2012 CPHST Laboratory Report: Gulfport/Biloxi Station

United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Center for Plant Health Science and Technology

2012 CPHST Laboratory Report Gulfport/Biloxi Station

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Realignment and Redirection of CPHST Gulfport Laboratory

CPHST Laboratory, Gulfport, MS Closed September 7, 2012

CPHST AQI Lab, Biloxi Station (MS) Opened September 10, 2012

The CPHST Gulfport Laboratory in Gulfport, MS, consisted of two sections: the Analytical Chemistry section, and the Imported Fire Ant (IFA) section. The analytical chemistry section conducted routine sample analysis for detecting the presence of pesticide residues and toxic substances directly supporting ongoing APHIS Operational and Emergency programs including; Imported Fire Ant, Asian Longhorned Beetle, Boll Weevil, Grasshopper/Mormon Cricket, and Fruit Fly. In addition, the chemistry laboratory supported APHIS projects by providing chemistry based options for PPQ field operations concerning the identification and detection of prohibited commodities, or the detection of invasive insect species.

The IFA section developed methods and tools for the survey, detection, regulation, and control (both chemical and biological control) of the imported fire ant. Technology developed by the IFA section was utilized by PPQ, State Plant Regulatory Officials (SPROs), the nursery industry, chemical industry, farmers, homeowners, and other stakeholders.

In 2012, the closure of the Laboratory in Gulfport, MS was completed and the staff and activities were redirected and relocated. All carryover field and laboratory operations were completed in February-April 2012, and all activities were transferred to the CPHST Miami Laboratory or outsourced. The main activities of 2012 were associated with the closure of a 3.5-acre government facility that has been in existence for over 60 years. The primary PPQ mission related activities that remain with the Biloxi staff include:

- oversight of the pesticide residue analysis program for environmental monitoring samples associated with PPQ treatment programs
- oversight of development of imported fire ant (IFA) regulatory/quarantine treatments to support the Federal IFA Quarantine and the rearing and release of phorid flies (biocontrol agents of IFA)

Laboratory Closing Highlights:

- The Gulfport facility officially closed on September 7, 2012 when remaining PPQ staff relocated to new office space in Biloxi MS (includes S&T, FO and PM staff)
- Worked with tenants of Gulfport facility to assist in their relocation as needed; disposal of their excess property, relocating their staff to Biloxi site, etc.
- Over 90% of inventoried property has been transferred or will remain with property and over 80% of non-inventoried property has been transferred or placed on GSA for sale; remaining property will be listed for sale with GSA in 2013
- 18-wheeler loaded by Gulfport staff and shipped to Mission by Mission RMS staff
- 18-wheeler loaded by contractor and shipped to Miami as well as smaller rental truck loaded and shipped by CPHST facility technician
- Final safety review/audit for closure required by APHIS/CPHST was completed in August 2012
- All hazardous waste was removed by contractor in August 2012
- Contracts were cancelled or modified throughout the year to provide as much savings as possible (maintenance agreements on analytical instruments cancelled or decreased to only 6 months); lawn service cancelled March 2012 saving ca. $10,000 by obtaining a mower from Mission FMS for Facility Tech to use; janitorial modified from daily to 3X/week mid-year (saving ca. $3000), then cancelled Sept. 30, 2012; guard service cancelled Sept. 30, 2012; trash pickup decreased to 2X/week and will decrease to 1X/week by early 2013.
- All paperwork required by APHIS property staff prior to submission to GSA has been completed for both the Gulfport facility property and the county farm property.
- APHIS-ES must complete soil remediation prior to GSA accepting property for disposal. Remediation not expected to be completed until at least 3rd qtr FY13 and the state must then release the property. Unknown impact of sequestration on remediation plans. CPHST will continue to be responsible for protection and maintenance for up to 15 months after GSA accepts property.
- The facility technician will remain on the Gulfport property until transfer to GSA to provide protection and maintenance as required by GSA (anticipated through end of 2014)

Gulfport Laboratory— Google Earth

Publications and Scientific Meetings Attended by Gulfport/Biloxi Staff

2012 Publications Gulfport/Biloxi staff – none

2012 Scientific Meetings attended Gulfport/Biloxi staff

- Imported Fire Ant and Invasive Pest Ant Research Conference
    - ◊ Overview of the APHIS Imported Fire Ant Program – 2012. Anne-Marie Callcott, Charles Brown, Katherine Hough, and Ronald Weeks
    - ◊ Development of cold temperature techniques for certifying bulk soil for movement: Large scale trials. Karen Vail (University of Tennessee, Entomology & Plant Pathology, Knoxville, TN), Jennifer Chandler, Jeremy Shoop, Anne-Marie Callcott, Kevin Hoyt, and Richard Evans
- Southern Section of the AOAC International
- North American Chemical Residue Workshop (formerly Florida Pesticide Residue Workshop)

2012 Chemical Residue Sampling/Analysis Program Service Summary

Robert D. Smith

The Gulfport/Biloxi analytical chemistry section conducted routine sample analysis (or outsourced the analysis) for detecting the presence of pesticide residues and toxic substances directly supporting ongoing APHIS Operational and Emergency programs including; Imported Fire Ant, Asian Longhorned Beetle, Boll Weevil, Grasshopper/Mormon Cricket, and Fruit Fly. In addition, the chemistry laboratory supported APHIS projects by providing chemistry based options for PPQ field operatives concerning the identification and detection of prohibited commodities, or the detection of invasive insect species. Chemistry based work for PPQ field operations were transferred to the new CPHST AQI Laboratory in Miami in 2012.

Major Accomplishments Gulfport/Biloxi Chemical Analysis Program

- Changes to routine environmental monitoring sample analysis procedures
  - ◊ An interagency agreement was established with AMS (Gastonia, NC) for analysis of routine PPQ program environment samples with pre-determined per sample costs. A new reporting system was designed with AMS and EDP.
  - ◊ Environmental sample supply request forms were redesigned and staff worked with EDP to educate PPQ samplers on new request and shipping procedures; particularly new shipping address.
  - ◊ APHIS discontinued funding of sample analysis for the IFA program once the Gulfport lab closed
- Analysis of routine environmental monitoring samples
  - ◊ CPHST-Gulfport
    - October 1, 2011 - March 15, 2012
    - 152 samples (Table 1)
    - Quality Assurance and sample custody support provided
    - Notes: Samples run during shut down period with staff of 4-6, this resulted in slower than typical response times for ALB backlog.
  - ◊ AMS-SD-Gastonia (NC)
    - March 16, 2012 - September 30, 2012
    - 144 samples (Table 1)
    - Test sample analysis on known samples provided by Gulfport, between January-March 2012. Test results within expected parameters.
    - 4/16/12: Fiscal year start-up Interagency Outsource program based on fixed per samples analysis costs
    - Quality Assurance and sample custody support provided
    - Total per sample cost for all work conducted at Gastonia on FY12 program samples = $16,434.00
    - Additional start-up costs for establishing sampling custody and methods = $25,000 (1st year only).
    - Notes: All results reported well within agreement specifications. Reports provided noted related QC and noted LOD/LOQ pairs for analysis. Quality of data as expected. Pending APHIS-EC comments on data supplied in FY12.
- Other analytical chemistry support
  - ◊ Project with DHS Savannah Lab to determine origin of mangoes by elemental analysis successfully completed a second year in early FY12 and was renewed late in FY12 with AQI funds (not FB). R. Smith was able to successfully obtain mango samples from Dominican Republic, Puerto Rico and California on very short notice. All data adds to the model to make it more robust and effective in determining origin of mangoes. Due to a fire in the DHS laboratory in August 2012, they have delayed their final report on 2012 collected samples until 2013.

Table 1. Environmental Monitoring Samples Analyzed in 2012 to Support PPQ Programs

Unit conducting Analysis	Time Frame	Boll Weevil		ALB		GH/MC*		IFA	
		No. samples	Avg. turn around (days)	No. samples	Avg. turn around (days)	No. samples	Avg. turn around (days)	No. samples	Avg. turn around (days)
CPHST-Gulfport	10/1/11-3/15/12	7	3.5	72	14.6	-	-	73	na
AMS-Gastonia	3/16/12-9/30/12	8	7.75	119	37.9	17	13.4	-	-
Total		15		191		17		73	

* extremely light year for the GH/MC program which submitted <20 samples vs. 100-300 in an average year

Overview of the APHIS Imported Fire Ant Program – 2012

Presented at the 2012 IFA Research Conference

Anne-Marie Callcott, Charles Brown, Katherine Hough, Ronald Weeks

The Federal Imported Fire Ant Quarantine was implemented in 1958 and is cited in the Code of Federal Regulations, Title 7, part 301.81 (7CFR301.81). The goal of the present day quarantine is to prevent the artificial spread of imported fire ants. Regulated items include nursery stock, grass sod, hay, soil and other items that can transport IFA. The current regulated area includes all or part of 14 states and Puerto Rico (AL, AR, CA, FL, GA, LA, MS, NC, NM, OK, SC, TN, TX, VA) and approximately 366 million acres. Numerous models indicate the potential range of the IFA is greater than those areas currently infested with IFA, primarily in the western states (OK, TX, NM, AZ and CA).

Since the late 1980's, the federal IFA quarantine program has been implemented by the states. States are responsible for inspecting nurseries, issuing compliance agreements, surveys, and conducting blitzes with USDA. Oversight of the program is by USDA-APHIS-PPQ and includes development of quarantine treatments, transfer of information to states and enforcement, including investigations and fines associated with violations.

Oversight and management of federal IFA quarantine program is a team effort of PPQ led by the National Program Manager (PPQ-EDP), with team members from PPQ-ER, PPQ-WR and PPQ-CPHST. The PPQ-CPHST Lab in Gulfport MS is responsible for the development of methods and tools used in the IFA Quarantine for survey, detection, regulation and control. The group oversees the APHIS Phorid fly (*Pseudacteon* spp.) rearing and release program (biological control of IFA). Florida Department of Agriculture, Division of Plant Industries (FL-DPI) mass rears the phorid flies and state cooperators conduct releases. ARS-CMAVE, Gainesville, FL develops the rearing techniques and transfers them to FL-DPI.

Recent accomplishments of the APHIS, PPQ IFA program included several new IFA quarantine treatments that have been developed and validated over the last few years with data from PPQ-CPHST, Tennessee State University and University of Arkansas. These include a new treatment for balled-and-burlapped nursery stock using a bifenthrin dip/immersion which is awaiting APHIS approval, and a new treatment for grass sod using bifenthrin also awaiting APHIS approval. In addition, APHIS has funded ARS to develop a rapid IFA identification field kit and trap, and a potential cold treatment for contaminated soils is under development with data from PPQ-CPHST and the University of Tennessee. To date, four species of phorid flies are in rearing and are being released. *P. tricuspis* and *P. curvatus* are established in more than 65% of the IFA quarantined area in the southern U.S. and releases of *P. obtusus* and *P. cultellatus* will continue through 2013. If no other phorids are available for rearing on red IFA, we will phase out the rearing and release program in 2013-2014. Special thanks to ARS-CMAVE (S. Porter), FL-DPI (G. Schneider) and all the state cooperators for making this program a success.

So, where is the APHIS-PPQ IFA Program going and what is changing in 2012?

APHIS will be closing the Gulfport Facility (MS) in 2012. Existing CPHST operations and staff will be outsourced or relocated to other facilities. All other APHIS or PPQ staff that work at that facility will continue to work out of other locations in the local commuting area. The CPHST Analytical chemistry unit will transfer operational work to other units or will outsource the work. Environmental monitoring to support routine PPQ programs will be outsourced to other labs through cooperative agreements or contracts and managed by a CPHST scientist. Project work to support CPHST and PPQ will be relocated to CPHST Miami Lab and staff will be relocated to CPHST Miami or other CPHST or PPQ units. Most importantly for IFA interests, PPQ-CPHST will NO LONGER provide analytical support for IFA soil samples. The CPHST Imported Fire Ant unit will be outsourcing all methods development work through cooperative agreements and managed by a CPHST scientist. Anne-Marie Callcott will remain as CPHST IFA coordinator/contact and located in Biloxi MS. As soon as new contact information is available it will be distributed. Staff will be relocated to other CPHST or PPQ units.

Where can you get IFA soil samples analyzed for pesticide residue?

There is no federal requirement to analyze routine soil samples annually at nurseries under compliance agreements, however it is encouraged. Historically, these analyses were conducted either by the USDA, APHIS, PPQ-CPHST lab in Gulfport MS or by a local state pesticide lab. In late 2011, a letter was sent to all PPQ-SPHDs in impacted states to share with their SPROs. States may use their state pesticide lab or a neighboring state lab. States may enter into an agreement with USDA-AMS-National Science Lab in Gastonia, NC to conduct the analyses for them. AMS NSL-Gastonia has provided cost estimates for the IFA samples for FY12 (ca. $125/sample for a single pesticide analysis; ca. $38/sample for bulk density determination). However, states MUST negotiate with AMS directly. CPHST staff is available to discuss/provide analytical methods to state labs, and states may contact CPHST for contact information for AMS NSL. State inspectors will need to notify all growers in the state about this change since every year there are +20 samples submitted independently to the CPHST Gulfport Lab by nurseries (not through their state inspector).

What are we working on?

- Environmental Assessment (EA): getting EA into the Federal Register
- Once EA is final
  - ◊ Add new quarantine treatments for B&B and grass sod to PPQ Treatment Manual and IFA Program Manual
  - ◊ Update Program Aid "IFA 2007: Quarantine Treatments for Nursery Stock and Other Regulated Articles"
  - ◊ Update IFA Program Manual
    - Adding new quarantine treatments
    - Adding section on "policing the quarantine"
- PPQ staff will be working to update/modify all existing publications (including online sites) regarding submission of IFA soil samples
- Continuing development of regulatory treatments
  - ◊ Grass sod: bait + contact insecticide treatments
  - ◊ Field grown stock: replacement for infield bait+chlorpyrifos treatment
  - ◊ Combined infield and B&B treatment for long term stored B&B
  - ◊ Continue/validate cold treatments of small containers of bulk soils
  - ◊ Continuing releases of *P. obtusus* and *P. cultellatus*

Evaluation of Imported Fire Ant Quarantine Treatments in Commercial Grass Sod: Arkansas 2012

PIs – Kelly Loftin and John Hopkins, University of Arkansas
11/12-8100-1325-CA

INTRODUCTION: Imported fire ants (IFA) originated from South America and were accidentally introduced into the United States in the early to mid-1900's. IFA are now widespread across the Southeastern United States. Movements of this pest are regulated through a system of Federal and State quarantines. Products regulated by the IFA quarantine include but are not limited to hay, nursery plants and other landscape materials including grass sod.

When treating sod in compliance with Federal and State quarantine regulations, sod producer's options are limited. One option is treatment using the active ingredient chlorpyrifos at a rate of eight pounds of active ingredient per acre. Currently, no products are registered for IFA in sod at that required rate. The other option is to use two separate applications of fipronil at 0.0125 pounds per acre about one week apart. Fipronil can be too expensive to apply and the Environmental Protection Agency has indicated their intention to review the registration and possibly remove it when the 5-year conditional registration expires for broadcast granular products containing fipronil for imported fire ants. The removal of products containing fipronil which are labeled for use against IFA, at the rate required for quarantine, will leave no options for sod producers when selling their products to non-quarantined areas and will also prevent the movement of those products across state lines because of the Federal quarantine regulations.

Because of limited or costly options available to sod producers, a field study was conducted to evaluate the efficacy of other insecticides for use in the IFA quarantine. Liquid bifenthrin has been very effective in treating for IFA in grass sod, however, two applications one week apart, for a total of 0.4 lb ai/acre are required for quarantine level control. This treatment is currently in the APHIS approval process. Using a bait as the first treatment, followed by 0.2 lb ai/acre of bifenthrin has shown promise as a quarantine treatment, and this trial will add to the data to support that treatment. We also added an insecticide synergist to see if it would enhance/increase the residual activity of the bifenthrin or allow an even lower rate of bifenthrin to be applied. All of these options, if effective, will allow a treatment with lower costs to the grower than the current fipronil treatment or the proposed bifenthrin 0.4 lb treatment rate (two applications of 0.2 lb ai/acre, applied 1 week apart).

MATERIALS AND METHODS: The study was conducted on an irrigated sod farm in Fulton, AR (Hempstead Co.) beginning in May 2012 and ending in August 2012. Plots were square, measured ½ acre in area, and treatments (three treatments and an untreated control) were arranged in a Randomized Complete Block Design (RCBD) with three replications. Plots used in the study had a range of 12-32 active fire ant mounds per acre when the study began. An active fire ant mound is defined as a mound with 25 or more ants in the colony which is the USDA standard for classifying active mounds. Treatments within the same plot were separated by one week.

Spray applications were made using a towed boom sprayer applying @ 20 gal/A (15 ft. boom with ten 8003FF nozzles on an 18" spacing at 20 psi and 5.2 MPH). Granular bait applications were made using an Earthway 2750 hand operated seeder calibrated to apply 1.5 pounds per acre. Treatment numbers, insecticide rates and the total amount of active ingredients applied per acre are provided in Table 1.

The number of active mounds per plot was determined by counting the mounds in a circle at the of the center plot. This circle had a diameter of 58.9 ft which corresponds to a circle with an area of 0.25 acre. The mounds are counted by anchoring one end of a 58.9 ft. rope at the center of the plot and moving the free end along the circumference of the circle. Each mound encountered anywhere along the length of the rope is disturbed by probing with a small rod and estimating the number of imported fire ants exiting the mound within 20 seconds (Jones et al 1998).

The number of active mounds in each plot was determined before any treatments were applied and then at seven days after the last application (DALA) then weekly up to 28 DALA, at which time evaluations were made every 14 days until the study ended.

All data were analyzed using Gylling's Agriculture Research Manager Software (ARM 7.0.3. 2003). An analysis of variance was performed and Least Significant Difference (p=0.05) was used to separate means only when AOV Treatment P(F) was significant at the 5% level (ARM 2003).

RESULTS: The data are summarized on Table 2 and Figure 1. Before applying treatments, there were no significant differences in the number of active mounds in any of the plots to be used in the study. At seven DALA, all treatments had zero active mounds per acre. At 56 DALA there was one active mound detected in the bait and bifenthrin 0.1 lb ai/acre + synergist plots. By 70 DALA, active mounds were present on plots in each of the treatment regimes. Untreated controls maintained excellent activity all summer, probably due to routine irrigation on the plots.

Table 1. Insecticide applications, rates and total amount of active ingredients.

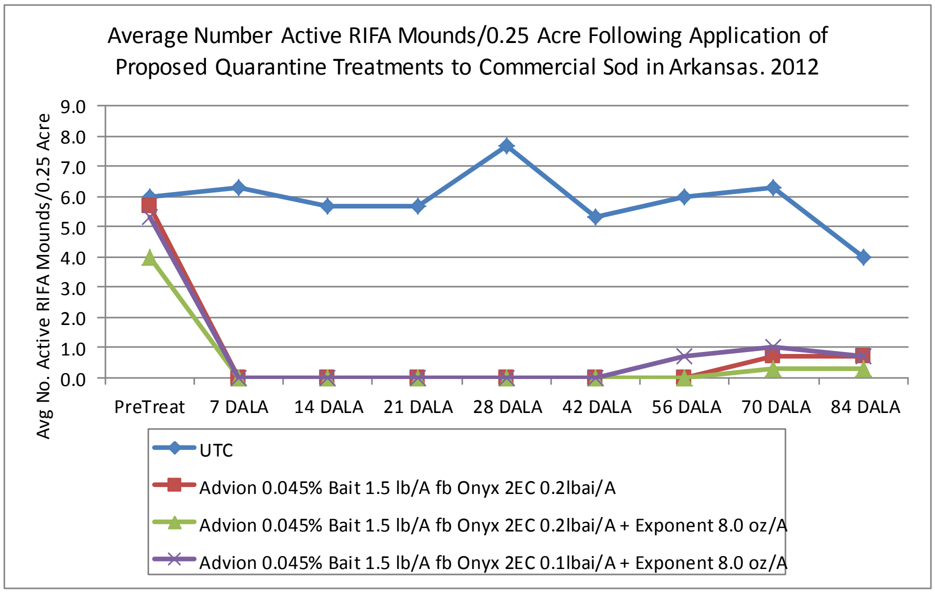
Treatment Number	Insecticide Application	Total active ingredients/acre
1	None – Untreated Control	None
2	1 application Advion® bait 1 application of OnyxPro® EC applied 1 wk later	0.000675 lb ai/A indoxacarb 0.2 lb ai/A bifenthrin
3	1 application Advion® bait 1 application of OnyxPro® EC 0.2 + Exponent® (tank mix) applied 1 wk later	0.000675 lb ai/A indoxacarb 0.2 lb ai/A bifenthrin 8 oz material/A piperonyl butoxide
4	1 application Advion® bait 1 application of OnyxPro® EC 0.1 + Exponent® (tank mix) applied 1 wk later	0.000675 lb ai/A indoxacarb 0.1 lb ai/A bifenthrin 8 oz material/A piperonyl butoxide

Table 2. Average number of active RIFA mounds/0.25 acre for each treatment on commercial grass sod in Arkansas 2012.

Treatment	Avg No. Active mounds/0.25 Acre								
	PreTreat	7 DALA	14 DALA	21 DALA	28 DALA	42 DALA	56 DALA	70 DALA	84 DALA
UTC	6.0a	6.3a	5.7a	5.7a	7.7a	5.3a	6.0a	6.3a	4.0a
Advion Bait 1.5 lb/A followed by OnyxPro 0.2 lb ai/A	5.7a	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.7b	0.7a
Advion 0.045% 1.5 lb/A followed by OnxPro 0.2 lb ai/A + Exponent 8.0 oz/A	4.0a	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.3b	0.3a
Advion 0.045% 1.5 lb/A followed by OnxPro 0.1 lb ai/A + Exponent 8.0 oz/A	5.3a	0.0b	0.0b	0.0b	0.0b	0.0b	0.7b	1.0b	0.7a

Means followed by the same letter do not significantly differ (P=0.05, LSD)

Figure 1. Average number of active RIFA Mounds/0.25 acre for each treatment on commercial grass sod in Arkansas 2012.



Alabama Grass Sod Treatments for IFA Quarantine, 2012

Kelly R. Palmer, Auburn University

PI – L.C. Graham, Auburn University
11-8130-0073-CA

INTRODUCTION: This study compared the efficacy of four different insecticidal combinations for the control of red imported fire ant (RIFA) in turfgrass. Turfgrass is a federally regulated item under the Federal Imported Fire Ant Quarantine (7CFR 301.81) and therefore has to be treated in an approved manner prior to shipment outside of the quarantined area. The goal of this study was to provide additional approved treatment options for turfgrass producers.

MATERIALS AND METHODS: The study site, Cottonwood Golf Club, Montgomery, AL, was selected because of an abundance of RIFA. Plots measuring 0.5 acre in size were laid out with each having a ¼ acre permanently marked circular area in the center that was used as the evaluation area. Three plots/replicates were used per treatment. RIFA populations (presence or absence; number per 1/4 acre evaluation area) were evaluated pre-treatment and at 3 days, 1, 2, 3, 4, 6, 8, 10, 12 and 16 week post-treatment intervals. Treatments consisted of:

- Extinguish Plus Fire Ant Bait (hydramethylnon + methoprene) applied at 1.5 lb/acre followed in 7 days by Onyx Pro EC (bifenthrin) at 0.2 lb AI/acre;
- Extinguish Plus Fire Ant Bait (hydramethylnon + methoprene) applied at 1.5 lb/acre followed in 7 days by Onyx Pro EC (bifenthrin) at 0.2 lb AI/acre + Exponent synergist;
- Extinguish Plus Fire Ant Bait (hydramethylnon + methoprene) applied at 1.5 lb/acre followed in 7 days by Aloft SC (clothianidin + bifenthrin) at 0.2 lb AI/acre cloth:0.1 lb AI/acre bif;
- Extinguish Plus Fire Ant Bait (hydramethylnon + methoprene) applied at 1.5 lb/acre followed in 7 days by Aloft SC (clothianidin + bifenthrin) at 0.2 lb AI/acre cloth:0.1 lb AI/acre bif + Exponent synergist;
- untreated control.

The first treatment (bait) was applied on 4 May 2012, and the liquid spray treatments were applied on 11 May 2012 for all plots except the untreated controls. The first data collection was on 14 May 2012 (3 DAT).

RESULTS: RIFA mound numbers were significantly higher in the control plots on the 3 DAT data collection. No mounds could be found in the treated plots and this trend continued for the 1, 2, 4 and 6 WAT collection periods (Fig 1&2). The 8 and 10 WAT counts showed no RIFA mounds in any of the treatment plots or in the control plots; however, at this time we were experiencing very hot and dry conditions at the golf course. The hot and dry conditions combined with the frequent mowing of the turf inhibited RIFA mound rebuilding activities. None of the fairways where our test plots were located were watered during this time. In the 12 WAT count the control plots again had significantly higher mound numbers than the treatment plots since there were still no mounds found in any of the treatment plots. The final data collection period at 16 WAT showed significantly higher RIFA mound numbers in the control plots. All treatment plots were re-infested with RIFA but there was no significant difference in mound numbers between the treatments.

Figure 1. IFA mound control in grass sod for various proposed treatments. Alabama 2012.

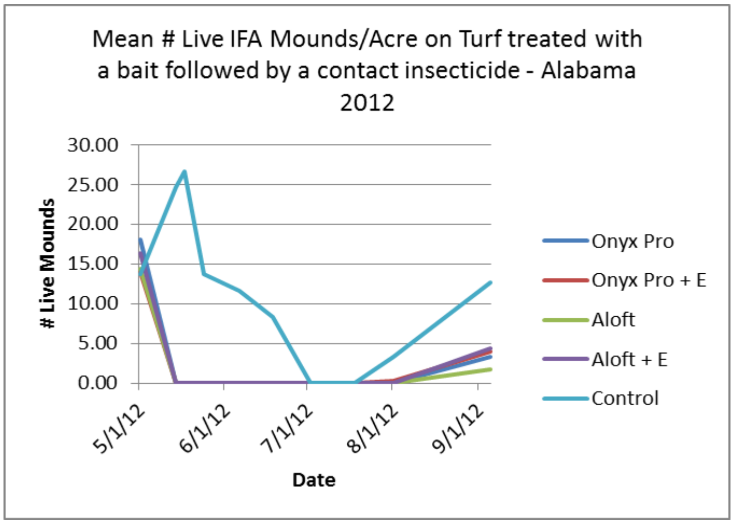
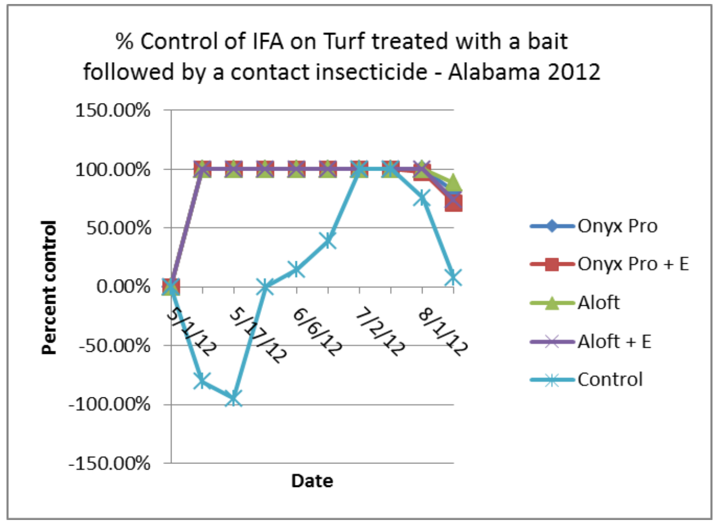


Figure 2. Control of IFA mound populations in grass sod for various proposed treatments. Alabama 2012.



Summary of Grass Sod Treatments 2009-2012

Anne-Marie Callcott, Lee McAnally, Craig Hinton, Xikui Wei, Richard King (APHIS-PPQ-CPHST)

Kelly Loftin (Univ. of AR cooperator), Fudd Graham (Auburn Univ. cooperator)

INTRODUCTION: Currently there are two treatments available for sod growers to certify grass sod for movement outside the IFA regulated area: chlorpyrifos applied at 8 lb ai/acre (6 weeks certification after 48 hour exposure) and fipronil applied at a total of 0.025 lb ai/acre applied in two applications ca. 1 week apart (20 weeks certification after a 4 week exposure). In 2008, the only chlorpyrifos labeled product, Dow Dursban® 50W, discontinued the grass sod IFA quarantine rate of application and therefore only the fipronil product was available for growers. This product does require 2 applications and a 30 day exposure period, both of which are not cost effective for growers. Additionally, many growers take orders with short turnaround times, needing to ship sod within days of receiving an order, which makes a 30 day exposure period (to insure insect mortality) prior to shipping, unacceptable. We do have an additional treatment option in the APHIS approval process and hope to have it available for growers sometime in 2013: bifenthrin liquid applied in two applications of 0.2 lb ai/acre (1 week apart) for a total application rate of 0.4 lb ai/acre. There is a 28 day exposure period for this treatment, again not optimal for many growers, and then a 16 week certification period (currently only one label will allow this treatment rate). This product is more economical in terms of material cost than fipronil, but does not allow short term shipping for growers.

Many earlier trials have determined that many contact insecticides alone do not provide quarantine level efficacy at labeled rates, therefore we have focused on treatments utilizing a bait product prior to application of the contact insecticide. In addition, most contact insecticides cost 2-4x that of a bait for the material to treat 1 acre. Therefore a reduced rate of application of a contact insecticide combined with a bait application could provide significant cost savings for the growers provided these types of treatments are effective.

MATERIALS AND METHODS: Test sites for these trials were in southern Mississippi, southern Arkansas and central Alabama. Treatments were applied in the spring/early summer months or in the late summer/early fall months; in some areas sod is harvested almost year round so treatments need to be effective year round. Plots were generally 0.52-acre square in size for all treatments (150' x 150'). On plots receiving bait plus a contact insecticide, the bait was applied to 147' x 150' of the plot to accommodate the bait spreader used in MS trials. The contact insecticide application on the same plot was applied to the full 0.52 acre area. All plots contained a permanently marked ¼-acre circular efficacy plot in the center. This is the area that was evaluated for active IFA mounds, allowing a minimum of 15 ft treated buffer

Table 1. summary of grass sod treatments evaluated from 2009-2012.

Bait option	Contact insecticide	Formulation	Rates of application	Promising Yes/No
Bait	Bifenthrin	EC or F	0.2 lb ai/acre	Yes
Bait	Bifenthrin Bifenthrin+zeta-cypermethrin combo product	G Gx	0.2 lb ai/acre bif 0.2 lb ai bif+0.05 lb ai zeta	No Yes
No bait	Bifenthrin+clothianidin combo product	SC	0.1 lb ai bif+0.2 lb ai cloth 0.2 lb ai bif+0.4 lb ai cloth	No No
Bait	Bifenthrin+clothianidin	SC	0.1 lb ai bif+0.2 lb ai cloth	Yes
No bait	Lambda-cyhalothrin single app rate now limited to 0.069 lb ai/acre	GC	0.069 lb ai/acre 0.13 lb ai/acre 0.13+0.069 lb ai/acre 0.13+0.13 lb ai/acre	No No No No
Bait	Lambda-cyhalothrin this rate no longer on label	GC	0.13 lb ai/acre	Yes

between the evaluation area and untreated areas. There were 3 plots per treatment and controls. Prior to treatment and at 1, 2, 3, and 4 weeks after treatment and bi-weekly or monthly thereafter, IFA populations in each efficacy plot was evaluated. Due to the weekly evaluations, we used a minimal disturbance method to evaluate the IFA populations. Instead of using a shovel to excavate each mound to determine worker numbers and presence or absence of brood, a stick/ rod (ca. ¼-inch diameter and 3 ft. long) was used to "poke" each mound several times to disturb the workers. A rating was then given based on activity; 1= <100 workers, 2=100-1,000 workers, 3=1,000-10,000, 4=10,000-50,000, 5= >50,000 workers. Trials in 2012 reverted to simply enumerating the number of live colonies within the evaluation area; a live colony consisted of more than 10-20 worker ants when disturbed. Therefore all results presented here are on mortality of colonies.

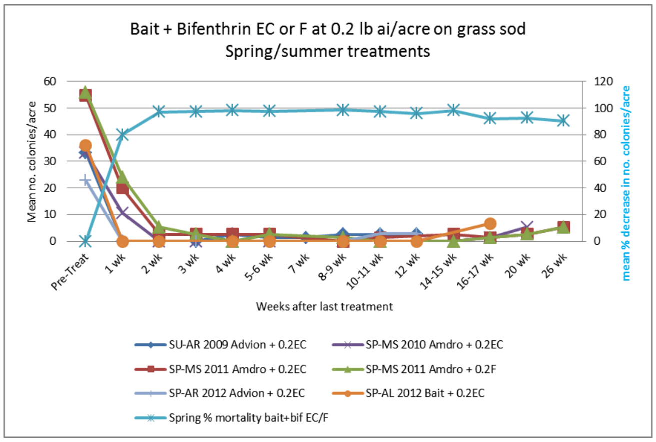
For specific application equipment, please see the individual reports for each of the trials. In general baits were applied with either a shop built spreader or a Herd bait spreader. Liquid treatments were applied with a typical small tank spray rig (ca. 50 gal tank) with spray swaths of 10-12 ft at a rate of ca. 20-35 gallons of finished solution per acre. Granular contact insecticides (only applied in MS) were applied with a Herd GT-77 granular applicator mounted to a farm tractor. Bait applications were made a minimum of 3 days prior, but sometimes up to 14 days (depending on weather) prior to the contact insecticide application to allow the toxic bait to be passed throughout each colony. We suggest a 3-5 days period between bait and contact application. Specific treatments shown in Table 1.

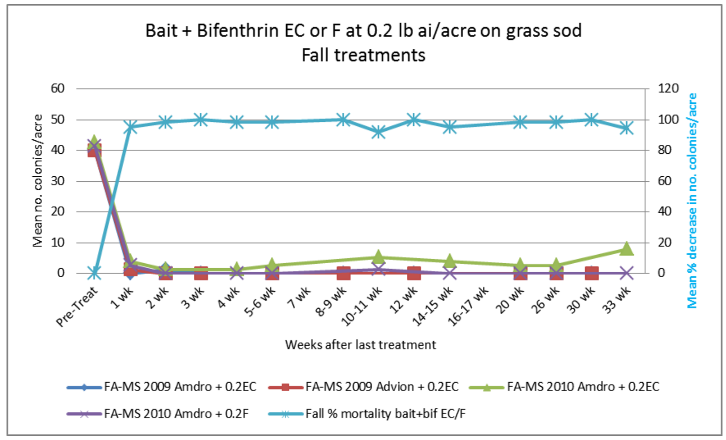
RESULTS:

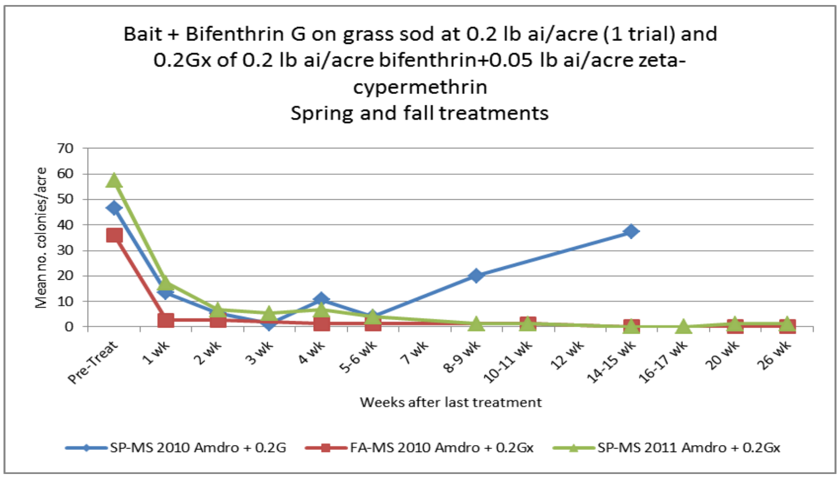
Bifenthrin: Historically, we have seen that bifenthrin products at the 0.2 lb ai/acre rate of application do not provide consistent quarantine level efficacy when used alone on grass sod. The liquid products, when applied at 0.2 lb ai/acre plus a second application of 0.2 lb ai/acre do provide excellent quarantine level efficacy and this treatment as noted in the introduction, is in the APHIS approval process for use in the IFA quarantine.

Between 2009 and 2012 at least one trial per year has been conducted using a bait followed by a 0.2 lb ai/acre liquid bifenthrin treatment. There have been excellent results with the liquid bifenthrin treatments, with the late summer/early fall treatments (August/Sept) providing longer residual activity than the spring/early summer (April-June) treatments. There was one 2010 fall treatment which was not very effective – this could have been an application issue since the other liquid formulation applied on the same property the same time was very effective. This longer residual in the fall has been noted by several researchers and is attributed to less degradation from sunlight, rainfall and microbial activity in the winter months. In general, the spring/summer treatments are achieving an average of 80% control at 1 week after final application, 97% control at 2 weeks and maintaining >95% control for an additional 12-13 weeks (14-15 weeks after final application). Out of six spring/summer application trials, 3 achieved 100% mortality in 1 week and 4 in 2 weeks. The fall treatments are achieving an average of 95% control at 1 week, 98% control at 2 weeks after the final insecticide application and maintaining >92-95% control for an additional 28 weeks (30 weeks after treatment); maintaining >97% control if the odd 2010 trial is eliminated. Speed of control of fall applications was similar to spring applications. Out of four fall applications, 2 achieved 100% control at 2 weeks and 3 in 3-4 weeks (2 trials did not have a 3 week evaluation due to weather). Additional fall/late summer applications are needed to validate these results, especially the length of control.

In 2012, we conducted trials initiated in the spring/summer months in Arkansas with bait + bifenthrin EC at 0.1 lb ai/acre tank mixed with an insecticide synergist (Exponent®: piperonyl butoxide) and bifenthrin at 0.2 lb ai/acre tank mixed with the synergist. In Alabama the same year, bifen-





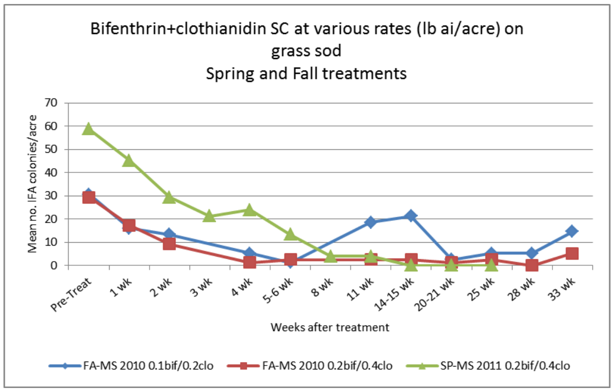


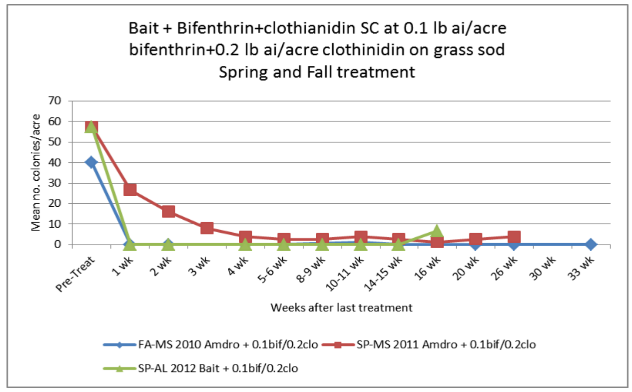
thrin EC at 0.2 lb ai/acre was tank mixed with the same synergist. In both of these trials, there was no difference in speed of control or length of control between synergist and non-synergist treatments when tank mixed with bifenthrin at 0.2 lb ai/acre (following bait treatment). However, in both of these trials, all treatment rates, with or without synergist, achieved 100% control in 1 week, faster than any of the other spring/summer trials. One of these trials had artificial irrigation throughout this very dry summer and the other did not.

Granular bifenthrin alone, even at 0.4 lb ai/acre, is not a consistent quarantine level treatment. It is very dependent on rainfall or irrigation to insure activation. Limited trials have been conducted on combining a bait treatment with a granular bifenthrin treatment. One spring/summer trial using bait + 0.2 lb ai/acre of granular bifenthrin showed fairly quick and effective control of IFA, but colonies either rebounded or new colonies moved in between 6-8 weeks after treatment. In the fall 2010 and spring 2011, we used a new product from FMC called Talstar® Xtra which contains bifenthrin and zeta-cypermethrin. One spring and one fall trial using the product (with a bait) at 0.2 lb ai/acre bifenthrin and 0.05 lb ai/acre zeta-cypermethrin provided an average of 89% control at 2-3 weeks after the last treatment, reaching 95% at 4 weeks and then provided >95% control for ca. 22 weeks (very limited data). Additional trials during both seasons are needed to validate these results.

In general, bait + liquid bifenthrin treatments (at 0.2 lb ai/acre) are providing very promising results and data from a few more fall trials might allow different certification periods for spring/summer vs. early fall treatments. At least one more set of spring/summer trials would hopefully confirm both the exposure period and the certification period. I would anticipate an exposure period of ca. 2 weeks after the last treatment (thus potentially 2½-3 weeks after the first bait treatment), thus providing a treatment option with a 1 week shorter exposure period than currently approved treatments. Bifenthrin granular does not appear to be an option for this quarantine use pattern when used alone with a bait. However, the new bifenthrin+zeta-cypermethrin product does show promise when applied after a bait for IFA grass sod use and additional trials in both seasons need to be conducted.

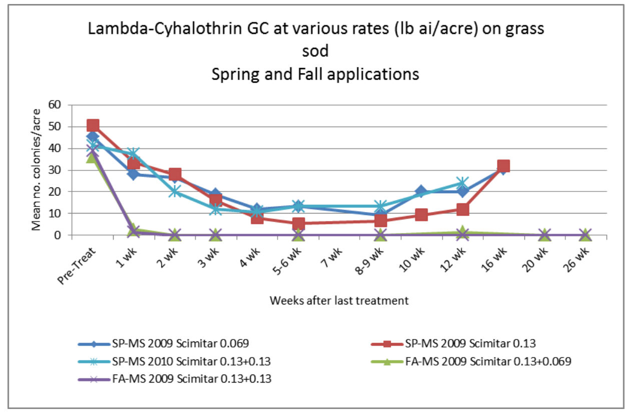
Bifenthrin+clothianidin: Aloft® is one of the newer products to combine bifenthrin with another insecticide, in this case, clothianidin (Arysta LifeSciences®). Several trials were conducted with the bifenthrin+clothianidin SC (liquid) product alone, with promising results at 2 rates of application, but very slow initial control. Therefore we tested the lower rate of application (0.1 lb ai/acre bifenthrin+0.2 lb ai/acre clothianidin) with a bait application. Very limited results are showing excellent results, and again there appears to be a longer residual when applied in the fall. Additional trials in both spring and fall will be needed to validate these results and confirm potential exposure periods and certification periods.

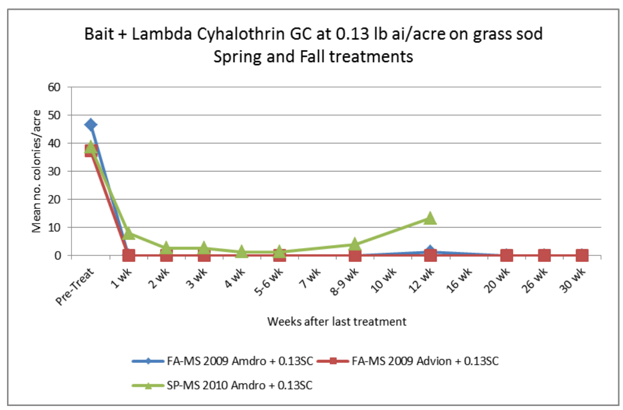




Lambda-cyhalothrin: Prior to 2010, lambda-cyhalothrin SC labels with turf uses included single treatments rates up to 0.13 lb ai/acre, but were cut to single applications of 0.069 lb ai/acre. Current labels do allow multiple applications 7 days apart up to 0.36 lb ai/acre/year. This product appears to be very susceptible to the degradation effects of the spring and summer months; these degradation factors may include high temperatures, rainfall and resulting microbial activity. Limited fall treatments, even applied without a prior bait, show the product to be fast acting, reaching ca. 95% control at 1 week after the final treatment and maintaining that control for an additional 25 weeks (26 weeks after last treatment). However, these 2 trials, with different rates of application (0.13 lb + 0.13 lb ai/acre; 0.13 lb + 0.069 lb ai/acre) using the pre-2010 labeled single application rate of 0.13 lb ai/acre, would now require 3 to 4 applications 7 days apart, adding significant labor and fuel costs to the growers.

Bait trials, using a single 0.13 lb ai/acre rate after a bait treatment, again showed quick control in both spring and fall treatments, with the only spring treatment allowing reinfestation about 8-9 weeks after the final treatment (Figure 3). Again, the fall trials were very promising with >95% control at 1 week after the final treatment, and maintaining that control for an additional 29 weeks (30 weeks after the final treatment). However, with the new label restrictions, these treatments would require at least 3 treatment trips over the grass sod, adding labor and fuel costs. Therefore testing of lambda-cyhalothrin was discontinued in 2010.





DISCUSSION: The use of a bait just prior to certain contact insecticide applications does provide effective control of IFA on grass sod. The use of the bait allows a lower rate of application for the contact insecticide decreasing the expense of sod treatments to comply with the regulations. At this time, bifenthrin, and bifenthrin combination products, are the most promising contact insecticides for this use pattern. We will have to consider seasonal certification periods, depending on time of initial treatment, for some treatments, a novel approach in the IFA quarantine. Additional data is needed for the following treatment to confirm/validate current data and determine final exposure and certification periods (Table 2).

Table 2. Future grass sod trials needed to validate efficacy for IFA regulatory use.

Bait +	Rate of application	Estimated No. of Needed trials
Bifenthrin EC or F	0.2 lb ai/acre	1-2 late spring/early summer trials 3 late summer/early fall trials
Bifenthrin Gx (Talstar Xtra®: bifenthrin+zetacypermethrin)	0.2 lb ai (bif) + 0.05 lb ai (zeta)/acre	3 late spring/early summer trials 3 late summer/early fall trials
Bifenthrin+clothianidin SC (Aloft®SC)	0.1 lb ai (bif) + 0.2 lb ai (cloth)/acre	3 late spring/early summer trials 3 late summer/early fall trials

Summary of Balled-and-Burlapped (B&B) Immersion/Dip Treatments for Potential Use in the Federal IFA Quarantine Program: 2002-2010

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SUMMARY: The most promising dip/immersion treatments for nursery plants grown in field soil and harvested as balled-and-burlapped nursery stock for IFA include bifenthrin at rates possibly as low as 0.0375 lb ai/100 gal water, but definitely at 0.05 lb ai/100 gal water, with the rate of application dictating the certification period or longevity of the treatment (2-6 mths). Seasonal impacts show up at rates of 0.025 and 0.0125, with spring applications having significantly shorter residual activity than fall applications. However, bifenthrin rates of 0.00625 and 0.0125 when combined with either carbaryl (0.125 lb ai/100 ga water) or dimethyl phosphonate/ trichlorfon (0.125 lb ai/100 gal water) show excellent activity through 4 months, though these data would need additional trials for validation (these 2 products alone were not effective as dips against IFA even at high rates of application). Also, while older carbaryl labels included IFA and nursery stock dip uses, current labels have removed the nursery stock dip uses (retain IFA and bedding plants soil treatments). Therefore, concerns over whether carbaryl would retain IFA uses on the label prompted us to look at other products, but this bifenthrin+carbaryl combination still remains high on the efficacy list. The currently approved chlorpyrifos dip at 0.125 lb ai/100 gal water was consistently effective for the approved 30 days, validating that treatment option.

Unlike IFA, Japanese beetle (JB) only need to be eliminated from root balls one time to certify plants from October to May because no additional reinfestation can occur at these times. For JB control in 12 inch diameter B&B, a number of products showed promising efficacy, but treatment effectiveness was affected by the time of the year. For example, bifenthrin is effective for IFA at rates as low as 0.05 lb ai/100 gal water, but JB requires 0.115 and 0.23 lb ai/100 gal in spring and fall applications, respectively, with the flowable formulation, but that rate lowers to 0.025 to 0.0375 with the EC formulation. Highly effective combination treatments for JB included bifenthrin + carbaryl and bifenthrin + trichlorfon at rates as low as 0.00625+0.125 (both fall and spring), again similar to rates effective for IFA. Thus, dip treatments for JB could be equally efficacious for IFA, allowing growers one treatment for 2 pests.

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 led to reduced production, consequently limiting its availability to growers and making compliance difficult. Thus, other treatment methods and additional approved insecticides are needed in order to insure imported fire ant-free movement of this commodity. In addition, growers in Tennessee and other states are also required to treat for Japanese beetle

Current certification options against imported fire ants for harvested B&B stock are immersion in a chlorpyrifos solution (dipping) or watering twice daily with a chlorpyrifos solution for three consecutive days (drenching) both at a rate of 0.125 pounds of active ingredient (a.i.) per 100 gallons of water. Likewise, the current treatment for Japanese beetle (*Popillia japonica* Newman) in B&B requires dipping in chlorpyrifos, but at a rate of 0.25 lb a.i./100 gal water (lowered from 2.0 lb ai/100 gal water in 2008). Thus, a cooperative research effort to screen other insecticides for inclusion in imported fire ant (IFA) quarantine treatments for B&B, with priority given to products also effective for Japanese beetle (JB), was initiated with the Tennessee State University Nursery Research Center (TSU-NRC). Trials conducted over the past few years indicated several chemicals could potentially be used in addition to chlorpyrifos in treatment of B&B nursery stock.

These trials have resulted in one additional treatment option for immersion to be submitted to the APHIS approval system. Bifenthrin at 0.115 lb ai/100 gal water is currently undergoing the USDA approval process to be added to the Federal Imported Fire Ant Quarantine as an immersion treatment for B&B nursery stock. The certification period for this rate will be 6 months. One bifenthrin label has already added this language to their label, but commercial use to comply with the IFA Federal Quarantine is awaiting final USDA approval. Approval is contingent on publication of a new Environmental Assessment for Pesticide Use in the Imported Fire Ant Program in the Federal Register. The EA was completed in March 2012 and is currently (Jan 2013) in the queue for publication.

Many products and product combinations have been tested in this use pattern and a summary was reported in 2007. This report summarizes all the numerous trials and products tested since 2002 specific to IFA with brief comments on JB efficacy. Note: This report does not provide details on nursery plant phytotoxicity. For some of the chemicals listed in this report, phytotoxicity was observed at some rates. If some rates are approved for usage in the IFA quarantine, additional dip testing on a broader assortment of nursery plant species may be needed to confirm general safety to plants.

MATERIALS AND METHODS: Specific materials and methods may be found in the 2002-2010 Gulfport Imported Fire Ant Annual Accomplishment Reports. In general, harvested root balls were obtained from a commercial grower ranging in size from 12” to 24”. Most trials utilized 12” root balls for economic and ease of handling reasons, but there have been a number of trials with larger root balls. Insecticides were mixed at the testing rates and placed in dip tank/ container large enough to dip the largest root ball being tested. Root balls were immersed in the liquid for approximately 1 minute or until bubbling ceased. Balls were then stored outdoors for aging. At specific intervals, soil samples were collected using a soil corer. Samples were taken either from the middle/core of the root ball, or from the surface/top of the root ball. There were generally 3-4 replicates (root balls) per treatment in each trial, and samples were generally collected at 0.5, 1, 2, 4, and 6 months after treatment, although there was some variation in sampling. Testing was initiated in both fall and spring months primarily in Tennessee with some trials in Mississippi.

All IFA bioassays were conducted in Gulfport, MS at the APHIS-PPQ-CPHST laboratory. Field collected red imported fire ant alate females were subjected to the soil samples and mortality was accessed at 14 days after continued confinement to the soil sample. While the number of females per sample varied due to changes in the protocol and resources, a minimum of 10 alate females per replicate, and thus a minimum of 30 alate females per treatment per sampling interval were always used. A few trials also included bioassays conducted against IFA worker ants. Untreated controls (usually dipped in water only) were included in every trial but that data is not included here.

All trials with Japanese beetle (JB) were done with third instars, the final larval stage that is the most difficult to control. These trials were conducted by TSU and all data reporting is being done by that group.

RESULTS: For the purpose of this summary report, all soil sample sites (inner center of ball, or outside/surface of ball) as well as life stages tested were combined. Because of the uniformity of treatment achieved with root ball dip treatments, we have seen no significant efficacy differences between soil samples collected in the outside surface or inside center of the root balls, so there was no reason not to combine results from different sampling sites. A summary of active ingredients tested, singly or in combination, rates and whether the product(s) was promising for IFA control as a B&B immersion treatment is noted in Table 1. Specific details for each active ingredient or combination follow in text.

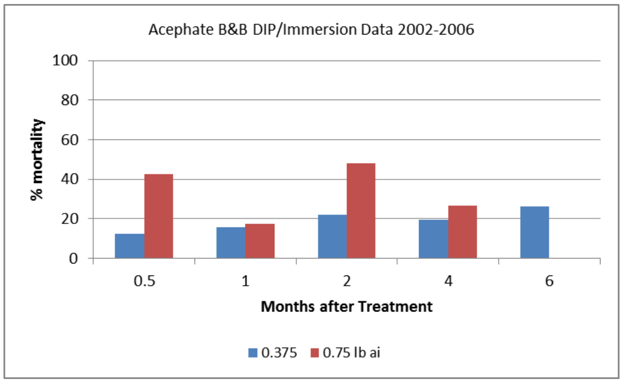
Table 1. Summary of active ingredients tested as B&B immersion/dip treatment for IFA control. Promising for IFA indicates potential for 1-6 months of residual activity. Promising for JB indicates potential in both spring and fall applications. Product/product combinations highlighted in blue show significant promise for use in both IFA and JB programs. Those not highlighted but with promise for one pest were inconsistent in trials for either IFA or JB. See text for more details.

Active ingredient(s)	Rate of Application lb ai/100 gal water	Promising for IFA B&B Dip (mth residual)			Promising for JB	No. IFA trials
		1-2 mth	4-6 mth	No		
Acephate	0.375			X	N	3
	0.75			X	Y	2
Bifenthrin	0.006			X	N	4
	0.012	X			N	6
	0.025	X			N	11
	0.0375		X		Y	2
	0.05		X		Y	13
	0.115*		X		Y	13
	0.23**		X		Y	6
Bifenthrin + carbaryl	0.006+0.125		X		Y	2
	0.0125+0.25		X		Y	8
	0.0125+0.5		X		Y	2
Bifenthrin + dimethyl phosphonate/ trichlorfon	0.00625+0.125		X		Y	2
	0.0125+0.25		X		Y	4
	0.0125+0.5		X		Y	2

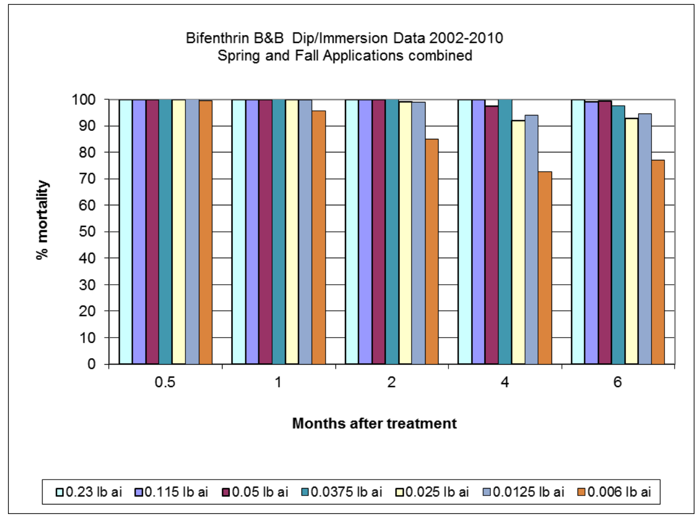
Active ingredient(s)	Rate of Application lb ai/100 gal water	Promising for IFA B&B Dip (mth residual)			Pro-mising for JB	No. IFA trials
		1-2 mth	4-6 mth	No		
Bifenthrin + Imidacloprid	0.0125+0.0156			X	N	2
either 2 products or Allectus combo product	0.025+0.03125	X			N	2
	0.05+0.0625		X		Y	6
	0.1+0.125		X		Y	6
	0.2+0.253		X		Y	8
Bifenthrin + various essential oils	Many see text					1-2
Carbaryl	4			X	Y	3
	8			X	Y	2
Chlorpyrifos	0.06	X			--	3
	0.125***	X			Y	7
	2		X		Y	9
Clothianidin	0.2	X			--	1
	0.4	X			Y	2
Deltamethrin	0.02			X	--	1
	0.04	X			N	5
	0.065	X			N	5
	0.13	X			Y	3
Dimethyl phosphonate/trichlorfon	4			X	Y	3
	8			X	Y	1
Dinotefuran	0.54			X	--	1
DPX-E2Y50/chlorantraniliprole	0.42			X	--	1
Essential Oils/Biopesticides (in text)	Many see text			X		1-2
Halofenozide	0.75			X	--	1
	1.5			X	--	1
Imidacloprid	0.15			X	N	1
	0.2			X	N	2
	0.3			X	N	2
	0.4			X	Y	3
Imidacloprid + cyfluthrin	0.1875+0.045			X	N	6
Discus combo product	0.25 + 0.06	X			Y	5
	0.5 + 0.12		X		Y	6
Lambda-cyhalothrin	0.017	X			--	3
	0.034	X			N	7
Thiamethoxam	0.065	X			N	5
	0.13	X			Y	5

* 0.115 rate in Federal IFA Quarantine B&B dip
** in JB Harmonization Plan as B&B dip
*** 0.125 rate in Federal IFA Quarantine as B&B dip; 0.25 rate in JB Harmonization Plan as B&B dip

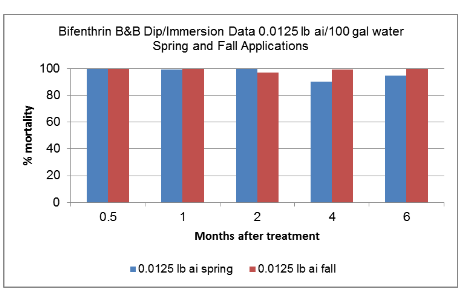
<u>Acephate</u>: Acephate was tested in TN during 2002-2006 at rates of 0.375 and 0.75 lb ai/100 gal water in both spring and fall trials. The 0.375 rate was tested in the spring of 2006 and the fall of 2003 and 2005. The 0.75 rate was tested in the spring of 2002 and 2004. Neither rate was effective in controlling IFA in B&B nursery stock when used as a dip treatment. Acephate did control JB during the spring at rates of 0.1875 to 0.75 lb ai/100 gal water, but was generally not effective in the fall.

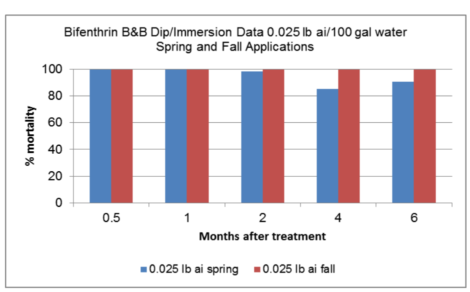


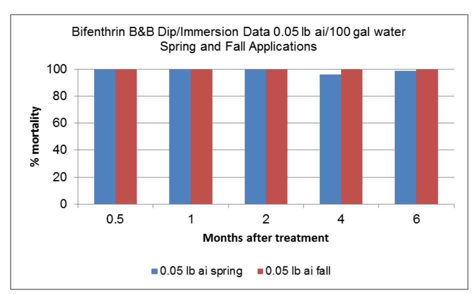
<u>Bifenthrin</u>: Bifenthrin has been tested at rates from 0.006 to 0.23 lb ai/100 gal water in both TN and MS in both spring and fall. The 0.115 lb ai/100 gal water rate has been submitted to USDA-APHIS for approval as a B&B dip/immersion treatment for IFA with a certification period of 6 months. Overall, bifenthrin used as a B&B immersion/dip treatment at rates of 0.05 lb ai/100 gal water or higher provided 97-100% control for 6 months, including one fall and one spring trial on 24" root balls and one 2 mth and one 4 mth inside center of the root ball sample on the 24" balls. The 0.0375 lb ai rate has only been tested in one fall and one spring trial, and thus needs additional testing to validate quarantine level efficacy. The 0.025 and 0.0125 rates were similar in multiple testing with efficacy dropping to below 95% control at 4 months after treatment. The lowest rate of 0.006 lb ai/100 gal water provided excellent control at 2 weeks, but dropped to below 95% at 1 month and continued to lose efficacy over time.



Seasonality has an impact on efficacy of bifenthrin at the lower rates of application. As noted in the graphs below, bifenthrin at 0.0125 and 0.025 lb ai/100 gal water, when applied in the fall, is very effective against IFA for 6 months (only a small decrease in the 0.0125 rate at 2 mths). However, the same rates of application when applied in the springtime drop to 85-90% control at 4 months after treatment. The 0.05 lb ai rate shows a small decrease in mean efficacy at 4 months to 96% control but rebounds to 98% at 6 months, thus this rate of application seems to be able to withstand the additional degradation pressures of the summer months; increased rainfall, increased sunlight, increased microbial activity. Seasonal differences in bifenthrin activity have been noted in several trials by other researchers as well (most reported in Proceedings of the Annual IFA Research Conferences). Pyrethroids have also been reported to have more insecticidal activity at lower temperatures, although this probably did not have any effect in laboratory bioassays performed at room temperature during winter months. However, greater pyrethroid activity in colder weather could enhance IFA control in treated B&B root balls stored outside during the winter months.



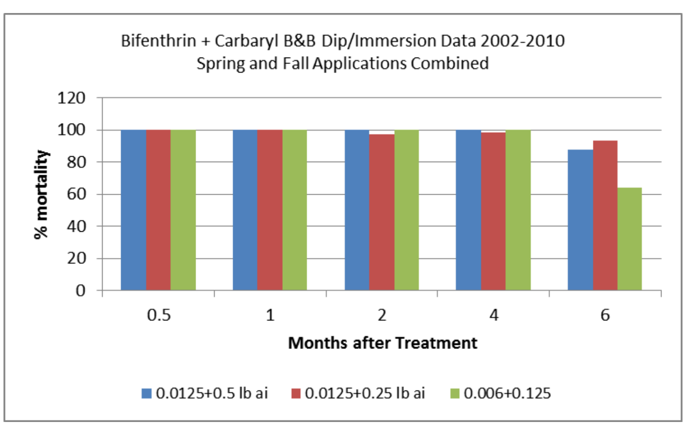




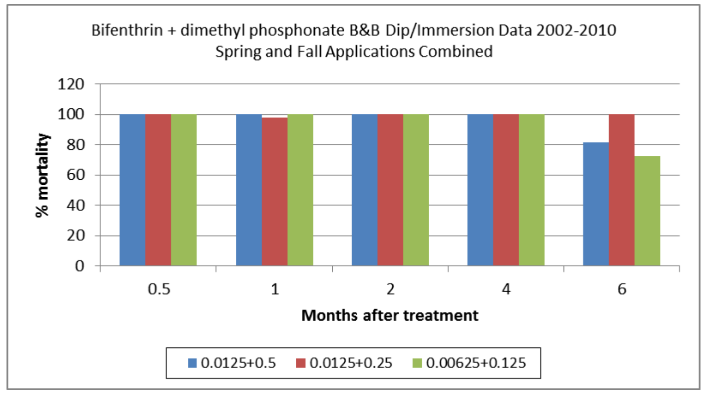
Differences in seasonality of Japanese beetle control was also noted, but with the reverse effect, with spring treatments generally being more effective than fall treatments. In addition, there was a difference in efficacy of bifenthrin formulations. Bifenthrin (Talstar F) was effective at 0.23 to 0.345 lb ai/100 gal water (fall) and 0.115 to 0.345 lb ai/100 gal water (spring). Another bifenthrin product (Onyx Pro EC) was more effective than Talstar F at much lower rates. Onyx was effective at 0.025 to 0.05 and 0.0375 to 0.05 lb ai/100 gal water in fall and spring trials, respectively. Currently, the JB Harmonization plan allows a 0.23 lb ai/100 gal water dip treatment for B&B stock which is more than effective for IFA control as well, allowing a single treatment to cover both pests. Lower rates for both IFA and JB would, of course, be much more economically feasible for growers.

While APHIS is pursuing approval of the 0.115 lb ai/100 gal rate for IFA certification for 6 months, the additional trials presented here would indicate we could drop that rate to 0.05 lb ai and maintain the 6 month certification for dip/immersion treatments of B&B stock. Based on the separate seasonal data at the 0.05 rate, continued testing of the 0.0375 rate is probably not warranted since the 0.05 rate appears to be at the cusp of the seasonality impact. We will also consider approving a lower rate for a shorter certification period; possibly the 0.025 lb ai/100 gal rate for a 2 month certification.

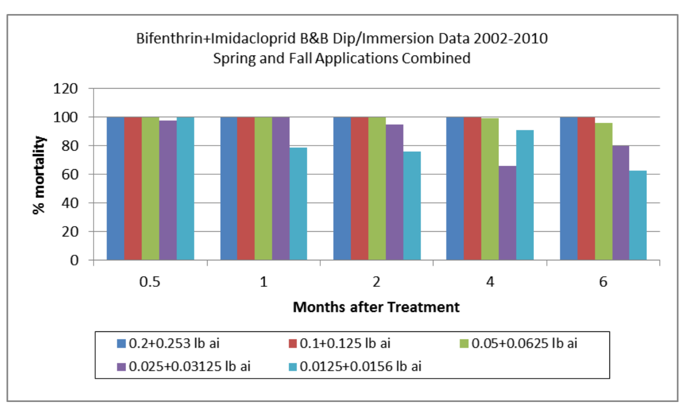
<u>Bifenthrin + Carbaryl</u>: The addition of carbaryl to bifenthrin does appear to increase the residual activity of the combined treatment, potentially providing up to 4 months of residual activity for a very low rate of bifenthrin (0.006 lb ai/100 gal water) combined with a low rate of carbaryl (0.125 lb ai/100 gal water). This rate has only been tested in one spring and one fall trial and would require additional testing to validate the results. While older carbaryl labels included IFA and dip uses, new labels have removed the nursery and dip language (but retained IFA for other uses including soil treatment of bedding plants). However, concerns over whether carbaryl would retain IFA uses on the labels have prompted us to focus on other products. The bifenthrin + carbaryl rates were also highly efficacious against JB in both fall and spring for 12 inch diameter B&B, but only the highest rate combination has been tested with 24 inch B&B in one fall and one trial, where 100% control was achieved.



<u>Bifenthrin + Dimethyl phosphonate/trichlorfon</u>: Dimethyl phosphonate or trichlorfon is primarily for control of grubs (Japanese beetle). The addition of dimethyl phosphonate to bifenthrin does appear to increase the residual activity of the combined treatment, potentially providing up to 4 months of residual activity for a very low rate of bifenthrin (0.006 lb ai/100 gal water) combined with a low rate of dimethyl phosphonate (0.125 lb ai/100 gal water). This rate has only been tested in one spring and one fall trial and would require additional testing to validate the results. The bifenthrin + trichlorfon rates were also effective against JB in both fall and spring for 12 inch diameter B&B, but for 24 inch B&B only the highest rate combination worked in the fall and both the highest and middle rate combinations in the spring.



<u>Bifenthrin + Imidacloprid</u>: Trials using this combination of active ingredients either used bifenthrin and imidacloprid as separate products combined in the tank mix or used a combination product, Allectus®. Several trials were initiated before we determined the lowest effective rate of bifenthrin alone against IFA. The addition of imidacloprid to the bifenthrin did not improve the efficacy or residual activity of the bifenthrin at the two lower rates. The 0.0125 rate of bifenthrin was less effective in the 2 trials with imidacloprid (1 spring and 1 fall trial) than as a standalone treatment; however, the fall trial was 97-100% effective for 2 months with the spring trial showing a significant decrease in efficacy compared to the standalone bifenthrin 0.0125 rate. One trial in each season however, does not confirm efficacy. For JB, bifenthrin + imidacloprid combinations were effective down to 0.05 + 0.0624 lb ai in 12 inch diameter B&B in both fall and spring, but for 24 and 32 inch diameter, only the 0.2 + 0.253 rate has been tested, and it was 100% effective both fall and spring.

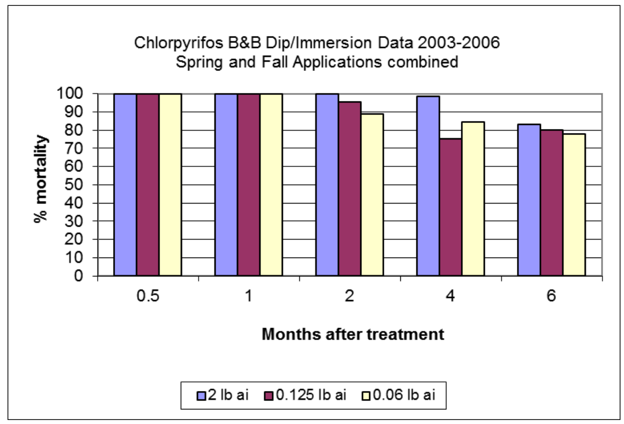


Bifenthrin + Various Essential Oils/Biopesticides: Bifenthrin at a rate of 0.025 lb ai/100 gal water was combined with various essential oil products. Only two trials were completed at each rate; one spring and one fall. Most combinations were similar to bifenthrin standalone treatments at this rate with the fall treatment effective in the fall through 4 months and the spring treatment losing efficacy at the 4 month evaluation, thus not offering any additional residue activity or efficacy than bifenthrin alone. A list of the essential oil products can be found in the Essential Oil section and specific results found in the 2006-2009 Gulfport IFA Annual Accomplishment Reports.

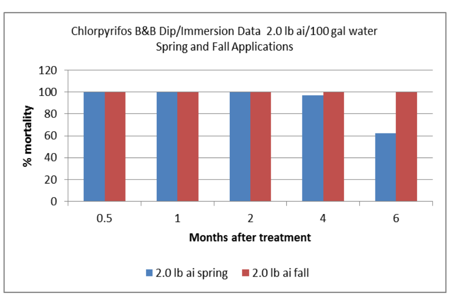
A number of biopesticide combinations were evaluated with conventional insecticides for third instar JB control in 12 inch diameter B&B. The most effective and consistent combinations were Armorex + bifenthrin, carbaryl, or trichlorfon, Azatin + bifenthrin, carbaryl, or trichlorfon, Triact + bifenthrin or trichlorfon, and Cinnacure + bifenthrin or carbaryl. The bifenthrin, carbaryl, and trichlorfon rates evaluated with biopesticides were below rates known to work for JB, but in many cases, were effective in combination with biopesticides. An issue with the biopesticide trials was that some of the biopesticide rates are likely higher than will be cost effective.

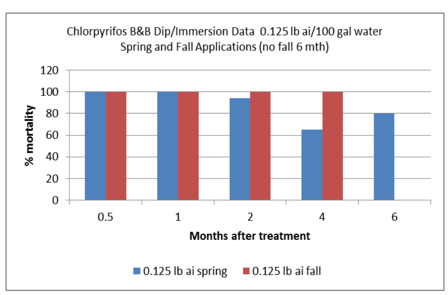
Carbaryl: Carbaryl as a standalone dip treatment for IFA at rates of 4 and 8 lb ai/100 gal water was not effective at any evaluation period (trials in 2002-2004). This is interesting in light of the potential for its use at much lower rates combined with low rates of bifenthrin, indicating some possible potentiation affects. Again, due to increased concern over the possible removal of IFA from the carbaryl label, we have not pursued any carbaryl uses. Carbaryl was effective on JB down to 0.25 lb ai in the fall and 0.0625 in the spring.

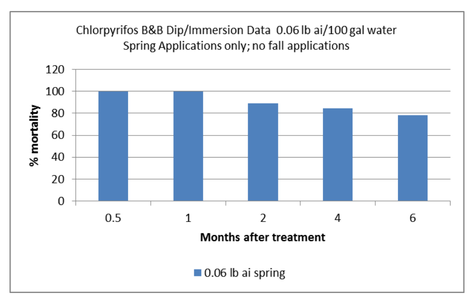
Chlorpyrifos: Chlorpyrifos is the current approved dip/immersion treatment for B&B nursery stock for certification in the Federal IFA Quarantine. The current rate of application is 0.125 lb ai/100 gal water for a 30 day certification period (original JB Harmonization plan dip rate of application was 2.0 lb ai/100 gal water and lowered to 0.25 lb ai/100 gal water in 2008). These trials confirm the current IFA treatment and indicate a possibility of lowering the rate to 0.06 lb ai/100 gal water although additional trials would be needed to verify this rate. However, with chlorpyrifos under scrutiny by EPA and IFA uses being removed from many labels, resources are better used looking at other insecticides.



Seasonality appears to have an impact on chlorpyrifos at the 0.125 and the 2 lb ai rates of application with spring applications showing shorter residual activity than fall applications. The 0.06 lb ai rate was only applied in spring months. Chlorpyrifos was 100% effective both fall and spring at these rates for JB, but other past trials have suggested 0.125 may be inconsistent at times, and therefore, the current JB Harmonization rate has been set at 0.25 lb ai.

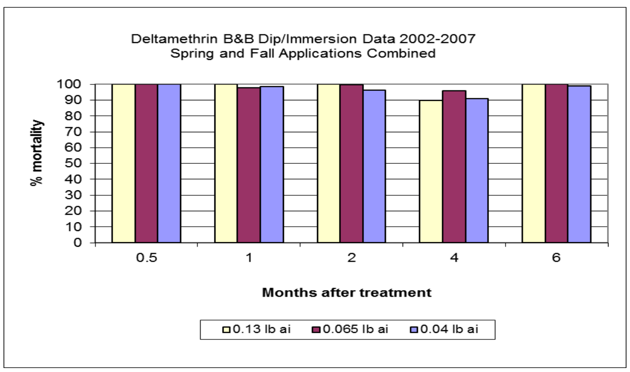






Clothianidin: Clothianidin was tested one spring at the 0.2 lb ai/100 gal water rate and provided 2 months of control. It was tested at the 0.4 lb ai rate one spring and one fall with the spring application providing 2 months of control and the fall application providing 4 months of control. The 0.4 lb ai rate was effective against JB in the spring in one trial with 12 inch B&B, but not the fall. This product provides good control of JB and is used in the JB Harmonization Plan on sod.

Deltamethrin: Deltamethrin is effective against IFA as a B&B dip/immersion, but is not as consistent as other products. There is some seasonality noted with this product, but it is not as evident as with some others noted in the text. These rates are higher than labeled ornamental rates (0.02625 lb ai/100 gal water) but similar to labeled soil treatment rates for other pests including JB adults (0.08-0.13 lb ai/acre). Deltamethrin did not provide consistent JB control as a dip treatment at any of the IFA rates tested.



Dimethyl phosphonate/trichlorfon: This is primarily an insecticide for control of turfgrass pests such as white grubs, mole crickets, cutworms, etc. This product was tested at rates of 4 and 8 lb ai/100 gal water on IFA. Acceptable control was achieved only at the 2 week evaluation and significantly decreased by 1 month. Therefore IFA testing as a standalone treatment was dis-continued. However, when combined with bifenthrin there does appear to be some potentiation affects as noted in that section above using very low rates of trichlorfon (0.125 lb ai/100 gal). Trichlorfon was 100% effective against JB in 12 inch diameter root balls at 4 to 8 lb ai (fall) and 0.0625 to 8 lb ai (spring), as well as in 24 inch root balls at 4 lb ai (fall) and 1 to 4 lb ai (spring).

Dinotefuran: Dinotefuran was tested one spring (2008) only at a rate of 0.54 lb ai/100 gal water. While it provided 88% control at 2 months further testing was not conducted. Dinotefuran provided 100% JB control in one spring trial with 12 inch B&B at the 0.54 lb rate.

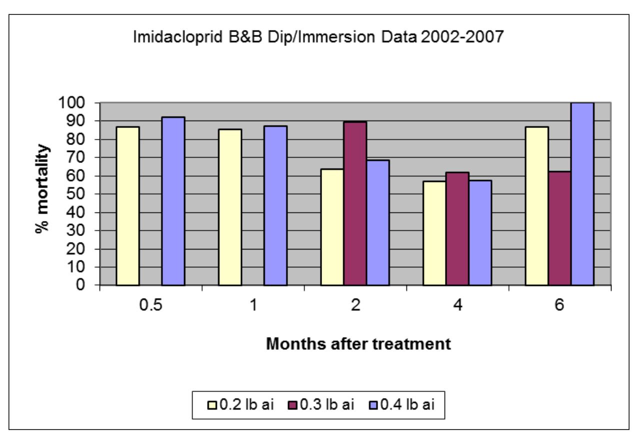
DPX-E2Y50/chlorantraniliprole: This was a numbered compound when tested against IFA as a dip/immersion treatment in 2008. It is now sold under the trade name Acelepryn (ai = chlorantraniliprole) and is currently approved for use in the JB Harmonization Plan as a sod treatment. Applied at a rate of 0.42 lb ai/100 gal water, it did not provide any activity against IFA (<17% mortality at any evaluation period). It did provide 100% JB control at this same rate in a single spring trial with 12 inch B&B, but was ineffective during the fall.

Essential Oils/Biopesticides: Essential oils were tested in fall 2005/spring 2006 and another group tested in fall 2008/spring 2009. No product in either set of trials provided significant control of IFA when used as a B&B dip/immersion treatment. Below is a list of essential oils tested.

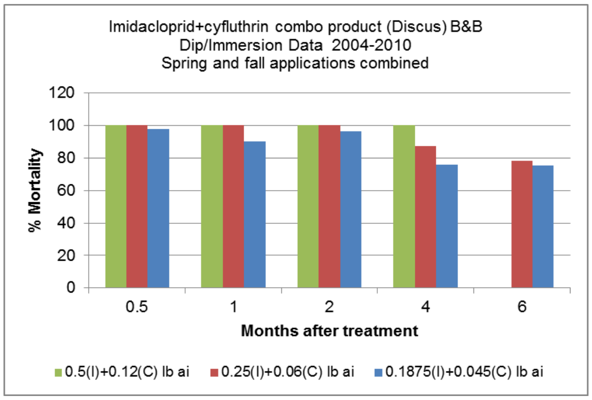
Oil Name	Active ingredients	Application Rate
Armorex	rosemary, garlic, clove, white pepper, sesame	12.5ml/gal water
Azatin XL	azadiractin/neem	17.5ml/gal water
Cinnacure	cinnemaldehyde	12.5 and 37.5 ml/gal water
Eco Trol	rosemary oil, peppermint oil	10.65 and 20 ml/gal water
Eco E-rase	Jojoba oil	75.7 ml/gal water
Muscle	unknown	37.85 ml/gal water
Triact	neem oil	37.85 ml/gal water

Halofenozide: Halofenozide was only tested one time in TN in the spring of 2002 at rates of 0.75 and 1.5 lb ai/100 gal water. Neither rate provided greater than 45% control and was not pursued.

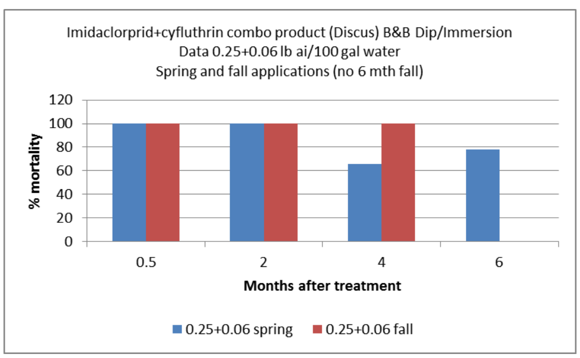
Imidacloprid: Imidacloprid is an excellent control product for JB and used in the JB Harmonization Plan on grass sod, containerized nursery stock (incorporated and drench) and preharvest soil treatment for B&B stock. However, it is very inconsistent for control of IFA as a B&B dip/immersion at the rates tested of 0.2, 0.3 and 0.4 lb ai/100 gal water. Even when combined with bifenthrin, there was no increase in residual activity; thus no apparent potentiation effects with imidacloprid for control of IFA (see text above on combined treatment). Seasonal differences in efficacy at these rates, with limited trials, were not readily apparent. Imidacloprid was consistent against JB as a dip on 12 inch root balls at a rate of 0.4 lb ai/100 gal water during both fall and spring.

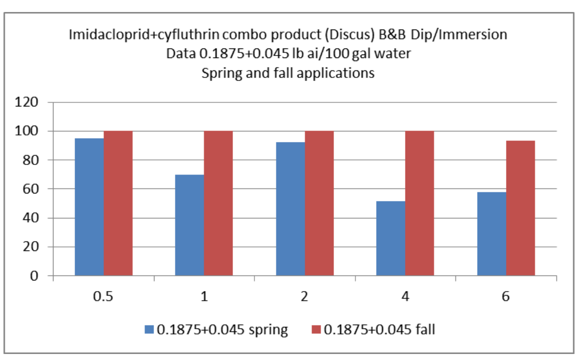


Imidacloprid + cyfluthrin: Imidacloprid+cyfluthrin is available as a combination product under the trade name of Discus® and is used in the JB Harmonization Plan as a pre-harvest soil treatment for B&B stock. The highest rate (0.5+0.12 lb ai) tested did provide good control for IFA for 4 months and the middle rate (0.25+0.06) provided 2 months of activity (no 6 month data for the higher rate). Although many Discus rates gave 100% JB control, the highest rate tested (0.5+0.12 lb ai) had a control failure in both spring and fall tests, making this product too inconsistent in this use pattern for usage against JB.

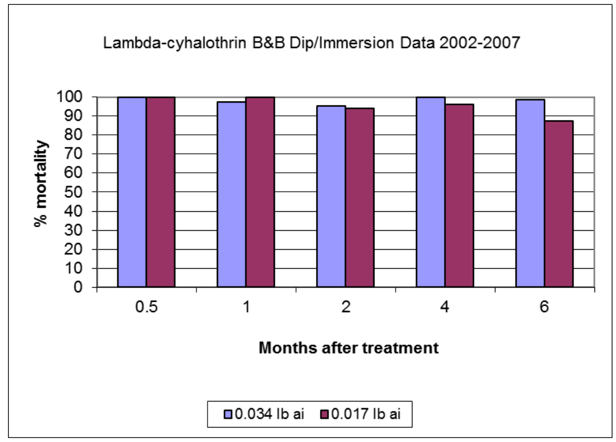


Seasonality appears to affect efficacy of this product as well at the 2 lower rates of application with spring applications losing efficacy sooner than fall applications.

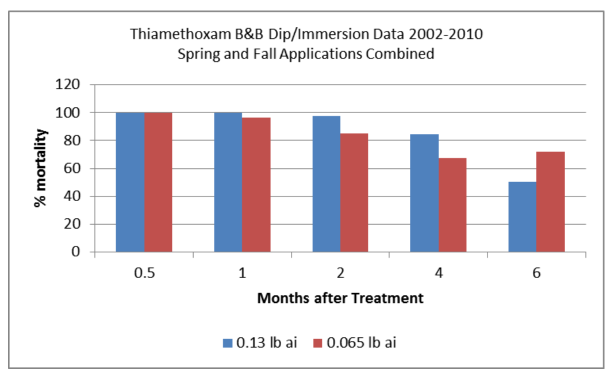




Lambda-cyhalothrin: Lambda-cyhalothrin shows promising results when used as a B&B dip/immersion for control of IFA but is a bit inconsistent. Only spring trials were conducted at the 0.017 rate, and the 0.034 rate showed very little difference in efficacy between spring and fall applications. However, 0.034 lb ai/100 gal water is the highest labeled rate at this time, and while the label allows for multiple treatments at 7 day intervals, this would be cost prohibitive and very labor intensive to do multiple dips. Lambda-cyhalothrin was ineffective against JB at the 0.034 rate in both a fall and spring trial.



Thiamethoxam: Thiamethoxam is an excellent JB insecticide, used in the JB Harmonization plan for sod, containerized drenches and pre-harvest soil treatment for B&B nursery stock. As an IFA dip treatment for B&B stock, there is some potential for a short term certification with the 0.13 lb ai rate providing 2 months of control (highest labeled rate). The lower 0.065 lb ai rate did not provide efficacy past the 1 month evaluation. While some seasonality was apparent at the lower rate (spring applications less effective than fall applications), the 0.13 rate did not show apparent seasonality in limited trials. Thiamethoxam provided near 100% control of JB at both rates in the spring using 12 inch B&B, but was not consistently effective in the fall.



DISCUSSION: The most promising and consistent performers for control of both IFA and JB when used as B&B dip treatments were as follows.

Bifenthrin alone at rates of 0.05 to 0.2 lb ai/100 gal of water depending on season and formulation (formulation had a bigger impact on JB than IFA); 4-6 months activity for IFA. A rate of 0.115 is in the APHIS approval system for use in the IFA program and the JB program currently allows use of a 0.23 lb rate. Lower rates will be considered.

Chlorpyrifos at 0.125 to 2 lb ai/100 gal of water; rates of 0.125 and 0.25 are both currently in use in the IFA (30 day certification) and JB programs, respectively.

Bifenthrin + carbaryl at rates of 0.006 to 0.0125 lb ai of bifenthrin plus rates of 0.125 to 0.5 lb ai of carbaryl. Only the 2 highest rates of each ai provided consistent control in 24-inch root balls for JB and all rates were consistently effective against IFA for 4 months.

Bifenthrin + trichlorfon at rates of 0.006 to 0.0125 lb ai of bifenthrin plus rates of 0.125 to 0.5 lb ai or trichlorfon. Only the 2 highest rates of each ai provided consistent control in 24-inch root balls for JB and all rates for IFA were consistently effective for 4-6 months (dose dependent).

The most promising and consistent performers for control of IFA when used as B&B dip treatments were:

- Bifenthrin
  - ♦ Data supports 0.05 lb ai/100 gal rate for 4 month certification
  - ♦ Data supports 0.025 lb ai/100 gal rate for 2 month certification
  - ♦ Pursue label changes and language changes in PPQ Treatment Manual
- Chlorpyrifos
  - ♦ No changes
- Bifenthrin + carbaryl
  - ♦ Talk with manufacturer about future of carbaryl and IFA uses
  - ♦ If label feasible, need additional trials
    - ◊ 0.0125 lb ai (b)+0.25 lb ai (c)/100 gal rate – 2 spring and 1 fall
    - ◊ 0.00625 lb ai (b)+0.125 lb ai (c)/100 gal rate – 3 spring and 3 fall
- Bifenthrin + trichlorfon
  - ♦ Talk with manufacturer about possibility of adding nursery uses
  - ♦ If label feasible, need additional trials
    - ◊ 0.0125 lb ai (b) + 0.25 lb ai (t)/100 gal rate – 3 spring and 3 fall
    - ◊ Lower rates of both ai's may be worth investigating as well
- Bifenthrin + imidacloprid
  - ♦ All combination rates effective against IFA included bifenthrin rates that were effective alone, thus no benefit from the addition of imidacloprid against IFA; combination rates that included ineffective standalone bifenthrin rates were not consistently effective against IFA

Most other products, either alone or in combinations, were not consistently effective at the rates of application we tested. Neonicotinoids are generally effective against JB, but less so against IFA, and did not improve efficacy or consistency even when combined with pyrethroids (bifenthrin or cyfluthrin) for IFA.

Alternative Drench Treatments for Balled-and-Burlapped (B&B) Nursery Stock Use in the IFA Quarantine, Spring and Fall 2012 in Tennessee

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Jason Oliver and Nadeer Youssef (Tennessee State University)
Chris Ranger and Jim Moyseenko (USDA-ARS)
David Oi (USDA-ARS-CMAVE)

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. Soil core bioassays for drenches conducted in 2002 and spring 2003 yielded erratic results over time and among replicates within treatments. Results from the same chemicals at equal or lower rates, when applied by immersion, were consistent, thus indicating insufficiency in application of the drench treatments. Doubling the volume of solution in drench application conducted in fall 2003 and spring 2004 failed to eliminate inconsistent results. The search for the cause of the inconsistency problem became narrower and has pointed to coverage and penetration of the drench solutions.

During drenching, B&B normally rests on one side of the root ball throughout the three-day drench process. This was true for all drench treatments done before fall 2004. This drench method possibly restricts treatment coverage on the resting side, while giving the surface of direct application a higher concentration of chemical and deeper penetration. The 2004 fall drench strongly suggested that rotating root balls during treatment, regardless of application frequency, improved the consistency of bioassay results and could potentially cut the number of days spent applying drenches from three down to one. Trials were repeated from spring 2005 to fall 2007 to examine whether changes in plant handling during application improve penetration and coverage and possibly allow reduction in the number of days required to complete a drench. Results of such trials can be found in our annual reports each year from 2005 to 2007. It is clear that rotating root balls during treatment application leads to a uniform coverage of the spray treatment and consistently effective bioassay results.

2012 drench trials in TN again focused on examining some promising insecticides and plant handling methods for 12” root balls. Multiple insecticides and their combinations, application frequencies, and plant handling methods (rotating) were investigated.

<u>MATERIALS AND METHODS</u>: In March 2012 and again in October 2012 TSU-NRC and USDA-ARS personnel completed drench applications on B&B plants with 12-inch diameter root balls at the TSU-NRC in Warren Co., TN. Treatments were applied at 0.82 gallons per drench using a regular garden sprinkler can (Figs. 1 & 2; Tables 1 & 2). Solutions were applied twice daily (once in the morning and again in the afternoon) and between these applications the root balls were rotated or flipped to expose a different side to the direct application. This plant handling methods are described as 1F1. This method requires minimum chemical solution and days of application for drench treatments. The regime 2F2, which was not used in this trial was to apply one drench in the morning and another in the afternoon on one side of the root balls for the first day. The next day, flip the trees and drench two more times (morning and afternoon) for the other side of the root balls. 2F2 treatments would receive twice as much drenching and thus twice as much a.i. as the 1F1 application. The regime 6NF was not used in this trial but as the currently approved drench application method it requires applying drenches twice a day for 3 consecutive days without flipping the root balls. The amount used per drench application was based on the amount needed to achieve “the point of runoff” required in the IFA quarantine.

After final treatment, the plants were maintained outdoors to weather naturally. Five replicate root balls were selected out of the 8 plants in each treatment group at 0.5, 1, 2, 4, and 6 months after final treatment for soil core sample collection. One soil core sample was taken from the mid-side area of each rootball at the initial bioassay day. On next sample day,

Table 1. List of treatments for 12 inch root ball drench trial in TN spring 2012

Product	Active Ingredient	Rate* lb ai/100 gal H_2O	Handling	
			1F1	2F2
Allectus	Imidacloprid+bifenthrin	0.0625+0.05	X	
Onyx 23%	Bifenthrin	0.05	X	
Onyx 23%	Bifenthrin	0.025	X	
Control		--		X

*all treatments applied true to listed rates without converting to 6NF first.

Table 2. List of treatments for 1 inch root ball drench trials in TN fall 2012

Product	Active Ingredient	Rate* lb ai/100 gal H_2O	Handling	
			1F1	2F2
Allectus	Imidacloprid+bifenthrin	0.0625+0.05	X	
Onyx 23%	Bifenthrin	0.05	X	
Onyx 23%	Bifenthrin	0.025	X	
Control		--		X

*all treatments applied true to listed rates without converting to 6NF first.

we rotated the rootballs for a quarter turn (as shown in Fig 3) and took a soil core from the mid-side of the rootballs at the new location. We rotated the rootballs again for a quarter turn and took the third soil core from the mid-side area and so on. Soil samples were collected from within the first four inches of soil depth for testing against red IFA. The soil samples were frozen and sent to the ARS-CMAVE Lab in Gainesville, FL where they were utilized in female alates bioassays. A single bioassay cup containing 10 female alates was utilized for each soil sample (replicate). Female alate mortality was recorded two times a week during the 14-day exposure period, and dead alates were removed from bioassay cups during these observations (Figs 4 & 5); (Appendix I – Standard Laboratory Bioassay).

RESULTS AND DISCUSSION: Similar to previous trials at these rates, all these rates are either inconsistent (imidacloprid+bifenthrin) or at the end of the efficacy range for the products (bifenthrin alone) (Figures 6 & 7). Higher rates of these products are more consistent and effective.

A summary of all B&B drench treatments will be provided in the 2013 annual report allowing us more focused testing and determination of any validation trials needed to move forward with approval of any treatments for inclusion in the federal IFA quarantine.

Fig. 3. Soil core sample collection sites

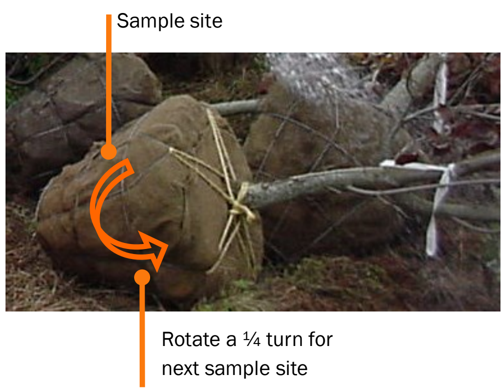


Fig. 4. A tray of alates mortality bioassay cups.

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

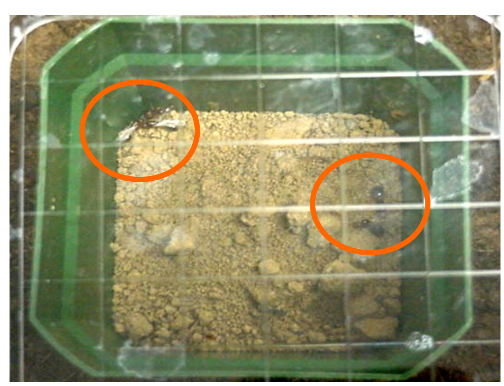


Fig. 6. IFA control achieved with soil samples treated with various insecticides at 0.5, 1, 2, 4 and 6 months after final drench application in TN spring 2012.

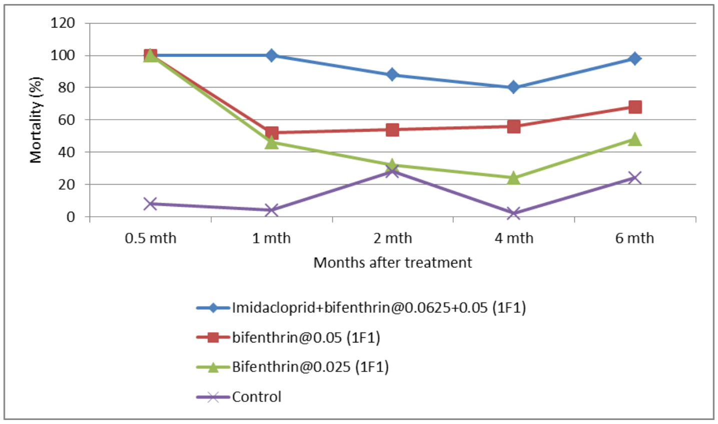
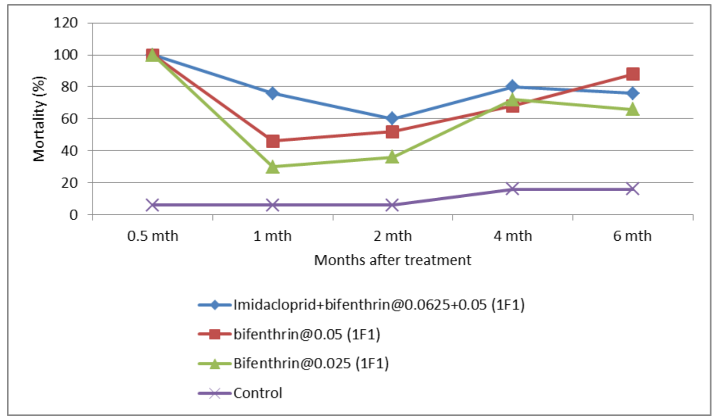


Fig. 7. IFA control achieved with soil samples treated with various insecticides at 0.5, 1, 2, 4 and 6 months after final drench application in TN fall 2012.



Individual Tree Drench Treatment Using 5-gallon Bucket or Tree Rings as a Potential Quarantine Treatment for Field Grown Nursery Stock – Tennessee Fall 2012

Jason Oliver and Nadeer Youssef (Tennessee State University)
Anne-Marie Callcott (APHIS-PPQ-CPHST)
David Oi (ARS-CMAVE)
Chris Ranger, Mike Reding, and Jim Moyseenko (ARS-ATRU)

INTRODUCTION: APHIS is responsible for developing treatment methodologies for certification of regulated commodities, such as field grown balled-and-burlapped nursery stock, for compliance with the Federal Imported Fire Ant Quarantine (7CFR 301.81). Current treatments for field grown nursery stock, as described below, are not only inefficient but also come with environmental and human health problems. Thus additional treatment methods, as well as additional approved insecticides, are needed to ensure IFA-free movement of this commodity.

The primary objective of a quarantine treatment for field grown nursery stock is to render the plants fire ant free. The currently available pre-harvest (in-field) treatment requires a broadcast of approved bait followed in 3-5 days by a broadcast application of granular chlorpyrifos. This treatment must extend 10 feet beyond the base of all plants to be certified. After a 30-day exposure period, plants are certified IFA free for 12 weeks. A second application of granular chlorpyrifos extends the certification period for an additional 12 weeks. The ten-foot radius requirement, due to row spacing, frequently includes plants and soil that otherwise need not be treated.

Various drench methods such as tree ring chemigation, multiple bucket drench, or other in-field drench application, coupled with burlap treated before or after harvest could provide a practical quarantine treatment option in addition to the currently available treatment methods such as post-harvest dip, drench, and pre-harvest (in-field) treatment. Tree-ring chemigation or other pre-harvest drench applications may penetrate the entire root ball area with a chemical solution to achieve results that are similar to the dip treatment, but do not require the use of heavy equipment or come with the problem of disposing a large volume of harmful chemical waste at the end of the treatment. Compared with post-harvest drench, the tree-ring or infield bucket method could reduce labor and chemical costs and have little or no run-off problem. Also, this method selectively treats the trees to be harvested, thus avoiding the unnecessary treatment to the entire field and eliminates the need to wait for a 30-day exposure period before harvesting. Bifenthrin treatment of the burlap wrapping before or after harvest may kill newly-mated fire ant queens that land on the rootballs through contact.

The objective of this study was to evaluate an alternative quarantine treatment method that uses various drench methods for individual tree (in-field) treatment combined with bifenthrin treatment to the burlap wrapping before or after harvest. Specifically, we wanted to find out the effectiveness of infield 5-gal bucket drench treatment method and also at normal aging conditions how long the treated-burlap and root ball soil could kill IFA before losing quarantine level efficacy. Our overall goal was to develop an IFA quarantine treatment method for field grown B&B nursery stock that is effective, easy to do, economical, environmentally friendly, and endangers neither nursery workers nor trees during treatment application.

MATERIALS AND METHODS:

Fall 2012: Individual tree drenches, using 5-gal buckets or 5-gal Tree Rings, were conducted in a nursery field with rows of redbud (*Cercis canadensis* L.; ~ 2 inch caliper) at Moore Nursery, McMinnville, TN on October 11, 2012. Trees included in the trial were selected with enough space in between so that drench solution from one treatment would not contaminate other nearby drenches. In areas of the field with sloping ground, a garden hoe was used to make furrows between trees outside of the treatment zone, just to ensure no chemical solution could run between trees. Three 5-gal buckets were placed close to the tree and equidistant from each other on three sides of each tree. Each bucket had three 1/16 inch diameter drain holes spaced 3 inches apart and ~ 1 inch above the base of the bucket. The center drain hole was pointed directly at the trunk of each experimental tree (Fig. 1 A&B). One 10-gal Tree Ring Jr. was placed around the trunk of each tree (Fig. 1C). A water tank mounted on the bed of a pickup truck was used to carry water to the treatment field. Drench solutions were first mixed in 5 gallon quantities in plastic containers then the appropriate amount poured into buckets or tree rings (see Table 1 for treatment details). Total finished solution volume was equally distributed between the 3 buckets. Four trees were used in each treatment.

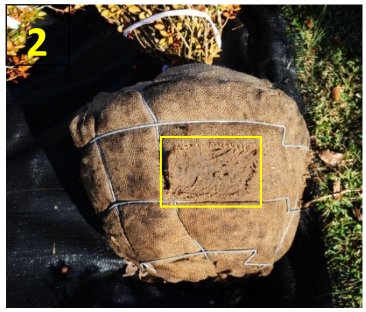
In past trials, trees were harvested at 24 hours after treatment, but significant rain the night after the all 2012 IFA treatments prevented entry into the site the day after treatments. Treated trees were harvested on October 13, 2012 at 48 hours post-treatment. Root balls had top and bottom diameters of ~60 cm and 30 cm respectively, and a ball height of ~50 cm. Trees were placed in metal baskets lined with burlap and wrapped, pinned, twined on the top and crimped according to standard nursery practices by the nursery grower. Trees were transported to an open field site at the TSU Lab on October 13, 2012. Due to late arrival at the lab, the burlap treatment was delayed by a day. Before treating the burlap, we determined that ~ 1 gallon of water was needed to wet the entire surface of the burlap on the control root balls. Control root balls only received water. On October 14, 2012, each treated root ball received 1 gallon of solution applied with a sprinkle can and mixed at a rate of 0.94 ml Onyx Pro per gallon of water (0.05 lb ai/100 gal of water). This same rate of bifenthrin solution was sprinkle drenched on all chemically treated trees regardless of what rates the trees had received at the previous bucket-drench in the field. One side of the root ball was treated with about half of the solution, then the root ball was rotated and the other side was treated with the remainder of the solution. During the drench process, care was taken to also treat the top part of the ball (where the tree exits) and

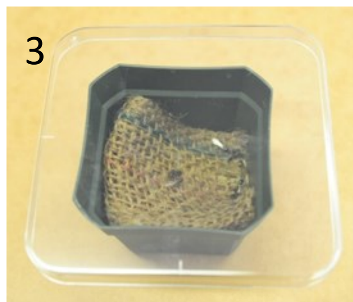
Table 1. Treatment list for individual tree drench application at Moore Nursery, TN fall 2012.

Season	Active Ingredient	Rate of Application (lb ai/100 gal water)	ml prod/gal	Method of App	Gal finished soln/tree	Total ml product/tree
Fall 2012	Bifenthrin (Onyx Pro®)	0.0125	0.237	3 buckets – 5 gal soln per bucket	15	3.56
		0.025	0.473	3 buckets – 5 gal soln per bucket	15	7.1
		0.025	0.473	3 buckets – 3.3 gal soln per bucket	10	4.73
		0.025	0.473	1 tree ring	5	2.37

the bottom part (opposite from the tree exit side). At the completion of the burlap treatment, root balls were rotated back to the original position and left undisturbed at that point. The trees were stored outdoors in full sunlight without straw, mulch or overwintering blankets, which is not a typical nursery practice, but did expose the chemical treatments to more solar degradation. Trees were initially watered as needed during the fall until dormancy (i.e., moisture loss from transpiration ceased); then no additional watering was required due to frequent winter rains.

In previous trials a surfactant was used to facilitate application. The product used in these trials was Suffusion®, a blend of three types of surfactants; wetter/ spreaders, penetrants and re-wetting agents, specifically for use on growing media during plant production.





Bioassay method: To evaluate the residual effect of bifenthrin-treated burlap over a 6-month aging period under outdoors conditions, a piece of burlap was cut from each of the root balls and sent to the ARS-Gainesville lab for efficacy evaluation (Fig. 2). The burlap piece was placed in a standard bioassay cup and covered with a clear square dish (Fig. 3). A few drops of water were added to moisten the burlap if needed.

Soil samples were also collected from the surface (about 1 cm deep) of the root ball where the burlap was removed (Fig. 2) to determine if the soil that has direct contact with the treated burlap would also kill the ant as the burlap does. The bioassay method for the soil samples was the same as that for burlap pieces. Both burlap and soil samples were frozen and shipped to the ARS-Gainesville lab for bioassay.

To do the bioassay, ten field collected female alates were used for each burlap or soil sample taken from a root ball. Female alates were placed on top of burlap or soil in the bioassay cup and allowed free contact with the material to be tested (Fig. 3). Alates were not given food, but water was added to moisten the burlap or soil if they were not sufficiently moist. Mortality data were taken at 4, 7, 10 and 14 days after exposure. To determine the residual effect of bifenthrin-treated burlap over time, burlap and soil samples were taken at 1, 2, 3, 4, and 6 months to monitor the degradation process.

<u>RESULTS</u>: Excellent results were obtained with all treatment rates and methods of application (Figs. 4 & 5). Multi-year data with the higher rates of application and 10-15 gal finished solution per tree have been consistently successful and we will complete testing of several treatment rates at 10-15 gal applications in 2013. We will also continue trials at several rates of application using the 5 gal of finished solution as this allows less water and time in the field. This type of testing does not test efficacy against infestation by whole colonies or the elimination of existing colonies. Other trials will be initiated in 2013 to determine the full range of protection provided by this type of treatment. A summary of all bucket and tree ring with post-harvest treated burlap will be compiled in 2013-2014.

Fig. 4. Mortality of IFA alate females when exposed to soil from bucket or tree ring drenched field grown nursery stock subsequently harvested and wrapped in burlap that was then sprinkle drenched with 1 gal of 0.05 lb ai bifenthrin/100 gal water (after wrapping). Various infield drench rates of application and amounts of finished solution. Tennessee fall 2012 trial

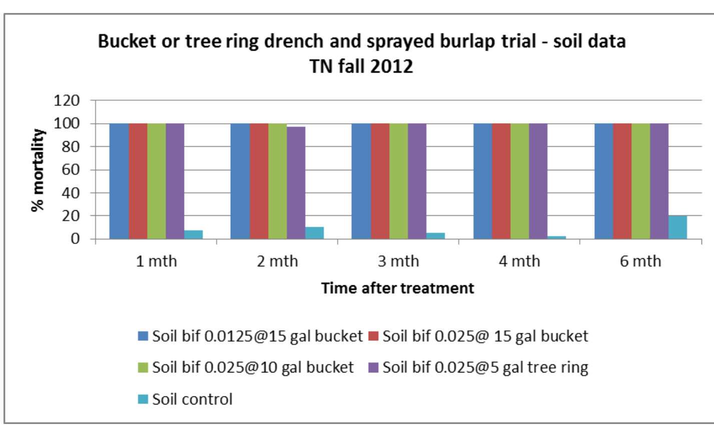
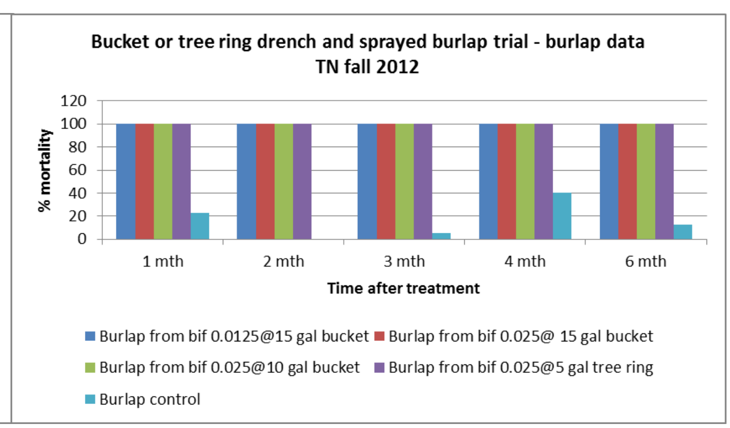


Fig. 5. Mortality of IFA alate females when exposed to burlap from bucket or tree ring drenched field grown nursery stock subsequently harvested and wrapped in burlap that was then sprinkle drenched with 1 gal of 0.05 lb ai bifenthrin/100 gal water (after wrapping). Bucket and tree ring drenches were of various rates of application and amounts of finished solution. Tennessee fall 2012 trial.



Drench Treatments for Balled-and-Burlapped Nursery Stock Use in the IFA Quarantine: Ability to Eliminate Live IFA Colonies Wrapped inside Harvested Root Balls, Mississippi Fall 2011

Xikui Wei, Anne-Marie Callcott, Craig Hinton & Lee McAnally (APHIS, PPQ, CPHST)

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

There have been numerous trials evaluating various insecticides, rates of application, and treatment method (flipping root ball between drench applications) in the past several years, but all treatments have focused on eliminating or killing IFA alate females exposed to the treated soil. The objective of this study was to determine if live fire ant colonies inside root balls of trees would be killed/eliminated by the most promising of these alternate insecticides; bifenthrin. To this end, we conducted a whole root ball bioassay with live ant colonies wrapped inside root balls of harvested trees.

MATERIALS AND METHODS: Trees with live ant colonies within each root ball were purchased from Deep South Nursery, Lucedale, George County; MS. Thirty-six shrubs were machine harvested on November 3, 2011. In order to keep live fire ant colonies in each root ball, digging and wrapping were done such that there was as little disturbance as possible to the tree bases where the fire ants nested. After trees were excavated by machine harvester, either bifenthrin-treated burlap or plain burlap was used to wrap the root balls with live ant colonies inside. Six balls were wrapped in plain burlap and each placed in a Plantainer™ pan painted on the inside surface with Fluon® to prevent ants from escaping during transport and during the trial. Since a previous trial indicated that bifenthrin-treated burlap would not immediately kill a colony inside a root ball, the remaining 30 root balls were wrapped with bifenthrin-treated burlap to insure ants remained inside the root ball during transport and handling prior to drench treatment.

To do the pre-treatment of the burlap, 30 burlap liners (10 oz weight burlap) were soaked in bifenthrin solution (12 gal at 0.01 lb ai per 100 gal of water) for 24 hours. Then the fully soaked burlap liners were taken out to dry in the green house and they were ready to use after drying. This was done a few days prior to harvesting of root balls.

Drench application: On November 4, 2011, 24 root balls were treated with bifenthrin at 0.1 lb ai/100 gallon water in a drench application plus a blue dye to help insure complete coverage of the root ball. Controls (6 in plain burlap and 6 in pre-treated burlap) were sprayed with water plus dye only. Water volume per drench was determined by measuring the root ball volume (7 gal per ball) and taking 1/5 of the volume (1.4 gal) to be used for the total drench volume of each root

ball. Since this total volume of 1.4 gal per root ball was for 2 drenches, each drench used 0.7 gal per root ball and 4.2 gal for a group of 6. When doing the application, this 4.2 gal solution (mixed in a 5-gal bucket) was sprayed and emptied onto 6 root balls. Insecticidal solutions were prepared in a 5-gal bucket and siphoned through a hose attached to a battery-powered pump which was connected to a sprayer nozzle. Our drench applications showed that this water volume was about right and it reached the point of run-off when finished drenching but without having too much run off to the ground. Root balls were drenched on one side, allowed to rest for 30 minutes, then flipped and drenched to the other side of the rootballs (referred as 1F1 in numerous other B&B drench project reports).

Evaluation: After final treatment, the plants were maintained outdoors to weather naturally and an irrigation schedule was set up to closely simulate outdoors nursery storage conditions. Root balls were destructively sampled at 4 week intervals. One root ball from each control group (plain burlap aged in containers and treated burlap aged on the ground) and 4 bifenthrin treated root balls were sampled at each evaluation period by splitting open the root balls and examining for live ants.

RESULTS AND DISCUSSION: Between 4 and 16 weeks after treatment, bifenthrin drenched root balls did not achieve greater than 50% control in this trial while the controls maintained live ants through 16 weeks indicating some effect of the drench on the ants (Table 1). While the treated root balls showed 75% and 100% mortality at 20-24 weeks, respectively, the controls were also showing 100% and 50% mortality, respectively, at the same periods, indicating probable natural mortality occurring.

In trials conducted in 2004 and 2006, bifenthrin applied as a drench (with no rotating) at 0.1 lb ai/100 gal to 16" root balls eliminated 90-100% of fragmented field collected IFA colonies (0.33 liter of workers and brood only) introduced onto the top of the root ball either 48 hours prior to or within 24 hrs after treatment. The 2011 results, using established IFA field colonies collected as a part of the root ball, showed that these established colonies were not easily eliminated at this rate of drench application.

These results indicate that we may need to re-evaluate the rates of application for B&B drench treatments. Higher rates of application or increasing number of drench applications and thus increasing amount of ai applied to each root ball may be needed to eliminate whole colonies vs eliminating newly mated queens (results of alate female bioassay trials). The alternative would be that a pre-harvest treatment to the base of trees to kill the ants in the root ball area using bucket drench, tree ring dripping, or other application methods may be a necessary step to "clean" the root balls before wrapping them up and treating the burlap. Additional studies looking at higher rates and multiple applications will be needed to validate drench research we conducted in the past years.

Table 1. Mortality of IFA colonies in root balls after treatment with bifenthrin root ball drench application

Date	Weeks after treatment	Control		Treated Bifenthrin 0.1 lb ai/100 gal water bifenthrin (1F1)			
		Plain burlap in container	Pre-treated burlap on ground				
		Rep 1	Rep 1	Rep 1	Rep 2	Rep 3	Rep 4
Dec. 02, 2011	4 WAT	live	live	live	live	live	dead
Dec. 29, 2011	8 WAT	live	live	live	live	dead	dead
Jan. 27, 2012	12 WAT	live	live	live	live	live	dead
Feb. 24, 2012	16 WAT	live	live	live	live	dead	dead
Mar. 23, 2012	20 WAT	dead	dead	live	dead	dead	dead
Apr. 23, 2012	24 WAT	dead	live	dead	dead	dead	dead

Development of IFA Quarantine Cold Temperature Techniques for Certifying Bulk Soil for Movement: Large Scale Trials

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11-8130-0086-CA

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INTRODUCTION: As a federally regulated item, under the Federal Imported Fire Ant Quarantine (7CFR 301.81), bulk soil must be treated in an approved manner prior to shipping outside the regulated area to prevent IFA from inadvertently being moved to a previously uninfested area. Currently, only heat treatment is approved for bulk soil. This is not a viable option for contaminated soils destined for burial since heating may produce dangerous volatiles from the contaminants. The goal of this work is to provide initial data in full-sized refrigerated containers to support cold treatment of bulk soil to meet the needs of all types of bulk soil. It is the ultimate goal of this research to provide data that will be used to support inclusion of cold-treatment options in the Federal Imported Fire Ant Quarantine (7CFR 301.81).

MATERIALS AND METHODS:

Collection and separation of ants. Imported fire ants (IFA) were collected on October 17, 2011 from a farm in Kingston TN and brought back to the lab to be separated from the soil. Fire ants were collected in 5 gallon buckets coated with fluon (BioQuip Products Inc., Rancho Dominguez CA). Four different colonies were collected. After fire ants were brought back to the lab, soil and ants were dumped into a plastic container coated with fluon. At least one stack of three nest cells made from plaster of Paris (DAP Inc, Baltimore MD) was put into each container. Nest cells were soaked in water before being added to the trays. Water tubes and frozen crickets were put into the trays. Plant lights (120w medium base plant light, Philips Lighting Company, Somerset NJ) were turned on to aid in the drying of soil. As soil dried and ants moved into the nest cells, soil was removed and more soil/ants added to the container. Soil was disturbed every day to speed drying. Nest cells were spritzed with water to help maintain moisture. Frozen crickets were added every day and water tubes added as needed. A WatchDog data logger (Model 150, Spectrum Technologies Inc, Plainfield IL) was placed on the lab bench to record temperature and humidity in the lab.

Trial 1. The first round of freezing was set up on October 25, 2011. That morning, nest cells with ants were removed and placed into an 18 quart fluoned container. Ants were scooped up with an index card, tapped into an 8 cup plastic container coated with fluon and then put into labeled 4oz snap seal cups (Corning Snap Seal Vials No. 1730) partially filled with nest tumulus. One set of controls from each colony was set up to be kept at room temperature and one set to keep in a refrigerator in the lab. Colonies of ants were randomly assigned to different containers and depths. A WatchDog data logger was put into the refrigerator to record temperature and humidity. The ants were then transported to the Rogers Group Quarry in Oak Ridge to be buried in the containers of soil.

Soil for containers was obtained from the University of Tennessee Forest Resources Research and Education Center (UT-FRREC). Four types of containers were filled with soil: a 55-gallon drum, super sack, B-25 box and a cubic yard box. Reconditioned 55 gallon open head steel drums (Volunteer Drum, Knoxville, TN) were used. The super sacks were a 38 X 38 X 38 inch duffle top, flat bottom 6 oz. woven polypropylene 3 ml liner (Vac-Pac, PacTec, Clinton, LA). The HazPak ™V cubic yard boxes (Containers Distributors, Venice, IL) had a five-wall construction of a double outer wall laminated to a triple inner wall and the liner was 8 mil LDPE. The B25s were constructed of steel and were roughly 4 X4 X 6 ft with butterfly clips used as locking mechanisms (Impact Services, Oak Ridge, TN). All containers except for the B-25 box were put onto pallets prior to being filled with soil using a tractor with a front end loader. Depths at which ants were buried were changed from 12”, 18”, 24” and 30” from the bottom of the container to 6”, 12”, 18” and 24” from the bottom of the container because soil containers were not filled all the way (Figure 1). All cups of ants were buried in the center of the containers. Cups of ants were also buried at 18” from the bottom on the left and right sides of the B-25. After containers were filled with soil, cups of ants were buried and a soil sample taken from each spot a cup was buried. The 24” depth cup of ants for the super sack was placed on top of the soil since there was not 24” of soil in the container. Soil samples were brought back to UT and taken to Dr. Jaehoon Lee in the Department of Biosystems Engineering and Soil Science to be analyzed.

A ~40’L x 8’W x 9’6”H cold storage trailer (refrigerated storage unit, A & M Cold Storage, LLC, Suwanee, GA) was placed on a concrete pad at the Rogers Group Quarry in Oak Ridge. Electricians from the quarry hooked up the 230/240volt, 3 phase power on a 60 amp breaker. The trailer’s minimum temperature setting was -20°F and was set at -15°F so as not to overtax it. The trailer had lockable double doors and durable, aluminum t-rail flooring rated for pallet jack and fork lift traffic. An electric Carrier unit cooled the trailer.

HOBO Pro v2 U23-003 data loggers (Onset Computer Corporation) with two 6 feet long external temperature sensors

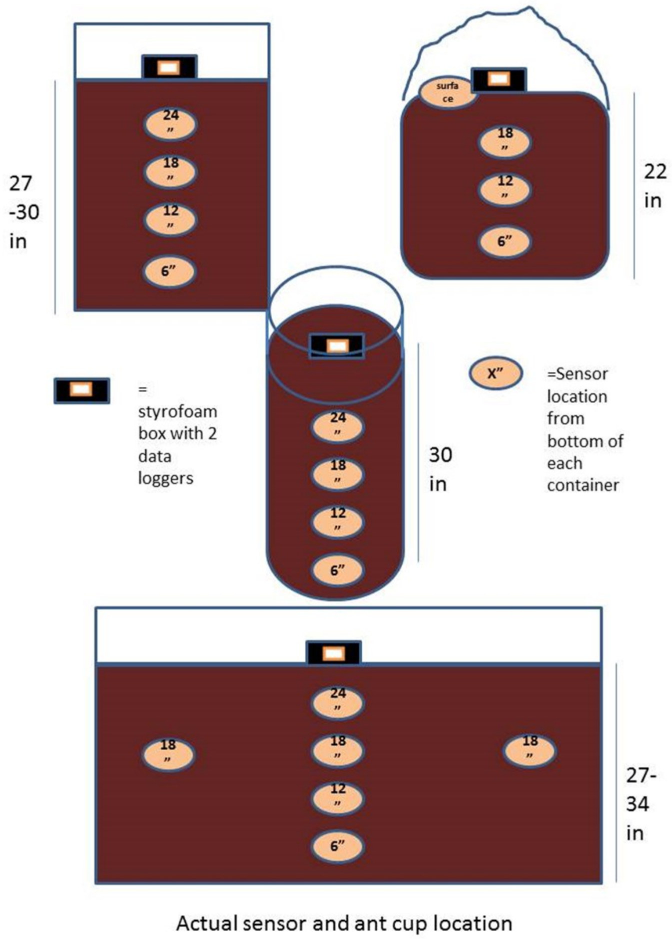

Figure 1. Location of sensors in each container (counter clockwise from top left: cubic yard box, Supersac, 55-gallon drum and B-25)

were used to record soil and ambient air temperatures inside the trailer. Data loggers were set to record temperatures every 15 minutes. There were only enough data loggers to put in one rep of containers so temperature probes were only put into the containers furthest from the condenser. Nine loggers with two temperature probes each were buried with each cup of ants and one logger with two temperature probes was suspended in the air. To protect the probes from damage caused by sharp objects in the soil or from digging implements, the probes were fed through a length of PVC pipe before being buried. The logger was put into a small insulated box with holes drilled in the side to feed the temperature probes through. The box was kept on top of the soil after ants were buried, the drill holes in the box were plugged with poster putty and the boxes sealed with tape. The container of soil was then sealed and put into the trailer with a fork lift operated by a Rogers Group employee. Containers were randomly assigned position within each of the areas in the trailer (Figure 2).

Containers were to be removed on October 31 if the low temperature of -5°C had been reached for 36 hours. When temperatures were checked at 9:15AM on October 31, the 18" temperature probe in the center of the B-25 box had not yet reached -5°C so soil containers were not removed. Data was downloaded from the loggers using a U-DTW-1 HOBO waterproof shuttle (Onset Computer Corporation). Containers were removed the morning of November 2. The freezer was turned off at 8:45AM. We started digging the cups of ants out of the soil before the pallets holding soil containers were removed from the trailer. At 11:15AM, the fork lift arrived and we started unloading containers filled with soil from the freezer. All cups of ants were retrieved by 1:30PM and no temperature probes were damaged. Frozen cups of ants were brought back to the lab and dumped into labeled 8 cup containers coated with fluon. The ants were monitored for at least 24 hours to check mortality. Ants were counted and the mean number of ants per cup per container type was calculated.

Trial 2. The second set up was also completed on November 2. Soil in all cups was spritzed with water prior to placing ants. Ants were counted that morning in the lab as they were for the October 25 set up with the exception of one extra cup from each colony (total of 4) being set up and taken to Oak Ridge with the ants that were to be frozen. These cups were then brought back to the lab and put into the refrigerator to see if transporting the ants had an effect on mortality. In this trial, the cups of ants were transported to Oak Ridge in a cooler to avoid temperature extremes before being buried in the soil.

Removing the frozen soil from the containers so they could be refilled proved to be a challenge. Because of this, only the rep to receive temperature loggers had cups of ants buried in them. The containers that did not have temperature probes were not emptied of soil from the first run but were put back into the trailer. Containers were filled with soil in the same manner as the first set up. The containers were refilled beginning at 2:00PM. At 2:45PM we began burying cups of ants. Burying the ants was simpler this time since Mike from UT-FRREC made a device using a large piece of PVC pipe with two holes drilled in one end with a small piece of rebar through the holes acting as a handle. The pipe was placed into the middle of the containers before filling with soil and the hole covered to prevent soil from entering. The containers were then filled with soil and the pipe removed leaving a hole that the cups of ants could be buried in. We measured from the bottom of the container and filled in the hole with soil, placed the probe/PVC pipe into the hole, covered it with a small layer of soil and then placed the cup of ants into the hole. This was continued until all cups of ants were buried. The 24" cup of ants was placed on top of the soil in the super sack since it did not contain 24" of soil. A representative soil sample was taken from each container instead of every place a cup of ants was buried. Soil samples were brought back to UT and given to Jaehoon Lee to analyze.

Based upon the previous set up, we planned to remove the containers of soil on November 10. When temperatures were checked on November 8, they had already reached -5°C but would not be at that temperature for 36 hours until later that evening. Removal of ant cups was planned for the following morning. We turned the freezer off at 10:00AM and began digging out the cups of ants. The second soil type was much more difficult to dig in. Because it was raining outside, containers of soil were not removed from the freezer. We stopped digging around 7:00PM and continued the following morning. Not all cups of ants were recovered, and we mistakenly cut four temperature probes. Frozen cups of ants were brought back to the lab and dumped into labeled 8-cup containers coated with fluon. The ants were monitored for at least 24 hours to check mortality. Ants were counted and the mean number of ants per cup per container type was calculated. Soil was removed from the containers by UT-FRREC employees at a later date after soil had defrosted.

RESULTS AND DISCUSSION:

Trial 1. The cold treatment was successful. All fire ants removed from the cold storage trailer at 7.71 days appeared to be dead. Twenty-four hrs after removal this was confirmed in the laboratory. Ants placed in rep 3 for the first trial, averaged 550±239 ants per cup, n=17) (Table 1). One cup was smashed by the shovel during removal and thus the ants were not counted.

All of the control ants in Trial 1 were dead when checked on day 9. Unexpected ant death may have been caused by 1) very dry soil; (2) new and unwashed cups; and (3) exposure to warm laboratory room temperatures. Preliminary observations of the cups during the first few days indicated that mortality had occurred rather quickly. If reasons one and two were correct, we cannot determine if the cold was responsible for the dead ants removed from the trailer. However, in trial 1, the location that remained the warmest throughout the study were the probes placed 12 or 18 inches from the bottom of the rep 3 B-25. At these two locations the lowest temperature achieved was -15.6 or 15.7°C (Figures 3-7). The mean super cooling point of Tennessee-collected hybrid imported fire ants

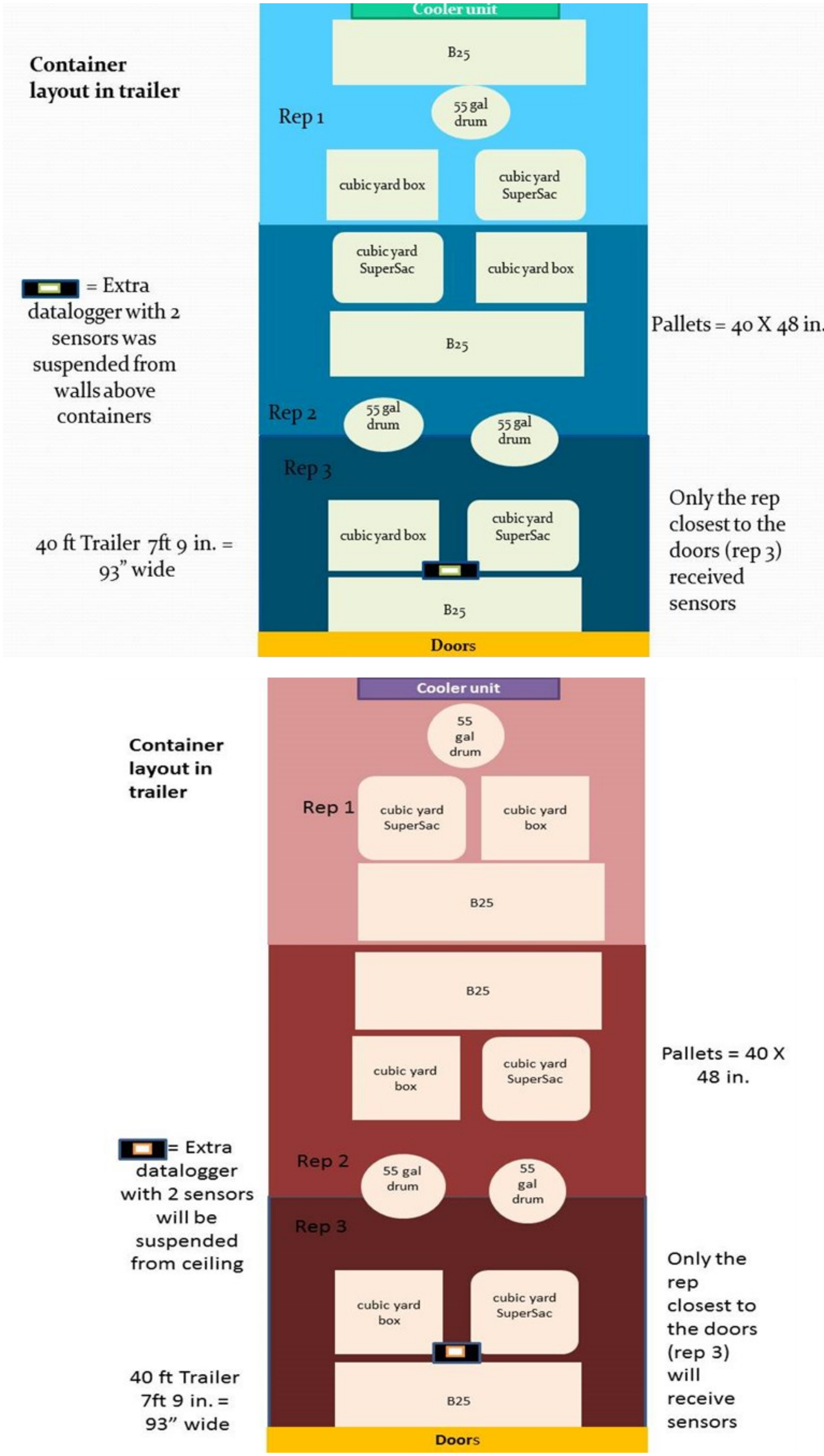

Figure 2. Trailer arrangement of containers for trial 1 (top) and trial 2 (bottom) not to scale.

Table 1. Number of ants in cups October 25 set up.

Container Type	Depth				Mean ± SD
	6”	12”	18”	24”	
B-25	206	879	Left 829 Center NA Right 503	487	580.8 ± 276.5
55-gallon drum	558	585	597	362	524 ± 108.9
Super sack	353	394	913	397	514.3 ± 266.6
Cubic yard box	419	1076	472	329	574 ± 339.8

Table 2. Number of ants in cups November 2 set up.

Container Type	Depth				Mean ± SD
	6”	12”	18”	24”	
B-25	583	*	Left * Center 1049 Right *89	753	795±235.8
55-gallon drum	*	276	482	356	371±103.8
Super sack	506	386	760	641	573±162.3
Cubic yard box	*82	583	423	880	492±332.5

was -6.9±0.23°C for small workers and -6.6±0.09°C for large workers (James et al. 2002). Alates, which were not present in our study, supercooled at -10±0.59°C. If ants in Trial 1 had survived in the cups before being introduced to the cold, they would have surely supercooled when exposed to the trailer temperatures. All container probes recorded temperatures less than -10°C after 186 hrs. Thus the ants were exposed to less than -10°C for 58 hours or more.

Trial 2. The cold treatment in trial 2 was also effective. Removal of ants and probes took considerably longer in the second trial and extended over 24 hours. Only two people were available to remove the probes and cups of ants for a majority of the work day on November 9. Removal was halted about 7 pm and continued the next day. A mean 590±223 (n=13, Table 2) ants per cup were placed in rep 3 for the second trial. The days in the trailer varied according to probe location. The mean time in the trailer was 7.0±0.4 days (Table 3). Five cups were smashed with shovels during removal and left or partially removed. In addition, four probes were cut during excavation. Twenty-four hours later all ants from the trailer cups were confirmed dead in the laboratory.

The soil cooled quicker in the second trial at all locations except three (12 and 18 inch cubic yard box and super sack 6 inch, Table 3, Figures 8 -12). The faster cooling soil in the second trial could have been due to soil type. The soil in the two trials appeared to be very different. Although we had assumed the soil was moister in the second trial, analysis revealed this was not true. Mean and SD of the soil moisture for trial 1 was 19.6 ±1.2% (n=51) and 17.3 ±1.2% (n=6) for the second trial. The mean percentage sand: silt: clay was 17.1: 15.2: 67.8 and 10.1: 28.8: 61.1 for trials 1 and 2, respectively, which classifies both soils as sandy loam. Unfortunately, due to our poor communication to Dr. Lee's lab, carbon analysis was conducted on five random samples from trial 1 and one sample from trial 2. The mean carbon content for trial 1 was 1.9±0.2 and 3.1 for trial 2. It's possible that carbon content was higher in the trial 2 soil, but lack of trial 2 soil samples for carbon analysis prohibits any statistical analysis.

Container configuration in the trailer was not the same in the second trial. The different fork lift used did not allow extension of a soil-filled B25, which could weigh up to 10,000 lbs., to reach the back of the trailer. Container location within each rep was randomized again. It is possible this new configuration affected air flow. The slower cooling experienced in the first trial may have been caused by the B25 placement at the back of the trailer next to the cooling unit, possibly reducing cold air circulation. Rep 3 containers, where the probes were placed, were in the same location for trial one and two.

Although all containers had been outside the trailer for several hours, only rep 3 received the new soil. Thus the containers in rep 1 and 2 held somewhat defrosted soil and were not as warm as that in rep 3. The containers of cooler soil may have helped lower temperatures more quickly in trial 2.

The rep 3 B25 cooled more quickly in the second trial than the first. This may have been due to the colder metal container cooling the soil more quickly than the warmer rep 3 B25 used in the first trial.

Container type and/or volume of soil appear to influence the cooling rate of the soil. In both trials, the 55-gallon drum cooled most quickly followed by the super sack. These two containers held a smaller volume than the cubic yard sac or the B25 and thus a higher surface to volume ratio. The insulated cubic yard box cooled more slowly in the second trial than the first.

The control ants survived better in trial 2. Some ants from one cup held at room temperatures were still alive on November 11. Some ants from all four cups held in the refrigerator, ~4°C, were still alive. The cooler control cups that were transported via a cooler to the trailer in Oak Ridge and brought back to the lab had excellent survival. These ants experienced the same treatment up to insertion into the trailer as the cold-treated ants and would account for any negative environmental effects caused by transportation to the trailer. Nearly 100% of the cooler control ants were still alive on November 11, over 24 hours after the other ants had been removed from the trailer (Figure 13). Ant survival gradually declined in the cooler control cups and all ants were dead 28 days into the study. Thus we were confident that the ants had died in the trailer due to cold exposure and not due to preparation of the cups. We had improved the ant preparation techniques. Washing the cups prior to use, adding water to the cup soil prior to ant placement and transporting the ants in a cooler reduced any negative environmental effects.

<u>CONCLUSIONS:</u> Freezing soil in bulk containers appears to be a viable alternative to ensuring soils are fire ant free prior to shipping. The cooling of two different soil types in 4 bulk soil containers effectively reduced the soil temperatures below the fire ant supercooling point. In addition, only 7 or 8 days were required for all soil to reach -5°C for greater than 36 hours which was a tentative threshold developed by Anne-Marie Callcott with USDA-APHIS-PPQ.

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Joseph Maples: UT E&PP, fire ant removal from frozen soil and lab support

Pat Parkman: UT E&PP, fire ant removal from frozen soil and transportation support

Table 3. Time ants were in cold storage trailer.

		Trial 1							Trial 2						
Con-tainer	Depth	Date first reached -5°C	Time first reached -5°C	Hours to reach -5°C	Hours at -5°C	Days at -5°C	Total time in trailer (h)	Total time in trailer (d)	Date first reached -5°C	Time first reached -5°C	Hours to reach -5°C	Hours at -5°C	Days at -5°C	Total time in trailer (h)	Total time in trailer (d)
55-gallon drum	6"	27-Oct	9:30	41	144	6.0	185.00	7.71	3-Nov	10:30	16.75	167.5	7.0	184.25	7.7
	12"	27-Oct	18:00	49.5	135.5	5.6	185.00	7.71	3-Nov	16:30	22.75	145.5	6.1	168.25	7.0
	18"	27-Oct	19:45	51.25	133.75	5.6	185.00	7.71	3-Nov	23:15	29.50	133.25	5.6	162.75	6.8
	24"	27-Oct	18:45	50.25	134.75	5.6	185.00	7.71	4-Nov	0:15	30.50	132.25	5.5	162.75	6.8
B-25	6"	31-Oct	11:00	138.5	46.5	1.9	185.00	7.71	5-Nov	0:30	54.75	111.75	4.7	166.50	6.9
	12"	31-Oct	23:45	151.25	33.75	1.4	185.00	7.71	6-Nov	10:15	88.50	96.25	4.0	184.75	7.7
	18"L	31-Oct	0:45	128.25	56.75	2.4	185.00	7.71	6-Nov	7:45	86.00	72	3.0	158.00	6.6
	18"C	31-Oct	18:15	145.75	39.25	1.6	185.00	7.71	7-Nov	6:15	108.50	57.75	2.4	166.25	6.9
	18"R	30-Oct	18:30	122	63	2.6	185.00	7.71	6-Nov	14:00	92.25	101.5	4.2	193.75	8.1
	24"	30-Oct	21:45	125.25	59.75	2.5	185.00	7.71	6-Nov	22:15	100.50	65	2.7	165.50	6.9
Cubic yard box	6"	30-Oct	20:45	124.25	60.75	2.5	185.00	7.71	6-Nov	16:45	95.00	73	3.0	168.00	7.0
	12"	29-Oct	3:30	83	102	4.3	185.00	7.71	7-Nov	11:45	114.00	49.75	2.1	163.75	6.8
	18"	30-Oct	13:00	116.5	68.5	2.9	185.00	7.71	7-Nov	18:00	120.25	46.25	1.9	166.50	6.9
	24"	30-Oct	19:00	122.5	62.5	2.6	185.00	7.71	7-Nov	11:45	114.00	50	2.1	164.00	6.8
Super sack	6"	27-Oct	16:15	47.75	137.25	5.7	185.00	7.71	4-Nov	22:30	53.25	112.5	4.7	165.75	6.9
	12"	28-Oct	11:15	66.75	118.25	4.9	185.00	7.71	5-Nov	0:45	55.50	108.25	4.5	163.75	6.8
	18"	28-Oct	16:30	72	113	4.7	185.00	7.71	5-Nov	0:30	55.25	109.5	4.6	164.75	6.9
	24"	27-Oct	15:00	46.5	138.5	5.8	185.00	7.71	3-Nov	16:15	22.25	141.75	5.9	164.00	6.8

Trial 1 initiated 10-25-11 at 10:30 am; Trial 2 initiated 11-2-11 at 17:45. No ants survived in either trial.

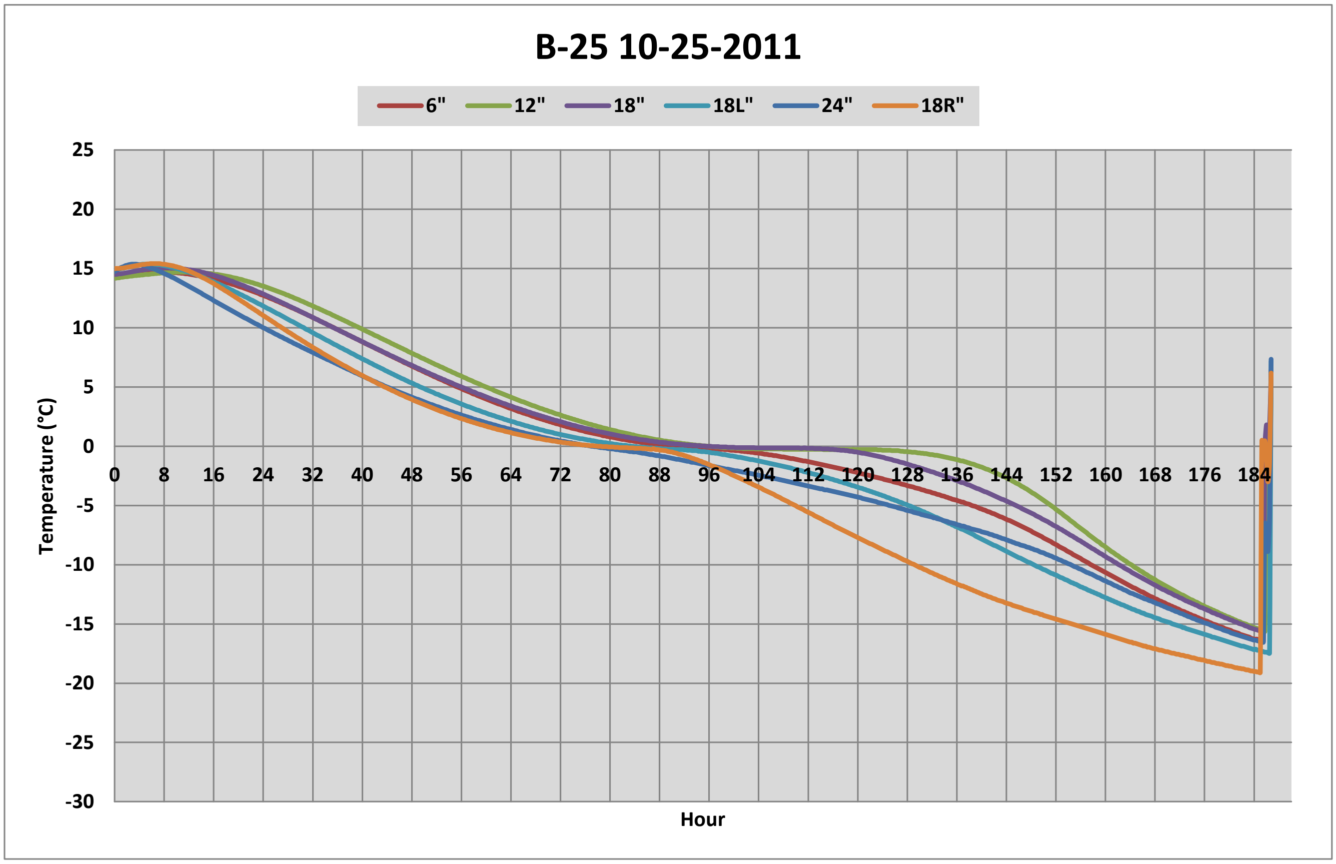

Figure 3. Temperatures (°C) for the six probes buried in the B-25 soil on October 25, 2011 (Trial 1)

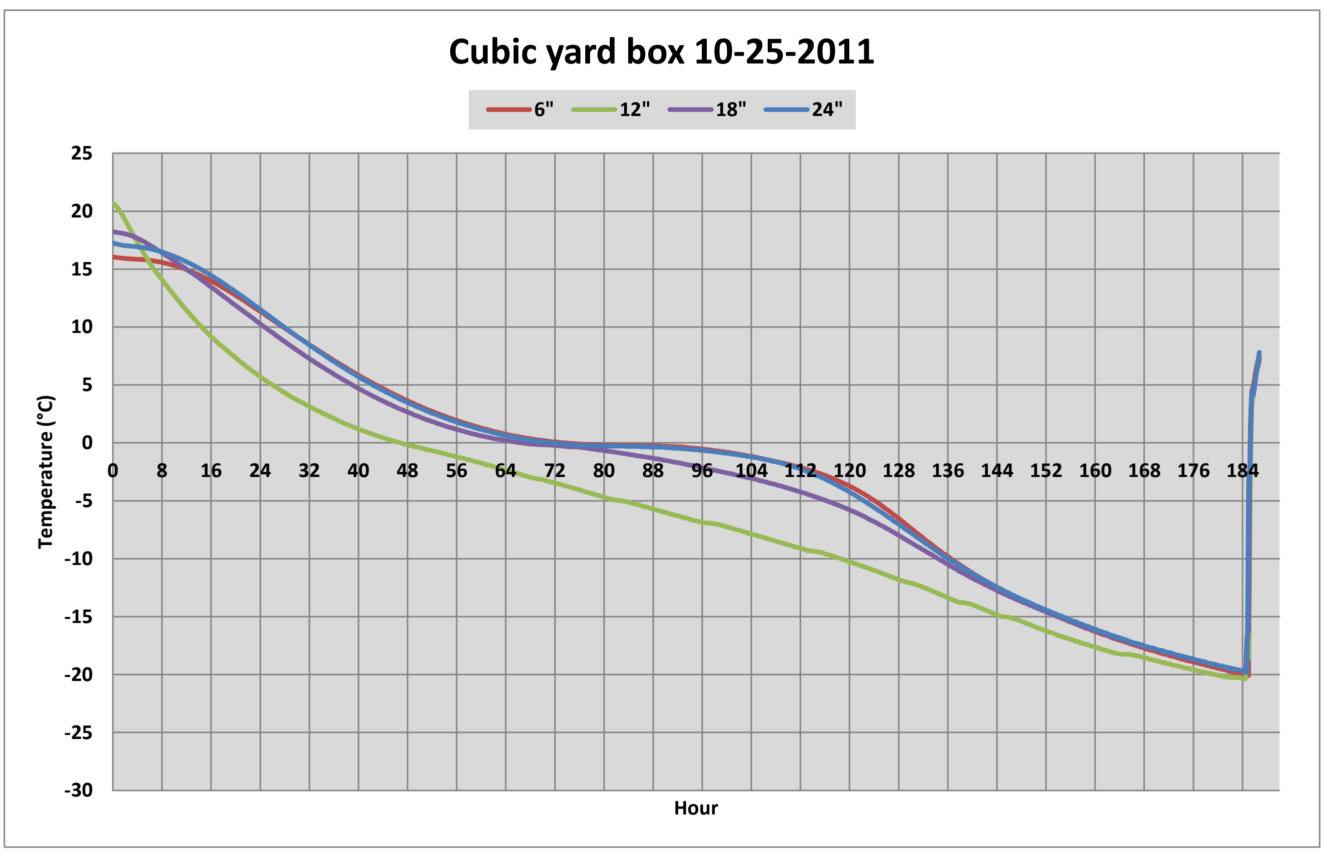

Figure 4. Temperatures (°C) for the four probes buried in the cubic yard box soil on October 25, 2011 (Trial 1)

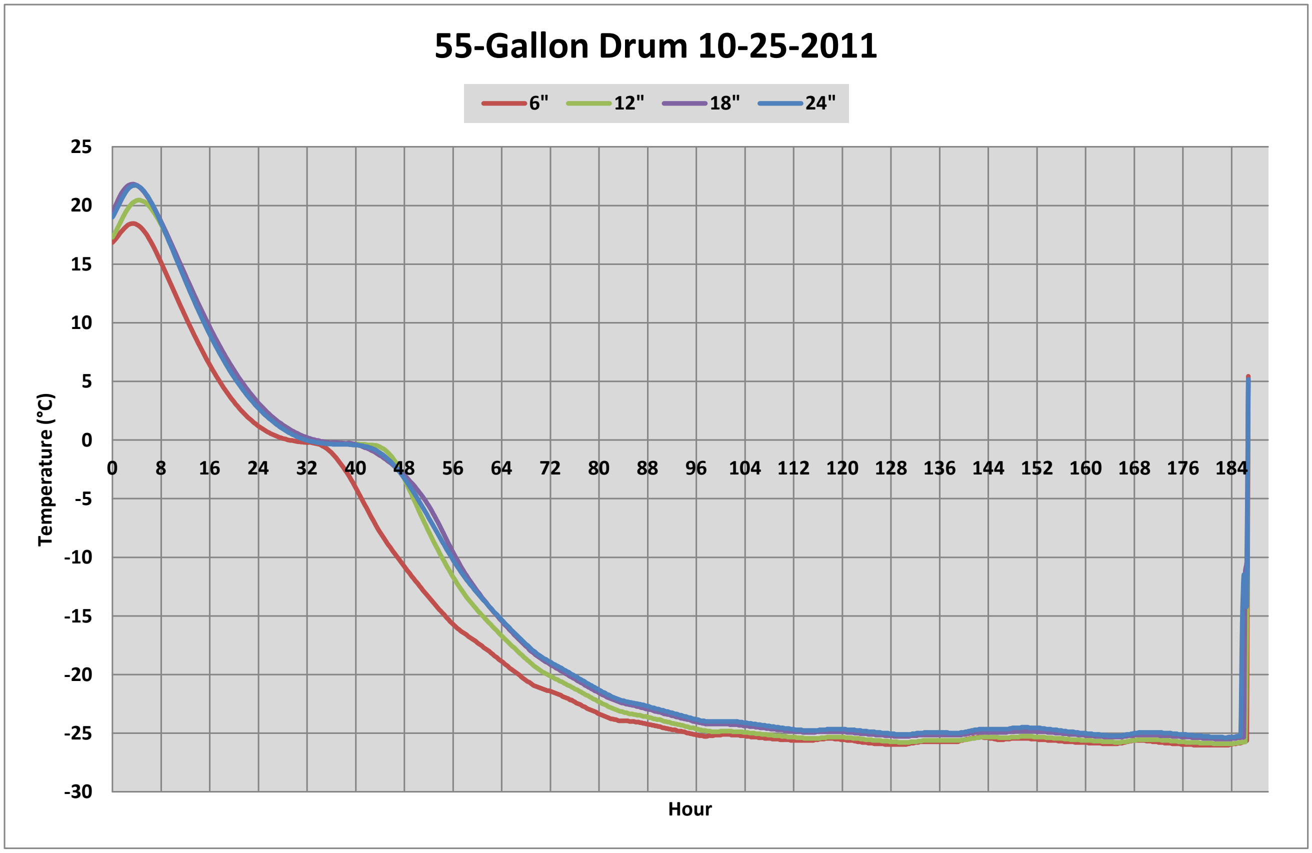

Figure 5. Temperatures (°C) for the four probes buried in the 55-gallon drum soil on October 25, 2011 (Trial 1)

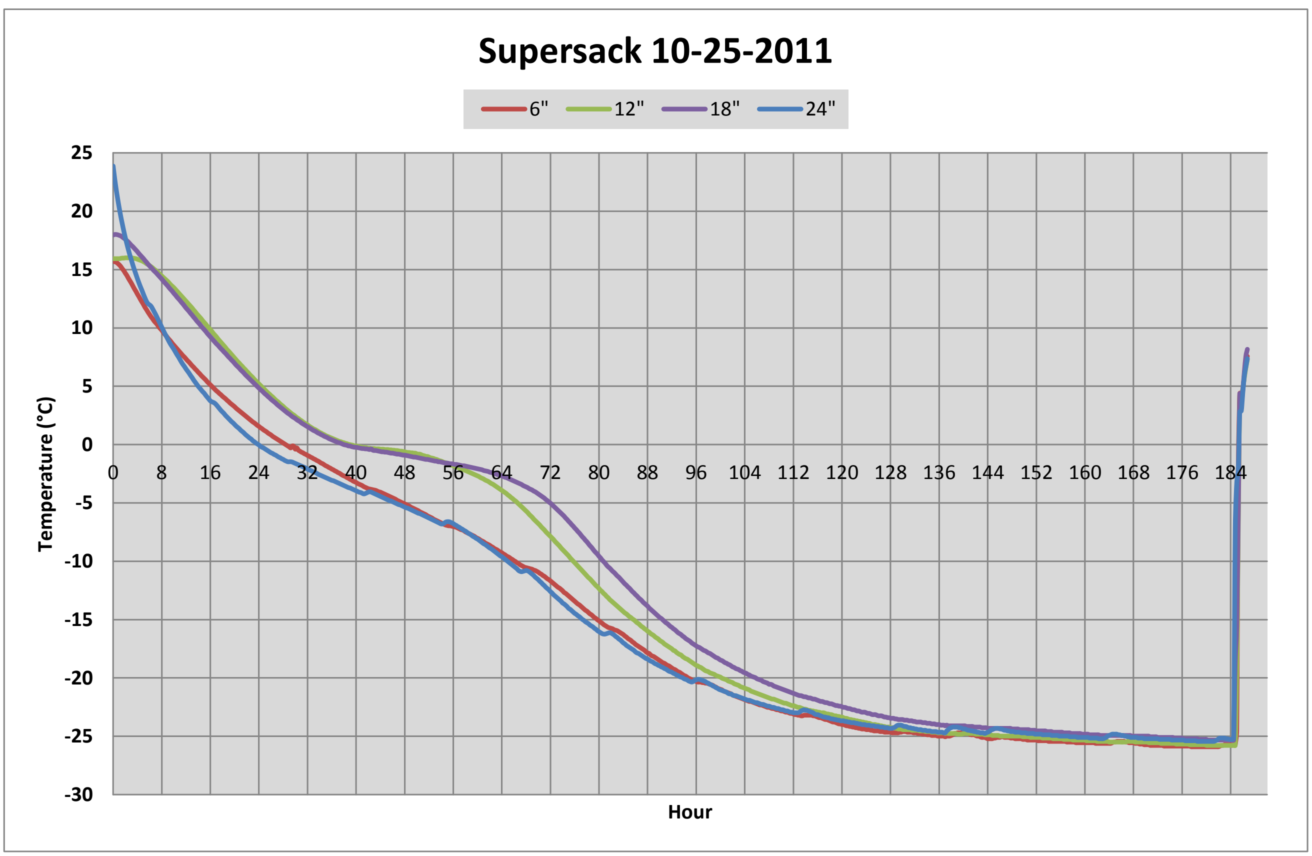

Figure 6. Temperatures (°C) for the four probes buried in the super sack soil on October 25, 2011 (Trial 1)

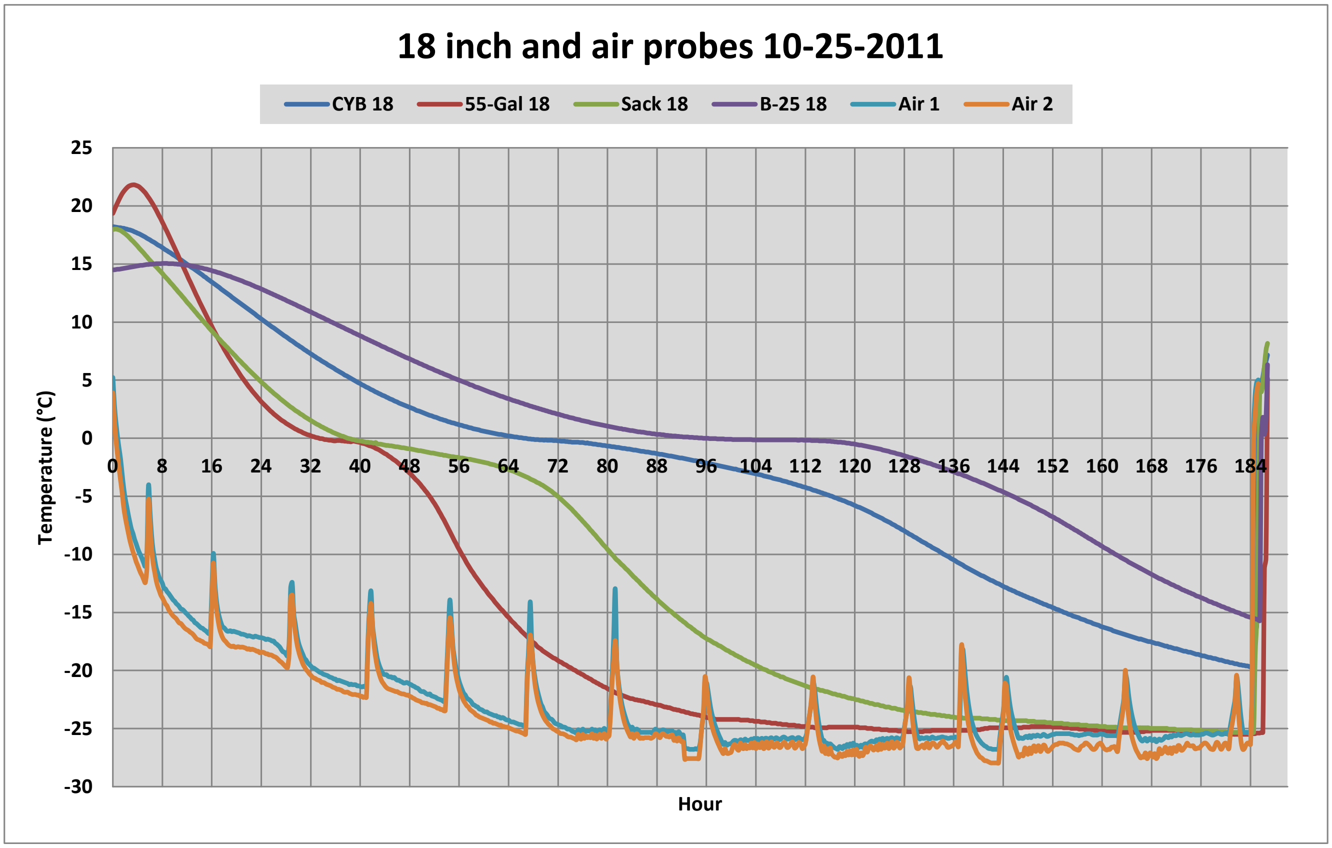

Figure 7. Temperature (°C) comparison of the 18-inch from bottom probes for all container types and the trailer air (Trial 1)

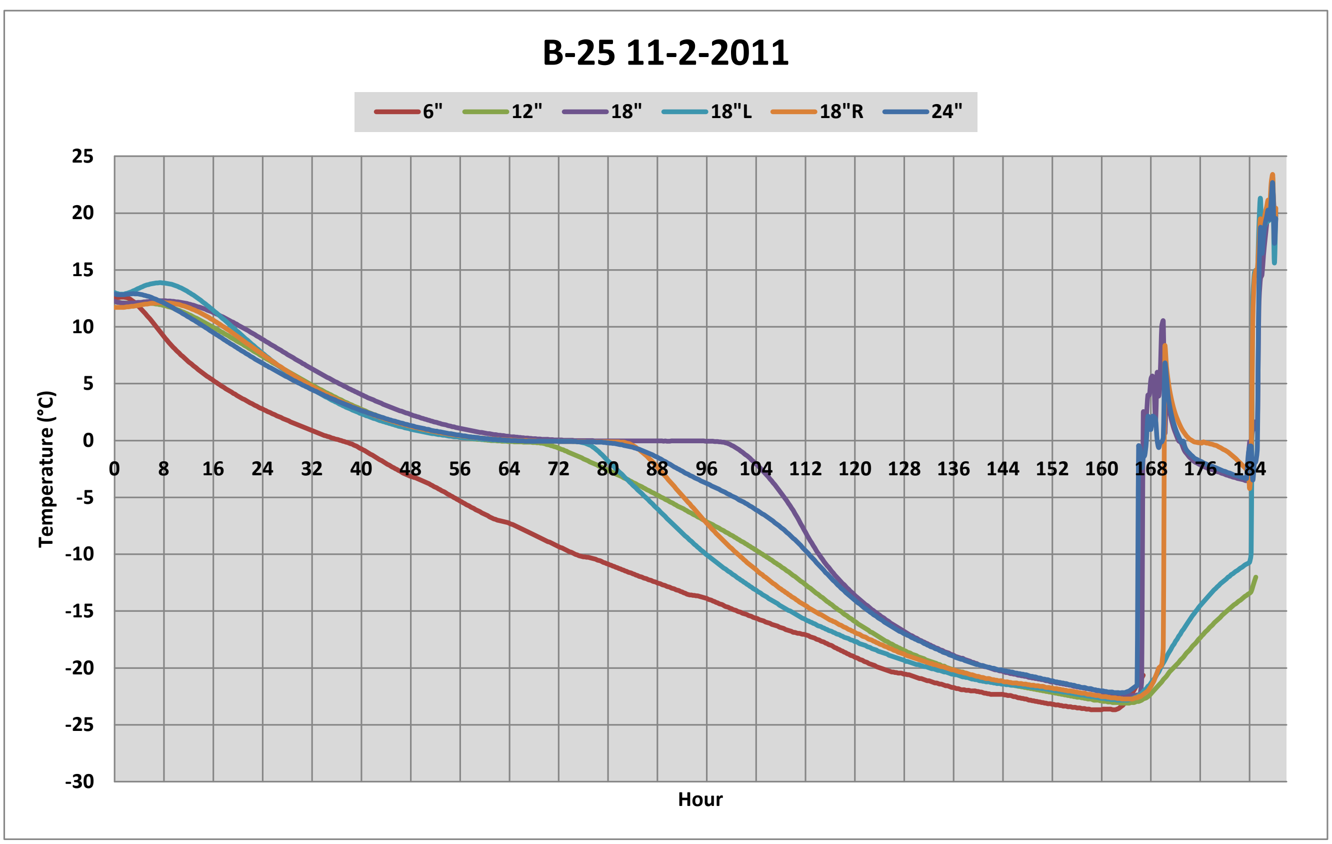

Figure 8. Temperatures (°C) for the six probes buried in the B-25 soil on November, 2011 (Trial 2)

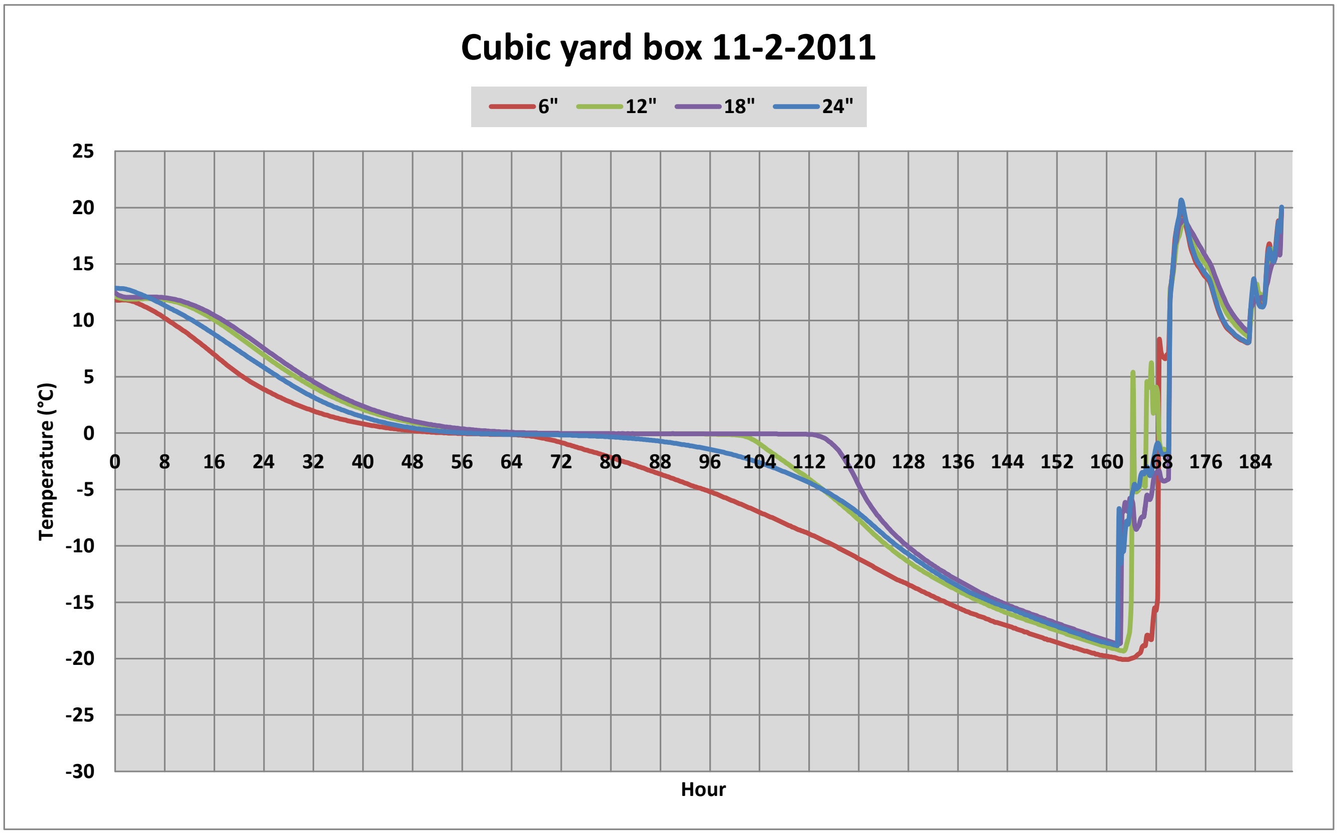

Figure 9. Temperatures (°C) for the four probes buried in the cubic yard box soil on November 2, 2011 (Trial 2)

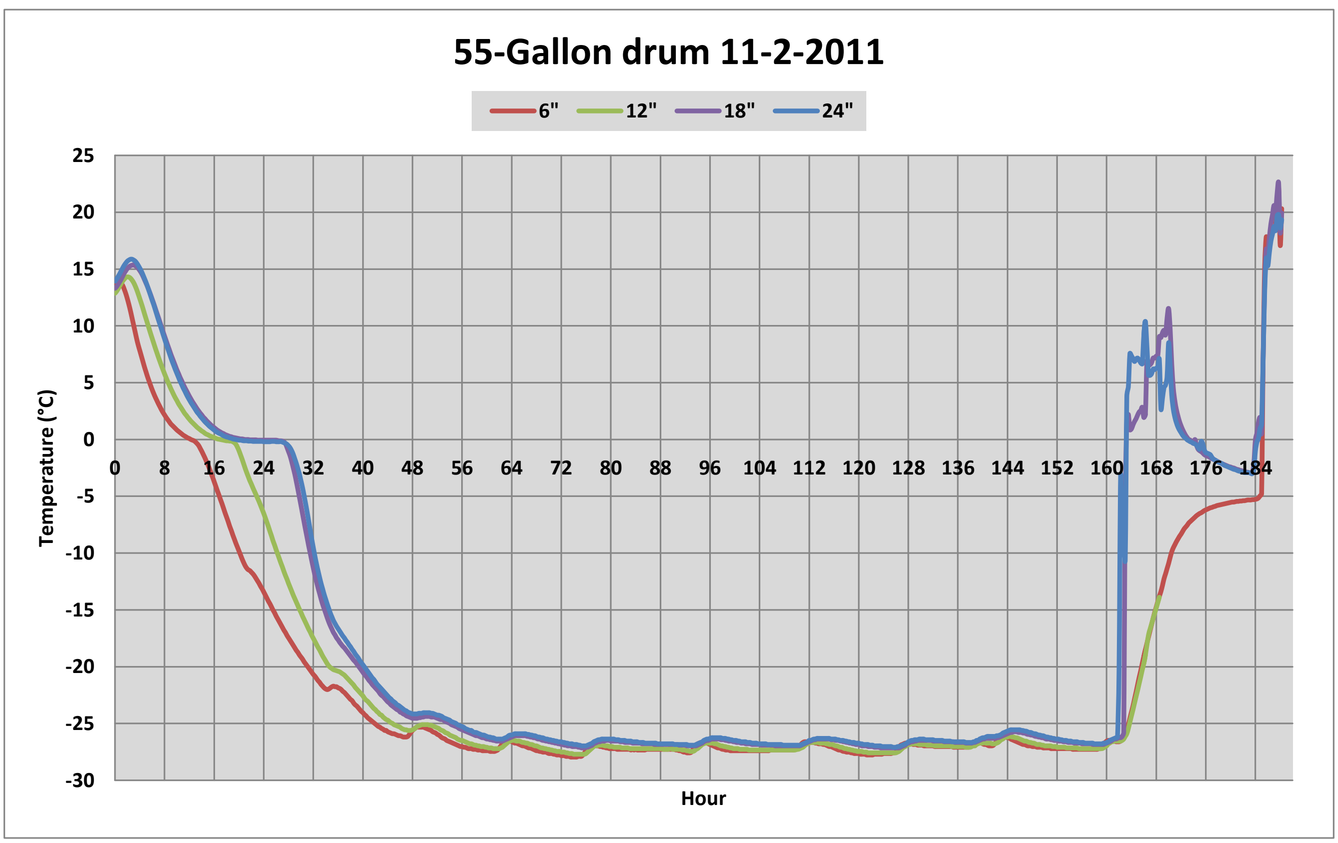

Figure 10. Temperatures (°C) for the four probes buried in the 55-gallon drum soil on November 2, 2011 (Trial 2)

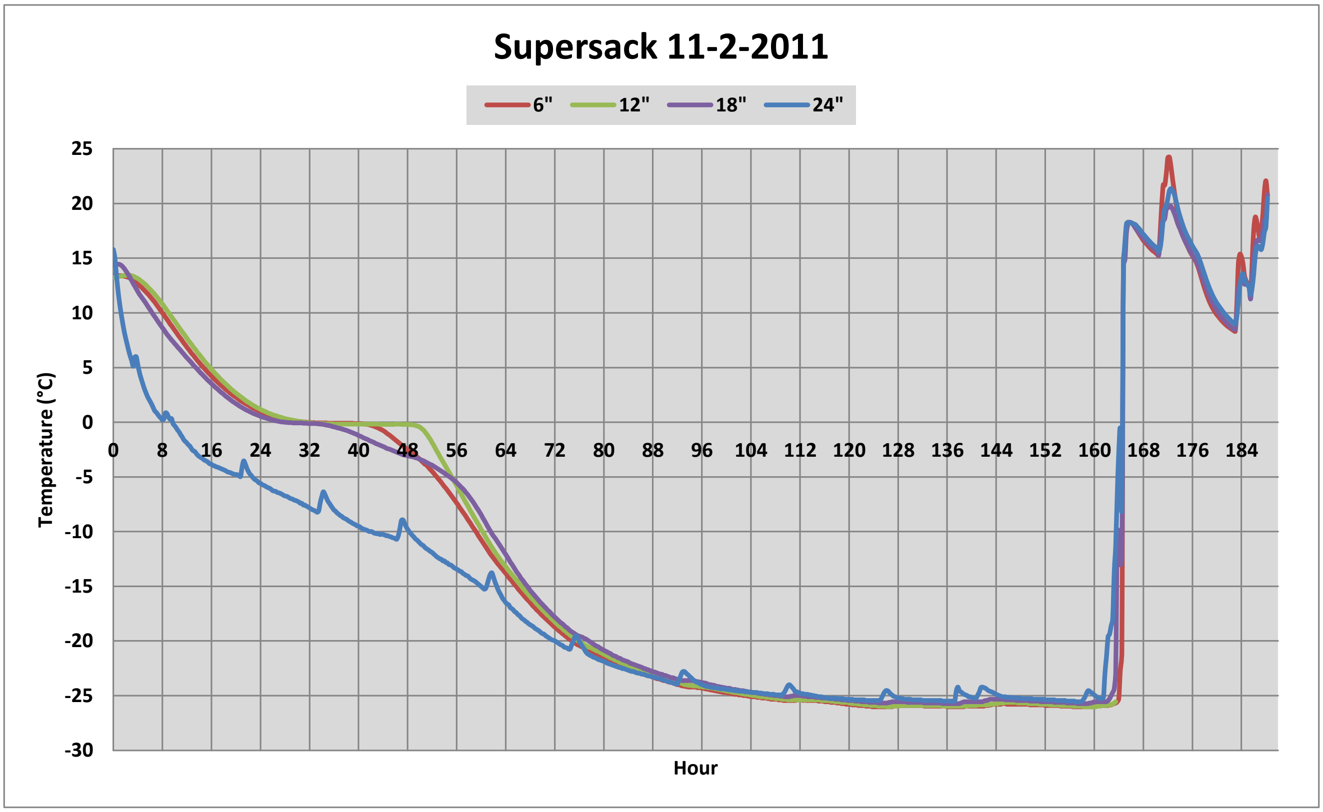

Figure 11. Temperatures (°C) for the four probes buried in the super sack soil on November 2, 2011 (Trial 2)

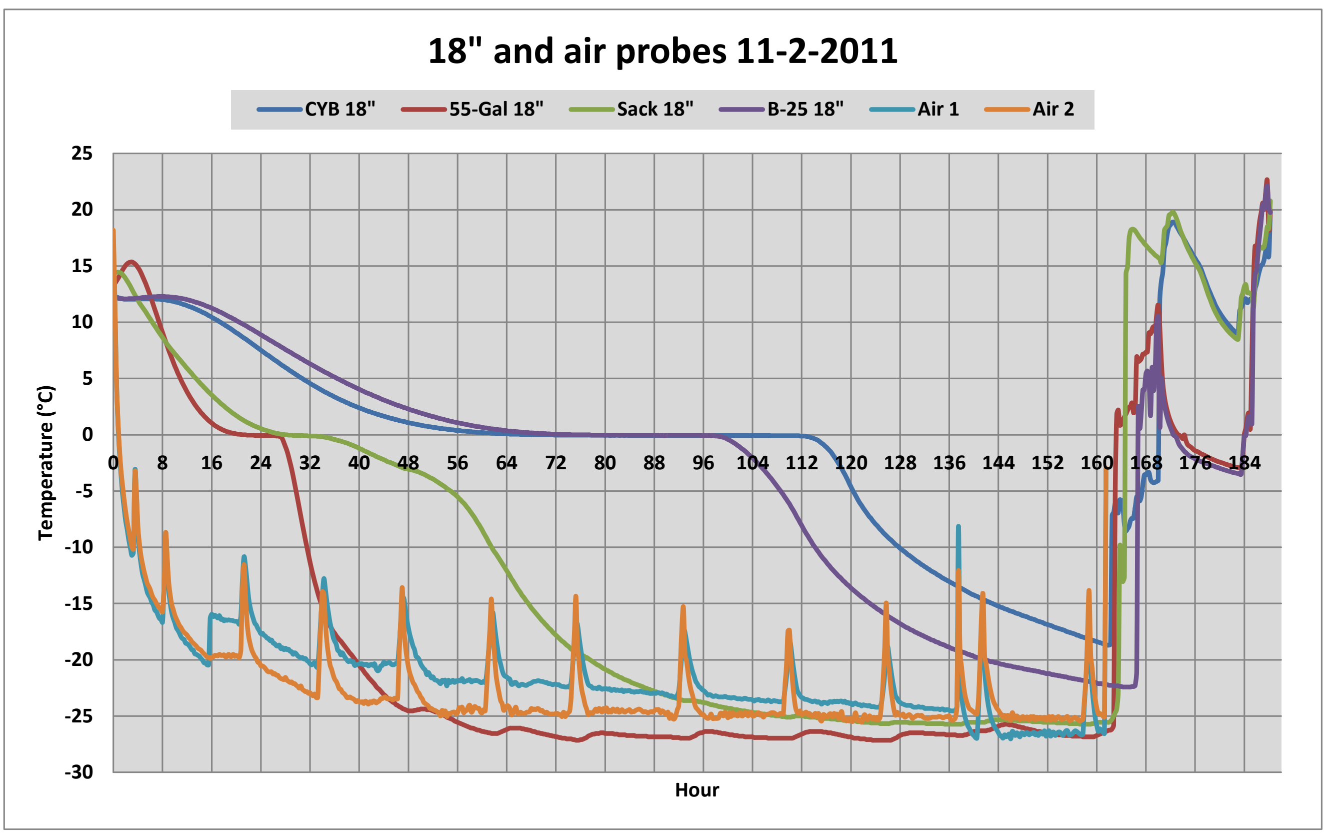

Figure 12. Temperature (°C) comparison of the 18-inch from bottom probes for all container types and the trailer air (Trial 2)

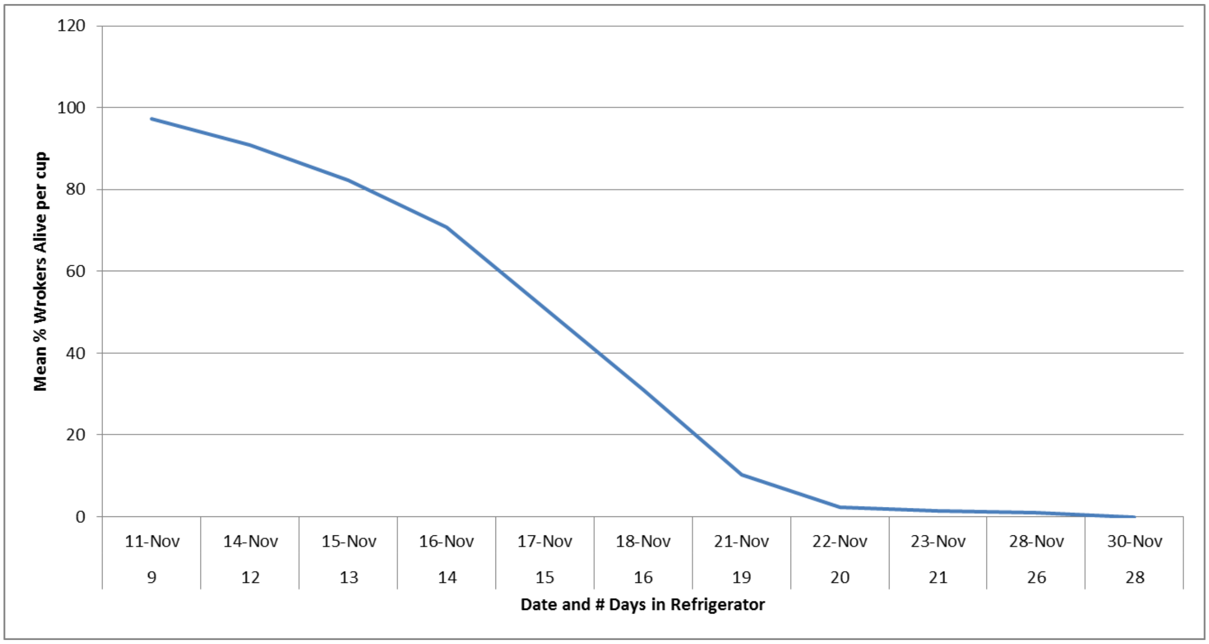

Figure 13. Survival of cooler control ants (Trial 2). These ants were prepared the same as the ants buried in the soil except they were transported back to the lab in a cooler and placed in the refrigerator. Survival is shown starting at day 9 which is about 24 hours after the last cups were removed from the cold containers.

Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing and Release Project, 2011 (*Pseudacteon tricuspis, P. curvatus, P. obtusus* and *P. cultellatus*)

Anne-Marie Callcott (APHIS-PPQ-CPHST)
Sanford Porter (ARS CMAVE),
George Schneider and staff at FL DPI,
State Departments of Agriculture and their designees

SUMMARY: The phorid fly rearing and release project is a great success. Since 2002, two to three species of *Pseudacteon* sp. flies have been released at multiple sites in all imported fire ant quarantined states in the contiguous southeastern states and Puerto Rico (no releases in NM and only one species released in CA). Field releases with a fourth species, *P. cultellatus*, began in 2011. From 2002 through 2012 there have been 138 field releases in IFA quarantined states in the contiguous southeastern states and Puerto Rico (no releases in NM and only one species released in CA) and more than 1.5 million potential flies released or used in demonstration/research projects. Of these 138 releases, 67 were *P. tricuspis*, 45 were *P. curvatus*, 23 were *P. obtusus*, and 3 were *P. cultellatus*. Through APHIS releases, along with other federal and university releases, *P. tricuspis* is well established in the southern areas of the IFA regulated area covering over 50% of the IFA regulated area. To date, *P. tricuspis* is not known to be established in CA, OK or TN. The second species, *P. curvatus*, is well established in all southern IFA regulated states and PR, covering about 65% of the regulated area. *P. curvatus* has not been released in CA. Overwinter establishment of *P. obtusus* has been confirmed with very limited expansion at this time, and overwintering of *P. cultellatus* has not yet been confirmed.

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

<u>MATERIALS AND METHODS</u>: Preliminary research and rearing techniques have been developed by USDA, ARS for four species, with others under development. ARS will continue to evaluate other phorid fly species for potential use in the U.S., and transfer rearing techniques to the rearing facility as the new species are ready for mass rearing. Mass rearing of flies is being conducted by the Florida Department of Agriculture, Dept. of Plant Industries (DPI), in Gainesville, FL. The CPHST biological technician position assigned to the rearing facility was transferred to the cooperative agreement when the position was vacated in early 2008. The position was refilled by one of the FL-DPI qualified and experienced technicians as a promotional opportunity. This position will continue to coordinate the shipment of phorid flies to field cooperators as well as assist in production duties and perform methods development experiments to improve rearing techniques or solve problems as needed.

Rearing of these flies is extremely labor intensive, requiring 1-1.5 person(s) to maintain every 2 attack boxes. These flies cannot be reared on a special diet or medium but require live fire ants to complete their life cycle. An excellent pictorial and text description of the rearing technique is available online from the FL DPI at: http://www.freshfromflorida.com/Divisions-Offices/Plant-Industry/Science/Biological-Control/Phorid-Fly-Rearing.

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

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

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

Release techniques for the third and fourth fly species, *P. obtusus* and *P. cultellatus*, are utilizing a combination of the above techniques. This fly species parasitizing the largest of the worker ants, and many cooperators are having difficulty collecting enough large workers for a full release. Therefore, if the cooperator cannot collect enough large workers, fly pupae (ant heads) are shipped to the cooperator as in the *P. tricuspis* release technique, and upon release of the adult flies, allowing the flies to find the large workers in the field. This has decreased our average number of potential flies for each release. In 2011 and 2012, IFA workers collected by cooperators were sized in the lab and parasitized by two fly species if appropriate. This allowed several states to get *P. obtusus* and *P. curvatus* in a single shipment, and did not "waste" ants that were collected.

Monitoring the success of the fly releases was originally conducted at a minimum annually and involved returning to the original release site, disturbing several IFA mounds and visually looking for attacking phorid flies over a set period of time. If flies were found at the original release site, the cooperator moved a set distance away from the release site along the four cardinal positions and monitored for flies. Personnel continued moving away from the original release site until no flies were found. In 2007, changes to the monitoring protocols were developed due to the availability of a phorid fly trap and the number of releases that had occurred. Our primary focus changed from monitoring release sites and spread from individual sites to determining fly presence by species at the county level. The use of the trap has enabled personnel to monitor many sites in a very short period of time – place the trap and retrieve it 24 hours later. Instructions for making the traps and site selection for monitoring are sent to cooperators involved in the trap monitoring. Traps were usually sent to the Gulfport Lab for fly identification until 2012; after this time traps or specimens are being sent to the ARS-CMAVE lab in Gainesville FL for identification.

RESULTS:

Highlights of the APHIS project:

- APHIS funding initiated through CPHST-NBCI in 2001 and supported by PPQ-HQ, ER, WR, CPHST
- Cooperative agreement initiated with FL-DPI to conduct rearing in 2001
- 2001 – *Pseudacteon tricuspis* rearing initiated
- 2002 – *P. tricuspis* releases begun
- 2002 – *P. curvatus* rearing initiated
- 2004 – *P. curvatus* releases begun
- 2006 – *P. obtusus* rearing initiated
- 2008 – *P. obtusus* releases begun
- 2010 – *P. cultellatus* rearing initiated
- 2011 – *P. cultellatus* releases begun
- 2012 – *P. tricuspis* releases ceased (small population retained for demo and research purposes)

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. Rearing of *P. tricuspis* was at its peak in 2003 and 2004 with ca. 1.6 million flies being produced annually with production gradually decreased to allow increased production of the *P. curvatus* and *P. obtusus* flies. *P. tricuspis* field releases ceased in 2012 and a small population was retained for demonstration and research projects. Production will be phased out completely in 2013. *P. curvatus* rearing was initiated in late 2002, with the initial 1-2 boxes again seeded by flies from the ARS-CMAVE facility. Production of this species was at its peak in 2006 and 2007 with 7 boxes in production and has subsequently decreased as *P. obtusus* production increased. Production of this species will begin to phase out in 2013, with anticipated elimination of rearing in 2014. In 2006, the third species, *P. obtusus*, was brought into production with the first releases of this species in 2008. In 2010, rearing was initiated on the fourth species, *P. cultellatus*, with the first releases conducted in 2011. Except for 2009 when production levels were above 3,000,000, total fly production levels have remained fairly constant in the last several years (Table 1).

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

Releases began in spring 2002. In most cases, the cooperator made the release at one site, however, in a few cases the cooperator split the release and released flies at more than one site. Also, there are several sites were multiple releases over several years have occurred. From 2002 through 2012 there have been multiple releases in each of 13 states and Puerto Rico, with a total of 138 field releases and more than 1.1 million potential flies released. Of these 138 releases, 67 were *P. tricuspis*, 45 were *P. curvatus*, 23 were *P. obtusus*, and 3 were *P. cultellatus*. (Table 1). The average number of potential flies per release is about 6,000-10,000 flies. In 2012, the average number of potential flies released decreased primarily due to the large number of *P. obtusus* releases (5 of 9 releases were *P. obtusus*). *P. obtusus* releases require extremely large worker ants, which are a very small percentage of workers in a colony; thus many fewer ants are collected and parasitized for this species. In 2008, the changing economy had an impact on our cooperators' abilities to conduct releases, and due to lack of resources in many states the number of overall releases in 2008 was less than in previous years. In 2009, we were able to increase our releases from 2008 and have maintained that level through 2011. However, again in 2012, cooperator resources, as well as drought conditions in some of our release areas, adversely impacted our release numbers.

In addition to field releases, the equivalent of 3 *P. tricuspis* shipments went to Louisiana to seed their own rearing facility, the equivalent of 2 releases went to New Mexico for research purposes, one *P. curvatus* release was abandoned due to site issues, and numerous small numbers of flies have been supplied to cooperators for research or educational purposes, such as state fair exhibits and field days. Louisiana completed its first release from LA-reared flies in 2005, conducted a few releases and then abandoned rearing flies in 2006-2007 and is now releasing APHIS reared flies only. Over 400,000 potential flies have been shipped for these varied uses since 2002.

Success of the program was originally measured by successful overwintering of fly populations at release sites. However, resources do not allow all cooperators to conduct the intensive monitoring surveys needed to determine success at this level. Of the 56 releases conducted in 2002-2005, flies were found after a winter at 27 of these sites, a 48% success rate; 19 *P. tricuspis* sites (AL, AR, FL, GA, LA, MS, NC, PR, SC, TX) and 8 *P. curvatus* sites (FL, LA, NC, OK, SC, TX). In 2007 we also realized that we could no longer determine the true source of flies present in an area due to the large number of established and spreading fly populations and so the attempt to determine individual site establishment of flies was abandoned. Since 2007 the use of the phorid fly trap and a monitoring protocol for surveying for fly presence at the county level has provided a wealth of information regarding establishment and spread of the flies. Through APHIS releases, along with other federal and university groups which are also releasing flies, *P. tricuspis* is well established in the southern areas of the IFA regulated area (AL, FL, GA, LA, MS, TX and PR), and moderately established in AR, NC and SC. To date, *P. tricuspis* is not known to be established in CA, OK or TN. The second species, *P. curvatus*, is also well established in all southern IFA regulated states and PR (AL, AR, FL, GA, LA, MS, NC, OK, SC, TN, TX, and PR), and appears to be better suited to life in the U.S. than *P. tricuspis*. *P. curvatus* has not been released in CA. Overwinter establishment of *P. obtusus* has been confirmed, but overwintering for *P. cultellatus* has not yet been confirmed.

The current rearing facility in Gainesville FL is limited to production of fly species that can be reared on red imported fire ants. While there are other fly species specific to red IFA, not all are suitable for mass rearing and distribution. Other phorid fly species must be reared on black IFA which are limited to a small range in TN, and northern areas of MS, AL and GA. Therefore, we will be reviewing this program during 2013 and 2014 to determine its future.

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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. Production and field release numbers for IFA-phorid fly program. Does not include flies shipped for research and demonstration projects.

Species	Year	No. flies produced	Approx. no. shipped*	No. field releases**	Mean flies/ release
tri,cur	2002†	950,063	58,750	12	4,895.83
tri,cur	2003	1,746,383	81,450	15	5,430.00
tri,cur	2004	2,280,039	128,602	12	10,716.83
tri,cur	2005	2,765,291	179,813	17	10,577.24
tri,cur,obt	2006††	2,448,798	178,259	17	10,485.82
tri,cur,obt	2007††	2,614,655	137,381	12	11,448.42
tri,cur,obt	2008	2,524,047	80,813	8	10,101.63
tri,cur,obt	2009	3,335,019	88,109	12	7,342.42
tri,cur,obt,cul	2010†††	2,571,357	76,221	12	6,351.75
tri,cur,obt,cul	2011	3,322,028	92,148	12	7,679.00
tri,cur,obt,cul	2012	3,612,325	37,119	9	4,124.33
Total		28,170,005	1,138,665	138	8,104.84

* approx. no. potential flies shipped for release

** does not include multiple shipments to LA for initiating their own rearing facility, NM for research, and multiple shipments to cooperators for educational purposes or small research projects when flies were available

† only tricuspis shipped in 2002

†† only tricuspis and curvatus shipped in 2006 and 2007

††† only tricuspis, curvatus and obtusus shipped in 2010

APPENDIX I - LABORATORY BIOASSAY PROCEDURE

Protocol for Bioassay of Insecticide Treated Potting Media/Soil with Alate IFA Females

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

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

Test chambers: Test chambers are 2.5" x 2.5" plastic flower pots which have been equipped with a Labstone® bottom. Labstone is generally available through dental supply firms such as Nowak Dental Supplies, 8314 Parc Place, Chalmette, LA 70043 (800-654-7623). The labstone bottom prevents the queens from escaping through the drain holes in the bottom of the pot and also serves as a wick to absorb moisture from an underlying bed of wet peat moss. Ants are susceptible to desiccation so humidity/moisture levels must be optimized. Pots should be soaked in water to moisten the labstone prior to placing potting media in the pots. The peat moss bed should be watered as needed to maintain a constant supply of moisture to the test chamber. Plastic petri dishes are inverted over the tops of the pots to prevent escape from the top of the test chambers (Figure 2). Prior to placing queens in the test chamber, 50 cc of treated potting media is placed in the bottom of each pot. Each test chamber with test media and queens is placed in a tray with a bed of wet peat moss (Figure 3). Due to possible pesticide contamination, test chambers are discarded after use.

Replicates: Traditionally, each treatment to be evaluated is subdivided into 4 replicates; with one test chamber per replicate. Five to ten 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.

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.

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.

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

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