless of application technique (Figures 6). The chlorpyrifos treatment was 100% effective through the 4 months regardless of application technique, however it needs to be reiterated that this is the Japanese beetle rate of application of 2.0 lb ai/100 gal $H_2O$, not the IFA rate of 0.125 lb ai/100 gal $H_2O$ (Figure 7).

A comprehensive compilation of all drench studies will be accomplished in 2007 depending on resources.

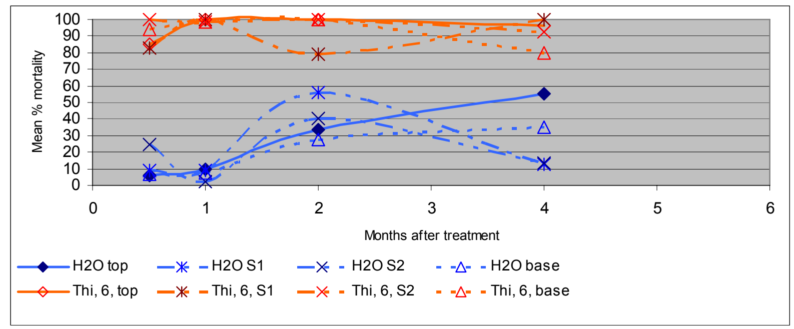
Portions of this project performed by TSU-NRC were partially funded through a research grant from USDA-CSREES Pest Management Alternatives Program Project 2003-34381-13660.

Figure 5. IFA control achieved in thiamethoxam (0.26 lb ai/100 gal $H_2O$) treated soil samples collected at four surface sites from four application regimes at various sampling intervals after final drench application. A – plants rotated once during 2 drench applications in a single day (F2); B - plants rotated twice during 4 drench applications over 2 days (F4); plants rotated 3 times during 6 drench applications over 3 days (F6); plants not rotated during 6 drench applications over 3 days (NF).

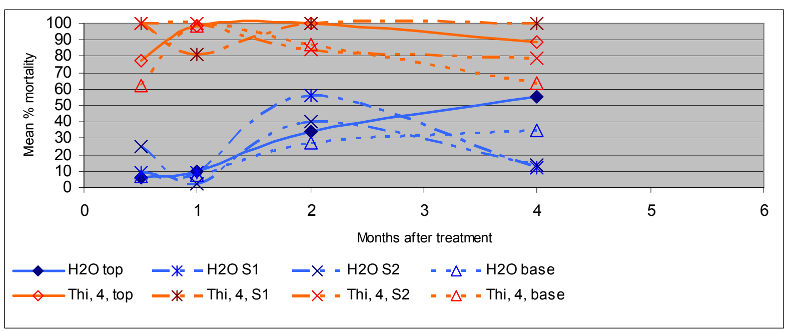
**A**




**C**




**B**




**D**

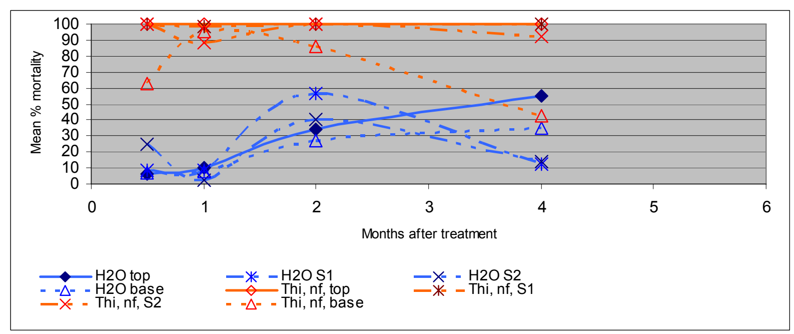


Figure 6. IFA control achieved in bifenthrin (0.23 lb ai/100 gal $H_2O$) treated soil samples collected at four surface sites from four application regimes at various sampling intervals after final drench application. A – plants rotated once during 2 drench applications in a single day (F2); B - plants rotated twice during 4 drench applications over 2 days (F4); plants rotated 3 times during 6 drench applications over 3 days (F6); plants not rotated during 6 drench applications over 3 days (NF).

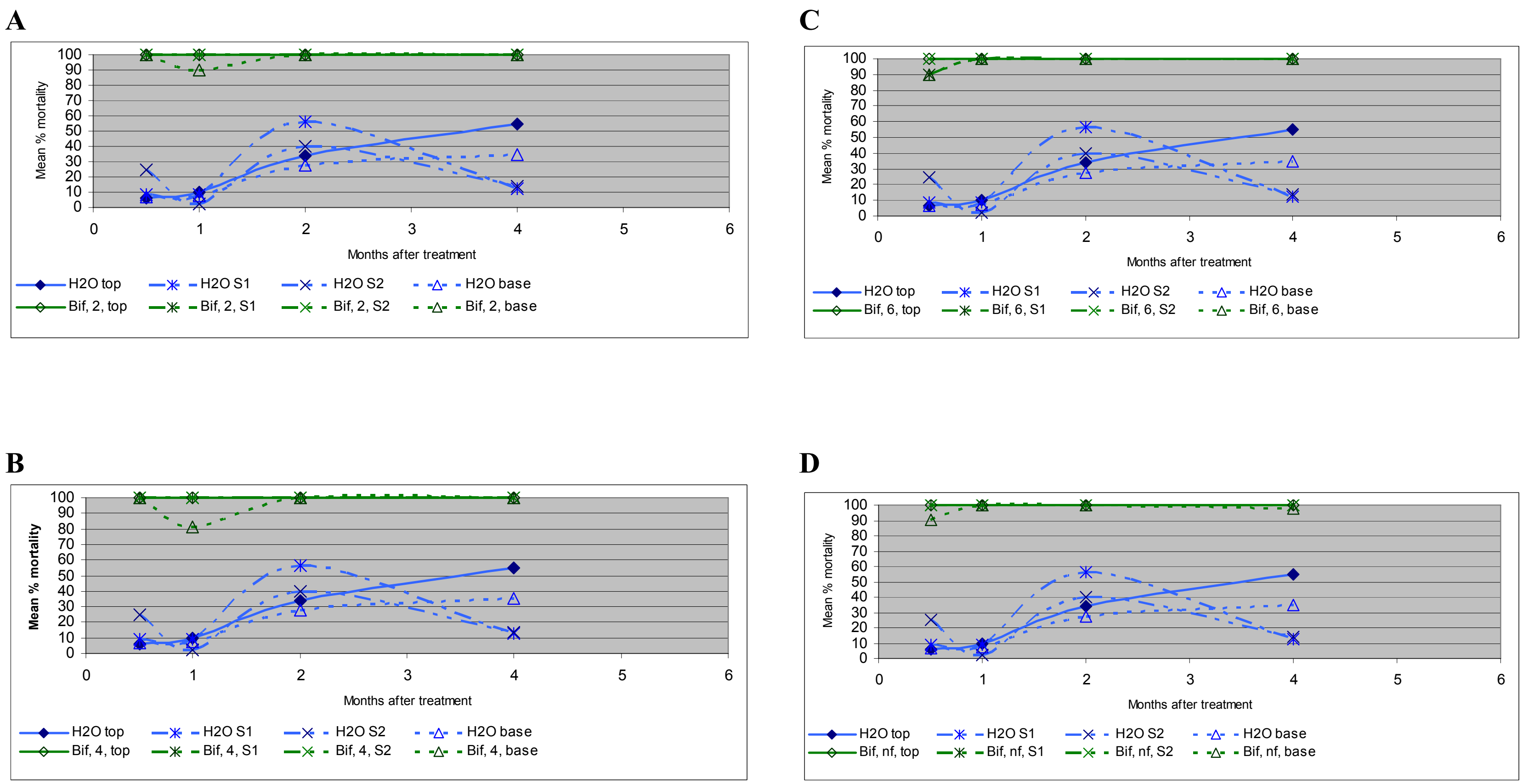
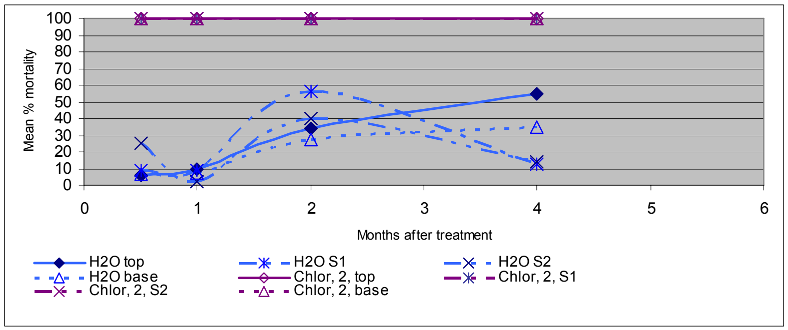
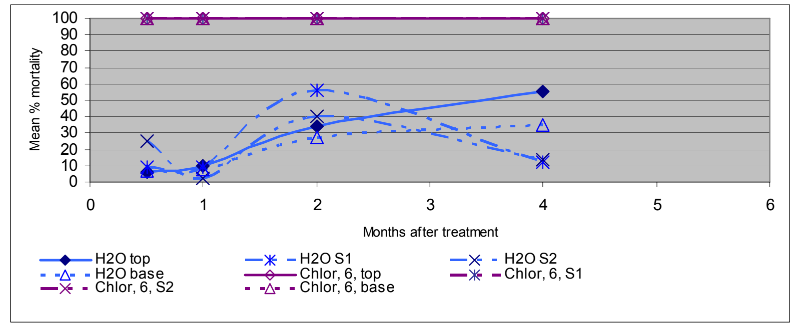


Figure 7. IFA control achieved in chlorpyrifos (2 lb ai/100 gal $H_2O$) treated soil samples collected at four surface sites from four application regimes at various sampling intervals after final drench application. A – plants rotated once during 2 drench applications in a single day (F2); B - plants rotated twice during 4 drench applications over 2 days (F4); plants rotated 3 times during 6 drench applications over 3 days (F6); plants not rotated during 6 drench applications over 3 days (NF).

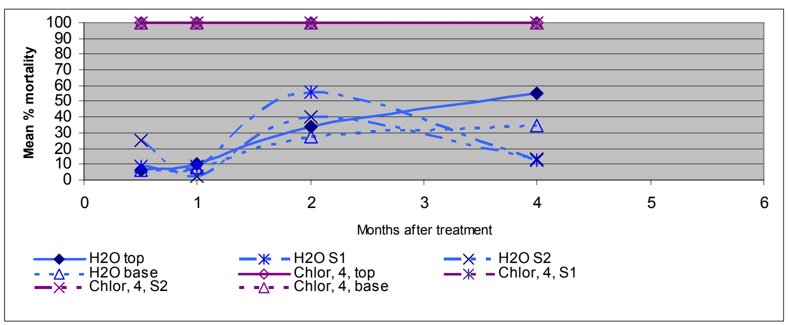
**A**



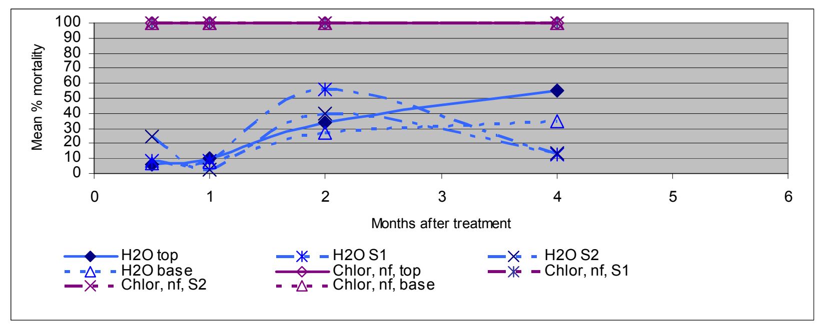

**C**




**B**




**D**



CPHST PIC NO: A1F04/A1M04

PROJECT TITLE: Intact Root Ball Bioassay Tests on Alternative Drench Treatments for use in the IFA Quarantine, 2006

REPORT TYPE: Final

LEADER/PARTICIPANT(s): Shannon James, Lee McAnally, Anne-Marie Callcott, and Jennifer Lamont

## INTRODUCTION:

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

Current certification options for harvested B&B stock are immersion in a chlorpyrifos solution (dipping) or watering twice daily with a chlorpyrifos solution for three consecutive days (drenching). Drench applications do not penetrate into the root ball as deep or with the same evenness as immersion, which can lead to inconsistent results within the trials if test soil samples are collected beyond the depth the insecticide penetrated. However, if a drench application can render root balls free of fire ants by penetrating and eliminating established colonies and providing a barrier to new infestation, then it is effective despite absence of the insecticide in deeper soil. Standard testing of chemical treatments for either application method has been through female alate bioassays on soil core samples from the treated root balls (Appendix I). Erratic results from soil core bioassays for drenches conducted in 2002 and 2003 indicated insufficiency in either pesticide application or the mode of testing. Therefore a new type of bioassay using small colonies to infest intact root balls either before or after treating was developed and first used in 2004.

Drench application of pesticides in the 2002 – 2003 trials followed the pattern of the current certification procedure in being applied twice daily for three consecutive days (i.e., six drenches of each root ball to complete a treatment). The amount applied to each root ball at each application in these trials appeared possibly to be too little to adequately cover the surface of the plant. When dry, the burlap used on the plants also appeared to restrict penetration by liquids. The 2006 drench trial in Mississippi mimicked a trial in 2004, testing the two new application styles developed to address the observed issues; rotating or flipping the root balls between drench applications, and decreasing the number of drenches to complete a treatment thus increasing the amount of liquid per drench.

MATERIALS and METHODS:

Treatment for this trial was initiated on September 15, 2006 at the Gulfport, MS USDA-APHIS facility. One-hundred twenty-five box wood shrubs (*Buxus microphylla*) with 16" diameter root balls grown in sandy loam soil from George Co., MS were each treated with one of the following chemicals or control.

| Active Ingredient | Trade Name | Rate (lb ai/100 gal H2O) |
|---|---|---|
| Bifenthrin | Bifenthrin Pro (flowable) | 0.100 |
| Lambda-cyhalothrin | Scimitar | 0.034 |
| Deltamethrin | Delta-Gard | 0.065 |
| Chlorpyrifos | Dursban 4E | 0.125 |
| Water | Control | -- |

Three application techniques were employed: twice daily for three days (2 x 3), twice on one day (2 x 1), and single application (S). Total volume of solution applied was the same regardless of application style. The amount of total finished solution applied to each root ball was determined from Appendix II. The surfactant Indicate 5 was added to the diluting water prior to chemical introduction and all root balls were watered a few days prior to treatment to ensure the soil was moist at time of treatment.

Each treatment (chem. x app) had 10 replicates. Five were tested within days after treatment and the other five were tested two months after treatment. All ten were treated at the same time. The two-month test plants were left on the ground in groups segregated by chemical to weather. Groups of plants stored on the ground were arranged to prevent rain runoff from one treatment to another.

Wild collected IFA were removed from their nest soil by dripping (Banks et al. 1981), and at infestation approximately a $^{1}/_{3}$ ℓ volume ball of workers with brood was placed on top of each root ball. Plants at the time of testing were kept in 26" diameter by 7" deep (66 x 18 cm) plastic Plantainer™ pans (Mac Court, Denver, CO) which were painted on the inside surface with Fluon (AGC Chemicals Americas Inc., Bayonne, NJ) to prevent ant escape (Figure 1).

Figure 1. An infested root ball

Figure 2. Splitting a root ball at the final observation to determine infestation

Manual readings of air and soil temperatures were taken at the times of ant infestation and at both the 7 and 14 day observations. Observations of IFA infestation of treated plants were conducted at 7 and 14 days after treatment application. At these observations the side of the root ball was firmly hit and the plant stem shaken to agitate any live IFA in the soil. If workers appeared within a minute of this disturbance, the plant was considered to have an active infestation. Plants that did not show active infestation at day 14 were split open and searched for live IFA (Figure 2).

## RESULTS AND DISCUSSION:

Bifenthrin, deltamethrin and lambda-cyhalothrin eliminated introduced IFA colonies within the 14 day observation period (Figures 3 and 4). Only one lambda-cyhalothrin replicate in the single application treatment of newly drenched root balls had ants visually present at the 7 day observation period. Splitting the root ball at the 14 day observation period verified the visual observations that all ants were eliminated within 14 days. The chlorpyrifos treatments were less consistent. The traditional 2x3 application technique provided 100% elimination of IFA within the 14 day test period. In the single drench application technique, one replicate in those tested immediately after treatment did not eliminate the IFA colony, but in the 2-month old replicates all treatments were effective. The 2x1 application technique allowed IFA to survive in 3 out of 5 replicates in those tested immediately after treatment, and in 2 out of 5 replicates in the 2-month old replicates. IFA colonies survived in all control root balls.

## REFERENCES CITED:

Banks, W. A., C. S. Lofgren, et al. (1981). Techniques for collecting, rearing, and handling imported fire ants, USDA, SEA, AATS-S-21, 9 p.

Figure 3. Survival of IFA colonies introduced onto newly drench-treated root balls (14 day exposure).

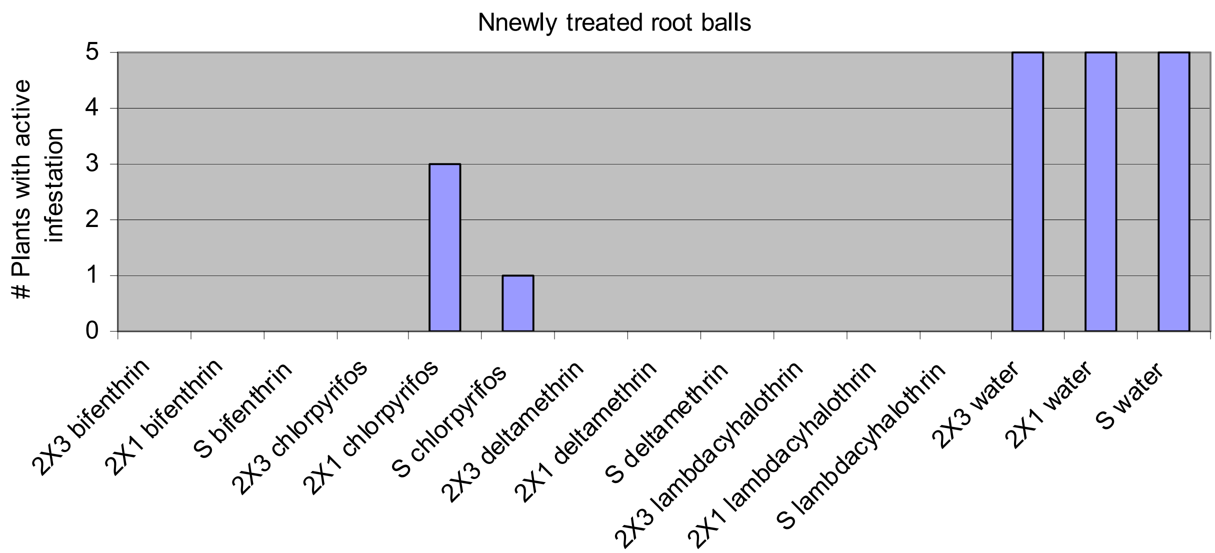
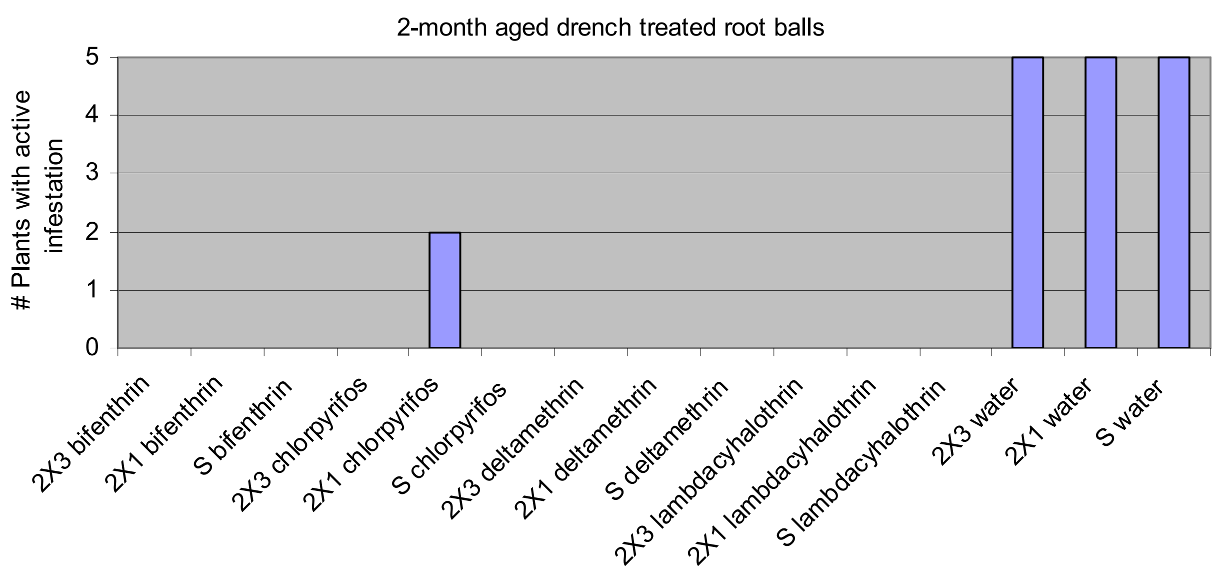



Figure 4. Survival of IFA colonies introduced onto 2-month aged drench-treated root balls (14 day exposure).



CPHST PIC NO: A1F04 /A1M04

PROJECT TITLE: Development of Alternative Quarantine Treatments for Field Grown Nursery Stock – Broadcast Bait plus Surface Band Application, Spring and Fall 2006

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Shannon James, Lee McAnally, Anne-Marie Callcott, Jennifer Lamont, Shannon Wade, Bill Guyton (chemist)

INTRODUCTION:

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

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

Preliminary testing initiated in Sept. 2001 assessed several liquid and granular insecticides against individual IFA mounds in the field. Results of this trial indicated promising results with acephate, bifenthrin, and deltamethrin. Tests against individual mounds continue to provide direction for insecticides utilized in the larger scale band treatments. The first two band trials applied in the fall of 2001 and spring of 2002 tested five to six-foot wide bands of bifenthrin and deltamethrin. Both liquid and granular formulations showed promising results but demonstrated that in band treatments contact insecticide alone was not effective enough for use in the IFA quarantine. Subsequent band trials have included a broadcast application of bait 3-5 days prior to the contact insecticide application. The inclusion of bait in the treatment procedure has facilitated quarantine level control for several contact insecticides in these trials (see 2002-2005 IFA Annual Accomplishment Reports). The trials in this report continue to explore alternative insecticides and provide supporting data for those previously seen to perform well.

There is some evidence of longer residual activity of the contact insecticides during the winter months vs. the spring/summer months. Literature indicates there may be more microbial activity/degradation as well as chemical degradation during the summer months of some insecticides; higher temperatures and moisture contributing to greater biotic and abiotic degradation (Baskaran et al. 1999; Getzin 1981; Tingle et al. 2000). However, the biology of the ant may also be a factor in this phenomenon. Chemical analysis of soil samples collected from treated areas in both spring and fall applications were initiated in 2006. Analyses were conducted by the ANPCL-Chemistry Section.

## MATERIALS AND METHODS:

*Spring 2006 Band Trial*:
The Bobby Chain Municipal airport in Hattiesburg, MS (Forrest Co.) was selected as the test location for this fall trial. Plots consisted of 800-foot long strips of land containing at least five active fire ant mounds within a 4-foot wide (two feet on both sides of a center line) observation strip that ran the length of the band (Figure 1). Plot center lines, which simulated rows of plant stock, were set a minimum of twenty feet apart side to side and end to end to provide a buffer zone between plots. Wooden stakes with plot identification numbers were planted at the plot ends and Pramitol®, an herbicide, was sprinkled around them to keep the grass from obscuring the stakes. Fluorescent orange spray paint marked the center line of each plot and was repainted as needed.

Figure 1. Plot arrangement diagram

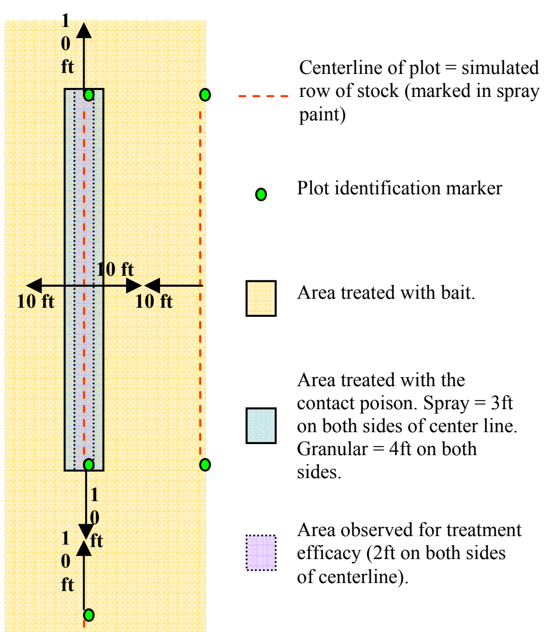



Figure 2. Application of contact insecticide to the plots of simulated stock

On May 12, 2006 hydramethylnon bait was applied at a rate of 1.5 lb/acre through the use of a shop built spreader mounted to a farm tractor. Control plots were not treated with bait. Contact insecticide application occurred on May 16-17, 2006. Granular treatments were applied using a Gandy 48" granular drop spreader attached to a farm tractor. Liquid treatments were applied using a roller pump boom sprayer equipped with two standard flat spray tips (8015-SS; TeeJet Corp.) to provide a 36" band spray and a total spray volume equivalent to ca. 76 gal/acre. Treatments were applied on both sides of the centerline producing a band size, depending on formulation used, either 800'x 8' or 800'x 6' in each plot. There were 3 replicates per treatment. Many liquid chemical labels suggest the use of a surfactant or buffer, and in this trial, the adjuvant Indicate® 5 was used for the first time primarily to buffer the water to pH 5. To insure the adjuvant did not have an effect on IFA populations, a set of replicates of this treatment were included. Treatments consisted of the following.

| Chemical | Formulation | Rate of Application |
|---|---|---|
| chlorpyrifos | granular 2.5% | 6 lb ai/acre |
| chlorpyrifos | granular 2.5% | 3 lb ai/acre |
| lambda-cyhalothrin | SC 9.7% | 0.068 lb ai/acre |
| fipronil | granular 0.0143% | 0.0125 lb ai/acre |
| Indicate 5 | liquid | buffered to pH5 |
| control | --- | --- |

Active IFA colonies in each plot's observation area were recorded prior to bait application and after contact insecticide application at 1, 2, 4, 6, 8, and 12 weeks and every four weeks thereafter. Mounds were evaluated using as little disturbance as possible, usually through insertion of a wire flag into the mound. Mounds were considered active if any workers appeared. Temperature was recorded during observation by use of air and soil thermometers.

Additional plots were treated to use for chemical analysis of contact insecticides. These plots, one for each treatment, were separate from those used for IFA evaluation and were treated only with the contact insecticide, not the bait. Five soil core samples were collected from each treated plot and composited for a single sample. Core samples were 2" diameter and 2" in depth. Samples were collected at 2, 4, 6, 8, and 12 weeks and every four weeks thereafter and submitted to the ANPCL-Chemistry Section for analysis. Spring treatments included:

| Chemical | Formulation | Rate of Application |
|---|---|---|
| chlorpyrifos | granular 2.5% | 6 lb ai/acre |
| chlorpyrifos | granular 2.5% | 3 lb ai/acre |
| chlorpyrifos | liquid 44.8% | 1 lb ai/acre |
| lambda-cyhalothrin | liquid 9.7% | 0.069 lb ai/acre |
| fipronil | granular 0.0143% | 0.0125 lb ai/acre |
| bifenthrin | granular 0.2% | 0.4 lb ai/acre |
| bifenthrin | liquid 7.9% | 0.2 lb ai/acre |
| deltamethrin | liquid 4.5% | 0.128 lb ai/acre |

Chemical analyses were conducted by the ANPCL-chemistry section. Procedure for the analytical method can be obtained by request.

*Fall 2006 Band Trial*:
Stennis Airport near Kiln, MS (Hancock Co.) was selected as the test location for the spring trial due to the large amount of IFA infested land available. Plots were set up and marked as described in the fall trial. Treatments were as follows.

| Chemical | Formulation | Rate of Application |
|---|---|---|
| bifenthrin | granular 0.2% | 0.2 lb ai/acre |
| chlorpyrifos | granular 2.5% | 3 lb ai/acre |
| chlorpyrifos | granular 2.5% | 1 1b ai/acre |
| chlorpyrifos | liquid 44.8% | 1 lb ai/acre |
| lambda-cyhalothrin | flowable 9.7% | 0.069 lb ai/acre |
| lambda-cyhalothrin | flowable 9.7% | 0.137 lb ai/acre |
| control | --- | --- |

Hydramethylnon bait was applied to insecticide treatment plots on October 26, 2006 at a rate of 1.5 lb/acre through the use of a shop built spreader mounted to a farm tractor. Granular contact insecticide application was done on October 31, 2006. However, due to weather and construction at the airport, the liquid treatments were done on Nov. 13, 2006. The equipment utilized to apply the insecticides in the fall 2006 trial was the same as used in the spring.

Active IFA colonies in each plot's observation area were evaluated and recorded as previously described with observations occurring prior to bait application and at 1, 2, 4, 6, 8, and 12 weeks and every four weeks thereafter.

Additional plots were treated to use for chemical analysis of contact insecticides. These plots, one for each treatment, were separate from those used for IFA evaluation and were treated only with the contact insecticide, not the bait. Five soil core samples were collected from each treated plot and composited for a single sample. Core samples were 2" diameter and 2" in depth. Samples were collected at 2, 4, 6, 8, and 12 weeks and every four weeks thereafter and submitted to the ANPCL-Chemistry Section for analysis. Spring treatments included:

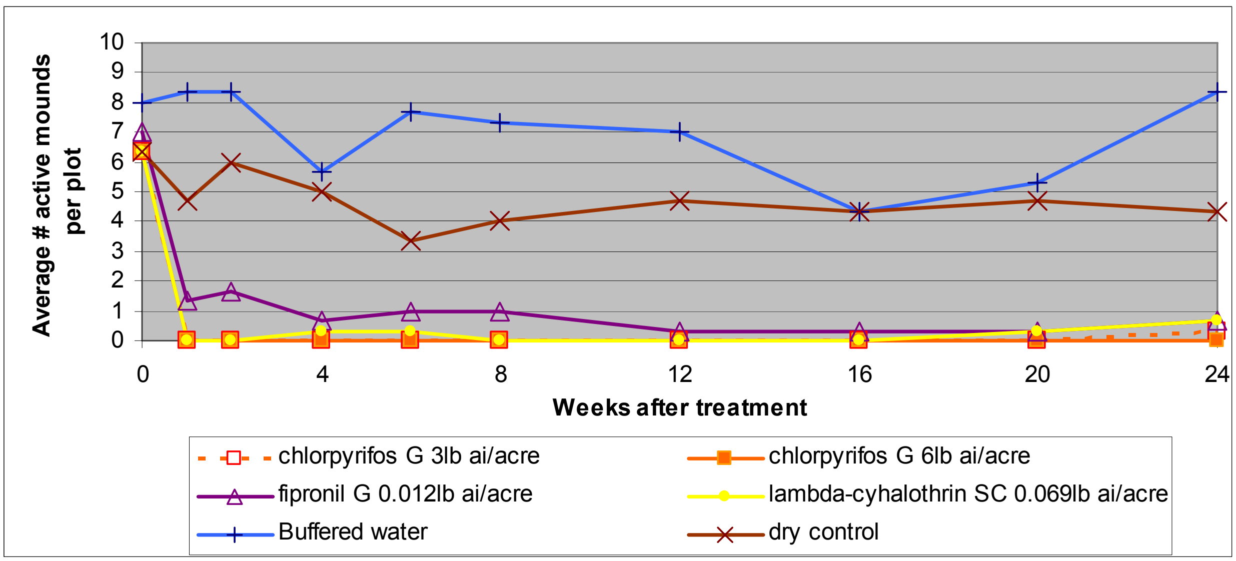
| Chemical | Formulation | Rate of Application |
|---|---|---|
| chlorpyrifos | granular 2.5% | 6 lb ai/acre |
| chlorpyrifos | granular 2.5% | 3 lb ai/acre |
| chlorpyrifos | granular 2.5% | 1 lb ai/acre |
| chlorpyrifos | liquid 44.8% | 1 lb ai/acre |
| lambda-cyhalothrin | liquid 9.7% | 0.13 lb ai/acre |
| lambda-cyhalothrin | liquid 9.7% | 0.069 lb ai/acre |
| fipronil | granular 0.0143% | 0.0125 lb ai/acre |
| bifenthrin | granular 0.2% | 0.4 lb ai/acre |
| bifenthrin | granular 0.2% | 0.2 lb ai/acre |
| bifenthrin | liquid 7.9% | 0.2 lb ai/acre |
| deltamethrin | liquid 4.5% | 0.128 lb ai/acre |

Chemical analyses were conducted by the ANPCL-chemistry section. Procedure for the analytical method can be obtained by request.

RESULTS:

*Spring 2006 Band Trial*:
Both granular chlorpyrifos treatments achieved 100% mortality by the 1 week post-treatment evaluation (Figure 3). The 6 lb ai/acre rate maintained 100% efficacy through the 24 week evaluation period. The 3 lb ai/acre rate maintained 100% efficacy through 20 weeks. Lambda-cyhalothrin at the 0.069 lb ai/acre rate eliminated all colonies by the 1 week evaluation period, but one plot had one mound appear within the evaluation area at weeks 4 and 6, but disappeared again by week 8. Complete control was then maintained through the 16 week evaluation period. Fipronil granular provided 80% control at 1 week, reaching a maximum control of 95% at 12-20 weeks.

Figure 3. Spring 2006 trial – Mean colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide.




Chemical analyses were limited by the extremely low theoretical rates of application. Limits of detection for the analysis were approximately 0.13 ppm. In this initial test on the 2 week samples, the chemist attempted to quantify results for those results falling between the limit of quantification (LOQ = 0.4ppm) and the limit of detection (LOD = 0.13ppm). In subsequent analyses, the results between these limits were captured as below quantifiable limits (BQL = 0.13-0.4ppm). Theoretical initial rates of application (ppm) were determined, but this number is a true best guess, and admittedly not accurate, but it gave a ballpark range for the chemists to look for. Many assumptions were made including bulk density of top soil in the area, and use of a 2-inch acre for calculations. The fipronil initial theoretical was below the detection limit of the analysis and was not detected in any analyses.

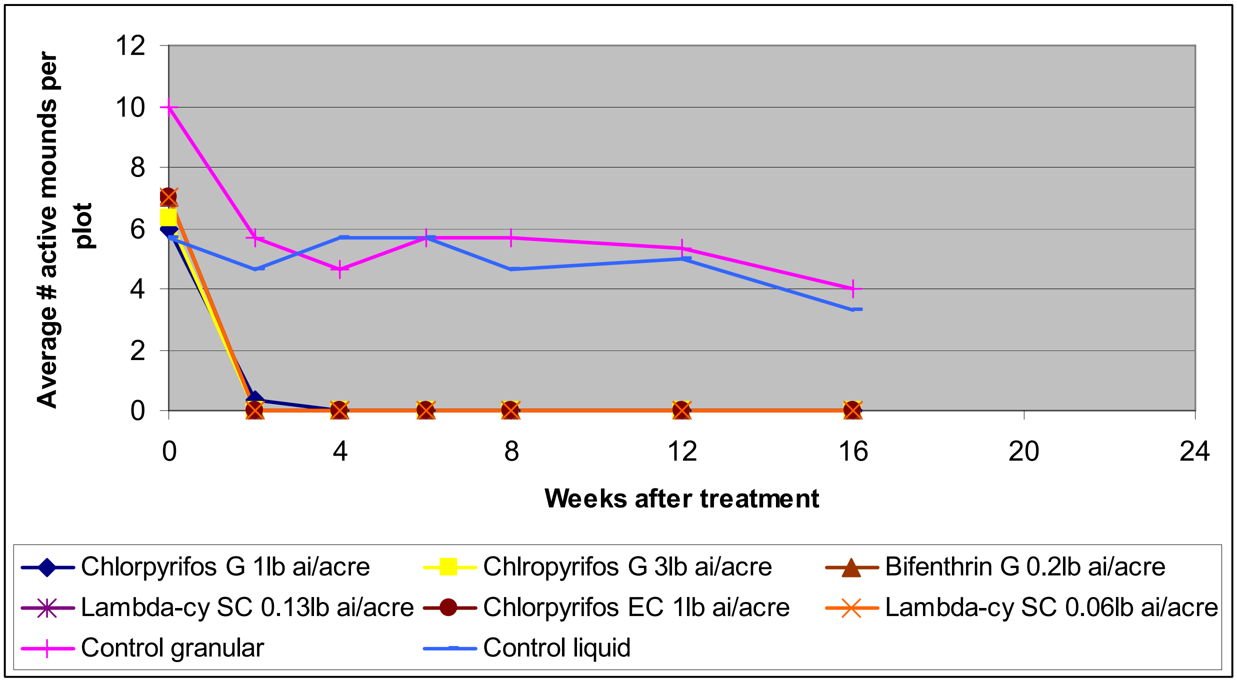
The results of the chemical analyses did detect chemical in all the 2 week samples except the fipronil sample (Table 1). Those chemicals that had multiple rates were detected in proportions similar to the application proportions (1x bifenthrin and 0.5x bifenthrin detected at 4.2 and 2.5 ppm, respectively). However, by 8 weeks all of the spring samples were not detected in the chemical analyses, indicating less than 0.13 ppm present in the samples.

Table 1. Results of chemical analyses of soil samples from spring 2007 band treatments (ND=not detected, or below limit of detection).

| Treatment | Application Rate | Theoretical Initial ppm | ppm by chemical analysis at weeks PT | | | | |
|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 8 | 12 | 20 |
| Chlorpyrifos G | 6 lb ai/acre | 14.66 | 4.27 | 0.31 | ND | ND | ND |
| Chlorpyrifos G | 3 lb ai/acre | 7.33 | 2.59 | bql | ND | ND | ND |
| Chlorpyrifos EC | 1 lb ai/acre | 2.4 | 0.28 | bql | ND | ND | ND |
| Lambda-cyhalothrin SC | 0.069 lb ai/acre | 0.167 | 0.22 | bql | ND | ND | ND |
| Fipronil G | 0.012 lb ai/acre | 0.03 | ND | ND | ND | ND | ND |
| Bifenthrin G | 0.4 lb ai/acre | 0.97 | 0.45 | bql | ND | ND | ND |
| Bifenthrin F | 0.2 lb ai/acre | 0.49 | 0.20 | bql | ND | ND | ND |
| Deltamethrin SC | 0.128 lb ai/acre | 0.31 | 0.13 | bql | ND | ND | ND |

*Fall 2006 Band Trial*:
Due to weather and construction at the test site, 1 week evaluations were not conducted. As noted in the materials and methods section, liquid contact treatments were conducted 2 weeks after the granular treatments, therefore the control evaluations were adjusted accordingly. All treatments achieved 100% control of IFA in the evaluation areas by the 2 week evaluation except the chlorpyrifos granular 1 lb ai/acre rate (Figure 4). One plot in this low chlorpyrifos rate had one active mound at the 2 week evaluation, but this colony was no longer present by the 4 week evaluation. All treatments have provided IFA colony free areas throughout the 16 week evaluation.

Figure 4. Fall 2006 trial – Mean colony mortality after a broadcast treatment of bait followed by a band treatment of contact insecticide.




Chemical analyses were limited by the extremely low theoretical rates of application. Limits of detection for the analysis were approximately 0.1 ppm. The limit of quantification (LOQ) for this trial was 0.5ppm and the limit of detection (LOD) was 0.16ppm. Results between these limits will be captured as below quantifiable limits (BQL = 0.13-0.4ppm). As in the spring 2007 trial, theoretical initial rates of application (ppm) were determined, but this number is a true best guess, and admittedly not accurate, but it gave a ballpark range for the chemists to look for. Many assumptions were made including bulk density of top soil in the area, and use of a 2-inch acre for calculations. The fipronil initial theoretical was below the detection limit of the analysis and was not detected in any analyses.

Presence of all chemicals, except fipronil, was detected by chemical analysis at the 2 week sample date (Table 2). With the exception of the 4 week bifenthrin flowable treatment, all treatments are being detected at rates of 0.13ppm or greater through the 8 week sample date. The low rate of lambda-cyhalothrin had fallen to non-detection levels at the 12 week sample date and the low granular chlorpyrifos treatment fell to non-detection levels at the 16 week sample date. The chlorpyrifos granular treatments of 3.0 and 6.0 lb ai/acre have continued to give quantifiable results through the 16 week sample date, unlike in the spring trial where quantifiable results were lost at the 8 week sample date. Chemical analyses will continue until at least 2 sampling dates of ND determination are made.

Table 2. Results of chemical analyses of soil samples from fall 2007 band treatments (ND=not detected, or below limit of detection of 0.5ppm; bql = between 0.16-0.5ppm).

| Treatment | Application Rate (lb ai/acre) | Theoretical Initial ppm | ppm by chemical analysis at weeks PT | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 8 | 12 | 16 | 20 |
| Chlorpyrifos G | 6.0 | 14.66 | 11.6 | 11.9 | 15.7 | 9.3 | 5.0 | |
| Chlorpyrifos G | 3.0 | 7.33 | 14.4 | 3.5 | 2.2 | 3.0 | 2.1 | |
| Chlorpyrifos G | 1.0 | 2.4 | 2.9 | 3.7 | 2.0 | 1.6 | ND | |
| Chlorpyrifos EC | 1.0 | 2.4 | 2.6 | 1.6 | bql | bql | bql | |
| Lambda-cyhalothrin SC | 0.13 | 0.33 | 1.8 | bql | bql | bql | bql | |
| Lambda-cyhalothrin SC | 0.069 | 0.167 | 0.54 | bql | bql | ND | ND | |
| Fipronil G | 0.012 | 0.03 | ND | ND | ND | ND | ND | |
| Bifenthrin G | 0.4 | 0.97 | 1.8 | 0.69 | 1.3 | 0.68 | 0.93 | |
| Bifenthrin G | 0.2 | 0.49 | bql | 0.54 | bql | 0.61 | bql | |
| Bifenthrin F | 0.2 | 0.49 | 1.9 | ND | bql | 0.58 | 0.6 | |
| Deltamethrin SC | 0.128 | 0.31 | 0.67 | 0.56 | bql | bql | bql | |

DISCUSSION:

The biological results of this trial, both spring and fall data, is supportive of past trials with these chemicals in this use pattern. A compilation of all band trials will be presented in the 2007 IFA Annual Report. Due to limited resources a full discussion of the interpretation of the chemical analyses will not occur in this report, but will be done at a later date. A limited literature search indicates that these results do reflect the normal activity of many insecticides as noted in the introduction; i.e. longer residual under cooler temperatures and drier conditions such as in the winter due to less microbial degradation, photodegradation, hydrolysis, etc.

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CPHST PIC NO: A1F03/A1M03

PROJECT TITLE: Exclusion Methods for Imported Fire Ants (IFA) in Hay-Transport Operations

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Ronald D. Weeks, Lee McAnally

## INTRODUCTION:

As a federally regulated item, under the Federal Imported Fire Ant Quarantine (7CFR 301.81), baled hay stored in direct contact with the ground cannot be moved outside the quarantine area. This poses significant limits on commodity transportation and access. Currently, there are no quarantine treatments approved for assuring that transported hay bales are imported fire ant (IFA) free. Previous research (see Annual Report 2003 – 2005) suggests that a Best Management Practices (BMP) approach which focuses on control at both the field and commodity level is useful in developing steps and applications to use against IFA. Broadcast bait applications are useful for reducing IFA populations in half-acre to larger field areas. Contact insecticides may be applied strategically around or under the commodity with few caveats. Previous research examining BMP have used individual hay bales as replicates. This was advantageous to complete several screenings of chemical formulations, application rates, methods, and efficacy. These studies have shown significant promise. In most hay production systems hay is stored in large groups or stacks in a designated area of the field, this creates significant challenges to managing IFA (Fig. 1). In 2006 individual hay bale treatments were extended to multiple "stacked" hay bales. Also, 2006 treatments included methods development for evicting IFA from hay bales that may be infested through the baling process.

## MATERIALS AND METHODS:

Hay trials were conducted at the White Sands Mississippi Agriculture and Forestry Experiment Station in Pearl River County. Trails were conducted in a 5.5 acre field, infested with monogyne red imported fire ants *Solenopsis invicta* Buren. Hydramethylnon bait was broadcast applied at the labeled rate to the field 4 weeks prior to trials. Previous SIPS studies have shown that broadcast applications of commonly applied ant baits may kill up to 90% of colonies from the treatment area. Post treatment counts of mounds along four equal distant transects (200 ft) show that the untreated side of the field had 10 mounds while the treated side had 5. Hay for this evaluation was cut and baled on October 4, 2006 in an adjacent field and moved within 24 hours to the experimental field.

The percentage of hay stacks infested by IFA was compared for two chemical barrier application methods at a half labeled-rate of permethrin (0.0042 lb A.I./gallon of water). Barrier methods comprised; 1) applying permethrin to an 18 $ft^2$ area directly under a "stack" of hay then placing a permeable landscaping ground cloth between the hay bales and chemically treated soil, and 2) application of permethrin to a 3.28 ft wide band around each stack of hay (Fig. 2). Soil applications of chemicals consisted of 6 gal finish solution for each 100 sq ft application area. Control and treatment stacks were placed in a block design spaced approximately 30 ft apart. Four replicates of each treatment were set up (n = 12). Routine ant sampling of each bale was completed 3-4 days post hay placement and at weekly intervals. A SIPS formulated attractant was placed on the outward sides of each hay bale comprising a stack (4 attractants per stack) to determine the presence of foraging workers (Fig. 3, n = 48 samples). Baits were placed within 8 inches of the base of each bale and allowed to sit for 30 minutes.

RESULTS and DISCUSSION:

Sampling began 24 hrs after hay placement within treatments. Ants were detected foraging on control bales the first sampling period and each subsequent week of the 7 week study. Ants were detected foraging on one bale within each of two stacks in the soil area treatment. These ants may have come from colonies that escaped the initial broadcast bait application, hitchhiked in with bale, or they could have been moving in from outside the treated area. To eliminate these nuisance ants, 4 vials containing 1 tsp. of indoxycarb bait were placed at the base of each hay bale within the infested stacks. There were significant differences between control and contact insecticide barrier treatments (Table 1). After indoxycarb treatment no other IFA were found on protected hay stacks until the end of the 7 week study. Both barrier methods provided protection to bales from IFA infestation for up to six weeks. Windows of opportunity for IFA-free hay transport may provide best management practices that hay operators may use to temporarily store hay bales on the ground before shipping to non-quarantined areas.

Table 1. Percent of hay stacks infested by imported fire ants in treatments during each of the 7 weeks of the study (number of hay stacks = 12, with four replicates in each treatment). Strip applications comprised a 3.28 ft wide strip of permethrin applied around hay stacks. Area applications comprised a 18 $ft^2$ treated area with permethrin and a 18 $ft^2$ cloth cover between the hay and treated surface. The application rate of permethrin used was 0.0042 lb A.I./gallon of water with 6 gal finished solution applied per 100 square feet.

| **Treatment** | **Week 1** | **Week 2** | **Week 3** | **Week 4** | **Week 5** | **Week 6** | **Week 7** |
|---|---|---|---|---|---|---|---|
| Strip application | 0 | 0 | 0 | 0 | 0 | 50 | 75 |
| Area application | 50 | 0 | 0 | 0 | 0 | 0 | 50 |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

| | | |
|---|---|---|
|  |  |  |
| Figure 1. Typical “in-field” storage practices | Figure 2. Spraying chemical barrier around hay bales. | Figure 3.  SIPS bait used to sample for IFA on hay bales. |

CPHST PIC NO: A1F03/A1M03

PROJECT TITLE: Exclusion Methods for Imported Fire Ants (IFA) in Pine Straw

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Ronald D. Weeks

## INTRODUCTION:

Baled pine straw has been implicated in the movement of imported fire ants in recent years. Under the Federal Imported Fire Ant Quarantine (7CFR 301.81), baled hay and straw stored in direct contact with the ground cannot be moved outside the quarantine area. This poses significant limits on commodity transportation and access. Currently, there are no quarantine treatments approved for assuring that transported pine straw is imported fire ant (IFA) free. Previous experience and research (see Annual Report 2003 – 2005) on both baled hay and pine straw, to a limited extent, suggests that a Best Management Practices (BMP) approach which focuses on control at the storage site in the field and commodity level is useful in developing steps and applications to use against IFA. The objectives of this project are to evaluate several BMP for pine straw that prevent or eliminate IFA infestation.

## MATERIALS AND METHODS:

Pine straw experiments were started at the SIPS laboratory in Gulfport, MS., October 23, 2006, to evaluate methods to a rapidly "clean-up" infested pine straw bales through the use of spot delivery of a fast acting insecticide (Fig. 1). Indoxycarb bait is fast-acting and designated by the EPA to be a "reduced-risk" pesticide and is considered an organophosphate (OP) replacement. The experimental design consisted of infesting 16 pine straw bales with IFA (approximately 10,000 ants, some brood and alates, Fig. 2). Once infested, pine bales and ants were allowed to sit in-situ for 7 days prior to insecticide treatments. Spot applications of indoxycarb bait (1 tsp per bale) were placed on 8 randomly chosen bales. The remaining bales (8) served as control units and received an equal portion of SIPS formulated non-toxic bait attractant (*see* Annual Report 2005 - Attractants).

IFA activity was monitored daily using the SIPS bait and looking under pine bales. SIPS bait attractant was placed on bales and foraging observed 30 and 45 minutes after attractant placement. The number of pine bales infested with IFA after indoxycarb application was compared between control and treatments daily.

## RESULTS and DISCUSSION:

Ants were observed foraging on all baits 45-minutes after initial placement. Observations at 24-hr showed that all baits except for two indoxycarb baits were more than 50% removed. Results of sampling 48-hrs after insecticide treatment show that no active foraging was detected on 50% of treated bales (4). Active foraging was observed on all control bales. Active foraging continued on the remaining 4 treated bales and controls for two-weeks after initial insecticide application.  Ants in the remaining treated bales were eliminated with a second application of indoxycarb.  Ants were observed foraging on these baits 45-minutes after initial placement.  Sampling at 48-hrs after the second application showed no active foraging on treated bales and active foraging on all untreated bales.  These results suggest that a fast-acting insecticide bait such as indoxycarb may be useful in a BMP to evict or eliminate IFA from infested pine straw bales.  However, a single application may not complete the job and a follow-up sampling and repeat applications may be needed.  Future research is being planned to examine if the dose application of 1 tsp/bale was sufficient to kill colonies and examine other insecticide delivery options.

Figure 1. Spot application of insecticide (1 tsp/bale)


Figure 2. Pine Straw bale infested with IFA colony

CPHST PIC NO: A1F02/A1M02

PROJECT TITLE: Efficacy of Dupont DPX-MP062-502 Fire Ant Bait when Formulated on Tast-e-Bait® Carrier

TYPE REPORT: Final

LEADER/PARTICIPANTS: Anne-Marie Callcott, Lee McAnally, Shannon Wade, Jennifer Lamont, Ronald D. Weeks, Shannon James

## INTRODUCTION:

Baits are an important part of the Federal Imported Fire Ant (IFA) Quarantine (7CFR301.81) and are an environmentally friendly treatment method for both the quarantine affected industry and the general public. Testing of new bait formulations for inclusion in the IFA Quarantine is an ongoing process with new baits routinely added to the list of approved insecticides. Fire ant baits are utilized in the IFA Quarantine 1) in combination with a granular chlorpyrifos treatment for certification of field grown nursery stock, and 2) as an environs treatment within the Fire Ant Free Nursery Program. Traditional IFA baits are formulated on a pregelled corn grit carrier with soybean oil. Over the years, there have been some concerns in the industry regarding the availability of corn grit. Therefore, new carriers need to be tested to determine efficacy of fire ant baits on any new carrier. Tast-e-bait® is a carrier product formulated from the by-products of bakery products by Advanced Organics. This carrier has been tested by this laboratory for several years and has proven to be an acceptable carrier. Several active ingredients have been tested in the past using this carrier, with similar efficacy as using the traditional corn grit carrier. We tested the efficacy of Dupont's DPX-MP062-502 fire ant bait formulated on Tast-e-Bait carrier on field colonies of imported fire ants.

## METHODS AND MATERIALS:

Dupont provided DPX-MP062-502 fire ant bait on the Advanced Organics' Tast-E-Bait carrier. Commercially available Amdro® fire ant bait was used as a standard. Test plots were set up in Pearl River county Mississippi. Each plot was one acre in size with a ¼-acre efficacy subplot located in the center of the test plots. There were 3 replicates per treatment as well as 3 replicates for untreated controls. Treatments were applied on May 18, 2006 using a shop-built spreader on a farm tractor at a rate of 1.5 lbs/acre. Prior to treatment and at 4 week intervals thereafter, evaluations of IFA populations are made in each ¼-acre efficacy subplot using the procedures described by Lofgren and Williams (1982) and Collins and Callcott (1995). Evaluations continue until reinfestation is observed. Means subjected to ANOVA and t-test ($\alpha$ = 0.05).

## RESULTS:

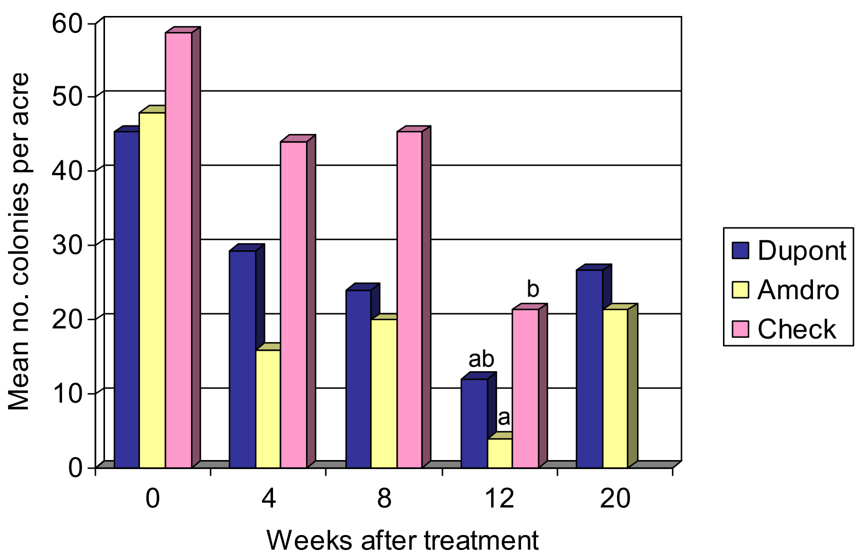
This was an extremely dry year for south Mississippi, with record drought conditions, particularly in the spring months. Due to the dry conditions, we did wait to treat until after a small rainfall, and colonies appeared healthy during treatment. Rainfall totals in the area throughout the entire trial, which ran from May until mid-October, were about 20 inches below normal. At the 12 week count, evaluations in all plots were difficult due to very high and dense grass (especially in 2 of the 3 check plots), as well as the continued drought conditions. A 16 week count was not done because of tall grass. The cooperator cut in the treated field prior to the final 20 week count, but did not cut in the control field and therefore the controls were not counted at week 20. However, observation of other untreated IFA colonies on the same property as the treated areas indicated that while colonies were still affected by the drought (colonies difficult to find in the dry sandy soil), the observed untreated colonies were active when disturbed and most did contain worker brood.

Results were extremely unusual for bait treatments (especially for Amdro), with only about 30-50% reduction in colony numbers at 4 and 8 weeks after treatment (Table 1). By 12 weeks, there was a 73% reduction in colony numbers in the Dupont bait plots. Significant differences were difficult to determine due to high variation in the plots, especially in the control/check plots.

Population indices, which measure reproductive viability, for the Dupont bait treatment decreased by >90% at 8-12 weeks after treatment, indicating that the bait had significantly affected worker production (Figure 1).

The trial was terminated after the 20 week evaluation since colony numbers and population indices indicated reinfestation was occurring.

Figure 1. Change in mean numbers of colonies per acre in plots treated with Dupont DPX-MP062-502 and Amdro® fire ant baits – initiated May 18, 2006; Pearl River Co., MS




Means within a time period followed by the same letter are not significantly different (t-test; α = 0.05). Unless noted means are not significantly different. Controls not counted in week 20; no statistics for week 20.

Table 1. Mean percent change in population index per acre in plots treated with Dupont DPX-MP062-502 and Amdro® fire ant baits – initiated May 18, 2006; Pearl River Co., MS

| Treatment | Pre-treat mean pop. index/acre | Mean change in population index at indicated weeks after treatment | | | |
|---|---|---|---|---|---|
| | | (4) | (8) | (12) | (20) |
| Dupont | 805.32a | -68.19a | -90.74a | -93.52a | -69.66 |
| Amdro | 829.32a | -81.81a | -93.56a | -98.68b | -78.58 |
| Check | 866.68a | -48.66a | -34.15b | -66.30c | ----- |

Means within a column followed by the same letter are not significantly different (t-test; $\alpha = 0.05$)
---- no controls counted at 20 weeks due to extremely tall grass; no stats in week 20

<u>DISCUSSION</u>:

A broadcast application of Dupont DPX-MP062-502 bait provided control of IFA in a field situation similar to a standard Amdro bait application under extremely unusual environmental conditions. Generally, we expect to see high decreases in population indices early in evaluations and slower colony kill with insect growth regulator (IGR) baits. Since both the Dupont test product as well as the standard bait performed in a similar manner, both very slow to show significant activity in colony kill, but both showing significant decreases in population indices, this would indicate more of an environmental impact on the biology/susceptibility of the insect to the baits, rather than the Dupont test product being an IGR. However, since mode of action on this numbered material was not disclosed, these comments are speculative only. Additional testing under more normal environmental conditions next year would be warranted to provide better data for this Dupont product since it did perform very well considering the conditions.

<u>REFERENCES CITED</u>:

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

Lofgren, C. S. and D. F. Williams. 1982. Avermectin $B_1a$, a highly potent inhibitor of reproduction by queens of the red imported fire ant. Jour. Econ. Entomol. 75: 798-803.

CPHST PIC NO: A1F02/A1M02

PROJECT TITLE: Efficacy of BASF BAS 320 04 I Fire Ant Bait

TYPE REPORT: Final

LEADER/PARTICIPANTS: Anne-Marie Callcott, Lee McAnally, Shannon Wade, Jennifer Lamont, Ronald D. Weeks, Shannon James

## INTRODUCTION:

Baits are an important part of the Federal Imported Fire Ant (IFA) Quarantine (7CFR301.81) and are an environmentally friendly treatment method for both the quarantine affected industry and the general public. Testing of new bait formulations for inclusion in the IFA Quarantine is an ongoing process with new baits routinely added to the list of approved insecticides. Fire ant baits are utilized in the IFA Quarantine 1) in combination with a granular chlorpyrifos treatment for certification of field grown nursery stock, and 2) as an environs treatment within the Fire Ant Free Nursery Program. We tested the efficacy of BASF BAS 320 04 I fire ant bait on field colonies of imported fire ants.

## METHODS AND MATERIALS:

BASF provided BAS 320 04 I fire ant bait. Commercially available Amdro® fire ant bait was used as a standard. Test plots were set up in Pearl River county Mississippi. Each plot was one acre in size with a ¼-acre efficacy subplot located in the center of the test plots. There were 3 replicates per treatment as well as 3 replicates for untreated controls. Treatments were applied on May 18, 2006 using a shop-built spreader on a farm tractor at a rate of 1.5 lbs/acre. Prior to treatment and at 4 week intervals thereafter, evaluations of IFA populations are made in each ¼-acre efficacy subplot using the procedures described by Lofgren and Williams (1982) and Collins and Callcott (1995). Evaluations continued until reinfestation was observed. Means subjected to ANOVA and t-test ($\alpha = 0.05$).

## RESULTS:

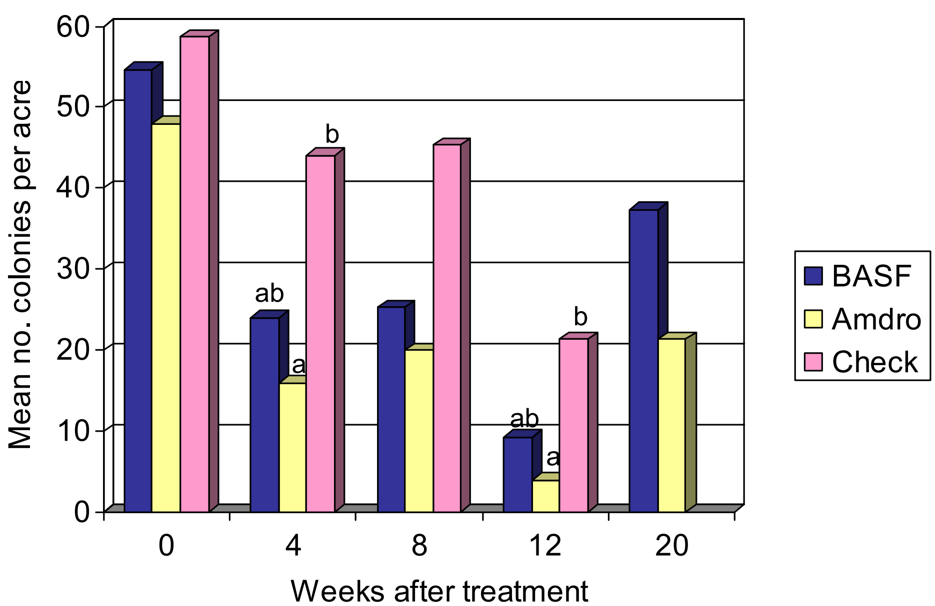
This was an extremely dry year for south Mississippi, with record drought conditions, particularly in the spring months. Due to the dry conditions, we did wait to treat until after a small rainfall, and colonies appeared healthy during treatment. Rainfall totals in the area throughout the entire trial, which ran from May until mid-October, were about 20 inches below normal. At the 12 week count, evaluations in all plots were difficult due to very high and dense grass (especially in 2 of the 3 check plots), as well as the continued drought conditions. A 16 week count was not done because of tall grass. The cooperator cut in the treated field prior to the final 20 week count, but did not cut in the control field and therefore the controls were not counted at week 20. However, observation of other untreated IFA colonies on the same property as the treated areas indicated that while colonies were still affected by the drought (colonies difficult to find in the dry sandy soil), the observed untreated colonies were active when disturbed and most did contain worker brood.

Results were extremely unusual for bait treatments (especially for Amdro), with only about 50% reduction in colony numbers at 4 and 8 weeks after treatment (Table 1). By 12 weeks, there was an 83% reduction in colony numbers in the BASF bait plots. Significant differences were difficult to determine due to high variation in the plots, especially in the control/check plots.

Population indices, which measure reproductive viability, for the BASF bait treatment decreased by >92% at 8-12 weeks after treatment, indicating that the bait had affected worker production (Figure 1).

The trial was terminated after the 20 week evaluation since colony numbers and population indices indicated reinfestation was occurring.

Figure 1. Change in mean numbers of colonies per acre in plots treated with BASF BAS 320 04 I and Amdro® fire ant baits – initiated May 18, 2006; Pearl River Co., MS




Means within a time period followed by the same letter are not significantly different (t-test; $\alpha = 0.05$). Unless noted means are not significantly different. Controls not counted in week 20; no statistics for week 20.

Table 1. Mean percent change in population index per acre in plots treated with BASF BAS 320 04 I and Amdro® fire ant baits – initiated May 18, 2006; Pearl River Co., MS

| Treatment | Pre-treat mean pop. index/acre | Mean change in population index at indicated weeks after treatment | | | |
|---|---|---|---|---|---|
| | | (4) | (8) | (12) | (20) |
| BASF | 993.32a | -85.70ab | -92.67a | -95.84a | -63.81 |
| Amdro | 829.32a | -81.81a | -93.56a | -98.68a | -78.58 |
| Check | 866.68a | -48.66b | -34.15b | -66.30b | ---- |

Means within a column followed by the same letter are not significantly different (t-test; $\alpha = 0.05$)
---- no controls counted at 20 weeks due to extremely tall grass; no stats in week 20

## DISCUSSION:

A broadcast application of BASF BAS 320 04 I bait provided control of IFA in a field situation similar to a standard Amdro bait application under extremely unusual environmental conditions. Generally, we expect to see high decreases in population indices early in evaluations and slower colony kill with insect growth regulator (IGR) baits. Since both the BASF test product as well as the standard bait performed in a similar manner, both very slow to show significant activity in colony kill, but both showing significant decreases in population indices, this would indicate more of an environmental impact on the biology/susceptibility of the insect to the baits, rather than the BASF test product being an IGR. However, since mode of action on this numbered material was not disclosed, these comments are speculative only. Additional testing under more normal environmental conditions next year would be warranted to provide better data for this BASF product since it did perform very well considering the conditions.

## REFERENCES CITED:


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

Lofgren, C. S. and D. F. Williams. 1982. Avermectin $B_1a$, a highly potent inhibitor of reproduction by queens of the red imported fire ant. Jour. Econ. Entomol. 75: 798-803.

CPHST PIC NO: A2F02/A2M02

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

TYPE REPORT: Final

LEADER/PARTICIPANTS: Anne-Marie Callcott and Lee McAnally

## INTRODUCTION:

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

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

## MATERIALS AND METHODS:

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

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

RESULTS:

Initial aerial applications by AEO occurred in 2002 in 3 states, with a second application done in one of the original states. SIPS is providing coordination of pilot and airplane, technical expertise, and ground support for the aerial treatments. Due to complicated state regulations APHIS is not aerially treating in Florida and in 2003 SC decided to use a local applicator for logistical reasons. SIPS personnel assisted with initial aerial applications at all sites treated by AEO, and assisted with follow up applications when needed. Aerial treatments were completed in spring 2002 in MS and TX; and during the fall in TX and SC. In 2003, spring aerial applications were completed in MS and OK; and repeated in the fall in OK and MS. Weather precluded a fall 2003 treatment in TX. 2004 treatments were greatly impeded by weather during the spring. Bait applications were made during 2004 in TX and OK in the spring and in MS in the fall. In 2005, treatments were made in OK and TX in the spring, and in OK and MS in the fall. In 2006, final aerial applications were made in OK and TX. The large scale treatments for this project have been completed, and data for the project are being collected by state cooperators and compiled by ARS. No detailed results have been released by ARS at this time.

Loading airplane with IFA bait.

Bait application – calibration is conducted at the airport prior to treating test sites.

CPHST PIC NO: A1F01/A1M01

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

TYPE REPORT: Interim

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

INTRODUCTION:

In a recent USDA-APHIS survey, seven southern states ranked IFA as a top priority target organism for biological control. Most research on phorid flies has been under the direction of ARS in Gainesville, FL. Phorid flies (*Pseudacteon spp*.) from South America are promising biological control agents of IFA because they are relatively specific to IFA, are active throughout most of the year, and through suppression of fire ant activity, may allow native ants to compete with IFA for food and territory (Porter 1998). Potentially, there may be as many as 15 species or biotypes of the fly that will have an impact on IFA, and thus are candidates for rearing and release in the U.S. Phorid flies will not be a stand-alone biological control agent for IFA. A homeowner will not be able to release a few flies in their back yard and see a significant decrease in IFA mounds in the yard. However, the flies will be an important tool in IFA management programs. It is anticipated that if several species of flies are established in the IFA infested area of the U.S. over the next 10 or more years, the added stress caused by these flies on the IFA colonies will allow native ants to compete better for food and territory. This fly-native ant-IFA interaction will hopefully allow homeowners, municipalities, and others, to make fewer chemical control product applications annually to suppress the IFA to acceptable tolerance levels, lessening the impact of the IFA on humans, livestock, wildlife and the environment. USDA, APHIS, PPQ began funding a cooperative project in 2001 to rear and release this potential biological control agent for imported fire ants.

MATERIALS AND METHODS:

Preliminary research and rearing techniques have been developed by USDA, ARS for two species, with other types under development. ARS will continue to evaluate other phorid fly species for potential use in the U.S., and transfer rearing techniques to the rearing facility as the new species are ready for mass rearing. Mass rearing of flies is being conducted by the Florida Department of Agriculture, Dept. of Plant Industries (DPI), in Gainesville, FL. The CPHST biological technician assigned to the rearing facility will continue to conduct small methods development projects aimed at improving efficiency of fly production and shipping (see CPHST PIC NO: A1F01/A1M01: Progress Report of IFA-Phorid Fly Rearing Lab, Gainesville, FL 2006). In 2003, a second species of fly was transferred to the FL-DPI rearing facility, but the rearing of the first species will continue for another few years for complete distribution. Currently (winter 2006) 5 attack (rearing) boxes are online producing one species of fly, *P. tricuspis*, 7 boxes are online producing the second species, *P. curvatus*, and 2 boxes were seeded with a third species of fly, *P. obtusus*, from ARS-CMAVE in August 2006 . Funding supplied in FY04 and sustained through FY06 through all sources enabled an increase from 12 boxes to 14 boxes. A total of 16 boxes are available for rearing, however 1-2 boxes are maintained for research purposes to improve rearing techniques such as those described in the report mentioned above.

Rearing of these flies is extremely labor intensive, requiring 1-1.5 person(s) to maintain every 2 attack boxes. These flies cannot be reared on a special diet or medium but require live fire ants to complete their life cycle. Excellent pictorial and text descriptions of the rearing technique is available online from the FL DPI at: http://www.doacs.state.fl.us/pi/methods/fire-phorid.html.

Very simply, imported fire ant workers and brood are placed in a pan (from which they cannot escape) within a large attack box where adult flies are allowed to emerge, mate and lay eggs within the worker ant. The parasitized worker ants are then maintained for ca. 40 days with food and water. As the immature fly develops, the larval stage migrates to the ant's head capsule. The head capsule of the ant falls off and the larva then pupates within the head capsule. Head capsules are collected by hand and either prepared for shipping to the field for release or are used to maintain and/or increase production. Adult flies live only a few days and are very fragile, therefore it is impractical to ship and release adult flies.

Release techniques for the first fly species, *P. tricuspis*, are also labor intensive. Originally, approximately 5000-6000 parasitized worker ant head capsules were shipped to the cooperator for each release. In 2004, numbers of head capsules shipped per release were increased to ca. 10,000. The cooperator must then place the head capsules in an enclosed emergence box and allow the adult flies to emerge daily over 10-14 days. Adult flies are then aspirated into vials, carried to the field and released over IFA mounds. The mounds are disturbed frequently for 2 hours to insure worker ants are available on the soil surface for the flies to attack. One "release" encompasses 10-14 days of daily fly collection and release over mounds. Detailed instructions are available on: http://cphst.aphis.usda.gov/projects/Phorid_rearing or http://cphst.aphis.usda.gov/projects/Phorid_monitoring/.

Release techniques for the second fly species, *P. curvatus*, are somewhat less labor intensive. Worker ants are field collected from marked mounds and sent to the Gainesville rearing facility. The worker ants are subjected to flies to become parasitized, and then returned to the collector to be re-introduced to their "home" mound to complete the fly's lifecycle.

Monitoring the success of the fly releases is conducted at a minimum annually. The best case scenario would be to monitor 2-3 times a years under optimum environmental conditions of temperature, wind, soil moisture, etc. Basically, monitoring involves returning to the original release site, disturbing several IFA mounds and visually looking for attacking phorid flies over a set period of time. If flies are found at the original release site, the cooperator moves a set distance away from the release site along the four cardinal positions and monitors for flies. Continue moving away from the original release site until no flies are found. Flies can be aspirated and submitted to this office for identification. Explicit instructions for fly monitoring can be found at the same CPHST websites mentioned above.

RESULTS:

*Rearing data*: Rearing was initiated in 2001 for *P. tricuspis*, seeded by flies from the ARS-CMAVE facility. The number of rearing boxes in *P. tricuspis* production has increased from the initial 1-2 boxes in 2001 to a high of ca. 10-12 boxes in 2003 to the current 5 boxes in 2006 to make room for an increase in *P. curvatus* production. Annual rearing of *P. tricuspis* was at its peak in 2003 and 2004 with ca. 1.6 million flies being produced, to the current 2006 production of 1.0 million (Table 1). *P. curvatus* rearing was initiated in late 2002, with the initial 1-2 boxes again seeded by flies from the ARS-CMAVE facility. By late 2006, 7 rearing boxes were in production. Production has dramatically increased from 121,000 in 2003 to 1.3 million in 2005 and 2006 (Table 2). Also in 2006, a third species, *P. obtusus* was brought into production. We expect the first releases of this fly in late 2007 or early 2008. Combined production for all 3 species in 2006 is shown in Table 3.

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

Releases began in spring 2002. In general, a release consists of ca. 5,000-10,000 potential flies (heads with pupae or infected worker ants) shipped to a cooperator in 2 or more shipments. In most cases, the cooperator made the release at one site, however, in a few cases; the cooperator split the release and released flies at more than one site. We have attempted to capture this information, but “releases” and “release sites” may not match at this time. From 2002 through 2006 there have been 2-10 releases in each of 13 states and Puerto Rico, with a total of 73 field releases (Table 3; Figure 1) and more than 629,000 potential flies released. Of these 73 releases, 53 were *P. tricuspis* and 20 were *P. curvatus*. A few releases were conducted at multiple sites, making the actual number of release sites closer to 75-76. Additionally, the equivalent of 3 *P. tricuspis* shipments have gone to Louisiana to seed their own rearing facility, the equivalent of 2 releases have gone to New Mexico for research purposes, one *P. curvatus* release was abandoned due to site issues, and numerous small numbers of flies have been supplied to cooperators for research or educational purposes, such as state fair exhibits and field days. Louisiana completed its first release from LA-reared flies in 2005. Over 111,000 potential flies have been shipped for these varied uses.

In the fall 2004, there were numerous hurricanes that impacted Florida, two of which impacted the phorid fly rearing facility. Electricity was off at the facility twice for 3 days each time during the 2004 hurricane season. This impacted the number of releases that occurred that fall. We anticipate 15-20 releases/shipments per year, and in 2004 only 12 releases were conducted (not including one that was terminated by the cooperator due to site problems). Despite hurricanes in 2005, only 2-3 potential releases in fall 2005 were impacted, with 17 releases that year, the best since the program was initiated. In 2006, 17 releases were made.

Success of the program is currently being measured by successful overwintering of fly populations. Of the 56 releases conducted in 2002-2005, flies have been found after a winter at

27 (48%) of these sites; 19 *tricuspis* sites (AL, AR, FL, GA, LA, MS, NC, PR, SC, TX) and 8 *curvatus* sites (FL, LA, NC, OK, SC, TX). Those sites at which flies have not been found have not been abandoned. Cooperators and others studying the flies are finding that it may take 2-4 years for flies to build populations that are easily detected in the field. Unfortunately, this was not known early in this program and many states have conducted multiple releases at the same site when they believed no flies were present a year after a release. As resources allow, all release sites will be monitored a minimum of yearly to determine fly presence. Once flies are found at a site, cooperators move out from the site and monitor to determine spread of the flies. Collection of fly data from cooperators is fairly good and new options on collecting and transmitting that data is becoming available. We have also asked that IFA populations at the original release site be monitored. This data is much slower coming in. Specific spatial data collected from releases and the subsequent monitoring of the ant and fly populations will be discussed in a future report.

Multiple releases of each fly species in each state are anticipated, depending on total acreage quarantined or generally infested within each state. Another CPHST project initiated in FY2003, utilizing spatial technology to assist in monitoring and evaluating the success of these fly releases (A3M02/A3F02), will hopefully allow us to more efficiently target sites and states where each fly species would be most successful in establishment.

REFERENCES CITED:

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

Table 1. Rearing and release data for APHIS phorid fly rearing project – *Pseudacteon tricuspis*.

| Species | Year | No. flies produced | No. pupae shipped* | No. field releases** | Mean flies/ release | Percent flies field released | Total flies shipped*** | Percent flies shipped |
|---|---|---|---|---|---|---|---|---|
| **P. tricuspis** | 2002 | 942,659 | 58,750 | 12 | 4,895.83 | 6.23 | 59,385 | 6.30 |
| | 2003 | 1,625,067 | 81,450 | 15 | 5,430.00 | 5.01 | 111,000 | 6.83 |
| | 2004 | 1,698,942 | 89,050 | 9 | 9,894.44 | 5.24 | 115,100 | 6.77 |
| | 2005 | 1,381,650 | 91,175 | 10 | 9,117.50 | 6.60 | 123,350 | 8.93 |
| | 2006 | 1,079,091 | 37,600 | 7 | 5,371.43 | 3.48 | 60,540 | 5.61 |
| | | | | | | | | |
| Total | | 6,727,409 | 358,025 | 53 | | | 469,375 | |

* approximate number of potential flies shipped for release
** does not include multiple shipments to LA for initiating their own rearing facility and NM for research purposes, nor multiple shipments to cooperators for educational purposes or small research projects when flies were available
*** shipped for all purposes: field release, initiate rearing, research, education, etc.

Table 2. Rearing and release data for APHIS phorid fly rearing project – *Pseudacteon curvatus*.

| Species | Year | No. flies produced | Approx. no. shipped* | No. field releases | Mean flies/ release | Percent flies field released | Total flies shipped*** | Percent flies shipped |
|---|---|---|---|---|---|---|---|---|
| **P. curvatus** | 2002 | 7,404 | 0 | 0 | 0.00 | 0.00 | 0 | 0.00 |
| | 2003 | 121,316 | 0 | 0 | 0.00 | 0.00 | 0 | 0.00 |
| | 2004 | 581,097 | 39,552 | 3 | 13,184.00 | 6.81 | 39,552 | 6.81 |
| | 2005 | 1,383,641 | 88,638 | 7 | 12,662.57 | 6.41 | 88,638 | 6.41 |
| | 2006 | 1,301,738 | 143,467 | 10 | 14,346.70 | 11.02 | 146,376 | 11.24 |
| | | | | | | | | |
| Total | | 3,395,196 | 271,657 | 20 | | | 274,566 | |

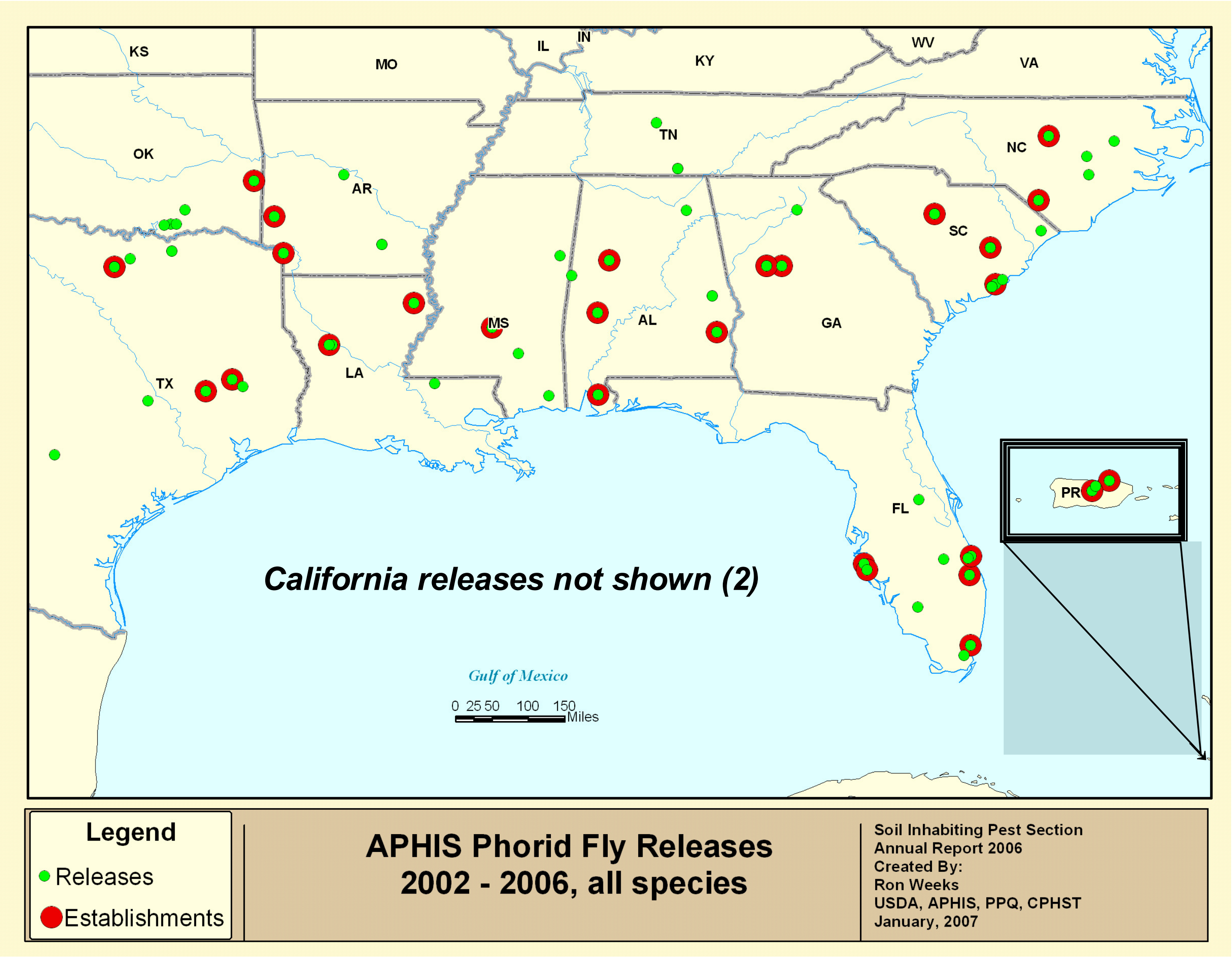
* approximate number of potential flies shipped for release
** does not include one attempted release that was abandoned
*** shipped for all purposes: field release, initiate rearing, research, education, etc.

Table 3. Rearing and release data for APHIS phorid fly rearing project – all species combined (*P. tricuspis, P. curvatus, P. obtusus*).

| Species | Year | No. flies produced | Approx. no. shipped* | No. field releases** | Mean flies/ release | Percent flies field released | Total flies shipped*** | Percent flies shipped |
|---|---|---|---|---|---|---|---|---|
| **tri,cur** | 2002† | 950,063 | 58,750 | 12 | 4,895.83 | 6.18 | 59,385 | 6.25 |
| **tri,cur** | 2003 | 1,746,383 | 81,450 | 15 | 5,430.00 | 4.66 | 111,000 | 6.36 |
| **tri,cur** | 2004 | 2,280,039 | 128,602 | 12 | 10,716.83 | 5.64 | 154,652 | 6.78 |
| **tri,cur** | 2005 | 2,765,291 | 179,813 | 17 | 10,577.24 | 6.50 | 211,988 | 7.67 |
| **tri,cur,obt** | 2006†† | 2,448,798 | 181,067 | 17 | 10,651.00 | 7.39 | 204,007 | 8.33 |
| | | | | | | | | |
| **Total** | | 10,190,574 | 629,682 | 73 | | | 741,032 | |

* approximate number of potential flies shipped for release
** does not include multiple shipments to LA for initiating their own rearing facility and NM for research purposes, nor multiple shipments to cooperators for educational purposes or small research projects when flies were available
*** shipped for all purposes: field release, initiate rearing, research, education, etc.
† only *P. tricuspis* shipped in 2002
†† only *P. tricuspis* and *P. curvatus* shipped in 2006

Figure 1. 2002-2006 phorid fly releases in APHIS program; both *P. tricuspis* and *P. curvatus* (multiple releases at some sites). Releases in CA (2 *P. tricupsis* and neither successful at this time) not shown on this map.



CPHST PIC NO:  A3F02/A3M02

PROJECT TITLE:  Geographic Information Systems (GIS) Program for Monitoring Decapitating Phorid Flies in Imported Fire Ant *Solenopsis* spp. Populations

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Ronald D. Weeks

## INTRODUCTION:

APHIS supports the rearing and distribution of phorid flies to state collaborators for releases in imported fire ant (IFA) infested states and Puerto Rico (see Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing Project A1M01/A1F01).  GIS (geographic information systems) is a useful tool that is used to organize and deliver phorid fly release and spread information.  This approach can be of immense value in targeting areas for efficient and effective phorid fly releases.  There are two components to this GIS project; 1) development of phorid fly tracking/data systems, and 2) a GIS based decision and management support program. Phorid fly release information collected for this project will provide regulatory officials a tool to monitor multiple phorid species releases, establishments, and spread.  Also, this tool will enable cooperators to select areas for effective releases and spread.

## MATERIALS and METHODS:

Data for this project have been collected in several ways; 1) via a web-based data entry site maintained at NCSU's Center for Integrated Pest Management, 2) handheld data collection units using GPS/PDA handheld units and, 3) paper datasheets (PDF docs) from the project website (http://cphst.aphis.usda.gov/projects/Phorid_rearing/).  Based upon data submission patterns and tendencies observed from cooperators, the web-based data entry site has been abandoned and taken down.  In its place SIPS has designed an in-house data management system to organize paper and email based information into an ArcGIS compatible format for basic reporting and data entry functions (Fig.1).  Several hand-held data entry devices (PDAs)  and digital survey forms in ArcPad's Mobile mapping Software ® are available for cooperators to use.  Using PDAs data can be entered into GPS/PDA units via customized application forms running in ARCPAD 7.1 (ESRI®).  Application forms were designed using ARCPAD Application Builder 7.1 (ESRI®).  Data are maintained using Microsoft Access® and ARCGIS 9 (ESRI®) software on a Dell® Precision 650 Workstation computer in Gulfport, MS at the Soil Inhabiting Pests Section (http://cphst.aphis.usda.gov/sections/sips/).

## RESULTS:

APHIS has released *Pseudacteon tricuspus and P. curvatus* at more than 70 locations throughout 12 IFA infested states and Puerto Rico between 2002 and 2006 (Fig. 2).  Of these releases, 53 were *P. tricuspus* and 20 *P. curvatus*.  Over-wintering, a measure of population establishment, has been detected at 19 of *P. tricuspus* locations and at 8 of *P. curvatus* release sites (Fig. 3).

Currently, all data related to APHIS phorid fly releases and surveys are being collected by state cooperators; state agricultural inspectors, university personnel, extension personnel, etc. Data from these organizations and state groups are being shared, complied and organized in this project (Fig. 4). As more phorid species are released and other organizations become involved, this program will provide regulatory officials a tool to monitor multiple phorid species releases, establishments, and spread. In the future, this GIS-Phorid program may be linked with other IFA control strategies or biological control agents, which would allow for estimation of their impact on IFA populations under different management scenarios.

Figure 1. Screenshot of SIPS Phorid fly data tool in Microsoft Access®.

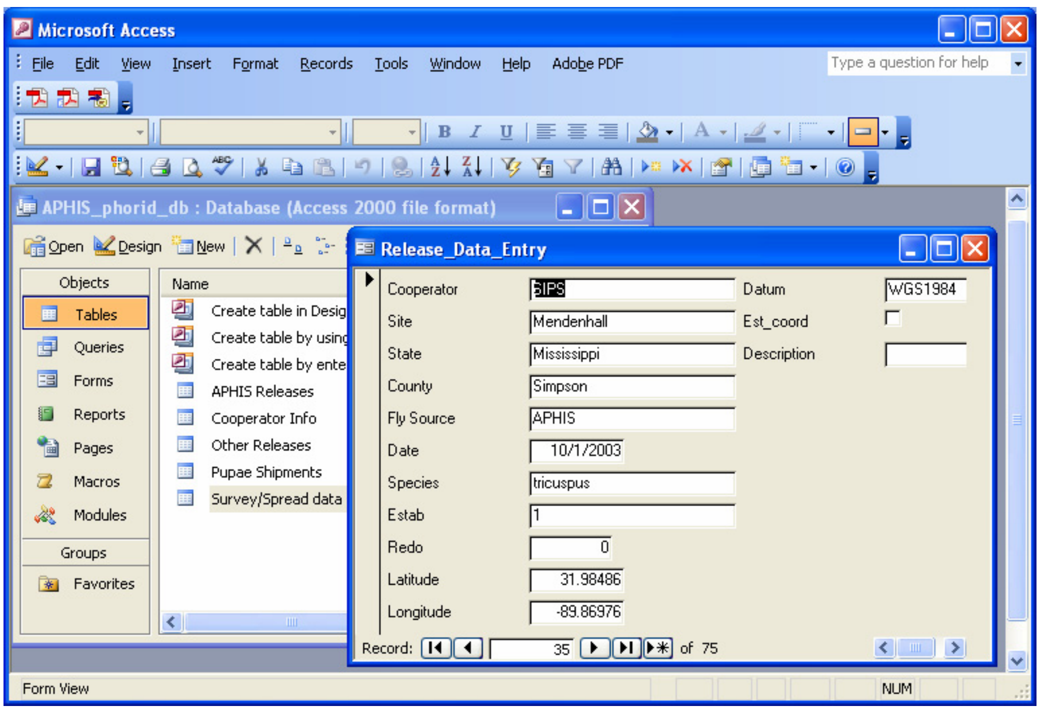


Figure 2. GIS Map of APHIS phorid fly release efforts (2002-2006).

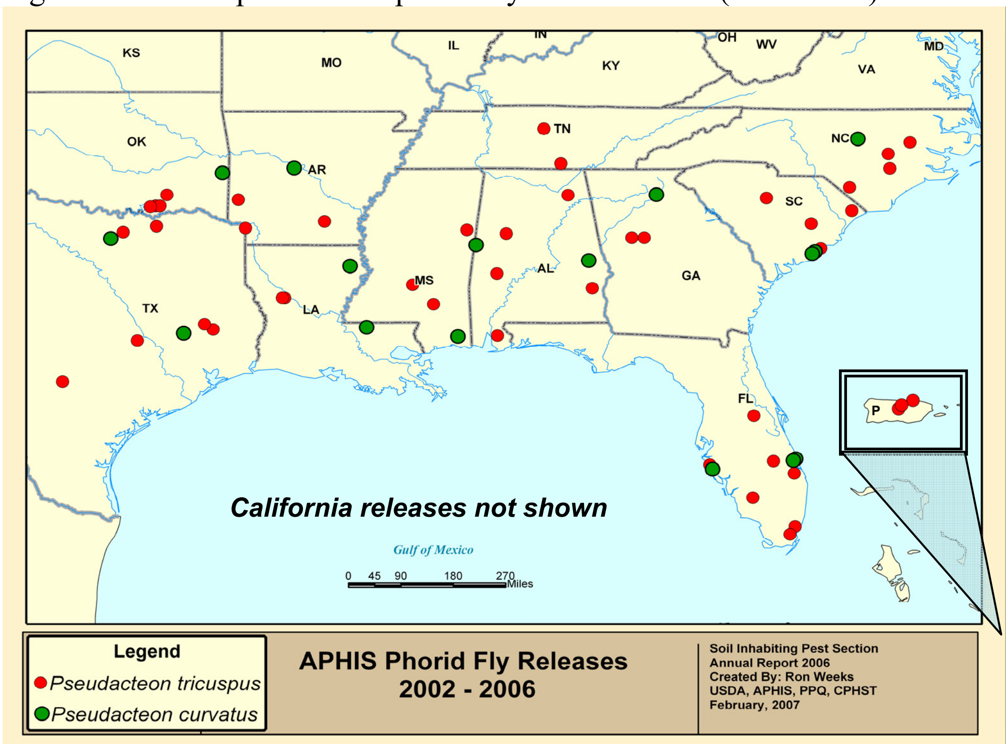



Figure 3. Established phorid fly populations from APHIS release efforts (2002-2006).

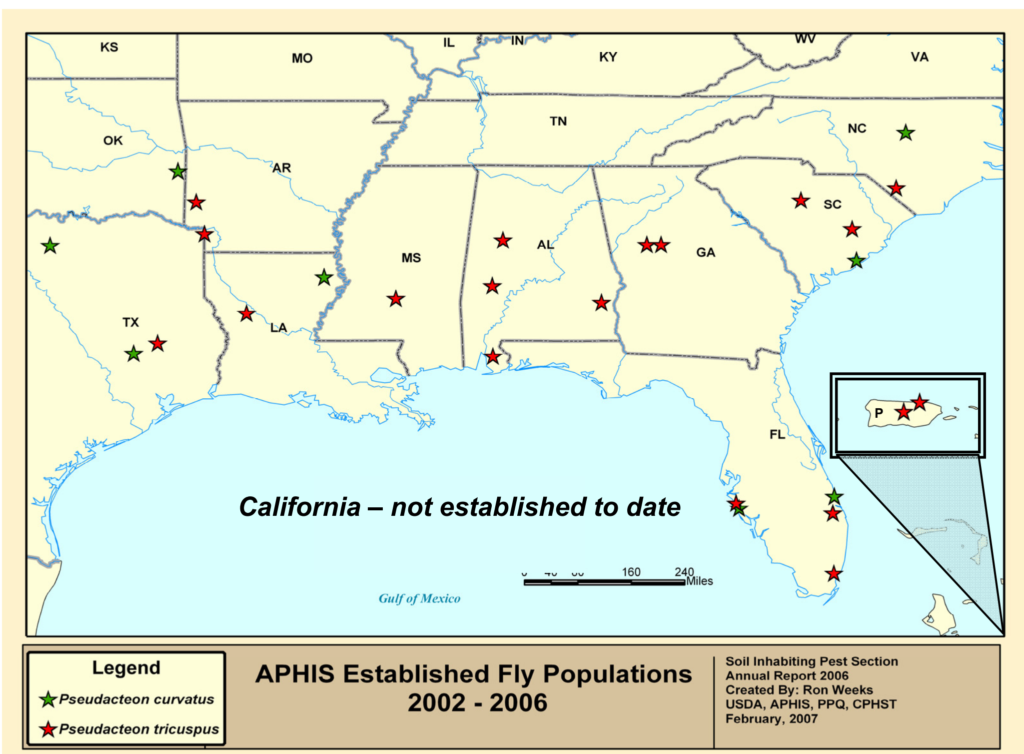
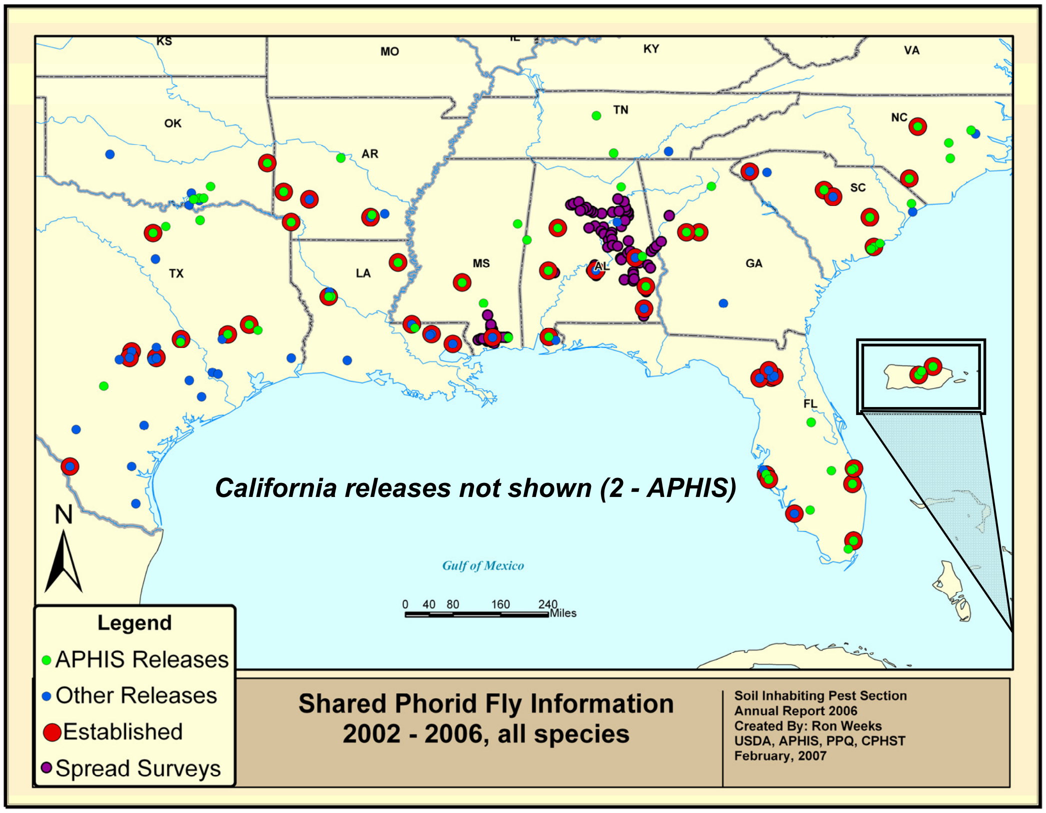


Figure 4. Phorid fly data reported by cooperators and USDA-APHIS personnel. Surveys in Alabama and Mississippi were conducted using GPS/PDA units. Continued survey effort, data reporting, and coordination are planned for other states.



CPHST PIC NO: A1F01/A1M01

PROJECT TITLE: Progress Report from IFA-Phorid Rearing Lab, Gainesville, FL 2006

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Debbie Roberts, George Schneider and FL-DPI personnel

2006 was a year of incredible difficulties due to the weather. In the absence of devastating hurricanes, we endured record breaking drought. Going into the month of December this region of north central Florida was still more than 15 inches behind on annual rainfall. Rivers turned into slow moving streams, ponds that were normally full were reduced to mud holes. Mounds were small and difficult to find. In addition we had our coldest weather of the year earlier than usual, with temperatures dipping below the freezing mark several times in early November. All this combined put a major strain on both the ant population and brood availability. Other regions as well were drought stricken and the ants we received for parasitizing were few in number or weakened by prolonged dehydration.

In an effort to decrease the demand on the already taxed IFA ant trailer group, six boxes were taken out of production in May (three per species). The trays were combined into the two corresponding boxes to boost the number of attacking phorids. The amount of brood that is normally put in with each pan of ants was reduced from 1 gm to 0.6 gm or as little as 0.4 gm in some cases. Brood production to this date still has not fully reached sufficient levels and the reduced amount is still being given.

Boxes were brought back into production slowly and only as the availability of resources allowed. As was done last year only this time on a much larger scale, ants were collected for the sole purpose of being exposed out in the field. This netted a total of 103,154 flies (15,481 *tricuspus* and 87,673 *curvatus*) that were put in to the production boxes. The following chart covers numbers over the span of the program to date.

| | Species | Production | Shipped |
|---|---|---|---|
| 2006 | *P. tricuspus* | 1,079,091 | 68,190 |
| | *P. curvatus* | 1,301,738 | 153,344 |
| | *P. obtusus* | 67,969 | 0 |
| 2005 | *P. tricuspus* | 1,381,650 | 125,580 |
| | *P. curvatus* | 1,383,641 | 79,008 |
| 2004 | *P. tricuspus* | 1,698,942 | 114,800 |
| | *P. curvatus* | 581,097 | 44,040 |
| 2003 | *P. tricuspus* | 1,625,067 | 111,810 |
| | *P. curvatus* | 121,316 | 0 |
| 2002 | *P. tricuspus* | 942,659 | 59,385 |
| | *P. curvatus* | 7,404 | 0 |

## Shipments

The following is a breakdown of the shipments of *P. tricuspus* and *P. curvatus* for this year in comparison to the four previous years.

| | | 2006 | 2005 | 2004 | 2003 | 2002 |
|---|---|---|---|---|---|---|
| Field Releases | | 7 | 9 | 9 | 15 | 12 |
| | | 10 | 7 | 4 | | |
| Fairs/Demos | | 3 | 9 | 4 | 6 | 3 |
| Research Projects | | 10 | 4 | 3 | 2 | - |
| | | 6 | | | | |
| Box Start-up | | 1 | - | 1 | 1 | - |
| Total | *P. tricuspus* | 68,190 | 125,580 | 114,800 | 111,810 | 59,385 |
| | *P. curvatus* | 153,344 | 79,008 | 44,040 | 0 | 0 |

The number of releases of *P. tricuspus* this year was only slightly fewer than that of the last year, but the total number of heads was significantly less. This can be directly correlated to the decrease in number of boxes and the effect of the drought. Whereas in the past we may have been pulling from a tray that contained 2000-3000 heads, we would have had trays with as few as 600 heads to pull from. Some trays would have only 200 or so heads; if a tray did not contain at least 600 heads it was not used. Effort was made to send as many parasitized heads as possible, yet not diminish the emergence in the box over any given time to a condition where we were only penalizing ourselves by removing them.

*P. curvatus* numbers on the other hand were way up, partly due to the availability of phorids outside of the laboratory. When we received a large shipment of ants we now had the luxury of splitting the amount going into an attack box and place some outside; if two shipments were received simultaneously they could be split. Frequently the number of attack phorids in the outside pans would be so great we could only assume these ants were better parasitized than those in the production boxes. This could change daily though, as production boxes strengthened and weather conditions would deteriorate. Slightly cooler temps, heavy winds or overcast skies would often preempt or end an outside exposure altogether. This method is not reliable and can only used to increase the number of releases during the optimal times.

In an effort to supplement the traditional method of "receiving" ants for a *P. curvatus* release method, we did a few trials of shipping out entire local colonies that retained the queen and had been exposed for several days to attacking phorids. Two states, Oklahoma and Mississippi, received three each of these parasitized colonies. Oklahoma has reported back that after some extremely cold weather, two of the three colonies were still surviving. This alternate method will be tried again in 2007, with more and larger colonies. If successful it could reduce overall costs, *i.e.*, shipping cost would go down, and time in the field by the releasers would be cut significantly.

**Updates on previous experiments**

Repairs to Rubbermaid #6 pans lids: Plasti-Dip has proven to be an excellent repair method over the long term. Periodically lids are sorted out post washing and the broken ones will have Plasti-Dip applied liberally to the new breaks. Occasionally old repairs are stripped off and a new rubber coating is applied. For a very small investment (approximately $7.95 a can) the life of the lids has been extended by years (these containers were all purchased in late 2001-early 2002).

Shipping Containers for *P .curvatus:* The Lock and Lock containers have not failed any time in the past year of shipments, by this I mean there have been no ant escapes. The one component that has proven inadequate is the nest tube binding wire. Plant tie wire, a very thin plastic coated wire was used to hold the nesting tubes in place. After only one or two uses, this wire would tend to break off at the point it came through the bottom of the container or where it was twisted together. Another wire was substituted…red and white bell wire purchased at Lowe's. It is relatively inexpensive, easy to work with and long lasting.

Nylon Eyebolts/Strings: The nylon eyebolts have been in the boxes over a year now. Not one has failed, as was expected. The longevity of the string (twine) has been extended well beyond the time it previously was had been. In the past we may have had to replace a string every month or so, now it could go upwards of three months before we do so. This has made it unnecessary to find an alternative.

Fluon Replacement: DuPont informed us about five months ago, that they would no longer be manufacturing Teflon PTFE T-30 due to the constituent C8 and its links to cancer. Teflon PTFE TE-3859 has now totally replaced the old product. Experiments were done to see if it would work as well. The only possible difference is it seems to fail somewhat more quickly after exposure to high humidity. Regardless there is nothing to replace this product, so we are stuck with what ever attributes it has. The change over was seamless.

**New Improvements**

Vacuum Lines: During the daily routine of "breaking down the attack boxes", one of the duties is to remove the trash by means of vacuuming out the pans; this reduces the amount of debris that gets caught up with the brood. Portable vacuum pumps carried from room to room and box to box are cumbersome and slow the process down. The main vacuum system is extremely strong and can handle many vacuum ports working at one time (there are four in place at the scopes and two in the dead pulling area). The addition of one more seemed unlikely to tax the system.

Initially long vinyl hoses (60-80 ft) were attached to one of the front work station vacuum ports. The process worked well, but impeded the start up of one of the technicians pulling the dead and caused a walking hazard. A design was drawn up to run a split off from the main line through the wall providing a vacuum port in the Attack Room 2 and then back through the wall into Attack Room 1. Maintenance was able to install the entire set up in only a couple of hours. Vacuuming out the attack boxes now takes about a quarter to a third of the time that it was taking.

Vacuuming Containers: There are several critical points in the work stream that involve the use of the vacuum system. The collection of parasitized ant heads is of utmost importance and has at times in the past been compromised by a hole in the organdy cloth filters *i.e.* heads being drawn through the entire vacuum system and ending up in the glass traps just prior to the pump. In order to prevent this from happening, a new type of filter was needed. First of all the glass tubing was replaced in all the rubber stoppers for the vacuuming flasks with ¼" copper tubing. This solved the problem of frequently broken tips.

After trying several different types of fittings, a copper 3/8" X ¼" adapter was selected. To this was soldered a very fine brass mesh that was acquired from Dr. Porter (it had been removed from a brass sieve). The mesh was carefully soldered to the wide end of the fitting, brass wire placed on the outside of the opening and folded down, and the non-lead solder was applied. On the opposite end a small piece of silicone hose was cut approximately ¾" long and inserted into the opening. This seats the filter snuggly on the copper tubing that runs through the rubber stopper where the vacuum hose is attached; the design makes it easily removable for cleaning. Only one of the nine filters has failed, due to someone scrapping it excessively while cleaning and tearing a hole in the brass wire. Four additional filter units were constructed for Dr. Porter.

Collecting Equipment: Due to the increased number of *P. curvatus* releases, a more efficient method of collecting ants in the field was looked at. A modification from the three stick method is still being evaluated.

This involves a 4 ½" wide piece of PVC pipe cut in to 8 ½" lengths. At one end two holes are drilled across from each other and a 5" long ¼" wide bolt is run through the holes and nut screwed on; this becomes the handle. Approximately seven inches is fluoned, by dipping, coating both the inside and outside. The pipe can be placed in an ant mound and removed several times without disturbing the mound too much. The ant covered PVC pipe is then banged into the bottom of a five gallon container that has been fluoned. Initial trials of the pipe proved it to be too slick for the ants to hang on to, so roughing up of the surface may be necessary. More trials will be performed this coming spring.

Castone Pyramids: It has been observed that some phorids prefer to attack ants on a vertical surface, be it the side of a cup or castone block or walls of an ant mound. In an effort to capitalize on this behavior, experiments were done with different types of material. We finally found that disposable conical paper cups made a great mold for a castone pyramid. This provides a tremendous amount of surface area as well as moisture to the ants while being attacked. These have been used extensively when exposing ants out in the field. Variations include a brass wire hoop that slides down over the top of the pyramid and leaving two stick like appendages. Chunks of apple can be stuck on this wire attracting more ants causing more phorid attacks.

Too Many Free Flies: When breaking down the attack boxes, the screen doors must be opened for an unspecified amount of time. This task is performed prior to the lights coming on; however preventing phorids from escaping the box is unavoidable. In an effort to try and recapture some of these free flying phorids we have undertaken the routine of placing extra ants into a white pan, providing them with water tubes and castone pyramids. The flies are attracted to the ants and are allowed to attack them for most of the day. At the end of the day, the flies are aspirated up and placed back into one of the attack boxes. The ants are utilized as well. If attacks were numerous, the ants are removed from the room and treated just as those in the attack boxes. If attacks were minimal, then they are saved until the next day and subjected to more attacking. It's a win/win situation. These ants have been used to supplement the boxes that may have been lagging in number.

<u>Brood Room:</u> The brood room was moved from the IFA trailer into the main building the last week of November. The new location is the southwest corner of the building through the *Diaprepes* rearing rooms. Morgan Swiers, supervisor of the IFA trailer noted that there was initially an improvement in production, followed by a drop; production is once again on the upswing. He thinks the temperature of this room is a little too unstable and that a secondary heat source must be added. At present besides the central air and heat which cannot support the elevated temperature need, there is a floor radiant heater.

<u>Drip System:</u> After a couple of years of use, the outside drip system had begun to fail. Many of the ports were clogged or not working for one reason or another. A new, expanded system utilizing brass fittings rather than the plastic lawn irrigation fittings has been installed increasing the drip capabilities from eight colonies to thirteen. Seven drip lines are now in place inside the trailer for utilization during the colder months.

<u>Increased Production:</u> Changing the time of setup from late afternoon to early morning has increased the number of *P. curvatus* sized ants for production. All other sieves are now set up by noon. These ants are all supplied with food and water during the sieving process, cutting die-off to a negligible amount.

In addition to the traditional method of extracting the brood, Morgan has added increasing their activity by stirring them up while trying to get the brood separated. Sieving excess dirt also increases the brood yield.

A dehumidifier and an air purifier were added to the trailer to improve the air quality and working environment.

<u>Vehicles:</u> For a brief period of time the state van that is utilized in collecting ants was broken down. Repairs included replacing a cracked rotor and a broken water pump along with some general maintenance. During this time technicians doubled up working with the USDA Colorado.

<u>Summary:</u> While there have been many setbacks, personnel voids and weather related problems, this year has also brought us a new species that is producing good numbers, given us many learning opportunities and ways to fine tune old methods and the satisfaction from hearing success stories from the field. While production has become very routine, there is comfort in knowing that there is security in the sameness of the job, and despite the adversities success is still attainable.

CPHST PIC NO: A9F03/A9M03

PROJECT TITLE: Mississippi Phorid Fly Release Project

TYPE REPORT: Interim

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

## INTRODUCTION:

Imported fire ants are pest of agricultural, environmental, urban and medical import throughout the southern half of the United States. Within their native range in South America fire ants are encountered less frequently, with fewer nest mounds per acre, and with fewer individuals per nest (Porter et al. 1992, 1997). It is speculated that lack of natural controls in the U.S., namely parasites and disease, have been responsible for this difference of abundance between the native and introduced populations of imported fire ants (Buren et al. 1978; Porter et al. 1997; Stimac and Alves 1994). The use of a complex of biological control agents through an integrated pest management program may be a successful long-term management tool for imported fire ant. Dozens of potential biological control agents have been identified in South America, and a few have been imported into United States to determine potential for release.

Species of *Pseudacteon* (phorid flies) are dipteran endoparasites of the *Solenopsis* genera of ants and are widely distributed throughout the *S. invicta* and *S. richteri* native range. Phorids impact fire ants both through parasitic destruction of individual ants and cessation of ant activities when the flies are present (Morrison 1999). Testing conducted by ARS-CMAVE determined *Pseudacteon tricuspis* safe for release in the United States. To assess their ability to establish in the wild, phorids were released by APHIS SIPS in the spring of 2000 in Harrison County, MS. Initial success of this release and others conducted in several IFA-infested states supports the rearing and distribution of these parasites, which is now conducted through the AHIS phorid fly rearing and release project, the activities of which are detailed elsewhere in this annual accomplishment report. Results from the initiation of new releases and continued observation of established releases, as described in this report, will be used to develop and improve methods for large scale release programs.

## MATERIALS & METHODS:

A release site near Saucier, MS (Harrison Co.) and a paired control at the Harrison County Work Farm were selected for the study. The sites were ca. 20 km apart. Each site was similar in habitat at the time of the release, consisting of grasslands with deciduous woods and a large pond adjacent. Both the release site and the control site had ca. 100 active mounds per hectare. Conversion of the release site into a pine tree farm in 2001 moved all subsequent checks of the release site to the adjacent roadside, consequently negating the relevance of the control site.

Emerged adult flies of *P. tricuspis*, supplied by S. Porter, were released daily, per the protocol supplied by S. Porter, at the Saucier site on 11 April, 2000 with the final release occurring on 20 April. A total of 2612 phorids were released on 45 separate imported fire ant colonies. The successful establishment of phorids in the first MS release and the development of the APHIS phorid fly rearing and release project have permitted subsequent releases at other locations in southern MS. In August 2002, over 2000 phorids were released on 42 IFA colonies at the Hattiesburg Airport (Bobby Chain Air Field – Forrest Co.). Another 3000 *P. tricuspis* were released in a pasture on October 2003 near Mendenhall, MS (Simpson Co.) in collaboration with the Mississippi Department of Agriculture. These two later releases both followed the open-mound release protocol available online at:

http://cphst.aphis.usda.gov/projects/Phorid_rearing/index.cfm

wherein each morning 20-30 flies are aspirated into individual vials and enough mounds are opened at the site to accommodate releasing two vials each.

Post-release monitoring is conducted by opening ten IFA mounds at the release site. Mounds are "opened" by removing half a shovel full of nest soil crating an open depression in the nest. Ants are intentionally mashed in this process to increase alarm pheromone release which attracts the flies. Flies at open mounds are counted over a 30-minute period. If flies are present then the process is repeated at sites 200 m along the cardinal points from the original release. If flies are present at these locations, then the distance from the initial site is doubled and checked again. Lack of flies at a remote check site requires traveling half the distance back to the last positive site and checking again. This process is repeated until range is established. A year after release, monitoring for flies is initiated at 2 km away from the release site or at the furthest positive sites from the previous check date. Initial post-release observations are conducted three months later; this is enough time that any flies observed should be second generation or later. Subsequent checks are conducted in spring and fall.

RESULTS:

Current survey methods are extremely labor intensive, requiring a great deal of time at each survey point. A new phorid fly trap that can be quickly set at a survey point and retrieved in 24 hours is under investigation by universities and ARS and we also hope to test its efficacy spring 2007 for use in the fall surveys. This trap will allow faster, more efficient phorid fly surveys.

Three months after release all three sites were confirmed to have flies present. Subsequent surveys of all sites have yielded different data. The Mendenhall site has only been surveyed once or twice since the original survey due to lack of resources by Mississippi Dept of Agriculture and limited other resources to pick up the survey. We do hope to re-survey the site and adjacent areas in 2007-2008.

Over the past seven years the Saucier release maintained fly presence and the area of fly coverage has increased. In fall 2006, extremely limited resources limited our ability to conduct a full survey of the south Mississippi area for phorid establishment and spread. Survey was only conducted due west of the Saucier, MS release site showing fly spread of ca. 65 km (40 mi) to the west (Figure 1). Spread in other directions for 2006 is extrapolated from the western spread and previous years. The most recent full survey was conducted in June 2005 and gave confirmation of fly spread 62 km (38 mi) north, 36 km (22 mi) east, 16 km (10 mi) south, and 34 km (21 mi) west of the original release site (the green area in Figure 1). This area for 2005 is approximately 4118 $km^2$ (1589 $mi^2$).

After the first monitoring date, no subsequent observations at the Hattiesburg site produced confirmation of fly presence. However, the furthest fly active point north of the Saucier site is at a greater distance than the other points for that site and is only about 9 km from the Hattiesburg release site. It is possible that the Hattiesburg release has been successful in establishing phorids in the area but due to unfavorable elements, such as an almost constant breeze, we have not detected flies at the release site itself. Conversely, about one hundred phorids were counted during the spring 2005 survey of the roadside at the original Saucier release site. One notable difference between the two locations is the Saucier roadside has a thick row of trees along it which provides a wind break. Similarly four out of five mounds abutting a wooded area at one remote site in the fall 2004 monitoring session had phorid activity while the remaining mounds next to an open field had none.

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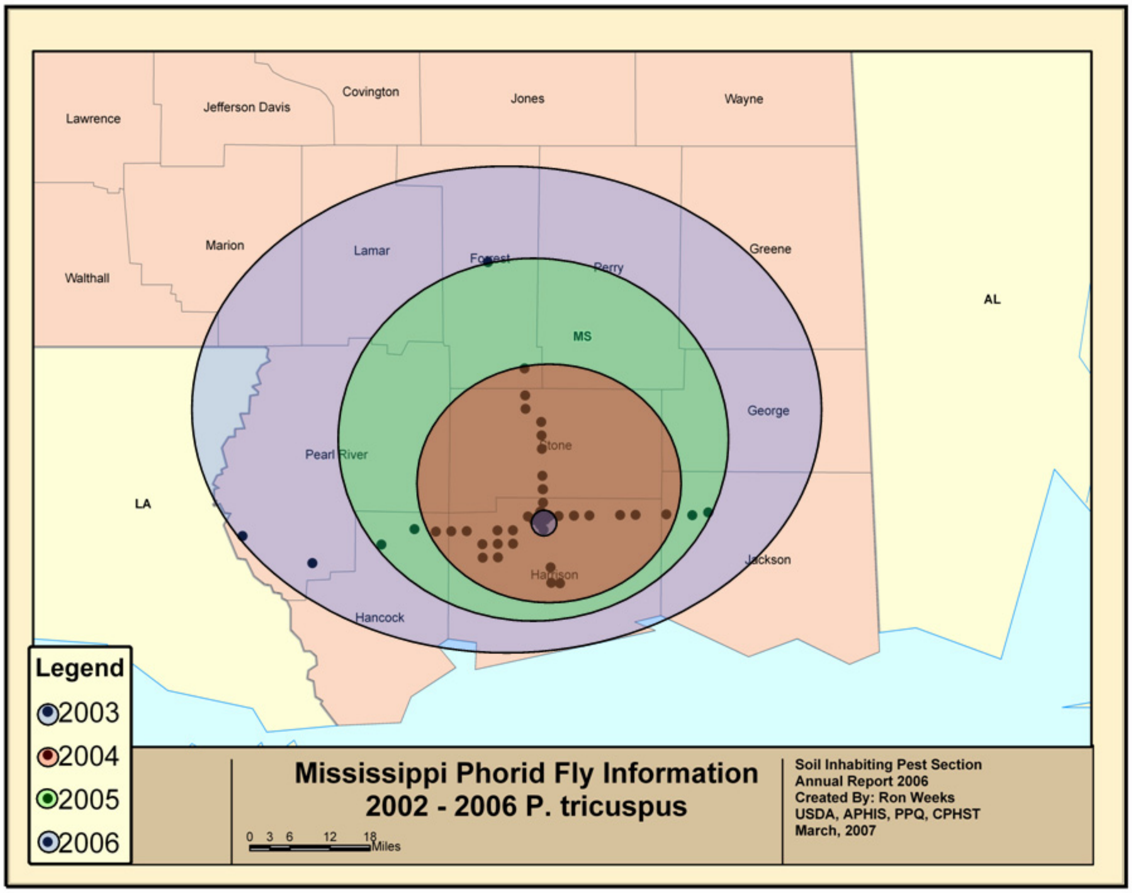
Stimac, J.L. and S.B. Alves. 1994. Ecology and biological control of fire ants. Pp. 353-380. *In* D. Rosen, F.D. Bennett, and J.L. Capinera [eds.], Pest management in the subtropics biological control: a Florida perspective. Intercept Ltd. Andover, Hampshire, UK.

Figure 1. Distribution of phorid flies, *P. tricuspis*, in south Mississippi 2000-2006.



CPHST PIC NO: None

PROJECT TITLE: Survey of Imported Fire Ant Populations in Areas Impacted by Hurricane Katrina Storm Surge in Harrison County, Mississippi

TYPE REPORT: Interim

LEADER/PARTICIPANTS: Anne-Marie Callcott and Tim Lockley (MDAC)

INTRODUCTION:

At the 2006 Imported Fire Ant Research Conference, Linda Hooper-Bui and Beverly Wiltz with LSU presented preliminary information on ant surveys in New Orleans post-Katrina. Their data showed a significant decrease in ant species, particularly IFA, in areas of the city that were flooded compared to surveys completed in the same areas several years prior to Katrina. The Mississippi coastal area was also flooded by storm surge from Hurricane Katrina, however the majority of the flood waters in Mississippi receded in about 8-12 hours, unlike parts of the city of New Orleans which were underwater for days or weeks due to levee system breaches and geography. To compliment the work in New Orleans, monthly surveys were conducted in Mississippi to monitor the populations of imported fire ants in Katrina flooded areas.

METHODS AND MATERIALS:

Due to limited available resources and other priority commitments a simple survey was initiated in April 2006. Three known flooded sites in close proximity to the Mississippi Sound were chosen as well as one known non-flooded control site, all within Harrison County, Mississippi. All sites were originally grassy, open public access areas that typically would have contained IFA pre-Katrina; parts, large rights of way, etc. The specifics are as follows:

Flooded sites – 3

2nd Street Park (1400 block of 2nd Street-corner of 2nd and Henderson; Gulfport, MS)
- Geographical coordinates = 89 04 34.452 W; 30 22 23.502 N
- ca. 600 ft. from Miss. Sound; homes adjacent to park standing but flooded
- E-W transect runs along north side of park
- prior to 9/5/2006 survey, park was "renewed" with tilling and shrubs planted in 70% of survey transect, irrigation added, and drainage adjacent/parallel to transect
- probably under water shortest period of time of 3 flooded sites

Episcopal Park (600 block of Magnolia Dr.-corner of Magnolia and East Ave.; Long Beach, MS)
- Geographical coordinates = 89 09 35.526 W; 30 20 39.450 N
- ca. 600 ft. from Sound; homes adjacent to park destroyed
- N-S transect runs south from northeast side of park

Scenic Dr. median (400 block E. Scenic Dr.-corner of Scenic and Fleitas Ave.; Pass Christian, MS)

Geographical coordinates = 89 14 22.530 W; 30 19 03.378 N

ca. 75 ft. from Sound; most homes destroyed/unlivable in this town

E-W transect in median between Hwy. 90 and Scenic Rd. starting just east of Memorial Park (Fleitas Ave.)

Probably under water longest of 3 flooded sites

Non-flooded/control site – 1

Bayou View Ball Park (4500-4600 block of Hewes Ave.-corner of Hewes and 47th St.; Gulfport, MS)

Geographical coordinates = 89 03 35.922 W; 30 24 24.816 N

N-S transect along road side area with crepe myrtle trees and ballpark fence

Bait transect surveys were conducted monthly. Transects were 200 feet long with 10 bait stations placed along the transect at 20 foot intervals. Stations were let in place for approximately one hour. Stations were collected, brought back to the laboratory, and frozen until identification and quantification could occur. Tim Lockley generously provided ant identifications for the survey. The bait attractant was the SIPS attractant developed by Dr. Robert Jones and reported in the 2005 Annual Accomplishment Report. Mound counts were initiated in May and made along the transect and 5 feet to either side of the transect, resulting in a 2000 sq. ft. evaluation area. Mounds were counted and evaluated by the USDA colony evaluation method (Lofgren and Williams 1982; Collins and Callcott 1995).

RESULTS:

In 2006, surveys were conducted April through November. No IFA were collected in any of the flooded sites in April 2006 (Table 1; Figures 1-4), but in May and June small numbers of IFA began to be collected at the bait stations gradually increasing to a peak collection in September 2006 for all flooded sites. The control site had a primary peak collection in June 2006 with a secondary peak in September 2006. One visible colony was detected at the Scenic Dr. flooded site at the May 2006 evaluation, but at the other flooded sites, visible colonies were not detected until July and August, even though workers were being collected at all sites by June 2006. Colonies initiated from newly mated queens require 2-3 months to produce more than 100 workers (Markin et al. 1973), and colonies of less than 100 workers are rarely visible in the field. This would account for workers being collected in bait stations even though colonies are not yet visible to the observer. Visible colony numbers in the flooded sites peaked in November 2006, while colony numbers for the control site peaked in May 2006 which is typical for south Mississippi.

Since we did not start collecting data until April 2006, more than 7 months after Katrina, we can not definitively state that the IFA were eliminated by the storm surge. However, since no IFA were collected in any of the flooded sites in April 2006 and numbers collected remained markedly lower than numbers collected in the control site, it is evident that IFA populations were significantly impacted by the storm surge. Collections will continue as resources allow in 2007.

Table. 1. No. IFA collected at various sites in Harrison county Mississippi.

| Date | Bayou view/Control | 2nd St. | Episcopal Park | Scenic Dr. |
|---|---|---|---|---|
| 4/14/2006 | 70 | 0 | 0 | 0 |
| 5/8/2006 | 201 | 3 | 0 | 0 |
| 6/13/2006 | 775 | 81 | 5 | 3 |
| 7/11/2006 | 348 | 83 | 114 | 66 |
| 8/8/2006 | 116 | 8 | 394 | 69 |
| 9/5/2006 | 563 | 349 | 1395 | 421 |
| 10/10/2006 | 265 | 46 | 93 | 0 |
| 11/9/2006 | 12 | 0 | 5 | 7 |
| | | | | |

Table 2. No. of non-IFA ants and non-IFA species collected per site.

| Date | Bayou view/Control | | 2nd St. | | Episcopal Park | | Scenic Dr. | |
|---|---|---|---|---|---|---|---|---|
| | No. ants | No. sp. | No. ants | No. sp. | No. ants | No. sp. | No. ants | No. sp. |
| 4/14/2006 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5/8/2006 | 0 | 0 | 36 | 3 | 0 | 0 | 0 | 0 |
| 6/13/2006 | 41 | 2 | 67 | 1 | 4 | 3 | 1 | 1 |
| 7/11/2006 | 597 | 1 | 232 | 1 | 16 | 1 | 323 | 2 |
| 8/8/2006 | 8 | 1 | 122 | 1 | 8 | 1 | 181 | 1 |
| 9/5/2006 | 306 | 1 | 41 | 1 | 0 | 0 | 0 | 0 |
| 10/10/2006 | 78 | 1 | 79 | 1 | 0 | 0 | 0 | 0 |
| 11/9/2006 | 10 | 1 | 94 | 1 | 0 | 0 | 0 | 0 |
| | | | | | | | | |

All non-IFA ants were *Brachymyrmex sp*. or *Pheidole sp*., and primarily *Ph. obscurithorax*.

Figure 1. Bayou View/Control site – IFA colonies vs. IFA workers collected in bait traps (no colony count in April 2006).

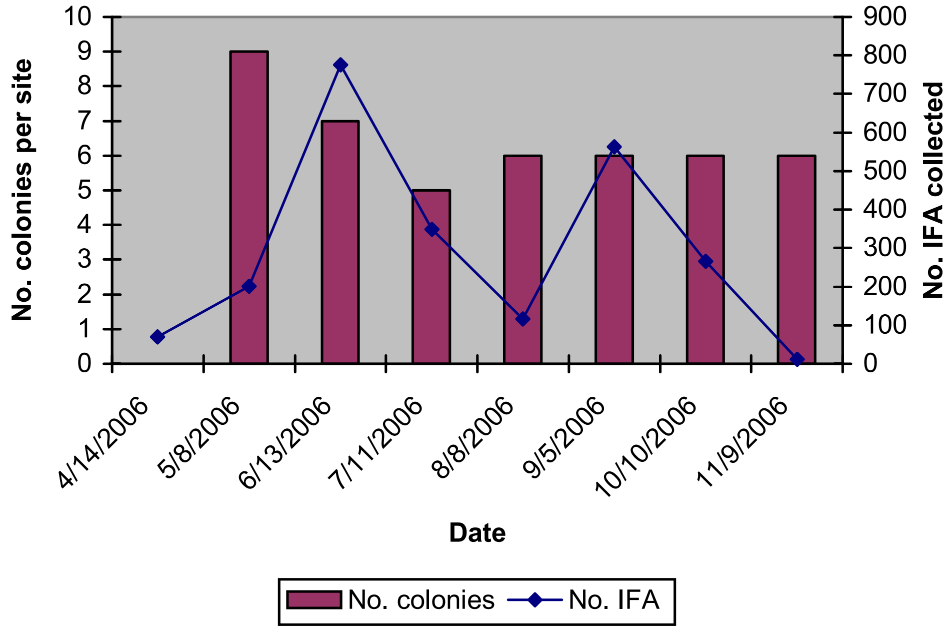



Figure 2. 2nd St. site – IFA colonies vs. IFA workers collected in bait traps (no colony count in April 2006).

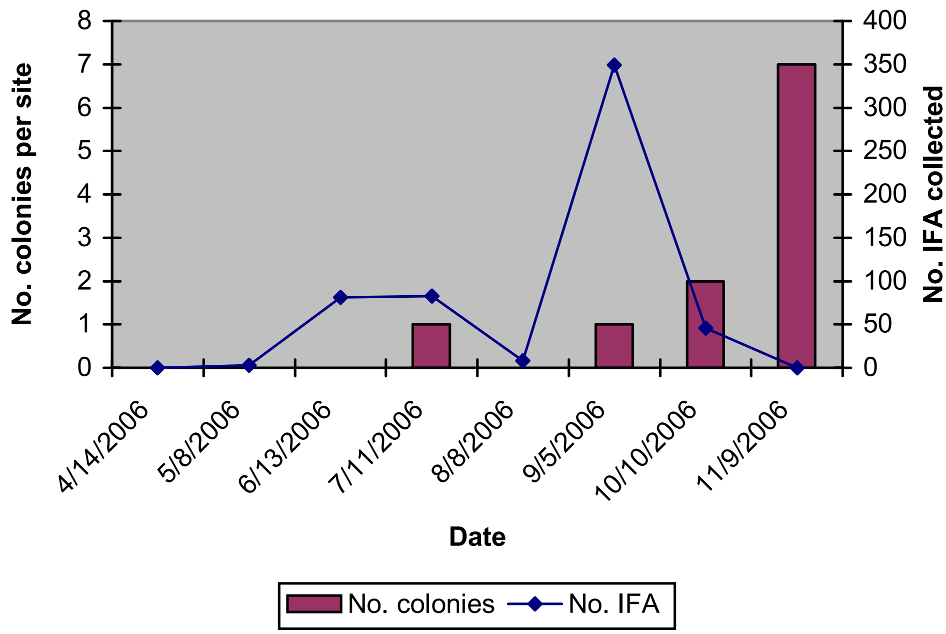


Figure 3. Episcopal Park site – IFA colonies vs. IFA workers collected in bait traps (no colony count in April 2006).

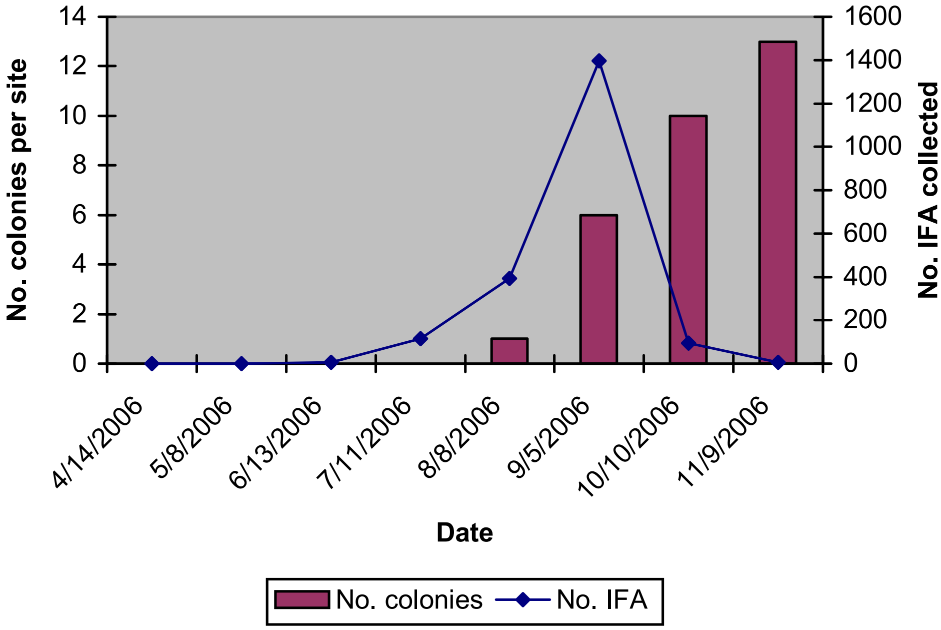
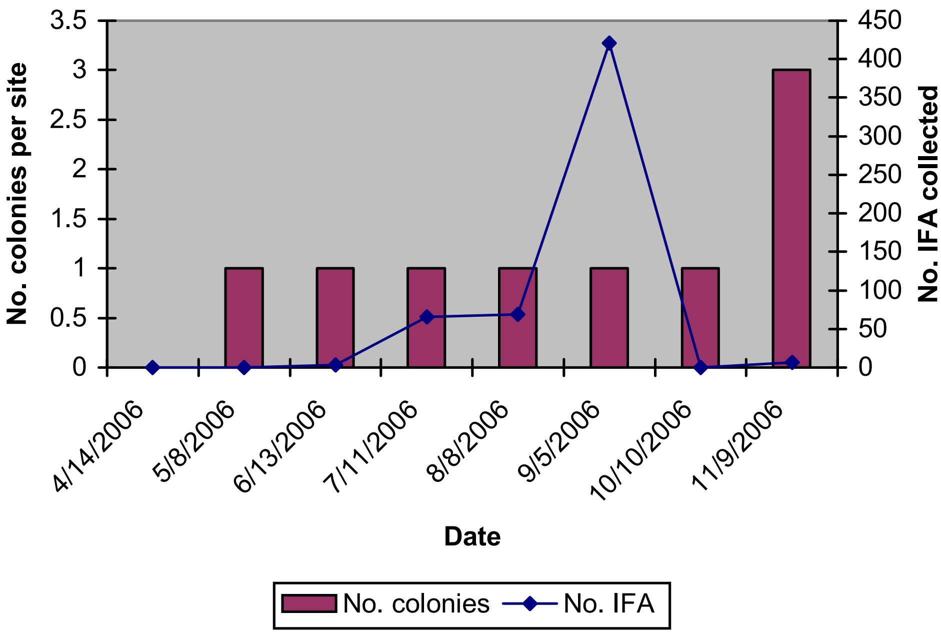



Figure 4. Scenic Dr. site – IFA colonies vs. IFA workers collected in bait traps (no colony count April 2006).



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CPHST PIC NO: None

PROJECT TITLE: Ad-hoc Projects

TYPE REPORT: Final

LEADER/PARTICIPANTS: Ronald D. Weeks

**Ad-Hoc Project Report "Development of Survey Collection Templates using ArcPad® for APHIS-IS Central American Pest Survey"**

Costa Rica is a key commodity trader of Central America's agricultural products and therefore is heavily involved in a number of key pest detection survey programs and initiatives to address a wide array of exotic pest threats and pathways. Many APHIS programs are in the process of identifying and adopting new technologies to assist ministry programs in the collection, maintenance, and distribution of pest detection related data. APHIS-IS is also working to reduce pests in shipments of agricultural commodities through a "Clean Stock Program". To that end, our goal was to streamline data issues for APHIS-IS and ministry survey programs in Costa Rica by developing an automated survey system to easily store and exchange key program data. Costa Rica possesses a great deal of talented individuals and resources that are poised to develop spatially explicit survey systems, however, they required some initial assistance and expertise in order to begin to fully utilize existing ArcPad technology.

For this project, CPHST scientist Dr. Ronald Weeks worked with lead APHIS-IS personnel in the design and development of several surveys using contemporary GIS (ESRI ArcPad® mobile mapping software) software and handheld PDA/GPS integrated tools. The goals of the project were 1) to provide onsite training and support for the development and integration of these state-of-the-art technologies and equipment, and 2) provide both the field users and the program specialist with a reliable, functional and user friendly tool to document and rapidly report pest survey activities to key personnel across agencies.

To accomplish these goals, Dr. Ronald Weeks worked directly with Mr. Hector Paniagua (APHIS-IS Program Specialist) and Mr. Marco Gonzalez (APHIS-IS Program Supervisor) under the leadership of Mr. John Stewart (APHIS-IS Liaison) in Costa Rica. Recently, Mr. Paniagua was tasked with learning the ArcPad® software to support survey development, distribution, and maintenance for several projects in Costa Rica.

The goal of this project was successfully accomplished through demonstration and step-by-step instruction of the technology used to develop several existing CPHST and PPQ surveys. Now, survey data is downloaded directly from the field and sent to the APHIS-IS database manager. If successful, this project will bring significant automation to the APHIS-IS survey and detection program in Costa Rica. To that end, APHIS-IS will realize significant time savings in the collection, management and dissemination of key survey data. Furthermore, APHIS-IS will greatly enhance the quality of data making their programs more efficient and effective. Also, this project will position APHIS-IS to be fully integrated in ministry survey programs which will provide valuable information regarding pests of U.S. concern meeting the mandates of the Safeguarding American Plant Health Resources initiative.

**Ad-Hoc Project Report "NAPIS SOD template for PDA based surveys"**

This report completes CPHST ad-hoc project "NAPIS SOD template for PDA based surveys". This survey template uses ESRI ArcPad® software a mobile-GIS application that operates on handheld digital devices (Personal Data Assistants – PDA'S) and was delivered to the project champions, Dan Williams and Jonathan Jones. The software may be customized to collect routine survey information more efficiently and accurately than typical paper based surveys. This SOD template captures and records data simultaneously between the field user, pre-loaded map layers, and GPS (Global Positioning Systems). NAPIS required field data is collected automatically off of pre-loaded state, county, and zip code maps on the PDA. Spatially explicit (e.g. latitude and longitude, GPS quality) information is collected and may be accessed continuously via GPS capabilities. The user interface is simplified through a series of pages and pre-defined pull-down menu choices. Menu choices use common survey terminology to convey and capture field data however, the data is recorded as properly coded NAPIS fields. The layout of the pages and how data is presented or entered can be modified as the program and end-user needs dictate. Field data is stored on secure digital cards and may be downloaded directly to a desktop database via ActiveSync® Microsoft.

This ad-hoc project has taken more time to develop than initially proposed. Most of the delay may be attributed to several programming challenges created by the project within the ArcPad programming environment. The main challenge was to collect multiple samples from hosts within a site and have each sample be represented in the database as a single line record without having to re-enter redundant site and surveyor information. This required a customized programming script to do this work automatically. Ms. Yu Takeuchi, a talented CPHST cooperator in Raleigh worked out the programming details and integration. Because this SOD survey relied on standardized CAPS and NAPIS defined data requirements this survey template may be shared with other CAPS surveys.

Field personnel interested in obtaining and using this SOD template can contact Dr. Ron Weeks, at CPHST for a copy. Included in this technology transfer package is a copy of the ArcPad template and associated files and maps, a general user guide, and power point presentation outlining the template's form and functions. This template should provide program managers and field users the conceptual knowledge and framework to modify this tool to might their needs more efficiently.

**Ad-Hoc Project Report "ArcPad Data Collection System for Florida CAPS Survey Technology Support Service"**

CPHST supported PPQ Florida state operations and lead personnel in the design and development of a survey template using contemporary GIS (i.e. ArcPad® mobile mapping software) software and handheld PDA/GPS integrated tools. Mr. Joe Beckwith, PPQ Pest Survey Specialist FL, served as spatial technology point of contact for this project. CPHST demonstrated working examples of surveys for phorid fly decision support and tracking (http://cphst.aphis.usda.gov/projects/Phorid_monitoring/) and CAPS (cactus moth). Useful features and routines included custom survey forms, application routines, and a PDA/GPS User's Guide. Other examples presented included NAPIS code integration, GPS functionality, data quality assurance standards, and some mapping capabilities. Ms. Yu Takeuchi developed several important sampling and mapping routines within the software to increase functionality and data quality. CPHST was available for several phone and email discussions for technical support and remains available for continued support and collaboration.

Under the tutelage of Mr. Beckwith, the PPQ survey staff in Florida has developed and deployed a survey system running GPS enabled mobile data mapping software (ArcPad®). Currently, they are capturing spatially explicit variables in response to an emergency regulatory action against citrus greening in both residential and orchard areas.

# APPENDIX I - LABORATORY BIOASSAY PROCEDURE

## PROTOCOL FOR BIOASSAY OF INSECTICIDE TREATED POTTING MEDIA/SOIL 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, and others may contain both males and queens, while some will contain only alate queens. Seasonal differences in the abundance of queens is quite evident; in the warmer months of the year 50% or more of the colonies in a given area may contain queens. However, in the cooler months, it is not uncommon to find that less that 10% of the colonies checked will contain an abundance of alate queens. Therefore, it is necessary to examine numerous colonies, selecting only those which contain large numbers of alate queens for collection. During winter, ants will often cluster near the surface of the mound facing the sun. Collection during midday on bright, sunny days is highly recommended for winter; whereas the cooler time of day is recommended for hot, dry days of summer. Once a colony (or colonies) has been selected for collection, the entire nest tumulus is shoveled into a 3-5 gallon pail. Pails should be given a liberal dusting with talcum powder on the interior sides to prevent the ants from climbing up the sides of the pail and escaping. Approximately 3-6" head room should be left to prevent escape. An effort should be made to collect as many ants as possible while minimizing the collection of adjacent soil which will contain few ants. Collected colonies are then transported to the laboratory for a 3-5 day acclimation period. The addition of food or water during this short acclimation period is not necessary. Alate queens are collected with forceps after placing a 1-2 liter aliquot of the nest tumulus in a shallow laboratory pan (Figure 1). Again, the use of talc on the sides of containers prevents escape while talced rubber gloves minimize the number of stings experienced by the collector. The forceps should be used to grasp the queens by the wings in order to prevent mechanical injury. An experienced collector can collect 200-300 queens per hour. It is generally advisable to place collected queens in a 500 cc beaker or other suitable vessel containing moist paper towels prior to being introduced into the test chamber.

Test chambers: Test chambers are 2.5" x 2.5" plastic flower pots which have been equipped with a Labstone® bottom. Labstone is generally available through dental supply firms such as Nowak Dental Supplies, 6716 Hwy 11, Carriere, MS 39426 (800-654-7623). The labstone bottom prevents the queens from escaping through the drain holes in the bottom of the pot and also serves as a wick to absorb moisture from an underlying bed of wet peat moss. Ants are susceptible to desiccation so humidity/moisture levels must be optimized. Pots should be soaked in water to moisten the labstone prior to placing potting media in the pots. The peat moss bed should be watered as needed to maintain a constant supply of moisture to the test chamber. Plastic petri dishes are inverted over the tops of the pots to prevent escape from the top of the test chambers (Figure 2). Prior to placing queens in the test chamber, 50 cc of treated potting media is placed in the bottom of each pot. Each test chamber with test media and queens is placed in a tray with a bed of wet peat moss (Figure 3). Due to possible pesticide contamination, test chambers are discarded after use.

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

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

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

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

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

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

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

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

Figure 1. Alate females being removed from nest tumulus.

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

Figure 3. Set up of bioassay test procedure.

Appendix II. Chart used to determine volume of balled-and-burlapped root balls.

**B&B Wire basket dimensions**

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

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

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