Proceedings of the 2007 Annual Imported Fire Ant Conference

April 23-25, 2007

Gainesville, Florida, USA

Hosted by:

Imported Fire Ant and Household Insects Research Unit,
USDA-ARS, Center for Medical, Agricultural,
and Veterinary Entomology

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Imported Fire Ant and Household Insects Research Unit
U.S. Dept. Agriculture, Agricultural Research Service,
Center for Medical, Agricultural, and Veterinary Entomology
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Proceedings Disclaimers

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Copies of the Annual Imported Fire Ant Conference Proceedings are available as pdf files on the USDA, APHIS, CPHST, Soil Inhabiting Pest Section website.

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Greetings all fire ant customers and partners,

That was the salutation on the invitation letter that we sent out to prospective attendees to the 2007 Annual Red Imported Fire Ant Conference, held at the Best Western Gateway Grand April 23-25. Conference success depends on your attendance and participation and I am very pleased to tell you that we had over 175 registered attendees (including many international participants). By that measure the conference was a great success and illustrates the interest in fire ants at virtually every level. Our Keynote speaker, Dr. Walter Tschinkel (Florida State University), gave an excellent presentation that generated a lot of active discussion – his proceedings contribution should do the same. Dr. Rick Brenner (Assistant Administrator, Office of Technology Transfer) gave a very interesting historical perspective on ARS technology transfer as our after dinner speaker at the banquet. Increasing the breadth of the Conference was the Areawide Project Core member meeting and the workshop organized by Kathy Flanders for the Imported Fire Ant e-Xtension group. I am sure all of you are being kept up-to-date on the progress of this interesting and extensive project. We had many comments about the excellent job carried out by the Best Western Gateway Grand – the food, facilities, and hospitality were top of the line!

What was really exceptional were the 175+ participants, who created a great interactive atmosphere that promoted discussion and exchange of ideas that went far beyond the formal presentations. Maybe that's not unexpected; after all we are all working on a **social** insect. The other exceptional happening was the superb support the Conference received from Sponsors – check out the Sponsors page in these Proceedings. I want to send a personal THANKS to all mentioned above, and those who gave the opening remarks: CMAVE Director, Ken Linthicum; National Program Leaders, Dan Strickman and Herb Bolton; and Mary Chiles, staff member for Senator Bill Nelson. Lastly, a special thanks to the members of the Imported Fire Ant and Household Insects Research Unit who made everything happen!

Be on the look out for the 2008 Fire Ant Conference announcement to be hosted by Clemson University and Tim Davis in Charleston, SC!

Bob Vander Meer
Chairperson, 2007 Annual Imported Fire Ant Conference

Acknowledgements

The organizers were very grateful for the generous support of the 2007 IFA Conference provided by the following sponsors:

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We thank Elaine Connolly, Jorge Negron, and staff of the Best Western Gateway Grand for the excellent accommodations and service in meeting needs of the conference participants and organizers.

Organizers also wish to acknowledge the dedicated assistance of staff from the Imported Fire Ant and Household Insects Research Unit and other units in preparation of and during the conference, and with the proceedings publication: Kristy Simmons, Kathleen Smitherman, Peggy Zelonka, Michael Brooks, David Milne, Becky Blair, Euripides Mena, Chelsea Winikor, and Pamela Wheeler. In addition, the advice from the 2006 IFA Conference organizers Fudd Graham and Vicky Bertagnolli-Heller were most helpful.

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Keynote Address

Fire Ants and Native Ants: a Cautionary Tale

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The belief that fire ants competitively suppress populations of native and co-occurring ants has attained the level of religious dogma among fire ant researchers, and is the basis of two major approaches to fire ant management. However, until now, this belief has been based solely on non-experimental, correlational evidence, and has been fraught with several flaws of design and interpretation. Among the flaws are the use of relative abundance as evidence of suppression of native ants, and the failure to account for the effects of habitat disturbance. Careful reinterpretation of the literature suggested that habitat disturbance suppresses native ants, while at the same time and independently favoring fire ants. These two independent and opposite effects create a negative correlation between fire ants and native ants, a correlation that is not causal.

Joshua King and I have tested the effect of fire ants and disturbance on native ants in a large, landscape-scale experiment. Removal, using hot water, of fire ants from pasture had no effect on co-occurring ants, either by individual species or in the aggregate. There is thus no competition between fire ants and other ants. In our forest plots where we applied two levels of disturbance (mowing, plowing) in all possible combinations with transplanted fire ant colonies (and soil controls), our preliminary results show that whereas fire ants have little or no effect on native ants, disturbance (plowing) greatly reduces native ant abundance. All together, this suggests that habitat, not competition structures these ant communities.

The belief that fire ants are "the competitors from hell" is a major girder in the belief structure of many who study fire ants, and has now been shown to be false. Unfortunately, this is only one of several false beliefs that keep the hysteria about fire ants high. It is perhaps understandable that the pesticide industry has an interest in maintaining a high level of anti-fire-ant fervor among the public, because the industry is in the business of selling pesticides. However, it is appalling that so many researchers and managers at universities and in our government laboratories play such an active and willing role as handmaidens to industry, developing products, making false promises, and helping to maintain public hysteria. These university and government researchers and managers have failed in their duty to tell the public the plain truth about fire ants, a truth that would greatly reduce the emotional temperature (and possibly pesticide sales). The "fire ant-industrial-university-government complex" has become a vested interest block that is not serving the best interests of the public, but seems focused primarily on profit and funding.

Extension Fire Ant Web-site Launch at http://www.extension.org

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One of America's most important exotic insect pests has a new enemy—an online resource dedicated to providing information on the control and eradication of the imported fire ant. eXtension's Imported Fire Ants Web site puts a wealth of research-based information directly on consumers' computer screens. It's an excellent resource for anyone needing information about imported fire ants and how to control them. To take full advantage of the site, register at www.extension.org and choose Imported Fire Ants. This new tool was launched April 24-26 at the Annual Imported Fire Ant Conference in Gainesville, Fla. The eXtension Imported Fire Ants Web site features the following:

- Frequently Asked Questions allows users to submit queries about imported fire ants. If an answer is not already available in the FAQ section, the question is directed to Ask the Expert where local contacts provide requested information.
- Learning Sessions titled "Managing Imported Fire Ants in Urban Areas" and "Managing Imported Fire Ants in Cattle Production Systems" target unique situations facing homeowners and livestock producers.
- News & Upcoming Events keeps the news and calendar of events current at the local, state and national levels.
- Imported Fire Ant Management Decision Module, to be added soon, asks users a series of questions and then offers suggestions to help them decide what to do about fire ants in their urban landscapes or cattle operations.
- The eXtension Imported Fire Ants Web site has been developed through the collaboration of experts in entomology and pest management at land grant universities, federal, state, county, and municipal employees, and communications and information technology specialists, who formed a Community of Practice to develop a nationwide, Web-based site on imported fire ant management.

This site will be regularly maintained and kept current with new features and dates of events. For homeowners and producers needing fire ant information, this site will be a valuable resource.

eXtension is an educational partnership of more than 70 land-grant universities helping Americans improve their lives with access to timely, objective, research-based information and educational opportunities. eXtension's interactive Web site, at www.extension.org is customized with links to local Cooperative Extension Web sites. Land-grant universities were founded on the ideals that higher education should be accessible to all, that the university should teach liberal and practical subjects and share the university's knowledge with people throughout their states.

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Field trial of Fire Ant Bait Products for Controlling Fire Ants

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Introduction

Imported fire ants (*Solenopsis invicta* Buren) are a serious pest in urban environments, agriculture, and forestry. In recent years, the application of bait products has become a popular treatment option in fire ant management programs. Fire ant baits are designed to control imported fire ants. Compared to many non-bait products, fire ant baits are cost effective, safe, and easy to use. In fact, baits are the most effective way to control fire ant problems in most situations. However, bait products do require special conditions and handling for the best results. They are susceptible to UV light, heat, water, and chemical odors. Therefore, bait products are best when used fresh. Baits should not be used in contact with water or when fire ants are inactive due to either too hot or too cold. Nor should baits be applied prior to rain or irrigation.

Fire ant baits consist of three main components: 1) an insecticide that affects the ants; 2) an attractant that carries the insecticide; and 3) a medium that distributes the insecticide and the attractant. The insecticide can be an insect growth regulator, an organic toxicant, or a stomach insecticide which may be toxic on contact.

Two new active ingredients derived from the pyrazoline family have been registered as insecticides: metaflumizone and indoxacarb. Metaflumizone, developed by BASF, belongs to the chemical class of semicarbazone. Indoxacarb, developed by DuPont, is in the chemical class of oxadianizes. Both active ingredients are sodium channel blockers and are relatively physically and chemically stable. Their insecticidal activity occurs primarily after ingestion, rendering them suitable for bait.

In 2006, a field trial was conducted to evaluate the performance of the two chemicals in granular bait formulation against fire ant colonies. Control effectiveness was measured by declining speed of colony activity, foraging activity reduction, and the number of new colonies that re-invaded treated plots.

Materials and Methods

Siesta fire ant bait containing metaflumizone (0.063% a.i.) was provided by BASF (Research Triangle Park, NC, USA). Advion fire ant bait containing indoxacarb (0.04% a.i.) was provided by DuPont (Wilmington, DE, USA). Amdro® (0.073% hydramethylnon from BASF) was used as a standard treatment.

The field trial was conducted from October 2006 through January 2007 at E. V. Smith Research Center of Auburn University in central Alabama. The site had high density monogyne colonies of the imported red fire ant. Sixteen plots were selected at 15 m apart from each other. Although the sizes of the plots ranged from 100 to 350 m^2, each plot had at least 10 visible fire ant mounds. The fire ant mounds in each treatment were flagged and marked. The baits were broadcasted evenly using a hand-held spreader (Scotts® handy Green II, Cinnaminson NJ) at the label rate of 1.5 lbs/acre (=84.8 g/500 m^2) in the early morning of a clear day at 24^{o}C. The test design was a completely randomized, so each of the 3 treatments and the control had 4 replications.

Fire ant activities were monitored pre-treatment (1 day before the treatment) and post-treatment at 3, 7, 14, 30, 60, and 90 days at the time when temperature was above 22^{o}C and fire ants were active. Two monitoring methods (hotdog disk and metal probe) were used. The hotdog disk method was used to measure the level of fire ant foraging activity. A single hotdog disk (3-5 cm thick) was placed at the base of a fire ant mound and estimated fire ant activity rating was recorded 20 minutes later (0 = 1; 1 = 1-10; 2 = 11-20; 3 = 21-30; 4 > 31). The metal probe method was used to measure mound activity by inserting a metal probe (3-mm diameter and 2 feet long) carefully into the top of the fire ant mound (5 cm deep) and recording the presence of fire ants in 10 seconds (0 = no fire ant crawling up; 1 = fire ant crawling up).

At 90 day post-treatment, the presence of brood and queen was assessed by digging into each of the original mounds. The number of new fire ant mounds that appeared was recorded at each monitoring date.

Results and Discussion

Figure 1 shows the mean number of active mounds measured by the %metal probe method. Broadcasting Siesta and Advion fire ant baits resulted in >90% reduction of active mounds within 1 week, compared with <40% and 0% reduction in Amdro treatment and control, respectively. This result indicates that metaflumizone and indoxacarb in bait formulations were equally effective against fire ants and killed faster than hydramethylnon.

Figure 2 shows the mean estimated activity ratings measured by the hotdog disk method. Corresponding to mound deactivation, broadcasting Siesta and Advion fire ant baits also significantly suppressed fire ant foraging activity within 1 week post-treatment. The rate is faster than the Amdro application which gradually reached maximum suppression at about 60 days post-treatment.

At 3 days post-treatment, the two monitoring methods showed opposing measurement results. The hotdog disk method revealed lower estimated foraging activity while the metal probe method measured more active mounds in indoxacarb than in metaflumizone treated plots. Closer observation revealed that the foragers in indoxacarb treated plots had no propensity to locate or to feed on hotdog disks, indicating a somewhat restrained feeding/foraging behavior.

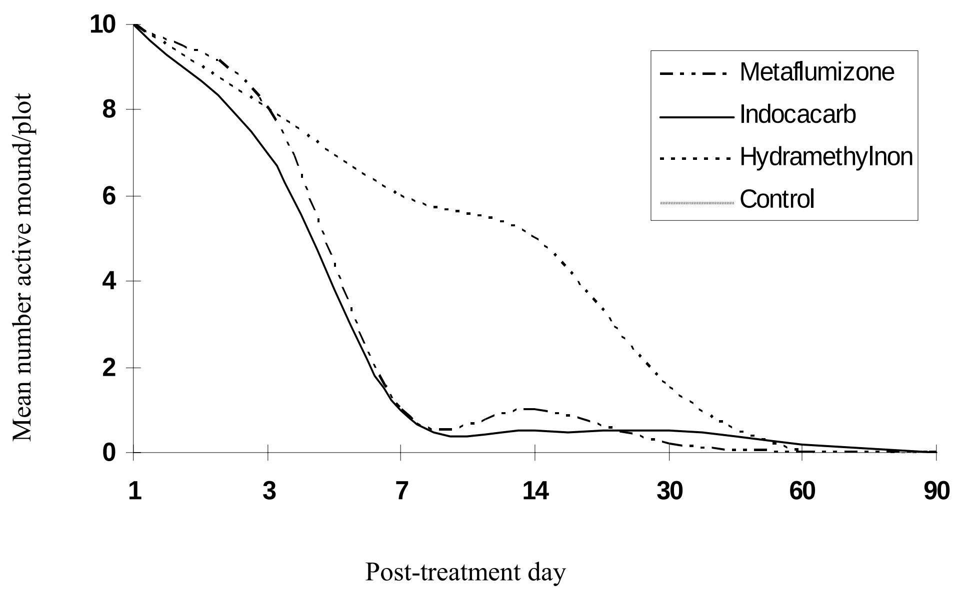

Figure 1. Fire ant mound activity in treated and control plots

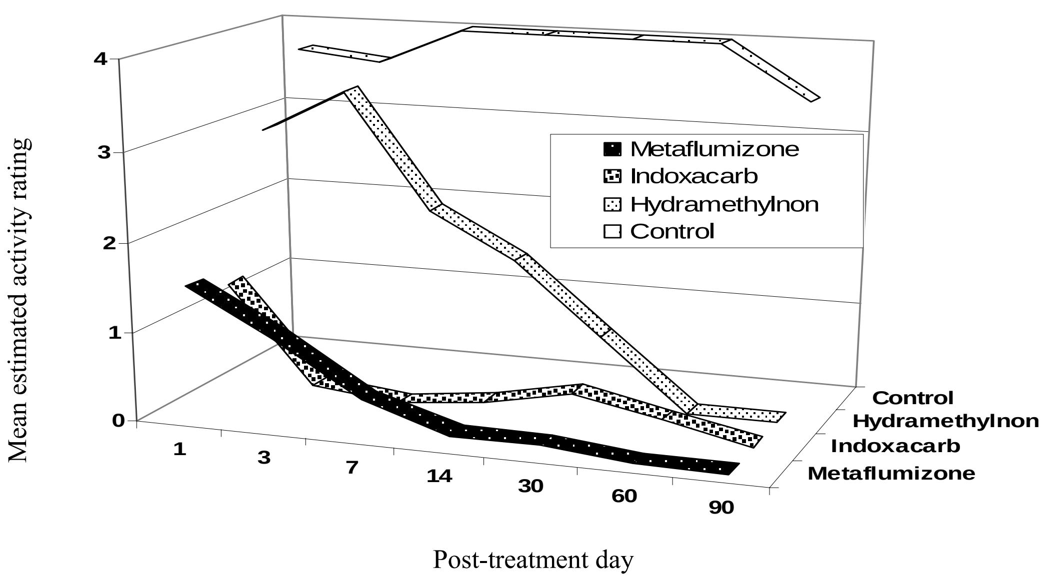

Figure 2. Estimated fire ant forager activity in treated and control plots

Figure 3 shows the mean number of new fire ant mounds that appeared. New fire ant colonies appeared later in October when the daily maximum temperature fell below 26^{o}C. Fewer new mounds appeared in plots treated with indoxacarb and metaflumizone baits, followed by plots treated with Amdro, while more new mounds appeared in control plots.

The mounds were examined 90 days post-treatment. No live queen or brood in any of the initial mounds treated with indoxacarb or metaflumizone were found. However, live larvae were found in 1 out of the 40 initial mounds in Amdro treated plots, but no live queen was observed. Live brood, queen, or both, were found in all the initial mounds in the control plot.

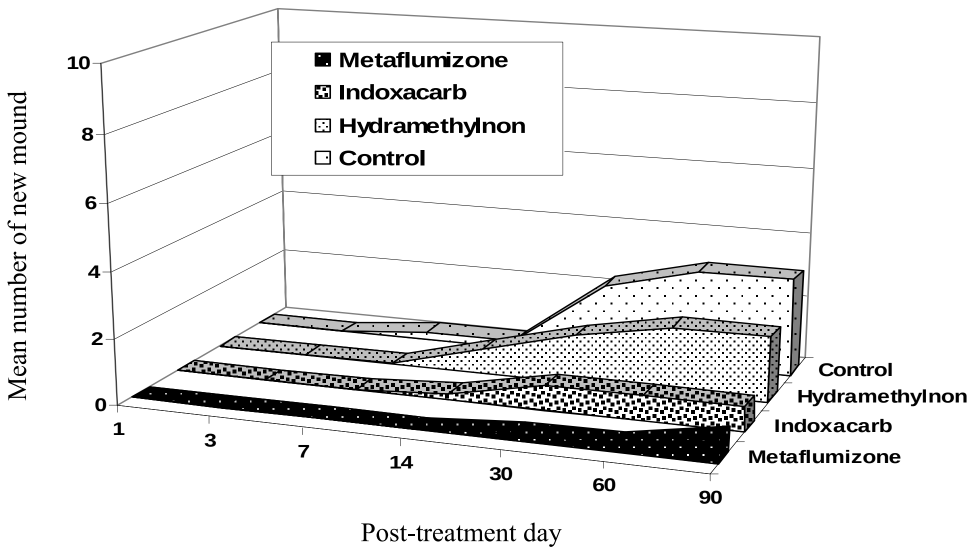

Figure 3. Re-invasion of fire ant colony in treated and control plots

The summer of 2006 was extremely dry and fire ant colonies remained underground without showing visible mounds. We were not able to start the field trial until late autumn when fire ant mounds began to appear. Considering there were 10 visible mounds in a plot about 100 m^2, the density of colonies in the test site was relatively high at that time. It was also possible that there were other colonies without visible mounds. Therefore, some of the new mounds may have originated from the existing underground colonies that did not show mounds at the time of treatment. Unfortunately, we did not monitor colony activity of these new mounds to determine if they were eventually affected by the treatment.

In summary, this study indicates that broadcasting the indoxacarb and metaflumizone baits can equally provide a quicker control of fire ant colonies than Amdro bait, and the control effect could last for at least 90 days.

Acknowledgement

We thank DuPont and BASF for providing product samples and support, Shizhu Li and E.V. Smith Research Center of Auburn University for assisting in data collection and maintaining the test site. We also thank Jon Andrew Isom and Lisha Li of Samford University for reviewing previous versions of this manuscript.

Best Management Practices (BMP) against Imported Fire Ants (IFA) in agricultural and forestry commodities known to harbor IFA

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Agricultural and forestry products such as baled hay used for bedding or forage, honey bees for pollination, and pine straw for landscaping can not be treated directly with existing chemicals and application labels. The consumptive and human handling issues associated with these non-horticultural commodities precludes most direct chemical applications. Fumigation may be possible; however a restriction on methyl bromide use and the cost of fumigation makes this option too limiting and expensive for these commodities. To combat IFA in these commodities, USDA is developing Best Management Practices (BMP) that focus on IFA control at both the field and commodity. Some components of a BMP may include; 1) field and commodity sanitation, 2) broadcast and targeted insecticide use, and 3) commodity surveillance. Sanitation at both the field and commodity level is useful for reducing the attractiveness of the commodity and production site to IFA infestation. Broadcast application of insecticides to fields or storage sites is useful for reducing IFA populations in half-acre to larger field areas. Chemical applications at the commodity level may include strategic placement of insecticides around sensitive commodities or onto supporting materials with few caveats. Insecticide delivery systems such as individual bait stations may be placed on the shipment or commodity while ensuring food or feed safety. Surveillance for IFA during commodity transportation is valuable in assessing pre-shipment control practices and providing an early warning to shippers prior to entering non-infested regions. Best Management Practices provide field producers a predictable window of opportunity to move clean products from quarantined areas (Table 1).

Table 1. Window of opportunity for moving IFA free hay is shown in gray cells. Percent of hay bales infested by imported fire ants in treatments during each of the 7 weeks of the study (n = 20, with four replicates in each treatment). Barrier applications comprised 1.0 m wide barrier of permethrin applied around hay bales. Area applications comprised a 3.5 m2 treated area with permethrin and a 3.5 m2 cloth cover between the hay and treated surface. Permethrin was applied at the labeled rate of 0.0084 lb A.I./gallon of water.

Treatment	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7
Strip Barrier							
Full-rate	0	0	0	0	0	25	25
Area							
Full-rate	0	0	0	0	0	25	0
Control	0	25	100	50	75	75	100

Comparison of Retail Market Broadcast Fire Ant Control Products

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Introduction

The retail market for fire ant (*Solenopsis invicta* Buren) control products has expanded in recent years with the introduction of such products as Amdro Yard Treatment®, Once and Done® and Over 'N Out®. Most of these products are variations of professional products that are applied at consumer-friendly rates of over 20 lbs. per acre and make some claim of "season long" or some months of control. Even the term "control" can be interpreted as anything from "no ants" to complete re-invasion depending on the claimant's tolerance level. Strictly speaking, the EPA defines "control" as 90% or greater reduction in active mound numbers for 30 days versus an untreated control. This trial was conducted to collect replicated field data on several of the more popular products to determine their speed and duration of control. The name of the sponsoring company and brand names of the products used were withheld at the request of the sponsor.

Materials and Methods

The trial was conducted at the Fayette Regional Airport (LaGrange) in central Texas. Soil was a sandy loam. Vegetation was native grasses mowed two to three times per year. Within 20 feet of the runway, however, mowing occurred at least every week in the warmer months, making it comparable to managed turf in density and appearance.

A pre-count evaluation was conducted on June 9, 2006. This and subsequent evaluations were conducted using the minimal disturbance technique in which mounds were lightly disturbed with a pointed tool handle. A mound was considered "active" when a sufficient number of ants were seen at the surface, given the day's weather conditions. Mound numbers were arrayed from highest to lowest and divided into four equal groups (replications). Treatments were assigned within replications so that the total number of mounds per treatment were as equal as possible.

Plots were 200 x 55 feet (0.25 acres) rectangles, one edge adjacent to the runway pavement, with the runway lights used as plot end markers. Sample areas extended from the pavement out 20 feet. They began and ended 10 feet from the runway lights (3600 ft^2), providing 10-foot treated buffers on both ends and 35 feet on the long, outer edge.

Treatments included both broadcast baits and broadcast contact insecticides and are listed in **Table 1.** Treatments were applied on June 16 using a PlantMates spreader with a shaking gate agitator. Granular treatments were applied with an Earth-Way spreader with a T-bar agitator. Both spreaders were of the "belly-bumper" hand-cranked type. Products were irrigated appropriately as described in the Results and Discussion section.

Evaluations were made every three days for the first two weeks, then at gradually increasing intervals through six months post-treatment. Data were analyzed using SAS analysis of variance procedures with means separated using Tukey's Studentized Range (HSD) Test, $P < 0.05$.

Results and Discussion

One of the concerns of this test was how to equalize the initial performance of the diverse products. Indoxacarb bait was expected to provide its maximum period of ant mortality between day two and three. Two granular pyrethroid products, were expected to cause ant mortality beginning within 24 hours, but *only if* they were irrigated. Conversely, the baits would be ruined by any irrigation within the first few hours.

Table 1. Treatments (and abbreviations) applied to LaGrange Airport June 16, 2006.

Active Ingredient	Product Type	Application Rate
untreated	-	-
0.36% hydramethylnon	Broadcast bait	20 lbs./plot
0.016% indoxacarb	Broadcast bait	5 lbs/plot
0.0103% fipronil	Broadcast granular	20 lbs/plot
0.1% lambda-cyhalothrin	Broadcast granular	23 lbs/plot
0.2% bifenthrin	Broadcast granular	20 lbs/plot

To address this concern, a spray rig including a 500 gallon tank with a gasoline-powered pump was brought to the site the day of application to irrigate the granular treatments. In a stroke of remarkable luck, strong thunderstorms were moving across the area that day. Granular treatments were applied first, beginning about 9:00 a.m.. As the last plot was being applied, heavy rain began moving up the runway and persisted, off and on, for a few hours. It is estimated that between 0.25 and 0.5 inches of rain were received, thoroughly irrigating all the granular-treated plots. By 3:00 p.m., the thunderstorms had moved through the area and the vegetation had dried. Broadcast baits were then applied and the area remained rain-free for several days.

Table 2 shows the results of the trial. One of the most unusual aspects of the data is that the number of untreated mounds never dropped below pre-count levels. This is quite unusual considering the summer-spanning duration of the test. Active mound numbers often drop by 50% or more during a Texas summer, but this site received very well-timed rains and held mound structures well, despite frequent mowing. Each product will be discussed as they appear in the table. **Figure 2** shows data from the first month of the test.

Hydramethylnon bait began to show significant mound suppression by Day 2 and reached a maximum by Day 12, continuing through Day 28. Reinfestation occurred rapidly over the second month. Initial activity was unusually rapid for a hydramethylnon bait, which usually takes about a month to reach maximum suppression. Reinfestation was also quite rapid considering the second month of the test was mid-summer, which is usually a period of very poor mound formation.

Indoxacarb bait showed slightly faster than expected mound reduction beginning on Day 2 and dropping to zero, or 100% control, by Day 6. This level of control lasted through Day 28, then a slow reinfestation began which lasted through test conclusion.

Fipronil, though a granular contact insecticide, exhibited a typical initial control profile like that of a hydramethylnon bait - maximum control over the course of a month. It reached 100% control at the 28 day evaluation then leveled out at about 90% control for the duration of the test. This low level infestation is characteristic of granular fipronil. The product has no repellent effects, but does have extremely long, true chemical residual activity. As new colonies migrate into treated areas, they are affected by the residual toxicant and take two to four weeks to die, as directly-treated colonies would.

Lambda-cyhalothrin is a more traditional, fast-acting granular contact insecticide. After its thorough irrigation, maximum control was more-or-less achieved by the first evaluation at Day 2. This roughly 85% control persisted until the 60 day evaluation when mound numbers increased dramatically in a pattern similar to that of hydramethylnon.

Granular bifenthrin reached maximum control in six days. This roughly 95% control persisted until the 60 day evaluation where it showed a similar jump in mound numbers. Statistically, however, bifenthrin maintained significantly lower mound numbers than untreated up to the 90 day evaluation.

The sudden jump in mound numbers in mid-August experienced by one of the baits and the two fast-acting contact insecticides is a very interesting phenomenon. To construct a plausible explanation, one must look at the other treatments. **Figure 2** illustrates data from Day 28 - 180. The 60 day evaluation was the *low point* of post-treatment untreated mound numbers. Though a relatively minor dip for a Texas summer, it is representative. Conversely, hydramethylnon, lambda-cyhalothrin and bifenthrin treatments showed a three to ten-fold *increase* in mound numbers, giving hydramethylnon and lambda-cyhalothrin no statistical difference from the untreated control.

Indoxacarb bait, on the other hand, showed a much lower increase in mound numbers. No bait has any true (chemical) residual activity, so indoxacarb's increase from zero to 3.5 mounds per plot could be considered the natural reinfestation rate for this site during this period.

These comparisons suggest, though by no means prove, that some colonies may not have been completely killed by hydramethylnon, lambda-cyhalothrin and bifenthrin. Some hydramethylnon-treated colonies may have suffered considerable worker mortality, but queen survival. The colonies then took two months to regain a visible size. It is also possible that worker mortality was incomplete, they rebuilt mounds, then died back due to natural mortality to the same level as indoxacarb plots - the natural reinfestation rate.

Some lambda-cyhalothrin and bifenthrin-treated colonies may have also suffered worker mortality and either taken two months to grow to visible size or may have lain underneath the toxic pesticide layer until it "broke" in the heat and the ants could safely rebuild on the surface.

In summary, indoxacarb bait and granular fipronil performed much as expected. Hydramethylnon, lambda-cyhalothrin and bifenthrin worked as fast as or faster than normal, but reinfestation occurred much more quickly than expected. Keep in mind that this was one test during one summer at one site, but it illustrates how product performance can vary. Though individual tolerance levels vary tremendously, 20 - 55% control at two months is unlikely to impress customers when other, similarly priced products maintained control of 79 -95%. Reasons for poor long-term performance could range from soil type to that summer's rainfall, but it should be a warning against broad statements of control duration.

Table 2. Mean number of active mounds per sample area, 4 replications. LaGrange Airport. Test applied June 16, 2006.

	Mean number of active mounds per sample area, 4 replications													
Treat.	Pre	Day 2	Day 3	Day 6	Day 9	Day 12	Day 15	Day 21	Day 28	Day 60	Day 90	Day 123	Day 150	Day 180
Untrt.	15.25a	20.25a	22.25a	20.50a	17.25a	18.50a	16.75a	18.75a	20.25a	16.75a	22.00a	23.50a	19.50a	23.25a
hydra.	14.75a	10.00bc	6.75bc	2.75b	0.25b	0.50b	0.75b	0.50b	0.75b	10.00abc	8.00bc	12.25bc	12.50ab	16.25ab
indox.	15.25a	2.75c	0.50c	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	3.50cd	7.25bc	10.25bc	10.25abc	14.00bc
fipro.	14.75a	11.25b	11.00b	7.50b	4.50b	4.75b	4.25b	1.25b	0.00b	1.00d	0.50c	1.75c	2.50c	2.50d
lambda	14.75a	3.75bc	3.75bc	3.00b	3.25b	3.50b	4.50b	3.50b	4.00b	12.25a	15.25bc	15.75ab	18.75a	11.50bc
bifenth.	15.00a	6.50bc	3.75bc	1.00b	2.00b	1.50b	1.00b	1.00b	0.75b	7.50bcd	9.75bc	6.00bc	7.00bc	6.75cd
F	12.26*	10.10	11.33	14.17	11.66	11.76	9.54	13.52	13.82	8.85	7.46	8.75	7.85	11.34
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0005	0.0002	0.0004	0.0001
MSD	4.5199	8.1152	8.7782	7.5334	7.0384	7.70287	7.7294	7.2689	7.9758	8.4411	11.204	10.827	9.7047	8.6935

* Treatment only: F=0.0.06, *P*=0.9968

Means in the same column with different letters are significantly different, *P*<0.05, using SAS ANOVA procedures and Tukey's Studentized Range (HSD) Test.

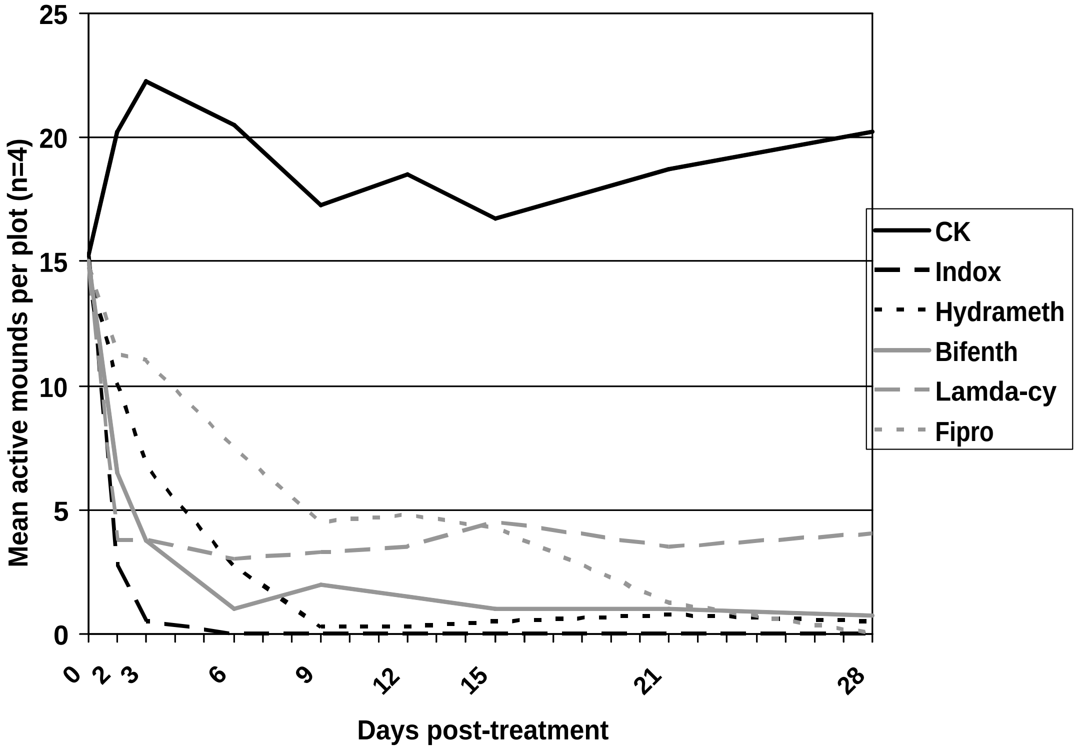

Figure 1. Initial control, 0 –28 days

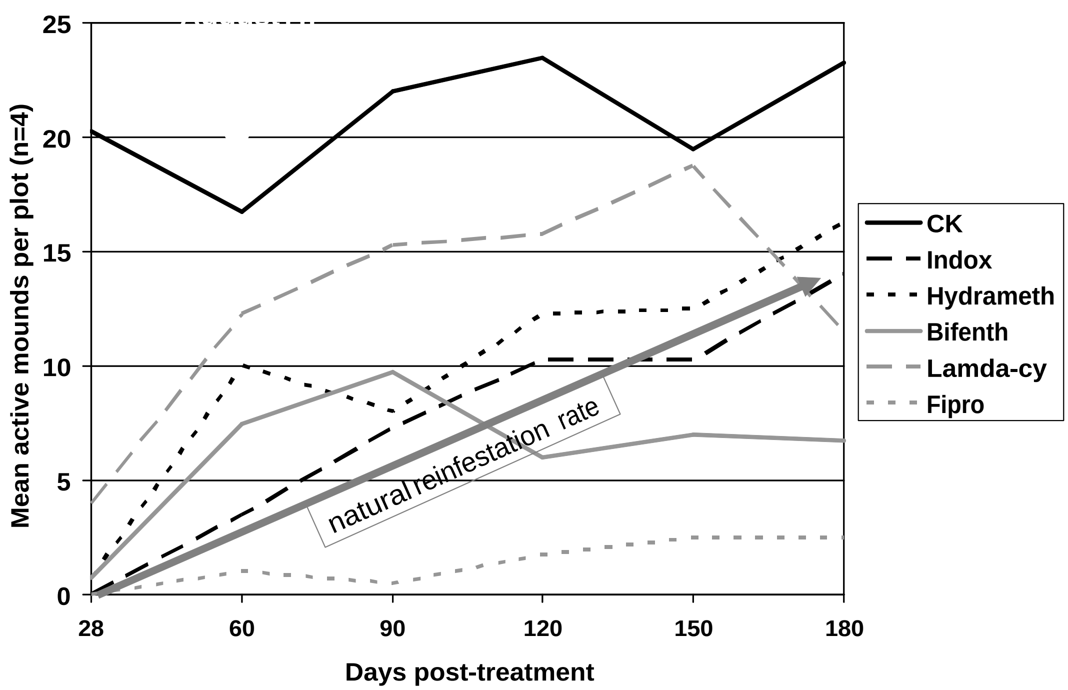

Figure2. Duration of control, 28 –180 days

Behavioral Interactions of the Black Imported Fire Ant (*Solenopsis richteri* Forel) and its Parasitoid Fly (*Pseudacteon curvatus* Borgmeier) as Revealed by High-Speed Video.

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Introduction:

Some species of phorid flies inject an egg into the thorax of fire ants. Previous accounts based on human visual observations and standard video (30-60 frames/second) suggest that fire ants can easily detect their relentless fly parasitoids and often display a particular posture when flies are nearby. W. R. Tschinkel states in the treatise "The Fire Ants" (The Belknap Press, Cambridge, Massachusetts, 723 pp.) "On the scale of the ant's world, detecting a fly hovering a few millimeters overhead, its wings beating several hundred times per second, is probably no more difficult than would be detecting an Apache helicopter in our world.". However, our observations using high-speed video suggest the opposite.

Materials and Methods:

We used the Photron FASTCAM Ultima APX high-speed camera at 2,000 to 10,000 frames per second. All video was taken in the field on real ant mounds in northeast Mississippi. We recorded flies attacking fire ants during natural ant swarming and at disturbed mounds during spring, summer and fall 2006.

Results and Discussion:

Phorid flies are extremely fast agile fliers that can hover and fly in all directions. Wingbeat frequency recorded with microphones and high-speed video is around 422 beats per second. Flies often hover very near to the ant and often touch the ant as they check it out. A phorid fly has been observed flying beneath the outstretched antennae of an ant, and flies often fly among a large number of ants with no apparent reaction from the ants. Ants, unless attacked, do not seem to detect the flies or at least do not seem to react to the flies. Once attacked, then fire ants may assume a defensive posture, but often do not. Attacks can be extremely short in duration from about 0.03 seconds to 0.3 seconds and longer, also some attacks are aborted after mounting the ant. Phorids may spend up to a second or more focused on a single ant. Some ants are attacked at least twice by the same fly. Phorid flies can hover in a very small area, at least up to 14.5 seconds for one recorded phorid female (recorded at 2000 fps). Flies may hover, make several passes or choose an ant very quickly. Sizing of ants by flies may be based on the fly's wingspan. While almost touching the ant and while the fly is horizontal to the ground and perpendicular to the ant's thorax, the fly rotates to an upright position by swinging down its "ice-pick" like ovipositor. The legs spread open as the legs contact the ant and its wings stop moving. The fly's head is usually over the ant's thorax. The sclerotized ovipositor is then moved to the thorax and may be jabbed to the thorax up to eight times. The actual injection of the egg has not been observed. The fly may "explosively" fly off the ant (forwards, backwards) and may hit the ground, other ants and other phorids. Flies sometime attack the wrong area, such as the abdomen. The ant may fall over during the attack with the fly still attached. The ant usually recovers immediately and often acts normally (in contrast to observations in the literature).

Solenopsis Invicta Virus 1 Tissue Tropism and Intra-Colony Infection Rate in the Red Imported Fire Ant: a Quantitative PCR-Based Study

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Solenopsis invicta virus 1 (SINV-1) is the first isolated virus from the red imported fire ant, *Solenopsis invicta* Buren. We have carried out quantitative real-time PCR to measure the SINV-1 genome load in tissues, individuals, and among colonies infected with SINV-1. Among body parts and tissues examined from SINV-1-infected workers and larvae, the midgut consistently had the highest number of SINV-1 genome copies (91.1 and 99.9 %, respectively). Negative staining of a fraction of the midgut homogenate demonstrated the presence of spherical virus particles with a diameter of 30 – 35 nm, consistent with SINV-1. Workers exhibited the highest SINV-1 genome copy number (2.1×10^9 copies/worker) and pupae exhibited the lowest (4.2×10^4 copies/pupa). Overall, SINV-1 genome copy number increased through larval development, sharply declined during pupation, then sharply increased in adults. The number of SINV-1 genome copies in infected larvae and workers from the same queenright colonies were similar, indicating the infection rate was consistent among both developmental stages. No significant differences were observed in mean SINV-1 genome copy number and intra-colony infection rate among the infected colonies collected in summer and winter, although our previous study had shown that inter-colony infection rate of summer-collected colonies was six-times higher than winter-collected colonies, which may indicate that limited inter-colony interaction occurs in winter. However the viral load among winter and summer colonies appears to be similar. A positive correlation between mean SINV-1 genome copy number per ant and intra-colony infection rate was also observed. Based on these results, it is likely that SINV-1 replicates in midgut epithelia of *S. invicta* and is shed into the gut lumen where it may be transmitted to nestmates by trophallaxis and fecal contamination.

Study on the Repellency of Callicarpenal and Intermedeol against Workers of Imported Fire Ants

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Abstract

Repellency of callicarpenal and intermedeol, two terpenoids isolated from American beautyberry and Japanese beautyberry were tested against workers of red imported fire ants, *Solenopsis invicta* Buren, black imported fire ants, *Solenopsis richteri* Forel, and a hybrid of these two species using digging bioassays. Both callicarpenal and intermedeol showed repellency against all tested colonies and intermedeol showed significantly greater repellency than callicarpenal. However, no significant difference was found among species and their hybrid.

Introduction

Imported fire ants are serious medical and agricultural pests in the United States. With the concern of potential environmental contamination of insecticides, there has been increasing interest in research on non-toxic or less-toxic treatments for fire ant control, such as fire ant repellents. Repellents can potentially be useful in quarantine treatment by preventing fire ants from re-entering treated matters, including nursery stocks and soil-moving equipments. Fire ants infest electrical equipments, so repellents may also be useful to exclude fire ants from such equipments.

Callicarpenal and intermedeol are two terpenoids isolated from leaves of American beautyberry and Japanese beautyberry with mosquito deterrent activity (Cantrell et al. 2005). The repellency of these two compounds has never been tested against imported fire ants. The objective of this study is to evaluate the repellency of callicarpenal and intermedeol against workers of red imported fire ants, black imported fire ants, and their hybrid using the ant digging bioassay.

Materials and Methods

Multiple choice bioassays were conducted on two colonies for each species and the hybrid with a purpose of finding the minimum effective concentration (MEC), which was defined as the lowest concentration that showed repellency against both colonies. Two choice bioassays were also conducted on another 5 colonies for each species and the hybrid at 50 ppm which was found in the multiple choice bioassays to be effective to both species and hybrid. The purpose of two choice bioassays was to compare the repellency among two tested compounds. Callicarpenal and intermedeol were isolated from American beautyberry leaves following the method described by Cantrell et al. (2005). The bioassay developed by Chen (2005) was modified in this study. In multiple choice bioassays, four 2 ml centrifuge tubes were mounted under a 14.0 × 2.3 cm petri dish using glue (Fig. 1). A 3-mm diameter access hole went through the bottom of the petri dish and the cap of the tube. The inner side of the petri dish was coated

Figure 1. Bioassay apparatus for evaluating fire ant repellents.

with Fluon. Each of there tubes was filled with sand treated with different concentrations of a test compound and one with untreated sand as control. The final water content of sand used in the study was 8.0%. Six concentrations, including 0.75, 1.50, 3.15, 6.25, 12.50, and 25.00 ppm, were tested in two separate bioassays for all chemical and species combinations. The callicarpenal against red imported fire ant treatment had three additional concentrations, 50.00, 100.00, and 150.00 ppm, because callicarpenal did not show the repellency against red imported fire ants from both colonies at 25 ppm. Fifty fire ant workers were introduced into the center of the petri dish. The experiment was conducted at 22 ± 0.8 ˚C (mean ± SD) temperature and 45.4% ± 11.87% RH. After 24 h, sand in each tube was collected, dried at 150 ˚C for at least 4 h, and weighed. A total of six colonies, two colonies of each species and the hybrid, were tested. The experiment was replicated five times for each colony. The analysis of variance (PROC GLM) followed by LSD test ($\alpha = 0.05$) (SAS Institute 1999) were used to compare the amount of sand removed by ants among treatments and significance was determined at $P < 0.05$. In two choice bioassays, 5 other colonies were tested for each species and the hybrid. The bioassay apparatus are similar as in multiple choice bioassays except only two choices were presented in each bioassay arena: one was sand treated with callicarpenal or intermedeol and the other with solvent as a control. Two centrifuge tubes with access holes were mounted under a smaller petri dish (8.5 × 2.3) and the other two tubes without access holes were used to support the bioassay device. The procedure was the same as described above except 25 fire ant workers were used and each colony was replicated 5 times. Digging suppress index was used to compare the repellency, which was calculated using formula $I_s = (A_c - A_t)/(A_c + A_t)$, where I_s is the digging suppress index, A_c and A_t are the amounts of sand removed from control tube and treatment tube, respectively. The analysis of variance (PROC GLM) followed by LSD test ($\alpha = 0.05$) were used to compare digging suppress indices between two chemicals and among species and significance was determined at $P < 0.05$.

Results and Discussion

Both callicarpenal and intermedeol were repellents of imported fire ants. The minimum effective concentrations for each species and the hybrid are listed in Table 1. At 50 ppm, there was a significant difference in the digging suppress index among callicarpenal and intermedeol ($F_{1,146} = 41.35$, $P < 0.001$) and intermedeol was a stronger repellant than callicarpenal against both species and the hybrid. No significant difference was found among species for both compounds ($F_{2,146} = 0.34$, $P = 0.72$) (Table 2).

Table 1. Minimum effective concentration (MEC) of callicarpenal and intermedeol against imported fire ant workers.

Chemical	Species	MEC (ppm)
Callicarpenal	*S. invicta*	50.00
	S. richteri	6.25
	Hybrid	6.25
Intermedeol	*S. invicta*	1.50
	S. richteri	6.25
	Hybrid	6.25

Table 2. Digging suppress indices of 50 ppm callicarpenal and intermedeol in the two choice digging bioassays.

Chemical	Species	Colony	Mean of Digging Indices (SE)
Callicarpenal	*S. invicta*	1	0.52 (0.17)
		2	0.65 (0.12)
		3	0.35 (0.14)
		4	0.74 (0.18)
		5	0.83 (0.10)
	S. richteri	1	0.84 (0.02)
		2	0.61 (0.16)
		3	0.27 (0.05)
		4	0.87 (0.12)
		5	0.41 (0.09)
	Hybrid	1	0.67 (0.10)
		2	0.65 (0.19)
		3	0.70 (0.16)
		4	0.60 (0.15)
		5	0.82 (0.07)
Intermedeol	*S. invicta*	1	0.91 (0.06)
		2	0.80 (0.14)
		3	0.91 (0.06)
		4	0.98 (0.02)
		5	0.96 (0.04)
	S. richteri	1	0.85 (0.09)
		2	0.84 (0.10)
		3	0.84 (0.10)
		4	0.99 (0.01)
		5	0.97 (0.03)
	Hybrid	1	0.85 (0.07)
		2	0.94 (0.03)
		3	0.83 (0.16)
		4	0.89 (0.10)
		5	0.96 (0.01)

Vander Meer et al. (1993) have proposed applications of fire ant repellents in the following potential areas: 1) protection of biological control agents, 2) protection of electrical circuitry and switches, 3) exclusion of fire ants from hospitals, and 4) protection of trees using repellent tree wrap. Evidence of strong repellency of intermedeol and callicarpenal against fire ant workers was found in this laboratory study. Further investigation on the practical application of these two compounds is warranted in proposed areas defined by Vander Meer et al. (1993).

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The Effects of Indoxacarb on Foraging Behavior of *Solenopsis invicta*

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The red imported fire ant (RIFA), *Solenopsis invicta* Buren, is an important economic pest dominating the southeastern United States. In the 1930s, the RIFA entered the United States through Mobile, AL (Wilson 1951, Williams et al. 2001). Today, the RIFA can be found throughout the southeast United Stated and Puerto Rico as well as in parts of California and New Mexico (Tschinkel 2006). The potential for the spread of RIFA is much greater, covering all of the southern United States and Hawaii (Morrison et al. 2004).

Introduction of the RIFA is believed to cause an estimated $5.6 billion dollars in damage every year (Lard et al. 2005). Injuries and death due to numerous fire ant stings are known to occur within the United States. As of 2004, there were reports of 6 cases of fire ant stings in health care facilities, four of which resulted in the victim dying within 1 week of their attack (deShazo et al. 2004). Therefore, proper control of RIFA is essential in areas frequented by sensitive people such as elderly, children, and the mentally ill.

The increasing expansion of RIFA and its associated problems has led to several attempts at eradication or control using baits. Baits are believed to be the most cost effective and simplest methods for RIFA control. In the past, toxic baits needed to (a) be slow-acting, (b) have a wide range of effective dosages, and (c) be mixable with a carrier (Williams et al. 2001). Generally, the carrier and attractant are the same for most RIFA baits in the market today and consist of pregelled, defatted corn grits and soybean oil (Williams 1983).

Slow-acting baits were thought to be necessary in order to have the active ingredient spread throughout the colony killing the fire ant queen. Several different slow-acting ingredients are currently present in the market including: methoprene, fipronil, and hydramethylnon. These toxicants were pre-screened and possessed all of the characteristics of a good toxicant including delayed toxicity (Williams 1983, Collins and Callcott 1998). Field studies using 15 µg/mg of fipronil eliminated 80% of RIFA colonies at 6 weeks (Collins and Callcott 1998). Hydramethylnon can reach its highest level of control at 3 months achieving 98.3% mound mortality. Baits with the most delayed toxicity are insect growth regulators. One growth regulator, methoprene, resulted in 57% RIFA mound mortality 4 months after bait application (Oi et al. 2004).

A different chemical, indoxacarb, found in a more recent RIFA bait, has been shown to differ from traditional slow-acting baits. Previous experiments show that indoxacarb produces 100% ant mortality within 48 hrs after application, which is significantly faster than traditional baits (Oi and Oi 2006). There was no mortality observed with indoxacarb within the first 8 hrs.

<u>Experimental Purpose</u>. The purpose of this experiment is to evaluate indoxacarb and its effect on *S. invicta* foraging behavior. Information on the foraging behavior of RIFA after bait exposure can help in understanding how fast-acting baits effectively reduce RIFA colonies.

Materials and Methods

Insects. Red imported fire ant colonies, *S. invicta*, were field-collected from the lawn surrounding the University of Florida's Entomology and Nematology Building (Gainesville, FL). Colonies were collected by shoveling the entire ant mound into 20 liter buckets coated with Fluon® (AGC Chemicals, Bayonne, NJ) to prevent ant escape. Colonies were extracted from the soil by dripping water into the bucket. When the water level went above the soil, the ants were scooped up and placed in a plastic rearing tray (56 by 44 by 12 cm; Panel Controls, Greenville, SC) coated with Fluon®. Colonies were maintained in the laboratory for 3 to 8 months and reared at 12:12 (L:D) photoperiod, at 25^{O}C, and ~50% RH. Colonies contained a queen and brood.

Nest cells prepared from Petri dishes (150 by 15 mm; Becton Dickinson and Company, Franklin Lakes, NJ) filled to a depth of 2 mm of dental plaster (DentSply International, Saint Louis, MO) were placed in each rearing tray. A hole (3 mm) was placed either into the nest-cell lid or on two opposite sides of the bottom dish wall to allow ant access into the nest-cell. Colonies were provided water and 10% w/v sugar water *ad libitum*, and cockroaches or crickets 3 days a week.

Pheromone extraction. The trail pheromone was used to induce trailing in the trailing behavior bioassay. The pheromone was extracted by cutting off 30 forager ant abdomens and placing them in a micro-centrifuge tube containing 1 ml of hexane (Fisher Scientific Company L.L.C., Fair Lawn, NJ). The ants used to extract the trail pheromone were taken from the same colony as the ants used in the trailing behavior bioassay. The ants' abdomens were crushed to speed up the extraction process and Parafilm® (American National Can™, Greenwich, CT) was wrapped around the micro-centrifuge tube to prevent the hexane from evaporating. The hexane was allowed to extract the trail pheromone from the abdomens for at least one hour.

Micro-colony setup. Two micro-colonies originating from the same field colony were set up the day of the experiment inside Fluon®-coated rearing trays. Each micro-colony contained 500 foragers, 500 nurses, and 500 mg of brood. Ants collected outside of the nest-cell in the rearing tray were considered foragers and adult ants found inside the nest-cell were considered nurse ants. All ants were collected using an insect aspirator attached to a 50 ml plastic tube. Nurse ants and brood were immediately placed into the nest-cell constructed from a polystyrene Petri dish (60 by 15 mm; Becton Dickinson and Company, Franklin, NJ) and dental plaster as described above. The dental plaster was dampened with distilled water prior to setup to create a humid environment for the ants.

Treatments. Each micro-colony was provided with one of two baits: Advion® Fire Ant Bait (fast-acting) [0.045% indoxacarb; DuPont, Wilmington, Delaware] or an untreated control. The control was a blank bait prepared with pregelled, defatted corn grits (Illinois Cereal Mills, Paris, Illinois) and once refined soybean oil (30% by weight), but no toxicant. This mixture is a common carrier for most commercial fire ant baits (Williams 1983).

Treatments were placed in a square pouch (50 by 50 mm) made from fiberglass insect screening (20 mesh; Phifer Wire Products, Inc, Tuscaloosa, AL). This construction allowed ants to feed on the bait without taking the granules back to the nest: Each pouch was filled with 1 g of bait. The mesh containers were left in the rearing tray allowing the ants to have continuous access for the duration of the experiment.

Trailing Behavior Bioassay. To measure trailing success, a bridge was set up in each rearing tray immediately before the experiment. Each bridge consisted of two wood block legs (8.7 by 4.9 by 3.7 cm) and a glass bridge (30 by 5 cm) covered with brown paper (Gainesville

Paper Co., Gainesville, FL). The leg farthest from the cell was set inside a deli cup (8 oz; American Plastic, Chattanooga, TN) coated on the inside and outside walls with unscented talcum powder. A series of lines were marked on the paper cover: one line down the center length-wise and two lines on each side of the center line at 5 mm and 10 mm from the center line. In addition to the lines, 2 points were marked on the center line 20 mm from each end of the bridge used as start and end points for behavior observations and limiting the observation area to a 25.8-cm long portion of the bridge. Immediately before the behavior trials start, the pheromone extract was laid onto the bridge's center line. The trail pheromone extracted earlier was spread using a micropipette as evenly as possible onto the entire bridge center line and down the center of the outer face of each leg. The bait pouch was placed inside the plastic deli cup so that the ants had to cross the bridge to reach the bait and then return to the nest-cell along the same trail . The experiment started immediately to prevent the trail pheromone from dissipating.

Data Collection. Foraging behavior was measured using only the trailing ants that were returning from the bait cup. Ants on top of the bridge, but clearly not following the trail were not considered for the observation. Data observations began 10 to 20 minutes after the bait was placed into the rearing tray.

Data was recorded every hour for a time interval necessary for 10 ants to successfully cross the bridge or 30 min if 10 successful crossings did not occur. While crossing the bridge, if the ants deviated from the marked trail (center line on the bridge) and touch the 10-mm line, an error was assigned to the ant.

Data recorded included: (a) time it took one ant to successfully cross between the two bridge points, (b) time for 10 complete successful crossing between the two bridge points, (c) number of 10 mm errors. After each behavioral observation, abnormal behavior and mortality was measured. Abnormal ants were any ants that did not perform normal tasks, but are not dead. Dead ants were considered any ant that did not respond to probing.

Results

The amount of time it took an ant to travel between the start and end point were averaged. The mean ant time of control ants was 18.95 sec 0 hours after treatment and 27.50 sec 6 hrs after treatment. Control ants did not significantly increase in the amount of time it took for an ant to cross the bridge (Figure 1). The amount of time it took ants fed indoxacarb to cross the bridge increased 3 hrs after treatment compared to control ants (Figure 1). At time zero, the average amount of time it took for an ant to cross was 19.15 sec compared 40.43 sec 6 hours after treatment.

Foraging rate was measured by dividing the amount of time it took for 10 successful ants to cross the bridge. If there were not 10 successful crossings, the number of successful ants in 30 minutes was recorded. The results show that the rate of ants fed the control bait decreased over time (Figure 2). The mean number of ants per minute for control ants was 1.67, 0 hrs after treatment and 0.53 ants/min at 6 hrs after treatment. The rate of ants fed indoxacarb decreased faster than the control ants (Figure 2). The mean number of ants per minute for indoxacarb fed ants was 1.55, 0 hrs after treatment and 0.12 ants/min, 5 hrs after treatment. There were no foragers for the indoxacarb fed ants 6 hrs after treatment; therefore, no data was collected.

Percent of ants committing a 10-mm error was measured to assess how well an ant followed a pheromone trail. Ants fed the control bait increased in the number of times an ant crossed the 10-mm error as time increased (Figure 3). At time 0, 10% of successful ants made a 10-mm error compared to 30% of ants committing an error 6 hours after treatment. Percentage of ant errors increased more than control ants 4 hours after treatment (Figure 3). At time 0, 0% of successful ants made a 10-mm error compared to 43% of ants committing an error 5 hrs after treatment. Ant foraging completely stopped 6 hours after treatment.

The cumulative number of dead ants gradually increased in control ants (Figure 4). The mean cumulative number of dead ants for the control at 0 hrs was 1 and had increased to 12.5 ants 6 hrs after treatment. There was no significant difference in the cumulative number of dead ants between indoxacarb and control bait (Figure 4). The mean cumulative number of dead ants for the indoxacarb bait at 0 hrs was 0.5 and increased to 20 ants 6 hrs after treatment.

Abnormal ants were not common among the control ants (Figure 5). However, 3 hrs after treatment there was a large increase in the amount of abnormal ants present in indoxacarb-treated ants (Figure 5). Indoxacarb-fed ants did not have any abnormal ants 0 hrs after treatment, but the number of abnormal ants increased to 32 ants 6 hrs after treatment.

Discussion

Preliminary results suggest that even though there is no significant amount of mortality within the first 6 hours after treatment, there are indications of toxic effects beginning 3 hours after treatment. Ants fed indoxacarb stopped foraging within 6 hours. Other effects on indoxacarb ants include slower trail following, decreased recruitment to a food source, and decreased ability to follow a pheromone trail. Future work will compare foraging behavior of indoxacarb ants to ants fed a slow-acting bait such as hydramethylnon.

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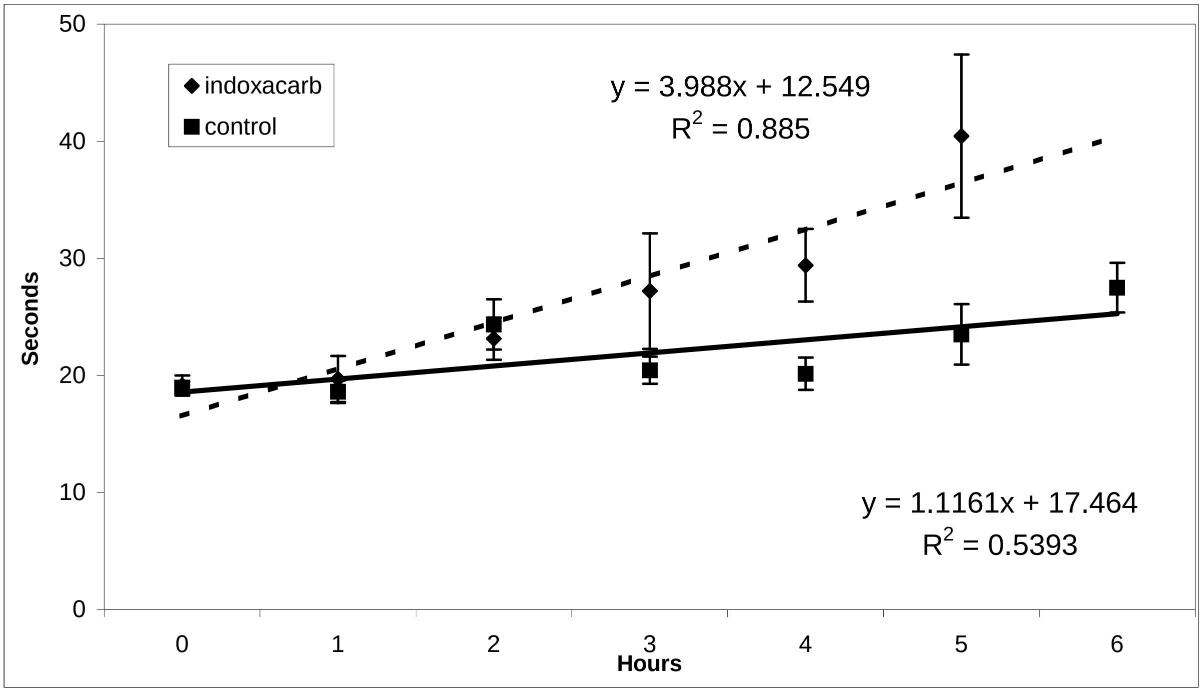

Figure 1. Average time for an ant to cross the bridge between the start and end point.

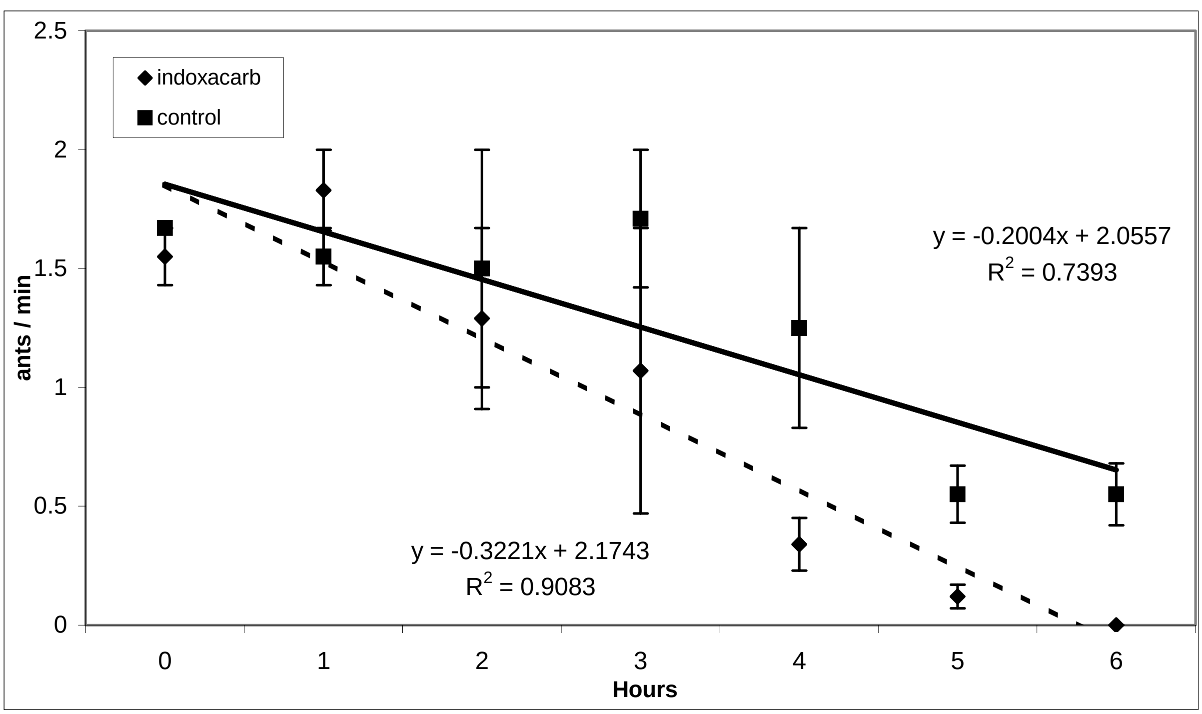

Figure 2. Foraging rate measured by the amount of successful foragers divided by the total amount of time

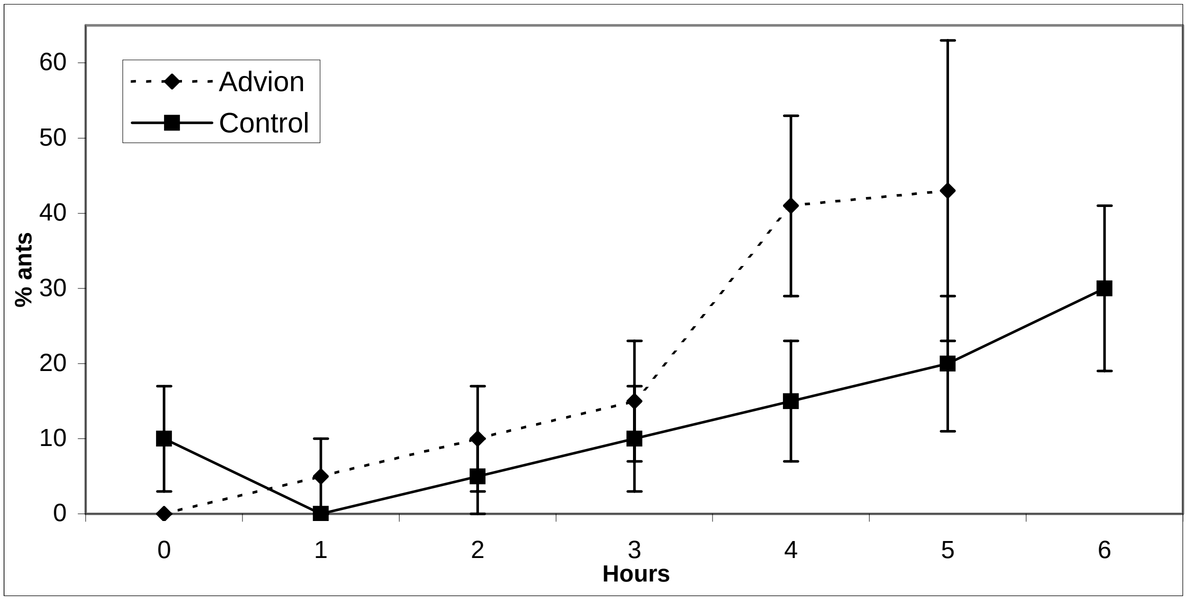

Figure 3. Percentage of ants committing a 10-mm error

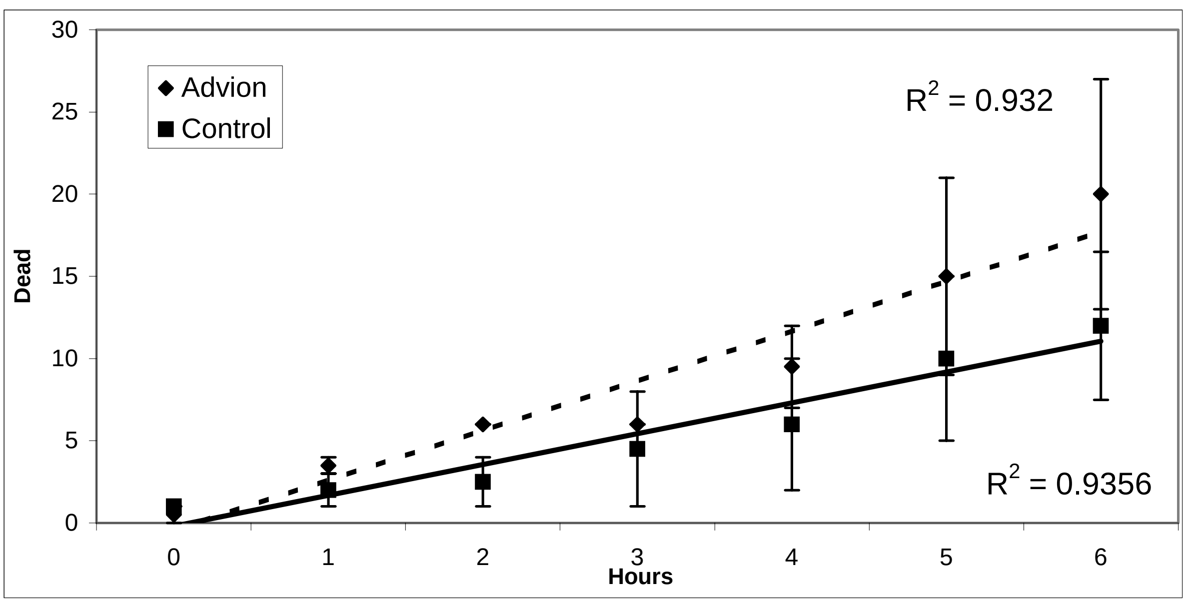

Figure 4. Cumulative number of dead ants

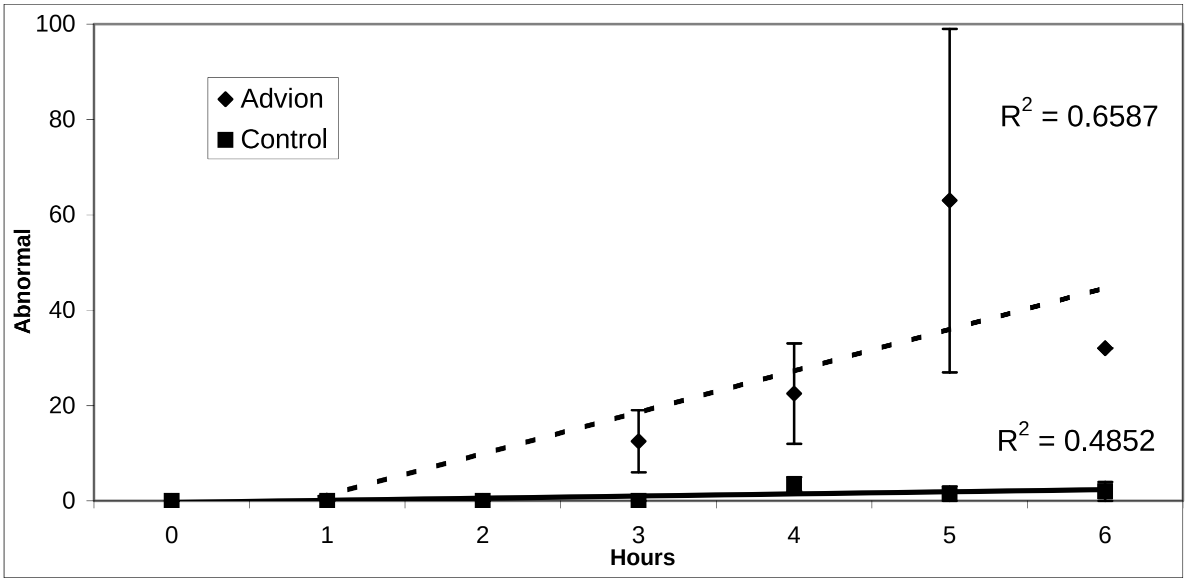

Figure 5. Number of abnormal ants

Influences of Imported Fire Ant (Hymenoptera: Formicidae) Bioturbation on Soils and Turfgrass in a Sod Production Agroecosystem

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Abstract. Mound-building imported fire ants (IFA) actively modify the biogeochemical and physical properties of soil. Soil alterations result from worker ants' nest construction and foraging activities as well as colony-wide food sharing and metabolic functions. However, their influence on nutrient levels in surrounding vegetation is poorly understood. Element enrichments as well as depletions were simultaneously documented for both ant-affected as well as undisturbed soils and warm-season turfgrass in a sod production agroecosystem. Collection of soils and turfgrass was timed to coincide with peak IFA biomass. Total C, total N, C/N ratios, organic matter (OM), and Zn^{2+} concentrations as well as pH of mound soils were significantly higher than control soils. Turfgrass harvested from ant mound perimeters exhibited elevated N, P, Ca^{2+}, S, Cu^{2+}, Fe^{2+}, and Na^{+} concentrations. The complexity of biogeochemical interactions within ant nests was most likely further enhanced by plant uptake and excretion in the rhizosphere coupled with continuous soil mixing by colony workers. Therefore, further study of the intrinsic complexities of soil ecosystem dynamics of IFA nests across seasons is warranted.

Opposable Spines Facilitate Fine and Gross Object Manipulation in Fire Ants

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Abstract Ants inhabit diverse terrestrial biomes from the Sahara Desert to the Arctic tundra. One factor contributing to the ants' successful colonization of diverse geographical regions is their ability to manipulate objects when excavating nests, capturing, transporting and rendering prey or grooming, feeding and transporting helpless brood. This paper is the first to report the form and function of opposable spines on the foretarsi of queens and workers used during fine motor and gross motor object manipulation in the fire ant, *Solenopsis invicta*. In conjunction with their mandibles, queens and workers used their foretarsi to grasp and rotate eggs, push or pull thread-like objects out of their way, or push excavated soil pellets behind them for disposal by other workers. Opposable spines were found on the foretarsi of workers from seven of eight other ant species, suggesting that they might be a common feature in the Formicidae.

Cassill, D.L., Anthony Greco, Rajesh Silwal and Xuefeng Wang. 2007. Opposable spines facilitate fine and gross object manipulation in fire ants. Naturwissenschaften 94:326-332. Online in 2006 (http://www.springerlink.com/content/a08132x3718p236p/).

Semiochemical Mediated Responses of the Phorid Fly, *Pseudacteon tricuspis* (Diptera: Phoridae) to Red Imported Fire Ants

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Abstract

We investigated the electroantennogram (EAG) and behavioral responses of *Pseudacteon tricuspis* of different sex and mating status to red imported fire ant, *Solenopsis invicta* host-related odor stimuli including live fire ant workers, extracts of worker whole body, head, thorax, and abdomen, and (*E*, *E*)-α-farnesene, a trail pheromone component of fire ants. In EAG experiments, female and male *P. tricuspis* showed significant EAG response to extracts of worker whole body, head, and abdomen, and to a less extent, thorax extract, but not to (*E*, *E*)-α-farnesene. Females showed slightly greater EAG response than males, but EAG response was not affected by mating status. Results from behavioral (Y-tube olfactometer) bioassays demonstrated the attraction of mated female *P. tricuspis* to live *S. invicta* workers. In addition, extracts of *S. invicta* worker whole body and thorax elicited strong olfactometer response in mated and unmated female flies and mated males, but not in unmated males. No significant attraction of *P. tricuspis* of different physiological states was elicited by extracts of *S. invicta* worker head and abdomen, or by (*E*, *E*)-α-farnesene, suggesting that fire ant thorax is likely the source of kairomones used by *P. tricuspis* to locate host fire ant workers.

Introduction

Pseudacteon phorid flies (Diptera: Phoridae) are parasitoids of ants, and many species are specific to imported fire ants, *Solenopsis* spp. (Porter *et al*., 1995; Morrison, 2000). At least two species, *P. tricuspis* and *P. curvatus* have been released in the last decade for biological control of the invasive imported fire ants, *Solenopsis* spp. in southern United States (Gilbert, 1996; Porter *et al*., 1999). Although several flies in the family Phoridae are known to utilize ant host-related semiochemicals for host location (Brown and Feener, 1991; Morehead and Feener, 2000), little is known about the cues used by *Pseudacteon* phorid flies to locate host imported fire ants. Olfaction has been suggested as the long range cue used by *Pseudacteon* phorid flies in locating host fire ants (Orr *et al*., 1997; Porter, 1998), however this has not been experimentally confirmed. In this study, we demonstrate the electrophysiological and behavioral responses of *P. tricuspis* to red imported fire ant, *S. invicta* odor.

Materials and Methods

Insects. *Pseudacteon tricuspis* used in this study were supplied by the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, U.S.A. Parasitized fire ant worker heads were received in batches and held in a plastic jar (25 × 13 cm) with a lid until emergence. Twice daily, newly-emerged flies were removed with an aspirator, sexed immediately, and placed in groups of 2 individuals either of the same sex (unmated individuals) or of opposite sex (mated individuals) in a 6-cm diameter plastic Petri dish. Petri dishes were kept in an incubator at 25 ± 1ºC, LD 14:10 h and 70 ± 5% r.h. Sugar (25% sucrose) solution and water were provided in the Petri dishes as previously described (Chen *et al*., 2005). Adult phorid flies utilized in the experiments were 1-2-day-old. Red imported fire ants, *S. invicta* used in this study were collected on the campus of Auburn University (Auburn, Alabama). Ants were raised in the laboratory using standard rearing procedures (Banks *et al*., 1981).

Chemical stimuli. Chemical stimuli consisted of live *S. invicta* workers, extracts of dissected body parts of *S. invicta* workers, and (*E*, *E*)-α-farnesene, a synthetic trail pheromone component of imported fire ants. Tests of extracts of *S. invicta* body parts included whole body, head, thorax, and abdomen (gaster). Fire ant workers from different colonies were chilled to -20 °C for 15 min before body parts were separated using micro dissecting scissors. For each body part, 100 dissected individual parts were extracted in hexane to obtain 0.1 worker equivalent per μL (WE/μL) solutions. (*E*, *E*)-α-farnesene (Sigma-Aldrich, St. Louis, MO) was diluted in hexane to give a 0.1 μg/μL solution.

Electroantennogram (EAG) recordings. The EAG techniques used in this study are described in details in Chen and Fadamiro (2007). Ten-microliter aliquot of each odor stimulus was applied to a piece of filter paper strip which was then inserted into a glass Pasteur pipette constituting an odor cartridge. The control stimulus was a similar pipette containing a filter paper strip impregnated with a 10 μL aliquot of hexane. In the first experiment, we tested the EAG response of mated and unmated *P. tricuspis* of both sexes to three doses of fire ant worker whole body extract, (*E*, *E*)-α-farnesene (1 μg dose), and hexane control, for a total of five odor stimuli treatments. Recordings were obtained from 12 individuals for each sex and mating combination. In the second experiment, we compared the EAG response of mated females to 1 worker equivalent of whole body, head, thorax, or abdomen extracts, and to (*E*, *E*)-α-farnesene (1 μg dose) and hexane control, for a total of six treatments. Twelve individual mated females were used to obtain EAG recordings. Data were first analyzed by using the standard least squares fit model to determine the effects of odor stimuli, sex, mating, and interactions among these factors on EAG response. Further analysis of EAG data was by ANOVA followed by Duncan's multiple comparison test ($P < 0.05$; SAS Institute, 2004).

Behavioral bioassays. A Y-tube olfactometer was used to test the attraction of mated and unmated individuals of both sexes of *P. tricuspis* to host-related odor. In the first experiment, we tested the attractiveness of live *S. invicta* (~250-300 mg live workers) to *P. tricuspis.* In the second experiment, we tested the attractiveness of body extracts of *S. invicta* workers and (*E*, *E*)-α-farnesene (trail pheromone) to *P. tricuspis*. A total of five stimuli was tested: extracts of fire ant worker whole body, head, thorax, and abdomen, and (*E*, *E*)-α-farnesene. Each stimulus was delivered as 10-μL sample (resulting in 1 worker equivalent) placed on filter paper strips. For each experiment, 30 adult *P. tricuspis* of each sex and mating status were bioassayed and flies were used only once. Data analysis was by chi-square ($P < 0.05$, SAS Institute, 2004).

Results

Electroantennogram (EAG) recordings. Significant effects of odor stimuli and sex were recorded on EAG responses of *P. tricuspis,* but mating was not significant. Thus, data from unmated and mated individuals of each sex were pooled and analyzed by using ANOVA. Both sexes of *P. tricuspis* showed dose-dependent EAG responses to *S. invicta* whole body extract with the 1 whole body extract (WBE) dose eliciting the greatest EAG responses in females and males (Fig. 1). Response of females to 1 WBE of fire ant whole body extract was significantly greater than response of males to the same odor stimulus (Fig. 1). However, (*E*, *E*)-α-farnesene failed to elicit any significant EAG response in both sexes. In the second experiment, significant differences were recorded in EAG response to the different odor treatments. The greatest EAG amplitude was elicited by whole body extract followed by head and abdomen extracts (Fig. 2). The EAG response evoked by thorax extract was significantly greater than the EAG evoked by

(*E*, *E*)-α-farnesene or control (hexane) stimulus, but significantly less than the EAG elicited by extracts of whole body, head, and abdomen (Fig. 2). The EAG response to (*E*, *E*)-α-farnesene was not significantly greater than the EAG elicited by the control stimulus hexane (Fig. 2).

Behavioral bioassays. Data from the first experiment showed that mated female *P. tricuspis* were significantly attracted to live workers of *S. invicta* (Fig. 3). However, no significant attraction to live workers was recorded for unmated females, mated males, or unmated males. In the second experiment, fire ant worker whole body extract and thorax extract elicited significant attraction in mated females (Fig. 4), as well as in unmated females and mated males. However, no significant attraction of *P. tricuspis* was recorded to head extract, abdomen extract, or (*E*, *E*)-α-farnesene (Fig. 4). Also, none of the odor stimuli was significantly attractive to unmated males (see Chen and Fadamiro, 2007 for full data).

Discussion

Our data demonstrate the attraction of *P. tricuspis* to host *S. invicta* odor as suggested in previous studies (Orr *et al*., 1997; Vander Meer and Porter, 2002; Morrison and King, 2004). Furthermore, our results showing no behavioral or EAG response of *P. tricuspis* to (*E*, *E*)-α-farnesene suggest that fire ant worker trail pheromones are not likely used by *P. tricuspis* for host location, as previously proposed by Vander Meer and Porter (2002) and Morrison and King (2004). However, the results showing behavioral attraction of *P. tricuspis* to *S. invicta* thorax extract but not to head and abdomen extracts do not appear to support the popular notion that phorid flies use fire ant alarm pheromones, which are produced in the abdomen and head, for host location (Vander Meer and Porter, 2002; Morrison and King, 2004).

Fire ant worker thorax elicited significant EAG response supporting data from behavioral bioassays. However, head and abdomen extracts which did not induce attraction of *P. tricuspis* in olfactometer bioassays, elicited even stronger EAG response. This data showing disparity between EAG and olfactometer results is not unusual. An electroantennogram does not distinguish between attraction or inhibitory effects, and the magnitude of EAG response does not always correlate with behavior (Bjostad, 1998). The strong EAG response of *P. tricuspis* to fire ant head and abdomen extracts may be due to the presence of defensive secretions in the head (e.g., mandibular gland secretions) and abdomen (e.g., secretions from Dufour's gland and venom gland), which although may elicit strong EAG response, but may not constitute attractants to phorid flies. Our results showing no attraction of phorid flies to abdomen and head extracts suggest that ant defensive compounds (including alarm pheromones), which are typically produced in the abdomen and the head, are not likely to be used as attractants by phorid flies. The significantly greater EAG response to fire ant odor recorded for females compared to males may be related to the observed sexual dimorphism in antennal morphology of *P. tricuspis*: the female antenna bears an arista which is not present on the male antennae (unpublished data). Further studies will investigate the glandular sources and chemical identity of the attractant compound(s) and possibly elucidate the disparity between our olfactometer and EAG results.

Acknowledgements

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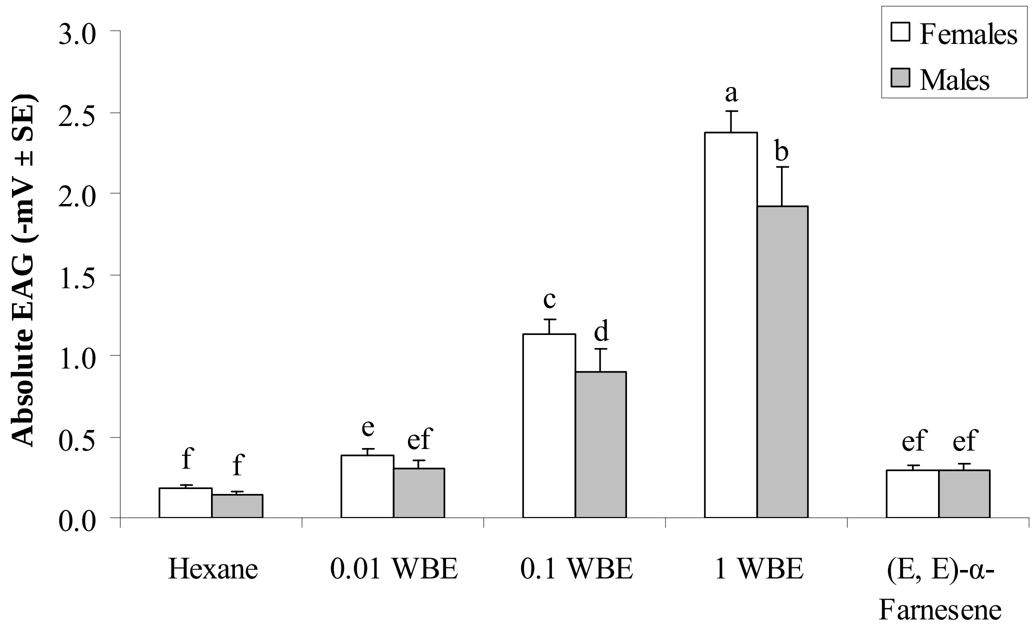

Figure 1. Electroantennogram (EAG) responses of female and male *Pseudacteon tricuspis* to different doses of *Solenopsis invicta* worker whole body extracts (0.01, 0.1, 1 worker whole body equivalent = WBE) and (*E*, *E*)-α-farnesene (1 μg dose). Means followed by different letters are significantly different.

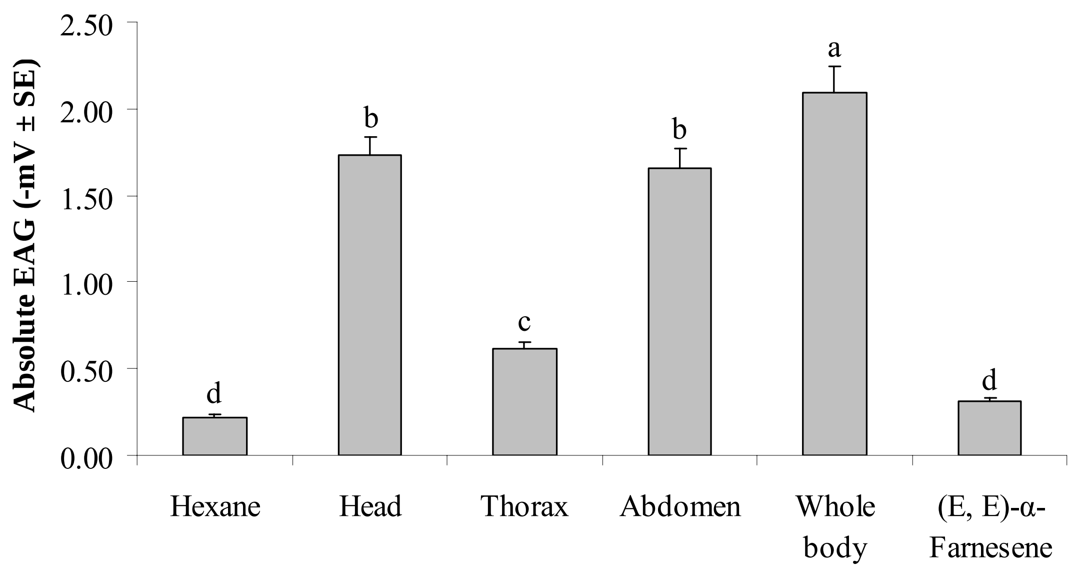

Figure 2. Electroantennogram (EAG) responses of mated female *P. tricuspis* to *S. invicta* worker extracts (whole body, head, thorax, or abdomen) and (*E*, *E*)-α-farnesene. Worker extracts were tested at 1 worker equivalent dose, while (*E*, *E*)-α-farnesene was tested at a 1 μg dose. Means followed by different letters are significantly different.

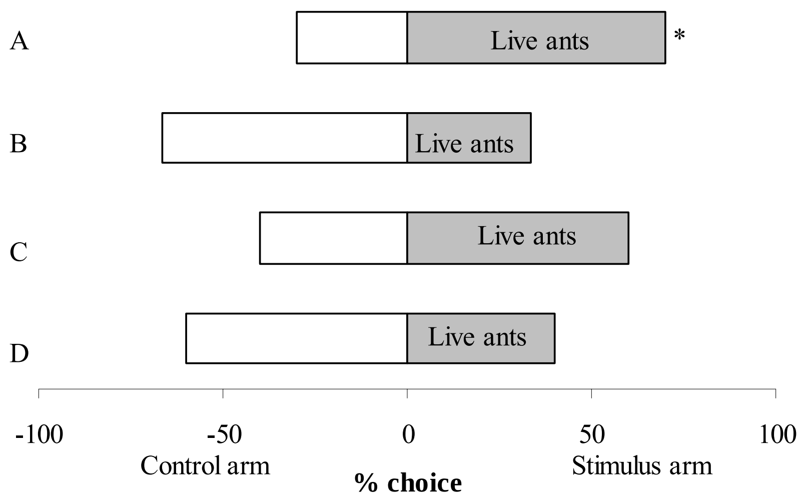

Figure 3. Response of *P. tricuspis* in a Y-tube olfactometer when given a choice between clean air (control) and live *S. invicta* workers (odor stimulus). (A) Mated female, (B) Unmated female, (C) Mated male, (D) Unmated male. Approximately 250-300 mg of live workers was used as odor stimulus. * indicates significant difference within a choice test.

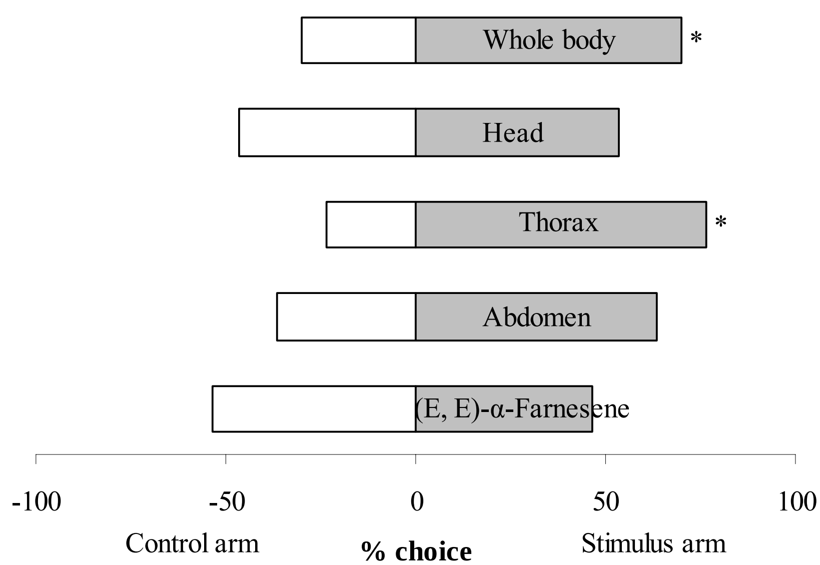

Figure 4. Response of mated female *P. tricuspis* in a Y-tube olfactometer when given a choice between hexane (control) and *S. invicta* worker extracts (whole body, head, thorax, or abdomen) or (*E*, *E*)-α-farnesene (1 μg dose). Worker extracts were tested at 1 worker equivalent dose. * indicates significant difference within a choice test. Similar results were obtained for unmated females and mated males.

Revised Data Showing Alternative Distribution of Two Forms of Red Imported Fire Ant in Taiwan

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Abstract
In order to understand the distribution of two social forms of red imported fire ant that recently were found in Taiwan (Taoyuan and Chiayi County, which are separated by ~250km apart), nest cluster based sampling procedures were addressed in this study. The number of queens within the nest and Gp-9 multiplex PCR were used to determine the social forms of fire ants sampled. The number of polygyne nest clusters is significantly much more than monogyne in the central region of Taoyuan County; but monogyne stands absolute dominance in outlier region. However, only five monogyne nests (6%) were discovered in Chiayi. The distribution pattern in Taoyuan confirms the possibility that fire ant might first arrive at Taoyuan International Airport with subsequent spreading out. Mosaic distribution of extremely few monogynes in Chiayi implies that the initial colonizer likely consisted of polygyne, and monogynes were most likely derived from existing polygyne nests.

Introduction
The occurrence of *Solenopsis invicta* in Taiwan was first reported in two counties, Taoyuan and Chiayi which separated by ~250km apart, in October 2003. They are estimated to have arrived Taiwan for 3~5 years earlier based on the size and distribution of colonies (Chen *et al*., 2006). Notably, both two forms (monogyne and polygyne) of *S. invicta* are found to be present in both of the infested areas (Chen *et al*., 2006). Since two forms differ in many features of social habits, physiology and genetics (Bourke, 2002; Krieger & Ross, 2002), previous studies have suggested that the distribution of two social forms as a critical part of drawing up the control strategies (Porter & Savignano, 1990; Porter *et al*., 1991). Therefore, the detailed surveillances on the spatial structure of both forms in Taiwan might be necessary for a successful eradication before the large scale bait treatment. In the present study, an alternative sampling procedure with higher resolution for reflecting the distribution of social forms would be addressed. Through intensive field surveillances and subsequent identifications by genotype of Gp-9 locus which highly correlated with transition of two social forms, combined with the GPS data, the distribution of two forms would be mapped. This study is of interest not only for development of effective integrated management strategies for control and eradication of fire ants in Taiwan, but also provides the opportunity for dynamics of two forms in a short period of invasion.

Materials & methods
Sampling procedure
In Chiayi County, because of its smaller size and patchy distribution, nests were collected intensively in each patch. The different sampling strategy was carried out in Taoyuan County with relatively huge area of infestation. According to the National Red Imported Fire Ant

Control Center (NRIFACC, http://www.fireant-tw.org/) annual report, we divided the study site of Taoyuan into two parts: the region located in central which was reported high density of fire ant mounds was treated as the core infestation; peripheral region which harbored fragmentary fire ant mounds was treated as an outlier infestation. Polygyne nests are believed that they expand the colony by budding process, resulting in new nests located just near the natal nest by 5~10m (Tschinkel, 1998). Based on this characteristic, if all the nests in such microgeographic scale were collected, determining the distribution of polygyne by nest as a sampling unit may have bias. In order to minimize the bias, in the core infestation we sampled by the nest cluster, an idea from Ross *et al.* (1997), which a group of nests of the same form located within 10 m of one another and separated from other such group by > 10 m**.** Furthermore, the outlier region was divided into 250 X 250 meters grids and a bait station was set with potato chip in each grid, representing almost 3000 grids in total. The subsequent surveillance was conducted in a grid with appearance of fire ant in bait station. GPS of each sampled nest was recorded and used as a measurement of inter-nest distance. We hope this kind of action could comprehend all the current infested area and reveal the real situation.

Determination of social forms

We identified the social forms in the field by counting the number of queens within the nests. After carrying back laboratory, 10-15 workers were pooled and extracted in bulk using the Puregene DNA extraction kit. These bulk-extracted samples were used in Gp-9 assays for social form determination (Valles & Porter, 2003). The method mentioned above cannot detect the b' allele which also expresses polygyne form in the native fire ant population, an additional method must be considered. We employed b and b' alleles multiplex PCR diagnosis developed by Mescher *et al.* (2003) to clarify the real monogyne nest. Each run includes positive, negative controls and blank.

Mapping

The nest confirmed by field survey and two Gp-9 assays will be mapped with the GPS data by using the computer software ArcView.

Results

Both Gp-9 diagnoses confirms the field data, that is the nest headed by more than two queens invariably harbor the b allele, resulting in a ~400bp band from Valles & Porter's assay (2003) and a ~200bp band from Mescher *et al.* (2003); but individuals from monogyne nests produce only B allele resulting in a ~500bp band from Valles & Porter's assay (2003) and no banding pattern from Mescher *et al.* (2003). In Taiyuan County, the number of polygyne nest clusters is significantly much more than monogyne in the core (Table 1), but monogyne stands absolute dominance in outlier infestation (Table 1). If we subsequently imported the GPS data of these nest clusters on the map, obviously high proportion of polygyne located in the central region and outlier region presented a tendency of much more monogyne (Fig. 1). On the other hand, only five monogyne nests were found and polygyne nests seem a local dominant form in Chiayi. Considering the GPS data for nest locality, these five nests mosaic distribute in each patch within large proportion of polygyne population (Fig. 2).

Discussion

The sampling procedures in this study do show relatively more comprehensive and intensive than the previous study performed by Chen *et al.* (2006), which several counties represent no collection of polygyne or monogyne, although some of them with severe infestation. Furthermore, townships in Taoyuan differ much in the shape according to the administrative boundary with some elongated but some long and narrow, the bias therefore occurs with township-based sampling methods. Our methods broke the administrative boundary and treat entire infested area as two separated units which not only comprehend current distribution of fire ant in Tayuan but also provide a large scale level to understand overall trends of distribution.

The overall data suggest most polygyne nest clusters locate central region but high proportion of monogyne nest clusters present in outlier region of Taoyuan, which was contrast with the previous point of view proposed by Chen *et al.* (2006) that most townships have a tendency to have more polygyne colonies than monogynes. Taking the biology of polygynous nests into consideration, the satellite nests resulting from colony budding process might locate within 5~10m of each other. If all the satellites nests derived from the same original nest were sampled, the number of polygyne nests must be much higher than monogyne whose newly mating queens conduct long-distance flight to found independently and nests located within certain distance because of the territory defense (DeHeer & Tschinkel, 1997; Tschinkel, 1998). Therefore, the pattern they found might be the bias resulted from the dispersal mode of polygyne.

The distribution pattern of two social forms we discovered in two infested area may imply something important for our control strategy or the evolutionary dynamics of two forms. First, theoretically speaking, newly mating queens from monogyne nests have large amount of fat reserve that enables them to fly long distance and imitate their reproduction, showing a potential to quick spread out; polygyne nests reserve low fat and shows a relatively slow oogenesis, resulting in a slow outward expansion (Bourke, 2002). According to alternative dispersal biology conducted by two forms, the polygyne nests seem to gradually spread outward from the north part of Lujhu and Dayuan township where are just near to the Taoyuan International Airport by 3~5km (Fig. 1). This speculation likely confirms the viewpoint that the fire ants may first arrive at airport and hitch hike by the air-transportation (Huang *et al.*., 2004). Second, monogyne located in outlier region of Taoyuan County generates the difficulties in our eradication program. Newly mating queens with superior flight ability from monogyne nests located in outlier have great potential to continue expanding outward and make nearby townships outside the Taoyuan County stand at high risk to be colonized by flying monogyne alates. This also suggests that our control treatment should be therefore carried out from outside to inside, rather than from inside to outside. Third, only five monogyne nests found in Chiayi and their mosaic distribution may indicate that initial invasion may only consist of polygyne and the monogynes are most likely derived from existing polygyne nests. Finally, monogynes reside in the outlier act as a pioneer to seek the suitable area to fund a new nest and precondition the environment and native fauna for the subsequent arrival of polygyne. It seems that two forms cooperate perfectly to reach their ultimate goal: becoming a successful invasive species.

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Tables & figures

Table 1 Distribution of nest cluster of two social forms in Taoyuan and Chiayi

Population	Infestation type	No. of polygyne nest cluster	No. of monogyne nest cluster
Taoyuan	core infestation	73	37
	outlier infestation	18	66
Chiayi	total infested area	80*	5*

*Asterisks represent that the unit for determining distribution of two social forms was nest in Chiayi because of relatively smaller infested area.

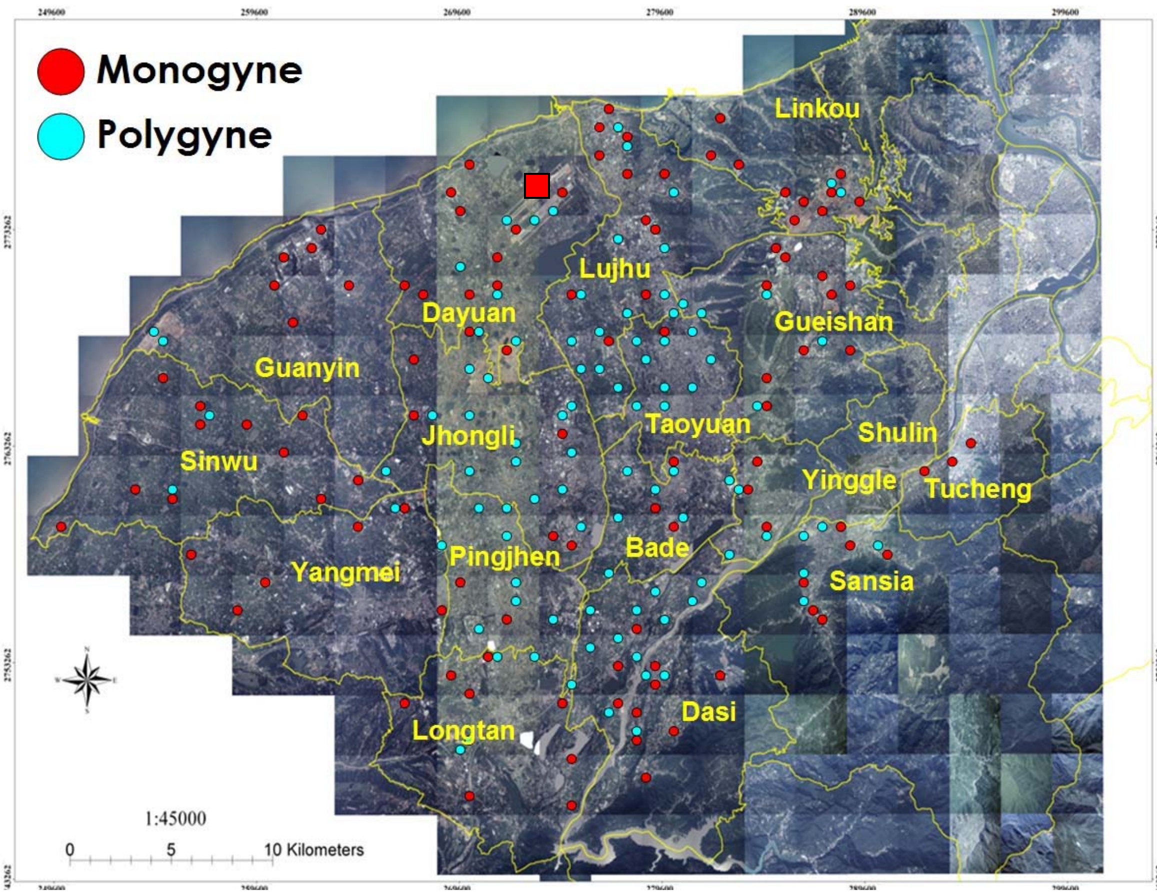

Fig. 1 The map showing the geographical distribution of polygyne and monogyne fire ants in the Taoyuan County with red and blue spots representing monogyne and polygyne nest cluster, respectively. The red square denotes the locality of Taoyuan International Airport.

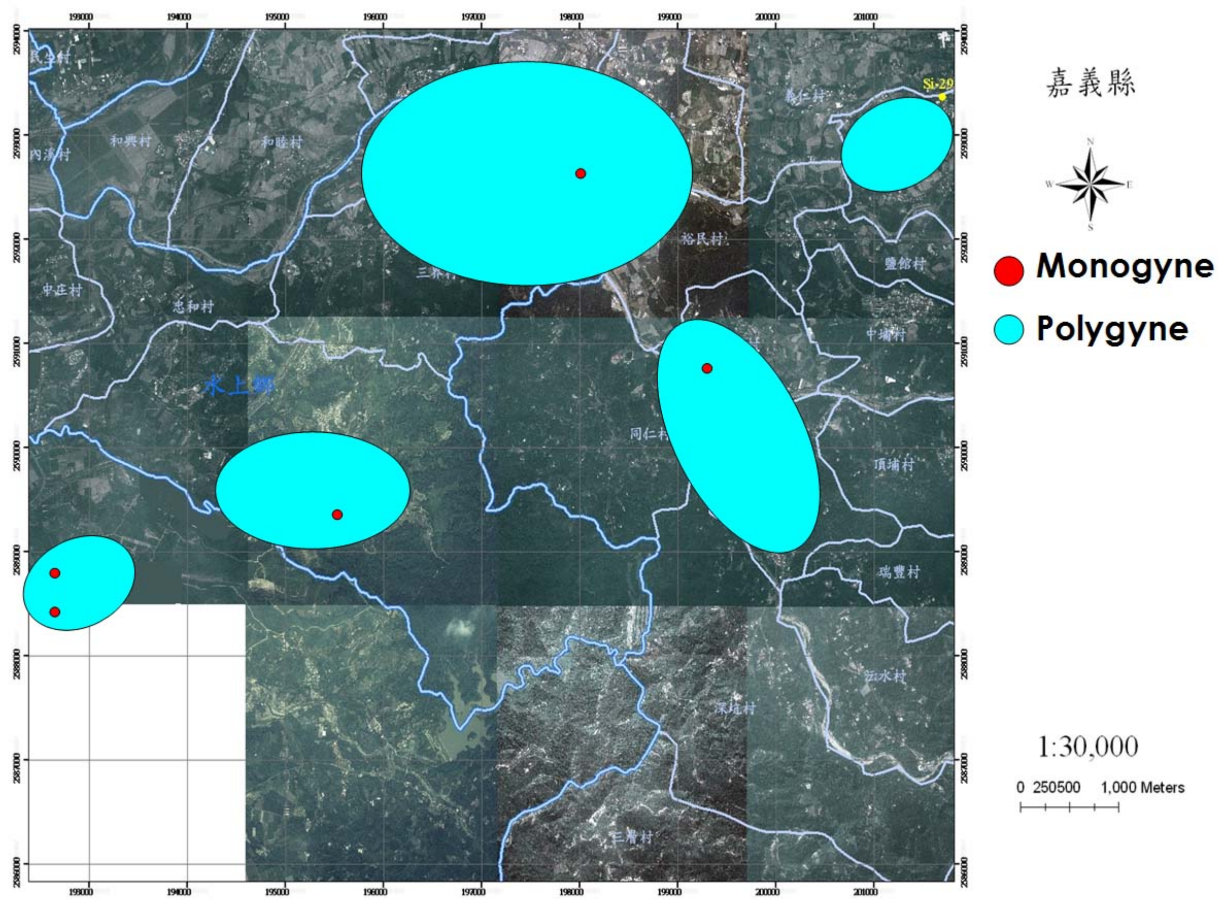

Fig. 2 The map showing the geographical distribution of polygyne and monogyne fire ants in the Chiayi County with red and blue spots representing monogyne and polygyne nest cluster, respectively.

Bacterial Microbiology of the Red Imported Fire Ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), Midgut

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Endosymbiotic bacteria have been associated with several physiological processes in insects, including digestion. However, the development of new molecular tools has allowed the discovery of microorganisms that manipulate insect reproduction, development and even provide defense against parasitoids and pathogens. In this study we investigated the presence of bacteria inside the Red Imported Fire Ant (*Solenopsis invicta*) midgut using different methods.

Electron Microscopy provided evidence of a possible role of these bacteria in the red imported fire ant (RIFA). Scanning Electron Microscopy (SEM) images of the fourth instar larva, extraorally digesting the solid food, support the idea of enzymatic activity in the saliva. After completing their development, the larva excretes the meconium which is fed to the queen or queens, indication of an important nutritional role. Presence of bacteria in the midgut lumen and inside specialized tissues was investigated using Transmission Electron Microscopy (TEM). No specialized structures or tissues had been found yet, but further investigation will be required.

Microorganisms were also isolated and cultured for molecular analysis. The small-subunit ribosomal RNA gene was amplified from bacterial genomic DNA using the Polymerase Chain Reaction (PCR) and consensus sequence primers. Restriction Fragment Length Polymorphism (RFLP) analysis revealed ten unique profiles. These isolates were identified as *Enterococcus* sp., *Enterobacter* sp., *Kluyvera cryocrescens, Lactococcus garvieae,* uncultured bacterium #38, *Pseudomonas aeruginosa, Achromobacter xylosoxidans, Bacillus pumilus, Listeria* sp., and *Serratia marcescens* when queried against the NCBI database.

Three of the strains, including the species closely related to well known symbionts of other insects, were genetically modified with the plasmid vector pZeoDsRed and successfully reintroduced into fire ant colonies. Strong fluorescence of DsRed was detected throughout the larval stage up to seven days after introduction. The transformed bacteria can still be rescued after pupal emergence; however most were passed out in the meconium. We further demonstrated that nurses contributed to the spread of the engineered bacteria within the colony by feeding the meconium to naive larvae. The role of these bacteria is being investigated by measuring the effects on the larvae and colony following antibiotic treatment. Molecular tools are also being used to determine the abundance and diversity of bacteria in samples from different counties and states.

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No Evidence for *Wolbachia* Phenotypic Effects in the Fire Ant *Solenopsis invicta*

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Wolbachia are intracellular bacteria that induce phenotypic effects in many arthropod hosts that enhance their own spread within host populations, despite sometimes also having deleterious effects on host fecundity and viability. The most common phenotypic effect observed is cytoplasmic incompatibility (CI). CI-inducing *Wolbachia* modify the sperm so that infected males are incompatible with uninfected females, effectively sterilizing the uninfected females they mate with and providing an advantage to infected females, who can mate with either class of males. Previous studies have shown that the *Wolbachia* variant wSinvictaA typically occurs at low prevalence in native *Solenopsis invicta* populations, has likely been horizontally transmitted into *S. invicta* on three or more times, and has been frequently lost from lineages over time. The instability of wSinvictaA infections in host lineages raises the possibility that these *Wolbachia* have negative fitness effects on their hosts. The objective of the present study was to identify the phenotypic effects, if any, that wSinvictaA has in *S. invicta*, and to use the data generated to better understand the distribution of wSinvictaA in South America, as well as provide insight into the utility of *Wolbachia* for biological control of *S. invicta*. To accomplish our objective, we collected infected and uninfected newly mated *S. invicta* queens from mating flights in Puerto Iguazú, Argentina and measured queen productivity and brood production patterns in the laboratory during colony founding.

Wolbachia infection had no effect on either the weight of queens at the beginning of observations, queen body size (head width), or on queen survival during simulated claustral colony founding. Production of all stages of offspring by uninfected queens was similar to that of infected queens, and there were no significant effects of *Wolbachia* infection on any measure of queen productivity. During claustral colony founding queens rear the first generation of workers on trophic eggs produced solely from their own fat stores, thus the quantity of weight lost is closely related to productivity. Not surprisingly, weight loss by queens during the pre-emergence period was also not significantly different for the two classes of queens. In addition, we used multiple regression to determine whether *Wolbachia* infection affected the efficiency with which queens converted their fat stores into offspring. As expected, regression of counts of total brood on queen weight loss yielded a significant model, but infection status had no effect on either the model slope or intercept. Thus *Wolbachia* infection had no effect on the efficiency with which queens converted fat stores into brood numbers.

In addition to studying putative direct fitness effects, we evaluated brood production patterns to determine whether wSinvictaA induces CI in *S. invicta*. In our sample of uninfected individuals, three produced no viable brood and seven produced at least one male, possibly consistent with mating incompatibility. However, this observed proportion (0.044 of uninfected females) was significantly less than the expected value of 0.16 given the population wSinvictaA prevalence of 0.16. (16% of uninfected females should mate with infected males and exhibit CI.) Of the infected queens, one individual also failed to rear any brood, which we would not predict if this phenotype was induced by CI. An additional infected queen produced males. In total we observed production of at least one male by 0.029 of our queens (7 uninfected queens + 1 infected queen). A likely explanation for the male production by these queens is that they were diploid-male producers, rather than exhibitors of CI.

Wolbachia are primarily transmitted via the egg cytoplasm, and fidelity of maternal transmission is important for *Wolbachia* population dynamics. We measured fidelity of maternal transmission for wSinvictaA in mature *S. invicta* colonies and found that it was less than perfect, with 3% of 145 workers (29 colonies) lacking infection.

Our results strongly suggest that wSinvictaA has no meaningful phenotypic effects on *S. invicta* queens. Therefore this *Wolbachia* variant is clearly unsuitable for the biological control of *S. invicta*. If wSinvictaA do not induce CI, and have imperfect maternal transmission, it is not clear how infections either spread or are maintained in host populations. One possible scenario for the persistence of wSinvictaA with imperfect transmission fidelity and no phenotypic effects is that infections are maintained in the native range by frequent horizontal transmission events which compensate for infection loss. Current prevalence as observed in Puerto Iguazú, and elsewhere, could persist if infection loss takes place at roughly the same rate as horizontal transmission, or be ephemeral if loss takes place at a higher rate. Clearly, *Wolbachia* infections are lost from populations, since in the colonization of the USA almost all *S. invicta* populations have shed these microbes, and *Wolbachia* infections have been repeatedly lost from host lineages in the native range. Lending further support to this scenario, previous studies have concluded that the *Wolbachia* infection dynamics in the native range have involved multiple ancient horizontal transmission events.

Proteomics as a tool for developing IFA control strategies

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The complete set of proteins expressed by a tissue or organism is called the proteome. We present two recent proteomic analyses of fire ants which provide us with information relevant to possible control strategies.

1) Longevity. Social insect queens have much longer lifespans than solitary female insects from the same order, or workers from the same colony (Keller & Genoud, 1997). Queen lifespans longer than 20 years have been documented in numerous ant species (Wilson & Hölldobler, 1990). Queen fire ants (*Solenopsis invicta*) live about seven years, workers about one year, and males for a few weeks. Caloric restriction and protection from oxidative damage are two mechanisms known to extend lifespans in other animals. Clearly queens are not calorie-restricted, due to the high metabolic rate required for continuous egg production. There is some indirect evidence against greater oxidative damage protection in queen ants compared with ant workers (Parker et al., 2004) and queen honeybees compared with honeybee workers (Corona et al., 2005). Nevertheless, previous studies have not directly compared the overall levels of oxidative damage to queen and worker proteins. We used two-dimensional gel electrophoresis and dinitrophenylhydrazine (Talent et al., 1998; Conrad et al., 2001) to measure the amount of oxidative damage to proteins in RIFA workers and queens. The results show widespread oxidative damage to queen proteins, qualitatively at higher levels than for workers. Therefore, protection from oxidative damage to proteins does not explain fire ant queen longevity.

2) Pheromone-binding proteins. Chemosensory proteins (CSPs) are expressed in many insect sensory appendages (Pelosi et al., 2005) and are thought to be involved in chemical signaling by ants (Ozaki et al., 2005). Using a pattern search, we identified at least eleven unique CSP sequences in the Lausanne fire ant EST library. We compared the RIFA CSP sequences with other known hymenopteran CSPs (see figure). The phylogenetic tree suggests that the fire ant protein SICSP7 is similar to a bee CSP expressed only the antenna. Thus, it is a good candidate for a pheromone-binding protein. However, one group of five RIFA CSP sequences has no close hymenopteran homolog. Using 2-dimensional gel electrophoresis and protein sequencing, we examined the fire ant worker antennal proteome and found SICSP1 to be expressed at high levels in the worker antenna. Therefore, SICSP 1 and its close homologs, SICSP2, 3, 5 and 10 may also be candidate pheromone-binding proteins.

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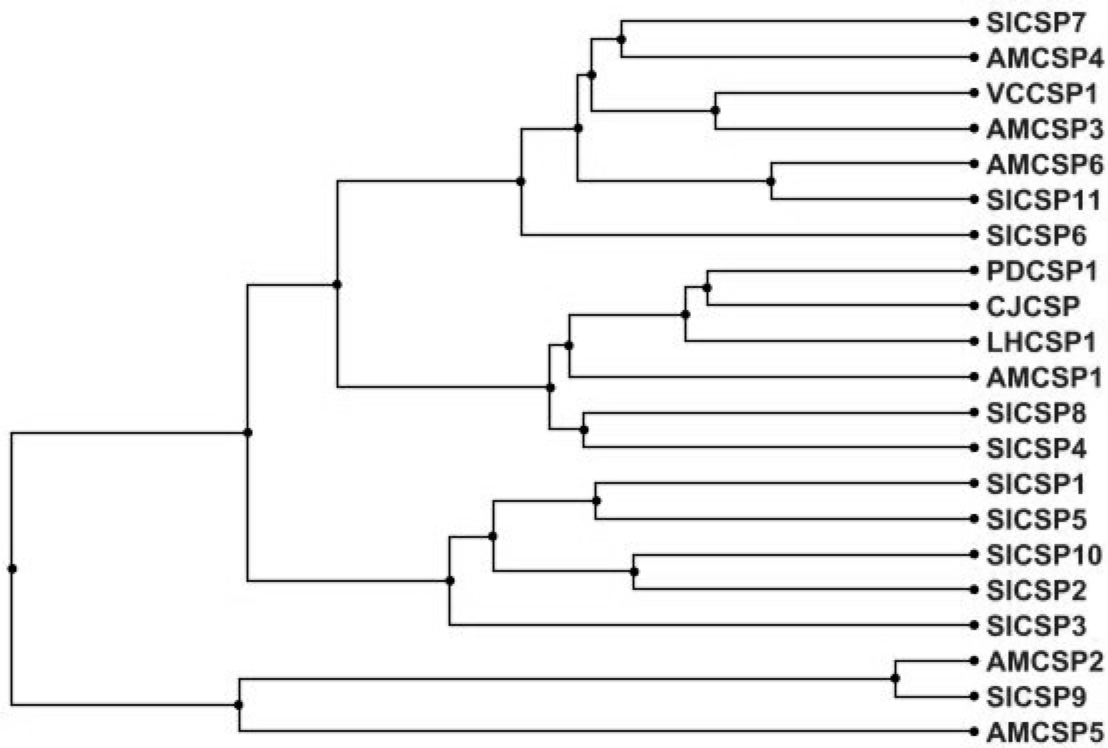

<u>Phylogenetic tree of chemosensory proteins (CSPs).</u> Eleven new fire ant CSP sequences which we identified in the Lausanne fire ant EST library are compared with other hymenopteran CSPs: SI, *Solenopsis invicta*; AM, *Apis mellifera*; VC, *Vespa crabro*; PD, *Polistes dominulus*; CJ, *Camponotus japonicus*; LH, *Linepithema humile*. The fire ant antennal protein SICSP1, along with SICSP2, 3, 5 and 10, have no close hymenopteran homologs.

Imported Fire Ant Responses to Interspecies Gland Extracts

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Imported fire ants use venom gland contents to subdue their prey, for defense against predators and as antimicrobials. The venom is composed predominantly of piperidine alkaloids, biosynthesized in the poison gland, stored in the poison sac and dispensed by the sting apparatus. We conducted species specificity studies on the extracts from poison glands of *Solenopsis invicta*, *S. richteri* and the *S. invicta* × *S. richteri* hybrids. *S. invicta, S. richteri* and the hybrids were respectively collected from Williamson, Maury and Sequatchie Counties in Tennessee. Ant species and venom alkaloid profiles were confirmed using gas chromatography (GC) analysis. In petri dish choice tests, we evaluated intra- and interspecies responses of venom gland extracts. *S. invicta* workers were attracted to their own poison gland extracts but repelled by extracts from *S. richteri* and hybrids. *S. richteri* were weakly attracted to own and S. invicta, but indifferent to hybrid gland extracts. Hybrid workers were repelled by *S. richteri* gland extracts, but indifferent to their own or to *S. invicta* extracts.

Evaluation of a water tolerant bait for red imported fire ant *Solenopsis invicta* (Hymenoptera: Formicidae) in Taiwan

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Abstract

To develop a water tolerant bait for the control of red imported fire ant (RIFA) *Solenopsis invicta*, evaluation of the attractiveness of microencapsulated (MC) pyriproxifen based corn grit bait (PCGB) "Distance" to the RIFA and a modified hot dog as a new carrier were conducted in the laboratory conditions 25 ± 2°C and 55 ± 2% RH. The PCGB gained 5.5 times more than its original weight when that was soaked in water for 3 h. In addition, the water soaked PCGB attracted significantly less number of RIFA workers (1.4 ± 0.24) than the normal dry one (2.6 ± 0.4). The 1 and 3% MC PCGB gained slightly less weight than the normal one when both baits were soaked in water for 3 h. Moreover, the total numbers of RIFA workers attracted by 1 and 3% MC PCGB were statistically similar with the normal one in the water soaked (5 h) condition. The modified hot dog could tolerate water 1.7 times more than the normal PCGB and could attract significantly higher number of RIFA workers (8.67 ± 0.33) than the normal one (0.67 ± 0.33) when both were soaked in water for 3 h. Although the MC PCGB could tolerate water better than the normal one, it could not attract significantly higher number of RIFA than the normal PCGB. Field studies of the performance of modified hot dog and control efficacy of MC PCGB are still under the study.

Key words: bait, water tolerance, microencapsulation, *Solenopsis invicta*

Eradication of Fire Ants (*Solenopsis invicta*) Using Liquid Nitrogen and Heated Gas Pulses

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William Can-Jen Ma[2], and C.C. Lin[3]
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In 2004, Red Imported Fire Ants (RIFA) were imported into Taiwan, several physical methods were developed for the RIFA control, methods such as asphyxiation, electrical shock, liquid nitrogen (LN_2) freezing and heated gas pulses heating were tested and evaluated for the eradication of fire ants. It was found that the liquid nitrogen (LN_2) freezing can eradicate the whole population of RIFA in the nest instantly and effectively. Although, the heated gas pulses heating is not as efficient as LN_2 freezing for RIFA control, due to its less cost than LN_2 freezing, the heated gas pulses heating is proved to be a useful method for RIFA control in large infested areas. Several merits of the two physical methods in RIFA control were approved. For examples, no toxic or chemical residual left in the soil, no drug-resistant side effect, treatment is limited in the area of nests, rapid and high effectiveness, and not influenced by bad weather. The LN_2 freezing and heated gas heating are proved to be suitable for RIFA control in the local areas such as home yards, public parks, golf courses, high population communities and the places where the RIFA have to be removed instantly or the non-toxic result is the highest priority, such as for pastures and farms. For large area RIFA control, a combination of insecticide and LN_2 freezing can be applied. After application of insecticide, the residual colonies those are hard to be eradicated by insecticide, can then be removed by LN_2 immediately. In addition, LN_2 freezing is also an excellent sampling tool for biology research.

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Methods and Results

LN_2 can rapidly freeze nests and kill RIFA, it gives no chance to queen for escape (Fig. 1). A pile of dead ants including queens were removed to outside of the nest by residual workers later (Fig. 2.). LN_2 freezing was used to sample the nest for biology research (Fig. 3, 4). Hot air pulses were injected into RIFA nest, mass destruction of RIFA was observed in the open nest (Fig. 5). All the ten nests in a field experiment showed no fire ant activity after three weeks of LN_2 treatment (Fig. 6). In average, 6-18 liters of LN_2 is required to clean a nest, the actual amount depends on the real size of nest.

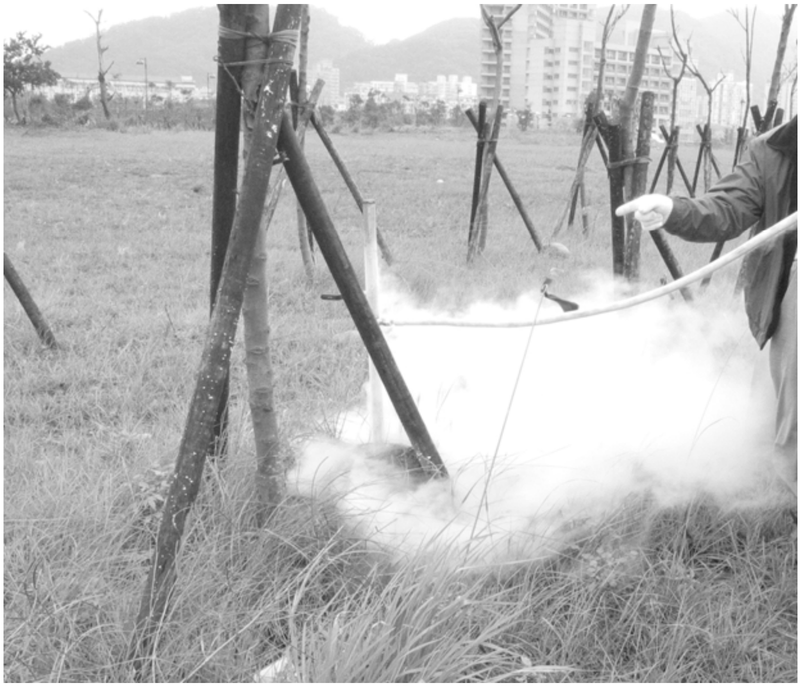

Fig. 1. LN_2 was introduced into a RIFA nest in the campus of National Taipei University, the nest was frozen and all RIFA were killed immediately. The mist is the nitrogen gas escaped from the nest as LN_2 vaporizes.

Fig. 2 A pile of dead ants was removed to outside of the nest by residual workers, those went out to search food during LN_2 treatment.

Fig.3 The nest treated by LN_2 treatment was dug out from ground for biology study

Fig.4 The fresh dead bodies obtained from the nests treated by LN_2 for biology study.

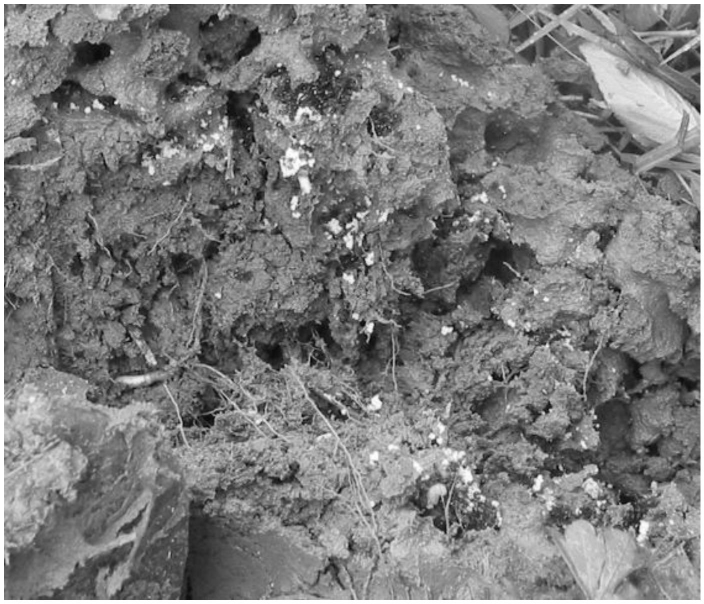

Fig. 5 The dead fire ants, pupa, and larva are founded in a nest treated by heated gas pulses.

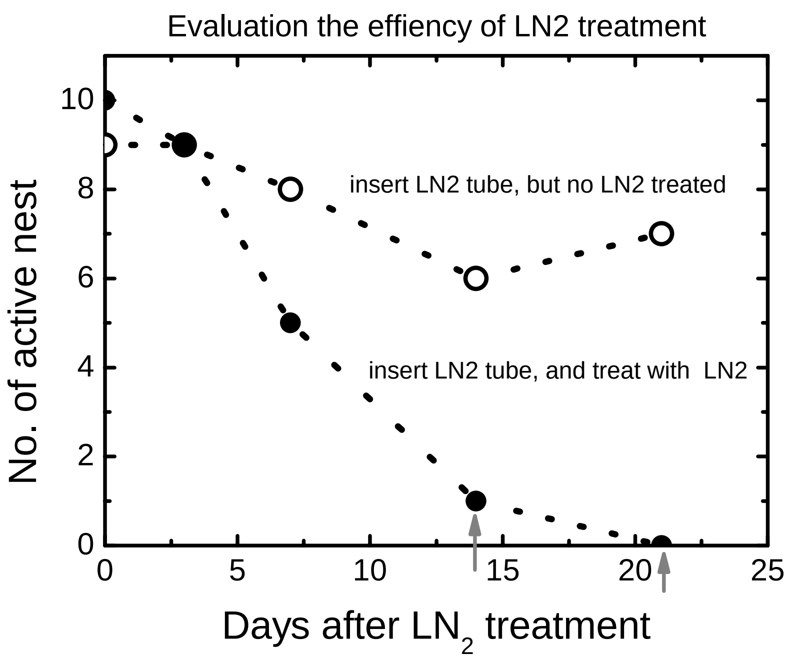

Fig 6 All the ten nests, represented by solid circles, showed no fire ant activity three weeks after LN_2 treatment; the open circles representing the control group only shows a small fluctuation.

Reference

Tschinkel, W. R., Howord, D. F., 1980. A simple, non-toxic home remedy against fire ants. J Georgia Entomol. Soc. 15(1), January, 102-105.

Utilization of odor sensibility of dogs in detecting the red imported fire ant (*Solenopsis invicta*) in Taiwan

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To use sniffer dogs to detect the red imported fire ants and nests in Taiwan, the test of minimal odor or number of red imported fire ants was made in Detector Dog Training Center (DDTC), NPUST. Nine quantification samples containing 80, 40, 20, 10, 5, 4, 3, 2 ants and 1 ant were used in this test. The quantification sensibilities are 95 to 100 % for five ants and above; it drops to 80 and 65% for four ants and one ant respectively. The experimental result showed that the sniffer dogs had very excellent sense of smell and scent association with the red imported fire ants, it was proved that sniffer dog is a potential bio- detection tool for fire ants. In addition, a preliminary standard procedure of operating was established and the utilization of canine for long-term monitoring and control of the red imported fire ant was proposed and tested in Taiwan.

Methods and Results:

The flow chat shows the standard procedure of fire ants detection carried out by a sniffer dog (Fig. 1). In Fig. 2 the dog was detecting the fire ants in a sugar can field. Once the sniffer dog pinpointed the spot of a fire ant nest (Fig. 3), the nest was marked by GPS (Fig. 4). Then identification of red imported fire ant will be conducted and followed by the necessary treatment of eradication. A pest relief will be announced subsequent to the complete fire ant elimination. Sniffer dogs will keep on monitoring the area for any fire ant re-infestation. In Fig. 6 the percentage of successfully spotting the fire ants versus the number of fire ants.

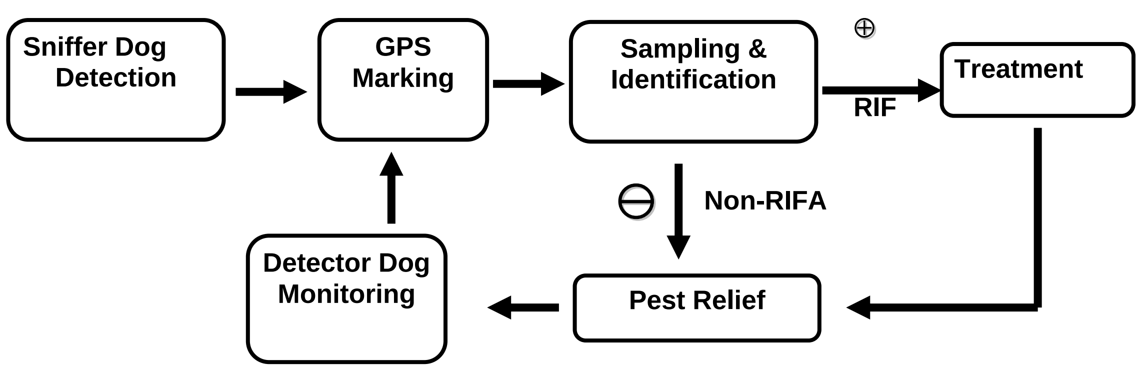

Fig. 1. The standard procedure of fire ant detection using a sniffer dog.

Fig. 2. Sniffer dog worked in sugar can field.

Fig. 3. Dog pinpointed a RIFA nest.

Fig. 4. Using GPS to mark the location of the nest founded

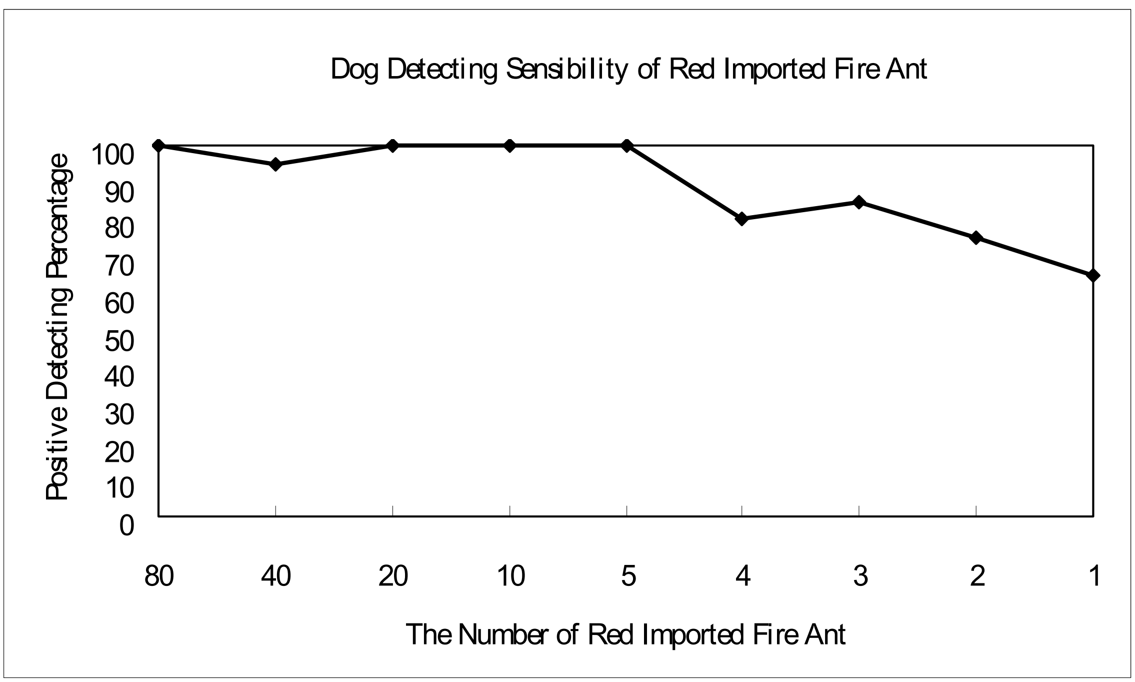

Fig. 5. The percentage of sniffer dogs can successfully spot the fire ants. Two dogs were used for the experiment.

Fire Ant Pesticide Trials at Knott's Berry Farm, 2006

Dr. Les Greenberg, Ph.D.[1] and Charlie Cassidy[2]

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Introduction

We began a study at Knott's Berry Farm on May 4, 2006, to determine the efficacy of new red imported fire ant pesticides. Metaflumizone belongs to a new class of pesticides called semicarbazones. This pesticide inhibits the sodium channel of the insect nervous system, causing "relaxed paralysis" of the insect. The compound is also a candidate for reduced risk status by the US EPA. It is a fast acting pesticide that can give a rapid reduction in ant activity. This product will be available in California in 2007 under the name "Siesta".

Objectives

Our goal is to make new low toxic products available for fire ant control by generating efficacy data, and to compare these new compounds with others already on the market. For this study we decided to compare metaflumizone with another product called Advion (indoxacarb). We want to know if they give similar results over time.

Methods

At Knott's Berry Farm we selected several areas for our trials. First, Orange Co. Vector Control staff surveyed for fire ant mounds. The locations of mounds were plotted on aerial maps so that plots could be chosen with similar ant densities. A parking lot that had fire ants living in the vegetated islands and grassy areas was divided into 4 plots that were at least 50 ft apart. Half of these plots received the metaflumizone, and half Advion. Areas outside the plots were treated with Amdro (hydramethylnon). An auxiliary parking area that was not in use contained grass and was infested with fire ants. We divided this area into 2 more plots. Finally, one more area near a sidewalk on the edge of a parking lot contained fire ants and was left as an untreated control for the duration of the experiment. Thus, there were 3 plots for each treatment and one untreated plot.

Within each plot ant numbers were monitored by putting on the ground every 10 ft a 9-dram plastic vial containing a corn chip as ant bait. Where birds were a problem, wire cages were placed over the vials to make them inaccessible to the birds. The vials were left on the ground for about 1.5 hrs. Vials containing fire ants were then capped and brought back to the laboratory where the ants were counted. Vials with chips were placed at the same locations at each sampling period, so that each vial served as its own control over time.

Pretreatment ant numbers were determined on May 4 using the corn chips. Advion and metaflumizone fire ant baits were then broadcast in the plots using a hand spreader at the recommended 1.5 lbs/a. The next day we returned to see whether foraging activity had decreased after 24 hrs. We also did follow-ups at 1 week, 1 month, and approximately monthly thereafter through November. Due to a resurgence of ants in June and July, a second application of the pesticides was done on August 31.

Results

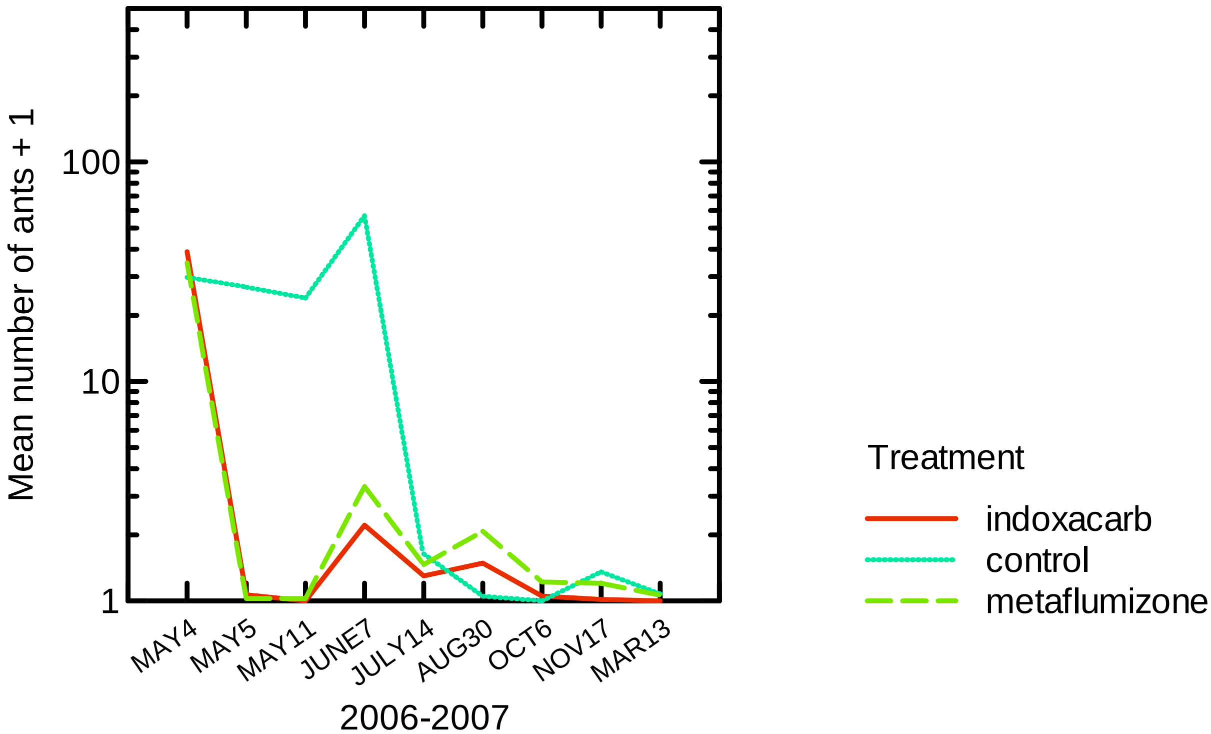
Figure 1 shows mean values for ant counts on corn chips over time. Table 1 shows the percent reduction in ant numbers from the pre-treatment values. 24 hrs after application the Advion and metaflumizone had both reduced ant foraging activity to nearly 0. One week later the same situation was seen. However, we began to see some resurgence of ant activity one month post-treatment on June 7. A second application of the baits was done on Aug. 31. Ant numbers then continued to decline in October and November. The control plot was apparently treated by a landscaper, as it declined after June 7. There was no significant difference between the Advion and metaflumizone treatments whether we looked at their post-treatment grand means, or within any single week. Both treatments showed reductions in ant numbers > 98% in October and November.

Discussion

Both products gave very rapid knockdown of the fire ants; either one would be a good product for situations where immediate reduction of ant numbers is essential (for example, where an event is to be held in an area with fire ants). Furthermore, both products are considered low toxic and are very safe in the bait formulation that was used. Although some resurgence in ant numbers was observed after 1 month, ant numbers continued to decline after that period. We also noticed that the ants at the baits during the resurgence were extremely large workers. The absence of small workers may indicate that brood production in the colonies had ceased. Large workers live longer and their presence may therefore indicate a dying colony. We also observed the reappearance of other ant species at the corn chip baits after the fire ant treatments had gone out. These other species, including Argentine ants and *Monomorium* sp. ("little black ants") will attack dying fire ant colonies and hasten their disappearance.

In conclusion, both of the products we tested will be useful in fire ant control, particularly where very rapid control in necessary. In other trials the metaflumizone has out-performed Amdro in reducing ant numbers. We still need to see the long term outcome of these treatments going into next spring and summer.

Figure 1. Plot of data from Knott's Berry Farm. Treatments were done on May 4 and Aug. 31, 2006.



Treatment	Day1 (May 5)	Week 1 (May 11)	Week 5 (June 7)	Week 10 (July 14)	Week 17 (Aug. 30)	Week 22 (Oct. 6)	Week 28 (Nov. 17)	Week 44 (Mar. 13)
Meta-flumizone	99.95	99.95	56.95	95.52	89.22	98.46	99.31	99.7
Indox-acarb	99.86	100.00	86.54	96.54	98.17	99.85	99.98	100
Control	30.93	33.36	-65.95	93.04	99.89	100.00	98.42	99.8

Table 1. Percent reduction in ant numbers from pretreatment values. A negative value indicates an increase in ant numbers. Treatments were done on May 4 and Aug. 31.

Lethal Dose-Response Curves of Several Ant Species to Fire Ant Venom

L. Greenberg, J. N. Kabashima, C. Allison, M. K. Rust, J. H. Klotz, and T. D. Paine
Department of Entomology, University of California, Riverside

Abstract

We tested the lethality of venom from *Solenopsis invicta* against several ant species. Venom droplets were collected from the ant's stinger into 1 µl capillary tubes. The venom was then emptied into 0.25 ml PCR tubes and stored at -30° F until it was used. For lethality trials the venom was diluted with methanol. One 0.2 µl drop of this solution was then applied to the gaster of a test ant using a microapplicator. Ten ants were tested at each venom concentration. A control group of 10 ants was treated with 0.2 µl of pure methanol. After 2 hrs, mortality or morbidity of the test ants and controls was recorded. The lowest dose of venom applied was 0.025 µg for Argentine ants, and the highest dose was 255 µg for *S. invicta*. For comparison, 0.2 µl of undiluted venom would weigh 170 µg.

We tested *S. invicta* venom against the Argentine ant, *Linepithema humile*, the bicolored pyramid ant, *Dorymyrmex bicolor*, the California harvester ant, *Pogonomyrmex californicus*, and against small to medium-sized *S. invicta* workers. Taking into account the weight of the ants, the Argentine ant was most susceptible to the fire ant venom, followed by the pyramid ant, the harvester ant, and the red imported fire ant. The fire ant LD50 to its own venom was about 350 times higher than that of the Argentine ant (in terms of µg venom/mg wet body weight). As expected, the fire ant has a high tolerance for its own venom. On the other hand, the Argentine ant, which overlaps in range with the fire ant, is very susceptible to even tiny droplets of venom.

Application of Monoclonal Antibodies on Detection of Red Imported Fire Ant Venom Proteins and Antibodies in Mouse

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Abstract

Red imported fire ant (RIFA), *Solenopsis invicta* Buren, is one of important invasive insect species. It seriously impacts the wildlife, agriculture, public health, and economy. They usually attack creatures which have similar habitats with them. Rodents living on or under the ground probably encounter the RIFA and become victims. The venom of RIFA contains four major protein allergens and might cause serious allergy reaction. These proteins may remain in rodent body for a retention period then induce the production of antibodies against these proteins. We tried to test the serum of the injured rodents to investigate the retention of RIFA venom proteins and induction of antibodies, which may be applied to monitor the spreading of RIFA. Before the detection of mouse blood, we established the survey methods with the monoclonal antibody Rf-E7 against RIFA to study several ecological factors for RIFA. The results demonstrated that the most efficient antibody dilution rate for the DAS-ELISA (double antibodies sandwich enzyme-linked immunosorbent assay) of WBE (whole body extracts) assays is 10^5X while the efficacy of pure venom assay is similar between dilution rates 10^5 to 10^6X. The comparative assays of venom proteins were conducted among different social forms, worker sizes, and worker tasks by DAS-ELISA and SDS-PAGE. We found slight difference in the venom proteins of polygyne major workers with monogyne workers and polygyne minors in the results of SDS-PAGE. We also found that Sol i 4 made up the largest part of the venom proteins instead of Sol i 2 according to the gels. When analyzing the mouse serum, the venom proteins might be qualitatively and quantitatively detected according to the ELISA values and the induced antibodies were detected by Western blot.

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Endres Processing Presents Tast-E-Bait

David Vander Hooven and Darrell Courtney
Endres Processing, Ohio, LLC
1124 Fort Street, Maumee, Ohio 43537

Bakery waste has been around for years, but never thought of as anything other than a high quality livestock and poultry feed ingredient. However, when you think about it, cookies, bread, cake, donuts, etc., all attract insects, as well as snack food waste like corn, potato chips, cheese curls, caramel corn, candy bars and cereal wastes. Blend these wastes together in large uniform quantities (300,000,000+ pounds/year) then pellet or granulate them to obtain a powerful insect attractant and active ingredient carrier (Figs. 1-5). It is so well liked by insects, we call it Tast-E-Bait.

Tast-E-Bait (TEB) was also designed to be easily spread in cyclone type spreaders with excellent particle distribution and broad widths. TEB's density increases the pounds you may carry in all makes of spreaders, including aerial. Less downtime is required between application runs. An example of spreadability is shown in the Herd Spreader test data and data from The University of Louisiana (available upon request). There is much we can do to TEB, including adding non-toxic ingredients for you in our manufacturing process. Sugar aromas, molasses, and other enhancements can be added. TEB is made to be water repellent without hurting its attractiveness to imported fire ants, Mormon crickets, slugs, snails, mole crickets, etc (Fig. 6). In a controlled 2006 dog feeding trial, we found that dogs will not consume our product; therefore, TEB offers no taste attractiveness to dogs (Fig. 7).

To summarize, TEB has the following characteristics:

1) Easy spreader calibration – 20 foot swaths not uncommon.
2) Fill your spreader half as often as you do conventional bait/carriers.
3) Lower oil inclusion means fewer oil spots on driveways and sidewalks.
4) More weather resistant than other bait/carriers.
5) Lasts longer – works longer.
6) Dust free.
7) Dogs do not eat this bait/carrier.
8) Ultra uniform bait/carrier.
9) Highly acceptable to red imported fire ants.
10) Highly resistant to rancidity
11) Bait/carrier average analysis has a standard deviation of <1.2% (over data collections of 10 one-half year periods). Supporting the active ingredients without fail.
12) Handling and spreading this bait/carrier does not break it down.

Fig. 1.

Tast-E-Bait®

A Feast Fit For A Queen

An Ag-Chem Carrier Granular Bait

Easy calibration, 20' wide swath, refill of spreader half as often as with pregelled defatted corn.

Tast-E-Bait®		Tast-E-Bait®
Weather Resistant Granules		Weather Resistant Pellets
Grade -8+30		1/8 inch diameter
+8	<1%	Length: 1/4-3/8 inch
-8+20	96.7%	
-20+30	2.7%	Fines: <3%
-30	<1%	
Resistance to Attrition		Resistance to Attrition
98-99% (ASTM.E728-91)		98-99% (ASTM.E-7728-80)
Density: 34-36 lbs/ft.3		Density: 40-44 lbs/ft.3
Moisture: <10%		Moisture: <10%

Fig 2

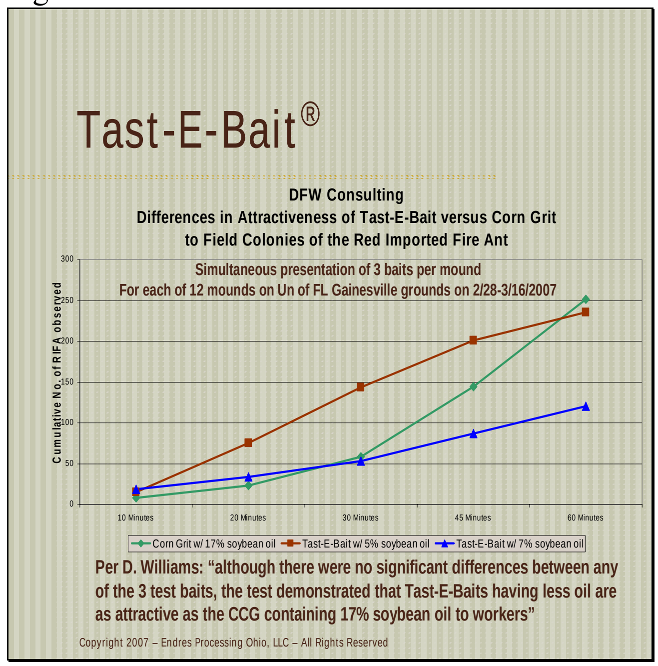


Fig. 3.

Tast-E-Bait®

Technical Data – Average Analysis

Sample Dates	Moisture %	Protein %	Fiber %	Ash %	Fat (AH) %	Sugars & Starches (NFE) %
January-June 2006						
Average	9.501	11.585	3.050	4.312	9.100	62.244
Standard Deviation	.687	.310	.506	.401	.814	1.399
July-December 2006						
Average	10.551	11.605	2.949	4.180	8.584	62.130
Standard Deviation	.875	.439	.632	.245	.687	1.176
	Fructose	***Glucose***	***Sucrose***	***Maltose***	***Lactose***	***Starch***
Average Sugars & Starch %	1.130	3.090	4.530	1.220	0.280	41.70
Standard Deviation %	0.350	1.180	4.340	0.270	0.050	1.25

All Testing done by Iowa Testing Laboratories, Inc. 4/1/07

Fig. 4

Tast-E-Bait®

Acceptability of various aged TAST-E-Bait samples, March 2003

Candidate	Standard	Bait Acceptance Ratio ± SD	Mean amt. of bait removed (g)	
			Candidate	Standard
TEB – 7/22/01	TEB – 7/30/03	1.00 ± 0.00	4.00	4.00
TEB – 5/9/03	TEB – 7/30/03	1.00 ± 0.00	4.00	4.00
TEB – 7/22/03	TEB – 7/30/03	1.00 ± 0.00	4.00	4.00
TEB – 7/30/03+4% soybean oil	Pregel corn grit standard	1.48 ± 1.07	3.54	3.33

Pregel corn grit contained 30% soybean oil as standard. TAST-E-BAIT contained 5% soybean oil.

Tests performed at USDA/ARS, Gulfport, Mississippi

Fig 5

Tast-E-Bait®

Tast-E-Bait®

David I.D. Vander Hooven
Director, Product Development, Tast-E-Bait®
Home Office: 1124 Fort St., Maumee, OH 43537
Home Phone: 419-893-1540
Home Email: byprodvh@buckeye-access.com

ENDRES PROCESSING OHIO, LLC
Innovative Processing of Food Waste
Ohio Office: 701 W. Johnson St., Upper Sandusky, OH 43351
Ohio Phone: 419-209-0216
Ohio Fax: 419-209-5010
Ohio Email: mkolarik@endresprocessing.com

DOG FEEDING TRIAL 5/10/06

A local dog shelter was used to conduct a feeding trial to identify the acceptability of 1/8" Tast-E-Bait pellets. The dogs are currently fed a commercially produced dry dog food once a day at approximately 8:30 A.M. which has been done like this for over 10 years. The dogs are conditioned to know when they are fed. Three (3) dogs out of the group of twelve (12) were used with the Tast-E-Bait pellets. These three (3) large eaters were given the same amount of pellets for 45 – 90 minutes to observe them eating the pellets and then it was removed and replaced with dog food. This was done for three (3) consecutive days - May 3rd, 4th, and 5th. The dog warden who feeds and is the care taker of the dogs observed the dogs. This was the only person present which is the normal routine.

Each dog on each day walked up to the dog food dish containing the Tast-E-Bait pellets which was the same dish that is used daily, smelled the product, and then walked away. None of the dogs tasted the product nor was any product consumed during the three (3) days by any of the three (3) dogs. After the test period noted above, the Tast-E-Bait pellets were replaced with dog food and the dogs consumed it as normal. The Tast-E-Bait WR-3 product had been produced less than 30 days prior to May 3rd.

/s/ Andy A. Niederkohr

Andy A. Niederkohr, Wyandot County Dog Warden

Fig 6

Tast-E-Bait®

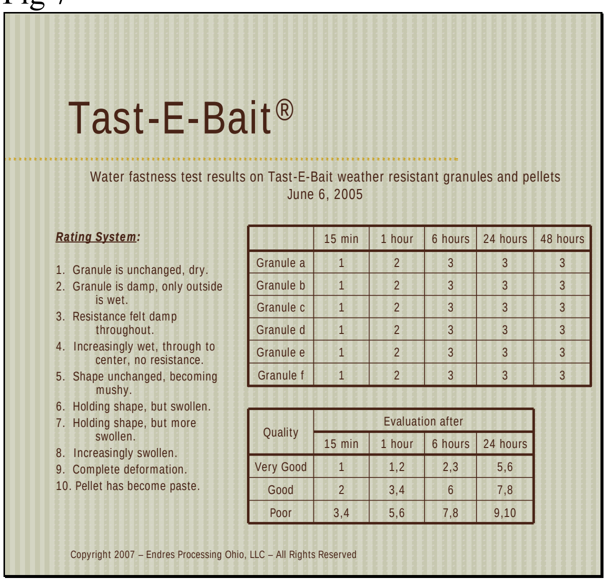
EPA Approval for use in Pesticide Formulations

From: Gandi.Bipin@epamail.epa.gov
To: byprodvh@buckeye-access.com
Sent: Tuesday, August 23, 2005 – 7:42 am
Subject: Tast-E-Bait WR-3

This is to confirm that the product Tast-E-Bait WR-3 based on the information you have submitted on August 3, 2005, to the Agency, can be used as an inert ingredient in the pesticide formulations applied to the growing crops only under 40 CFR 180.920 and applied to animals under 40 CFR 180.930.

Bipin C. Gandi
Inert Ingredient Assessment Branch
Registration Division (7505C)
(703) 308-8380 (voice)
(703) 305-0599 (fax)
Email address: gandi.bipin@epa.gov

Fig 7



Tast-E-Bait®

Water fastness test results on Tast-E-Bait weather resistant granules and pellets
June 6, 2005

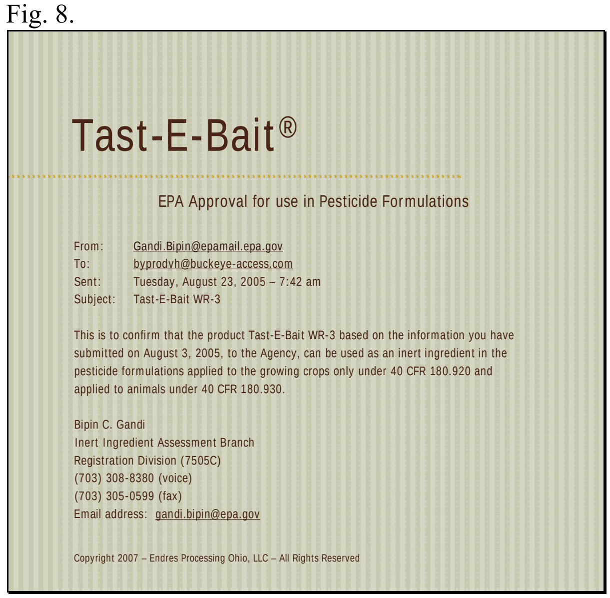
***Rating System*:**

1. Granule is unchanged, dry.
2. Granule is damp, only outside is wet.
3. Resistance felt damp throughout.
4. Increasingly wet, through to center, no resistance.
5. Shape unchanged, becoming mushy.
6. Holding shape, but swollen.
7. Holding shape, but more swollen.
8. Increasingly swollen.
9. Complete deformation.
10. Pellet has become paste.

	15 min	1 hour	6 hours	24 hours	48 hours
Granule a	1	2	3	3	3
Granule b	1	2	3	3	3
Granule c	1	2	3	3	3
Granule d	1	2	3	3	3
Granule e	1	2	3	3	3
Granule f	1	2	3	3	3

Quality	Evaluation after			
	15 min	1 hour	6 hours	24 hours
Very Good	1	1,2	2,3	5,6
Good	2	3,4	6	7,8
Poor	3,4	5,6	7,8	9,10

Fig. 8.



Tast-E-Bait®

EPA Approval for use in Pesticide Formulations

From: Gandi.Bipin@epamail.epa.gov
To: byprodvh@buckeye-access.com
Sent: Tuesday, August 23, 2005 – 7:42 am
Subject: Tast-E-Bait WR-3

This is to confirm that the product Tast-E-Bait WR-3 based on the information you have submitted on August 3, 2005, to the Agency, can be used as an inert ingredient in the pesticide formulations applied to the growing crops only under 40 CFR 180.920 and applied to animals under 40 CFR 180.930.

Bipin C. Gandi
Inert Ingredient Assessment Branch
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Seasonal Shifts in the Hyperspectral Characterization of Imported Fire Ant (Hymenoptera: Formicidae) Mound Features in Turfgrass

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Abstract. Safe, expedient, and cost-effective field- to landscape-scale treatments of imported fire ant (IFA) infestations require technological developments that exploit the use of remotely-sensed contrasting features to detect cryptic mounds in heavily-managed turfgrass. Ground-based implementation of hyperspectral techniques in the field-scale quantification and seasonal monitoring of IFA colony distributions is a prerequisite for either designing ground-based sensor arrays or for equipping airborne multispectral digital cameras with appropriate band-pass filters to maximize mound detection for multiple-scale surveys. The objectives of this study were twofold: (1) examine seasonally-acquired spectral reflectance characteristics of ant-affected versus undisturbed turfgrass and soils; and (2) identify bandwidths that enhance the detection of cryptic fire ant mounds in intensively-managed turfgrass areas. Reflectance data (N=22,000 full-range spectra collected August-September 2006), for sparsely-covered ant mounds (≤50% vegetation) from Mississippi sites in the North Central Hills and Delta physiographic regions, indicated that mean reflectance values for targets (i.e., bermudagrass, mound soil, and undisturbed bare soil) averaged over 50 nm bandwidths were most distinctive (P<0.001) from each other at 650-700 nm, 1450-1500 nm, and 2000-2050 nm during peak summer season. Reflectance data collected during the Summer-Fall transition displayed shifts in mound feature recognition in the visible (VIS) and near-infrared (NIR) regions, with distinctive bandwidths constrained to just the VIS region ranging from 600-700 nm (P<0.001). The development of new remote sensing monitoring tools, employing seasonally-acquired spectroradiometric data in turf as a model system, will aid site-specific management of fire ant infestations in perennial, warm-season turfgrass settings, help foster sustainable reduction of IFA populations, and benefit a broad base of stakeholders.

Comparative Electroantennogram Response of Two Phorid Fly Species to Different Species of Imported Fire Ants

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Abstract

In the past decade, two species of *Pseudacteon* phorid flies, *P. tricuspis* and *P. curvatus* have been released in many parts of southern United States for biological control of the invasive imported fire ant (IFA) complex consisting of red, black and hybrid IFA, *Solenopsis invicta*, *S. richteri*, and *S. invicta* × *S. richteri*, respectively. In a recent study, we demonstrated response of *P. tricuspis* to odor of *S. invicta* workers (Chen and Fadamiro 2007), suggesting semiochemical mediated host location. To determine possible differential olfactory sensitivity of phorid fly species to different species of IFA, we compared the electroantennogram (EAG) responses of both sexes of *P. tricuspis* and *P. curvatus* to body extracts of red, black, and hybrid IFA. The data showed a significant effect of sex on EAG response of both phorid fly species. Females of both species generally showed significantly greater EAG response than conspecific males to body extracts of the three IFA species. In general, *P. tricuspis* showed greater EAG response than *P. curvatus* to body extracts of all three IFA species. Body extracts of black and hybrid IFA elicited significantly greater EAG response in both phorid species than did body extract of red IFA.

Introduction

Pseudacteon phorid flies (Diptera: Phoridae) are parasitoids of imported fire ants (IFA), *Solenopsis* spp. (Porter 1998). Two species, *P. tricuspis* and *P. curvatus* have been released since 1995 in southern U.S. for biological control of invasive imported fire ants (IFA). Both species show strong preference for ants in the *saevissima* complex (consisting of red, black, hybrid IFA, *S. invicta*, *S. richteri*, and *S. invicta* × *S. richteri*, respectively) over closely-related *geminata* complex (including *S. geminata*) (Gilbert and Morrison 1997).

Despite the interest in phorid flies as biological control agents of IFA, little is known about their host location cues. Ants have evolved elaborate intraspecific chemical communication systems involving the use of pheromones for nestmate recognition, recruitment of nestmates (trail following), alarm signaling, mating, and attraction of workers to brood and the queen (Wilson 1962, Vander Meer and Porter 2002). These pheromonal signals may be exploited by parasitoids of ants as kairomones for host location (Morehead and Feener 2000).

In a recent study, we demonstrated the semiochemical mediated response of *P. tricuspis* to *S. invicta* workers using electroantennogram (EAG) and behavioral techniques (Chen and Fadamiro 2007). Other authors have previously reported specificity/preference of different species of *Pseudacteon* phorid flies to different IFA species (Gilbert and Morrison 1997, Porter 2000). In order to determine possible differential olfactory sensitivity of phorid fly species to different species of IFA, we compared the EAG responses of both sexes of *P. tricuspis* and *P. curvatus* to body extracts of red, black, and hybrid IFA.

Materials and Methods

Insects. *Pseudacteon tricuspis* and *P. curvatus* flies used in this study were reared on workers of red IFA (*S. invicta*) at the fire ant rearing facility of the USDA-ARS, CMAVE, Gainesville, Florida, U.S.A. Parasitized fire ant worker heads were received in batches and handled as described in Chen & Fadamiro (2007). Adult phorid flies utilized in the experiments were 1-2-day-old.

Red, black and hybrid (*S. invicta* × *S. richteri*) IFA used for extraction were collected from southwest Tennessee and north Mississippi, and raised in the laboratory using sugar solution with some crickets provided .

Extraction. Fire ant workers of different species were chilled to -20 °C for 15 min and then extracted with hexane under laboratory conditions for 24 h. The supernatant of each extraction was withdrawn into a glass vial and additional appropriate amount of hexane was added to result in 0.1 worker equivalent per μL (WE/μL) solutions. Further dilutions were made to give 10-4, 10-3, 10-2 WE/μL solutions for dose-response study. These solutions were kept in a freezer at -20 °C until used.

EAG recording. The EAG technique used in this study was same as previously described (Chen and Fadamiro 2007). A test series of odorant extracts of the same dose (0.001, 0.01, 0.1, or 1 WE) were applied to 12 antennae per sex per fly species.

Statistical Analyses. Data were analyzed by using the standard least squares fit model method (SAS Institute 2004) to determine the effects of fly species, sex, fire ant species (stimuli), dose and interactions on absolute EAGs. Further analysis of EAG data was performed by using analysis of variance (ANOVA) followed by Duncan's multiple comparison tests (SAS Institute, 2004). The effect of species and sex on EAG response was compared by using the Student's t-test (SAS Institute 2004).

Results

Sex exerted a significant effect on EAG response of both fly species. Body extracts of all three fire ant species elicited significantly greater EAG in females of both fly species than in conspecific males at 0.1 and 1 WE doses (Table 1). In general, body extracts of black and hybrid fire ants elicited greater EAG response in females of both fly species than did body extract of red fire ant, although this was not always significant (Table 1; Fig. 1A). Comparing the two fly species, both female and male *P. tricuspis* showed greater EAG response than counterpart *P. curvatus*, although this was significant only at some doses (Table 1; Fig. 1B).

Discussion

The results of this study showed that female of both phorid fly species are significantly more responsive than conspecific males to body extracts of workers of different fire ant species, consistent with the results previously reported for *P. tricuspis* (Chen and Fadamiro 2007). In general, body extracts of black and hybrid fire ant workers elicited significantly greater EAG response in both phorid fly species than did body extract of red fire ant. Assuming a correlation between EAG and behavioral response, these results may explain, at least in part, the observed preference of *P. curvatus* for black and hybrid imported fire ants (Porter 2000). Although *P. tricuspis* is commonly reared on red fire ant, preference of this species for red fire ant over black and/or hybrid fire ants has not been demonstrated. Our current data suggest that this is unlikely.

Both sexes of *P. tricuspis* showed greater EAG response than counterpart *P. curvatus* to extracts of all three fire ant species. This observed greater olfactory sensitivity of *P. tricuspis* may be related to its relatively larger size (Porter 1998) and/or larger antennae. Preliminary examinations of the antennae of *P. tricuspis* and *P. curvatus* using scanning electron microscopy (SEM) reveal no major morphological difference in the antennae of both species (Chen & Fadamiro, unpublished data). Ongoing and future studies will further characterize differential olfactory sensitivity of both phorid fly species to different species of imported fire ants.

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Table 1. EAG dose response of two *Pseudacteon* fly species to body extracts of three fire ant species (-mV ± SE, absolute EAG, *n* = 12/fly species/sex). Means across the same column for each species and dose followed by different letters are significantly different ($P < 0.05$). Means across the same row for each species marked with an asterisk (*) are significantly different between both sexes of the same species. WE = worker equivalent.

Dose	Ant	*P. tricuspis*		*P. curvatus*	
(WE)	species	Female	Male	Female	Male
	Red	0.20 ± 0.05	0.10 ± 0.03 b	0.12 ± 0.03	0.10 ± 0.03
0.001	Black	0.23 ± 0.06	0.15 ± 0.04 ab	0.14 ± 0.02	0.13 ± 0.03
	Hybrid	0.32 ± 0.03 *	0.21 ± 0.04 a	0.14 ± 0.02	0.11 ± 0.02
	Red	0.33 ± 0.05 b	0.25 ± 0.02 b	0.14 ± 0.02	0.15 ± 0.03
0.01	Black	0.50 ± 0.07 a	0.35 ± 0.04 a	0.15 ± 0.03	0.18 ± 0.02
	hybrid	0.53 ± 0.04 a *	0.41 ± 0.04 a	0.16 ± 0.02	0.16 ± 0.02
	Red	0.67 ± 0.09 b *	0.45 ± 0.04 b	0.32 ± 0.04 b *	0.22 ± 0.02
0.1	Black	1.08 ± 0.07 a *	0.77 ± 0.05 a	0.52 ± 0.05 ab *	0.25 ± 0.03
	hybrid	1.09 ± 0.12 a *	0.72 ± 0.06 a	0.46 ± 0.05 a *	0.26 ± 0.04
	Red	1.33 ± 0.19 b	1.01 ± 0.08 b	1.13 ± 0.19 b *	0.59 ± 0.04
1	Black	2.02 ± 0.20 a *	1.54 ± 0.11 a	1.89 ± 0.30 ab *	0.68 ± 0.05
	hybrid	2.17 ± 0.17 a *	1.57 ± 0.08 a	1.68 ± 0.28 a *	0.63 ± 0.04

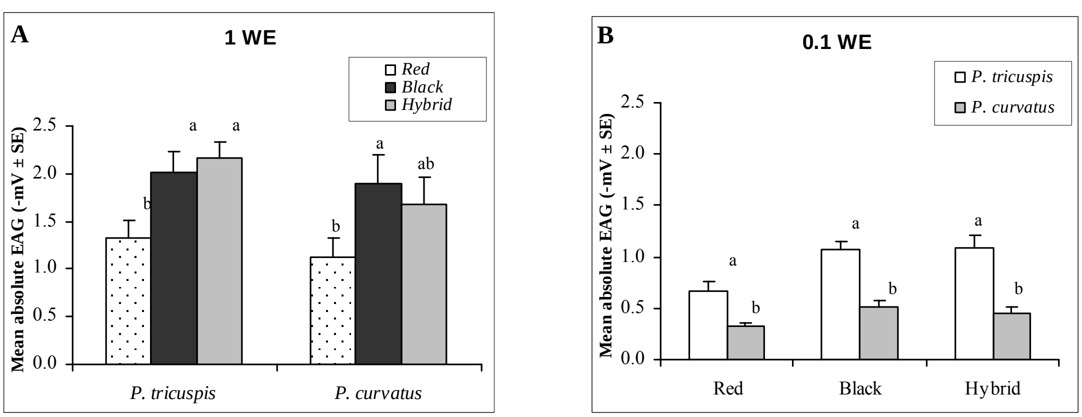

Figure 1. Comparative EAG responses of *P. tricuspis* and *P. curvatus* females to body extracts of red, black and hybrid imported fire ants. (A) Comparing response of each fly species to 1 worker equivalent (WE) dose of body extracts of different fire ant species; (B) Comparing EAG response between both fly species to 0.1 WE dose of body extracts of different fire ant species. Figure shows mean ± SE absolute EAG.

Developing Methods to Evaluate Reproductive Rates of *Pseudacteon curvatus* (Diptera: Phoridae) in *Solenopsis richteri* (Hymenoptera: Formicidae)

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Introduction

Imported fire ants are serious pests that infest 300 million acres in the United States. *Pseudacteon curvatus* Borgmeier is one species of phorid fly parasitoid currently approved for release in the U.S. as a self-sustaining biological control agent for suppression of the black and hybrid imported fire ants. Phorid flies show promise as biological control agents because of their specificity, broad distribution across habitat and season, and their interference with normal ant activity (Porter et al., 1997). Previous phorid fly studies have described their biology, host-parasite interactions and larval development (Porter et al., 1997, Folgarait et al., 2002, Consoli et al., 2001).

Little or no information exists on the reproductive rates of any of the phorid fly species currently reared on imported fire ants. We developed a rearing system to culture *P. curvatus* in *Solenopsis richteri* Buren in an effort to calculate reproductive rate values. Preliminary calculations of egg/day, survival, gross and net fecundity, reproductive rate, finite and intrinsic rate of increase, mean generation time, and double time were determined for *P. curvatus* parasitizing *S. richteri*. In addition, flight attack responses of *P. curvatus* during the adult life span were evaluated.

Materials and Methods

This study was conducted at the USDA, ARS, National Biological Control Laboratory, Stoneville, MS. The *P. curvatus* were obtained from a colony kept in continuous culture since 2003 (Vogt et al. 2003), and the *S. richteri* were field collected near Grenada, Mississippi. Hosts and parasitoids were maintained in environmental rooms at 25 ºC with 85% RH and 12L: 12D photoperiod.

Three groups each comprised of 10 *P. curvatus* pupae were placed on the cardboard lid of an emergence container (Solo Cup Company No. PL 1), which contained 5 cc of 1.5% benomil solution. Each emergence container with the fly pupae was put inside an attack container (Pioneer-plastic container 22.5cm x 10cm diameter/depth) with about 120 *S. richteri* and maintained in a large petri dish (Pioneer-plastic container 22.5 cm x 2.5 cm diameter/depth) at 23 ºC, 85% RH and 12L:12D photoperiod. Each attack container had a plastic vial with a perforated snap-cap covered with a cotton ball (35.5 ml Crystal-Thornton Plastic). The plastic vial contained water: sugar solution (50:50) as an ant food source. The attack container had a 10 cm diameter opening covered with nylon to provide ventilation. A drop of water: sugar (50:50) was placed over the nylon as required as a feeding source for the phorids. Each group of 120 ants were removed daily and replaced with a fresh group of ants until all flies died. Groups of attacked ants were kept in the same petri dish and placed in a Percival environmental chamber (MODEL: RE-16) at 23 ºC, 85% RH and 0L: 24D photoperiod for 40 days. Dead ants were removed daily from each container following the second week of parasitism and stored separately in a plastic container (Solo Cup Company No. PL 1). The containers were covered with a perforated sealed snap-cap (6 holes 1 mm diameter). The containers contained 10 mm of moistened castone to increase RH. A 2.0 ml solution of 1.5 % benomil was injected in the cup each week until the initial observation of a *P. curvatus* pupa.

Phorid attack attempts were observed for 10 hours each day until the last fly was killed or dead. Flight time, number of attack attempts, number of attacks per flight, and number of *P. curvatus* pupae were recorded and used to construct the fertility tables. These tables were calculated by assuming a 1:1 sex ratio and 0% immature mortality for *P. curvatus*.

Results and Discussion

Preliminary reproductive rates of *P. curvatus* demonstrated a gross fecundity of 32.12 progeny/female, 3.82 days of survival, 40.69 day mean developmental time, 13.35 increases in individuals per female fly each generation, and a 1.06 individual increase each day per female fly. According to these preliminary results, a fly can lay 8.4 eggs per day and double its population in 11.02 days (Table 1).

Our main objective was to construct life and fertility tables in an effort to calculate the productive rate values of *P. curvatus*. These preliminary rate values give us a better understanding of the contribution to the future population that an individual female of *P. curvatus* can make using this rearing system.

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Table 1. Preliminary Calculation of Reproductive Rates of *Pseudacteon curvatus* in *Solenopsis richteri*.

Formulae	Parameters	Calculated Values by DMNA *	Calculated Values by DMNPH *
	Eggs/day	11.2	8.4
l_x	Survival (days)	3.88	3.82
M_x	Gross Fecundity (Progeny/female)	43.55	32.12
m_x	Net Fecundity (Females/female)	21.75	16.42
$R_0 = \sum_{x=\alpha}^{\beta} l_x m_x$	Reproductive Rate (Adults/females) Population which increase each generation.	18.20	13.35
$\lambda = e^{r_m}$	Finite Rate of Increase (Adults/Female) Population which increase each day.	1.07	1.06
$r_m = \sum_{x=\alpha}^{\beta} e^{-r(x+0.5)} l_x m_x = 1$	Intrinsic Rate of Increase	0.071	0.066
$T = \sum_{x=\alpha}^{\beta} l_x m_x / \sum_{x=\alpha}^{\beta} x l_x m_x$	Mean generation Time (Mean interval separating the births of one generation from those of the next).	40.68	40.69
$(Log_e 2)/r_m$	Doubling Time Time required for a newborn female to replace herself	9.76	11.02

* Computation assuming a sex ratio of 1:1 and 0% immature mortality
DMNA = Daily Mean No. of Attacks
DMNPH = Daily Mean No. of Parasitized ants

Enumeration, Isolation and Characterization of Heterotrophic Bacteria in RIFA Habitat of in Taiwan

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Red Imported Fire Ant (RIFA) (*Solenopsis invicta*) caused devastating damage in Taiwan. Distance Ant Bait® is the major pesticide used to eradicate RIFA. The effects of pesticides on the soil flora of heterotrophic bacteria were investigated in this study. The soil texture of the ant nest was about 70% of fine sand particles which was defined as the soil between loam and sand. The pH in ant nest soil ranged from 6.86 to 8.35. The bacterial cells were 1.3×106-2.5×107 CFU/wet wt of soil. There were about 9.2×10-2.9×103 CFU on the body surface of an ant. About 200 strains of bacteria were isolated from the surface of RIFA. They could be clustered into several genotypes based on the analysis of restriction patterns of PCR-amplified 16S rDNA digested with various restriction enzymes. About 65% isolates were classified into *Bacillus, Pseudomonas, Arthrobacter, Acinetobacter* and *Staphylococcus* on RIFA. The mounds treated with pesticide, contained *Streptomyces* (6.0%) and *Burkholderia* (6.0%) which were different from mounds without pesticide (*Virgibacillus* 8.0% and *Pseudomonas* 8.0%). In soil, *Bacillus* (76.4%) and *Pseudomonas* (15.2%) were two dominated genus. After the treatment of Distance Ant Bait®, the bacterial density remained the same, but the proportions of major bacteria did change along the time.

Potential Impact of Imported Fire Ants *(Solenopsis invicta)* on Loggerhead Sea Turtles *(Caretta caretta)*

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Introduction-Loggerhead sea turtles *(Caretta caretta)* nest annually on Sea Island's 3.5 miles of beach. During an average year, 70 nests are laid from May to September. Documentation by Sea Island sea turtle technicians in the past several years has shown an incidence of fire ant depredation in sea turtle nests. The following study was undertaken to determine: (1) if *S. invicta* are capable of entering *C. caretta* eggs, (2) if *S. invicta* will tunnel to depths representing an actual nest chamber, and (3) if *S. invicta* depredation on *C. caretta* nests can be prevented using Advion (Indoxacarb).

Materials and Methods-The field section of this project was conducted on Sea Island, Georgia, USA. Sea Island (Latitude: 31.18N, Longitude: 81.35W) is located East of St. Simon's Island, and Brunswick, GA and 60 miles south of Savannah, GA. Nests were located on the beach of the eastern side of the island

Loggerhead sea turtle nests on Sea Island were randomly selected to serve as Advion (Dupont Corp) treated nests (n=15) and untreated nests (n=15.) Advion fire ant bait was applied to the area around the treated nests at the onset of the study, and was reapplied on an as-needed basis to ensure the elimination of fire ants in the area surrounding the turtle nests. The bait was applied around the turtle nests at the labeled rate of 0.5 oz. per 1000 sq ft. Sampling for imported fire ants was conducted weekly by placing bait (hot dog slice) in each of the cardinal directions 10 feet from the center of each turtle nest. The bait was left for 1 hour, then the number of fire ants on each slice was counted and assigned a rating based on a predetermined scale. Three days after hatching, turtle nests were excavated according to Georgia Department of Natural Resources protocol. Finally, data was analyzed with analysis of variance and, where significant, means were separated with LSD (using PROC GLM of SAS).

Three foraging arenas were set up in the lab on the Tifton Campus of the University of Georgia. Loggerhead Sea Turtle eggs (n=3) were buried in 5" deep cups that were filled with sand brought from Sea Island and ants were provided access. The eggs were excavated after one week and ant depredation (breaching of the egg and destruction of the embryo) was recorded.

In a second lab experiment, 3 eggs were buried in each of three foraging arenas and ants were provided access. Each arena was two feet deep and filled with Sea Island sand. Eggs were buried 20", 12" and 3" below the surface. Eggs were excavated after three weeks, and ant depredation was recorded.

The ant colonies used in both lab experiments were collected in Tifton County, GA and allowed to acclimate in the lab. They were provided food and water to satiation prior to the initiation of the experiments. During the trials, the ants were provided water daily in the foraging arenas. Additional food was not provided to the ants.

Results-Field experiments demonstrated that Advion fire ant bait was effective at decreasing the number of fire ants in the vicinity of treated nests (Fig. 1). The Advion treatment effect was statistically significant at all 4 post treatment counts. After the preliminary application of Advion, only one (out of 14) of the nests in the treated area had fire ants in the proximity. A second application of bait eliminated this threat.

Laboratory experiments demonstrated the ability of *S. invicta* to penetrate *C. caretta* eggs and depredate embryos at depths up to and including those of an actual nest; 92% (11 out of 12) of the eggs were depredated when fire ants were provided access. The lone un-penetrated egg was determined to be non-viable. Preliminary field studies indicate that penetration of eggs is not a regular occurrence. Fire ants were discovered in only 2 of the 27 study nests. The ants did not damage any of the eggs in these 2 nests.

Discussion-Laboratory studies showed imported fire ants have the ability to breach loggerhead sea turtle eggs and to totally consume the developing embryo. In addition to direct mortality, fire ants may potentially inflict injuries that affect the survival of hatchlings later in life (Parris et al. 2002). These injuries could include flipper deformities, and weakened immune systems caused by envenomation. Even though ants are capable of depredating eggs at depths equal to that of a normal nest, this study showed that ants did insignificant amounts of damage to eggs in the nests on Sea Island. However, damaged sea turtle eggs examined by one of the authors in previous years resemble those breached in the current laboratory experiments, suggesting this does occur in the field. Therefore, ants may only depredate healthy sea turtle eggs when there is a lack of other food sources.

Interestingly, the one unviable egg was the only egg not penetrated by the fire ants in the lab. This egg was not penetrated in the first experiment conducted in the lab; it was re-buried in the second trial and attached to a different colony. Again, it was not penetrated. This suggests fire ants are able to determine which eggs are viable and which are not.

It appears that under normal field conditions, ants probably harm loggerheads while the hatchlings are resting below the surface of the sand after hatching but prior to emergence from the sand. Further research into these dynamics is warranted.

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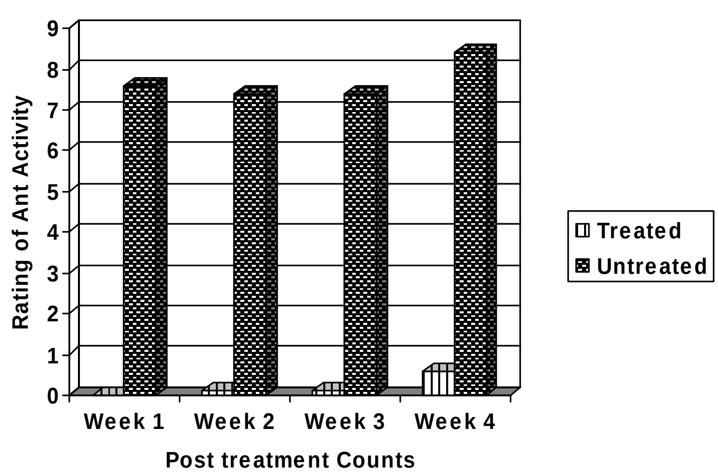

Fig. 1. Comparison of imported fire ant activity in Advion treated and untreated areas.

Phorid Flies – USDA, APHIS Rearing and Release Program: Overview of current USDA, APHIS efforts to release phorid flies (*Pseudacteon* spp.) into imported fire ant populations in the U.S. and Puerto Rico

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Introduction:

In a recent USDA-APHIS survey, seven southern states ranked imported fire ant (IFA) as a top priority target organism for biological control. 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. 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. While phorid flies will not be a stand-alone biological control agent for IFA, 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 for 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.

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

Overview:

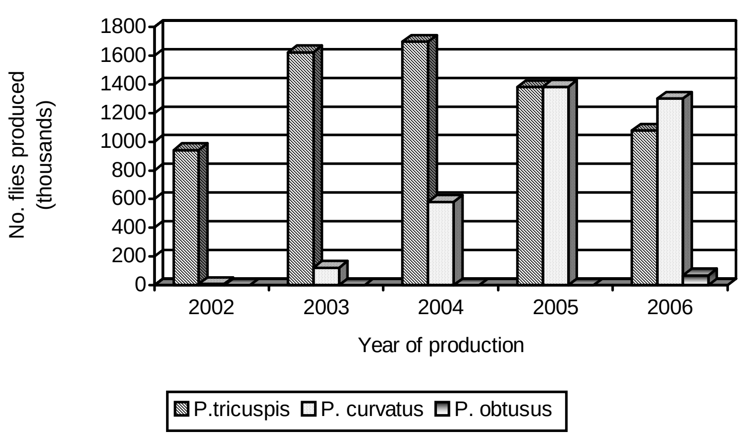
- APHIS, PPQ coordinates activities
- USDA, ARS, CMAVE (Gainesville, FL)
    - imports; develops rearing methods; preliminary releases; transfers rearing technology to FL-DPI

- Florida DPI rears flies (Gainesville, FL)
    - *Pseudacteon tricuspis*
        - 5 boxes in rearing
        - 53 releases 2002-2006
    - *Pseudacteon curvatus*
        - Biotype that prefers smaller red IFA (polygyne)
        - 7 boxes in rearing
        - 20 releases 2004-2006
    - *Pseudacteon obtusus*
        - 2 boxes in rearing
        - First releases anticipated fall 2007
- Cooperators
    - State cooperators handle releases and monitoring of releases
- Other phorid rearing and releases
    - ARS researchers
    - Universities, etc.

Results:

Rearing data: Rearing was initiated in 2001 for *P. tricuspis*, seeded by flies from the ARS-CMAVE facility. The number of rearing boxes in *P. tricuspis* production has increased from the initial 1-2 boxes in 2001 to a high of ca. 10-12 boxes in 2003 to the current 5 boxes in 2006. Annual rearing of *P. tricuspis* was at its peak in 2003 and 2004 with ca. 1.6 million flies being produced, to the current 2006 production of 1.0 million (Figure 1). *P. curvatus* rearing was initiated in late 2002, with the initial 1-2 boxes again seeded by flies from the ARS-CMAVE facility. By late 2006, 7 rearing boxes were in production. Production has dramatically increased from 121,000 in 2003 to 1.3 million in 2005 and 2006. Also in 2006, a third species, *P. obtusus* was brought into production.

Figure 1. No. phorids produced by year and species: FL-DPI rearing facility



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

Figure 2. Number of fly releases per year conducted by state cooperators and mean number of flies per release

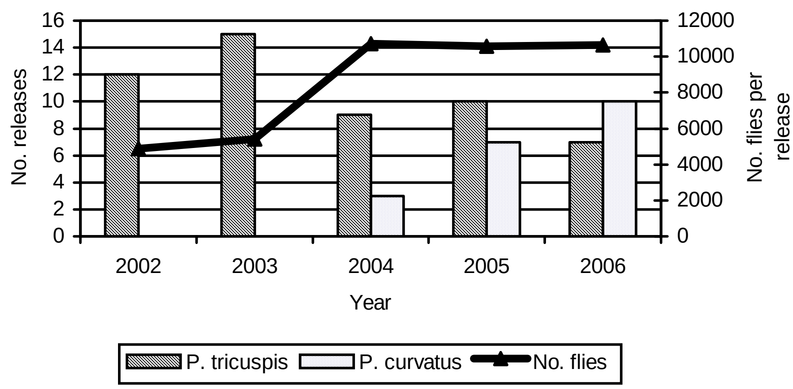
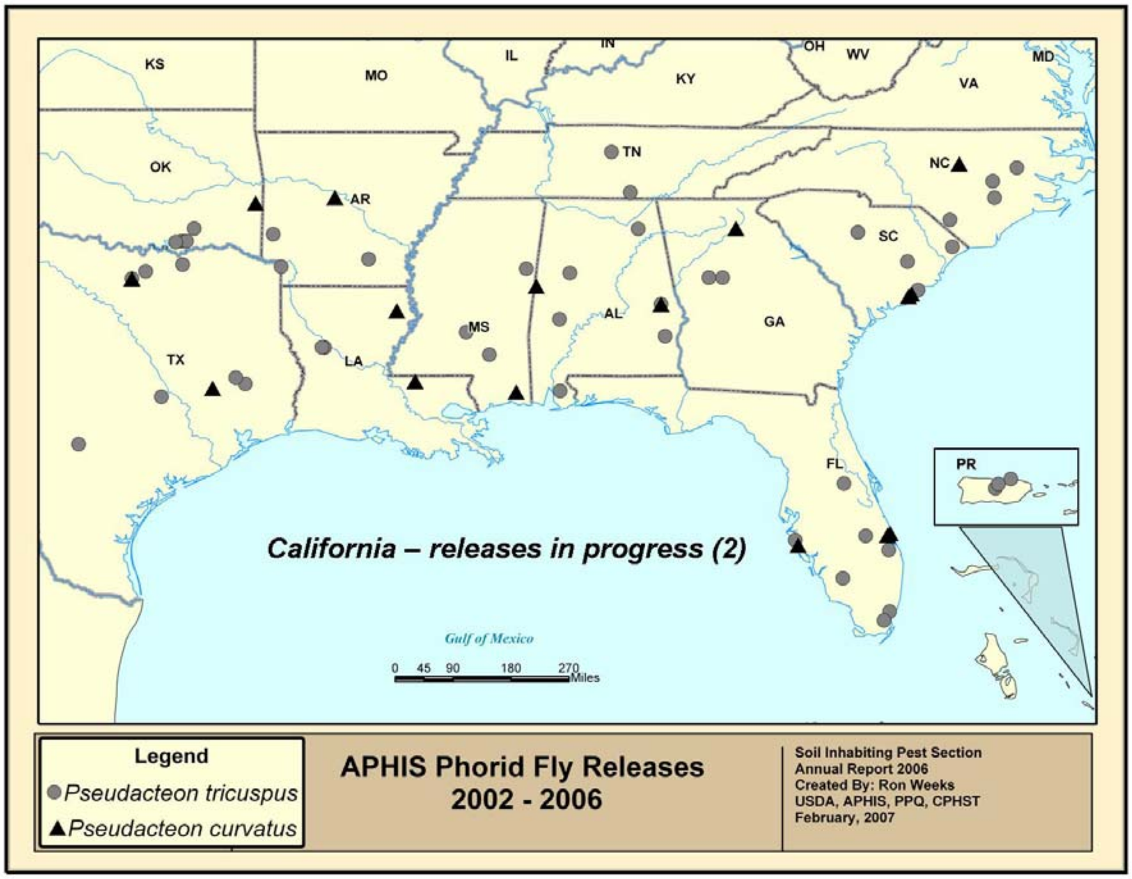


Figure 3. 2002-2006 phorid fly releases from APHIS program; both *P. tricuspis* and *P. curvatus* (multiple releases at some sites). Releases in CA (2 *P. tricupsis* and neither successful at this time) not shown on this map.



Survival data: Success of the program is currently being measured by successful overwintering of fly populations. Of the 56 releases conducted in 2002-2005, flies have been found after a winter at 27 (48%) of these sites; 19 *tricuspis* sites (AL, AR, FL, GA, LA, MS, NC, PR, SC, TX) and 8 *curvatus* sites (FL, LA, NC, OK, SC, TX) (Figure 4). Those sites at which flies have not been found have not been abandoned. Cooperators and others studying the flies are finding that it may take 2-4 years for flies to build populations that are easily detected in the field. Unfortunately, this was not known early in this program and many states have conducted multiple releases at the same site when they believed no flies were present a year after a release. As resources allow, all release sites will be monitored annually to determine fly presence. Once flies are found at a site, cooperators move out from the site and monitor to determine spread of the flies. Collection of fly data from cooperators is fairly good and new options on collecting and transmitting that data is becoming available. We have also asked that IFA populations at the original release site be monitored. Several cooperators have provided spatially explicit data from all releases in their state, not just APHIS funded releases (Figure 5).

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

Figure 4. Established phorid fly populations from APHIS release program 2002-2006

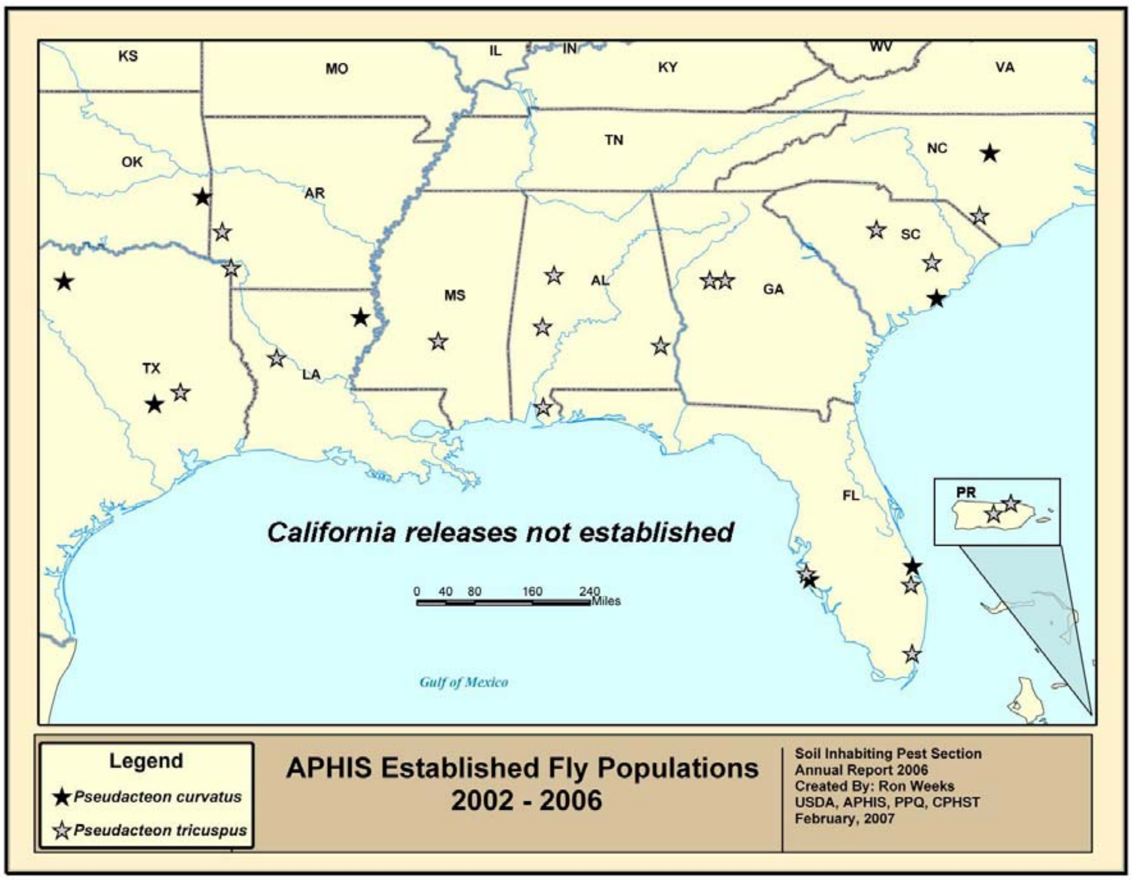
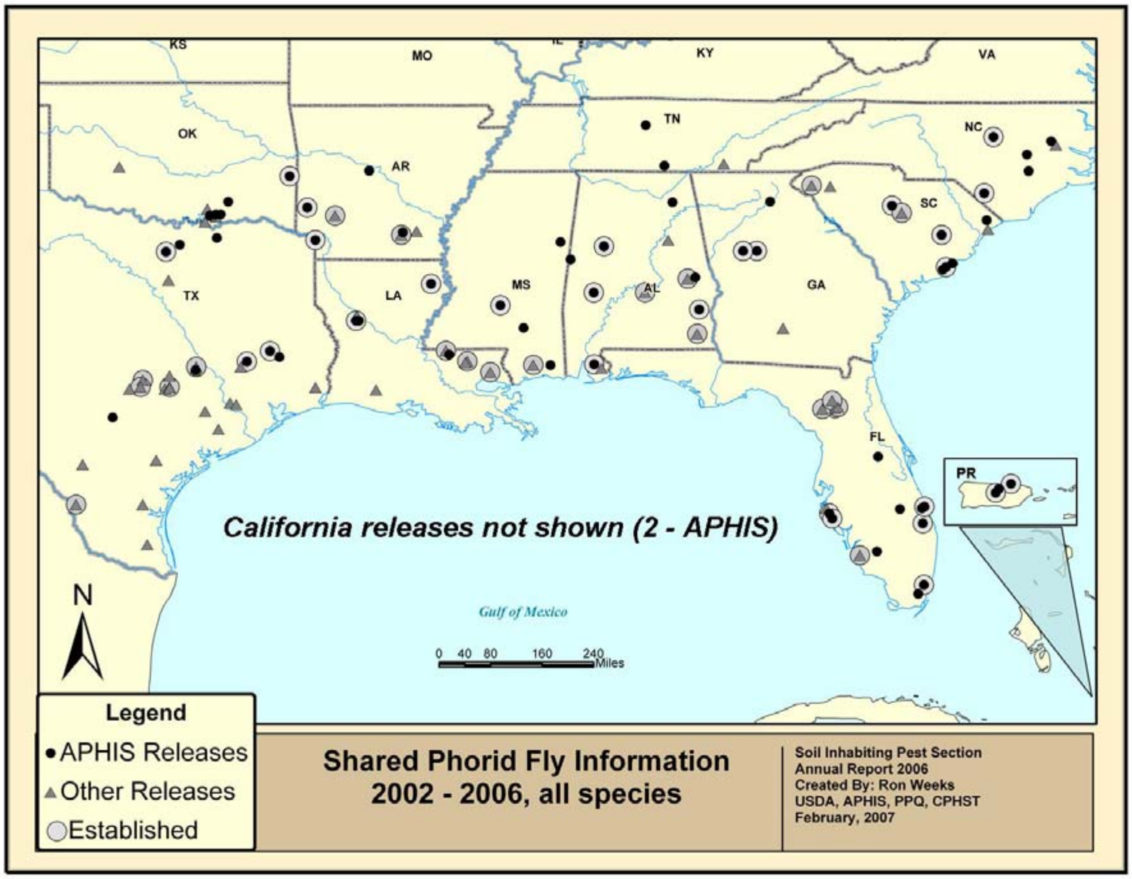


Figure 5. Phorid fly data, establishments and spread, reported by cooperators and USDA-APHIS personnel. Continued survey effort, data reporting and coordination are planned for all states.



Laboratory Host Specificity Testing of the Microsporidian Pathogen *Vairimorpha invictae*

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Summary

Vairimorpha invictae is a microsporidian pathogen of fire ants that was first described in 1986 by Jouvenaz and Ellis from red imported fire ants, *Solenopsis invicta*, collected in Brazil. *V. invictae* infections alone and in combination with another microsporidian pathogen of fire ants, *Thelohania solenopsae,* have resulted in dramatic declines (53-100%) in *S. invicta* populations in Argentina (Briano 2005). *V. invictae* is currently being evaluated for release in the U.S. which includes determining its host specificity. Examinations by microscopy of ants collected at baits and from sampling nests in Argentina and Brazil by Briano et al. (2002) revealed infections of *V. invictae* in *S. invicta, Solenopsis richteri, and Solenopsis macdonaghi*. Infections were most prevalent in *S. invicta*, and *V. invictae* infections were not observed in 10 non-*Solenopsis* genera. Similarly, infections were not detected by PCR in 235 non-ant arthropods (10 orders, 43 families, 80 spp.) and 509 non-*Solenopsis* ants (12 genera, 19 species) collected at baits in *V. invictae* sites in Argentina (Porter et al.). Here we report the results of laboratory inoculations with *V. invictae* on two fire ants species found in North America, the tropical fire ant, *Solenopsis geminata*, and the southern fire ant, *Solenopsis xyloni*.

Queen-right, laboratory colonies of *S. xyloni, S. geminata,* and *S. invicta* were inoculated with brood from *V. invictae* infected *S. invicta* colonies collected in Argentina. Brood inocula consisted of an arbitrary mixture of live larvae, pupae, and eggs that had an average infection rate of 86%. Infected brood has been used to transmit *V. invictae* to uninfected *S. invicta* colonies (Oi et al. 2005). Infections were determined by microscopy or PCR 12-24 weeks after inoculation. *V. invictae* was detected in 60% (3/5) of the *S. invicta* colonies while infections were not observed in *S. xyloni* (0/5) and *S. geminata* (0/4) colonies. All control colonies (*S. invicta* n=5; *S. xyloni* n=5; *S. geminata* n=4) were uninfected.

V. invictae infections can be transmitted from infected adult workers to larvae that they tend. Groups of *V. invictae*-infected *S. invicta* workers (ca. 125 workers/group, infection rate 30-80%) were given access to 50 uninfected 2nd/3rd instar and 50 4th instar larvae of either *S. xyloni, S. geminata,* or *S. invicta*. Transmission of *V. invictae* was determined by PCR of melanized pupae that developed from tended larvae. *V. invictae* was found in *S. invicta* pupae in 40% (4/10) of the worker groups. Infections were not detected in any of *S. xyloni* and *S. geminata* pupae that were reared by the worker groups (n=8 and n=7 groups, respectively) and in the controls. Recovery of melanized pupae from larvae tended by worker ants from different colonies and species was limited, especially with larvae exposed to adults from a different species. Pupae were recovered from 90-100% of worker groups tending the same species of larvae. In contrast, *S. geminata* pupae were recovered from only 0 and 14% of the groups with infected and uninfected *S. invicta* workers, respectively. Similarly, *S. xyloni* pupae were recovered from 25 and 43% of the groups. Thus, the host specificity of *V. invictae* may be a combination of both physiological and behavioral factors. In summary, laboratory inoculations

of *V. invictae did not result in infection* in either *S. xyloni* or *S. geminata*, while infections were detected in 47% (7/15) of the *S. invicta* inoculations.

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Efficacy and Residual of BAS 320I (Metaflumizone) Granular Bait for Fire Ant Control using Broadcast Application in Urban Settings in Arkansas

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Introduction

The objective of this trial was to evaluate the biological activity of a metaflumizone granular bait treatment against red imported fire ants (RIFA), *Solenopsis invicta* Buren, using an area broadcast application technique in the urban environment around man-made structures. Evaluations were conducted by monitoring foraging fire ants using hot dog baited traps and by evaluating active mounds in each plot throughout the duration of the project. The efficacy and residual activity of metaflumizone was compared to an industry standard (Award) and an untreated control.

Materials and Methods

Evaluations were conducted in Texarkana, Arkansas (Miller Co.) on the lawns of the Miller County Courthouse, Beech Street First Baptist Church, Central Baptist Church, and Lonoke Baptist Church. Average density of red imported fire ant mounds by treatment ranged from 52-68 mounds/acre (Table 1). Lawns used in the test were mowed on a weekly basis. This trial consisted of 3 treatments arranged in a Randomized Complete Block Design (RCBD) with 4 replications.

Table 1. Mound density by treatment in the metaflumizone EUP broadcast RIFA control study. Miller Co. AR. 2006.

	RIFA Mounds / Acre		
Replicate Address	**Award 1% Granular Bait**	**BAS 320 I 0.063% Granular Bait**	**Untreated Control**
Miller County Court House, 400 Laurel, Texarkana, AR 71854	34.1	54.9	23.7
Beech Street First Baptist Church, 601 Beech Street, Texarkana, AR 71854	121.7	60.9	93.1
Central Baptist Church, 2117 E. 35th St., Texarkana, AR 71854	60.0	42.9	51.8
Lonoke Baptist Church, 1841 Lonoke Ave., Texarkana, AR 71854	55.1	48.0	40.2
Average Number of RIFA Mounds/Acre by Treatment	**67.7**	**51.7**	**52.2**

Prior to the initiation of the study, ten active mounds within each plot were permanently marked by staking small color coded metal discs (Figure 1.) approximately two inches from the base of the mound (on the north side) to minimize disturbance. The GPS coordinates of each metal disc were recorded so that the same mounds could be relocated and monitored throughout the test. Metal discs were used so as not to impede regular mowing and to facilitate marker location with a metal detector if necessary. Treatments were applied on July 11, 2006 with a Model 2700-A Earthway EV-N-SPRED Broadcast Seeder/Spreader (Figure 2). Insecticidal baits and application rates for all treatments are listed in Table 2.

Figure 1. Color coded metal discs used to mark RIFA mounds in the metaflumizone EUP broadcast RIFA control study. Miller Co. AR. 2006.

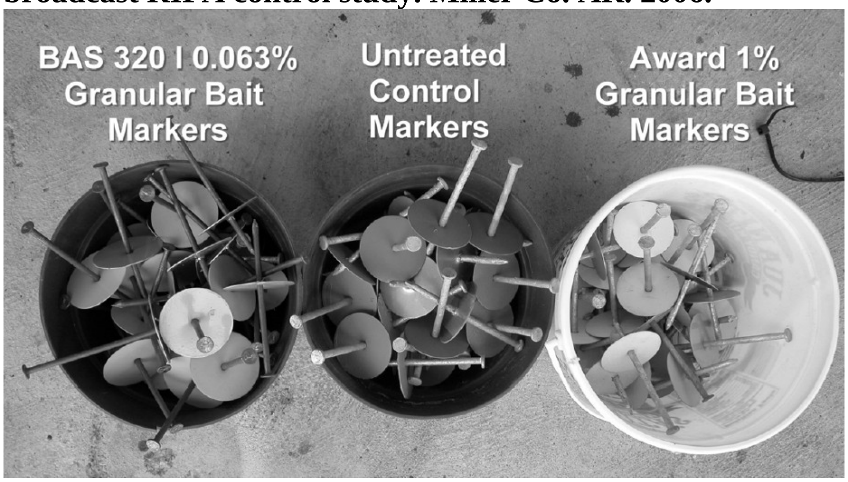

Figure 2. Model 2700-A Earthway EV-N-SPRED Broadcast Seeder/Spreader used in the metaflumizone EUP broadcast RIFA control study. Miller Co. AR. 2006.

Table 2. Insecticide bait treatment, formulation, and application rates for the metaflumizone EUP broadcast RIFA control study. Miller Co. AR. 2006.

Insecticide	Formulation	Applic. Rate
UTC	---	---
BAS 320 I (metaflumizone)	0.063% Granular Bait	1.5 lb/Acre
Award Fire Ant Bait (fenoxycarb)	1.0% Granular Bait	1.5 lb/Acre

Evaluation of fire ant populations was conducted during the morning prior to treatment application (pretreatment, 0DAT), 3 days after treatment (3 DAT), 7 DAT, 29 DAT, and 59 DAT. Pre and post treatment evaluations were conducted using baited stations to collect foraging ants within each plot following the methods of Jones et al. (1998). Bait stations consisted of a 0.25 inch hot dog cube placed on a snap vial lid and marked with a wire survey flag. Ten bait stations were arranged randomly throughout each plot, but never within 5 feet of a mound. Bait was made available to foraging ants for approximately 30 minutes. The number of foraging RIFA collected from each station in each plot was determined to evaluate effects on ant foraging. To avoid false negative readings due to daytime heating, bait station evaluations were conducted prior to 12:00 pm.

Treatment efficacy was also evaluated by determining RIFA activity in marked mounds prior to treatment and at all post treatment intervals. Mound activity was determined by gently probing mounds with a small diameter probe (minimal disturbance technique) and estimating the number of ants responding within 20 seconds. To avoid a false negative reading, mound activity was determined prior to 12:00 pm.

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

Results and Discussion

The mean number of foraging RIFA collected from bait stations is given in Table 3.

Table 3. Efficacy of BAS 320 I and Award granular baits in reducing foraging activity of RIFAs in turf. Miller Co., AR. 2006.

	Mean Number of Foraging RIFA (30 min. post hotdog baiting)				
Treatment	**Pre Treat**	**3 DAT**	**7 DAT**	**29 DAT**	**59 DAT**
UTC	67.6 a	65.5 a	67.9 a	54.8 a	81.4 a
BAS 320 I 0.063% Granular Bait @ 1.5 lb/A	70.3 a	3.8 b	18.3 b	19.0 b	13.4 b
Award 1.0% Granular Bait @ 1.5 lb/A	78.1 a	73.1 a	63.6 a	39.0 ab	19.3 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.

There were no statistically significant differences among treatments with regard to RIFA foraging when rated (pretreatment) prior to the application of control materials and RIFAs were actively foraging in all plots. Foraging ants were observed approximately 10 hours after treatment and RIFAs in BAS 320 I 0.063% granular bait (metaflumizone), treated plots were having obvious difficulties maneuvering. Ants were having difficulty walking on narrow blades of grass and would fall frequently. These symptoms were not evident in RIFAs foraging in the Award 1.0% granular bait (fenoxycarb) and untreated control plots.

A significant reduction in the number of foraging RIFA was noted at 3 and 7 DAT for the BAS 320 I 0.063% granular bait, while the Award 1.0% granular bait treatment did not differ significantly from the untreated control. At 29 DAT, BAS 320 I 0.063% granular bait provided a significant reduction in foraging RIFAs compared to the untreated control but did not differ significantly from the Award treatment. At 59 DAT, both BAS 320 I 0.063% granular bait and Award 1.0% granular bait provided as statistically similar reduction in RIFA foraging compared to the untreated control.

Results of this test indicate that the homeowner can expect a much quicker reduction in RIFA foraging activity following an application of metaflumizone bait than with a similar application of fenoxycarb bait.

The mean number of RIFAs responding to a probe as an indication of mound activity is given in Table 4.

Table 4. Efficacy of BAS 320 I and Award granular baits in reducing the number of active RIFA mounds In turf. Miller Co., AR. 2006.

	Mean Number of RIFA responding to probe in 20 seconds				
Treatment	**Pre Treat**	**3 DAT**	**7 DAT**	**29 DAT**	**59 DAT**
UTC	94.4 a	66.9 a	84.9 a	66.8 a	79.1 a
BAS 320 I 0.063% Granular Bait @ 1.5 lb/A	93.1 a	16.3 b	15.5 c	8.1 b	11.9 b
Award 1.0% Granular Bait @ 1.5 lb/A	78.1 a	48.9 a	46.9 b	21.4 b	13.0 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.

There were no statistically significant differences among treatments with regard to RIFA mound activity when rated (pretreatment) prior to the application of control materials and all marked RIFA mounds were active. The BAS 320 I 0.063% granular bait treatment significantly reduced RIFA mound activity compared to the untreated control at 3 DAT, while the Award 1.0% granular bait treatment did not differ significantly from the untreated control. At 7 DAT, both the BAS 320 I and the Award bait treatments resulted in a significant reduction in mound activity compared to the untreated control. However, the BAS 320 I treatment at 7 DAT also significantly out performed the Award bait treatment in reducing mound activity. At 29 and 59 DAT, the BAS 320 I 0.063% granular bait and the Award 1.0% granular bait treatments provided a statistically similar reduction in mound activity compared to the untreated control.

Based on the results of this trial, BAS 320 I 0.063% granular bait (metaflumizone) will provide a faster reduction in numbers of foraging RIFAs and a faster reduction in RIFA mound activity compared to Award 1.0% granular bait (fenoxycarb) which works as an insect growth regulator and requires a longer period to provide control.

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Appendix: Temperature (ºF) and Rainfall (inches) Data for Texarkana, AR.

Weather Source: http://www.weather.gov/climate/index.php?wfo=shv

2006 July Day	MAX	MIN	AVG	RAINFALL	
1	97	67	82	0.00	
2	94	71	83	0.00	
3	92	69	81	0.00	
4	89	74	82	T	
5	85	73	79	0.00	
6	89	65	77	0.00	
7	89	61	75	0.00	
8	95	60	78	0.00	
9	97	74	86	0.00	
10	97	74	86	T	
11	97	76	87	0.00	0 DAT
12	100	78	89	0.00	
13	101	76	89	0.00	
14	101	78	90	T	3 DAT
15	101	78	90	T	
16	103	74	89	0.00	
17	104	76	90	0.00	
18	104	75	90	0.00	7 DAT
19	104	79	92	0.00	
20	102	72	87	0.00	
21	102	78	90	0.00	
22	93	77	85	0.00	
23	93	69	81	0.00	
24	96	63	80	0.00	
25	98	74	86	0.00	
26	89	73	81	T	

27	81	73	77	2.81R	
28	95	77	86	T	
29	96	76	86	0.00	
30	96	77	87	0.00	
31	95	76	86	0.00	

2006 Aug Day	MAX	MIN	AVG	RAINFALL	
1	95	77	86	0.00	
2	94	76	85	0.00	
3	95	75	85	0.00	
4	96	77	87	0.00	
5	97	73	85	0.00	
6	97	73	85	T	
7	93	73	83	0.00	
8	**Sorry, no records are currently available**				
9	98	79	89	0.00	29 DAT
10	**Sorry, no records are currently available**				
11	98	78	88	0.01	
12	95	77	86	T	
13	98	75	87	0.00	
14	100	77	89	0.00	
15	102	75	89	0.00	
16	101	76	89	0.00	
17	101	74	88	0.00	
18	102	72	87	0.00	
19	101	77	89	0.00	
20	102	80	91	0.00	
21	102	77	90	0.08	
22	94	73	84	0.00	
23	89	73	81	0.03	
24	92	72	82	0.02	
25	100	77	89	0.00	
26	99	76	88	0.00	
27	96	72	84	0.56	
28	89	72	81	0.03	
29	90	67	79	0.00	
30	86	64	75	0.00	
31	91	62	77	0.00	

2006 Sept Day	MAX	MIN	AVG	RAINFALL	
1	91	62	77	0.00	
2	90	66	78	0.00	
3	88	66	77	0.00	
4	84	63	74	T	
5	89	70	80	T	
6	87	62	75	0.00	
7	88	57	73	0.00	
8	88	57	73	0.00	59 DAT
9	94	62	78	0.00	

Areawide Suppression of Fire Ants: Demonstration Project in Mississippi, 2006

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Introduction

The USDA-ARS demonstration project for the suppression of imported fire ants has entered its sixth year. In 2005, Mississippi State University joined the project monitoring sites in Clay and Grenada Counties. The Areawide project integrates biological control agents with the chemical bait products hydramethylnon and methoprene. Mississippi's involvement in the project has focused on black/ hybrid imported fire ants (Vogt et. al 2003). The following is a report on the status of the USDA-ARS Areawide Suppression of Fire Ants Demonstration Project in Mississippi as of 2006.

Materials and Methods

Two sites located in Grenada County were selected for chemical bait treatment and two sites located in Clay County were selected for the combined biological and chemical bait applications. Treatment areas consisted of at least 220 acres with a 280 acre minimum untreated contiguous border. *P. curvatus* was first released in two pastures in Clay County, Mississippi during the spring of 2002 and 2003.

Amendments to the 2005 protocol reduced monitoring from 50 to 25 (0.1ha) plots; 10 plots in the treated areas and 15 in the boundaries. The amendment also called for the establishment of additional small high value satellite) monitoring sites. The smaller sites selected in 2005 included the MSU Golf Course (MSUGC) and Memorial Gardens Cemetery (MGC), Oktibbeha County, with 10 and 18 (0.1ha) plots, respectively. The 2006 sites include Tik-A-Witha Girl Scout Camp (Tik-A-With a) in Chickasaw County and Columbus Air Force Base (CAFB) in Lowndes County. Mound counts and hotdog bait attractants were conducted every sampling period with pitfall samples collected only in the spring and fall except in satellite areas.

In cooperation with USDA-APHIS, Gulfport, Mississippi, aerial applications of Extinguish® (A.I. methoprene 0.75 lbs/acre) and Amdro Pro® (A.I. hydramethylnon 0.75lbs/acre) were applied when a threshold level was exceeded in the treatment areas (satellite sites are chemically treated with vehicle broadcast applications). Mound counts are no longer used as a method for determining reapplication. The current reapplication threshold is the presence of ant foragers at 35-40% of the hotdog bait attractant stations.

Results and Discussion

The Woodland and Knox site were subjected to a spring 2006 chemical bait application (Figure 1). Imported fire ant presence at Tik-A-Witha was overall low, however, high traffic areas did exceed the reapplication threshold and those areas were treated. Memorial Gardens Cemetery (MGC) was treated with an IGR purchased by the cooperator. No bait applications were made at the MSU Golf Course (MSUGC) or CAFB.

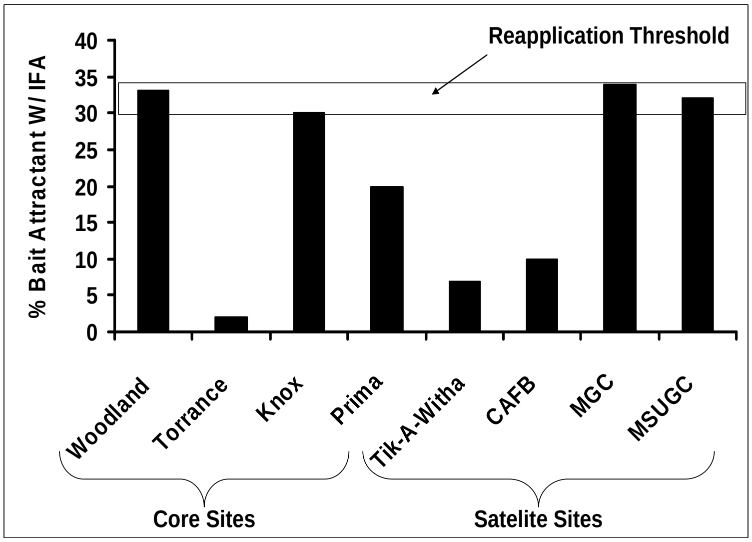

Figure 1. Percentage of hotdog bait attractants containing imported fire ants at each study site. Core sites exceeding 35-40% are subject to chemical bait application.

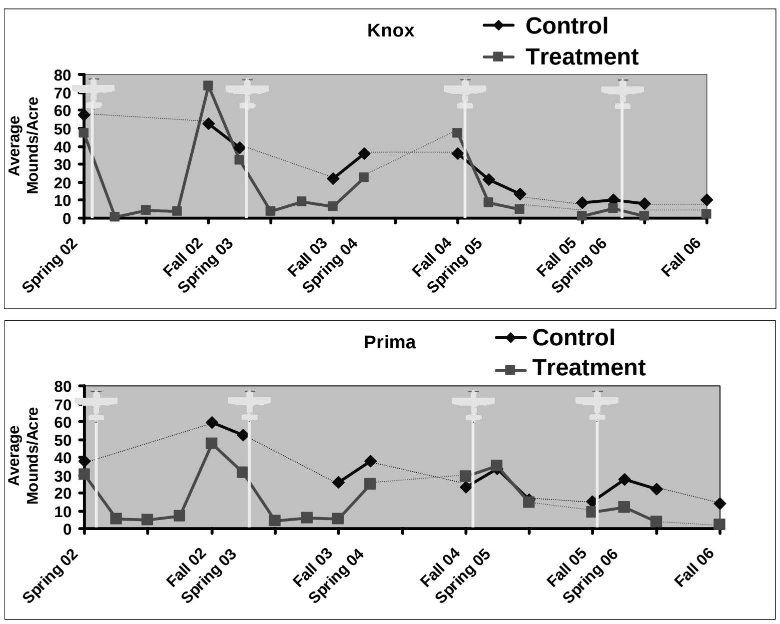

Figure 2. Linear graphs showing the average mounds per acre throughout the study period. Each white line with airplane represents an aerial bait application event (application of methoprene and hydramethylnon; 1.5 lbs/acre total). The diamond-shaped markers represent the boundary area (Control) of the site and did not receive bait applications. The square-shaped markers represent the treated area (Treatment) of the site and received the aerial bait application. Markers along each line designate a sampling date. Dotted lines denote periods of time with noncontiguous data collection and therefore may not represent actual field conditions.

A total of 13 ant species have been identified from the project sites with all thirteen species captured at the Torrance site (Table 1). *Crematogaster liniolata* (Knox) and *Pyramica membranifera* (Torrance) were the only new species captured in 2005 and 2006, respectively. No new species were captured after the initial 2002 spring sampling at Woodland or Prima. Thirty-eight percent of the species captured were via pitfall trap only. No new species were captured after the initial 2002 spring sampling at Woodland or Prima. Thirty-eight percent of the species captured were via pitfall trap only.

Table 1. List of ant species captured throughout the study at each of the core areawide study sites. Species in regular font were captured in both pitfall traps and bait attractants over the entire study and prior to the 1st treatment application in 2002. Names in bold represent additional ant species captured after the 1st treatment application. Species with an asterisk were only captured in pitfall traps.

Torrance	Woodland	Prima	Knox
Imported Fire Ants	Imported Fire Ants	Imported Fire Ants	Imported Fire Ants
Monomorium minimum	*Monomorium minimum*	*Monomorium minimum*	*Monomorium minimum*
Solenopsis molesta	*Solenopsis molesta*	*Solenopsis molesta*	*Solenopsis molesta*
Forelius pruinosus	*Forelius pruinosus*	*Forelius pruinosus*	*Forelius pruinosus*
Paratrechina vividula	*Paratrechina vividula*	*Paratrechina vividula*	*Paratrechina vividula*
Hypoponera opacior*	*Hypoponera opacior**	*Hypoponera opacior**	**_Hypoponera opacior*_**
Pheidole bicarinata	*Pheidole bicarinata*		**_Crematogaster liniolata_**
Pheidole tysoni*	*Pheidole tysoni**		
*Pheidole dentata**	*Tapinoma sessile**		
*Tapinoma sessile**			
Paratrechina arenivaga			
Pyramica membranifera*			
Crematogaster liniolata			

The phorid fly has become established on black and hybrid imported fire ants, *Solenopsis richteri* and *S. invicta* X *richteri,* respectively. In 2004, *P. curvatus* had dispersed from its initial release sites to an area of more than one half million acres (Thead et al. 2004). Dispersal continued and in 2006 *P. curvatus* was found to have spread to over three million acres in Mississippi (Figure 4).   Flies have been captured as far west as Holcomb, MS and include the Woodland Plantation, one of the core sites in Grenada County.

In 2004, *P. litoralis* was released at Knox Farms in Clay County, Mississippi. *P. litoralis* was not found after 11 survey days in the fall of 2004 and four survey days in the spring of 2005. No additional releases of *P. curvatus* or *P. litoralis* were made in 2005 or 2006.

Figure 3. Establishment and spread of *P. curvatus* represented by shaded area in 2006.

Data are currently being analyzed from the core sites in the project which received either a chemical bait treatment or a combined biological and chemical bait treatment. The project was unsuccessful in establishing *Thelohania* in Black/hybrid fire ants, but was successful in establishing the phorid fly in Mississippi.

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Fire Ant Management in Urban Landscapes with Broadcast Treatments

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Introduction

The red imported fire ant (RIFA), *Solenopsis invicta* Buren, is one of the most destructive insect pests in the urban/suburban landscape. RIFA thrive in disturbed habitats and quickly invade these areas whether the disturbance is natural or man-made.

RIFA was introduced into the Mobile, AL area from South America in the 1920s and rapidly spread across the Southeastern U.S. It has now spread from coast to coast across the Southern States and infests over 133.5 million hectares (330 million acres). It continues to spread world wide and now infests parts of Mexico, Australia, New Zealand, Taiwan and China. The total annual cost due to damages and expenditures for control for RIFA within Texas alone was estimated at $1.2 billion for 1998 and the cost is increasing each year (Lard et al. 2001). This figure includes expenditures of $702 million for residential households, $42 million for schools, $47 million for golf courses, $45 million for commercial businesses and $64 million for cemeteries. These figures include the impact of unsightly mounds, damage to turf maintenance equipment, human health issues, damage to electrical equipment and the impact on recreation and tourism and on environmental quality.

Fire ant baits are normally effective as mound control because the workers collect the bait and distribute it within the entire colony to all stages including the queen (Drees et al. 2002). Baits containing indoxacarb have been shown to cause colony decline within 24-78 h, (Barr 2002). Fire ant control is generally aided by both passive and active contact with the chosen insecticide (Chen 2006). Currently marketed insecticides for the management of fire ants do not repel fire ants and therefore allow both passive and active contact as workers forage and construct colonies. Comparisons between long-lasting-slow-acting control methods and fast-acting-short-residual control products are needed to compare the efficacy of each product versus the time to begin control and the length of time of actual control. These types of studies provide efficacy data to make recommendations for nurserymen, homeowners and turf managers on golf course and sports field based on the type of control immediately desired to meet their environmental and sociological needs.

This experiment was initiated to compare the effectiveness of broadcast treatments of several fire ant control products in the urban landscape. The test compared a bait formulation of indoxacarb (Spectracide - Once 'N Done) with contact granular formulations of fipronil and bifenthrin (Over N' Out and Ortho Max Fire Ant Killer, respectively). Also, three alternate formulations of Over N' Out (fipronil) were compared with the commercial Over N' Out product.

Materials and Methods

This experiment was established on the grounds of a community college in the north Dallas, TX metroplex on 1 June 2006. Formulations and rates of application for the six treatments are given in Table 1. Plot sizes ranged from 240 to 2,860 m^2 [mean = 780 m^2 (8,400 ft^2)] and was delineated due to the number of active mounds. Each plot had at least 10 (up to 18) active mounds (mean = 13.75; mode = 13). To delineate the boundaries of a plot, all mounds were first flagged and the plot was terminated either ca 4.6 m past the last mound or at the nearest artificial barrier such as a sidewalk. The initial flagging of mounds was done within 2 d of a double irrigation cycle of 2 inches so that nearly all active colonies were visible. Plots were delineated with white turf marking paint on two sides and aall plots were bordered on at least two sides by concrete curbs along parking lots or streets.

Table 1. Treatments and rate of application.

Fire Ant Control Product	Active ingredient	Formulation %	Application Rate[c] kg product/ha
Over N' Out (Sub 1: Sol E) [ab]	fipronil	0.0103 G	97.6
Over N' Out (Sub 1: Sol D) [a]	fipronil	0.0103 G	97.6
Over N' Out (Sub 2: Sol E) [a]	fipronil	0.0103 G	97.6
Over N' Out (Sub 2: Sol D) [a]	fipronil	0.0103 G	97.6
Ortho Max Fire Ant Killer	bifenthrin	0.2 G	112.2
Once & Done (Spectracide)	indoxacarb	0.016 B	24.4
Untreated Check			

[a] Substrate 1 = Biodac; Substrate 2 = Ecogran; Solvent E and D are proprietary formulations.
[b] Commercial standard for Over N' Out.
[c] To convert from kg/ha to lbs/1000 ft^2 divide kg/ha by 48.91.

Granular treatments were applied using walk-behind fertilizer spreaders of either a Scott's Pro Turf Professional Drop Spreader (The Scotts Co., Marysville, OH 43041) and a Spyker Cyclone Spreader Model # 34B7 (Spyker Spreaders, Urbana, IN 46990). Both spreaders were calibrated immediately before applications were made using blank granule formulations of each product. A belly bumper was used to apply the bait treatments. For all plots receiving a granular treatment, the perimeter of the plot was first ringed with one pass (ca. 1 m wide) application from the Scotts drop spreader, and the remainder of the plot was then treated with the Spyker Cyclone Spreader.

In this way the area adjacent to the curb was carefully treated by dropping the granules directly on the interface of the curb with the turf. This approach was important since well over half of the RIFA mounds in these plots were established at the curb/turf interface. No irrigation was applied the evening after treatments, but all plots were irrigated with ca. 0.84 cm (0.33 inch) of water the following evening. Irrigation was applied weekly thereafter throughout the duration of the experiment; however, this area of the city was put on 'Stage 3' water rationing and only ca. 0.84 cm of water was applied weekly instead of the 1.3 cm (0.5 inch) or more that was required to maintain good turf cover.

A pre-count of the number of live mounds and an assay of the foraging activity was completed before treatments. Each live colony was marked ca. 30 cm from the mound by spraying a white X on the turf, so the colony could be easily found for the next assay. Mounds were recorded as active if RIFA workers expressed or emerged from the mound when it was probed with the wire of a red flag. Pretreatment foraging activity of the ants within each plot was assayed by placing five, 8-dram shell vial traps near the central area of the plot for ca. 30 to 50 min exposure. These vials were baited with ca. 1/10 (ca. 1.5-1.8 g) of hot dog (processed meat sausage) and placed no closer than ca. 1 m from the nearest active RIFA colony. After exposure, the labeled vials were collected, closed with a rubber stopper, and transported to the laboratory for counting. Only the three tubes with the largest number of ant were counted for each plot. Vials then were flooded with 95% ethanol, emptied into 10 cm diam. Petri dishes, and the ant counts recorded as data. All vials with greater than 200 ants were recorded as only 200 for the sample. Plots were divided into 4 replicates based upon pretreatment counts of foraging ants and treatments were randomly assigned within each replication. This method assured that plots with the highest foraging activity were in Rep 1 with the next highest group comprising Rep 2, and so forth.

Foraging activity was assessed 1 or 2 d before treatments were applied and at 3 days after treatment (DAT), 1, 2, 3, 6, 12 and 52 weeks after treatment (WAT). Individual mound mortality was determined as the number of active colonies before and at 5, 10 and 50 WAT by probing each mound with a wire flag. To assist with these mound assays when the test area was so dry, a 1.5 g piece of hot dog was also dropped on those mounds that did not express workers and this bait was observed 20-30 min later to confirm whether a mound was really dead or just quiescent. This secondary procedure was done since the test site became very dry as the test continued. If the colony was active, workers would forage for the hot dog pieces within this time period. The plots in this experiment were monitored for ca. one year post-treatment to confirm residual activity of test treatments.

Statistical Analysis

Transformations (arcsine n + 0.01) were used on each data set to achieve normality and homoscedasticity before analysis (Steel et al. 1997) but untransformed means are presented. Analysis of variance (ANOVA) for a randomized complete block design was performed to test the differences between treatments, and means were compared at the 5% level of significance using Fisher's least-significant difference (LSD) multiple range test (SAS Institute 2003).

Results and Discussion

Foraging at Bait Tube Stations:

Among the six treatments, the Spectracide Once & Done (formulation of indoxacarb) provided immediate suppression and control of the foraging ants (Table 1). Bait trap samples were reduced to an average of less than two ants per bait tube/plot within 3 DAT and the trap samples remained ≤ 17.7 ants until 6 WAT (30.3 ants per bait tube/plot) when trap samples began to increase and remained at 43.5 ants per bait tube/plot at 52 WAT. All four formulations of the Over N' Out (formulation of fipronil) significantly reduced the numbers of foraging ants trapped. Initially the highest reduction in foraging ants was produced by the formulations with Substrate-2 with little difference in the level of control due to the two Solvents by 12 WAT. However, by 52 WAT, all four formulations of Over N' Out had reduced the number of foragers to ≤ 5.3 ants per bait tube/plot and the two formulations with Solvent-D each reduced the number of foragers to zero. Plots treated with Ortho Max Fire Ant Killer (formulation of bifenthrin) never reduced to number of foragers below 100 ants per bait tube/plot during the first 12 WAT but populations of foragers were reduced to 50.8 ants per bait tube/plot by 52 WAT. In a previous study, both broadcast and individual mound treatments with Talstar, another formulation of bifenthrin provided excellent control for at least 6 WAT (Reinert and Maranz 2001). Populations trapped in the untreated check plots remained ≥ 150 ants per bait tube/plot throughout the 52-week test period. The reduction in time of RIFA foraging activity for each treatment is presented in Fig. 1.

Table 2. Mean number of foraging RIFA foragers per bait tube/plot at pre-treatment and at WAT.

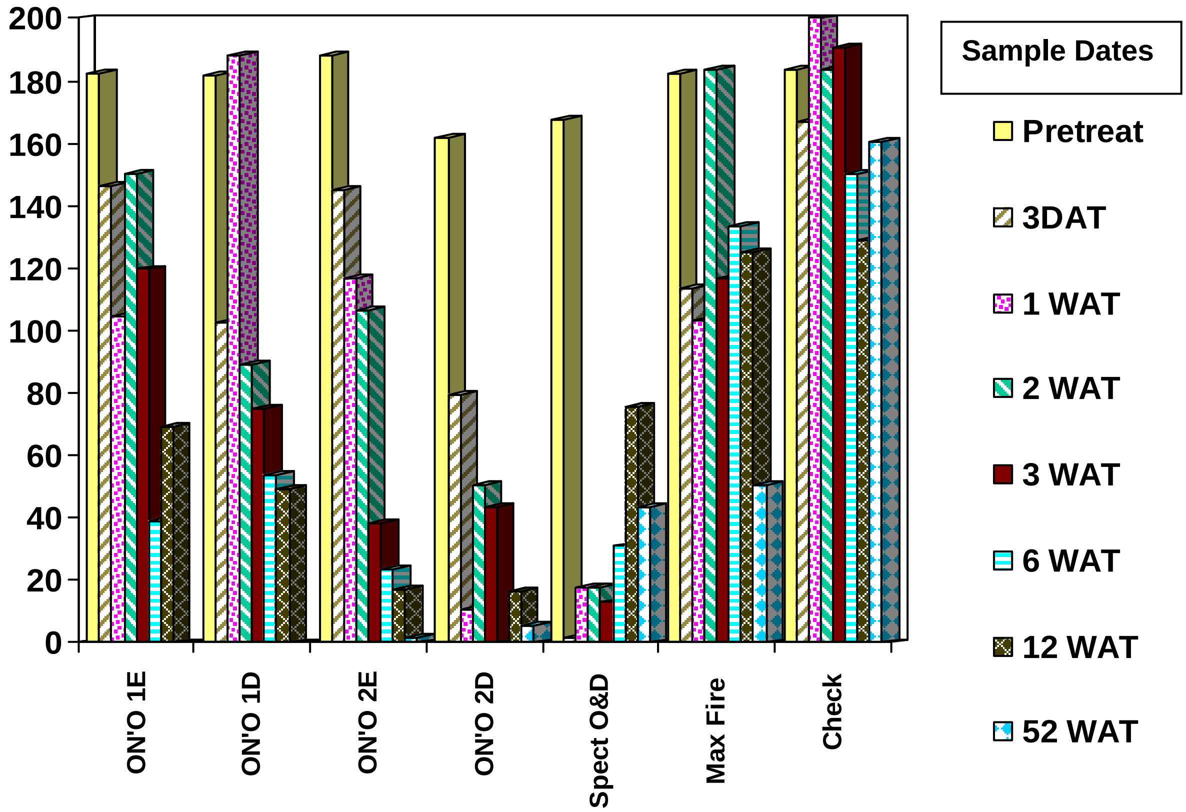
	Mean no. RIFA foragers per bait tube/plot							
Treatment [a]	Pre-treat	3 DAT	1 WAT	2 WAT	3 WAT	6 WAT	12 WAT	52 WAT
ON'O 1E [b]	182.5 a [c]	146.4 b	104.6 ab	150.0 b	119.6 cd	38.8 abc	69.2 [ns]	5.3 a
ON'O 1D	181.7 a	102.5 b	188.3 b	89.2 ab	75.0 bcd	53.8 abc	49.2	0.0 a
ON'O 2E	188.3 a	144.8 b	116.8 ab	106.7 ab	38.3 ab	23.3 ab	17.1	1.8 a
ON'O 2D	161.7 a	79.5 ab	10.4 a	50.7 a	43.3 ab	0.0 a	16.7	0.0 a
Spect O&D	167.7 a	1.9 a	17.5 a	17.5 a	12.9 a	30.3 abc	75.6	43.5 ab
Max Fire	182.1 a	113.3 b	103.3 ab	183.5 ab	116.9 cd	133.5 bc	125.0	50.8 b
Untreated Ck	183.3 a	166.9 b	200.0 b	183.8 ab	190.4 d	150.0 bc	128.8	160.5 c

[a] Legend: ON'O 1E = Over N' Out (Sub 1: Sol E) (fipronil); ON'O 1D = Over N' Out (Sub 1: Sol D); ON'O 2E = Over N' Out (Sub 2: Sol E); ON'O 2D = Over N' Out (Sub 2: Sol D); Spect O&D = Once & Done (Spectracide) (indoxacarb); Max Fire = Ortho Max Fire Ant Killer (bifenthrin). Substrate 1 = Biodac; Substrate 2 = Ecogran; Solvent E and D are proprietary formulations.

[b] Commercial standard for Over N' Out.

[c] Means in the same column followed by the same letter are not significantly different by Duncan's mean separation ($P = 0.05$).

Figure 1. Mean number of ants trapped per bait tube/plot for each treatment at pre-treatment, 3 DAT and at WAT.



Legend: ON'O 1E = Over N' Out (Sub 1: Sol E) (fipronil); ON'O 1D = Over N' Out (Sub 1: Sol D); ON'O 2E = Over N' Out (Sub 2: Sol E); ON'O 2D = Over N' Out (Sub 2: Sol D); Spect O&D = Once & Done (Spectracide)(indoxacarb); Max Fire = Ortho Max Fire Ant Killer (bifenthrin).

Reduction in Number of Active Colonies:

At the initiation of this study, there were an average of 13.8 active colonies per plot with a range of from 10 to 21 colonies depending on the colony density and size of the plot. In contrast to the continually high numbers of ant foragers in the Ortho Max Fire Ant Killer (bifenthrin) treated plots, this treatment, however, provided 56.9% reduction of active colonies at 5 WAT, but only a 10.6 and 28.9% reduction at 10 and 50 WAT, respectively. The four formulations of Over N' Out (fipronil) provided from 52.0 to 72.2% reduction of live colonies at 5 WAT, but each provided > 81% by the 50 WAT rating. The Spectracide Once & Done (indoxacarb) provided the highest initial colony reduction of 95.1% at 5 WAT, but fell to 69.4% by 50 WAT. During the test period, 37.6 and 7.9% of the colonies in untreated check plots were lost at 5 and 10 WAT respectively, but by 50 WAT only 60 % could be found that were still alive. Throughout the test period, the turf in these plots received minimal irrigation and it was extremely dry. An accurate assay of mound activity could not be recorded after 10 WAT until we had substantial rainfall in May 2007. These heavy rains allowed us to make a good assay of mound that were still alive within the plots at 50 WAT. This data confirmed the long residual control provided by the fipronil in other experiments.

Table 3. Mean number of active fire ant colonies in plots at pretreatment and at WAT and the percentage reduction provided by each treatment. [d]

Treatment [a]	Live colonies per/plot Pretreatment	(Live colonies/plot) % reduction 5 WAT [b]	10 WAT [b]	50 WAT [b]
ON'O 1E [c]	13.0	(3.7) 72.2 b [d]	(11.3) 13.1 bc	(3.0) 75.6 ab
ON'O 1D	14.7	(7.3) 52.0 bc	(14.7) 2.0 c	(2.7) 81.2 a
ON'O 2E	14.7	(5.3) 64.3 bc	(13.0) 11.4 bc	(1.3) 90.2 a
ON'O 2D	14.7	(7.0) 53.3 bc	(11.3) 22.8 b	(2.3) 82.4 a
Max Fire	14.3	(6.3) 56.9 bc	(13.0) 10.6 bc	(10.3) 28.9 c
Spect O&D	14.3	(0.7) 95.1 a	(9.0) 37.1 a	(4.3) 69.4 ab
Untreated Ck	13.7	(8.3) 37.6 c	(13.0) 7.9 c	(8.7) 39.7 bc

[a] Legend: ON'O 1E = Over N' Out (Sub 1: Sol E) (fipronil); ON'O 1D = Over N' Out (Sub 1: Sol D); ON'O 2E = Over N' Out (Sub 2: Sol E); ON'O 2D = Over N' Out (Sub 2: Sol D); Spect O&D = Once & Done (Spectracide) (indoxacarb); Max Fire = Ortho Max Fire Ant Killer (bifenthrin). Substrate 1 = Biodac; Substrate 2 = Ecogran; Solvent E and D are proprietary formulations.
[b] Percentage reduction = (no. of active colonies at WAT / no of active colonies at start of test).
[c] Commercial standard for Over N' Out.
[d] Means in the same column followed by the same letter are not significantly different by Duncan's mean separation (P = 0.05).

Test Conditions and Impacts:

The slowness of control activity exhibited by treatments in this experiment can partially be attributed to the severe drought and watering restrictions experienced in the Dallas area and throughout the Southwest during 2007. Just after the test was established, this area of the metroplex was put under 'Stage 3' water rationing (watering of turf and landscapes only once-a-week), which significantly limited the amount of irrigation that could be applied throughout the test period. Also, essentially no rainfall was recorded during most of the test period. This lack of adequate soil moisture may have limited the release of toxicant from the granular formulations and either curtailed or delayed potential control of the RIFA until the rainfall at the end of the test.

Another factor, and a recommendation supported by previous experiments (JAR) by this experiment and likely also to be supported by future research, is to treat RIFA colonies along concrete curbs, sidewalks and other hard surfaces or against buildings or other structures in a different manner than colonies that are surrounded entirely by turf areas. These colonies require special attention either by applying an increased application rate (double pass) of the granular insecticide treatments along the hard surfaces, applying an additional bait application in these areas or by applying a follow-up retreatment of the granules. In urban areas, such as this test area, quite often a high percentage of the RIFA colonies (well over half in this experiment) are situated at the curb/turf interface and are much more difficult to bring under control, compared to colonies situated in an open turf area where the granular treatment can be applied on all sides of the colony. Along these hard surfaces, unless one is careful to make full coverage applications to these interface areas, many of the colonies will escape full exposure to the toxicants. **Another reason for increasing the application rate or modifying the application procedures along the hard surfaces is that these colonies are essentially only being treated on one/half of the mound perimeter and much of the RIFA foraging activity may continue across the hard surface and away from the treated area with little on no exposure to the toxicant.** This factor alone could explain why the ants in many of the colonies within these test plots were not killed by the contact insecticide treatments.

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Asked and Answered: The Imported Fire Ant eXtension FAQs

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Attendees of the annual Imported Fire Ant Conference have a history of working together on research and educational projects. Recently, fire ant experts, together with communications and computer technology specialists, formed a Community of Practice to develop a nationwide Web-based site on imported fire ant management: http://www.extension.org/fire+ants. This effort is part of a larger initiative called eXtension (pronounced E-Extension). More information about eXtension can be found at http://about.extension.org.

More than 100 frequently asked questions (FAQs) about fire ants are now in the eXtension database (http://faq.extension.org). These questions were provided by fire ant experts or were taken from existing FAQ resources on imported fire ants. The questions and answers were subjected to several rounds of peer review before they were uploaded to faq.extension.org. Then they were copy-edited and published. The published questions are accessible by Internet search engines and can be found at http://www.extension.org/fire+ants.

Now that Imported Fire Ant eXtension has been launched, we have access to information that wasn't available when the FAQ database was assembled. Existing FAQs can be revised to take advantage of this information, then republished. Spanish translations of the FAQ's are ready to be reviewed and published. As new questions are submitted, we will need to decide if they should be added to the database.

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Effective & Economical Control of Imported Fire Ants in Cattle Production Systems in Talladega, Alabama

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Introduction

Since their introduction in Mobile, AL in the early 1900's imported fire ants (IFA) have become the scourge of the southland. In Talladega County, AL the primary IFA infestation is a hybrid of *Solenopsis richteri* and *Solenopsis invicta.*

Most fire ants in Alabama live in highly territorial single-queen colonies which may produce on average about 40 mounds per acre, however, in many portions of Talladega County, fire ants live in multiple-queen colonies which are more tolerant of each other and may produce in excess of 200 or even 300 mounds per acre.

In 2003, the USDA estimated production losses to exceed $38 million in livestock operations. However, many livestock producers do not attempt to control fire ants because of a perceived high cost and low effectiveness of treatment options. To the contrary, effective and economical control of fire ants in Alabama pastures and hayfields is very easy to achieve.

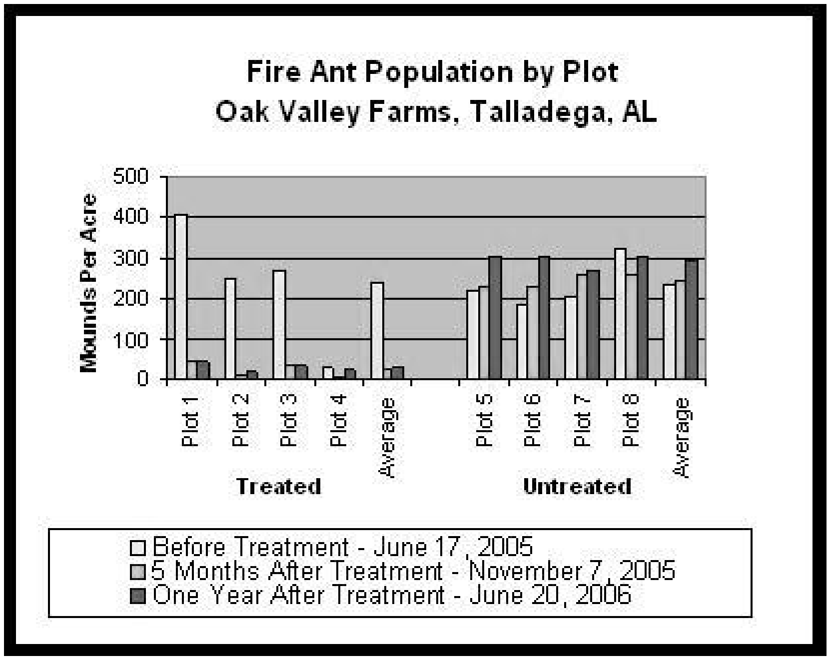
Oak Valley Farms in Talladega, AL is a purebred Angus cattle operation. A survey of pastures on the farm revealed an average of 236 live mounds per acre. It is easy to assume that such a fire ant population could disrupt cattle grazing and cause problems during the calving season. Using a State Beef Check-off grant from the Alabama Cattlemen's Association, a demonstration was initiated to evaluate the effectiveness and economics of treating cattle pastures with a fire ant bait product.

Project Methods

Using a Herd GT-77 seeder calibrated to spread 1.5 pounds of bait per acre, a 50/50 blend of Amdro Pro® and Extinguish® fire ant bait was applied to approximately 12 acres of pasture using a skip-swath method. An adjacent twelve acres were not treated for comparison. Four one-quarter acre sampling plots were established in each of the two areas. Plot centers were marked and located using a handheld GPS device. IFA populations were measured by using mound counts in each plot before treatment and again at 5 months and one year post-treatment.

Results

The 50/50 bait blend was applied on June 17, 2005. At the time of application, the treated and untreated areas had an average of 239 and 234 mounds per acre, respectively. Evaluation of the project 5 months after treatment revealed an overall 90% reduction in the average number of active fire ant mounds in the treatment area while the untreated area had a 5% increase. One year later, an evaluation demonstrated the beginning of reinfestation as the control level dropped slightly to 87% of the pre-treatment population, but remained at 90% when compared to the untreated area at one year.



In the treated area the average population was reduced from 239 to 24 mounds per acre five months after treatment and increased slightly to 30 at one year. An increase in population was observed in the untreated area as mound counts rose from 234 to 245 at five months and 296 at one year.

Conclusions

The effectiveness of the bait blend in the treated area is striking when considering favorable environmental conditions that resulted in a population growth in the untreated area during the same evaluation period.

Even more noteworthy is that by using the skip-swath method the effective rate of bait application was reduced to 0.75 lbs/acre or one-half the recommended label rate, resulting in a very economical cost of application at $5.94 per acre.

Based on the results of this project it is easy to understand how effective and economical control of fire ants is simple to achieve and can have a beneficial impact on any livestock operation. Publication of the results from this demonstration has led many livestock producers in Talladega and surrounding counties to take appropriate measures to control fire ants on their properties.

OUCH! Who bit me? – IFA in Alabama

Vicky Bertagnolli[1], L.C. "Fudd" Graham[1], Robert K. Vander Meer[2], Kenneth E. Ward[3] and Rufina N. Ward[3]

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Introduction

The black imported fire ant, *Solenopsis richteri* Forel, was the first imported fire ant identified in the United States (Loding 1929) and was originally referred to as *Solenopsis saevissima* (Fr. Smith). Wilson (1951, 1953) indicated that the population consisted of a "dark phase" that corresponded to the southernmost geographic variant of the South American population.

By 1957, Wilson and Brown reported a significant decline in the "dark phase" and an increase in the "light phase" in almost every locality except Lowndes and Noxubee counties in Mississippi. In 1972, Buren's revision of the *S. saevissima* complex renamed the "light phase" *Solenopsis invicta* and the "dark phase" *Solenopsis richteri*. Range maps by Buren et al. (1974) show *S. richteri* located in north central to northeastern Mississippi and northwestern Alabama (Fig. 1). More recent maps (Taber, 2000) show a similar range, but shifted somewhat further north and east into Alabama, now extending to the Tennessee border (Fig. 2).

Vander Meer et al. (1985) first detected a hybrid between the two species, *S. invicta* and *S. richteri*, in Mississippi. Diffie et al. (1988) reported the hybrid from 10 counties in western Georgia and 22 counties in north central Alabama in surveys in 1985-6. Streett et al. (2006) reported the hybrid in 25 counties in northern Mississippi.

The purpose of this study is to determine the approximate location of each imported fire ant species in Alabama. This will allow us to release biological control agents on their preferred host.

Figure 1. *S. richteri* range by Buren, 1974

Figure 2. *S. richteri* range by Taber, 2000

Methods and Materials

A grid was superimposed on a map of Alabama (Fig. 3). The grid is an extension of the one used by Diffie et al. (2002) in Georgia. The squares are approximately 27 km x 27 km. Worker ants were collected from three mounds at or near an intersection on the grid. These sites were surveyed during 2003, 2004, 2005, and 2006.

Ants were collected from each site by inserting a 30 x 80 mm plastic vial into a mound and capping it after at least 25 ants fell into the tube. Ants were chilled and 20 to 50 were removed from the sample tube. The ants were then placed into seven ml glass scintillation vials and covered with hexane. After 24 hours, the hexane was removed, added to a clean seven ml scintillation vial, and allowed to evaporate. These vials containing cuticular hydrocarbon residues from the ants were shipped to CMAVE in Gainesville, FL for species determination (Vander Meer et al. 1985).

Results and Discussion

We assumed that the southernmost line of the hybrid range would extend into central Alabama. Instead, the range remains north of Birmingham in the middle of the state, appears to approach the central portion of Alabama from the north in eastern Alabama (Randolph Co.) and in western Alabama it appears the hybrid extends much further south than anticipated (Fig.3).

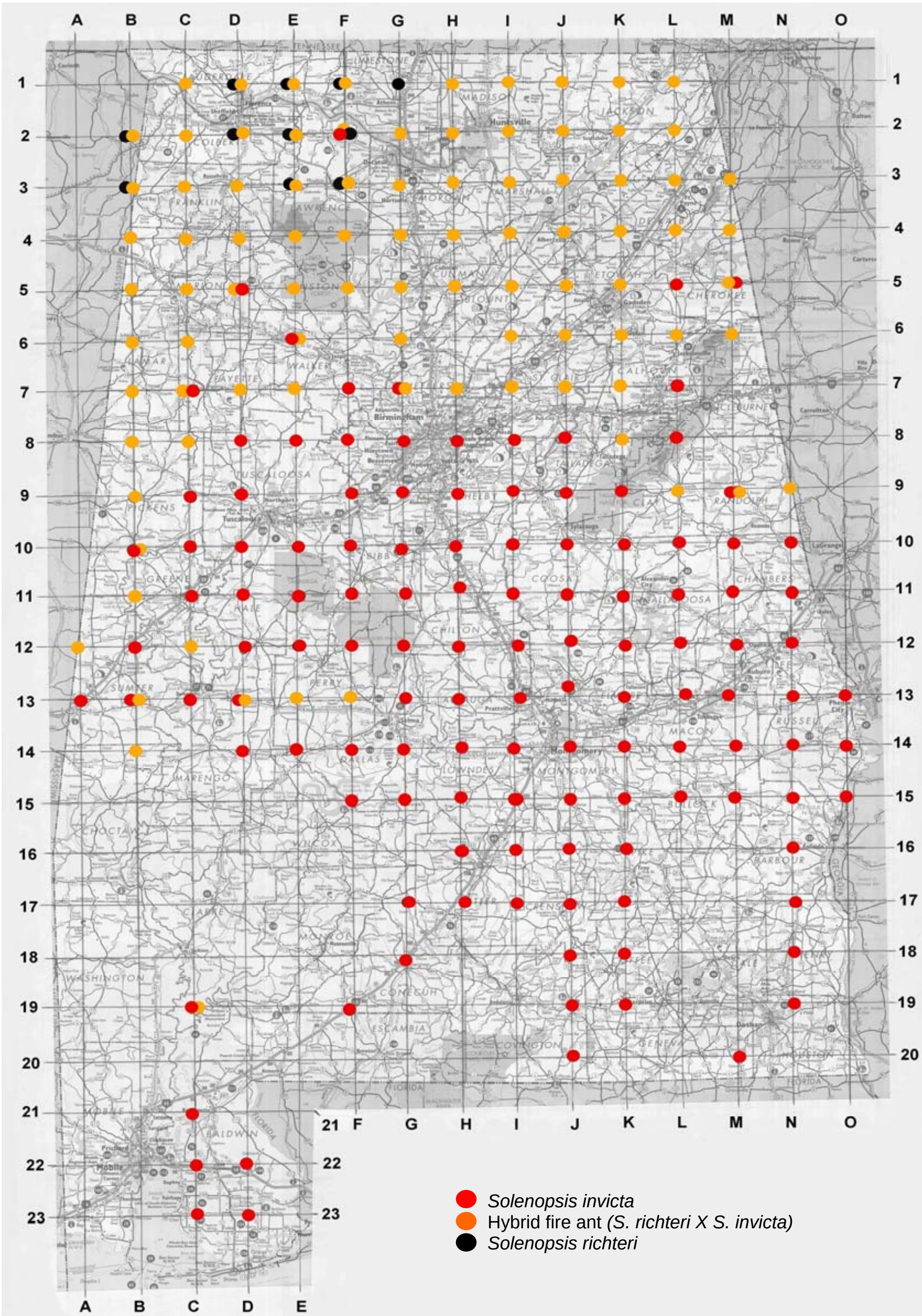
S. richteri has only been found in the northwest and north central portions of the state, extending west from near Athens (Limestone Co.). Only three mounds were collected from at each site or point on the grid. One site near Courtland in Lawrence Co. had all three ants at the site.

Buren (1972) speculated that the northward progression of *S. invicta* would be limited by winter kill conditions and that the species could be more successful in progressing northward in the eastern costal plans. It follows that *S. richteri,* which comes from cooler regions in S. America, and the hybrid fire ant would be more vigorous in cooler ecological regions here.

Streett et al. (2006) suggest that RIFA and hybrid will eventually replace BIFA. The hybrid now occupies many areas in cooler regions of Georgia and Alabama where BIFA have never been reported. However, in southwest Alabama, the hybrid appears to be competing with the RIFA and is either extending its distribution to the south or being pushed to the north.

We plan to continue mapping the ranges in the southern third of Alabama summer 2007 to determine the range of the hybrid especially in southwest Alabama.

Figure 3. Grid Map of Alabama



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A New Addition to Our Arsenal of Ant WMD's

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Introduction

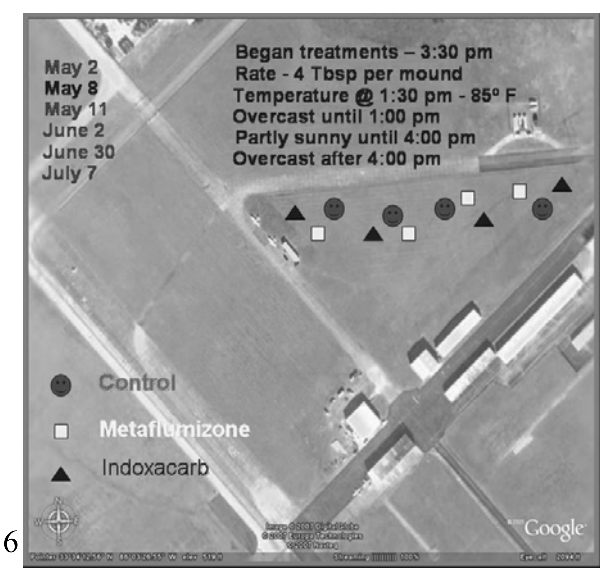
Probably the only people in Alabama that have not tangled with fire ants are visitors that go directly from their car to the hotel room and back. Most people who have had the opportunity to stray off concrete or asphalt have been attacked by fire ants, or will be.

There are many products used for fire ant management. Most are effective if used properly. Fast acting contact insecticides target foragers and may have limited activity on the queen and the colony as a whole. Slow acting residual insecticides are taken back to the colony and slow reinfestation. Baits are taken back to the colony and target the queen. In addition, we have discovered many unconventional and novel management tools that stakeholders use for fire ant control that usually do not work (Fig. 1).

Figure 1. Unconventional and novel management tools used by homeowners

Methods and Materials

Two efficacy trials were established at the Talladega Airport near the Talladega Superspeedway near Lincoln, AL. Initial population data for both trials were collected on May 2, 2006. Bait treatments were applied on May 8. Temperatures at 1:30 pm were 29.5º C. Skies were overcast until 1:00 pm and after 4:00 pm. Data were collected from all plots on May 11, June 20, June 30 and July 7.

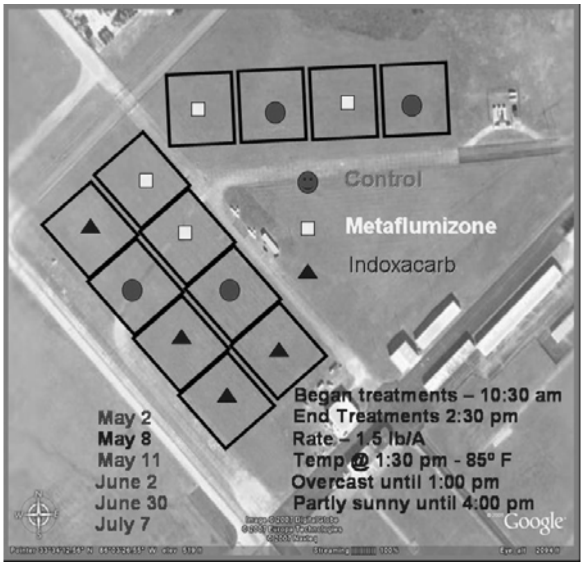


Individual Mound Treatments

Over 120 mounds were located and assigned in groups of 10 in a randomized complete block design. There was an approximately 10 meter buffer between the groups of mounds. Before treatments were applied, the areas were again scouted for mounds. Due to dry conditions, several new mounds were located in the grouping and were included in the study.

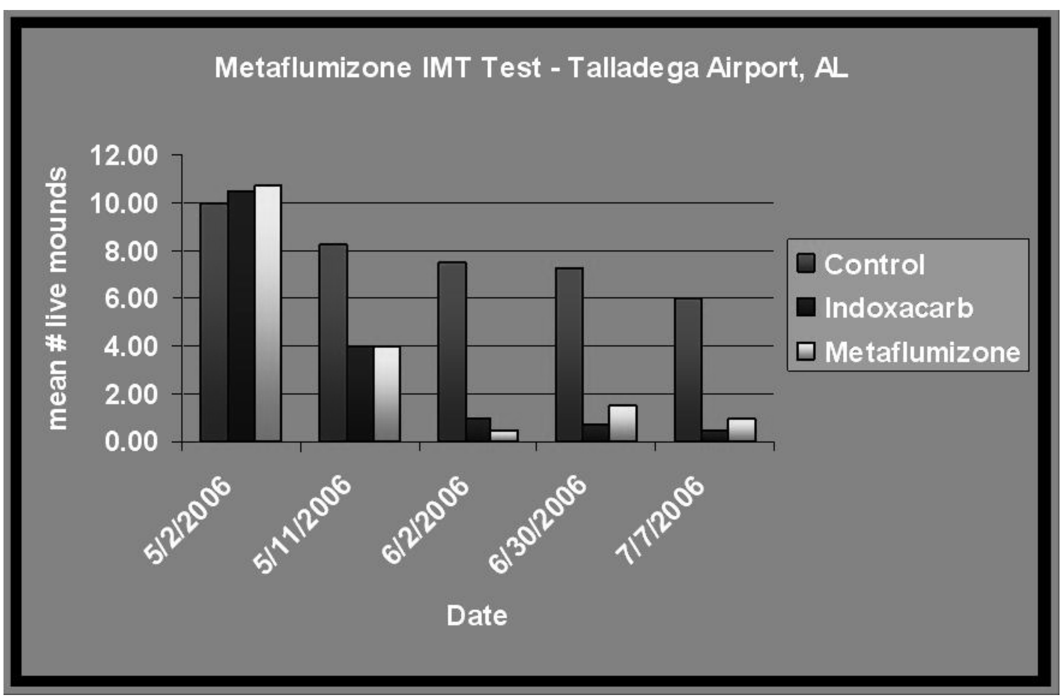
Broadcast Treatment

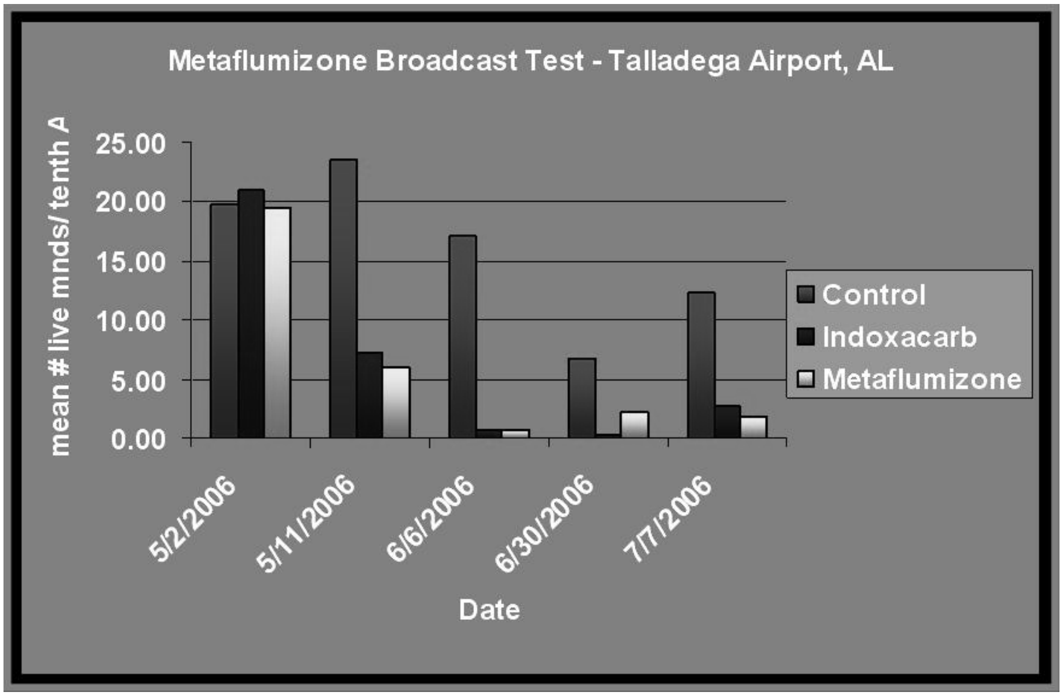
Plots were approximately 0.4 hectares with 0.1 hectare circles near the center. Data were collected from the circles in the middle of the plots. Plots were ranked by population size on 5 May to reduce variation. Treatments were then applied in a randomized complete block design.



Treatment	Plot #	# Mounds 5/2/2006
Indoxacarb	**101**	**10**
Control	**102**	**13**
Metaflumizone	**103**	**14**
Indoxacarb	**201**	**18**
Metaflumizone	**202**	**18**
Control	**203**	**19**
Metaflumizone	**301**	**21**
Indoxacarb	**302**	**23**
Control	**303**	**23**
Control	**401**	**24**
Metaflumizone	**402**	**25**
Indoxacarb	**403**	**33**

Results and Discussion





Results were similar in both trials. There was approximately a 90% reduction in fire ant mounds after one and two months in plots treated with indoxacarb and metaflumizone. Mound numbers were reduced by slightly more than 60% by both treatments in the individual mound trial after two weeks. In the broadcast trial, mound reductions after two weeks were 65% in the indoxacarb treated plots and 69% in the metaflumizone treated plots.

References

Oi, David H. and Faith M. Oi 2006. Speed of efficacy and delayed toxicity characteristics of fast-acting fire ant (Hymenoptera: Formicidae) baits. J. Econ. Ent. 99: 1739-1748.

2007 Fire Ant Conference

Agenda
23—25 April
Gainesville, Florida

Hosted by:

Imported Fire Ant and Household Insects Research Unit
Center for Medical, Agricultural and Veterinary Entomology
USDA, Agricultural Research Service
1600 SW 23rd Drive
Gainesville, FL 32608

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AGENDA
2007 IFA Conference
Gainesville, Florida

Presentations

Note: Affiliations listed are for presenter only

Monday, April 23

5:00 PM – 7:00 PM: Registration (Hotel Lobby)
5:00 PM – 7:00 PM: Poster Setup (San Felasco Meeting Room)
7:00 PM – 10:00 PM: Reception (Pool Deck/Mezzanine)

Tuesday, April 24

7:00 AM – 8:30 AM: Registration (Hotel Lobby)

8:30 AM – 9:00 AM: Welcome & Opening Remarks (Springhills Ballroom):
Bob Vander Meer, Research Leader USDA, ARS Imported Fire Ant & Household Insects Unit
Ken Linthicum, Director USDA, ARS Center for Medical, Agricultural, & Veterinary Entomology
Herb Bolton, USDA, CSREES National Program Leader, Army Environmental Programs
Dan Strickman, USDA, ARS National Program Leader, Veterinary, Medical, & Urban Entomology
Mary Chiles, Office of Senator Bill Nelson

9:00 AM – 10:00 AM: Keynote Address (Springhills Ballroom):

Dr. Walter Tschinkel
Florida State University
"Fire Ants and Native Ants: A Cautionary Tale"

10:00 AM – 10:20 AM: Break

10:20 AM – 11:30 AM: Presentations (Springhills Ballroom)
Session 1: Extension/Management/Range Expansion
Moderator: ***David Oi***

10:20 AM – 10:25 AM: Announcements

1) 10:25 AM – 10:40 AM: **Bastiaan "Bart" M. Drees**, Kathy Flanders, Henrietta Ritchie.
Texas A&M University.
eXtension fire ant web-site launch at http://www.extension.org.

2) 10:40 AM – 10:50 AM: **Xing Ping Hu**, Dunlun Song.
Auburn University.
Field trials of fire ant bait products for controlling fire ants.

3) 10:50 AM – 11:00 AM: **Seigo Higashi**, Shyuhei Koulo.
Hokkaido University, Japan.
Increasing threats of invasive ants in Japan.

4) 11:00 AM – 11:15 AM: **Ronald D. Weeks, Jr**.
USDA, APHIS, PPQ, CPHST, Soil Inhabiting Pest Section.
Best management practices (BMP) for guarding against imported fire ants (IFA) in agriculture and forestry commodities known to harbor IFA.

5) 11:15 AM – 11:30 AM: **Charles Barr**.
Barr Research and Consulting.
Speed, effectiveness and duration of consumer bait and contact insecticides.

11:30 AM – 1:00 PM: LUNCH (provided)

1:00 PM – 2:30 PM: Presentations
Session 2: Biological Control and Behavior
Moderator: ***Sanford Porter***

1:00 PM – 1:05 PM: Announcements

6) 1:05 PM – 1:15 PM: **Don Henne**, Seth Johnson.
Louisiana State University, Agriculture Center.
Zombie fire ant workers: behavior controlled by decapitating fly parasitoids.

7) 1:15 PM – 1:30 PM: **Tom Fink**, Vijay Ramalingam, Shirong Du, Lichuan Gui, Doug Streett, John Seiner.
University of Mississippi, National Center for Physics and Acoustics.
Behavioral interactions between black imported fire ants (*Solenopsis richteri* Forel) and their "stealthy" parasitoid Phoridae flies (*Pseudacteon curvatus* Borgmeier) as revealed by field high-speed videography.

8) 1:30 PM – 1:40 PM: **Yoshifumi Hashimoto**, Steven M. Valles.
USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology.
Solenopsis invicta virus-1 tissue tropism and intra-colony infection rate in the red imported fire ant: A quantitative PCR-based study.

9) 1:40 PM – 1:55 PM: **Jian Chen**, C.L. Cantrell, S.O. Duke, M.L. Allen.
USDA, ARS, National Biological Control Laboratory.
Repellency of callicarpenal and intermedeol against workers of imported fire ants.

10) 1:55 PM – 2:05 PM: **Patricia Toth**, Phil Koehler, Roberto Pereira, Dave Williams.
University of Florida.
Effects of indoxacarb on foraging behavior of *Solenopsis invicta*.

11) 2:05 PM – 2:20 PM: **L.M. Hooper-Bùi**, B. Wiltz, L.A. Womack, M.A. Seymour.
Louisiana State University.
FAST (Fire Ant Surge Threat) prevention program in south Louisiana after hurricanes Katrina and Rita.

12) 2:20 PM – 2:30 PM: **Sanford Porter**.
USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology.
Distribution of the Formosa strain of the fire ant decapitating fly *Pseudacteon curvatus* three years after releases in North Florida.

2:30 PM – 2:50 PM: BREAK

2:50 PM – 5:00 PM: Presentations
Session 3: Biology/Ecology
Moderator: ***DeWayne Shoemaker***

2:50 PM – 2:55 PM: Announcements

13) 2:55 PM – 3:10 PM: **Sherri L. DeFauw**, James T. Vogt, Debbie L. Boykin.
USDA, ARS, National Biological Control Laboratory.
Influences of imported fire ant (Hymenoptera: Formicidae) bioturbation on soils and turfgrass in a sod production agroecosystem.

14) 3:10 PM – 3:20 PM: **Deby Cassill**, Anthony Greco, Rajesh Silwal, Xuefeng Wang.
University of South Florida.
Opposable "thumbs" facilitate object manipulation in fire ants.

15) 3:20 PM – 3:35 PM: **Murali M. Ayyanath**, Gerald T. Baker, Peter W. K. Ma.
Mississippi State University.
Partial characterization of PBAN-like immunoreactivity in the hybrid-imported fire ant, *Solenopsis invicta* Buren X *Solenopsis richteri* Forel (Hymenoptera: Formicidae).

16) 3:35 PM – 3:45 PM: **S. Bradleigh Vinson**, M Walker Hale, J. Bernal.
Texas A&M University.
Effects of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) infection on *Solenopsis invicta* colony fitness components and queen adoption.

17) 3:45 PM – 4:00 PM: **Henry Y. Fadamiro**, Li Chen.
Auburn University.
Semiochemical mediated responses of phorid fly *Pseudacteon tricuspis* to imported fire ant odor.

18) 4:00 PM – 4:10 PM: **Chin-Cheng Yang**, Wen-Jer Wu, Cheng-Jen Shih.
National Taiwan University.
Revised data showing alternative distribution of two social forms of red imported fire ant, *Solenopsis invicta*, in Taiwan.

19) 4:10 PM – 4:25 PM: **Freder Medina**, Haiwen Li, Brad Vinson, Craig J. Coats.
Texas A&M University.
Bacterial microbiology of the red imported fire ant (*Solenopsis invicta* Buren) midgut.

20) 4:25 PM – 4:40 PM: **Andrew M. Bouwma**, D. DeWayne Shoemaker.
University of Florida.
No evidence for *Wolbachia* phenotypic effects in newly mated queens of the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae).

21) 4:40 PM – 4:50 PM: **Robert Renthal**, Otis Blanchard, Daniel Gonzalez.
University of Texas, San Antonio.
Proteomics as a tool for developing IFA control strategies.

22) 4:50 PM – 5:00 PM: **Samuel A. Ochieng**.
Tennessee State Nursery Research Center.
Imported fire ant responses to interspecies gland extracts.

5:00 PM – 6:00 PM: Poster Presentations (San Felasco)
Presenters available at posters from 5:00 PM – 6:00 PM, 24 April 2007.

6:30 PM – 7:00 PM: Cocktails (Springhills Ballroom).

7:00 PM – 10:00 PM: Banquet (Springhills Ballroom).

Guest Speaker: Dr. Richard J. Brenner
Assistant Administrator
USDA, ARS Office of Technology Transfer

Wednesday, April 24

8:00 AM – 10:15 AM: Presentations (Springhills Ballroom)
Session 4: Extension/Management/Prevention
Moderator: ***Steven Valles***

8:00 AM – 8:05AM: Announcements

23) 8:05 AM – 8:20 AM: Nate Royalty, **Jeff Michel**, Britt Baker, Tom Macom.
Bayer Environmental Science.
MaxForce FC fire ant bait and TopChoice on fertilizer—the latest developments in fire ant management from Bayer.

24) 8:20 AM – 8:30 AM: **Lekhnath Kafle**, Wen-Jer Wu, Yi-You Huang, Cheng-Jen Shih.
National Taiwan University.
Evaluation of a water tolerant bait for red imported fire ant *Solenopsis invicta* (Hymenoptera: Formicidae) in Taiwan.

25) 8:30 AM – 8:45 AM: **Yang-Yuan Chen**, Hui-Min Lin, Yu-Ching Tseng, C.T. Chen, C.C. Lin, C.J.W. Maa.
Institute of Physics, Academia Sinica, Taiwan.
Eradication of fire ants using liquid nitrogen and heated gas pulses.

26) 8:45 AM – 9:00 AM: **Eva Yi-Fan Huang**, Po-Yung Lai, William Wei-Lien Chyi, Kevin Tsair-Bor Yen, Yang-Yuan Chen.
Institute of Physics, Academia Sinica, Taiwan.
Utilization of odor sensibility of dogs in detecting the red imported fire ant, *Solenopsis invicta* Buren.

27) 9:00 AM – 9:10 AM: **James Saulnier**, Phil Boeing.
Coachilla Valley Mosquito and Vector Control District, California.
RIFA control in the Coachilla Valley, California.

28) 9:10 AM – 9:25 AM: **Charlie Cassidy**, Les Greenberg.
Orange County Vector Control District, California.
Field trials using reduced risk fire ant baits.

Session 5: Biology/Ecology
Moderator: ***Steven Valles***

29) 9:25 AM – 9:40 AM: **James Anderson**, Jake Marquess.
University of Mississippi.
Particle velocity measurements inside IFA tunnels: Was Hickling right?

30) 9:40 AM – 9:50 AM: **Les Greenberg**, John Kabashima, Mike Rust, John Klotz.
University of California, Riverside.
Lethal dose-response curves of several ant species to fire ant venom.

31) 9:50 AM – 10:05 AM: **Alejandro A. Calixto**, Marvin K. Harris, Charles L. Barr.
Texas A&M University.
Interference competition by *Solenopsis invicta* displaces native ants at broadcast baits: Implications for management of *Solenopsis invicta* and restoration of native ants.

32) 10:05 AM – 10:15 AM: **Kuo-Hsun Hua**, Wen-Jer Wu, Ting-Hsuan Hung.
National Taiwan University.
Application of monoclonal antibodies on detection of red imported fire ant venom proteins and antibodies in mouse.

33) 10:15 AM – 10:30 AM: **David Vander Hooven,** Darrell Courtney.
Endres Processing, Ohio, LLC.
Endres processing presents Tast-E-bait.

10:30 AM – 10:45 AM: BREAK.

10:45 AM – 11:30 AM: Business Meeting to choose location and coordinators for the 2008 IFA Conference.

11:30 AM – 2:30 PM: Luncheon and Tour, Butterfly Museum, University of Florida (Optional).

3:00 PM – 5:00 PM: Areawide Core Meeting, USDA, Center for Medical, Agricultural and Veterinary Entomology.

Thursday, April 25

8:00 AM – 12:00 PM: eXtension Work Day (Springhills Ballroom)

Posters

►Biology/Ecology◄

1. Seasonal shifts in the hyperspectral characterization of imported fire ant (Hymenoptera: Formicidae) mound features in turfgrass.
Sherri L. DeFauw, James T. Vogt.
USDA, ARS, National Biological Control Laboratory.

2. The assembly of communities in a heterogeneous landscape: Are ant assemblages a product of local or regional environmental variation?
JoVonn G. Hill, Keith S. Summerville, Richard L. Brown, Joe A MacGown.
Mississippi State University.

3. Comparative electroantennogram response of two phorid fly species to different species of imported fire ants.
Li Chen, Samuel Ochieng, Henry Y. Fadamiro.
Auburn University.

4. Developing methods to evaluate reproductive rates of *Pseudacteon curvatus* (Diptera: Phoridae) in *Solenopsis richteri* (Hymenoptera: Formicidae).
Maribel Portilla, Doug Streett, J.T. Vogt.
USDA, ARS, National Biological Control Laboratory.

5. Enumeration, isolation, and characterization of heterotrophic bacteria in RIFA habitat in Taiwan.
Hsiu-Hui Chiu, How-Jing Lee, Wung Yang Shieh.
National Taiwan University.

6. Potential impact of imported fire ants on loggerhead sea turtles
Stan Diffie, Jeannie Miller, Stacia Hendricks.
University of Georgia.

7. Investigation of fire ant extract with significant activity against the infectious pathogen *Mycobacterium tuberculosis*.
John J. Bowling, James B. Anderson, Mark T. Hamann.
University of Mississippi.

►Biological Control and Behavior◄

8. Current USDA, APHIS efforts to release phorid flies (*Pseudacteon* spp.) into imported fire ant populations in the U.S. and Puerto Rico.
Anne-Marie Callcott, Ronald D. Weeks, Jr.
USDA, APHIS, PPQ, CPHST, Soil Inhabiting Pest Section.

9. Laboratory host specificity testing of the microsporidian pathogen *Vairimorpha invictae*.
David H. Oi, Steven M. Valles, Juan A. Briano.
USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology.

10. *Pseudacteon* decapitating flies: Potential vectors of a fire ant virus?
Steven M. Valles, Sanford D. Porter.
USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology.

11. Stridulation behavior of *Solenopsis invicta* and *Solenopsis richteri* during non-nestmate interactions.
J.R. Marquess, J.B. Anderson, T. Fink.
University of Mississippi.

12. Passive traps for monitoring *Pseudacteon* parasitoids of *Solenopsis* fire ants.
Robert T. Puckett, Alejandro Calixto, Charles Barr, Marvin Harris.
Texas A&M University.

►Extension and Management◄

13. Efficacy and residual of BAS 320I (Metaflumizone) granular bait for fire ant control using broadcast application in urban settings in Arkansas.
John D. Hopkins, Kelly Loflin, Douglas Petty.
University of Arkansas.

14. Areawide suppression of fire ants: Demonstration project in Mississippi, 2006.
D.A. Streett, A.M. Pranschke, J.T. Vogt, Jack T. Reed, Anne-Marie Callcott.
USDA, ARS, National Biological Control Laboratory.

15. Fire ant management in urban landscapes.
James A. Reinert, Bart Drees, Joe McCoy, Kimberly Engler.
Texas A&M University.

16. Asked and answered: The imported fire ant eXtension FAQs.
Kathy Flanders, Patricia Beckley, Carol Whatley, Virginia Morgan, Bart Drees.
Auburn University.

17. Effective and economical control of imported fire ant in cattle production systems.
Henry Dorough.
Alabama Cooperative Extension System.

18. Ouch! Who bit me? – IFA in Alabama.
V. E. Bertagnolli-Heller, L.C. "Fudd" Graham.
Auburn University.

19. Evaluation of eradication of fire ants using liquid nitrogen and heated gas pulses.
Hui-Min Lin, **Yu-Ching Tseng**, Yang-Yuan Chen, C.T. Chen, C.C. Lin, C.J.W. Maa.
Institute of Physics, Academia Sinica.

20. Utilization of odor sensibility of dogs in detecting the red imported fire ant, *Solenopsis invicta* Buren.
Eva Yi-Fan Huang, Po-Yung Lai, William Wei-Lien Chyi, Kevin Tsair-Bor Yen, Yang-Yuan Chen.
Institute of Physics, Academia Sinica.

21. A new addition to our arsenal of ant WMDs.
L.C. "Fudd" Graham, Vicky Bertagnolli-Heller.
Auburn University.

Notes:

Acknowledgements

Organizing Committee:

- Matt Aubuchon
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- Margaret Martin
- David Oi
- Sanford Porter
- DeWayne Shoemaker
- Steven Valles
- Robert Vander Meer

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  - –Jorge Negron

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