2015 - 2017 CPHST
Imported Fire Ant Report
Biloxi Station

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Summary of Balled-and-Burlapped (B&B) DrenchTreatments for Potential Use in the Federal IFA Quarantine Program through 2015

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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). Treatment of balled-and-burlapped nursery stock must eliminate any existing colonies from the root ball, as well as provide a barrier to invasion by newly mated queens or relocating colonies. We can more easily test for efficacy against newly mated queens using IFA alate females, and thus trials focus on this life stage and only move to whole colony testing later in the project.

In 2013 a new dip treatment for B&B stock using bifenthrin was added to the list of approved treatments, however current treatments for drenching B&B stock are limited to a single insecticidal choice, chlorpyrifos. Furthermore, restrictions on chlorpyrifos 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 ensure imported fire ant-free movement of this commodity. In addition, growers in Tennessee and other states are also required to treat for Japanese beetle. The Japanese Beetle Harmonization Plan does not currently allow drenching of B&B nursery stock, but does allow drenching of container stock of a limited size. 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 as a drench treatment of B&B nursery stock.

During drenching, B&B stock normally rests on one side of the root ball throughout the traditional three-day drench process. This restricts treatment coverage on the resting side, while giving the surface of the root ball direct application of chemical. Trials in 2004-2005, designed to examine whether changes in plant handling during application would improve insecticide efficacy, showed that flipping or rotating the root ball between drench applications improved consistency of the insecticide treatment. Several rotation and drench schedules were tested. It was determined that flipping the root ball one time between 2 drench applications, applied on a single day, provided an effective drench treatment. Subsequently, from 2005-present, all B&B drench treatments have included rotating or flipping the root ball between drenches to ensure adequate coverage. In 2013, language adding a root ball rotation during B&B drench treatments was added to the PPQ Treatment Manual and in 2015 the application technique was changed from drenching twice a day for 3 consecutive days to drenching twice in one day with a root ball rotation between drenches.

Drench treatments for B&B nursery stock, even with rotating the root ball between drench applications, are not as effective as dip treatments at similar rates of application. Dip treatments penetrate the root ball much more efficiently than drenches, even with multiple drench applications per treatment. However, the worker safety and environmental concerns associated with dip applications (especially waste disposal issues) make drench treatments an acceptable treatment option.

Prior to fall 2007 in Mississippi and fall 2009 in Tennessee all B&B drench application rates were calculated based on the existing chlorpyrifos drench which required drenching twice a day for 3 consecutive days, thus applying a drench 6 times to a root ball. New terms were created to define the flipping and drench application techniques. **1F1:** one drench in the morning; then in the afternoon flip the trees and drench the other side of the balls. This method requires minimum chemical solution and days of application for drench treatments. **2F2**: one drench in the morning and another in the afternoon on one side of the root balls. The next day, flip the trees and drench two more times (morning and afternoon) for the other side of the root balls. **6NF**: this is the conventional and currently approved drench method of twice a day drenching for 3 consecutive days with no flipping. In order to compare the effectiveness of different handling methods (1F1, 2F2, 6NF), we kept the amount of chemical applied to each root ball the same by varying the concentrations of the individual drench solutions so that at the completion of the full drenching treatment, each root ball would receive exactly the same amount of chemical regardless how many drench applications a root ball received. To achieve this, trees with 1F1 treatment method were drenched with chemical solution that was doubled the rate of trees receiving 2F2 treatment method. As a consequence, trees receiving the same listed treatment rate could be applied with drench solution that is ⅓ or ½ the insecticide concentration of other trees at any single drench application, which was not only confusing for applicators but also creating other issues of concern. Once we determined that flipping the root balls between drench applications was an effective treatment, we were able to eliminate the difficult calculations required by using the true listed rate for drench trial and by 2011 we were using the same total volume of finished solution for each root ball (i.e. 1/5 volume of root ball), thus all root balls received the same amount of active ingredient regardless of application technique (1F1 vs 2F2).

The 1F1flip drench in our trials in Mississippi and in Tennessee as mentioned above was completed in one day. Between the two drenches, there was usually a “drying period” of a few hours. Although not specifically designed to have a fixed-length “wait period” in between, it is a natural way of doing drench treatments especially for small scale experimental treatments. However, it was unknown if this drying period between drenches was truly necessary from the standpoint of treatment efficacy. Nursery growers expressed that they would rather finish the drenches to both sides of root balls in a row without having to wait for a few hours before flipping and drenching to the other side. Also, when they do drench application to a large number of harvested trees, it usually will take them quite a while to drench one side before they can come back to flip and drench the other side of the root balls. Therefore, in the fall 2008 we did an investigation to see if waiting for a few hours before flip and drench would make a difference on treatment efficacy. We included in our trial a wait period of 0.5 hour (representing drenching in a row) and 5 hours between two drenches. It was determined that the waiting period between rotating/flipping and applying drenches did not impact efficacy of the overall drench treatment.

Non-flip drench trials conducted before fall 2004 indicated that several of the insecticides tested did not show promising results against IFA at the rates tested. These included acephate, carbaryl, and imidachloprid. Therefore, insecticides used in the flip trials were focused on those chemicals or formulations that show promising for IFA quarantine treatment. Chlorpyrifos was included in many trials as a chemical standard because it is still the only insecticide approved as an IFA quarantine treatment for B&B root ball drenches.

Numerous products and product combinations have been tested in this use pattern and a summary was reported in 2010 that primarily focused on trials using the old method of calculating a drench dose rate. This report summarizes all the numerous trials and promising products tested since 2002, but focuses on the 2008-2015 trials. 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 drench testing on a broader assortment of nursery plant species may be needed to confirm general safety to plants.

MATERIALS AND METHODS:

Specifics can be found in previous annual reports in the individual project trial reports. Root balls were obtained from various nurseries in both Mississippi and Tennessee for testing and ranged in size from 12-inch to 24-inch diameter. Root balls were drenched according to various handling methods. After drenching root balls were then stored outdoors for aging. Usually irrigation was set up to simulate nursery storage conditions. 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/sides of the root ball. There were generally 5 replicates (number of root balls) per treatment in each trial, and soil 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.

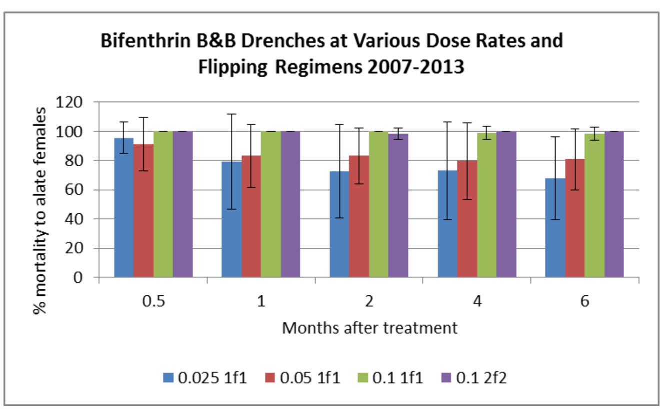
Insecticidal solutions were prepared either in 30-gal drums with polypropylene liners or in 5 gal bucket according the total volume of solution needed. Drench solution was pumped through a hose attached to a shower-headed nozzle using a Shur-Dri battery-powered pump (or similar type pump). The amount used per drench application was based on the amount needed to achieve "the point of runoff" required in the IFA quarantine; aiming to use a total of approximately 1/5 volume of the root ball for each complete drench treatment.

All IFA soil bioassays utilizing alate females were conducted in Gulfport, MS at the APHIS-PPQ-CPHST laboratory through 2011, after which time the ARS-CMAVE laboratory in Gainesville, FL conducted the bioassays. 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 (drenched with water only) were included in every trial but that data is not included here.

In addition to these trials utilizing alate females on soil samples collected from drench treated root balls, a few trials were conducted to determine efficacy of B&B drenches on whole IFA colonies established in the root ball. In two trials (2004 & 2006), field collected colonies were brought back to the lab, the workers and brood separated from the nest tumulus, and a fragmented colony introduced to the root ball either prior to drenching or after drenching (immediately after or 2 months after drenching); however no flipping of the root ball between drench applications occurred in these two trials. Treatments included bifenthrin, chlorpyrifos, deltamethrin and lambda-cyhalothrin. Mortality was assessed at 7 and 14 days, with destructive sampling at the 14-day assessment. Another whole colony trial was conducted in 2011 whereby IFA field colonies were "harvested" into a root ball and then subjected to 1F1 bifenthrin drench treatment. Root balls were destructively sampled at 4 week intervals to determine colony survival. A similar trial in spring 2014 also utilized root balls with IFA field colonies harvested in the root ball treated with bifenthrin at various rates and both 1F1 and a modified 2F2 schedule. Modified 2F2 treatments received one drench (1/4 total drench volume) and then a second drench (1/4 total drench volume) 15-30 minutes after the first. After 15-30 minutes the rootball was rotated and the process repeated. In the 2011 and 2014 trials all root balls got the same total amount of finished solution regardless of drenching regimen, and thus all received the same amount of active ingredient (a.i.) per dose rate. Finally in 2015, one trial was conducted to determine efficacy of drenched treatments to exclude/prevent IFA from infesting a treated rootball. Rootballs were treated with bifenthrin or lambda-cyhalothrin and aged under normal conditions (irrigation added as needed). One rootball was placed in an arena and a field collected colony with nest tumulus was placed in the arena with the rootball (treated or untreated) and allowed to either infest the rootball or die and mortality assessed daily for 7 days. Evaluation periods were 1, 4, 8 and 16 weeks after treatment.

RESULTS:

Bifenthrin: Numerous trials at various rates of application show that a dose rate of 0.1 lb ai/100 gal water applied twice in a single day with one rotation or flip between the drench applications is very effective against potential invasion of a root ball by a newly mated queen for up to 6 months. There is no difference between the 1F1 and the 2F2 with this rate. This would allow root balls to be treated, stored, and shipped as needed for a long period of time without re-treating.



In trials between 2004 and 2006, using fragmented IFA colony trials artificially introduced onto B&B root balls, bifenthrin treated root balls (0.1 lb ai/100 gal) provided 100% elimination of IFA using a single drench application (25/25 reps) and 93% after 14 days when using a traditional twice daily over 3 consecutive days drench regimen without flipping/rotating (14/15 reps). In the one root ball with ants present 14 days after treatment, IFA were not detected by external examination, but required destruction of the root ball to find the active ants (ca 50 ants found).

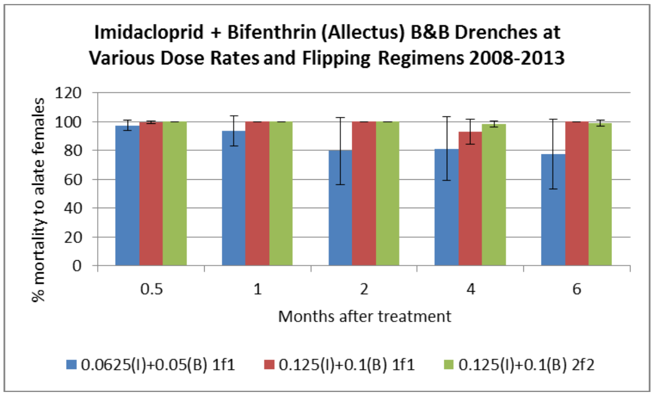
In subsequent trials in 2011 and 2014, where active IFA colonies were field harvested within the root ball and then subjected to drenching, results were much less promising, even with increased rates of application. However, the 0.2 lb ai/100 gal rate did cause significant mortality in the spring 2014 trials and thus these trials, using the 0.2 lb ai/100 gal rate were repeated in the fall of 2014, and the evaluation period extended to 14 days with visual and destructive sampling to see whether more time will provide 100% mortality of a whole colony. Unfortunately, while visual evaluations looked promising, once destructive evaluations were conducted, ants were found in some rootballs. Surviving ants were generally near the center of the rootball indicating poor penetration of the insecticide. Thus, visual examination of root balls is not a good indicator of presence or absence of ants if any treatment has been applied to the root ball.

Year	Bifenthrin rate (lb ai/100 gal)	App	reps	IFA Colony Mortality in Root Balls						
				2d	4d	7d	4 wk	8 wk	16 wk	20 wk
2011	0.1	1F1	4	--	--	--	25%	50%	50%	75%
spring 2014	0.15	1F1	4	50%	25%	50%	All active and numerous ants			
	0.2	1F1	4	75%	25%	50%	Mostly large ants and moderately active			
	0.2	2F2	4	100%	50%	50%	<100 ants and slow			
	controls		4	25%	0%	0%	All active and numerous ants w brood			

Year	Bifenthrin Rate (lb ai/ 100 gal)	App	Mortality											
			2-day visual		7-day visual		7 day destroy		10 day visual		14 day visual		14 day destroy	
			Reps	% mort	Reps	% Mort	Reps	% Mort	Reps	% Mort	Reps	% Mort	Reps	% Mort
fall 2014	0.2	1F1	8	87.5	8	87.5	4	25	4	75	4	100	4	50
	0.2	2F2	8	87.5	8	87.5	4	75	4	100	4	100	4	75
	controls	1F1	4	25	4	25	0		4	25	4	25	4	25

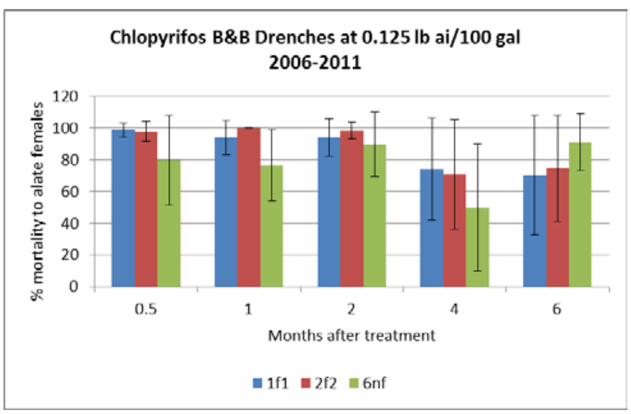
<u>Bifenthrin + Imidacloprid:</u> Imidacloprid is very effective against grubs, such as Japanese beetle, and bifenthrin, as noted above, is very effective against IFA. Trials using this combination of active ingredients either used bifenthrin and imidacloprid as separate products combined in a tank mix or used the combination product Allectus®. The addition of imidacloprid did not improve the efficacy or residual activity of the bifenthrin at the 0.05 lb rate. The 0.1 lb bifenthrin

+ 0.00625 lb imidacloprid rate was similar to the 0.1 lb bifenthrin rate alone against potential invasion by a newly mated queen.



Chlorpyrifos: Chlorpyrifos is currently approved for use as a post-harvest root ball (balled-and-burlapped) plant drench treatment. The rate of application is 0.125 lb ai/100 gal water and each treatment involves drenching the root ball twice a day for three consecutive days. The certification period is 30 days (plants must be shipped within 30 days or retreated).

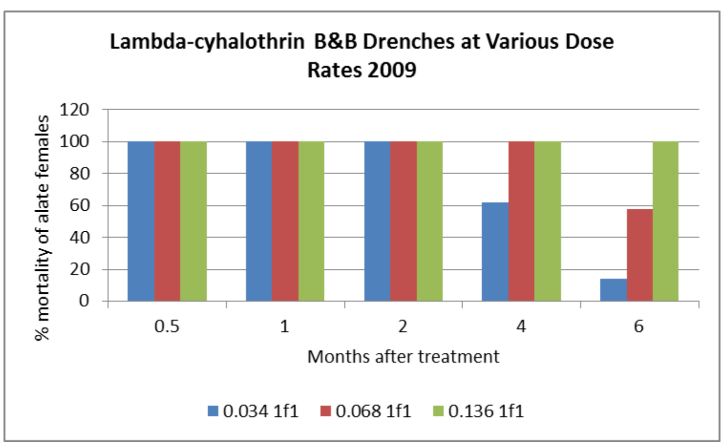
Results from numerous trials showed that rotating (flipping) the root ball between drench applications increased efficacy of treatments against alate females (surrogate for newly mated queens starting a new colony in a root ball). In addition, drenching the root ball twice in one day with a rotation between the drench applications (1f1), or drenching twice a day for two consecutive days (with a rotation between days-2f2), actually provided more consistent mortality to alate females over a 2 month period, than the traditional 3 day drenching regimen (6nf). These results would seem to indicate shortening the treatment regimen to a single day with 2 drench applications and a root ball rotation between the drenches would be acceptable.



In trials between 2004 and 2006, using fragmented IFA colony trials artificially introduced onto B&B root balls, chlorpyrifos treated root balls (0.125 lb ai/100 gal) provided 88% elimination of IFA using a single drench application (22/25 reps) and 80% after 14 days when using a traditional twice daily over 3 consecutive days drench regimen without flipping/rotating (12/15 reps). Of the combined 6 root balls with IFA after 14 days, 5 were not detected by external examination, but required destruction of the root ball to find the active ants.

Chlorpyrifos is currently approved as an IFA Quarantine B&B drench treatment at 0.125 lb ai/100 gal for 30 day certification when applied twice a day for three consecutive days. However, the data collected over the last several years indicates that this treatment is not as effective as normally desired for a quarantine treatment. In addition, using a single drench treatment or a 1F1 drench treatment is as effective, or more effective, than the longer 3 day treatment done without rotating the root ball. Until there is an approved alternative drench treatment, the IFA CFWG feels the IFA Quarantine would be best served by modifying this treatment option to two drenches in a single day, with a minimum of 30 minutes between drenches and rotating the root ball between drenches to insure all sides and the bottom are drenched. However, the total volume of the treating solution must be 20 percent (1/5) the volume of the root ball, thus not changing the amount of active ingredient applied to the root ball. This modification would significantly lower grower labor costs, by eliminating 2 days and 4 drench applications, as well as shortening the time required for the treatment.

Lambda-cyhalothrin: Limited B&B drench testing has been done with lambda-cyhalothrin for two reasons: 1) in 2010 the label rates decreased to single application of 0.034 lb ai/100 gal and 2) at the time we were testing for long term residual activity on B&B nursery stock (6 mth residual activity). However, with new interest in short term drench treatments for B&B plants, lambda-cyhalothrin is a very promising candidate for IFA quarantine use. The highest rate tested in 2009, 0.136 lb ai/100 gal in a 1F1 application regimen provided 100% control of alate females for 6 mth in one trial, while the 0.069 lb rate provided 100% control for 4 mths in two trials. The lowest tested rate (and currently the highest allowable rate on the label), 0.034 lb ai/100 gal, provided only 2 months of residual activity. Since, at the time, we were looking for long term treatments and the two high rates were removed from the label in 2010, testing was ceased. However, with the new interest in fast, short term B&B drench treatments, trials with lambda-cyhalothrin will be expanded in 2014-2015.



In a single trial in 2006, using fragmented IFA colony trials artificially introduced onto B&B root balls, lambda-cyhalothrin treated root balls (0.034 lb ai/100 gal) provided 100% elimination of IFA using both a single drench application (5/5 reps) and a traditional twice daily over 3 consecutive days drench regimen without flipping/rotating (5/5 reps). With the new interest in fast, short term B&B drench treatments, we initiated a trial using active IFA colonies harvested within the root ball and then subjected to drenching, in 2014-2015. Similar to the bifenthrin trials, while visual evaluations looked promising, once destructive evaluations were conducted, ants were found in some rootballs. Surviving ants were generally near the center of the rootball indicating poor penetration of the insecticide. Thus, visual examination of root balls is not a good indicator of presence or absence of ants if any treatment has been applied to the root ball.

Chemical	**Rate (lb ai/ 100 gal)**	**App**	**Mortality**											
			2-day visual		**7-day visual**		**7 day destroy**		**10 day visual**		**14 day visual**		**14 day destroy**	
			Reps	**% mort**	**Reps**	**% Mort**	**Reps**	**% Mort**	**Reps**	**% Mort**	**Reps**	**% Mort**	**Reps**	**% Mort**
Lambda-cyhalothrin	0.034	1F1	8	75	8	100	4	0	4	100	4	100	4	50
Controls	Water	1F1	4	25	4	25	0		4	25	4	25	4	25

DISCUSSION:

An informal survey of field production growers in Tennessee in 2014 indicated that growers are more interested in short and fast treatment for B&B stock, rather than a long residual treatment that allows one treatment and storage of root balls for shipping throughout the winter/early spring season. This is a shift in need than that expressed by most growers 5-7 years ago when we started many of these trials. For the last several years we focused on long residual treatments and eliminated many potential treatments due to residual activity of less than 2 months. However, as noted in the text, we will be looking at some of the promising short residual products.

Bifenthrin was extremely effective at eliminating IFA alate females (surrogate newly mated queens) with a long residual of up to 6 months at a rate of 0.1 lb ai/100 gal water when applied twice in one day with a rotation/flip between drench applications. While this rate was effective at eliminating fragmented IFA colonies introduced into root balls, it was not consistently effective at eliminating IFA colonies harvested within a root ball. Trials at the higher 0.2 lb ai/100 gal water rate to eliminate existing IFA colonies significantly reduced IFA numbers but in limited trials did not completely eliminate existing colonies from all root balls. In addition, it was found that visual examination was not a good indicator of ant presence in treated rootballs.

Lambda-cyhalothrin, while not a drench candidate for long term certification of B&B plants, is very effective at eliminating IFA alate females for up to 2 months after treatment at a rate of 0.034 lb ai/100 gal water. This rate was also effective at eliminating fragmented IFA colonies introduced into root balls. However, as we saw with bifenthrin, the elimination of IFA colonies harvested within a root ball (as opposed to artificially introduced) is more difficult to achieve, and trials with field harvested IFA colonies were also unsuccessful in completely eliminating all ants from all rootballs.

Chlorpyrifos is the current approved B&B drench treatment, applied twice in one day with a rootball rotation between drenches. This change in the PPQ Treatment Manual under D301.81-10(3) for the drench application technique for B&B plants from the old drench twice a day for 3 consecutive days was based on the data mentioned in this summary. This change did not decrease the total amount of insecticide applied, but changed the application technique, thus shortening the treatment regime from three days to one day.

Efficacy of Rootball Injection Treatments to Eliminate IFA Colonies – Mississippi, 2015

Anne-Marie Callcott and Richard King

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 2015 treatments for B&B nursery stock include a chlorpyrifos drench (applied twice in one day with a rotation of the rootball between drench applications) or a chlorpyrifos or bifenthrin rootball dip treatment. While rootball dips are the most effective treatment option against IFA, they are impractical with both environmental and human safety concerns. Rootball drenches have been very effective against IFA alate females over time; alate females being the substitute life stage for newly mated queens, the only reproductively hazardous life stage and at excluding IFA colonies, but less effective at eliminating whole IFA colonies harvested within a rootball. A rootball injection paired with a drench may be more effective at eliminating IFA colonies inside a rootball and still provide exclusion efficacy. It will be labor intensive, but many growers are currently requesting fast killing treatments with little residual (a treat and ship type of treatment vs. a treat and store type of treatment).

MATERIALS AND METHODS:

A block of field grown boxwood shrubs at Deep South Nursery (Lucedale, MS) were surveyed for plants with healthy IFA colonies located at the base of the shrub and flagged for use (Figure 1). We purposely selected plants with small colonies that could be contained within a harvested rootball, and thus most colonies contained several hundred worker ants. On August 24, 2015, 30 flagged shrubs were machine harvested by the nursery's workers into 16" rootballs (Figures 2&3). In order to keep live fire ant colonies in each rootball, digging and wrapping were done such that there was as little disturbance as possible to the tree bases where fire ant nested. In addition, the rootballs were wrapped using bifenthrin-treated burlap. Previous trials showed that bifenthrin treated burlap would not kill IFA colonies already present in a rootball, but will deter ants and alates from moving into a rootball, thus we assume that treated burlap would help keep ants from leaving the rootball during transportation and trial implementation. To do the pre-treatment of the burlap, burlap liners provided by Deep South were sprayed with a 0.05 lb ai/100 gal water bifenthrin solution. A total of 0.25 gal finished solution was sprayed on each burlap liner; half on one side and half on the other side. The liners were allowed to air dry and then place in plastic bins and taken back to the nursery on harvest day.

Figure 1. Flagged shrub with IFA colony.

Figure 2. Machine harvesting of plants.

Figure 3. Partially wrapped rootball with IFA colony.

Infested rootballs were transported to the Biloxi facility the same day as harvest on a flatbed trailer. Rootballs were divided into 3 groups of 9 for insecticide treatment and one group of 3 for the control treatment. Treatments were conducted the following day on August 25, 2015. Drench and injection solutions were mixed in a 15-gallon spray tank equipped with a battery operated High-Flo Gold series pump (Model 5275704; 45 psi; 3.8 gpm) (Figure 4). A hose attached the spray tank to the application tool (spray wand or injector). Drench applications were applied with a common garden spray wand calibrated to an application rate of ca. 1 gal/minute (Figure 5). Injection applications were applied with a B&G 430 Versagun Termite rod applicator with the 40” x 5/8” rod and 360° tip. The injector was also calibrated to apply ca 1 gal/minute (Figure 6). Times were calculated to apply the correct amount of solution per drench or injection.

Figure 4. Application tank set up.

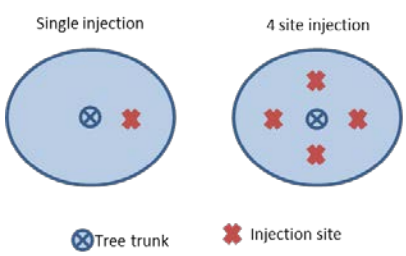
Figure 5. Drench application.

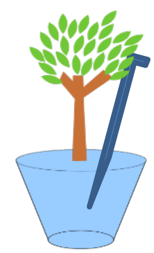
Figure 6. Injection app.

The total treatment volume for each rootball (drench + injection) was 1/5 the volume of the rootball. For these rootballs the calculated total treatment volume (drench + injection) was 1.35 gal/rootball. The total treatment volume was equally divided between drench application and injection application. The drench portion of the treatment was done first in an effort to contain the ants and we drenched all the rootballs first and then went back and did the injections. Each rootball was drenched with ½ the total treatment volume which was divided into 2 drench applications. Rootballs were placed on a side and drenched (1/2 total drench volume=0.34 gal) on one side of the rootball, hitting all exposed surfaces and after 15-30 minutes the rootball was rotated and the second drench applied to the now upfacing side, and all exposed surfaces. A stopwatch was used to time the drenches based on the pre-determined calibration rate.

Injections were either applied through a single injection point or through 4 injection points. The Chlorpyrifos 4EC weevil injection technique was used as a guide.

- Insert the injection rod at a 30-45° angle between the stem and the outer top perimeter of the rootball
- Insert to approximately the middle of the rootball and open the flow – we marked the rod with tape so injection depths were similar on all rootballs; if a rootball was significantly less deep than others we adjusted depth accordingly
- Single injection treatments applied the full injection amount through one site in approximately the middle of the rootball; more of a 45° angle to hit the middle of the rootball





- Four injection treatments applied ¼ the full injection amount in each of 4 sites equally spaced around the top of the rootball; more of a 30° angle so each injection saturates a different area of the center of the rootball
- A stopwatch was used to time the injections based on the pre-determined calibration rate

Treatments are found in Table 1. Nine rootballs were treated per treatment rate and 3 rootballs were treated with water only as controls. At 1, 2, and 7 days after treatment, 3 insecticide treated rootballs from each treatment and 1 control rootball were destructively sampled to determine survival of IFA colonies.

RESULTS:

All control rootballs had active ants present at 1 day after treatment when disturbed and therefore we decided to wait until day 2 and 7 to destructively sample (Table 2). At day 2, all still contained ants and we selectively sampled the one rootball with only a few ants present when disturbed. This rootball only had about 50 ants present, but no dead evident. At day 7, one of the remaining controls had 300+ active ants and the other had no ants, and again, no dead ants were present. The disturbance of the 4 injections may have prompted these colonies to move and in any future trials, the controls will be placed in Fluon®-ed Plantainer® to contain the colonies.

All treated rootballs eliminated the IFA colonies within 7 days (Table 2), with 1 lone ant found in one of the bifenthrin single injection site rootballs. However, none of the treatments eliminated ants within 2 days although in all cases, except 1 bifenthrin single injection replicate, the numbers of ants was significantly reduced from original numbers. At 2 days, most rootballs contained only 10-100 ants.

Table 1. Treatments for B&B rootball injection treatments to eliminate IFA whole colonies.

Treatment	Chemical	Amt chemical/gal	Total volume/ rootball	Drench first	Injection second
Drench + 1 Injection	Bifenthrin 0.2 lb ai/100 gal water	3.78 ml/gal	1.35 gal (5110 ml) split bet drench and inj	0.34gal (1277ml) on one side (20 sec), rotate, 0.34gal (20 sec) other side	0.68 gal (2555 ml) in 1 site (41 sec)
Drench + 4 Injections	Bifenthrin 0.2 lb ai/100 gal water	3.78 ml/gal	1.35 gal (5110 ml) split bet drench and inj	0.34gal (1277ml) on one side (20 sec), rotate, 0.34gal (20 sec) other side	bet 4 sites: 0.17 gal (643 ml) in each of 4 sites (10 sec each site)
Drench + 4 Injections	Lambda-cyhalothrin 0.034 lb ai/100 gal water	1.46 ml/1 gal	1.35 gal (5110 ml) split bet drench and inj	0.34gal (1277ml) on one side (20 sec), rotate, 0.34gal (20 sec) other side	bet 4 sites: 0.17 gal (643 ml) in each of 4 sites (10 sec each site)
Drench + 4 Injections	Water control	0	1.35 gal (5110 ml) split bet drench and inj	0.34gal (1277ml) on one side (20 sec), rotate, 0.34gal (20 sec) other side	bet 4 sites: 0.17 gal (643 ml) in each of 4 sites (10 sec each site)

Table 2. Efficacy of rootball injections against established IFA colonies – MS 2015. All were destructively sampled except controls as noted.

Chemical	Rate of application	Application technique*	Reps	Activity (ants present) at indicated hours/days after treatment		
				24 hours	48 hours	7 days
Bifenthrin	0.2 lb ai/100 gal water	Drench+single injection site	1	0	300+ active	1 ant
			2	100+ active at base of shrub	5 ants and 5 alates	0
			3	200+ active	0	0
		Drench+4 injection sites	1	0	20 ants and 5 alates	0
			2	50+ active	50+ active	0
			3	300+ active	50 active	0
Lambda-cyhalothrin	0.034 lb ai/100 gal water	Drench+4 injection sites	1	< 5 ants	100 active	0
			2	200+ active	0-did not see many dead	0
			3	0	0-did not see many dead	0
Control	Water only	Drench+4 injection sites	1	disturbed-active	50+ active-did not see many dead	---------
			2	disturbed-active	disturbed-active	300+ active
			3	disturbed-active	disturbed-active	no ants-did not see many dead

DISCUSSION:

Concerns over the true efficacy of B&B rootball drenches to eliminate existing IFA colonies harvested within rootballs prompted the investigation of insecticide injection as a possible application technique. All previous injection trials have been done on field grown nursery stock with IFA colonies or directly on IFA colonies and were small preliminary type trials. These in-field injection trials all used lower rates of bifenthrin, 0.01 to 0.05 lb ai/100 gal with 1 gal/mound; some injection only and some drench plus injection, and 1 to 5 injection sites. The injection only trials were variable and required 1 week to 4 weeks to eliminate colonies while the drench plus injection trials with both bifenthrin (0.01-0.05 lb ai/100 gal water) and lambda-cyhalothrin (0.034-0.069 lb ai/100 gal water) eliminated colonies within 1 week. In an attempt to provide even faster elimination of IFA colonies in rootballs, the trial reported here evaluated the highest labeled single application rate of bifenthrin (0.2 lb ai/100 gal water) and lambda-cyhalothrin (0.034 lb ai/100 gal water) and utilized the drench plus injection application technique. These rates did provide a significant decrease in IFA numbers in rootballs within 2 days and completely eliminated IFA within 7 days. However, no evaluation was done between the 2 and 7 day evaluations, therefore future trials will focus evaluations on those days between 2 and 7. In addition, trials will be conducted both in the field and on harvested B&B rootballs since the best use of this application technique may be pre-harvest while plants are still in the field.

Efficacy of Rootball Drench Treatments to Exclude IFA Colonies – Mississippi, 2015

Anne-Marie Callcott and Richard King

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 2015 treatments for B&B nursery stock include a chlorpyrifos drench (applied twice in one day with a rotation of the rootball between drench applications) or a chlorpyrifos or bifenthrin rootball dip treatment. While rootball dips are the most effective treatment option against IFA, they are impractical with both environmental and human safety concerns. A rootball drench, when rootballs are in the holding area, prior to shipment is the preferred method of treatment.

Previous trials have investigated numerous insecticides and drench application methods (reduce number of drench applications per treatment and rotating rootballs between drench applications, etc). Data from these trials has focusing on efficacy of treatment against IFA alate females over time; alate females being the substitute life stage for newly mated queens, the only reproductively hazardous life stage. No trials have been conducted to determine whether rootball drench treatments will exclude or keep IFA colonies from infesting treated rootballs stored for a period of time prior to sales/shipping.

MATERIALS AND METHODS:

Thirty-four Ligustrum were machine harvested as 12" rootballs (burlap only, no baskets) in early May by Barnhill Farms Nursery in Lucedale, MS. Plants were not treated with any insecticides prior to harvest and did not have any IFA colonies associated with them. Plants were transported to the Biloxi Station May 6, 2015 and set in groups on the ground: 2 groups of 12 each for insecticide treatments and 1 group of 8 each for the untreated controls. Rootballs were either watered prior to treatment to assist in drench penetration (bifenthrin treatment) or not watered if sufficient rainfall had occurred and burlap and rootball were damp (lambda-cyhalothrin treatment). After treatment, a sprinkler was set up to water as needed to supplement rainfall.

Due to limited "test arenas", different treatments were applied one week apart. Treatments included:

- Bifenthrin 0.2 lb ai/100 gal water – 1F1: treated May 11, 2015
- Lambda-cyhalothrin 0.034 lb ai/100 gal water – 1F1: treated May 18, 2015
- Control – water only – 1F1: all treated with water only on May 11, 2015

The total drench solution applied over the course of the treatment to each rootball was ca. 1/5 the volume of the rootball based on the average size of the root balls (12" top diameter x 10" bottom diameter x 14" height), thus a total of ca. 1.14 gallon of finished solution was applied to each root ball. The total drench solution was divided between the number of drenches each root ball would receive. 1F1 treatments received one drench (1/2 total drench volume) on one side of the rootball, hitting all exposed surfaces. After 15-30 minutes the rootball was rotated and the second drench applied to the now upfacing side, and all exposed surfaces. Controls were treated with plain water with a 1F1 process.

Plants were aged on the ground with supplemental irrigation as needed. Evaluations were conducted at 1, 4, 8 and 16 weeks after treatment. For each evaluation, 3 treated rootballs and 1 control rootball were used. Each rootball was placed at one end of a 2' x 4' x 6" test arena with Fluon® coated sides to prevent ant escape. A field collected IFA colony, with nest tumulus, was placed in the other end and spread out to facilitate drying of the soil to encourage ants to move into the rootball. Observations were made daily, and included estimated number of live and dead ants in the arena, and live ants in the rootball. On day 7 the rootball was removed and destructively sampled to more accurately quantify ants in the rootball. Test arenas were placed under cover (a small greenhouse type structure) and rootballs were watered by hand as needed. Test arenas were cleaned between evaluations with alcohol and Fluon® reapplied as needed. If ants penetrated Fluon® during an evaluation (escaped the test arena), talcum powder was used to supplement the barrier. There were 4 test arenas; 1 dedicated to control plants and the other 3 used for the insecticide treated plants.


RESULTS:

In all the control rootballs, the ants moved into the rootball within 1-2 days of introduction into the test arena. The control test arena was also the only arena that had ants escape, but even in the evaluations with ants escaping, there were still over 100 active, healthy worker ants in the control rootball at the day 7 destructive evaluation.

The bifenthrin rootball drench treatment was effective throughout the 16 week trial at excluding IFA colonies from infesting the treated rootballs (Table 1). Ants preferred to die in the test arena rather than move into the treated rootball. In general most of the worker ants were dead within 2-3 days after being confined to the test arena with the bifenthrin treated rootball.

The lambda-cyhalothrin drench treatment was effective at excluding IFA from infesting the treated rootball at 1 week after treatment (Table 1). At 4 weeks after treatment, 1 rootball contained about 50-100 worker ants with the remaining ants from the colony still in the test arena; about 50 live ants and more than 300 dead ants in the arena. By 8 weeks, 2 of the 3 treated rootballs contained over 200 ants and brood when they were destructively sampled at day 7. There were 300-500 workers ants dead in the test arena of these rootballs as well. At 16 weeks, all 3 treated rootballs contained IFA at the 7 day sampling period, with about 300 ants and brood in each rootball and about the same number dead in the test arenas.

Table 1. Efficacy of drench treatments at preventing IFA colony infestation of treated B&B root ball. Number of root balls with live IFA inside root ball at 7 days after treatment (3 reps/insecticide treatment; 2 reps/control).

Treatment	Rate of application (lb ai/100 gal water)	# rootballs with live ants at day 7 sampling of indicated weeks after treatment			
		1	4	8	16
Bifenthrin	0.2	0	0	0	0
Lambda-cyhalothrin	0.034	0	1	2	3
Control	--	2	2	2	2

DISCUSSION:

The 0.2 lb ai/100 gal bifenthrin drench treatment for B&B nursery stock appears to be effective in excluding IFA infestation for 16 weeks/4 months after treatment, allowing storage of rootballs for at least that period of time with minimal risk of re-infestation. The lambda-cyhalothrin treatment was only effective at the 1 week evaluation, showing a decrease in efficacy by the 4 week evaluation.

Efficacy of Rootball Injection Treatments to Exclude IFA Colonies – Mississippi, 2016

Anne-Marie Callcott and Richard King

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 2015 treatments for B&B nursery stock include a chlorpyrifos drench (applied twice in one day with a rotation of the rootball between drench applications) or a chlorpyrifos or bifenthrin rootball dip treatment. While rootball dips are the most effective treatment option against IFA, they are impractical with both environmental and human safety concerns. Rootball drenches have been very effective against IFA alate females over time; alate females being the substitute life stage for newly mated queens, the only reproductively hazardous life stage and at excluding IFA colonies, but less effective at eliminating whole IFA colonies harvested within a rootball. A rootball injection paired with a drench may be more effective at eliminating IFA colonies inside a rootball and still provide exclusion efficacy. It will be labor intensive, but many growers are currently requesting fast killing treatments with little residual (a treat and ship type of treatment vs. a treat and store type of treatment).

MATERIALS AND METHODS:

A variety of shrubs at Deep South Nursery (Lucedale, MS) were machine harvested by the nursery's workers into 16" rootballs (Figures 1) during late June 2016 and transported to the MSU-CREC facility for testing.

Figure 1. Machine harvesting of plants.

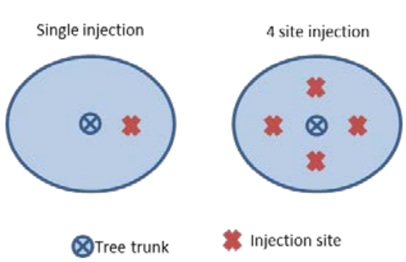
Rootballs were divided into 3 groups of 12 for insecticide treatment and one group of 12 for the control treatment. Treatments were staggered due to the limitation of test arenas for the colony exclusion testing. Drench and injection solutions were mixed in a 15-gallon spray tank equipped with a battery operated High-Flo Gold series pump (Model 5275704; 45 psi; 3.8 gpm) (Figure 2). A hose attached the spray tank to the application tool (spray wand or injector). Drench applications were applied with a common garden spray wand calibrated to an application rate of ca. 1.6 gal/minute (Figure 3). Injection applications were applied with a B&G 430 Versagun Termite rod applicator with the 40” x 5/8” rod and 360° tip. The injector was also calibrated to apply ca 1.6 gal/minute (Figure 4). Times were calculated to apply the correct amount of solution per drench or injection.

Figure 2. Application tank set up. Figure 3. Drench application. Figure 4. Injection app.

The total treatment volume for each rootball (drench + injection) was 1/5 the volume of the rootball. For these rootballs the calculated total treatment volume (drench + injection) was 1.2 gal/rootball. The total treatment volume was equally divided between drench application and injection application. The drench portion of the treatment was done first in an effort to contain the ants and we drenched all the rootballs first and then went back and did the injections. Each rootball was drenched with ½ the total treatment volume which was divided into 2 drench applications. Rootballs were placed on a side and drenched (1/2 total drench volume=0.3 gal) on one side of the rootball, hitting all exposed surfaces and after 15-30 minutes the rootball was rotated and the second drench applied to the now upfacing side, and all exposed surfaces. A stopwatch was used to time the drenches based on the pre-determined calibration rate.

Injections were either applied through a single injection point or through 4 injection points. The Chlorpyrifos 4EC weevil injection technique was used as a guide.

- Insert the injection rod at a 30-45° angle between the stem and the outer top perimeter of the rootball
- Insert to approximately the middle of the rootball and open the flow – we marked the rod with tape so injection depths were similar on all rootballs; if a rootball was significantly less deep than others we adjusted depth accordingly



- Four injection treatments applied ¼ the full injection amount in each of 4 sites equally spaced around the top of the rootball; more of a 30° angle so each injection saturates a different area of the center of the rootball
- A stopwatch was used to time the injections based on the pre-determined calibration rate

Treatments are found in Table 1. Twelve rootballs were treated per treatment rate and 12 rootballs were treated with water only as controls. Plants were aged on the ground with supplemental irrigation as needed. Evaluations were conducted at 1, 4, 8 and 16 weeks after treatment. For each evaluation, 3 treated rootballs and 1 control rootball were used. Each rootball was placed at one end of a 2' x 4' x 6" test arena with Fluon® coated sides to prevent ant escape (Figure 5). A field collected IFA colony, with nest tumulus, was placed in the other end and spread out to facilitate drying of the soil to encourage ants to move into the rootball. Observations were made daily, and included estimated number of live and dead ants in the arena, and live ants in the rootball. On day 7 the rootball was removed and destructively sampled to more accurately quantify ants in the rootball (Figure 6). Test arenas were placed under cover (a small greenhouse type structure) and rootballs were watered by hand as needed. Test arenas were cleaned between evaluations with alcohol and Fluon® reapplied as needed. If ants penetrated Fluon® during an evaluation (escaped the test arena), talcum powder was used to supplement the barrier. There were 4 test arenas; 1 dedicated to control plants and the other 3 used for the insecticide treated plants. Rootballs were watered during the arena testing period as needed.

Figure 5. Test arena.

Figure 6. Destructive sampling.

RESULTS:

In general, all treatments excludes IFA from moving into the rootballs over a 7 day period for 4 months after treatment (Table 2). In the daily evaluations during the 7 day test period, ants were seen climbing on the rootball or working the rootball early in the time frame but generally moved backed into the pan and died rather than remain in the treated rootball. By the 6 month evaluation, each treatment had at one rootball with a few ants inside or ants still alive in the pan.

Thus all these treatments were effective in excluding IFA colonies from relocating to the treated rootball for at least 4 months.

Table 2. Efficacy of drench + injection treatments at preventing IFA colony infestation of treated B&B root ball. Number of root balls with live IFA inside root ball at 7 days after treatment (3 reps/insecticide treatment; 2 reps/control).

Treatment	Rate of application (lb ai/100 gal water)	# rootballs with live ants at day 7 sampling of indicated months after treatment			
		1	2	4	6
Bifenthrin	0.1	0	0	0	0
Bifenthrin	0.2	1	0	0	1
Lambda-cyhalothrin	0.034	0	1	0	1
Control	--	2	3	3	3

Table 1. Treatments for B&B rootball injection treatments to eliminate IFA whole colonies.

Treatment	Chemical	Date Treated	Amt chemical/gal	Total volume/ rootball	Drench first	Injection second
Drench + 4 Injections	Bifenthrin 0.1 lb ai/100 gal water	July 6, 2016	1.89 ml/gal	1.2 gal (4542 ml) split bet drench and inj	0.3gal (1136ml) on one side (11 sec), rotate, 0.3gal (11 sec) other side	bet 4 sites: 0.15 gal (567 ml) in each of 4 sites (5.5 sec each site)
Drench + 4 Injections	Bifenthrin 0.2 lb ai/100 gal water	July 13, 2016	3.78 ml/gal	1.2 gal (4542 ml) split bet drench and inj	0.3gal (1136ml) on one side (11 sec), rotate, 0.3gal (11 sec) other side	bet 4 sites: 0.15 gal (567 ml) in each of 4 sites (5.5 sec each site)
Drench + 4 Injections	Lambda-cyhalothrin 0.034 lb ai/100 gal water	July 27, 2016	1.46 ml/1 gal	1.2 gal (4542 ml) split bet drench and inj	0.3gal (1136ml) on one side (11 sec), rotate, 0.3gal (11 sec) other side	bet 4 sites: 0.15 gal (567 ml) in each of 4 sites (5.5 sec each site)
Drench + 4 Injections	Water control	July 6, 2016	0	1.2 gal (4542 ml) split bet drench and inj	0.3gal (1136ml) on one side (11 sec), rotate, 0.3gal (11 sec) other side	bet 4 sites: 0.15 gal (567 ml) in each of 4 sites (5.5 sec each site)

2015 Alabama Grass Sod Treatments for IFA Quarantine

Fudd Graham

Objectives:

To determine the efficacy of a combination of a fire ant bait and combinations of liquid contact insecticides against Red Imported Fire ants (*Solenopsis invicta*)

Test Substance:

Treatment	Bait	Contact ai/acre	Contact material/acre	Notes
Bait + Aloft GC SC combo	Yes	Aloft SC @ 0.17 lb ai bifenthrin/A @ 0.35 lb ai clothianidin/A	20 oz/A	Combo product
Bait + tank mix of Bif & Carb	Yes	Onyx Pro EC @ 0.2 lb ai bifenthrin/A Carbaryl/Sevin SL (43%) @ 4 lb ai/A	Onyx Pro = 13.9 oz/A "Sevin" = 4 qts/A	Tank mix products
Bait + tank mix of Bif & Lambda-cy	Yes	Onyx Pro EC @ 0.2 lb ai bifenthrin/A Lambda-cy GC @ 0.069 lb ai L-cy/A	Onyx Pro = 13.9 oz/A Lambda-cy = 8.8-10 oz/A	Tank mix products
Tank mix of Bif & Carb	No	Onyx Pro EC @ 0.2 lb ai bifenthrin/A Carbaryl/Sevin SL (43%) @ 4 lb ai/A	Onyx Pro = 13.9 oz/A "Sevin" = 4 qts/A	Tank mix products
Control	No	No		

Test System:

Fire ant colonies were located within the area of the Farmlinks Research and Demonstration Golf Course and the home of David Pursell, the owner.

Method for Control of Bias:

There were 3 replicates of each treatment arranged in a randomized complete block design. Plots were arranged by numbers of mounds in the sample area and blocked using population size. Treatments were then randomized within the blocks.

Materials and Methods:

Red imported fire ant (RIFA): *Solenopsis invicta* Buren

This study compared the efficacy of five different insecticidal combinations and an untreated control for the control of red imported fire ant (RIFA). The study site was selected because of an abundance of RIFA and the cooperation of the course manager. Plots measuring 150 ft. X 150 ft.

were laid out with each having a ¼ acre permanently marked circular area in the center that was used as the evaluation area. Each evaluation area had at least 10 active RIFA mounds in pre-treatment count, which took place on Jun 16, 19 and 25. Three plots/replicates were used for each of the five treatments. RIFA populations (presence or absence; number per 1/4 acre evaluation area) were evaluated pre-treatment and at approximately 2-3 days, 1, 2, 3, 4, 6, 8, 10, 12 weeks post-treatment. Treatments are listed in the table above. Bait applications were made on Jul 15 with a Herd Seeder. Spray applications were made on Jul 21 with a GPS enabled sprayer calibrated to 32 GPA and set to prevent overlaps in the field, so no application was made out of the plots and no overlaps occurred . The first data collection post-treatment was on Jul 23 (1 WAT). Data were analyzed using SAS PROC MEANS/Tukey, SAS Version 9.2, Copyright® 2014 SAS Institute Inc., Cary, NC).

The following equations will be used to calculate percent control for the treated groups based on the untreated control numbers each data period (see graph and table).

$$\% \text{ Control} = \frac{\text{Control Mean No. Mounds} - \text{Treated Mean No. Mounds}}{\text{Control Mean No. Mounds}} \times 100$$

The following equations will be used to calculate percent control for the treated groups based on the pretreatment count in the treated plots (see graph and table).

$$\% \text{ Control} = \frac{\text{Pretreament Mean No. Mounds} - \text{Treated Mean No. Mounds}}{\text{Pretreatment Mean No. Mounds}} \times 100$$

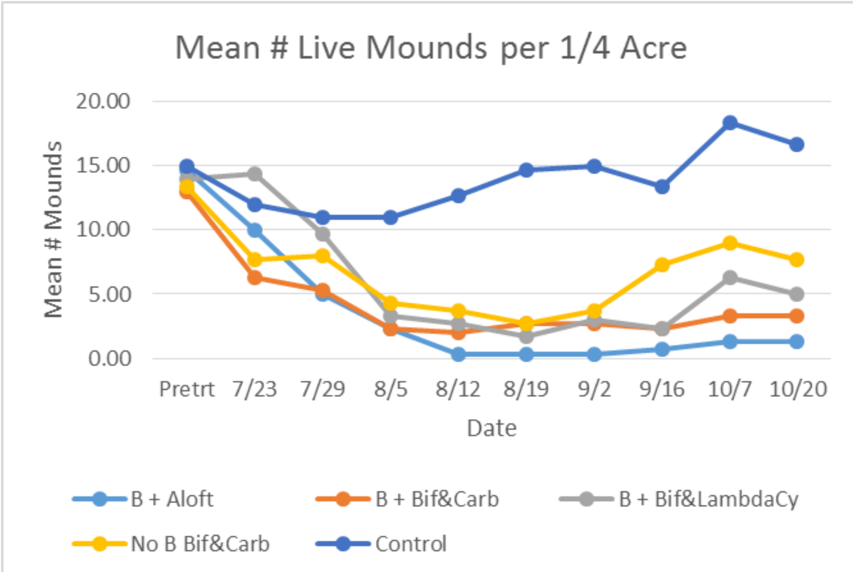
Results

Table 1

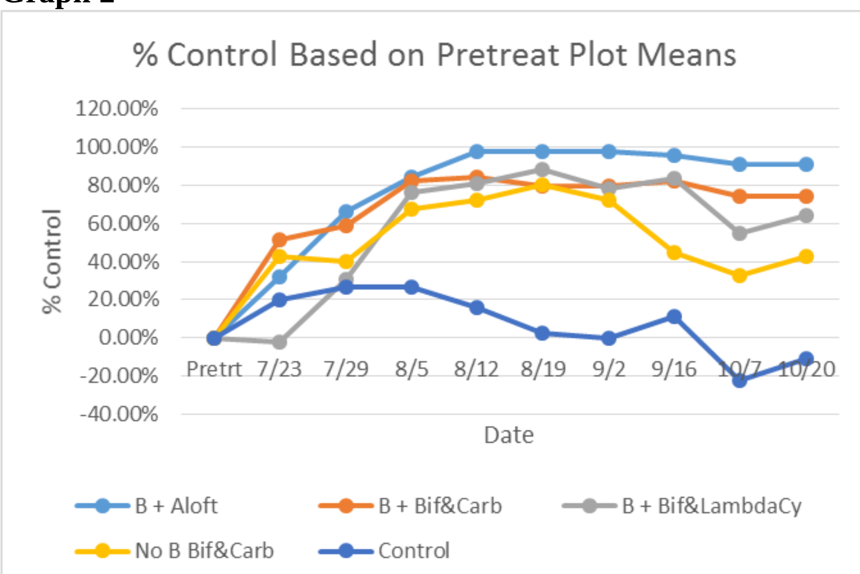
Date	Pretrt	7/23	7/29	8/5	8/12	8/19	9/2	9/16	10/7	10/20
Treatment	Mean Number of Mounds per 1/4 Acre									
B + Aloft	14.66a	10.00ab	5.00a	2.33b	0.33b	0.33b	0.33b	0.67b	1.33b	1.33a
B + Bif&Carb	13.00a	6.33b	5.33a	2.33b	2.00b	2.67b	2.67b	2.33b	3.33ab	3.33a
B + Bif&LambdaCy	14.00a	14.33a	9.67a	3.33b	2.67b	1.67b	3.00b	2.33b	6.33ab	5.00a
No B Bif&Carb	13.33a	7.67ab	8.00a	4.33ab	3.67b	2.67b	3.67b	7.33ab	9.00ab	7.67a
Control	15.00a	12.00ab	11.00a	11.00a	12.67a	14.67a	15.00a	13.33a	18.33a	16.67a

*Means within the same column with the same letter are not significantly different, Duncan's $P<0.05$.

Graph 1

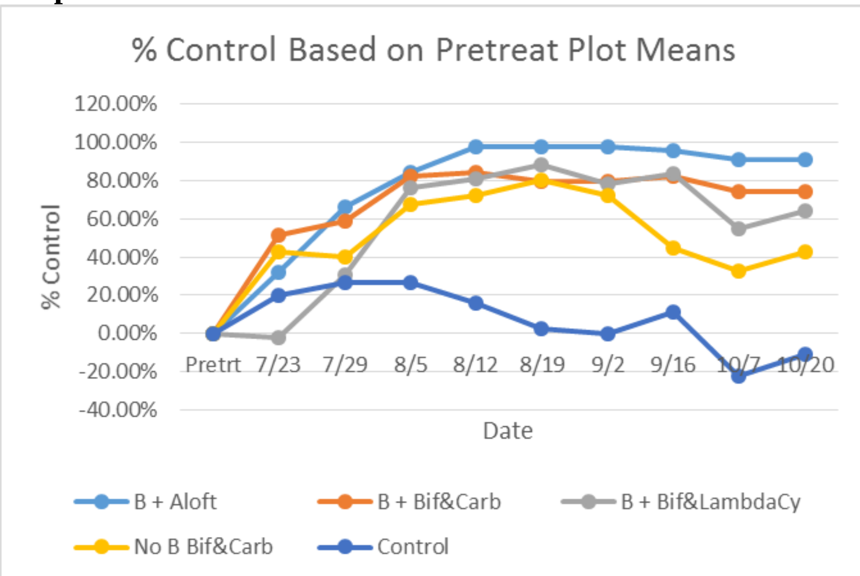


Graph 2



***Percent control based on the number of mounds in the plots before treatment and does not account for season variation that occurred in the untreated plots**

Graph 3



*****Percent control based on the number of mounds in the plots before treatment and corrects for the season variation that occurred in the untreated plots

Discussion

On 23 July, the B+Bif&Carb had significantly fewer mounds than all other treatments. On the next week, July 29, there were no differences between any of the treatments. On Aug 5, 12, 19 and Sep 2, there were significantly fewer mounds in all treatments than the control. On Sep 16, all treatments except the Bif&Carb with no bait had significantly fewer mounds than the control. On Oct 7 and 20, only the Bait+Aloft treatment still had significantly fewer mounds than the control

Conclusion

Even after the liquid treatments were applied, the mound numbers in the plots dropped slowly and with the exception of the Bait+Aloft treatment, none ever obtained over 90% control. The other treatment did reach a control level in the mid-80% range during late August and early September.

Alabama Grass Sod Treatments for IFA Quarantine, 2016-2017

L.C. 'Fudd' Graham, Ph.D.

Objectives:

To determine the efficacy of a combination of a fire ant bait and combinations of liquid and granular contact insecticides against red imported fire ants (*Solenopsis invicta*).

Test Substance:

Treatment	Bait	Contact ai/acre	Contact material/acre	Notes
Bait + Aloft GC SC combo	Yes	Aloft SC @ 0.17 lb ai bifenthrin/A @ 0.35 lb ai clothianidin/A	20 oz/A	Combo product
Bait + tank mix of Bif & Carb	Yes	Onyx Pro EC @ 0.2 lb ai bifenthrin/A Carbaryl/Sevin SL (43%) @ 4 lb ai/A	Onyx Pro = 13.9 oz/A "Sevin" = 4 qts/A	Tank mix products
Bait + Taurus Trio G	Yes	Taurus Trio G @ 0.0124 lb ai fipronil + 0.2 lb ai bifen + 0.051 lb ai lambda-cy	Taurus Trio G = 87 lb material/A	Combo product
Taurus Trio G	No	Taurus Trio G @ 0.0124 lb ai fipronil + 0.2 lb ai bifen + 0.051 lb ai lambda-cy	Taurus Trio G = 87 lb material/A	Combo product
Control	No	No		

Method for Control of Bias:
There were 3 replicates of each treatment arranged in a randomized complete block design. Plots were arranged by numbers of mounds in the sample area and blocked using population size. Treatments were then randomized within the blocks.

Data sorted by mound number to show replication

Date Pretrt	Plot	Mound #	Trt #	Trt name
7/18	2	10	101	Taurus only
7/18	3	10	102	Control
7/18	4	10	103	Bait+Aloft
7/20	6	10	104	Bait+Taurus
7/20	11	11	105	Bait+Bif+Carb
7/21	13	11	201	Control
7/21	15	11	202	Bait+Bif+Carb
7/20	10	13	203	Bait+Aloft
7/20	9	14	204	Bait+Taurus
7/21	14	15	205	Taurus only
7/20	8	15	301	Control
7/21	12	15	302	Bait+Taurus
7/18	1	15	303	Bait+Bif+Carb
7/18	5	16	304	Bait+Aloft
7/20	7	25	305	Taurus only

Materials and Methods:

Red imported fire ant (RIFA): *Solenopsis invicta* Buren

This study compared the efficacy of four different insecticidal combinations and an untreated control for the control of red imported fire ant (RIFA). The study site was located at the FarmLinks Research and Demonstration Golf Course and was selected because of an adequate number of RIFA and the cooperation of the course manager and owner. Plots measuring 150 ft. X 150 ft. were laid out with each having a ¼ acre permanently marked circular area in the center that was used as the evaluation area. Each evaluation area had at least 10 active RIFA mounds in pre-treatment count, which took place on Jul 18, 20 and 21. Each of the treatments were replicated three times. RIFA populations (presence or absence of live mounds; number per 1/4 acre evaluation area) were evaluated pre-treatment and at approximately 2-3 days, 1, 2, 3, 4, 6, 8, 10, 12 weeks post-treatment. Treatments are listed in the table above. Bait applications of Extinguish Plus were made on Aug 3 with a Herd Seeder at a rate of 1.5 lb product per acre. Spray applications were made on Aug 16 using a GPS enabled sprayer calibrated to 32 GPA and set to prevent overlaps in the field, so no application was made out of the plots and no areas were overlapped. Granular applications were made the same day using a Vicon pendulum spreader. Bait applications were delayed two weeks after pre-treatment counts due to rainy weather. The first data collection post-treatment was on Aug 18 (2 DAT). Mound number data per ¼-acre plot are presented in graph and table form and were analyzed using SAS PROC MEANS/Tukey, SAS Version 9.4, Copyright® 2016 SAS Institute Inc., Cary, NC). Data for percent control based on pre-treatment mound counts in a plot and percent control based on the mound counts in the control plot each date are presented in graph and table format, but were not analyzed. Data are also presented on mean mound numbers per ¼ acre plot omitting an outlier plot (plot 2, Taurus only, treatment 101).

The following equations were used to calculate percent control for the treated groups based on the pretreatment count in the treated plots (see graph and table).

$$\% \text{ Control} = \frac{\text{Pre-treatment Mean No. Mounds} - \text{Treated Mean No. Mounds}}{\text{Pretreatment Mean No. Mounds}} \times 100$$

The following equations were used to calculate percent control for the treated groups based on the untreated control numbers each data period (see graph and table).

$$\% \text{ Control} = \frac{\text{Control Mean No. Mounds} - \text{Treated Mean No. Mounds}}{\text{Control Mean No. Mounds}} \times 100$$

Results and discussion:

There were no significant differences between any treatments until the 14-day count on Aug 30 when all treatments had significantly fewer mounds than the control (P>0.05, Tukey). For some reason, Taurus Trio G failed in plot number 2 (treatment number 101). From that time on, all treatments except the Taurus Trio G alone treatment had fewer mounds than the control (P>0.05, Tukey). All treatments, other than the Taurus Trio G only treatment achieved greater than 90% control by Aug 1 and, all but the Bait+Taurus Trio G treatment on Nov 16 (87% control) maintained that level of control until the end of the trial.

Table 1 and Figure 1. Mean number of mounds found per ¼-acre plot

Date	Pretrt	8/18/16	8/23/16	8/30/16	9/6/16	9/12/16	9/29/16	10/13/16	11/16/16	12/14/16
Treatment	Mean Number of Mounds per 1/4 Acre*									
Bait+Aloft	13.67a	1.67a	1.33a	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b
Bait+Bif+Carb	12.33a	3.00a	2.00a	0.33b	1.67b	1.00b	0.00b	0.667b	1.00b	0.00b
Bait+Taurus	13.00a	3.67a	2.67a	1.67b	3.67b	0.00b	1.33ab	0.667b	1.667b	0.33b
Taurus only	16.67a	8.67a	7.00a	3.00b	8.33ab	6.00ab	10.00ab	6.33ab	5.33ab	5.67ab
Control	12.00a	9.00a	9.33a	9.33a	15.00a	12.67a	13.33a	12.33a	12.33a	13.00a
	*Means with the same letter not different P>0.05, Tukey									

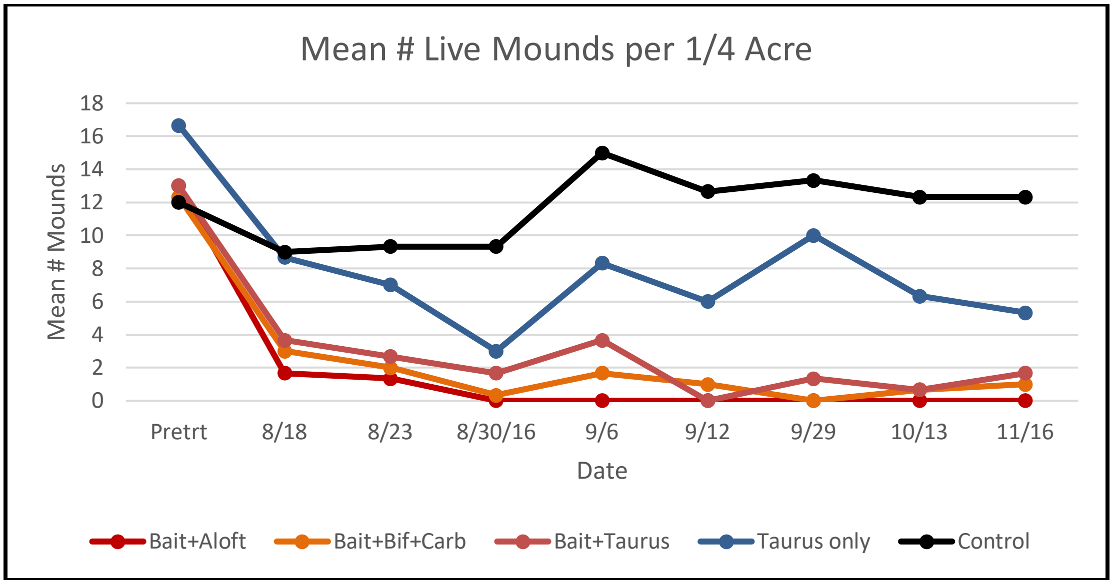


Table 2 and Figure 2. Percent control data based on pre-treatment mound counts in a plot

		% Control based on initial plot number								
	Pretrt	8/18	8/23	8/30	9/6	9/12	9/29	10/13	11/16	12/14
Bait+Aloft	0.00%	87.81%	90.25%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
Bait+Bif+Carb	0.00%	75.67%	83.78%	97.30%	86.48%	91.89%	100.00%	94.59%	91.89%	100.00%
Bait+Taurus	0.00%	71.79%	79.49%	87.18%	71.79%	100.00%	89.74%	94.87%	87.18%	97.44%
Taurus only	0.00%	48.01%	58.01%	82.00%	50.01%	64.01%	40.01%	62.01%	68.01%	66.01%
Control	0.00%	25.00%	22.22%	22.22%	-25.00%	-5.56%	-11.11%	-2.78%	-2.78%	-8.33%

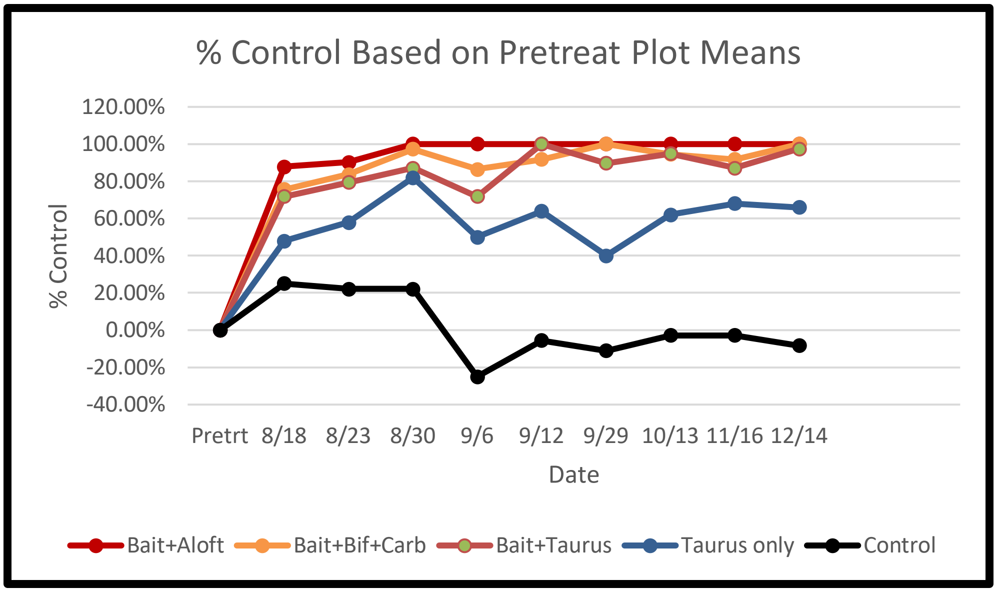
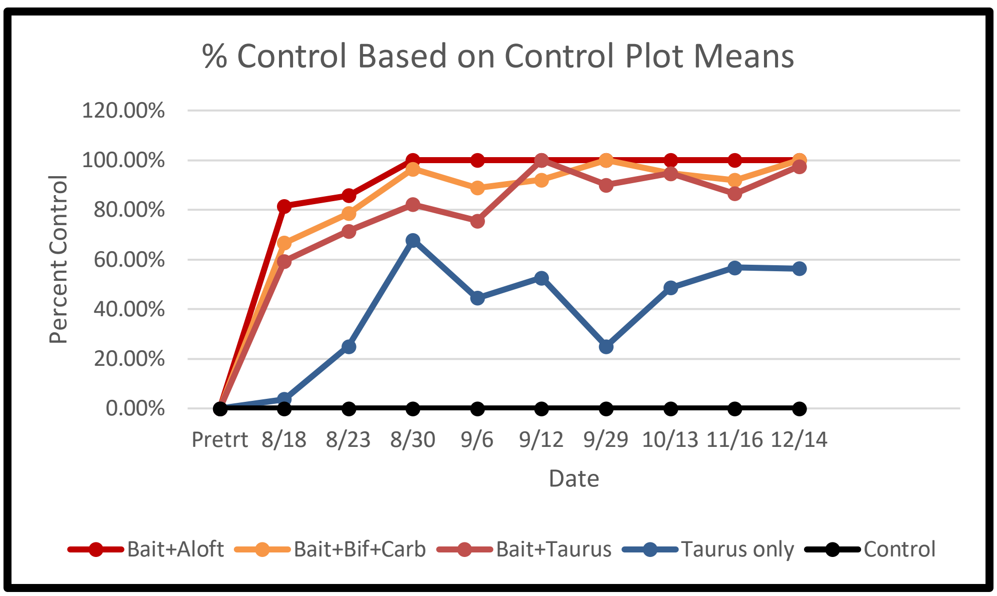


Table 3 and Figure 3. Percent control based on the mound counts in the control plot each date

	% Control based on control plot values									
	Pretrt	**8/18**	**8/23**	**8/30**	**9/6**	**9/12**	**9/29**	**10/13**	**11/16**	**12/14**
Bait+Aloft	0.00%	81.48%	85.71%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
Bait+Bif+Carb	0.00%	66.67%	78.57%	96.43%	88.89%	92.11%	100.00%	94.59%	91.89%	100.00%
Bait+Taurus	0.00%	59.26%	71.43%	82.14%	75.56%	100.00%	90.00%	94.59%	86.49%	97.44%
Taurus only	0.00%	3.70%	25.00%	67.86%	44.44%	52.63%	25.00%	48.65%	56.76%	56.41%
Control	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%



Alabama Grass Sod Treatments for IFA Quarantine, 2017-2018 December Progress Report

L.C. 'Fudd' Graham, Ph.D.

Objectives:

To determine the efficacy of a combination of a fire ant bait and combinations of liquid and granular contact insecticides against red imported fire ants (*Solenopsis invicta*).

Test Substance:

Treatment	Bait	Contact ai/acre	Contact material/acre	Notes
Bait + Aloft GC SC combo	Yes	Aloft SC @ 0.17 lb ai bifenthrin/A @ 0.35 lb ai clothianidin/A	20 oz/A	Combo product
Bait + tank mix of Bif & Carb	Yes	Onyx Pro EC @ 0.2 lb ai bifenthrin/A Carbaryl/Sevin SL (43%) @ 4 lb ai/A	Onyx Pro = 13.9 oz/A Sevin = 4 qts/A	Tank mix products
Bait + Taurus Trio G	Yes	Taurus Trio G @ 0.0124 lb ai fipronil + 0.2 lb ai bifen + 0.051 lb ai lambda-cy	Taurus Trio G = 87 lb material/A	Combo product
Control	None	None		

Method for Control of Bias:
There were 3 replicates of each treatment arranged in a randomized complete block design. Plots were arranged by numbers of mounds in the sample area and blocked using population size. Treatments were then randomized within the blocks.

Materials and Methods:

Red imported fire ant (RIFA): *Solenopsis invicta* Buren

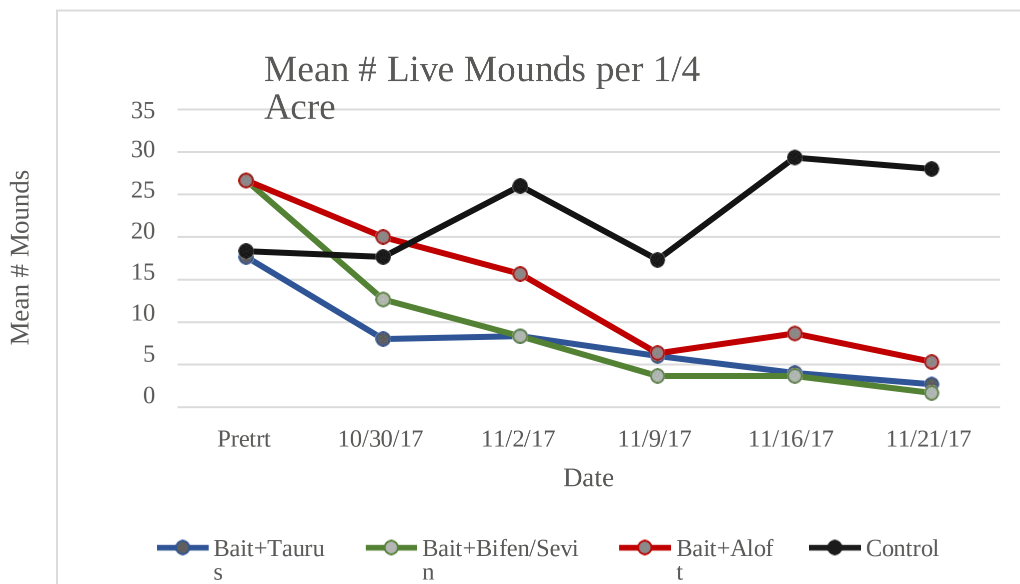
This study compares the efficacy of three different insecticidal combinations (see Table above) and an untreated control for the control of red imported fire ant (RIFA). The study site is located at the FarmLinks Research and Demonstration Golf Course in Fayetteville, AL and was selected because of an adequate number of RIFA and the cooperation of the course manager and owner. The test was scheduled to be initiated in May or June. Due to a change in land use patterns at the property, we were unable to start the test until early October. Plots measuring 150 ft. X 150 ft. were laid out with each having a ¼ acre permanently marked circular area in the center that was used as the evaluation area. Each evaluation area had at least 10 active RIFA mounds in pre-treatment counts, which took place on October 12, 13 and 16. Each of the treatments were replicated three times. Bait applications of Extinguish Plus were made on Oct 19 with a Herd Seeder at a rate of 1.5 lb product per acre. Spray applications were made on Oct 26 using a GPS enabled sprayer calibrated to 40 GPA and set to prevent overlaps in the field, so no application was made out of the plots and no areas overlapped. Granular applications were made the following day using a Vicon pendulum spreader. The first data collection post-treatment was on Oct 30 (3 DAT). RIFA populations (presence or absence of live mounds; number per 1/4 acre evaluation area) were evaluated pre-treatment and at approximately 2-3 days, 1, 2, 3, 4 weeks post-treatment. Mound number data per ¼-acre plot collected to date are presented in graph and table below. The six week data collection (due 12/6/17) was delayed one week due to cold rainy weather and will be collected the week of 12/11/17.

Results to date:

Data have not been analyzed. All three treatments have reduced the number of live mounds in the treated plots while numbers of mounds in the control plots have increased

Mean number of mounds found per ¼-acre plot

	Pretrt	**10/30/17**	**11/2/17**	**11/9/17**	**11/16/17**	**11/21/17**
Bait+Taurus	17.67	8.00	8.33	6.00	4.00	2.67
Bait+Bifen/Sevin	26.67	12.67	8.33	3.67	3.67	1.67
Bait+Aloft	26.67	20.00	15.67	6.33	8.67	5.33
Control	18.33	17.67	26.00	17.33	29.33	28.00



Evaluation of Imported Fire Ant Quarantine Treatments in Commercial Grass Sod: Arkansas 2015

PIs – Kelly Loftin and John Hopkins, University of Arkansas

Introduction

Imported fire ants (IFA) originated from South America and were accidentally introduced into the United States in the early to mid 1900s. IFAs 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 (USDA-APHIS 2006). One option was treatment using the active ingredient chlorpyrifos at a rate of eight pounds of active ingredient per acre. Currently, no chlorpyrifos products are registered for IFA in sod at that required rate. Another 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 longer required exposure period can be a logistical problem for sod producers. One newly approved quarantine option is two applications of 0.2 lb ai/acre bifenthrin, one week apart, for a total of 0.4 lb ai/acre. This option is less costly than fipronil and has a shorter exposure period than fipronil.

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. Using fire ant bait as the first application, followed by 0.2 lb.ai/acre of bifenthrin or the combination of 0.17 lb.ai/acre bifenthrin and 0.35 lb.ai/acre clothianidin has shown much promise as a quarantine treatment. This study is an evaluation on another combination with fire ant bait and a bifenthrin/carbaryl combination. All of these options, if effective, will allow a treatment with lower costs to the grower than the current fipronil treatment or the 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 late July 2015 and ending in late September 2015. Plots were square, measured ½ acre in area, and treatments (four 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 16-84 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. In plots receiving two treatments (bait*/application timing #1* followed by carbaryl/bifenthrin combinations*/application timing #2*), application timing #1 and application timing #2 were separated by six days. In plots receiving only the carbaryl combinations, treatments were applied at application timing #2.
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 a Herd fire ant spreader attached to a Kawasaki Mule ATV and calibrated to apply 1.5 pounds per acre. Granular bifenthrin/carbaryl (Duocide™) applications were made

using a tow-type granular applicator (Agri-Fab) towed by a Yamaha ATV and calibrated to apply 348 pounds per acre. Treatment numbers, insecticide rates and the total amount of active ingredients applied per acre are provided in Table 1.

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 Siesta® 0.063% bait (1.5 lb./A) and 1 application of Duocide™ 2.358% G 348lb/acre applied 6 days after bait	0.000945 lb ai/A metaflumizone 0.2 lb ai/A bifenthrin 8.0 lb ai/A carbaryl
3	1 application of Duocide™ 2.358% G 348lb/acre	0.20 lb ai/A bifenthrin 8.0 lb ai/A carbaryl
4	1 application Siesta® 0.063% bait (1.5 lb./A) and 1 application of tank mix Onyx Pro at 13.9 oz./A and Sevin SL at 128 fl oz/A applied 6 days after bait	0.000945 lb ai/A metaflumizone 0.2 lb ai/A bifenthrin 4.0 lb ai/A carbaryl
5	1 application of tank mix Onyx Pro at 13.9 oz./A and Sevin SL at 128 fl oz/A applied 6 days after bait	0.2 lb ai/A bifenthrin 4.0 lb ai/A carbaryl

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 were 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 was 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 used in the study. Throughout the remainder of the study, all insecticide treated plots had significantly ($p<0.05$) fewer active IFA colonies compared to the untreated control. At seven through 21 DALA, the Siesta bait plus bifenthrin/carbaryl tank mix treated plots had zero active mounds per acre. The

Duocide-only treated plots had no active mounds at 7 DALA, however by 14 DALA, an active mound was detected in one of the plots. Other treatments achieved zero colonies per acre later on in the study i.e. 14 and 21 DALA. Three treatments that achieved zero colonies per acre for three consecutive weeks were the Siesta bait plus the bifenthrin/carbaryl liquid tank mix, the bifenthrin/carbaryl only liquid tank mix and the Duocide only treatments. Untreated controls maintained reasonable fire ant activity all summer, probably due to routine irrigation of the test area.

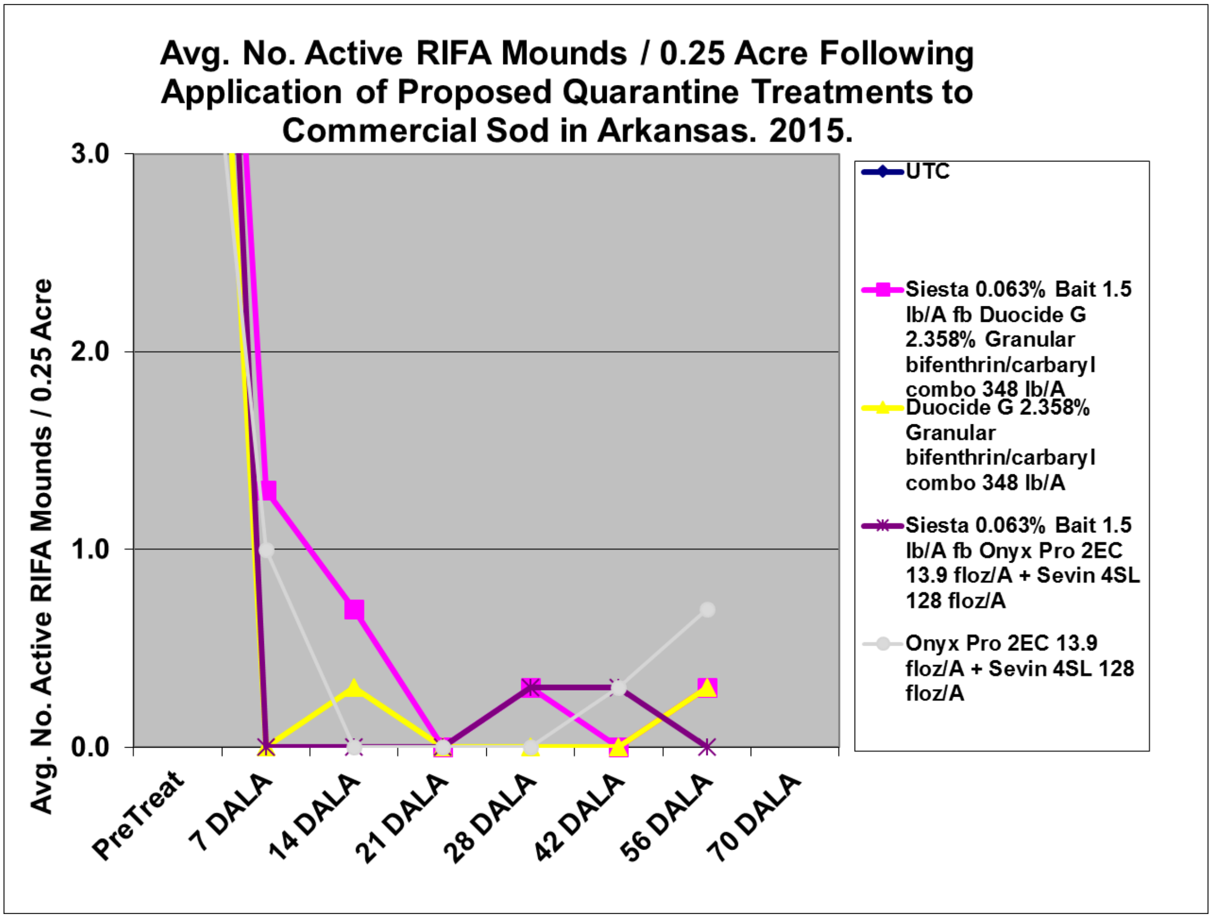
All insecticide treatments significantly reduced the number IFA colonies in treated plots. However, the duration of control (zero colonies per acre) was less than desired for quarantine treatment of commercial grass sod. Any of these options would likely be suitable for control in home lawns, parks or recreational areas but did not perform as well as some of the previously tested bait plus contact insecticides mixes e.g. bifenthrin/clothianidin mixture.

Table 2. Average Number of Active Mounds/0.25 acres for each treatment.

Treatment	**Average number of active colonies per 0.25 acre**						
	Pre Treat	**7 DALA**	**14 DALA**	**21 DALA**	**28 DALA**	**42 DALA**	**56 DALA**
UTC	11.7a	10.7a	6.0a	10.7a	7.0a	8.0a	10.7a
Siesta® 0.063% bait (1.5 lb./A) followed by Duocide™ 2.358% G 348lb/acre	8.7a	1.3b	0.7b	0.0b	0.3b	0.0b	0.3b
Duocide ™ 2.358% G 348lb /acre	7.7a	0.3b	0.3b	0.0b	0.0b	0.0b	0.3b
Siesta® 0.063% bait (1.5 lb./A) followed by tank mix - Onyx Pro at 13.9 oz./A and Sevin SL at 128 fl oz/A	9.7a	0.0b	0.0b	0.0b	0.3b	0.3b	0.0b
tank mix - Onyx Pro at 13.9 oz./A and Sevin SL at 128 fl oz/A	5.3a	1.0b	0.0b	0.0b	0.0b	0.3b	0.7b

Means followed by same letter do not significantly differ (P=.05, protected LSD)

Fig. 1. Average Number of Active Mounds/0.25 Acres for each treatment.



References

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Evaluation of Imported Fire Ant Quarantine Treatments in Commercial Grass Sod: Arkansas 2016

PIs – Kelly Loftin and John Hopkins, University of Arkansas

Introduction

Imported fire ants (IFA) originated from South America and were accidentally introduced into the United States in the early to mid-1900s. IFAs 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 (USDA-APHIS 2006). One option was treatment using the active ingredient chlorpyrifos at a rate of eight pounds of active ingredient per acre. Currently, no chlorpyrifos products are registered for IFA in sod at that required rate. Another 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 longer required exposure period (30 days) can be a logistical problem for sod producers. One recently approved quarantine option is two applications of 0.2 lb ai/acre bifenthrin, one week apart, for a total of 0.4 lb ai/acre. This option is less costly than fipronil and has a slightly shorter exposure period (28 days) than fipronil.

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. Using fire ant bait as the first application, followed by 0.2 lb.ai/acre of bifenthrin or the combination of 0.17 lb.ai/acre bifenthrin and 0.35 lb.ai/acre clothianidin has shown much promise as a quarantine treatment. The current study is to evaluate another commercial combination product – Taurus® Trio G (fipronil, bifenthrin and lambda cyhalothrin) with and without a prior (7 days) fire ant bait application with Advion. In addition, a tank mix of bifenthrin and carbaryl with a prior Advion fire ant bait application was evaluated.

Materials and Methods

The study was conducted on an irrigated sod farm in Fulton, AR (Hempstead Co.) beginning in late July 2016 and ending in October 2016. Plots were square, measuring 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 16-40 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. In plots receiving two treatments (bait*/application timing #1* followed by the carbaryl/bifenthrin combination or Taurus® Trio G*/application timing #2*), application timing #1 and application timing #2 were separated by seven days. In plots receiving only the Taurus® Trio G product, treatments were applied at application timing #2.

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 a Herd fire ant spreader attached to a Kawasaki Mule ATV and calibrated to apply 1.5 pounds per acre. Granular fipronil/bifenthrin/lambda cyhalothrin

(Taurus® Trio G) applications were made using a tow-type granular applicator (Agri-Fab) towed by a Yamaha ATV and calibrated to apply 87 pounds per acre. Treatment numbers, insecticide rates and the total amount of active ingredients applied per acre are provided in Table 1.

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® 0.045% bait (1.5 lb./A) and 1 application of Taurus® Trio G at 87 lb/acre applied 7 days after bait	0.000675 lb ai/A indoxacarb 0.0124 lb ai/A fipronil 0.2 lb ai/A bifenthrin 0.051 lb ai/A lambda cyhalothrin
3	1 application of Taurus® Trio G at 87 lb/acre	0.0124 lb ai/A fipronil 0.2 lb ai/A bifenthrin 0.051 lb ai/A lambda cyhalothrin
4	1 application Advion® 0.045% bait (1.5 lb./A) and 1 application of tank mix Onyx Pro at 13.9 oz./A and Sevin SL at 128 fl oz/A applied 7 days after bait	0.000675 lb ai/A indoxacarb 0.2 lb ai/A bifenthrin 4.0 lb ai/A carbaryl

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 were 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 was 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, through early October.

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 used in the study. Throughout the remainder of the study, all insecticide treated plots had significantly ($p<0.05$) fewer active IFA colonies compared to the untreated control. At eight through 42 DALA, the Advion® bait plus Taurus® Trio G treated plots had zero active mounds per acre. The Taurus®

Trio G-only treated plots had no active mounds at 14 through 56 DALA. For the Advion® fire ant bait followed by the bifenthrin/carbaryl tank mix treated plots, no fire ant colonies were found at the eight through 21 DALA evaluations. Fire ant colonies returned to the plots in the Advion® plus Taurus® Trio G and Taurus® Trio G-only plots at 56 and 70 DALA, respectively. Although the Advion® plus bifenthrin/carbaryl treated plots had significantly fewer active colonies than the untreated control, eight through 21 DALA were the only evaluation periods with no active colonies. Untreated controls maintained reasonable fire ant activity all summer, probably due to routine irrigation of the test area.

All insecticide treatments significantly reduced the number IFA colonies in treated plots throughout the study duration and are suitable for control in home lawns, parks or recreational areas. The Advion® plus Taurus® Trio G and the Taurus® Trio G treatments provided six and seven weeks of quarantine-level control, respectively (quarantine level control is defined as no active colonies). Data indicated that the addition of Advion® fire ant bait reduced the exposure period by one week. This study indicated that the Taurus® Trio G treatment demonstrates the level of control necessary for shorter-term (six weeks) fire ant quarantine treatment of grass sod.

Table 2. Average Number of Active Mounds/0.25 acres for each treatment.

Treatment	**Average number of active colonies per 0.25 acre**								
	Pre Treat	**8 DALA**	**14 DALA**	**21 DALA**	**28 DALA**	**42 DALA**	**56 DALA**	**70 DALA**	**84 DALA**
UTC	7.3a	6.3a	5.7a	8.0a	7.7a	5.0a	5.7a	9.7a	10.3a
Advion® 0.045% bait (1.5 lb./A) followed by Taurus® Trio G at 87 lb/acre	7.3a	0.0b	0.0b	0.0b	0.0b	0.0b	0.3b	0.3b	0.3b
Taurus® Trio G at 87 lb/acre	6.0a	0.3b	0.0b	0.0b	0.0b	0.0b	0.0b	0.3b	0.0b
Advion® 0.045% bait (1.5 lb./A) followed by tank mix of Onyx Pro at 13.9 oz./A and Sevin SL at 128 fl oz/A applied 7 days after bait	5.7a	0.0b	0.0b	0.0b	0.7b	0.3b	1.7b	1.0b	1.3b

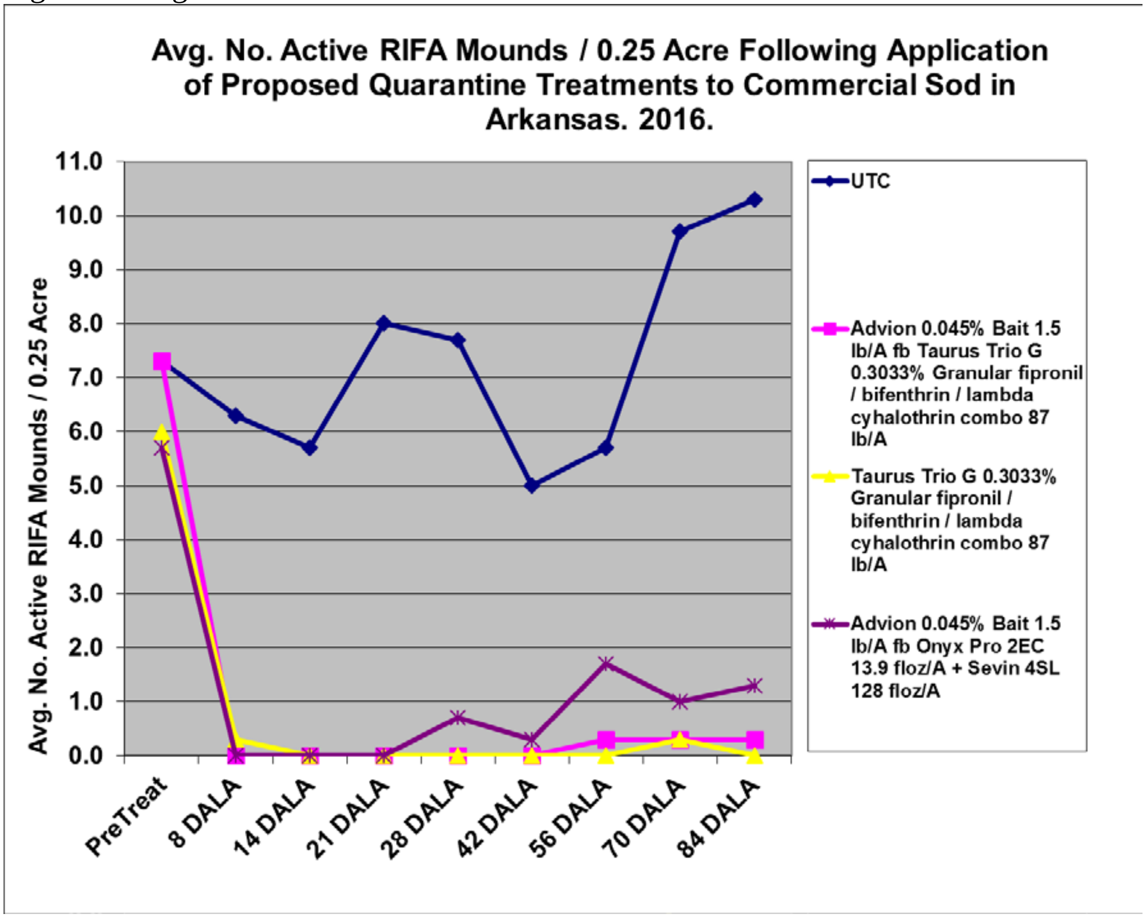
Means followed by same letter do not significantly differ (P=.05, protected LSD)

Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Data for Average Number of Active Mounds per Plot at 84 DALA failed Bartlett's test for homogeneity thus violating the AOV assumption of homogeneity of variance.

The Arcsine Square Root Percent Transformation was applied to this data and AOV was performed. Data reported has been detransformed.

Fig. 1. Average Number of Active Mounds/0.25 Acres for each treatment.



References

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Evaluation of Imported Fire Ant Quarantine Treatments in Commercial Grass Sod: Arkansas 2017

PIs – Kelly Loftin and John Hopkins, University of Arkansas
Progress Report

Introduction

Imported fire ants (IFA) originated from South America and were accidentally introduced into the United States in the early to mid 1900s. IFAs 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 (USDA-APHIS 2006). One option was treatment using the active ingredient chlorpyrifos at a rate of eight pounds of active ingredient per acre. Currently, no chlorpyrifos products are registered for IFA in sod at that required rate. Another 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 longer required exposure period can be a logistical problem for sod producers. One recently approved quarantine option is two applications of 0.2 lb ai/acre bifenthrin, one week apart, for a total of 0.4 lb ai/acre. This option is less costly than fipronil and has a shorter exposure period than fipronil. 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.

Materials and Methods

The study was initiated on an irrigated sod farm in Fulton, AR (Hempstead Co.) in mid-September 2017 and additional evaluations are planned. 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 22 to 42 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.

Granular bait applications were made using a Herd fire ant spreader attached to a Kawasaki Mule and calibrated to apply 1.5 pounds per acre. Spray applications were made using a towed boom sprayer applying @ 20 gal/A. Granular fipronil/bifenthrin/lambda cyhalothrin (Taurus® Trio G) applications were made using a tow-type granular applicator (Agri-Fab) towed by a Kawasaki Mule and calibrated to apply 87 pounds per acre. Treatment numbers, insecticide rates and the total amount of active ingredients applied per acre are provided in Table 1.

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® 0.045% bait (1.5 lb./A) and 1 application of Taurus® Trio G at 87 lb/acre applied 7 days after bait	0.000675 lb ai/A indoxacarb 0.0124 lb ai/A fipronil 0.2 lb ai/A bifenthrin 0.051 lb ai/A lambda cyhalothrin
3	1 application Advion® bait (1.5 lb./A) 1 application of Aloft GS SC (3.32 SC) (14.4 oz./A) applied 8 days later	0.000675 lb ai/A indoxacarb 0.12 lb ai/A bifenthrin 0.24 lb ai/A clothianidin
4	1 application Advion® 0.045% bait (1.5 lb./A) and 1 application of tank mix Onyx Pro at 13.9 oz./A and Sevin SL at 128 fl oz/A applied 7 days after bait	0.000675 lb ai/A indoxacarb 0.2 lb ai/A bifenthrin 4.0 lb ai/A carbaryl

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 were 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 was 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 54 DALA. Additional evaluations will be made after the 54 DALA evaluation.

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

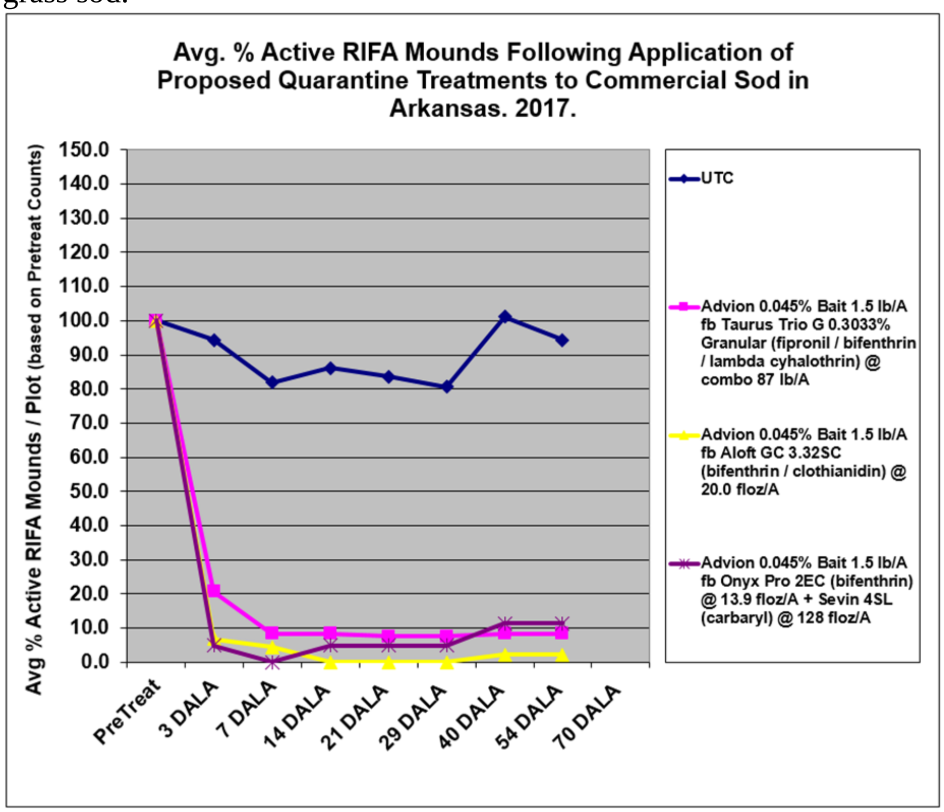
All plots with insecticide treatments had significantly fewer RIFA colonies than the untreated controls (Fig. 1 and Table 1) by 3 days after the last application (DALA) through 54 DALA. Only two treatments resulted in no active colonies – Advion bait/ bifenthrin-carbaryl tank mix and the Advion/Aloft treatments. For the Advion bait/bifenthrin-carbaryl tank mix treatment, the 3 DALA evaluation was the only evaluation in which no active colonies were observed. The Advion bait/Aloft GC treatment had no active colonies present in any plots from 14 -28 DALA.

Table 1. Efficacy of various insecticide treatment combinations against RIFA in commercial grass sod.

Treatment	Avg. % Active Mounds / Plot Pretreat		Avg. % Active Mounds / Plot 3 DALA		Avg. % Active Mounds / Plot 7 DALA		Avg. % Active Mounds / Plot 14 DALA		Avg. % Active Mounds / Plot 21 DALA		Avg. % Active Mounds / Plot 29 DALA		Avg. % Active Mounds / Plot 42 DALA		Avg. % Active Mounds / Plot 54 DALA		Avg. % Active Mounds / Plot 70 DALA	
	PreTreat		3 DALA		7 DALA		14 DALA		21 DALA		29 DALA		40 DALA		54 DALA		70 DALA	
UTC	100.0	a	94.4	a	81.9	a	86.1	a	83.6	a	80.6	a	101.1	a	94.4	a		
Advion 0.045% Bait 1.5 lb/A fb Taurus Trio G 0.3033% Granular (fipronil / bifenthrin / lambda cyhalothrin) @ combo 87 lb/A	100.0	a	20.6	b	8.3	b	8.3	b	7.5	b	7.5	b	8.3	b	8.3	b		
Advion 0.045% Bait 1.5 lb/A fb Aloft GC 3.32SC (bifenthrin / clothianidin) @ 20.0 floz/A	100.0	a	6.7	b	4.4	b	0.0	b	0.0	b	0.0	b	2.2	b	2.2	b		
Advion 0.045% Bait 1.5 lb/A fb Onyx Pro 2EC (bifenthrin) @ 13.9 floz/A + Sevin 4SL (carbaryl) @ 128 floz/A	100.0	a	4.8	b	0.0	b	4.8	b	4.8	b	4.8	b	11.4	b	11.4	b		

Means followed by same letter do not significantly differ (P=.05, LSD)
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Figure 1. Efficacy of various insecticide treatment combinations against RIFA in commercial grass sod.



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Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing and Release Project, 2015-2017 (*Pseudacteon tricuspis, P. curvatus, P. obtusus* and *P. cultellatus*)

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George Schneider and staff at FL DPI,
State Departments of Agriculture and their designees

SUMMARY:

The APHIS phorid fly rearing and release project is a great success. Since 2002, two to four species of *Pseudacteon* sp. flies have been released through this project at multiple sites in all imported fire ant quarantined states in the contiguous southeastern states, California and Puerto Rico (no releases in NM). From 2002 through 2017 there have been field releases at 176 sites in IFA quarantined states in the contiguous southeastern states, California and Puerto Rico and over 2.3 million potential flies released or used in demonstration/research projects. At these 176 sites, 146 sites received 1 species (67 *P. tricuspis*, 47 *P. curvatus*, 27 *P. obtusus*, and 5 *P. cultellatus*), while 2-3 species were released at 5 sites in 2013, 8 sites in 2014, 5 sites in 2015, and 6 sites in 2016 and 2017. Other federal agencies and universities have also been very active in releasing these 4 species as well as 2 additional species that were not suited to mass rearing. Through APHIS releases, along with other federal and university releases, *P. tricuspis* is well established in the southern areas of the IFA regulated area and is estimated to cover ca. 70% of the IFA regulated area, although populations have declined in recent years. To date, *P. tricuspis* is not known to be established in CA, NM, TN or VA. The second species, *P. curvatus*, is well established in most southern IFA regulated states and PR, and is estimated to cover more than 90% of the regulated area. Establishment of *P. obtusus* has occurred in small areas of GA and MS, and fairly large areas in FL and TX, and in 2014 it was established in CA. *P. obtusus* is estimated to cover about 25% of the regulated area. *P. cultellatus* has been confirmed in FL with limited expansion at this time. In 2017 this project came to its completion and the final releases were made in the summer of 2017.

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.

MATERIALS AND METHODS:

Preliminary research and rearing techniques have been developed by USDA, ARS for four species. 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.

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*, were labor intensive for the releaser. Originally, approximately 5,000-6,000 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 placed the head capsules in an enclosed emergence box and allowed the adult flies to emerge daily over 10-14 days. Adult flies were then aspirated into vials, carried to the field and released over IFA mounds. The mounds were disturbed frequently for 2 hours to insure worker ants were available on the soil surface for the flies to attack. One "release" encompassed 10-14 days of daily fly collection and release over mounds. This species is no longer being reared or released through this project.

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

Release techniques for the third and fourth fly species, *P. obtusus* and *P. cultellatus,* were originally utilizing a combination of the above techniques. *P. obtusus* parasitizes the largest of the worker ants, and many cooperators were having difficulty collecting enough large workers for a full release. Therefore, if the cooperator could not collect enough large workers, fly pupae (ant heads) were shipped to the cooperator as in the *P. tricuspis* release technique, and upon release of the adult flies, allowed the flies to find the large workers in the field. This decreased our average number of potential flies for each release. Therefore starting in 2011, if acceptable to the cooperator, IFA workers collected by cooperators were sized in the lab and parasitized by two or more fly species if appropriate. This allowed several states to get *P. obtusus, P. curvatus* and *P. cultellatus* in a single shipment, and did not "waste" ants that were collected. Most, if not all, releases in 2014-2017 were multiple species releases, primarily of *P. obtusus* and *P. cultellatus*. In 2017, as the project was coming to a close, the final pupae/head capsules from the rearing facility were sent out to various state cooperators for modified releases and pupae were "released" by distributing them around IFA mounds with the hope the adult flies would emerge and parasitize the local ants.

Monitoring the success of the fly releases was originally conducted 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.

<u>RESULTS</u>:

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 but completely eliminated by end of 2013)
- 2013 – *P. curvatus* general releases ceased (small population retained for demo and research purposes)
- 2014-2015 – *P. curvatus* released once each year in CA
- 2016 – *P. curvatus* rearing eliminated
- 2017 – the final releases of *P. obtusus* and *P. cultellatus* were made and the project concluded

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 and completely removed from production by the end of 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 subsequently decreased as first *P. obtusus* and then *P. cultellatus* production increased. Production of *P. curvatus* began to phase out in 2013, with anticipated elimination of rearing in 2015. We extended the production of this species into 2015 to allow releases in CA with a final elimination of *P. curvatus* in 2016. 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.

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 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 2017 there have been multiple releases in each of 13 states and Puerto Rico, with a total of 176 field releases and more than 1.6 million potential flies released (Table 1). At these 176 sites, 146 sites received 1 species (67 *P. tricuspis*, 47 *P. curvatus*, 27 *P. obtusus*, and 5 *P. cultellatus*) while 2-3 species were released at 5 sites in 2013, 8 sites in 2014, 5 sites in 2015, and 6 sites in 2016 and 2017 (Figure 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 2013, we had good cooperation and environmental conditions and releases were conducted at 10 release sites with 5 of the sites receiving multiple fly species. In 2014, 2 sets of releases were conducted in CA, utilizing all three species of flies currently in production.

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 700,000 potential flies have been shipped for these varied uses since 2002.

Distribution: 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 *tricuspis* sites (AL, AR, FL, GA, LA, MS, NC, PR, SC, TX) and 8 *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. However, in recent years, *P. tricuspis* populations have appeared to decline with sampling detecting primarily *P. curvatus* in many counties where *P. tricuspis* was originally found. LeBrun et al (2009) have found some evidence that *P. curvatus* can competitively displace *P. tricuspis* though the exact mechanism is uncertain. Callcott et al (2011) determined that *P. tricuspis* was most successful in the southern area of the U.S. IFA range with limited success in the cooler areas of the IFA range. To date, *P. tricuspis* is not known to be established in CA, NM, TN or VA, but is estimated to cover ca 70% of the IFA regulated area. The second species, *P. curvatus*, is also well established in all southern IFA regulated states except VA (AL, AR, FL, GA, LA, MS, NC, OK, SC, TN, TX, and PR), and has been more successful in establishing and spreading in all areas of the U.S. IFA range, covering about 95% of the IFA regulated area. Establishment of *P. obtusus* has occurred in small areas of GA and MS, and fairly large areas in FL and TX, and in 2014 it was established in CA. *P. obtusus* is estimated to cover about 25% of the regulated area. *P. cultellatus* has been confirmed in FL with limited expansion at this time.

In 2013, the Imported Fire Ant community of practice within the e-Xtension website began compiling information on phorid fly distribution from all sources. Using the data from Callcott et al (2011) as a starting point, they have continued adding data as federal and state cooperators, universities and others publish data or provide unpublished survey data, in an effort to keep fairly current maps of distribution of six species of phorid flies. These interactive maps are available at: http://articles.extension.org/pages/30546/natural-enemies-of-fire-ants. This site also provides information on some other potential biological control agents for IFA.

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Figure 1. Number of phorid fly releases per species per year. As of 2013, most release sites received 2-3 species per release date (see text).

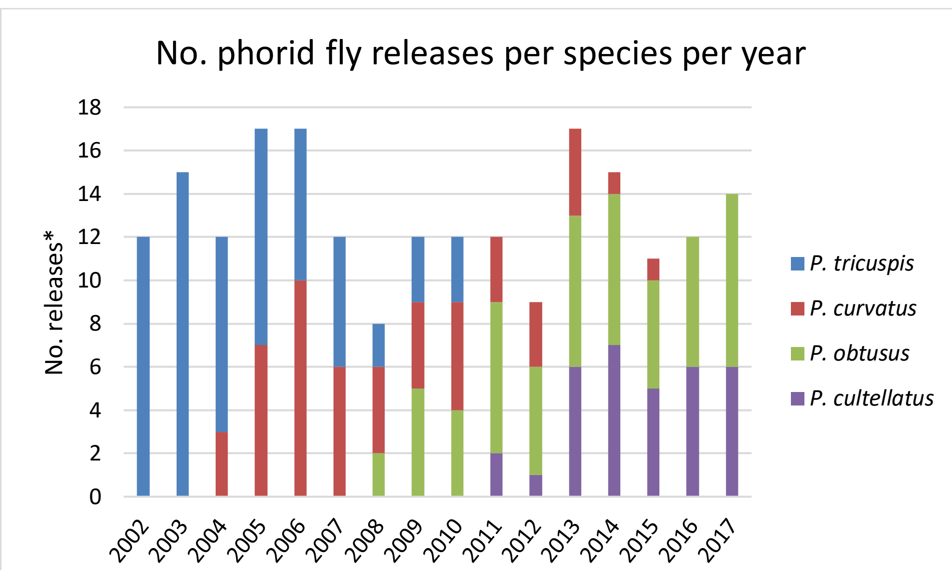

Table 1. Production and field release numbers for IFA-phorid fly program. Does not include flies shipped for research and demonstration projects.

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
tri, cur,obt,cul	2013#	3,182,354	180,645	10	18,064.50
cur,obt,cul	2014##	3,032,284	87,975	8	10,996.88
cur,obt,cul	2015##	3,229,199	76,458	6	12,743.00
cur,obt, cul	2016###	2,072,414	77,520	6	12,920.00
obt, cul	2017****	745,436	60,175	8	7,521.88
Total		40,431,692	1,621,438	176	9,462.47

* approx. no. potential flies shipped for release

** does not include multiple shipments to LA for initiating their own rearing facility and several universities for research purposes, nor multiple shipments to cooperators for educational purposes as flies were available

*** shipped for all purposes, field release, initiate rearing, education, etc.

† only tricuspis shipped in 2002

†† only tricuspis and curvatus shipped in 2006 and 2007

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

only curvatus, obtusus and cultellatus shipped in 2013 for releases; 5 sites with one species released; 5 sites with multiple species released

one site in CA where curvatus was release with obt; all other sites had obt & cul released

all releases obtusus & cultellatus mixed releases; curvatus to research only and production ceased Jun 2016

**** 6 releases obtusus & cultellatus mixed releases; 2 obtusus only releases; 3 releases traditional with parasitized ants; remaining 5 releases were shipped pupae scattered on mounds