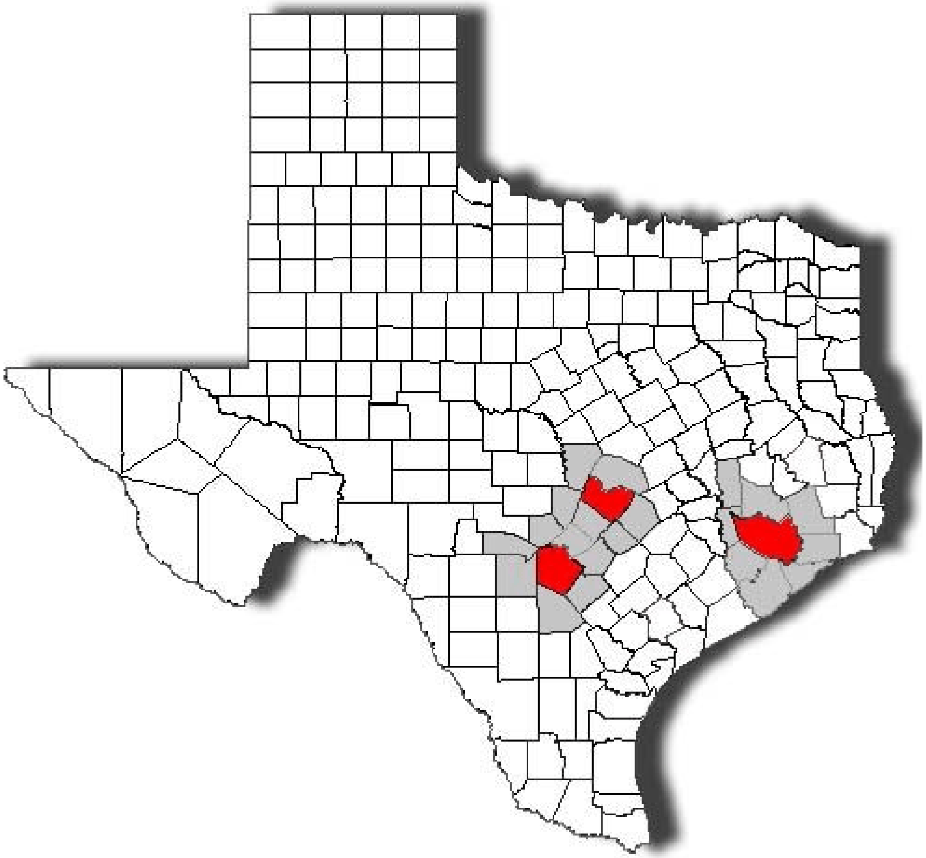
INTEGRATED PEST MANAGEMENT



Urban IPM Program 2011

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Introduction

The Urban Integrated Pest Management Program of the Texas AgriLife Extension Service supported by the Texas Imported Fire Ant Research and Management Plan of Texas AgriLife Research provides research-based information and education for citizens in the urban areas.

Technical support for the Urban IPM Program is provided by Extension Specialists from many disciplines (agronomy, horticulture, plant pathology, entomology, family and consumer science, agricultural engineering, agricultural economics) and Extension personnel at the statewide, district and county levels.

Urban IPM Program Specialists work with homeowners and others living in an urban environment to develop integrated pest management techniques in order to control pests found in and around structures. Texas AgriLife Extension Service Urban Integrated Pest Management program includes three professionals who serve major metropolitan areas, Paul Nester (Harris Co.), Wizzie Brown (Travis Co.), and Molly Keck (Bexar Co.) These urban areas make up the greater Houston, Austin, and San Antonio areas and include counties outside of the major cities. The specialists, support staff and graduate students who support the Urban IPM program are integral to its success. Integrated pest management in these areas is largely based on urban pests such as household, ornamental, garden, and turf pests. Fire ant IPM and education make up a large part of the urban IPM efforts in these areas.

Special thanks goes out to Bastiaan “Bart” Drees, Professor, Extension Entomologist and Regents Fellow, for helping with various urban IPM projects and contributing research to this handbook.

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Assessment of Direct Toxicity of Arinix I (8.56% Permethrin) and Arinix II (9.4% Ethofenprox) New and Aged Cylinder and Spiral Wrap Parts to the Red Imported Fire Ant

Bastiaan M. Drees, Professor and Extension Entomologist
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This trial was conducted to document direct toxicity of Arinix insecticide impregnated nylon parts to the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) after 4 years of aging under different conditions.

Materials and Methods

Solenopsis invicta colonies were collected, January 6, 2012, from USDA Pecan Breeding Orchard, Brazos Co., TX and extracted from the soil using slowly dripping water before being transferred to a laboratory colony tray. Ants were anesthetized with carbon dioxide gas and aliquots of 0.5 teaspoon of ants by volume were placed in plastic cups containing one of the following Arinix parts: 1) untreated control; 2) new Arinix I; 3) new Arinix II; 4) 4 year old laboratory aged Arinix I; 5) 4 year old laboratory aged Arinix II; 6) 4 year old outdoor sun exposed Arinix I; 7) 4 year old outdoor sun exposed Arinix II; 8) 4 year old outdoor shaded aged Arinix I; and 9) 4 year old outdoor shaded aged Arinix II. Each treatment was replicated 3 times. The trial was established, 3:45 p.m, January 10, 2012 and periodically (4:20, 4:50, 7:40 and 11:30 p.m.; 8:00 a.m., January 11) following initiation, ants were observed for morbidity (uncoordinated movements and inability to crawl) and mortality. This trial was conducted at 60°F. Only visual observations are reported.

Results

Within 35 minutes, as ants were recovering from being anesthetized, ants in cups containing Arinix parts showed signs of morbidity. Although some ants actually contacted the part placed in the cup, most did not and were affected by close proximity to the parts' surface evidently by insecticide vapor. Only ants in the cups containing sun aged 4-year old Arinix II parts appeared normal in 2 replications. Within 3 hours, ants in the new Arinix I treatment cups were dead and ants in other Arinix treatment cups were moribund, although in sun aged Arinix I and II cups ants were more mobile. Results were similar after 7 hours of exposure, with increased mortality evident and new parts producing faster mortality than aged parts. After 14 hours of exposure, all worker ants were dead in all treatment cups, although larger moribund winged reproductive male and female ants showed some movement.

Results clearly demonstrated the toxic effects of permethrin and etofenprox impregnated nylon parts to red imported fire ant workers. Although 4 year old sun exposed parts were observed to be slower to kill exposed ants, use of these parts as a barrier treatment should continue to have some effect over this period of time.

Acknowledgments

The author thanks Wizzie Brown, Molly Keck and Paul Nester for review of this report.

Description of a Technique Using of Arinix® Permethrin-Impregnated Nylon Spiral Wraps to Protect Airport Runway Lights at Northwest Regional Airport, Roanoke, TX

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Texas AgriLife Extension Service

Airport runway lights are critical for the success and safety of air transportation. Failure of these lights can be hazardous to people and property. Ants, including the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), can affect these and other electronic utilities (see http://www.extension.org/pages/30057/ants-and-their-affinity-for-electrical-utilities). The grounds maintenance volunteer of Northwest Regional Airport, Roanoke TX, Steve Trotter (personal communication, tmair@charter.net), estimates that 90% of runway light failures at this private airport occur due to fire ants. He spends roughly 120 hours per year (10 hrs/month) changing or replacing bulbs, re-scraping connections, repairing wiring connections and cleaning lenses to maintain the 50 lights along this airstrip. Runway light fixtures cost about $100 apiece and maintenance costs $600 to $1,000 per year.

Red imported fire ants are attracted to airport runway lights for several reasons: 1) they are warm nesting sites in cooler temperatures that attract ants due to their structure or location in raised areas where flooding is avoided; 2) lights attract June beetles and other insects which serve as a food source for the colony and attract foraging workers; 3) ants have an affinity for electrical utilities and if ants are electrically shocked they release chemicals (pheromones) that attract other worker ants that in turn get shocked and results in a large number of dead ants among circuitry that can result in equipment failure (**Fig. 1**). Thus, dead ants "pile up" at the base of light bulbs preventing electricity to flow into the bulb, and dirt brought into the bulb casing or lens blocks light emitted from functioning bulbs. Spiders also are found nesting in the support tubes of these fixtures where the nests are filled with insects attracted to the lights.

In 2005, Nix, Inc. (obtained registration of Arinix® by the U. S. Environmental Protection Agency (EPA): EPA Reg. No. 73745-2; EPA Est. No. 73745. This product consists of 8.56% permethrin-impregnated nylon plastic which has been manufactured in a wide variety of shapes for different uses, including grommets, spiral wraps, flat strips, L-shaped strips, and tackrolls (see http://nixofamerica.com/arinix.php). Data submitted for supporting product claims associated with product efficacy as a barrier treatment for elimination of the red imported fire ant was developed by Drees and Summerlin (2005, posted on http://fireant.tamu.edu). Laboratory tests conducted with Arinix, under accelerated aging conditions, showed that ARINIX™ is effective for five years and laboratory and field assessments have confirmed at least 4 years of effectiveness as of 2010. Sunlight, dirt and grime may reduce residual efficacy during use.

Figure 1. Airport runway light with red and blue lens (left), dead ants and nesting dirt in inoperable runway light (center) and in socket disrupting bulb connection (right).

Materials and Methods

On April 28, 2011, Arinix® parts were installed in 31of 50 airport runway lights which had been disassembled for repair, with 19 remaining lights serving as untreated controls. All lights were mapped and labeled (**Fig. 2**). Each Arinix treated light received two spiral wraps: 1) a larger (1 inch diameter spiral - 4inches long) spiral wrap was fitted to the inside surface of the support pole, and 2) a smaller (1/4 inch diameter) spiral was fitted around the wiring (**Fig. 3**). Thus, these two wraps served to make a continuous barrier preventing imported fire ant foraging workers and other arthropods like spiders from surviving contact with the permethrin surfaces, and thereby preventing problems with light function, wiring or importing dirt to obscure light shining through the lens.

Grounds maintenance workers (Terry Wheelock, NW Regional staff grounds maintenance at universalaerospace@sbcglobal.net, Steve Trotter and 3 volunteer pilots) have agreed to monitor all lights for at least 2 years and report any maintenance operations conducted for repair or replacement, including additional insecticide applications, identifying each light by the number or letter and recording service date (**Fig. 3**).

Results and Discussion

With two volunteers, three airport employees and authors working together, the 31 disassembled lights were repaired to incorporate Arinix® spiral wraps in roughly 3 hours (1:30 to 4:30 p.m.). Only one design was installed due to limited numbers of lights and available parts. However, use of just a single spiral and/or shorter lengths (1 inch minimum) may have dramatically reduced cost of Arinix installation without affecting effectiveness.

Acknowledgments

The authors wish to thank the personnel at the Northwest Regional Airport for access to the runway and providing the personnel to disassemble and re-assemble runway lights. We thank Yasushi Umeda, NIX, INC. for donating the Arinix spiral wraps used in this study. Finally, we thank the two Master Naturalists that helped with the installation were Mike Kmak and Bill Hammon for assisting with the Arinix applications. Steve Trotter, 310 Skyline Dr., Roanoke, TX 76262, 817/235-0004, tmair@charter.net. Review comments for this report from Dr. Paul Nester and Wizzie Brown are much appreciated.

Figure 2. Northwest Regional Airport, Roanoke TX – Arinix® treatments in runway lights, April 28, 2011.

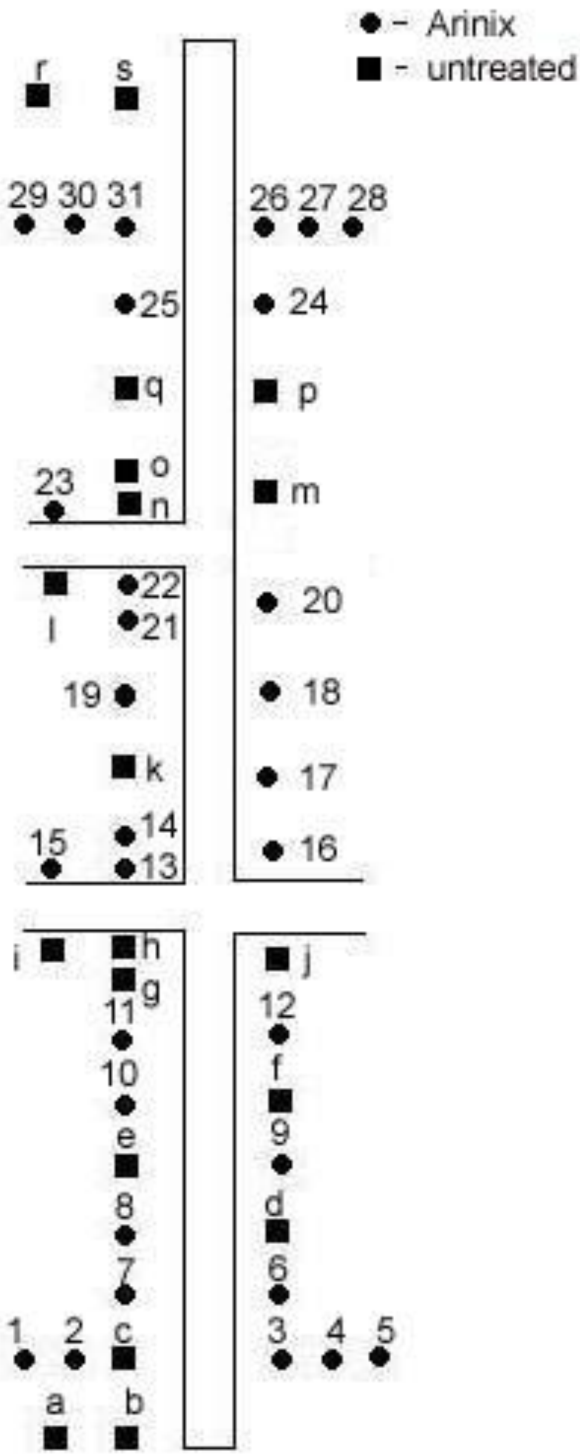

Figure 3. Installation of Arinix® spiral wrap parts into airport runway lights.

Statistical Analysis of 2008-2011 Annual Evaluations of Long-Residual Insecticidal Properties of Arinix™ Permethrin and Etofenprox Impregnated Nylon Cylinder and Spiral Wrap Parts as a Barrier Application for Protection of a Food Lure Target From Red Imported Fire Ant Foraging

Bastiaan M. Drees, Professor and Extension Entomologist,
Texas AgriLife Research and Extension, Texas A&M System

This report summarizes statistical analysis of 1 to 4 year aged cylinder and spiral wrap insecticide nylon parts to document the efficacy of Arinix® I (8.56% permethrin) and Arinix® II (9.4% ethofenprox) (Nix of America) affixed to the tops of dowel rods to deter foraging by the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), both in the laboratory and the field. Report of year 1 (2007) assessments and detailed description of methods and materials can be found at: http://fireant.tamu.edu/research/projects/pdf/urbanipmhandbook2007edited.pdf, pages 74-96.

Materials and Methods

Annually from 2007 to 2011, "new" Arinix parts were supplied by Nix of America (Yasushi Umeda, NIX, INC., y-umeda@nix.co.jp). Since 2010parts have been manufactured by a new firm and differ somewhat in design from original aged parts. Parts provided in 2007 were aged in: 1) closed storage units in the laboratory; 2) open "sun exposed" storage units outdoor; and, 3) closed "shaded" storage units outdoors. Annually in the fall, both laboratory and field trials to access the efficacy of new and aged parts were conducted using six replicates. Data were analyzed to compare mean numbers of ants and amount of peanut butter remaining on the tops of the dowel rods affixed with treatment parts. In the field, frames were used (**Fig. 1**) to support the treatment dowel rods and were moved from ant mound location to a new ant mound if ant activity on the mound ceased during the exposure periods. In the laboratory additional fire ants were added to assure maximum foraging pressure during the exposure periods. Numbers of imported fire ant foraging workers and amount of food lure (0.3 g or ¼ inch bead of Jiffy® Peanut Butter, smooth) remaining 24 to 265 hours following initial exposure were recorded. In 2010 and 2011, these trials were conducted in a covered carport to utilize higher temperatures and relative humidity to increase ant activity than what occurs in an air-conditioned laboratory. Results presented here include only the mean (average) amount of peanut butter remaining following initial exposure. Ant numbers associated with peanut butter food lure are available in raw data but not shown here. Data were analyzed using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ (SPSS 19.0).

Data for temperature and humidity were acquired for storage and both laboratory and field assessment conditions using a Hobo temperature and humidity monitoring devices. Following annual assessments, samples of the Arinix spiral wraps aged under various conditions and new parts were returned to Nix of America for chemical analysis annually.

Results and Discussion

After one year of aging (2007-2008) Arinix parts under various conditions, sun aged Arinix® I (permethrin) and Arinix® II (ethofenprox) cylinder parts began to show significant reductions in ability to protect a target food lure (0.3 g peanut butter on top of a dowel rod) from red imported fire ant foraging after 96 hrs of exposure (**Table 1**). Laboratory assessments, however, showed significant reduction of performance, while Arinix II showed numerical reduction of mean weight of food lure remaining (Sept. 28, 2008 trial, **Table 2**).

After two years of aging the Arinix parts (2007-2009), the field trial showed no statistical differences in performance between treatments, although mean weights of the food lure target were reduced for the untreated control and the 2 year sun aged Arinix I and II cylinder parts (**Table 3**). The laboratory trial found that after 96 hrs of exposure, all Arinix parts performed significantly better than the untreated control except for lab aged Arinix I, and with the exception of the sun aged and laboratory aged Arinix II, significant levels of protection continued through 265 hrs. (**Table 4**).

After three years of aging (2007-2010), the field trial documented significant mean differences of amount of peanut butter food lure remaining after 96 hrs of exposure for all Arinix I and II cylinder and new spiral wrap parts compared to the untreated control except for sun aged Arinix I and II parts (**Table 5**). However, new Arinix I spiral wrap parts, manufactured for this year's assessment by a different company, showed a significant reduction in performance compared to laboratory and shade aged Arinix I cylinder parts. The laboratory (carport) trial confirmed these results, although sun aged Arinix I performed significantly better than Arinix II after 96 hrs exposure to ant foraging (**Table 6**).

Field and laboratory trials conducted after 4 years of aging (2007-2011) provided inconclusive results. No statistical differences were found among treatments in the field trial (**Table 7**) after 96 hrs of exposure to foraging fire ants although initially (24 hrs of exposure), the laboratory aged Arinix II parts had significantly less peanut butter food lure remaining than all other treatments, and sun aged Arinix I and II parts has numerically less peanut butter remaining. As in 2009, the newly manufactured Arinix I and II parts provided numerically less protection of the target food lure after 96 hrs of ant foraging exposure than shade or laboratory aged Arinix I cylinder parts. The laboratory assessment confirmed these field trial results (**Table 8**): after 72 hrs of exposure the laboratory and outdoor shade 4 year old cylinder Arinix I cylinder parts had significantly more peanut butter food lure remaining that the untreated control while the outdoor sun and new Arinix I parts performed no better than the untreated control; Arinix II new, sun and shade aged cylinder parts performed significantly better than the untreated control.

Results of these trials support registration of 4 year laboratory and outdoor shade aged Arinix I (permethrin) cylinder parts (not the newly-manufactured, 2010 and 2011, spiral wraps) as capable of providing an effective barrier treatment to protect targets, such as the food lure used in these trials or others, from red imported fire ant foragers. They support modifying the current EPA insecticide label for this product to providing 4 years of residual control as opposed to the 1 year period of control stated on the current product label. For the most part, Arinix II performed similarly to Arinix I statistically.

Acknowledgement

The author is grateful for the support provided for these studies by Nix of America for these applied red imported fire ant research trials conducted by the Texas AgriLife Extension Service and Research. Review comments from Dr. Paul Nester and Molly Keck were appreciated and incorporated.

Fig. 1. Foraging structure housed in a red imported fire ant laboratory colony tray used to assess prevention of ant foraging on peanut butter placed on top of dowel rods with Arinix® Spiral Wraps or cylinders placed part way to the top of the rods.

Table 1. Mean amount of peanut butter (of 0.3 g) remaining after exposure to foraging red imported fire ants on dowel rods affixed with Arinix® I (permethrin) and II (ethofenprox) aged insecticide-impregnated spiral wraps, field trial, USDA Pecan Breeding Orchard, Brazos Co., TX, initiated Sept. 16, 2008.

		Peanut butter of 0.3 g remaining*			
Treatment		**24 h**	**48 h**	**72 h**	**96 h**
untreated		0.30	0.25	0.15b	0.05a
sun aged Arinix I		0.27	0.25	0.00a	0.00a
sun aged Arinix II		0.30	0.25	0.15b	0.05a
lab aged Arinix I		0.30	0.30	0.25bc	0.25b
lab aged Arinix II		0.30	0.30	0.25bc	0.25b
shade aged Arinix I		0.30	0.30	0.30c	0.29b
shade aged Arinix II		0.30	0.30	0.25bc	0.19b
new Arinix I		0.30	0.30	0.30c	0.28b
new Arinix II		0.30	0.30	0.30c	0.30b
	d.f.	8	8	8	8
	F	0.963	0.812	5.543	9.275
	P	0.476	0.596	0.00	0.00
	MSE	0.001	0.005	0.011	0.09

* Means of amount of peanut butter remaining at intervals following initial exposure of foraging red imported fire ant workers followed by the same letters are not significantly different using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT)(SPSS19.0).

Table 2. Mean amount of peanut butter (of 0.3 g) remaining after exposure to foraging red imported fire ants on dowel rods affixed with Arinix® I (permethrin) and II (ethofenprox) aged insecticide-impregnated spiral wraps, in the laboratory, initiated in Sept. 17 (top) and 29 (bottom), 2008.

Treatment		Peanut butter of 0.3 g remaining*		
		24 h	48 h	96 h
untreated		0.30	0.13a	0.05b
sun aged Arinix I		0.30	0.30b	0.30b
sun aged Arinix II		0.30	0.30b	0.25b
lab aged Arinix I		0.30	0.30b	0.30b
shade aged Arinix I		0.30	0.30b	0.30b
new Arinix I		0.30	0.30b	0.25b
	d.f.	5	5	5
	F	2.49	8.427	7.47
	P	0.53	0.00	0.00
	MSE	0.004	0.004	0.007

Treatment		Peanut butter of 0.3 g remaining*			
		24 h	48 h	72 h	96 h
untreated		0.20	0.14a	0.10a	0.05a
sun aged Arinix II		0.30	0.30b	0.26b	0.25b
lab aged Arinix II		0.30	0.30b	0.30b	0.30b
shade aged Arinix II		0.30	0.25ab	0.25b	0.25b
sun aged Arinix I		0.25	0.25ab	0.25b	0.17ab
new Arinix II		0.28	0.20ab	0.20ab	0.20b
	d.f.	5	5	5	5
	F	1.354	1.769	2.131	3.407
	P	0.269	0.15	0.089	0.15
	MSE	0.007	0.013	0.014	0.013

* Means of amount of peanut butter remaining at intervals following initial exposure of foraging red imported fire ant workers followed by the same letters are not significantly different using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT)(SPSS19.0).

Table 3. Mean amount of peanut butter (of 0.3 g) remaining after exposure to foraging red imported fire ants on dowel rods affixed with Arinix® I (permethrin) and II (ethofenprox) aged insecticide-impregnated spiral wraps, field trial, USDA Pecan Breeding Orchard, Brazos Co., TX, initiated Sept. 19, 2009.

		Peanut butter of 0.3 g remaining*				
Treatment		**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
untreated		0.25	0.20	0.20	0.20	0.20
sun aged Arinix I		0.25	0.20	0.20	0.20	0.20
sun aged Arinix II		0.25	0.20	0.20	0.20	0.20
lab aged Arinix I		0.30	0.30	0.30	0.29	0.29
lab aged Arinix II		0.30	0.30	0.30	0.30	0.30
shade aged Arinix I		0.30	0.30	0.30	0.25	0.25
shade aged Arinix II		0.30	0.30	0.30	0.30	0.30
new Arinix I		0.30	0.30	0.30	0.30	0.30
new Arinix II		0.30	0.30	0.30	0.30	0.30
	d.f.	8	8	8	8	8
	F	0.738	1.859	1.859	1.406	1.387
	P	0.658	0.091	0.091	0.22	0.228
	MSE	0.005	0.008	0.008	0.01	0.01

* Means of amount of peanut butter remaining at intervals following initial exposure of foraging red imported fire ant workers followed by the same letters are not significantly different using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT)(SPSS19.0).

Table 4. Mean amount of peanut butter (of 0.3 g) remaining after exposure to foraging red imported fire ants on dowel rods affixed with Arinix® I (permethrin) and II (ethofenprox) aged insecticide-impregnated spiral wraps, in the laboratory, initiated in Sept. 14, 2009.

		Peanut butter of 0.3 g remaining*							
Treatment		24 h	48 h	72 h	96 h	123 h	149 h	169 h	265 h
untreated		0.20	0.20	0.20	0.12a	0.05a	0.05a	0.00a	0.00a
sun aged Arinix I		0.30	0.30	0.30	0.25b	0.30b	0.30b	0.30c	0.15b
sun aged Arinix II		0.25	0.25	0.25	0.30b	0.30b	0.30b	0.20ab	0.00a
lab aged Arinix I		0.30	0.30	0.28	0.20ab	0.20b	0.20b	0.20ab	0.20bc
lab aged Arinix II		0.25	0.25	0.25	0.25b	0.25b	0.23b	0.12b	0.00a
shade aged Arinix I		0.30	0.30	0.30	0.30b	0.30b	0.30b	0.30c	0.30c
shade aged Arinix II		0.30	0.30	0.30	0.30b	0.30b	0.30b	0.30c	0.30c
new Arinix I		0.30	0.30	0.30	0.30b	0.30b	0.30b	0.30c	0.30c
new Arinix II		0.30	0.30	0.30	0.30b	0.30b	0.30b	0.30c	0.26c
	d.f.	8	8	8	8	8	8	8	8
	F	1.317	1.317	1.169	2.88	7.211	7.298	8.888	155.59
	P	0.26	0.26	0.339	0.011	0.00	0.00	0.00	0.00
	MSE	0.006	0.006	0.006	0.008	0.006	0.006	0.008	0.007

* Means of amount of peanut butter remaining at intervals following initial exposure of foraging red imported fire ant workers followed by the same letters are not significantly different using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT)(SPSS19.0).

Table 5. Mean amount of peanut butter (of 0.3 g) remaining after exposure to foraging red imported fire ants on dowel rods affixed with Arinix® I (permethrin) and II (ethofenprox) aged insecticide-impregnated spiral wraps, field trial, USDA Pecan Breeding Orchard, Brazos Co., TX, initiated Sept. 2, 2010.

		Peanut butter of 0.3 g remaining*		
Treatment		**24 h**	**48 h**	**96 h**
untreated		0.15a	0.05a	0.04a
sun aged Arinix I		0.10a	0.05a	0.05a
sun aged Arinix II		0.10a	0.05a	0.05a
lab aged Arinix I		0.30b	0.30b	0.30c
lab aged Arinix II		0.30b	0.30b	0.30c
shade aged Arinix I		0.30b	0.30b	0.29c
shade aged Arinix II		0.30b	0.30b	0.30c
new Arinix I		0.27b	0.20b	0.16b
new Arinix II		0.30b	0.30b	0.30c
	d.f.	8	8	8
	F	6.039	13.086	12.773
	P	0.00	0.00	0.00
	MSE	0.008	0.007	0.007

* Means of amount of peanut butter remaining at intervals following initial exposure of foraging red imported fire ant workers followed by the same letters are not significantly different using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT)(SPSS19.0).

Table 6. Mean amount of peanut butter (of 0.3 g) remaining after exposure to foraging red imported fire ants on dowel rods affixed with Arinix® I (permethrin) and II (ethofenprox) aged insecticide-impregnated spiral wraps, in the laboratory, initiated in Sept. 14, 2009.

Treatment		Peanut butter of 0.3 g remaining* 6 h	24 h	48 h	96 h
untreated		0.05a	0.00a	0.00a	0.00a
sun aged Arinix I		0.22b	0.20c	0.14b	0.14b
sun aged Arinix II		0.16b	0.11b	0.05a	0.05a
lab aged Arinix I		0.03c	0.30d	0.30c	0.30c
lab aged Arinix II		0.03c	0.30d	0.30c	0.30c
shade aged Arinix I		0.03c	0.30d	0.30c	0.30c
shade aged Arinix II		0.03c	0.30d	0.30c	0.30c
new Arinix I		0.03c	0.30d	0.25c	0.25c
new Arinix II		0.03c	0.30d	0.30c	0.30c
	d.f.	8	8	8	8
	F	7.886	14.316	14.484	14.753
	P	0.00	0.00	0.00	0.00
	MSE	0.006	0.005	0.006	0.006

* Means of amount of peanut butter remaining at intervals following initial exposure of foraging red imported fire ant workers followed by the same letters are not significantly different using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT)(SPSS19.0).

Table 7. Mean amount of peanut butter (of 0.3 g) remaining after exposure to foraging red imported fire ants on dowel rods affixed with Arinix® I (permethrin) and II (ethofenprox) aged insecticide-impregnated spiral wraps, field trial, USDA Pecan Breeding Orchard, Brazos Co., TX, initiated Sept. 14, 2011.

		Peanut butter of 0.3 g remaining*				
Treatment		**24 hr**	**48 h**	**60 h**	**72 h**	**96 h**
untreated		0.30b	0.15	0.18	0.18	0.12
sun aged Arinix I		0.25ab	0.20	0.17	0.15	0.15
sun aged Arinix II		0.24ab	0.15	0.12	0.12	0.12
lab aged Arinix I		0.30b	0.30	0.24	0.24	0.24
lab aged Arinix II		0.16a	0.15	0.18	0.18	0.12
shade aged Arinix I		0.30b	0.30	0.30	0.30	0.24
shade aged Arinix II		0.30b	0.25	0.24	0.24	0.20
new Arinix I		0.30b	0.25	0.24	0.24	0.12
new Arinix II		0.30b	0.25	0.24	0.24	0.18
	d.f.	8	8	8	8	8
	F	2.47	1.364	0.714	0.841	0.565
	P	0.305	0.238	0.676	0.573	0.799
	MSE	0.006	0.017	0.02	0.019	0.023

* Means of amount of peanut butter remaining at intervals following initial exposure of foraging red imported fire ant workers followed by the same letters are not significantly different using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT)(SPSS19.0).

Table 8. Mean amount of peanut butter (of 0.3 g) remaining after exposure to foraging red imported fire ants on dowel rods affixed with Arinix® I (permethrin) and II (ethofenprox) aged insecticide-impregnated spiral wraps, in the laboratory, initiated in Sept. 14, 2009.

		Peanut butter of 0.3 g remaining*		
Treatment		**24 hr**	**48 h**	**72 h**
untreated		0.20	0.17	0.05a
sun aged Arinix I		0.20	0.10	0.10ab
sun aged Arinix II		0.25	0.25	0.25b
lab aged Arinix I		0.25	0.24	0.24b
lab aged Arinix II		0.22	0.21	0.21ab
shade aged Arinix I		0.25	0.25	0.25b
shade aged Arinix II		0.26	0.26	0.25b
new Arinix I		0.25	0.25	0.20ab
new Arinix II		0.25	0.25	0.25b
	d.f.	8	8	8
	F	0.198	0.978	2.077
	P	0.99	0.465	0.058
	MSE	0.016	0.017	0.016

* Means of amount of peanut butter remaining at intervals following initial exposure of foraging red imported fire ant workers followed by the same letters are not significantly different using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT)(SPSS19.0).

Year 5: Evaluation of Long-Residual Insecticidal Properties of Arinix™ Permethrin-Impregnated Nylon Cable Wrap as a Barrier Application for Protection of a Food Lure Target for Red Imported Fire Ant Foraging

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Texas AgriLife Research and Extension,
Texas A&M System

This report summarizes the fifth year of a five-year evaluation of the efficacy of Arinix® I (permethrin) and Arinix® II (ethofenprox) (Nix of America) to deter foraging by the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), to the tops of dowel rods affixed with insecticide-impregnated nylon cylinders or spiral wraps both in the laboratory and the field. Report of year 1 assessments and detailed description of methods and materials can be found at: http://fireant.tamu.edu/research/projects/pdf/urbanipmhandbook2007edited.pdf, pages 74-96.

Materials and Methods

"New" 2010 Arinix parts were supplied by Nix of America (Yasushi Umeda, NIX, INC., y-umeda@nix.co.jp), September 2, 2011. New parts were recently manufactured by a new firm and differed somewhat in design from original aged parts. Aged parts are 4 years old (provided in 2007). Laboratory and field trials had six replicates and data were analyzed to compare mean numbers of ants and amount of peanut butter remaining on the tops of the dowel rods affixed with treatment parts. However, in the field trial, one replicate was lost due to armadillo destruction of the treatment frame after the 48 hr observation. The field trial was initiated September, 14, 2011 at USDA Pecan Breeding Orchard , Brazos Co., TXin a pecan orchard floor using nine treatments, including both Arinix® I containing permethrin and Arinix® II parts containing ethofenprox new and aged parts. Frames supporting the treatment dowel rods (**Fig. 1**) were moved from ant mound location to a new ant mound if ant activity on the mound ceased during the exposure periods. Numbers of imported fire ant foraging workers and amount of food lure (0.3 g or ¼ inch bead of Jiffy® Peanut Butter, smooth) remaining 24, 48, 60 and 96 hours following exposure were recorded. In addition, two laboratory trials were conducted in a similar manner: 1) initiated, in October, 12, 2011 and monitored 24, 28 and 72 hrs after initiation; and, 2) initiated October, 20, 2011 and monitored 24, 48, 72, 92 and 168 hrs after initiation omitting "new" Arinix I and II treatments due to excessive colony mortality affecting the first trial. As in 2010, these trials were conducted in a covered carport to utilize higher temperatures and relative humidity to increase ant activity than what occurs in an air-conditioned laboratory. Results presented here include only the mean (average) amount of peanut butter remaining following initial exposure. Ant numbers associated with peanut butter food lure are available in raw data but not shown here.

Data for temperature and humidity were acquired using a Hobo temperature and humidity recording device for storage and both laboratory and field assessment conditions. Following the assessments, samples of the Arinix spiral wraps aged under various conditions and new parts were returned to Nix of America for chemical analysis.

Fig. 1. Foraging structure housed in a red imported fire ant laboratory colony tray used to assess prevention of ant foraging on peanut butter placed on top of dowel rods with permethrin-impregnated nylon Spiral Wraps or cylinders placed part way to the top of the rods.

Results

Field trial. Results of the 2011 field assay conducted at the USDA Pecan Breeding Orchard, Brazos Co., TX initiated September, 14, 2011 were less clear than in previous years' assessments. Texas endured a record breaking hot, dry summer (The Eagle, Bryan College Station, Sept. 9, 2012, "Texas Summer Set Record High" by Maggie Kiely: "The state's 86.8 degree average for this summer beat the previous record of 85.5 degrees, which was set in Oklahoma in 1934, according to meteorologists.") and this trial was initiated prior to a rain event (Hobo data available but not included herein). Thus, ant foraging from weakened ant colonies was sub-normal, even though this orchard has a drip irrigation system. Due to excessive mortality to ants in active mounds on which the frames holding treated dowel rods were placed, they required frequent re-location to other active ant mounds in order to provide sufficient foraging pressure for ants to remove the peanut butter food lure. Shade and laboratory aged Arinix I (permethrin) retained more peanut butter than did other treatments. As in previous year's assessment, Arinix I and II parts aged for 3 years in direct sunlight were least effective at preventing loss of peanut butter food lure from dowel rods (**Fig. 1**). Again, the "new" (2011) Arinix I (permethrin) parts were somewhat less effective at protecting the ants from removing the food lure than were previously manufactured parts aged in the laboratory or in absence of direct sunlight.

Laboratory trials. Results of the first assay demonstrated that compared to the untreated control, the insecticide-impregnated nylon Arinix parts (new or aged 3 years initially containing the same percent active ingredient) continued to offer protection by preventing ants from consuming as much peanut butter (shown in percent remaining of 0.3 g provided). As in 2010 except for Arinix I and II parts aged in direct sunlight (**Fig. 2)**. However, in this trial Arinix II (ethofenprox) aged 3 years in direct sunlight performed better than did similarly aged Arinix I (permethrin). In the second field trial, conducted without new Arinix I or II treatments, all aged treatments seemed to similarly to the untreated control.

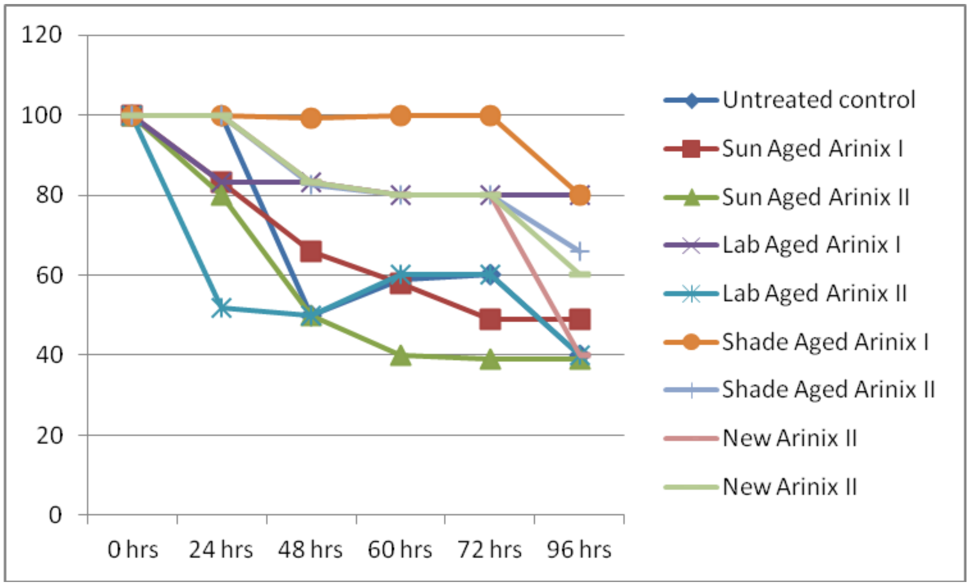
Discussion

A statistical analysis of results should indicate whether differences between treatments are significant. Provided graphs (**Figures 2-4**) indicate obvious trends. Temperature and humidity conditions of aging parts and conditions during laboratory and field trials are available as separate spreadsheet files.

Acknowledgments

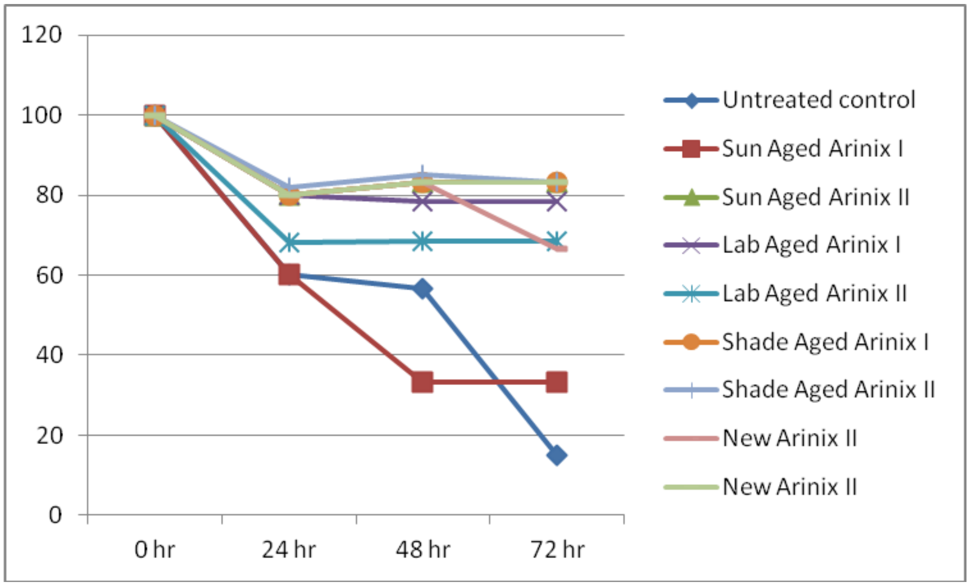
The authors appreciate support from Nix of America and review comments by Dr. Paul Nester and Molly Keck.

Figure 2. Amount of peanut butter (percent of 0.3 g) remaining after exposure to foraging red imported fire ants on dowel rods affixed with Arinix® I and II aged insecticide-impregnated spiral wraps, field trial, USDA Pecan Breeding Orchard, Brazos Co., TX, initiated September, 14, 2011: Percent peanut butter remaining (Y-axis) versus Time (X-axis).



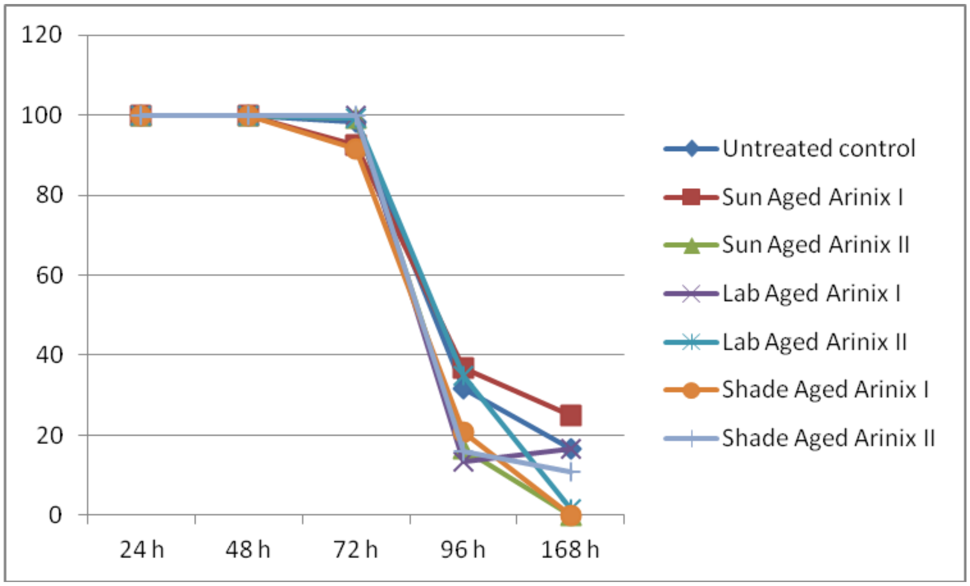
	0 hrs	24 hrs	48 hrs	60 hrs	72 hrs	96 hrs
Untreated control	100.0	100.0	50.0	59.0	60.0	40.0
Sun Aged Arinix I	100.0	83.3	65.8	58.0	49.0	49.0
Sun Aged Arinix II	100.0	80.0	50.0	40.0	39.0	39.0
Lab Aged Arinix I	100.0	83.3	83.3	80.0	80.0	80.0
Lab Aged Arinix II	100.0	51.7	50.0	60.0	60.0	40.0
Shade Aged Arinix I	100.0	100.0	99.2	100.0	100.0	80.0
Shade Aged Arinix II	100.0	100.0	82.5	80.0	80.0	66.0
New Arinix I	100.0	100.0	83.3	80.0	80.0	40.0
New Arinix II	100.0	100.0	83.3	80.0	80.0	60.0

Figure 3. Amount of peanut butter (percent of 0.3 g) remaining after exposure to foraging red imported fire ants on dowel rods affixed with Arinix® I and II aged insecticide-impregnated spiral wraps, laboratory (carport **Trial 1**, Center for Urban and Structural Entomology, Texas A&M University, Brazos Co., TX, initiated in October 12, 2011: Percent peanut butter remaining (Y-axis) versus Time (X-axis).



	0 hr	24 hr	48 hr	72 hr
Untreated control	100.0	60.0	56.7	15.0
Sun Aged Arinix I	100.0	60.0	33.3	33.3
Sun Aged Arinix II	100.0	80.0	83.3	83.3
Lab Aged Arinix I	100.0	80.0	78.3	78.3
Lab Aged Arinix II	100.0	68.0	68.3	68.3
Shade Aged Arinix I	100.0	80.0	83.3	83.3
Shade Aged Arinix II	100.0	82.0	85.3	83.3
New Arinix I	100.0	80.0	83.3	66.7
New Arinix II	100.0	80.0	83.3	83.3

Figure 4. Amount of peanut butter (percent of 0.3 g) remaining after exposure to foraging red imported fire ants on dowel rods affixed with Arinix® I and II aged insecticide-impregnated spiral wraps, laboratory (carport **Trial 2**, Center for Urban and Structural Entomology, Texas A&M University, Brazos Co., TX, initiated in October, 20, 2011: Percent peanut butter remaining (Y-axis) versus Time (X-axis).



	24 h	48 h	72 h	96 h	168 h
Untreated control	100.0	100.0	98.3	31.7	16.7
Sun Aged Arinix I	100.0	100.0	92.5	36.7	25.0
Sun Aged Arinix II	100.0	100.0	99.27	16.7	0.0
Lab Aged Arinix I	100.0	100.0	100.0	13.3	16.7
Lab Aged Arinix II	100.0	100.0	99.2	35.0	1.7
Shade Aged Arinix I	100.0	100.0	91.7	20.8	0.0
Shade Aged Arinix II	100.0	100.0	100.0	15.8	10.8

Community wide fire ant management at Belterra in Austin, TX

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Belterra is a 1,600 acre community in Travis County containing around 600 occupied homes as well as walking trails, 12 park areas and a recreation center that includes a playscape and swimming pool. The community also contains an elementary school situated on a 12.5 acre campus. The Belterra community is still in development, but 400 acres are reserved as greenbelts and open spaces and 106 acres are set aside as mixed use to allow for retail locations in the future.

In 2009, Texas AgriLife Extension Service was approached by Makar Properties to help initiate a community wide fire ant management program. After meeting with representatives, it was decided by the management company to designate a landscape company to be responsible for treating fire ants in greenbelts and common areas while residents would be responsible for treating fire ants on their property.

Over 560 residents have signed up to receive regular emails from the management company. Through this service, residents were sent information on fire ant management, community wide fire ant management and a spring and fall date directing them when to treat their property for fire ants.

Materials and Methods

Sixteen areas throughout the treated community were monitored for fire ant mounds and activity and data was collected. In each selected area, the lid of a 9 dram clear styrene tube containing a hot dog slice was left exposed for at least 45 minutes. After 45 minutes, the hot dog slices were inspected for foraging ants. If ants were present on the hotdog slice, the bait cup was capped and marked with the date and location. Bait cups were frozen, ants were identified and exact numbers recorded at a later time. Each location was monitored for active fire ant mounds and suspected nest or mound sites. Each were disturbed with a stick and counted as active if many (50+) worker ants were observed to emerge.

Four counts were taken in 2011- pre and post-baiting in both the spring and the fall. Community wide fire ant management days, or “Ant Out” days, were held on April 9, 2011 and October 29, 2011. Residents were responsible for treating their property for fire ants using the method and/ or product of their choice.

Monitored areas varied in size. The square footage of the areas was recorded and mound numbers adjusted to mounds per 1000 square feet so a true comparison could be made.

Results & Discussion

Monitored areas showed a low number of fire ant mounds throughout the year, but numbers were highest June through October (Fig 1). Foraging fire ant numbers increased until spring treatment and then decreased (Fig 2). It is possible that drought conditions suppressed locating mounds throughout the year and that high, day time temperatures decreased foraging activity during the day when the neighborhood was monitored.

Figure 1. Mean number of mounds of red imported fire ants found in selected areas of Belterra, Austin, TX during 2011 community wide fire ant management project.

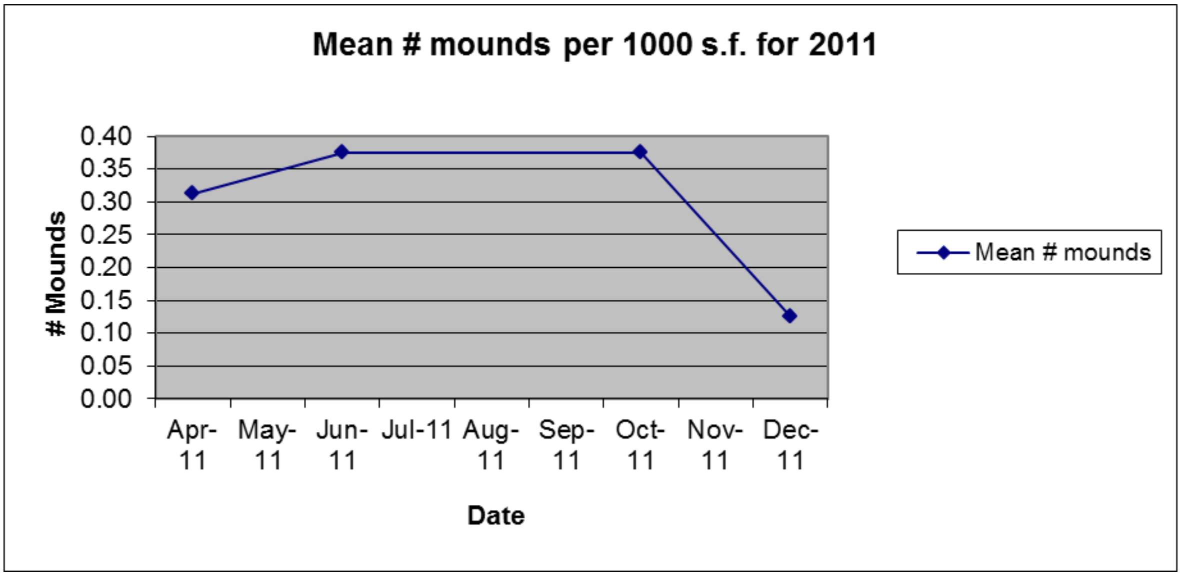
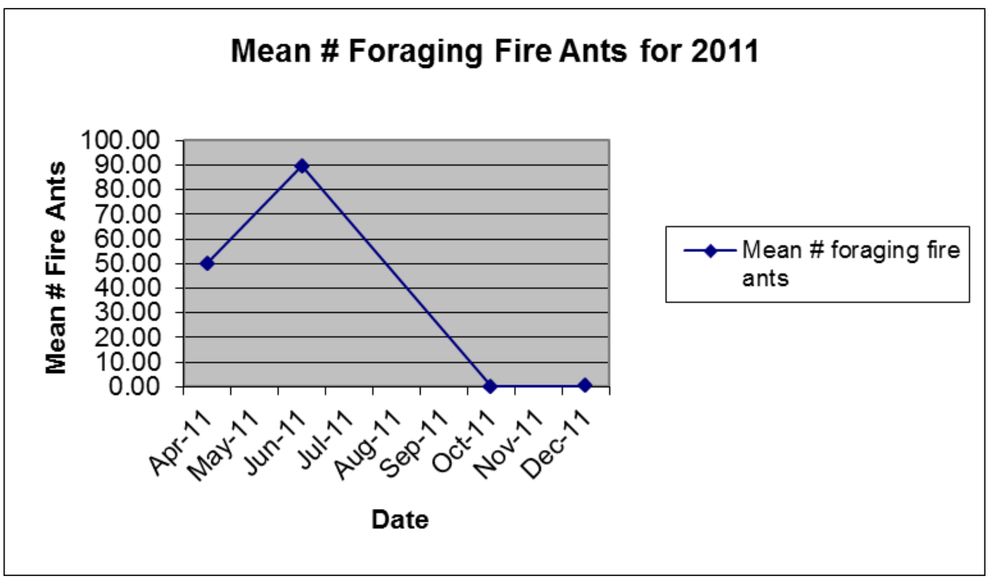


Figure 2. Mean number of foraging red imported fire ants found in selected areas of Belterra, Austin, TX during 2011 community wide fire ant management project.



Community wide fire ant management programs are a way for neighborhoods to reduce fire ant populations within their community. The Belterra approach allows residents to choose the method and product that they want to utilize for fire ant treatment which allows for differences of opinion. While this method may be better for some residents, fire ant management seems spotty throughout the neighborhood. Some monitored locations consistently either have active mounds or foraging fire ants while other monitored locations rarely have active mounds or foraging ants. While the management company is happy with the effort, I think the program would improve with a more aggressive strategy of baiting the common areas and green spaces within the neighborhood in spring and fall. While green spaces make up a large amount of the community, the landscape company spot treats mound they locate while mowing. Individual spot treatments may not kill all mounds and is time consuming. It would be more cost effective to broadcast bait.

Acknowledgements

The author would like to thank Denise Veselka for helping get information on fire ants and their management to the residents of Belterra.

Community wide fire ant management at Lindsey Place Neighborhood in San Antonio, TX

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Community wide fire ant management programs include participation by the entire community to treat for fire ants at a given time. This can reduce red imported fire ant populations and reduce pesticide costs for community residents, as demonstrated by Riggs et al. (2002).

Lindsey Place Neighborhood in northwest San Antonio, TX has been participating in a community wide fire ant management program for four years (2007, 2008, 2009 and 2011). Lindsey Place consists of 181 homes and a 1.5 acre easement that is used for community events. After not participating in the program in 2010, Lindsey Place Homeowners Association decided to participate in the program again in 2011.

Materials and Methods

One week before the community wide fire ant program baiting commenced, on May 26, 2011, 30 areas throughout the neighborhood were observed for fire ant foraging activity. This occurred only in front yards, and all front yards were observed for visible fire ant mounds.

In order to observe fire ant foraging a food lure consisting of a slice of hot dog was placed in the grass during the morning hours when fire ants were actively foraging for food during summer months. After 45 minutes, the hot dogs were observed and the presence of fire ants or native ants was recorded.

On June 1, 2011, every front yard in the neighborhood, the easement, and any common area (such as those around community mailboxes) were treated with Extinguish® Plus Fire Ant Bait using a Herd Seeder Bait Blower. This occurred approximately 10am to 10:30am.

June 3, 2011, neighbors were invited to pick up bait to treat their backyards for fire ants. Thirty three households took advantage of this opportunity. All households who picked up bait were asked to complete a questionnaire, asking for information about their current fire ant problem, management practices, and amount of money spent annually on management of fire ants.

Five weeks after the bait treatment, fire ant foraging and visible mounds were observed in 40 locations throughout the neighborhood. Hot dog slices were placed in the grass, and left out for 45 minutes before the presence of fire ants was observed and recorded. Any visible mounds were also recorded.

Two months after the community wide management program, a questionnaire was mailed to all individuals within the neighborhood. This questionnaire asked questions about their current fire ant problem and if management has been necessary since the program.

Results & Discussion

Fire Ant Observations

Of the 30 food lures that were placed out prior to the baiting event, 63.3% attracted fire ants, 20% attracted native ants , leaving 16.6% with no ants present. (Figure 1). There were 16 visible and active mounds found throughout the front yards and easement area of the neighborhood.

Five weeks post treatment, 42.5% of the hot dogs had fire ants actively foraging on them, 25% had native ants, and 32.5% had no ant species (Figure 1). One visible, active fire ant mound was observed.

Overall, there was a decrease in fire ant foraging activity and visible mounds and an increase in native ants, after the community wide fire ant management program. There was a 33% decrease of fire ant mounds and 25% increase of native ants attracted to hot dog food lures. Active fire ant mounds declined over 90% (16 active mounds to one).

Survey Results

Thirty three individuals picked up bait to treat their backyards and completed a pre-treatment survey. These surveys asked four questions related to current management practices:

1. How would you describe your current fire ant problem?
2. How much money do you spend each year on fire ants?
3. How often do you treat for fire ants?
4. When you treat for fire ants, what method do you use?

Results from the evaluation show that 52% of participants consider their fire ant problem to be moderate or severe. The average cost per year for managing fire ants in Lindsey Place is $73.57. This includes medical attention, pesticides, supplies, and pest management professionals. 39% treat for fire ants at least monthly and the preferred method of treatment is through the use of granules (53%). However, 41% prefer to use baits, which may be a direct result of this community participating in a community wide management program for four years, and seeing the results and cost benefits of using baits to manage fire ants.

Post-treatment surveys were returned at a 10% rate (17 of 169). These surveys asked four questions related to current management practices after the community wide fire ant management program:

1. Did you notice a decrease in fire ant activity or mounds after the fire ant treatment?
2. Have you changed your fire ant practices since the community wide fire ant program?
3. If you have changed your practices, how?
4. What treatments have you used since the community wide fire ant program?

Post treatment survey results showed that 65% of participants noticed a decrease in fire ant activity. 47% changed their fire ant practices, and of those 56% now treat less often. The treatments used since the community program included individual mound treatments using baits (6%), granules (56%), dusts (6%), drenches (6%) and using no treatments (38%). (Figures 5-7).

Community wide fire ant management programs are a way for neighborhoods to reduce fire ant populations within their community. Treating with fire ant bait products on the same day allows all foraging fire ants from visible mounds non-visible mounds access to bait particles which they take back to their respective mounds. The particles are fed upon by the entire fire ant colony and the colony dies from within. Since all visible and non-visble mounds over the whole neighborhood are affected, this can decrease the cost for the community by reducing the need for follow-up single mound treatments. There has been a trend in Lindsey Place from using baits to the use of granules for individual mound treatments. This may be a result from lower fire ant densities due to continued baiting from the community wide fire ant management program. While control was not as high from the 2011 program as anticipated, this may be a result from lack of participation in backyard treatments. In future years, a greater effort needs to be made to encourage all residents to treat backyards in addition to the provided front yard treatment.

An effort will be made to encourage Lindsey Place Neighborhood to participate in another community wide fire ant management program in 2012.

Figure 1. Percent of food lures with fire ants, native ants, or no ants attracted to hot dog slices pre-treatment and post-treatment.

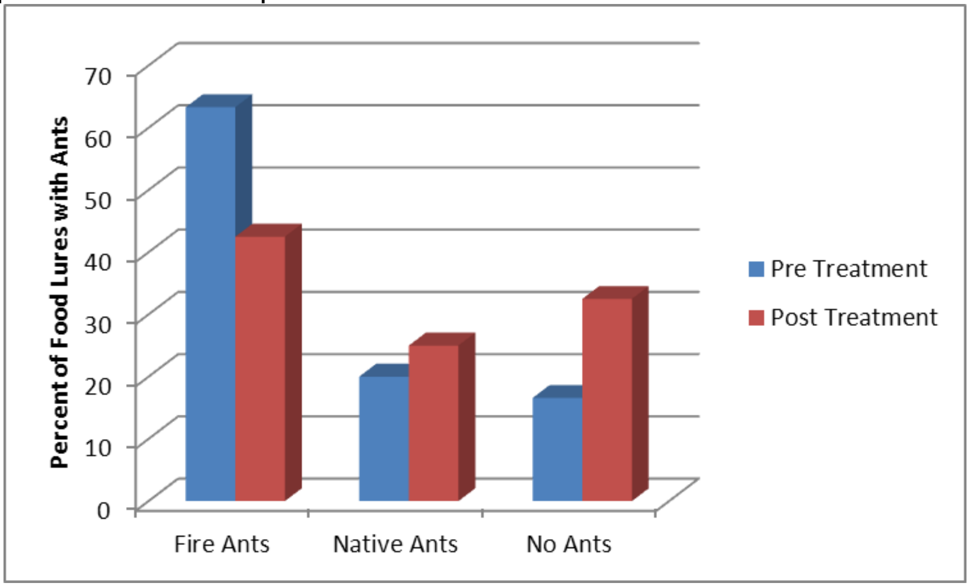


Figure 2. Results from pre-treatment survey. Self-description of current fire ant problem.

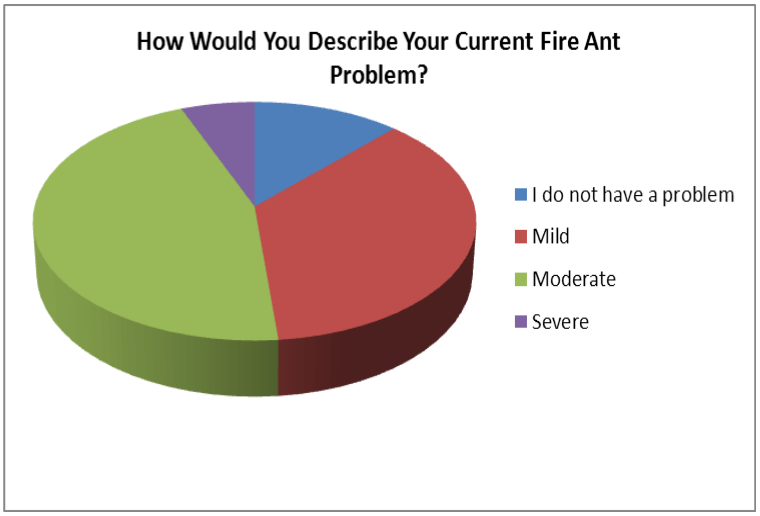


Figure 3. Results from pre-treatment survey. How often participants treat for fire ant before community wide fire ant management program.

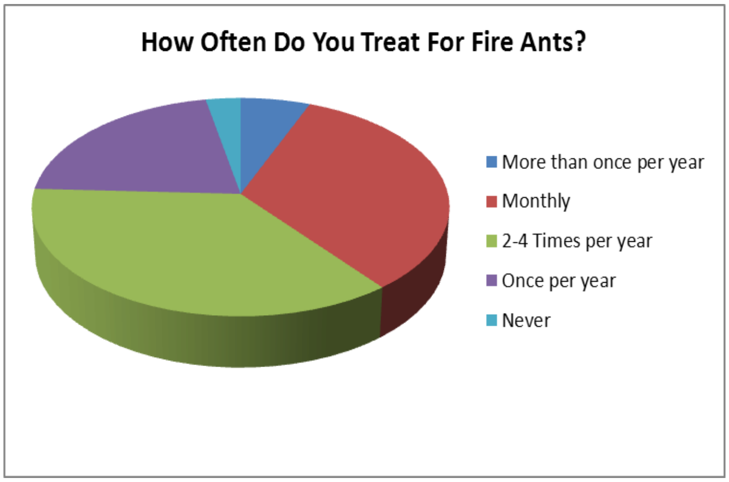


Figure 4. Results from pre-treatment survey. Method of fire ant management used by participants before community wide program.

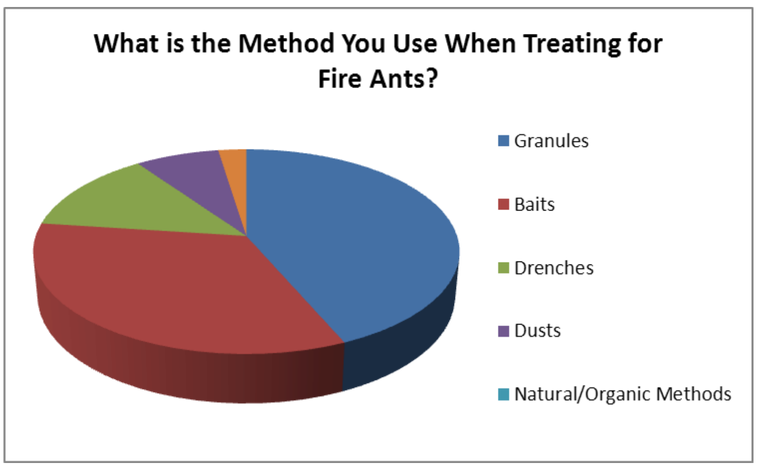


Figure 5. Results from post treatment survey. Participants determination of current fire ant activity.

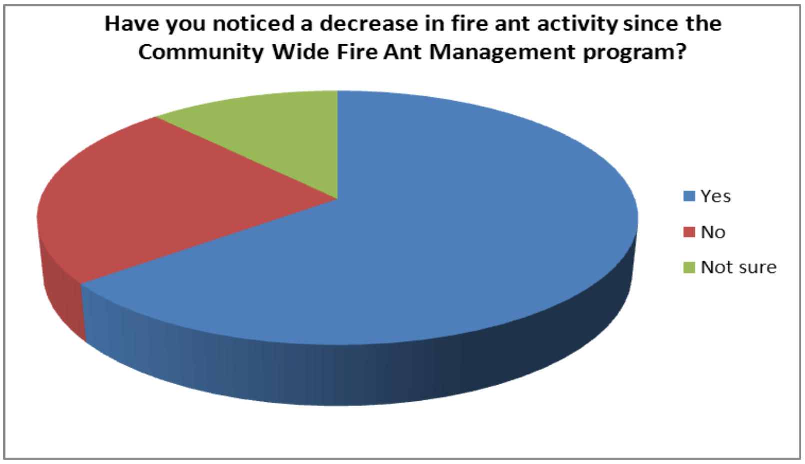


Figure 6. Results from post treatment survey. Participants determination of fire ant activity after community wide program.

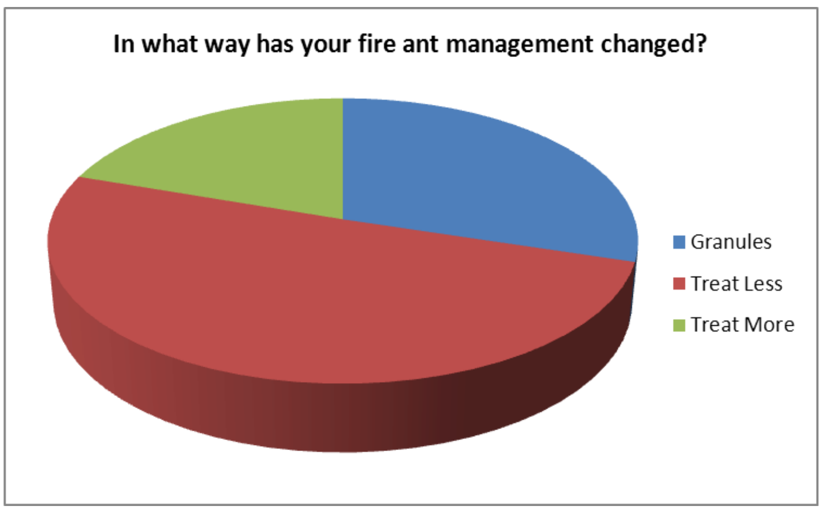
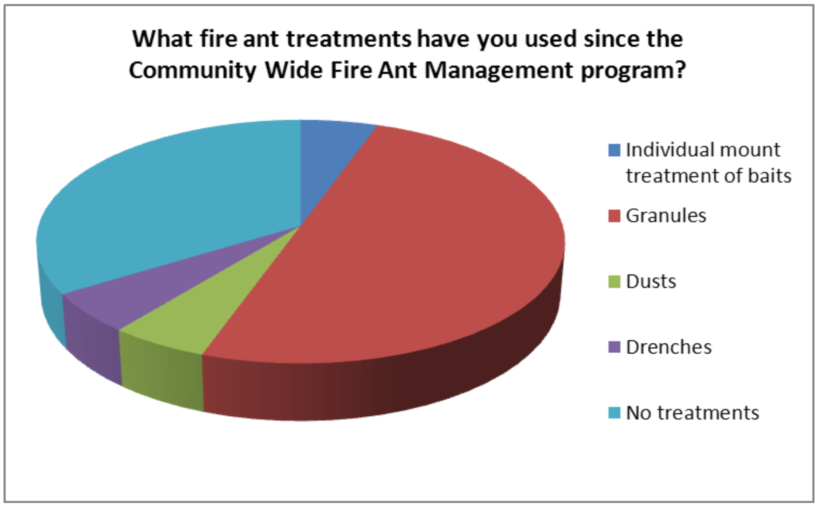


Figure 7. Results from post treatment survey. Participants determination of fire ant activity after community wide program.



Acknowledgements

The authors would like to thank Jesse Garcia, Lindsey Place Neighborhood Association president, for his help in this community wide fire ant management program.

Community wide fire ant management program at Wood Glen in Round Rock, TX

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Texas AgriLife Extension Service

Wood Glen subdivision in Round Rock, TX, Williamson County, began a community wide fire ant management program in the spring of 2005. Wood Glen is a 250 acre community with approximately 60 acres of green belt area. The Wood Glen Property Owners Association consists of nearly 550 homes. Common areas include areas such as a swimming pool, tennis courts, playground equipment and walking trails.

Riggs et al. (2002) demonstrated that community wide fire ant management programs can help reduce red imported fire ant (fire ant) populations and reduce pesticide costs for community residents. By developing community wide programs for larger treatment areas such as entire neighborhoods, fire ant re-infestation can be reduced or delayed.

Materials and Methods

Sixteen areas throughout the treated neighborhood were monitored for fire ant mounds and ant activity. One area outside the treatment area was selected and monitored for fire ant activity to serve as an untreated control. In each selected area, a hot dog slice was placed in the lid from a 9 dram clear styrene condiment cup and left exposed for at least 45 minutes. Thereafter, the hot dog slices were inspected for foraging ant workers. If ants were present on the hot dog slice, the bait cup was capped and marked with the date and location. Containers were frozen, ants were identified and exact numbers recorded at a later time. Each location was monitored for active fire ant mound sites. When fire ant mounds were located, they were disturbed with a stick and counted as active if many (50+) worker ants were observed to emerge. Four counts were taken in 2011: pre- and post-baiting in both the spring and the fall.

Extinguish® Plus Fire Ant Bait (0.365% hydramethylnon plus 0.25% s-methoprene) has been utilized for the neighborhood baiting program since its inception in 2005. The bait is broadcast at a rate of 1.5 pounds per acre. Since 2007, common areas, green belts and front yards were baited spring and fall by a pest management provider company that was contracted for this service by the homeowner's association.

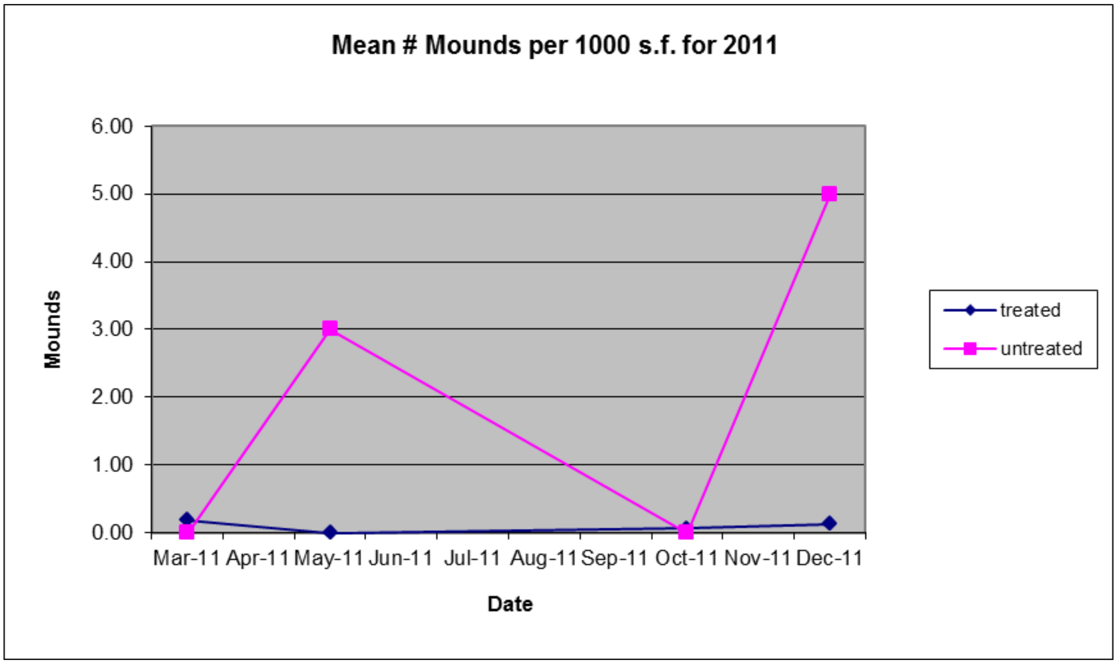
On April 16, 2011, a booth was set up in the common area of the neighborhood to provide information about fire ants and the community wide management efforts to anyone who chose to participate. Information about the bait handout date was sent out via email and signs were posted one week prior at each entrance. Residents were provided with pre-measured bait in a hand spreader. Residents supplied the approximate square footage of their backyard and the appropriate amount of bait was measured out into their spreader. Fall backyard bait handout occurred on October 8, 2011.

Monitored areas varied in size. The square footage of the areas was recorded and mound numbers adjusted to mounds per 1,000 square feet so a true comparison could be made.

Results & Discussion

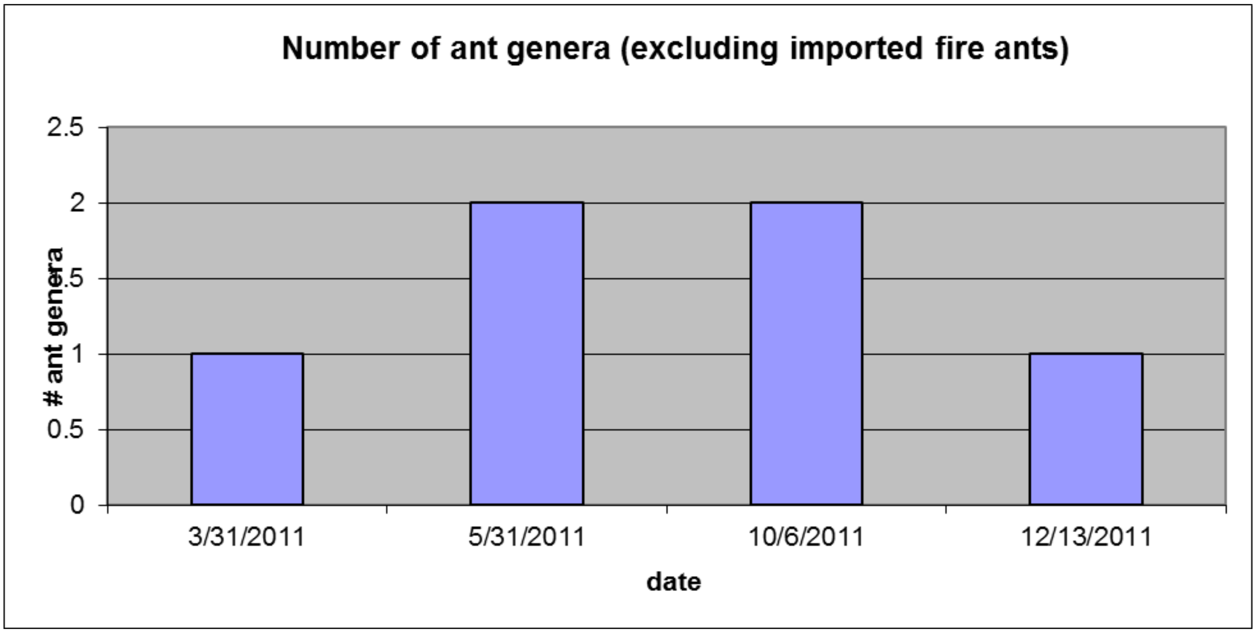
Treated areas showed a decrease in the mean number of mounds per square foot when compared to the untreated area (**Figure 1**). The mound numbers in the treated areas remain low as in previous years of the study, maintaining fire ants at a level that is satisfactory to residents of Wood Glen.

Figure 1. Mean number of mounds of red imported fire ants per 1,000 square feet found in selected areas of Wood Glen, Round Rock, TX during 2011 community wide fire ant management program.



Porter and Savignano (1990) found that native ant populations decreased when fire ants moved into an area. When fire ants are suppressed with community wide fire ant management, numbers of native ants can be increased (Riggs et al. 2002). The ant diversity in the Wood Glen neighborhood increased as fire ants have been suppressed by community wide fire ant management (Brown et al. 2007). This year, again, showed a continuation of native ants entering monitored areas (**Figure 2**).

Figure 2. Number of ant genera other than red imported fire ants found in selected areas of Wood Glen, Round Rock, TX during 2011 community wide fire ant management project.



Community wide fire ant management programs are a wonderful tool for neighborhoods to utilize for controlling imported fire ants and the problems they cause. Not only can this type of program reduce numbers of this pest species, but it can also increase native ant abundance which can provide biotic resistance to invading exotic pest ants such as red imported fire ants. Neighborhoods only need to have willing volunteers to formulate and implement a plan to garner the rewards of reduced fire ant population levels.

Acknowledgements

The author would like to thank Leslie & Sam Myers and for their efforts to continue the Wood Glen Fire Ant Management Project.

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“Fighting Texas’ Fire Ants: The Team Approach”
10 Years and Counting – Renewing interest in the Lago Santa Fe Fire Ant Project

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Bastiaan “Bart” M. Drees, Professor and Extension Entomologist
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Managing the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) through broadcast applications of fire ant bait products has been demonstrated to dramatically reduce the cost, insecticide use, maintain control of fire ants, and help eliminate problems caused by the fire ant (Riggs et.al, 2002). The Lago Santa Fe Fire Ant Project is a good example of a successful community-wide fire ant management program where the bi-annual broadcasting of a fire ant bait product (Extinguish® Plus) has resulted in continued suppression of a fire ant population. Lago Santa Fe is a Private Lake Community, situated 25 miles, South/Southwest of Houston, TX, in Galveston Co. The Community consists of 100 acres with four, ½ mile long X 200 ft wide lakes, with 48, one acre lots that border the lakes and 12 lots that do not border the lakes (Nester et.al, 2003).

Historical Perspective, 2001 – 2002. In 2001, Lago Santa Fe (**Figure 1**) was chosen as the site to host the 2002 National Water Ski Championships and the 2002 U.S. Open Water Ski Championships. This meant that in August of 2002, 1000 participants from all over the U.S. and the World, with up to 4,000 spectators would invade this 100 acre area for 7 days. Initial fire ant mound activity evaluations showed over 160 large (> 12” diameter) active fire ant mounds per acre (**Table 1**). The Lago Santa Fe Community realized they needed a coordinated approach to manage these pests. The Texas AgriLife Extension Service decided to work with the residents of Lago Santa Fe and develop an annual repeatable process for the management of the fire ants in this community. The Lago Santa Fe Fire Ant Project gave The Texas AgriLife Extension Service a chance to “showcase” various ideas, practices, products, and product uses developed by the Texas Imported Fire Ant Research and Management Plan which included:

1) Evaluation of the effectiveness of the "hopper blend" treatment (50:50 hydramethylnon plus s-methoprene ant bait later released as Extinguish® Plus Fire Ant Bait) at 1.5 lb blended product/acre.
2) Demonstration of application methods such as the truck-mountable industrial "bait blower"; and the ATV mounted Herd Broadcast Spreader.
3) Demonstration of the practicality of scheduling fire ant bait treatments to reach a goal of maximum control for the athletic events being hosted by the Lago Santa Fe Community, i.e., the 2002 and 2003 National Water Ski Championships, and U.S. Open Water Ski Championships.
4) Demonstration of how coordinating the efforts of the entire community resulted in an efficient process for managing fire ants over the Lago Santa Fe property.

Fire Ant mound activity counts showed that after a single spring 2002 hopper blend treatment, fire ant activity was reduced 85% in the community of Lago Santa Fe before the scheduled 2002 water ski events (**Table 2**). Full report can be found at http://www.extension.org/sites/default/files/w/0/03/2003_IFA_Conference_Proceedings.pdf.

2002 – 2004 ongoing program treatments: This community was also chosen to host these same events in the summer of 2003. In the succeeding years after the successfully implementation of the Lago Santa Fe fire ant project the community reduced the highs and lowered the lows of the yearly fluctuations in fire ant numbers (**Table 2**). After an additional fall 2002 and spring 2003 hopper blend treatment, fire ant mound activity was down 95%, before the scheduled 2003 water ski events. The community has continued to manage their fire ants. After 5 scheduled baiting events (April 2002 through May 2004) the fire ant mound activity reduction remained above 90% when compared to initial fire ant activity recorded in the spring of 2002 (**Table 2**).

2004 – 2008 ongoing program treatments: Lago Santa Fe residents continue their efforts to manage the fire ant with bi-annual applications of a fire ant bait product. Lago Santa Fe residents pay quarterly dues to the Property Owners Association (POA). Fire ant baiting is funded from the general dues just like mowing, lake dye, insurance, electricity etc. Fire ant bait is a line item in the budget that is approved annually by the Board of Directors (BOD). The most recent (2011) fire ant budget consists of seven 25 pound bags of fire ant bait, miscellaneous supplies and fuel for ATV on which a Herd G-77 Broadcast Seeder is mounted. It is estimated that each property owner will pay about $20 for fire ant management every 6 months for a total cost of $40/year/property (**Table 3**).

In a 2008, the Lago Santa Fe Fire Ant Project was re-visited and the residents surveyed as to their satisfaction of this project over the past years (Nester et.al, 2008). The residents (11 responses from 40 property owners, 28%) all responded (100%) that they felt the dollars spent by the POA for the Lago Santa Fe Fire Ant Project was cost-effective and all responded (100%) that they wanted it to continue. All (100%) ranked the project from good to excellent and 10 of the residents (91%) felt very positive about this fire ant baiting program being supported by the Texas AgriLife Extension Service. One of the residents was somewhat positive. One comment from a resident was, “Prior to moving to TX / Lago Santa Fe a few years ago, we had never lived anywhere that had a fire ant problem. We’ve only been exposed to the problem and solutions since living here but have heard numerous stories from neighbors as to how bad it was and how it is today. We certainly appreciate all the efforts made by Texas AgriLife Extension Service and Jay and Lydia Gilbert” (Note: all comments and survey results can be viewed at:
http://fireant.tamu.edu/research/projects/pdf/ipmmanual08a1.pdf).

2010 to present program activities: Extenuating circumstances kept the original organizers from coordinating the baiting event in the fall of 2010. Since other community members did not pick up the effort, the fall 2010 community-wide baiting event did not happen. As the year ended and the new (2011) year began, there was chatter in the community indicating that fire ant populations were increasing on the various properties, in numbers not seen in previous years (personal communication with Jay Gilbert, Lago Santa Fe resident, fall 2010). When communicating with Jay Gilbert, one of the original organizers of the Lago Santa Fe Fire Ant Project, it was decided to try and renew interest in the community-wide project by making a concerted effort in the spring of 2011 (10 years after the first fire ant baiting event) to make all residents aware of the importance of the bi-annual baiting by organizing an event and taking before and after observations of fire ant activity.

Materials and Methods

A date to spread the Extinguish® Plus Fire Ant Bait product (hydramethylnon + s-methoprene), donated by Central Life Sciences, Dallas, TX, over the Lago Santa Fe community was set for April 27, 2011. These are the only fire ant bait active ingredients that had been used on this property in previous years.

Pre-treatment fire ant mound activity observations were taken using the minimal disturbance method April 25, 2011 (**Table 4**) from 0.25 acre circles from 6 properties within the Lago Santa Fe community (a reduction in plot numbers due to houses constructed on previously vacant lots) . As in previous years, fire ant activity observations and ant mound counts were also taken from four 0.25 acre circles in an adjacent untreated pasture to serve as a control plot area. These same treated and untreated plot locations were checked 90 days later for fire ant mound activity. At the same time we conducted active ant mound counts in plots, we measured fire ant foraging activity by placing 20 hot dog food lures (0.25 inch thick slices of Bar-S Jumbo Franks) in the front yards of 20 of the interior properties that border the lakes. After 60 minutes, the total estimated number of fire ants per lure was recorded (**Table 5**). Food lures were also used 90 days later to check for fire ant foraging activity.

This time the Lago Santa Fe Fire Ant Project gave The Texas AgriLife Extension Service a chance to again:

1) Showcase The Community –Wide fire ant baiting concept promoted by the Texas AgriLife Extension Service.
2) Demonstrate how coordinating the efforts of the entire community can result in an efficient process for managing fire ants over the Lago Santa Fe property.
3) Evaluate if tolerance or resistance (behavioral or physiological) of the fire ants in this community to the active ingredients hydramethylnon and s-methoprene was occurring.

Results and Discussion

What started out to be a promising year for obtaining fire ant management data turned into a record setting year for Texas by having the hottest average temperature for June through August according to National Weather climatologists. The spring and summer of 2011 was extremely dry in south Texas. One would assume that the irrigation of lawns would attract fire ants. The lawns of Lago Santa Fe were no exception. Good fire ant activity was seen April 25, 2011 (**Table 5**). Of the 20 food lures, 19 (95%) of them had an average of 54 fire ants per lure. In April 2002 over 160 large (> 12" diameter) active fire ant mounds per acre were observed. The mounds (136/acre) observed in April 2011 were not overly large but they were present (**Table 1**). Fire ant mound activity counts taken in an adjacent pasture also showed good activity at 112 active fire ant mounds per acre.

The dry conditions did have an effect on mound survivability as seen by the 36% reduction in activity with no bait application in the adjacent pasture. Greater than 90% reduction of the fire ant mound activity was observed in the treated properties of Lago Santa Fe when compared to pre-treatment counts (**Table 4**), and a 74% reduction in food lure hits was observed (**Table 5**).

Were the residents of Lago Santa Fe satisfied with the 2011 effort? An email from Jay Gilbert, one of the originators of the Lago Santa Fe Fire Ant Project said, "Fall baiting was in October 2011 and was facilitated with 4 block captains. Utilizing block captains reduced the burden on the 2 of us and we are more willing to continue overseeing the semi-annual baiting. The Fall baiting appeared successful - as there were no complaints compared to lots of negative chatter last year. You will recall we skipped the fall baiting that year (2010) and the ants really were a problem. I have only spotted a couple of nuisance mounds following a 6.5" rain event Jan 9, 2012. We will go after them again in the Spring."

Block captains were chosen to help facilitate future fire ant management events. (**Appendix I**). Jay summed it up when he said, "We will go after them in the spring!"

Conclusions:

- The number of observation sites (sample size) was reduced for 2011 counts due to construction on previously empty lots but did not limit fire ant activity assessment.
- Despite drought, populations in untreated area (pastureland) remained unchanged, statistically.
- Percent reduction from April 25, 2011 to July 27, 2011 was 94.7% (versus 85.5% reduction after initial treatment, April 18, 2002 to July 7, 2002), indicating that after annual multiple application, Extinguish® Plus continues to perform over 10 years with no indication of resistance by imported fire ant populations.
- The Community-Wide fire ant management concept as promoted by the Texas AgriLife Extension Service remains a viable solution to the management of the red imported fire ants in a community setting.

Acknowledgements

The authors want to thank the residents of Lago Santa Fe for allowing them access to their community over the past years to document events and follow the effects of their fire ant management attempts. Special thanks go to Jay and Lydia Gilbert for their willingness to meet with the authors when necessary and help with fire ant activity evaluations. Central Life Sciences (Doug Van Gundy) is thanked for the generous donation of fire ant bait product when it was needed.

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Table1: Average active mounds per acre in 2011 before baiting (pre) and after baiting (post) based on number of active mounds in 0.25 acre circles, as compared to counts taken in 2002.

	Average active mounds per acre (percent reduction)			
Locations	**4/18/2002**	**7/17/2002**	**4/25/2011 (pre)**	**7/27/2011 (post)**
Lago properties	169	25	136	8 (94%)
Untreated adjacent pasture	87	75	112	72 (36%)

Table 2. Red imported fire ant mounds per 0.25 acre circle plot, Lago Santa Fe, Galveston Co., Texas, treated with a hopper blend of hydramethylnon fire ant bait (Amdro®Pro or Probait™) plus s-methoprene fire ant bait (Extinguish™) at 0.75 lbs of each product per acre through fall of 2003 or Extinguish® Plus Fire Ant Bait (s-methoprene + hydramethylnon) at 1.5 lb product/acre in 2004*. Observations on active fire ant mounds were taken at indicated intervals (weeks after treatment = WAT).

	Number of red imported fire ant mounds/0.25 acre				
Lot Number	I*	II	III	IV	V
Treated area:					
32	38	8	1	2	13
25	48	15	1	5	14
24	32	11	3	8	20
23	29	11	5	8	17
20	41	16	8	14	7
2	55	23	9	3	23
1	48	18	4	4	26
46	47	8	6	5	11
Mean ± Stand. Dev.	42.25 ± 8.88	13.75** ± 5.23	4.62** ± 2.97	6.13** ± 3.83	16.38** ± 6.37
T =		7.8243	11.3686	10.5679	6.6994
n = 8; d. f. = 14; *P* =		0.0000	0.0000	0.0000	0.0000
Percent reduction:		-67.46%	-89.07%	-85.50%	-61.23%
Untreated area (plot):					
1	34	27	28	-	24
2	27	28	17	-	39
3	12	10	13	-	15
4	14	15	17	-	15
Mean ± Stand. Dev.	21.75 ± 10.53	20.00*** ± 8.91	18.75*** ± 6.44		23.25*** ± 11.32
T =		0.2537	0.4859		-0.1940
n = 4; d. f. = 6; *P* =		0.4041	0.3221		0.4263
Percent reduction:		-8.10%	-13.8%		-6.8%

*I = 4/18/02 (pre-treatment), II = 5/28/02 (6 WAT), III = 6/12/02 (8 WAT), IV = 7/17/02 (12 WAT), V = 9/26/02 (22 WAT, pre-fall treatment)

** Mean significantly different ($P \leq 0.05$) from pre-treatment count (4/18/02) mean using the Student *T* test (Microstat).

*** No significant reduction in mean number of fire ant mounds per plot

Lot Number	Number of red imported fire ant mounds/0.25 acre				
	VI*	VII	VIII	IX	X
Treated area:					
32	0	0	6	3	0
25	4	0	2	0	1
24	13	0	4	0	0
23	13	0	3	7	2
20	5	1	20	3	2
2	8	1	20	46	10
1	2	0	8	9	5
46	5	0	11	4	2
Mean ± Stand. Dev.	6.25 ± 4.77	0.25 ± 0.46	9.25 ± 7.23	9.00 ± 15.27	2.75 ± 3.33
T =	-10.1033	-13.3654	-8.155	5.325	11.786
n = 8; d. f. = 14; *P* =	0.00**	0.00**	0.00**	0.00**	0.00**
Percent reduction:	-85.2%	-99.4%	-78.1%	-78.7%	-93.5%
Untreated area (plot):					
1	43	11	15	41	23
2	38	12	12	26	15
3	16	10	14	17	6
4	14	16	19	24	15
Mean ± Stand. Dev.	27.75*** ± 14.89	12.25*** ± 2.63	15.00 ***± 2.94	27.00***±10.10	14.75***±6.95
T =	0.6581	-1.7503	-1.2345	-0.720	1.110
n = 4; d. f. = 6; *P* =	0.2674	0.0653	0.1316	0.499	0.310
Percent reduction:	0.8%	-43.7%	-31.0%	+19.4%	-32.2%

* VI = 5/2/03 (pre spring treatment, 31 WAFT), VII = 7/1/03 (60 days after spring treatment, 5/3/03), VIII = 9/19/03 (pre fall application, 9/11/03), IX = 4/23/04 (pre spring application), X=9/17/04 (pre fall application)

** Mean significantly different ($P \leq 0.05$) from pre-treatment count (4/18/02) mean using the Student *T* test (Microstat).

*** No significant reduction in mean number of fire ant mounds per plot from pre-treatment counts (4/18/02)

Table 3: Example of total actual costs paid by Lago Santa Fe POA for fire ant management: (Includes 25# bags of Extinguish® Plus ant bait, misc. supplies, fuel for ATV).

Year	Actual Cost	Amount of product	Comments
2007	$2,015	2 X 6 bags	
2008	$2,042	2 X 6 bags	
2009	$2,043	2 X 6 bags	
2010	$1,217	1 X 7 bags	Spring treatment only, fall treatment skipped
2011	*$2,528*	2 X 7 bags	Estimated cost, increased rate from 1.5# to 2#/acre

Table 4. Red imported fire ant mounds per 0.25 acre circle plot, Lago Santa Fe, Galveston Co., Texas, treated with Extinguish® Plus Fire Ant Bait (s-methoprene + hydramethylnon, 1.5 lb product/acre) on 4/27/11*. Observations on active fire ant mounds were taken 91 days after application.

	Number of red imported fire ant mounds/0.25 acre	
Lot Number	XI*	XII
Treated area:		
32	4	0
28	11	0
22	39	3
11	63	3
2	31	5
46	58	0
Mean $\pm$ Stand. Dev.	34.33 $\pm$ 24.00	1.83 $\pm$ 2.14
T =	0.0867	10.817
n = 6; d. f. = ; *P* =	0.403	0.000**
Percent reduction:	18.74	95.67
Untreated area (plot):		
1	37	28
2	27	16
3	21	12
4	27	17
Mean $\pm$ Stand. Dev.	28.00 $\pm$ 6.63	18.25 $\pm$6.85
T =	-1.004	0.557
n = 4; d. f. = 6; *P* =	0.354***	0.598***
Percent reduction:	0.00	16.09

* XI = 4/25/11 (pre spring 2011 application), XII = 7/27/11 (91 days after spring 2011 application)
** Mean significantly different ($P \leq 0.05$) from pre-treatment count (4/18/02) mean using the Student *T* test (SPSS 0.19).
*** No significant reduction in mean number of fire ant mounds per plot from pre-treatment counts (4/18/02)

Table 5: Observations from 20 food lure stations (0.25 inch hot dog slices) placed in front yards of 20 properties lining the interior lakes of Lago Santa Fe Private Lake Community, Santa Fe, TX, Galveston County.

Evaluation dates	Total RIFA on lures	Avg. RIFA per Lure	% RIFA reduction on lures	Total RIFA hits on lures	Avg # RIFA on hit lures	% reduction lure hits
4/25/2011	1085	54	--	19	57	--
7/27/2011	300	15	72	5	60	74

Figure 1. Aerial view of Lago Santa Fe subdivision, Galveston Co., TX, site of a community wide fire ant management program, 2002-2012. Locations of 6 evaluation sites and 4 check sites for 2011 activity assessments are identified in image.

Appendix I: Example of advisory email sent to Lago Santa Fe Residents to alert them of the community fire ant baiting event in fall of 2011.

Email text:

Next weekend (Oct 14-16, 2011) is the community wide fall fire ant bait spreading time. Thanks again to our Fire Ant Captains: Jimmy Baker, Cathie Johnson, Danny LeBourgeois and David Pratt. Please contact them directly if you can help with some of the common areas or absentee neighbor lots. Keep in mind you may volunteer for an area with a different captain than the one from whom you receive your bait.

The captains are responsible for distributing the bait and lining up volunteers; they are not personally treating all the common areas and empty lots. We have had good support from the community volunteers in the past and the more volunteers we have, the more likely it is that all areas are treated and the baiting will be successful. If you have not volunteered previously, please consider signing up, more helpers makes the job easier for all.

Thanks,

Lydia and Jay Gilbert

Please look at the list and baiting tips below. If you are a lot owner or will be absent, please email your Captain to advise if you will arrange baiting on your own or if a volunteer needs to be arranged. Everyone else, please see if there is a specific empty lot or common area that you could treat, and email Jimmy, Cathie, Danny or David.

Jimmy Baker - Fire Ant Bait Captain-

Empty Lots
223
2 Lago Cove
9 Lago Cove
226

Common Areas
Peninsula between Lake 2 & 3
Lake 4 Dock
Bridge

Homeowners
1
6
10
13
218
222
303
306
307
314
318
403
===

Cathie Johnson – Fire Ant Bait Captain -

Empty Lots
135 –
142 -
214 –

Common Areas
Lake 2 Islands
Lake 2 Dock
Strip between 147 & 203

Homeowners
127
130
131
134
138
139
143 Crook
146
147
202
203
206
210
211
426
===

Danny LeBourgeois – Fire Ant Bait Captain -

Empty Lots
311
315

Common Areas
Lake 3 & 4 Islands
Triangle
Lake 3 Dock

Homeowners
319
322
323
326 Wehe
327
330
331
334
335
402
406
407

410
411
==

David Pratt – Fire Ant Bait Captain -

Empty Lots
102 –
122

Common Areas
Next to Cemetery Rd
Front Gate Flower Beds
Strip between 114 Gass & 423 Gonzalez
Lake 1 Islands

Homeowners
106
107
110
114
115
119
123
415
418
419
422
423

Remember the following tips:
* It is better not to mow or otherwise disturb mounds 48 hours before baiting as ants will rebuild their mound rather than take the bait. You can mow the following day.
* Bait should go on and stay dry - turn off sprinklers (lawn and septic) or place buckets over the heads. Please keep dry for 24 hrs if possible but 12 hours is OK. Wait for morning dew to clear before application.
* Ants generally eat in the evenings so it's optimal to put out bait later in the day so it does not spoil in the sun/heat before they take it that night.
*The opening of your hand held spreader (push spreader will not work) should be set at about 1/4 to 1/3 of an inch which is #1 or #2 generally.

Dr Nester confirmed that it is proper to bait near your vegetable gardens but not directly into the garden itself. He also advises starting by baiting the flower beds around the perimeter of your house. Next bait your property line and work in a spiral towards the center of your lot. This will make sure that if you run out of bait before you finish that you have all areas covered. We are providing 2# bags of Extinguish Plus ant bait, the recommended application is 1.5 to 2 lbs per acre.

Managing the Red Imported Fire Ant on a Green Roof in Friendswood, TX

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The use of green roof technology is gaining popularity among many real estate groups because of the marketable benefits which include energy conservation, storm water management, air pollution mitigation, scenic landscapes, wildlife habitat, and added recreational areas. In 2003 the United States Environmental Protection Agency cited reduced urban heat-island effects and lowered cooling costs as benefits for buildings utilizing this technology (USEPC 2003). Jacob White Construction Company, a Friendswood, TX based company (**Figure 1**), is a leader in the design and construction of green roofs atop of new "green" building projects in and around the Houston area.

During the early spring of 2011 while checking plant trials on a green roof at the Jacob White Construction Company Headquarters (2000 West Parkwood, Friendswood, TX) Dr. Camerino noticed active red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). In response, Dr. Nester and Dr. Camerino planned a fire ant management demonstration at the site. This report outlines some of the strategies used for the management of this pest.

Materials and Methods

The green roof located atop the Jacob White Construction Headquarters was assessed May 3, 2011 and nine subsequent dates (**Figure 2**) for the presence of the red imported fire ant. Foraging ant activity was checked using individual hot dog slice food lures (0.25 inch thick hot dog slices, Bar-S Jumbo Franks) that were placed in a grid across the green roof (**Figure 3**). Twenty three lures were used on 5/3/2011, while 34 lures were used on subsequent assessment dates. Food lures were checked after 60 minutes and total ants present on the lures were recorded (**Figure 4**).

DuPont™ Advion® ant bait arenas (30 arenas, 0.1% indoxacarb) were positioned in a grid pattern within the confines of the green roof (**Figure 5**). Bait stations were used so as not to directly apply a pesticide to the green roof growing media (**Figure 6**). Through a rainwater catchment system, all irrigation water applied to the roof is recycled and reapplied on site. The selection of bait arenas was to reduce the chance of pesticide movement from the target site. Additionally, irrigation water is applied several times per day as an energy saving passive cooling method. The frequent irrigation may have disrupted the integrity of a "unprotected" bait product. Since the roof was 11,000 sq. ft., the total active ingredient (0.059 g) contained within the 30 bait arena's was approximately equal to the active ingredient (0.052 g) in a 1.0 pound product per acre broadcast application of the DuPont™ Advion® fire ant bait (0.045% indoxacarb).

Since assessments of fire ant activity on the green roof indicated the continued presence of a population of fire ants (**Figure 2**), a fall broadcast application of the DuPont™ Advion®

fire ant bait (2 pounds product/acre) was planned for the grounds around the Jacob White Headquarters (**Figure 7**). Total mound counts were taken on September 22, 2011 (**Figure 8**) before fire ant bait applications and on 4 subsequent dates (**Figure 8**). To determine if a mound was active, visible fire ant mounds were checked using the minimal disturbance method, i.e., mounds were probed with a shovel and if no fire ants appeared after 15 seconds, the mound was considered inactive. The fire ant bait product was evenly spread with Scotts® HandyGreen® II Hand-Held Spreader set on smallest opening (**Figure 9**). In addition to the broadcast application on October 21, 2011, DuPont™ Advion® fire ant bait (0.5 oz/mound) was uniformly distributing around the active mounds with active brood.

We used a T-test statistical analysis to compare the mean numbers of worker ants observed at lures before and after the arena bait station treatment. We also estimated the mean and 95% Confidence Intervals (CI) for each sampling and display on a time series graph (**Figure 2**). No overlap among 95% CI indicates significant differences, and overlap indicates no significant differences. This approach allow us to compare post-treatment dates to pre-treatment numbers which in this case are consider a Control.

Results and Discussion

Results of the T-test (P <0.000, df: 327, F= 26.270) indicated that the mean number of worker ants recorded on food lures over the assessment period were significantly reduced compared to the initial pre-treatment values (**Figure 10**). The DuPont™ Advion® ant bait arenas did successfully reduce the ant population at all assessment dates (**Figure 2**), based on food lures, on the green roof atop the Jacob White Construction Company Headquarters.

Since some fire ant foraging activity was observed during the assessment period, and active fire ant mounds were found on the grounds surrounding the Jacob White Headquarters (**Figure 7**), DuPont™ Advion® fire ant bait was broadcast to the grounds and a 76% reduction in active fire ant mounds was observed 14 days after the treatment. Subsequent assessments of active fire ant mounds showed a continued increase in mound activity with no discernible reduction in activity after the additional single mound treatments with DuPont™ Advion® fire ant bait. A spring 2012 application of fire ant bait will be planned along with continued monitoring for fire ants on the green roof with placement of DuPont™ Advion® ant bait arenas as needed for continued management of the red imported fire ant on the green roof.

Literature Cited

United States Environmental Protection Agency. 2003. Cooling summertime temperatures: strategies to reduce urban heat islands. Publication No. 430-F-03-014.

Acknowledgements

The authors wish to thank Jeff Mickler, President /CEO, Jacob White Construction Company for allowing us total access to his facility to conduct this study and for DuPont for their gracious support of the project.

Figure 1. Headquarters of Jacob White Construction Company, Friendswood, TX, Galveston Co. 2011

Figure 2. Mean number of red imported fire ants (RIFA) per food lure at various assessment dates atop green roof in Galveston County, 2011. No overlap among 95% CI indicates significant differences, and overlap indicates no significant differences

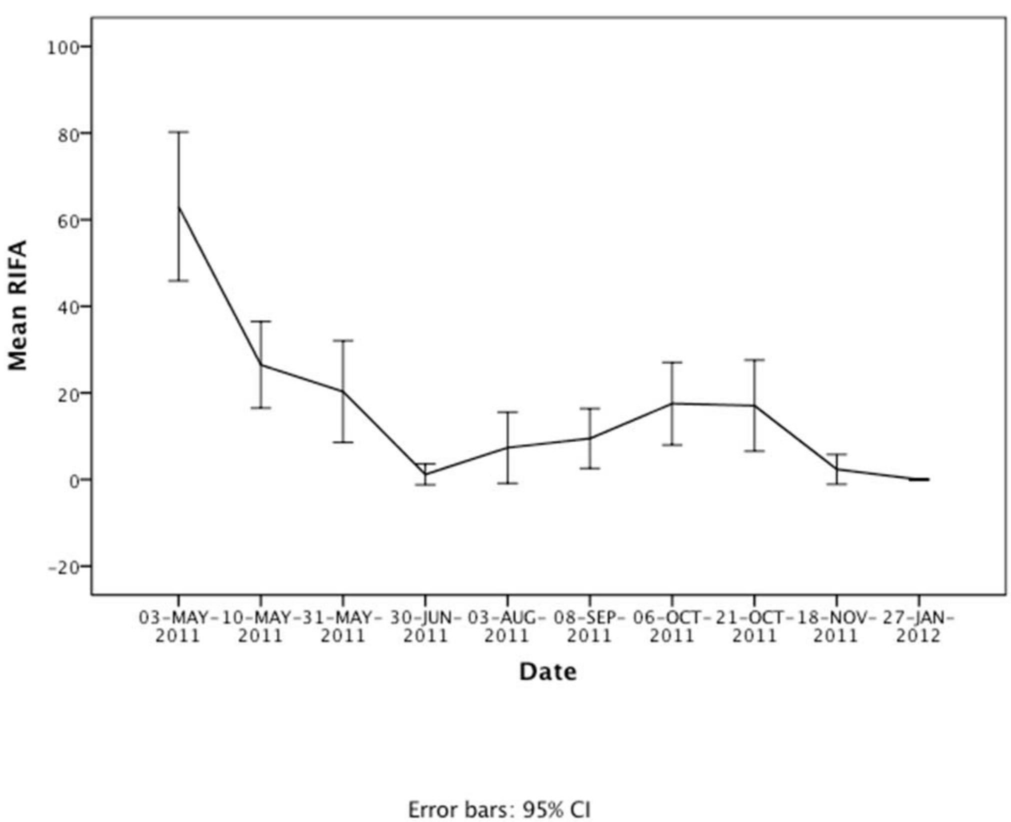


Figure 3: Approximate locations of 34 food lures for the assessment of red imported fire ant foraging on green roof, Galveston, Co. 2011.

Figure 4: Representative food lure with foraging fire ants as found on green roof during fire ant activity assessments. Galveston County, 2011

Figure 5: Approximate locations of 30 DuPont™ Advion® ant bait arenas on green roof, Galveston County, 2011.

Figure 6: Example of DuPont™ Advion® ant bait arenas placement atop green roof, Galveston Co., 2011

Figure 7: Grounds around Jacob White Construction Headquarters where the broadcast application of the DuPont™ Advion® fire ant bait was applied, Galveston, Co. 2011.

Figure 8: Total number of fire ant mounds found on grounds around Jacob White Construction Company Headquarters, before and after broadcast application of DuPont™ Advion® fire ant bait at 2.0 pounds product/acre, Galveston Co., 2011.

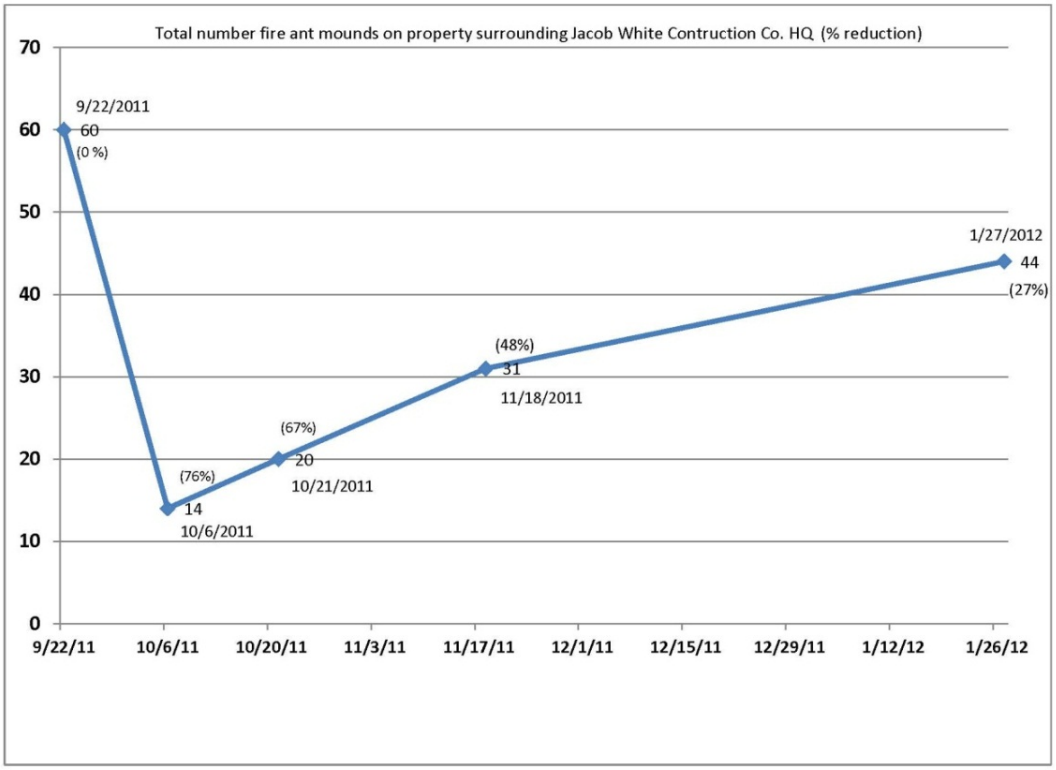


Figure 9: Scotts® HandyGreen® II Hand-Held Spreader used for broadcasting DuPont™ Advion® fire ant bait on grounds surrounding Jacob White Construction Headquarters, Friendswood, TX, Galveston County, 2011

Figure 10: Analysis of mean number of red imported fire ants (RIFA) on food lures over nine assessment periods (May 10, 2011 – January 27, 2012), Galveston County, 2011.

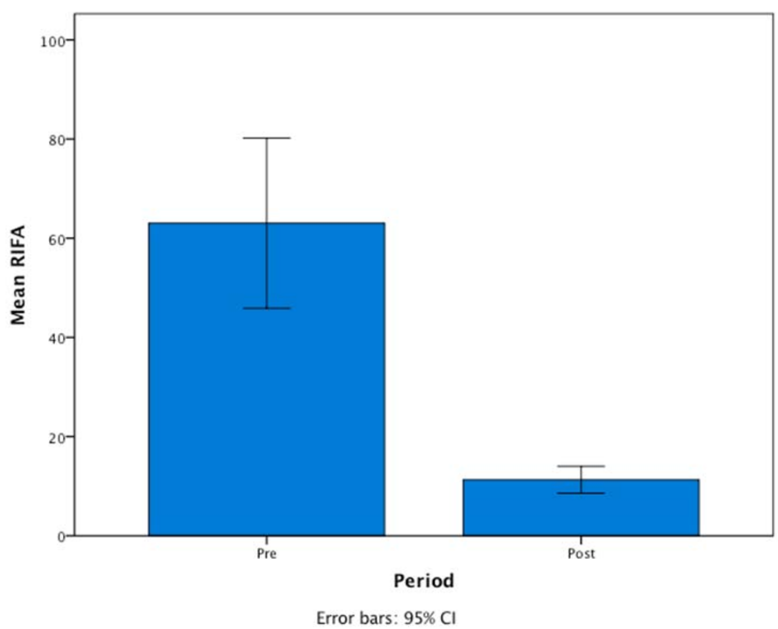
Group Statistics

	Period	N	Mean	Std. Deviation	Std. Error Mean
RIFA	Pre	23	63.04	39.707	8.280
	Post	306	11.30	24.245	1.386

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
RIFA	Equal variances assumed	26.270	.000	9.356	327	.000	51.743	5.531	40.863	62.623
	Equal variances not assumed			6.164	23.249	.000	51.743	8.395	34.387	69.098

T-test: P <0.000, df: 327, F= 26.270



Reducing the Impact of the Red Imported Fire Ant at the 2010 and 2011 Toyota Texas Bass Classic, Conroe, TX

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Mike Heimer, County Extension Agent – AG/NR - Montgomery County, Conroe, TX
Texas AgriLife Extension Service

The presence of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) any outdoor function can be unpleasant to those participating in the function, whether it is vendors or the general public. The Toyota Texas Bass Classic (TTBC) has been held in the Lake Conroe area the past few years (http://toyotatexasbassclassic.com/). It is advertised as the world championship of professional bass fishing and country music festival. The event is a three-day event that feature anglers from across all major fishing tours in the U.S., and features some of country music's premier artists. The event proceeds benefit the Texas Parks and Wildlife Department (TPWD) and its youth outdoor programs. The TTBC has become an annual community event that offers a wide range of activities that families, outdoor enthusiasts and music fans enjoy. It has generated over $1.2 million for the TPWD and the state of Texas (TTBC and TPWD estimates). The agenda of the tournament typically consists of daily tournament weigh-ins, and outdoor exposition and concerts.

Fire ants were an issue during the 2009 TTBC in Montgomery, TX. The organizers of the 2010 TTBC voiced their concern to the property managers of the Lone Star Convention Center, Conroe, TX, (property chosen for the 2010 TTBC), and asked that something be done. The Lone Star Convention wanted to secure the business of the TTBC in future years so in August of 2010, management of the Lone Star Convention and Expo Center (9055 FM 1484 Rd, Conroe, TX 77303) contacted the Texas AgriLife Extension Service – Montgomery County Office for advice on controlling the red imported fire ant before the 2010 Toyota Texas Bass Classic scheduled event in October of 2010. The Lone Star Convention Center is a Montgomery County property.

The Montgomery County AgriLife Extension Agent, Mike Heimer, thought this would be a great opportunity to showcase the Texas Two-Step Program for fire ant management to control fire ant populations on a large landscaped property. The Two-Step program is promoted by the Texas AgriLife Extension Service which utilizes broadcast applications of fire ant bait products. See AgriLife Bookstore publication L-5496, Fire Ant Control: The Two-Step Method and Other Approaches, (https://agrilifebookstore.org/publications_details.cfm?whichpublication=2577&orderby=pubnumber&SIMPLESEARCH=drees&criteriastring=SIMPLESEARCH%3Ddrees). This fire ant management program encourages owners of large tracts of property to plan fire ant bait treatments in advance of a scheduled event (minimum of 6 weeks) to reduce fire ant populations so they are not a nuisance to the event coordinators, vendors, or participants.

Methods and Materials

In late August 2010 and again in late September 2011 the fire ant bait product containing hydramethylnon plus s-methoprene, Extinguish® Plus Fire Ant Bait (donated by Central Life Sciences, Dallas, TX) was broadcast (1.5 pound product/acre) via a Herd GT-77 ATV Broadcast Seeder (Kasco Manufacturing Company, Shelbyville, IN, http://www.kascomfg.com/public/category/herd-seeders/seeders) over the Lone Star Convention Center property where the TTBC was to be held. Because of the success of the 2010 actions, this program was conducted again in 2011 to have sequential year's data on this type fire ant management program. Pre and post fire ant activity evaluations were made in both 2010 and 2011. Foraging ant activity was checked using 15 individual hot dog slice food lures (0.25 inch thick hot dog slices, Bar-S Jumbo Franks) that were placed in a grid across the property. After about 1 hour the hot dog slices were checked and the estimated number of ants recruited to each slice was recorded (**Table 1**).

The number of active fire ant mounds per acre was recorded utilizing 0.25 acre circles at four locations within the area to be frequented by vendors and participants on the property of the Lone Star Convention Center, Conroe, TX. Visible active fire ant mounds were checked using the minimal disturbance method, i.e., mounds were probed with a shovel and if fire ants appeared within ~ 15 seconds the mound was recorded as active (**Table 2**).

In 2010 a survey of the organizers (**Appendix 1**) as to their satisfaction with the fire ant management strategy was conducted. In 2011 a random face to face survey (**Appendix 2**) of the vendors as to their satisfaction of the fire ant management strategy was conducted.

Results and Discussion

In 2010 initial observations indicated a high level of fire ant foraging activity with 13 out of 15 lures hit with an average of 79 fire ants/hot dog slice. In 2011, 12 out of the 15 were hit with an average of 57 fire ants per hot dog slice (**Table 1**). In 2010 an average of 18 active fire ant mounds per acre were observed while in 2011 the active fire ant mound per acre count was down to 4 (**Table 2**). Because the goal of the organizers was for "zero" fire ant presence, baiting procedures were carried out in both 2010 and 2011 based on food lure observations.

The 2010 and 2011 bating program was a success. Six weeks after broadcasting the fire ant bait and one week before the scheduled Toyota Texas Bass Classic, no foraging activity and less than 1 active fire ant mound per acre was observed in both 2010 and again in 2011 (**Tables 1** and **2**). Resulting data calculated to greater than 94% reduction in activity for both years. Although we would like to think that the low mound count in 2011 was due exclusively to the fire ant baiting, Texas was experiencing a record drought at this time which could also have affected the number of active mounds visible on the ground. Foraging activity was still occurring at a high level. Organizers and vendors were completely satisfied with the effort as reflected in survey results (**Appendixes 1** and **2**).

The Conroe Convention Bureau estimated the economic impact of the 2010 TTBC on the Conroe area was $700,000.00 with 500 room nights rented and the 2011 impact to be equal or greater than that seen in 2010. It is noteworthy that the TTBC organizers would consider a fire ant baiting program (**Appendix 1**) as one of the negotiated items before signing a contract and the vendors would recommend (**Appendix 2**) that event organizers request that the hosting facility of future TTBC's to adopt a fire ant management program. Also, Negotiations for the 2012 TTBC are completed and the lone Star Convention Center will again host the event in 2012: (http://toyotatexasbassclassic.com/featured/ttbc-scheduled-for-sept-28-30-2012).

Acknowledgements

The authors would like to thank the managers of the Lone Star Convention Center and the organizers of the TTBC for unlimited access to the property before and during the 2010 and 2011 events. We also thank Doug Van Gundy, Central Life Sciences, Dallas, TX, for the generous donation of the Extinguish® Plus Fire Ant Bait product for this demonstration study.

Table 1: Observations of red imported fire ant foraging workers recruited to 15 food lures consisting of hot dog slices (0.25 inch thick) placed in a grid across the property of the Lone Star Convention Center, Conroe, Montgomery Co. TX, 2010 and 2011.

Observation/Date	8/26/2010	9/27/2010	9/12/2011	10/27/2011
Number of "hits"*	13	0	12	0
Total # ants	1180	0	860	0
Average # ants	79	0	57	0
Percent reduction	-	100	-	100

*number of hot dog slices of 15 with ants 1 hr after exposure

Table 2: Number of active red imported fire ant mounds per acre (converted from active ant mound numbers from on 0.25 acre circular subplots at four locations) on the property of the Lone Star Convention Center, Conroe, Montgomery Co., TX.

Date	Rep 1	Rep 2	Rep 3	Rep 4	Average	% Reduction
8/26/2010	24	12	8	28	18	-
9/27/2010	4	0	0	0	1	94
9/12/2011	0	12	0	4	4	-
10/27/2011	0	0	0	0	0	100

Appendix 1: 2010 Toyota Texas Bass Classic Satisfaction Organizer survey.

1) Have fire ants been an issue during previous events?
   In 2009 TTBC was held in a field in Montgomery, TX. Large population of fire ants present at this site.

2) Were they an issue with:
   a) Event staff
   b) Vendors
   c) Participants
   d) All of the above

3) Were fire ants an issue during The 2010 Toyota Texas Bass Classic? Yes No

4) Were you satisfied with the level of fire ant control given by the use of the fire ant bait product? Yes No

5) Would you consider requiring a hosting facility to adopt a fire ant management program before agreeing to hold an outdoor event at their location?
   Yes No

   Comment: It would not be on the top of the negotiation list (with host facility) but is important that a treatment plan be in place.

6) Would you consider recommending future hosting locations to utilize the Texas Two-Step control program to minimize fire ant issues during schedules events?
   Yes No

7) Additional comment:

   Based on our experience at the 2010 TTBC, the treatment seemed effective. In light of our previous challenges with fire ants, the vendors, patrons and staff all seemed satisfied with the treatment since no formal complaints were logged. Based on the overall positive experience (lack of fire ants included) we are hopeful to return the Fairgrounds at the Lone Star Convention & Expo Center in 2011.

Appendix 2: 2011 Toyota Texas Bass Classic Satisfaction Face to Face Vendor Survey conducted the second day of the tournament. Eleven of the participating vendors were approached. (Number of vendors responding)

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1) Have you participated in the Toyota Texas Bass Classic (TTBC) in previous years?

Yes	64%	(7)
No	36%	(4)

2) Have fire ants been an issue for you during previous TTBC?

Yes	0%	(0)
No	100%	(11)

3) Have fire ants been an issue for you during The 2011 TTBC?

Yes	0%	(0)
No	100%	(11)

4) Are you satisfied with the level of fire ant control obtained through the fire ant management strategy recommended by the Texas AgriLife Extension Service and adopted by the TTBC organizers and The Lone Star Convention Center?

Yes	100%	(11)
No	0%	(0)

5) Would you want the hosting facility of future TTBC's adopt a fire ant management program at their location?

Yes	100%	(11)
No	0%	(0)

6) Would you consider recommending to event organizers the need to request that the hosting facility of future TTBC's to adopt a fire ant management program?

Yes	100%	(11)
No	0%	(0)

Evaluation of baking soda as a mound treatment for red imported fire ant management

Wizzie Brown, Extension Program Specialist- IPM
Texas Agrilife Extension Service, Austin, TX

Over the past several years, I have tested various home remedies to see if they are a sound method of managing red imported fire ants, *Solenopsis invicta* (Buren). A recent item tested was actually by mistake on my part. I meant to test corn-starch but I grabbed baking soda instead. Hoping to salvage my data, I performed an internet search and found that many people on websites and online forums encourage baking soda as a method for killing fire ants touting that it is "poisonous to ants".

Materials and Methods

The trial was established on May 17, 2011 at Richard Moya Park in Travis County (10001 Burleson Rd Austin, TX 78617). Nine plots containing of the same width but varying lengths, all containing 5 active red imported fire ant mounds were established. A buffer space of 10 feet was placed between plots. Treatments were assigned randomly within each replicate.

Treatments:

1. Baking Soda (Arm & Hammer®) - 1 tablespoon sprinkled over each mound
2. Untreated control (check) – no treatment
3. Ortho® Orthene® Fire Ant Killer (standard Orthene® treatment) - 50% acephate; 1 tablespoon sprinkled over each mound

Prior to treatment, each mound marked with field paint was examined for ant activity using the minimal disturbance method whereby a mound was considered active if a dozen or more worker ants emerge en masse following mild disturbance by prodding the mound with a stick. This assessment method was also used to evaluate plots at 3, 6, 14, and 30 days post treatment.

Results and Discussion

At 3 and 6 days post treatment, the acephate (Ortho® Orthene® Fire Ant Killer) plots had significantly less fire ant mound activity than both the control and the baking soda treated plots (Table 1). The untreated control plots had significantly less fire ant mound activity than the baking soda treated plots at 3, 6 and 30 days after treatment.

At 30 days, the trial was concluded and all mounds that could be located within the plots were counted. The baking soda treated plot had significantly more fire ant mound activity than both the acephate and untreated control plots (Table 2). Due to extreme drought conditions mound activity was reduced, so while baking soda treated plots had more activity than the untreated control plots it is most likely not due to the baking soda treatment.

Table 1. Mean number of active marked red imported fire ant mounds (5 per plot) that was initiated on May 17, 2011, Travis County, TX.

	Mean no. Active Ant Mounds/5*			
Treatment	**3 days**	**6 days**	**14 days**	**30 days**
Baking Soda	4.00a	3.00a	2.67a	2.00a
Untreated Control	3.33b	3.00a	1.33ab	0.67b
Acephate (Orthene®)	0.00c	0.33b	0.00b	0.00b

*Means followed by the same letter within the same column were not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range test at $p \leq 0.05$ (SPSS, Windows 14.0).

Table 2. Mean number of red imported fire ant mounds per treatment plot area, treated on May 17, 2011, Travis County, TX.

Treatment	**Mean no. Active ant mounds/plot* 30 days**
Baking Soda	2.00a
Untreated Control	0.67b
Acephate (Orthene®)	0.00b

*Means followed by the same letter within the same column were not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range test at $p \leq 0.05$ (SPSS, Windows 14.0).

Acknowledgements

The author would like to thank Travis County Parks for cooperating with the trial.

Evaluation of used oak ash as a mound treatment for red imported fire ant management

Wizzie Brown, Extension Program Specialist- IPM
Texas Agrilife Extension, Austin, TX

In late 2010, I was contacted by an individual who wanted to test a "common item" as a method for killing colonies of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Through email correspondence, I discovered he was using oak ash on his property to manage fire ants. I agreed to run a field trial on the product if he would supply the oak ash and provide the proper application amounts. I secured the oak ash and his application tool, a 2 pound 1.9 oz. Folgers® coffee container and carried out the field trial once a site with enough mounds was located.

Materials and Methods

The trial was established on May 16, 2011 at Ben E. Fisher Park (600 W. Carrie Manor St. Manor, TX 78653). Nine plots of the same width but varying lengths containing 5 active red imported fire ant mounds were established. A buffer space of 10 feet was placed between plots to reduce movement of ant mounds between plots. Treatments were assigned randomly within each replicate.

Treatments:

1. Standard treatment: Ortho® Orthene® Fire Ant Killer - 50% acephate; 1 tablespoon sprinkled over each mound
2. Experimental treatment: Oak ash – 2 pound 1.9 oz. Folgers® coffee container sprinkled over each mound
3. Untreated control (check) – no treatment

Prior to treatment, each mound marked with field paint was examined for ant activity using the minimal disturbance method whereby a mound was considered active if a dozen or more worker ants emerge en masse following mild disturbance. This assessment method was also used to evaluate plots at 3, 7, 14, and 30 days post treatment. Data were analyzed using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range test at $p \leq 0.05$ (SPSS, Windows 14.0).

Results and Discussion

At 3 days post treatment, the acephate (Ortho® Orthene® Fire Ant Killer) plots had eliminated ant activity in all plots and had significantly less fire ant mound activity than both the control and the oak ash treated plots (**Table 1**). At 7 and 14 days post treatment the acephate treated plots, containing no active ant mounds of the five initially treated, were not significantly different but were numerically different than the oak ash and untreated control plots. At 30 days after treatment plots also showed no difference between treatments. However, the number of active ant mounds was the same in oak ash and untreated plots.

At 30 days, the trial was concluded and all mounds that could be located within the plots were counted. Mound numbers in all plots were not significantly different (**Table 2**) although they were half the number found in oak ash and untreated plots.

Travis County, where this field trial was carried out, was in an extreme drought and mounds were difficult to locate within plots except those that I originally marked. This made the final 30 day count difficult which may have bearing on test results.

Table 1. Mean number of active marked red imported fire ant mounds of five following treatments, initiated May 16, 2011, Ben E. Fisher Park, Travis County, TX.

		Mean no. Active Ant Mounds/5*		
Treatment	**3 days**	**7 days**	**14 days**	**30 days**
Acephate (Orthene®)	0.00a	0.00a	0.00a	0.00a
Oak ash	2.33b	2.67a	2.67a	0.67a
Untreated Control	4.00c	3.00a	2.67a	0.67a

*Means followed by the same letter within the same column were not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range test at $p \leq 0.05$ (SPSS, Windows 14.0).

Table 2. Mean number of red imported fire ant mounds per average (mean) treatment plot area, treated on May 16, 2011, Travis County, TX.

Treatment	**Mean no. Active ant mounds/plot*** **30 days**
Acephate (Orthene®)	0.33a
Oak ash	0.67a
Untreated Control	0.67a

*Means followed by the same letter within the same column were not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range test at $p \leq 0.05$ (SPSS, Windows 14.0).

Acknowledgements

The author would like to thank Travis County Parks for cooperating with the trial.

Evaluation of Various Fire Ant Baits and Hopper Blends for the Management of the Red Imported Fire Ant in Managed Turf

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Bastiaan "Bart" M. Drees, Professor and Extension Entomologist
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The fire ant has become an important economic problem in urban Texas, according to a 1998 study conducted by the Department of Agricultural Economics, Texas A&M University. Fire ant related costs in Dallas, Fort Worth, Austin, San Antonio, and Houston, have serious economic effects for these metro areas of Texas (Lard, Hall, and Salin 2000). Households experienced the largest costs among sectors examined with an average of $151 per household spent annually. These costs include repairs to property and equipment, first-aid, pesticides, baits, and professional services. A full damage assessment for Texas must include additional sectors, and the estimated costs of $581 million per year for the selected sectors underscore the impact of this pest. Treatment costs accounted for over 50% of the total cost. In Houston, the average medical treatment cost per household was $25.46. The duration of injury for children and adults was 6.6 days and 5.6 days, respectively. The fire ant limits outdoor activities and homeowners and businesses incur added costs in managing fire ants.

Management of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) on large mixed use land tracts using insecticide products is economically feasible when the economic impact of high fire ant population equals or exceeds the cost of control (Flanders and Drees 2004). Mixed use land tracts may include parks, sports fields, camp grounds, convention centers, health-care facilities, animal-care facilities, or a mixture of any of these. The use of these areas by large numbers of the general public exposes them to the risks of fire ants if no control measures are undertaken.

Fire ant bait products offer a means to treat large areas of managed turf to obtain a level of fire ant control and reduce the exposure of the general public to the fire ant (Barr et al. 2005). Fire ant bait formulations vary somewhat but most consist of de-fatted processed corn grit as a "carrier," soaked with soybean oil as an attractant in which the active ingredient is dissolved. The broadcasting of fire ant bait products allows foraging fire ants from visible or hidden mounds access to the bait particles that they pick up and take back to their respective mounds. When foraging ants return to the colony the product is fed ant-to-ant, ant-to-larva, larva-to-ant and ant-to queen(s) so that all members of the colony are affected. This is also why most bait ingredients must be rather slow to kill ants. If ants die too fast, the active ingredient fails to reach the queen or multiple queens.

This study evaluates various fire ant bait products and hopper blends of some of these products for use in managed turf.

Material and Methods

This study was established on managed Bermuda grass turf in the soccer fields of the Cris Quinn Memorial Soccer Complex, Beaumont, TX, (**Figure 1**). Thirty 200 ft X 250 ft (1.15.acre) plots were established, across this sports field complex on April 26, 2011. The area was mowed and trimmed once per week, weather permitting. Low rainfall occurred during the testing period. No supplemental irrigation was provided. All treatments (**Table 1**) were broadcast applied April 26, 2011, in the late afternoon, with an ATV (Kawasaki Prairie 700) mounted Herd GT-77 ATV Broadcast Seeder (Kasco Manufacturing Company, Shelbyville, IN, http://www.kascomfg.com/public/category/herd-seeders/seeders). The Herd GT-77 was calibrated to deliver 1.5 lb fire ant bait with a 20 ft swath while the ATV traveled at 10 mph. The Herd GT-77 was fitted with a Herd Seeder Co. #1 plate covering the agitator. After all the replications of each treatment were applied, the broadcaster hopper was vacuumed clean with a small industrial vacuum before the next treatment.

A pre-treatment assessment of the number of active red imported fire ant mounds within a 0.25 acre circle was conducted (**Table 2**). Plots were then arrayed in order from the plot containing the highest to the lowest number of ant mounds per plot. Replications were established by dividing the array into four blocks and randomly assigning eight treatments to each block and then adjusting to assure that pre-treatment mean differences between treatments in all replications or blocks were minimal. Untreated controls had to be located outside of the soccer field area. We adhered to an agreement with the grounds maintenance crew that all playing services would be treated. Extinguish® Plus was used on all fields and border areas not involved in the actual study.

Fifteen soccer fields were subdivided for this study. Each soccer field could be divided into two plots. Based on the array results, the treatments were replicated within 28 of the subdivisions (**Figure 1**).

At 0, 2, 4, 8, 12 and 16 weeks after treatment (WAT) fire ant mound activity was assessed. To determine if a mound was active, visible fire ant mounds were checked using the minimal disturbance method, i.e., mounds were probed with a shovel and if no fire ants appeared after 15 seconds, the mound was considered inactive. Total active fire ant mounds in each plot were counted, and the data was recorded as the number of active fire ant mounds per acre (Table 2). Data were analyzed using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test ($P \leq 0.05$) (SPSS 18.0).

Results and Discussion

What started out to be a promising year for obtaining fire ant management data turned into a record setting year for Texas by having the hottest average temperature for June through August according to National Weather climatologists. Even though reductions in fire ant mound activity looked good (**Figure 2**), the untreated areas also showed reduction in fire ant mound activity. Not until the 16 WAT observation date did the untreated control plots show a significant higher number of active fire ant mounds than the treated plots (**Table 2**). In summary, there are a few pertinent points that can be made about the data from this study:

1) There were no pre-count differences in means (analysis included check plots) between treatments.

2) Advion® + Distance® ant bait products did not provide significantly faster control (mound reduction) than Extinguish® Plus at the early evaluation dates.

3) There were no treatment differences for the first 4 weeks after treatment, including no difference compared to untreated plots.

4) At 8 WAT Extinguish® Plus, Distance® and the Distance® hopper blends performed significantly better than MaxForce® FC but otherwise there few only numerical treatment differences, even from the untreated control.

5) Only at 16 WAT did bait treatments result in mound numbers significantly lower than untreated plot numbers and the hopper blends with Distance® performing significantly better than MaxForce® FC, but with no other statistical differences between treatments.

Acknowledgments

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Table 1. Red imported fire ant bait products evaluated on 1.15 acre plots (200 ft X 250 ft) at the Cris Quinn Memorial Soccer Complex, Beaumont, TX.

Treatment	Rate # Product/acre
Extinguish® Plus (s-methoprene + hydramethylnon)	1.5
MaxForce®FC (fipronil)	1.5
Advion® (indoxacarb)	1.5
Distance® Fire Ant Bait (pyriproxyfen)	1.5
MaxForce®FC + Distance®	0.75 + 0.75
Advion® + Distance®	0.75 + 0.75
Advance® Granular Carpenter Ant Bait (abamectin)	1.5
untreated check	0

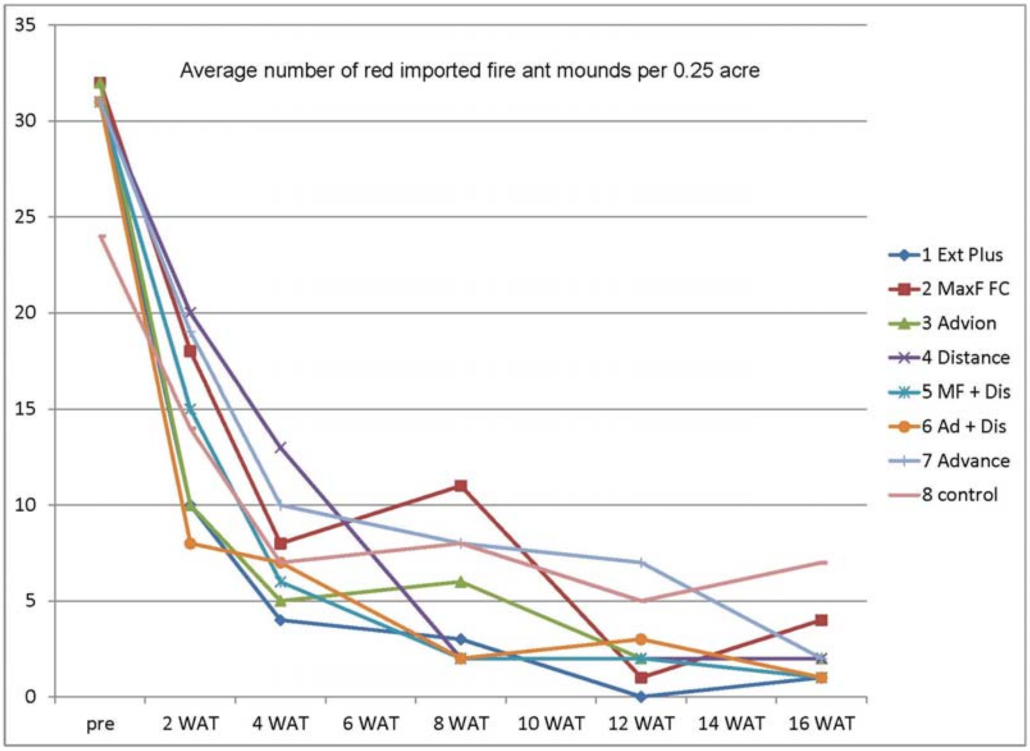
Table 2. Mean number of red imported fire ant mounds per 0.25 acre circle subplot before and following broadcast-applied fire ant bait product treatments, Cris Quinn Memorial Soccer Complex, Beaumont, TX initiated April 26, 2011, Jefferson Co., TX.

Treatment	Pre- 5/26/11	2 WAT 6/10/11	4 WAT 6/27/11	8 WAT 7/22/11	12 WAT 8/19/11	16 WAT 9/16/11
Extinguish® Plus	31.0	9.8	4.3	3 a	0.3 a	1.3 ab
MaxForce®FC	31.5	18.3	8.3	10.8 b	0.5 a	3.8 b
Advion®	31.5	10.3	4.5	6.3 ab	2.3 a	1.8 ab
Distance®	31.3	19.5	13.0	1.8 a	2.0 a	1.5 ab
MaxForce®FC + Distance®	31.0	14.5	6.3	2.3 a	1.8 a	0.8 a
Advion® + Distance®	31.3	7.5	6.5	2.0 a	2.8 ab	0.8 a
Advance®	31.0	19.3	10.3	7.5 ab	6.8 b	2.3 ab
untreated check	24.0	13.8	6.8	7.8 ab	4.8 ab	6.5 c
d.f. = 7						
F	0.101	0.707	0.759	3.232	2.388	5.835
P	0.997	0.667	0.627	0.015	0.053	0
M. Sq.	260.229	121.698	45.99	14.031	7.896	2.604

* Means followed by the same letter are not significantly different using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test ($P \leq 0.05$) (SPSS 18.0).

Figure 1. Aerial view of fire ant bait study plot randomization conducted at Cris Quinn Memorial Soccer Complex, Beaumont, TX. The soccer complex consists of fifteen acres. The facility has 30 large and small soccer fields with concession stands and restrooms. Boxes represent approximate location of the 1.15 acre treated areas. Each soccer field was subdivided into two plot areas. Fifteen soccer fields were used, which allowed for 30 plot areas. Active ant mound counts were taken from 0.25 acre circles from middle of plots.

Figure 2. Average number of imported fire ant mounds per 0.25 acre circle at 0, 2, 4, 8, 12 and 16 weeks before and following broadcast-applied fire ant bait product treatments applied April 26, 2011, Cris Quinn Memorial Soccer Complex, Beaumont, TX.



Evaluation of Red Imported Fire Ant Treatment Programs to Preserve Federally Endangered Species of Concern and Endemic Cave Adopted Arthropod Species at Camp Bullis, Bexar County, Texas: Final Report, December 2011

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Summary

Camp Bullis, in Bexar Co., TX, is a 11,331 ha U. S. Army training facility on which over 100 cave entrances have been located. Of these, 29 caves contain three federally listed endangered arthropods (*Rhadine exilis*, *Rhadine infernalis*, and *Cicurina madla*) and, in total, 79 contain additional species of concern not listed that are managed similarly. The accidentally introduced ant species, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), commonly called the red imported fire ant, is believed to pose a threat to these endangered species. Efforts have been ongoing since September 1999 to suppress their population levels around cave entrances using ant mound treatments including periodic (2-3 times per year) injection of very hot or boiling water into ant mounds and restricted application of an ant bait product followed by monthly assessment focused on counting the number of active *S. invicta* mounds.

Traditional methods of applying standard insecticides are not available within many areas at Camp Bullis due to the presence of endangered karst invertebrates and insects such as cave crickets which are needed to support the karst invertebrates' food cycle. Camp Bullis is regulated by US Fish and Wildlife Service under the federal Endangered Species Act through a succession of Biological Opinions which contain terms and conditions to protect these federally listed karst invertebrates. Although the karst invertebrates live deep in the caves and other karst features and do not emerge, cave crickets do routinely come out of the caves to forage and the materials they bring back into the features support the endangered karst invertebrates as a food source. The Biological Opinion requires suppression of *S. invicta* because of their predation on cave

crickets and subsequent indirect effect on karst invertebrates. The challenge is therefore to find methods to suppress *S. invicta* which do not also negatively affect the endangered karst invertebrates and species which support them such as cave crickets.

Since the fall of 2003, half of the sites treated for *S. invicta* in this manner have consistently been found to be below the critical density of 80 mounds per 7,850 m^2 or within a 0.8 ha or 2 acre circular area around each cave entrance with a radius of 50 m. This treatment program may be limited in success due to ants not forming the mounds characteristic of their surface nesting activity during hot, dry weather conditions and difficulty injecting hot water in often rocky situations and areas with dense tree/shrub vegetation.

The goal of this study was to develop, test and compare to current *S. invicta* management methods to an approach that is arguably less expensive, more environmentally-friendly and effective for the management of this exotic invasive pest ant around karst cave formations in Camp Bullis. We proposed a "fire-ant specific" approach called the Lure-Switch-Bait (LSB) method that primarily relies on behavioral features of the invasive *S. invicta*. Based on this background, we developed a method consisting in "luring" the ants with a diet (food lure consisting of hot dog slices) and switching the lures with a station containing a small amount of insecticide bait, Esteem® Ant Bait containing the IGR pyriproxyfen, calculated not to exceed the label allowable rate per area. These stations are exposed for 24 to 48 hours and then removed. The stations were closed and only allowed access to small insects (holes of ~5mm).

We tested this approach and compared to the currently used *S. invicta* specific treatment that consisted of detecting all active *S. invicta* colonies or ant mounds in the 0.8 ha circular area and injecting boiling water mixed with commercial dish soap into each colony. This was a labor-intensive and relatively expensive approach that required the use of heavy equipment and several people. Under hot, dry conditions, *S. invicta* colonies become less conspicuous because the ants move below the soil surface to regulate the temperature, thus making the detection of ant colonies or active mounds difficult. In contrast, the LSB method relied on foraging patterns of this invasive ant which is constantly foraging for food even during the warmer periods of the year.

To test the hypothesis that *S. invicta* control can best be achieved by the LSB treatment approach that relies on the ant's behavioral characteristics, we conducted a 3 year field trial in Camp Bullis. Our methodology consisted of locating new karst formation centered 0.8 ha (2 acre) field plots, similar to those in the ongoing treatment program but not known to host endangered arthropods of concern, and comparing three treatments: 1) boiling water ant mound injections; 2) Lure and switch bait station grid treatment (LSB); and, 3) untreated control. We used a total of 18 karst formations at the centers of 50 m radius plots. Pre-treatment assessment of foraging ant population levels allowed plots to be arrayed from highest to lowest in terms of fire ant activity and these were divided into six treatment blocks or replications. Within each replicate, treatments were assigned at random to each plot. We developed a 10 by 10 m grid system to deploy the LSB treatment consisting of 89 food lures (hot dog slices) that were placed on the ground and left for ~45 minutes. Afterwards, those food lure grid sites with *S. invicta* workers were replaced with a bait station holding the IGR bait product. Stations were exposed for 24 to 48 hours and then retrieved for their inspection. Zara Environmental LLC and Camp Bullis Environmental conducted the boiling water injection treatments. This consisted of applying commercial dish soap on the top of the colony and injecting the colony with boiling water for a few minutes. For each of the three treatments (LSB, boiling water injections and untreated control) plots were monitored for *S. invicta* three times in 2009, five times in 2010 and four times in 2011 using a combination of food lures and ant mound using a transect pattern of 17 food lure placement sites weighed towards the plot centers or whole plot active ant mound or colony counts.

Results of this study documented that the LSB approach provided superior control for all the 3 years of this study and that suppression of *S. invicta* was followed by a resurgence of native ant species. Presence of competitor ant species is considered critical to the success of implementing biological control of *S. invicta* using parasitic phorid fly species and the imported fire ant disease, *Kneallhazia solenopsae*. We also found that the number of bait stations that needed to be deployed and consequently the amount of insecticide bait required in subsequent applications declined following initial application while continuing to provide significant *S. invicta* control. Thus, the combination of the targeted treatment along with the increase of natural competitors potentially helps achieve sustainable control. In a separate laboratory study, we also provide preliminary data on the effects of *S. invicta* predation and the use of insecticide bait stations on laboratory colonies of the cave cricket, *Ceuthophilus* sp. Some cave crickets were able to enter bait stations and were observed to consume the bait. However, bait stations could be better designed to exclude cave crickets. Although IGR insecticides are known to affect female field crickets reproductive structures preventing egg formation, this trial failed to show similar effects of exposure, although the experiment used low numbers of cave crickets and should be repeated.

The boiling water injection treatment significantly reduced worker activity and colony numbers but *S. invicta* activity in these plots was always significantly higher than in LSB plots. Thus, the LSB approach performed better than the boiling water injection regime, provided better coverage, was environmental friendly to non-target ant species, less expensive and less time consuming. Based on the results of this this multiple year applied research program, we encourage the incorporation of the LSB approach for the management of *S. invicta* around karst cave formations in Camp Bullis.

Introduction

Solenopsis invicta Buren (Hymenoptera: Formicidae), native to South America, was accidentally introduced into the United States around the 1930’s, about 80 years ago, to Mobile, Alabama and has since spread throughout the Southern United States infesting more than 130 million ha (Callcott and Collins 1996, Taber 2000, Tshinkel 2006, Vinson 1997). Two colony forms are found in invaded areas: the monogyne (single queen) and the polygyne (multiple queens) form (Valles and Porter 2003). Monogyne colonies, characterized by a nest containing a single queen with strongly territorial workers, were predominant for decades following the original invasion in Alabama. In the early 1970's, polygyne colonies were detected and since have become the predominant form (Glancey et al. 1973, Porter et al. 1991). Each polygyne colony contains many queens and fewer workers than monogyne colonies but polygyne colony densities per ha are double or triple those of monogyne (Porter et al. 1997). Foraging territories and workers are shared among related polygyne colonies. *S. invicta* prefers disturbed habitats where they rapidly establish and proliferate. These key factors hinder the management of this invasive species, increasing the cost of management and increasing the risk to humans and animals (Macom and Porter 1996, Wojcik et al. 2001).

Management of *S. invicta* traditionally has involved suppression using broad-spectrum insecticides (Carson 2002, Drees and Gold 2003. Markin et al. 1974, Oi and Drees 2009, Wojcik et al. 2001, Tschinkel 2006). The absence of natural enemies and the lack of strong interspecific competition explain the dominance and higher abundance of *S. invicta* in the United States relative to that in their native South America (Porter et al.1988, Porter et al. 1991, Porter 1992, Porter et al. 1997).

One of the major factors regulating ant densities is interspecific competition (Hölldobler and Wilson 1990). Recent field studies conducted in South America indicate that interspecific competition with other ants appears to be a much stronger factor regulating *S. invicta* densities than mortality ascribed to parasitic flies (Diptera: Phoridae) (Porter et al. 1997, Morrison and Porter 2005, Holway 1999, Feener 2000, LeBrun et al. 2007). Relying on these findings and these concepts, improvements in *S. invicta* management practices should include the restoration and preservation of native ants in the United States. This is particularly important for the success of management programs that include the introduction of natural enemies (e.g., phorid fly species, imported fire ant microsporidian disease *Kneallhazia solenopsae*, fire ant viruses) from South America(Oi et al. 2008, Williams et al. 2003). This interspecific competition appears compatible with biological control and is expected to help regulate *S. invicta* densities to provide sustainable suppression of this exotic invasive pest ant.

Despite advances in management practices and the recent introduction of biological control agents, insecticides (particularly broadcast bait-formulated products) remain a primary tool for *S. invicta* control in the United States (Drees et al. 2002, Riggs et al. 2002, Barr et al. 2005, Aubuchon et al. 2006). Use of bait products is arguably the most cost-effective means of controlling *S. invicta*. Insecticidal baits available for *S. invicta* control are typically small granules (~1mm) derived from defatted corn grits and mixed with soybean oil and the active ingredient (an insecticide dissolved in the oil)(Williams et al. 2001). The most common active ingredients found in these baits are those carrying metabolic inhibitors, growth regulators and neurotoxins (i.e. abamectin, hydramethylnon, fenoxycarb, methoprene, pyriproxyfen, indoxacarb and fipronil). Under favorable temperature conditions (Drees et al. 2007) foraging worker ants are attracted to the bait and carry it back to the colony, where it is fed to the larvae, workers, and queen(s). Although active ingredients have different modes of action, they all serve to break the life cycle of the colony, resulting in its elimination. Fast-acting short residual baits kill the queen and, to varying degrees, worker ants (Barr et al. 2005). These baits pose very little toxic threat to people and animals as they are broadcast at very low rates (typically 0.6 kg/ha). These baits are not considered selective for *S. invicta* although their effects on non-target ants are relatively unknown. The fast-acting baits (metabolic inhibitors, neurotoxins) differ from slower acting insecticide baits (i.e. insect growth regulators or IGRs) in their mode of action. Understanding the effects these baits have on the ant community is critical for development of sustainable *S. invicta* management practices in the U.S. and elsewhere.

We proposed a “fire-ant specific” approach called the Lure-Switch-Bait (LSB) method that primarily relies on behavioral features of the invasive *S. invicta* and the slow-acting properties of Insect Growth Regulator (IGR) ant bait insecticide properties (Drees et al. 1992). The method we developed and compared in this study relies on the ability exhibited by *S. invicta* to rapidly discover and dominate food resources, a key feature presented in invasive ant species. This ability facilitates the use of an IGR bait that is detected as a food by ant foragers. *S. invicta* colonies contains up to 200,000 workers and studies have shown that nearly 30% of these workers are actively foraging (versus native ant colonies that contain nearly far fewer foraging workers), we assumed that the ability to discover and dominate resources exhibited by *S. invicta* would allow a species specific removal by using insecticide bait station applications (Calixto et al. 2011).

The goals for this project are: 1) to develop and assess alternate monitoring and treatment regime (LSB) for management of red imported fire ants and compare this approach to the currently used treatment program (injecting visible ant mounds with boiling water) compared to untreated control reference sites; 2) to establish a baseline of *S. invicta* natural enemies (and competitors or native ants) associated to this species around karst cave formations to help facilitate sustainable management and control involving potential release and monitoring of additional natural enemies; and, 3) determine the effects of *S. invicta* and insecticide baits on *Ceuthophilus sp.* cave crickets under laboratory conditions.

Methods and Materials

<u>Experimental approach</u>. To compare the efficacy of the an experimental Lure-Switch-Bait (LSB) targeted treatment approach to the current management practices (i.e., boiling water ant mound injections), we conducted a manipulative 3 year field experiment on previously untreated 18 karst formation centered 50 m radius circular 0.8 ha (2 acre) plots at Camp Bullis, Bexas Co., TX. Three management practices were compared: 1) boiling water ant mound injections (standard treatment regime); 2) the LSB approach using IGR bait insecticide selectively applied in bait stations; and 3) no treatment (untreated control reference cave) (**Figure 1**).

<u>Treatment plots assessments</u>. To assess *S. invicta* population levels, food lures were initially used on each of the designated 18 karst formation centered plots, April 13, 2009. None of the sites selected were known to harbor endangered cave arthropods of concern. Food lures (a single two-gram slice of Bar-S hot dog) were used to assess *S.*

invicta relative abundance. Each lure was deployed on the ground, one in the plot center, and the along 4 transects (North, South, East and West) radiating from the central cave formation at intervals of 4.5, 9, 18 and 36 m (5, 10, 20 and 40 paces, respectively) (**Figure 2**), and left exposed for 45-60 minutes (17 food lures were used per plot), before the number of worker ants on each food lure was estimated (0-100). Note that this pattern of food lure sites increased in density toward plot centers, the karst formation "target" of management efforts implemented. This method was also used to assess treatment effects periodically following treatment applications in addition to direct whole-plot ant mound count numbers. Plots were numbered and arrayed from those with the highest number of ants estimated on food lures to the lowest, and then grouped into three replicate blocks of six plots each. Thereby, one block or replicate of six contained plots with the highest numbers of ants recruited and one contained the lowest foraging ant numbers to eliminate pre-treatment plot mean differences (**Appendix I**).

<u>Treatments approaches</u>. Camp Bullis (initially outsourced to Zara Environmental) conducted monthly inspections to count mounds within a 50 m radius, 0.8 ha circular plot of the Karst formation centered plots. All mounds within a 10 m radius were treated within 15 days . (**Figure 3**) Monthly mound counts were tabulated and entered into a database to monitor infestation levels over time. Biannual hot water treatments were conducted with one treatment in spring and one treatment in the fall. Hot water treatments were conducted with a Hotsy high pressure washer and water heater. The high pressure washer was mounted on a trailer with a 225 gallon water tank. Equipment was pulled to plots in sometimes very remote locations without roads. The trailer was pulled as close as possible to the plot center and the hot water was dispensed through high pressure "wands" with up to 50m of hose. In areas unable to be reached with the trailer, rainwater was collected and stored on site. Propane and a large burner were hauled to the site to heat water in 5 gallon buckets to be carried to individual ant mounds. A small amount of dish soap was dripped onto the mound while the wand injected the hot water. The hot water directly killed the RIFA on contact and the soap allowed water to more effectively penetrate the mound and drown remaining ants. Treated mounds were excavated with picks to ensure any remaining ants were destroyed. Treated mounds were tabulated according to colony size from 100, 500, 1000, and 5000+ individuals. The data and the amount of water used at each site were entered into the database. The first scheduled application in spring 2009 was delayed. Problems with the contract for these treatments between the Department of Defense and Zara delayed treatments until fall, preventing direct comparisons between the standard and experimental treatments for the initial treatment. The first treatment by Zara was deployed in August 5, 9, 29 and 31 and the second one in October 20, 21, 23 and 27. In 2010 the treatments were applied in April and September. In 2011 treatments were applied in March. No treatments were applied in the fall.

The LSB management approach, developed by Texas AgriLife Extension Service and Research with the Texas A&M System, relied on foraging worker ants lured by using a food lure (i.e., hot dog slices) placed in a grid around the periphery of the karst formation entrance. We deployed each lure on the ground, one in the plot center, and others on a 10 by 10 meter grid to encompass the circular 50 m radium, 0.8 ha (2 acre) plot area (89 food lure stations per plot). Food lures were exposed for 45 to 60 minutes. Discovery and dominance by *S. invicta* workers on food resources occurs during this short period of time, allowing their relative abundance in the area to be estimated (**Figure 4**). In this study, the action level for treating *S. invicta* was a single live ant, although in other sites such as pastures a higher level (i.e., 30 or more foraging worker ants on 10 hot dog slices is equivalent to approximately 20 ant mounds per acre) is encouraged for use as an action threshold. At food lure stations where *S. invicta* worker ants were attracted, hot dog slices were replaced with professional Perimeter insect bait stations (B&G Equipment Company, 135 Region South Drive, Jackson, GA 30233, 678/688-5601, http://www.bgequip.com/HTML/pc_ipm/ipm_baitstations.html) containing 20.5 gr Esteem® Ant Bait (0.5% pyriproxyfen, http://www.valent.com/Data/Labels/2006-EAB-0001%20Esteem%20Ant%20Bait%20Form%201609-A%20-%20CA%20approved.pdf) (**Figure 5**). Thereby, no more than 907 g per 0.4 ha (2 lbs/acre) of product was applied per plot as directed on the product label.

The action level driven LSB approach was only delivered when *S. invicta* workers were present at the food lure, making it target-specific, and with the amount of active ingredient sufficient to provide significant control applied. The protocol intended for bait stations to be removed from the ground 24 hours later, but after the initial treatment they were left in the field for 48 hrs for subsequent applications. Remaining insecticide materials was later weighted to estimate the amount of bait removed by the ants. Pyriproxyfen, the active ingredient in Esteem® Ant bait mimics the insect's juvenile growth hormone and decomposes quickly in the environment when not consumed by the ants. The LSB treatment was applied on June 29-July 2 and October 15 in 2009; March 23, May 24 and October 3-7 in 2010; and on March 28-April 14, May 31-June 4 in 2011.

<u>*S. invicta* population level monitoring</u>. To assess treatment effect on *S. invicta* population levels, food lures were used on each of the designated 18 cave formation plots. Food lures consisted of a single two-gram slice of Bar-S hot dog. Each lure was deployed on the ground, one in the plot center, and the along 4 transects (North, South, East and West) radiating from the central cave formation at intervals of 4.5, 9, 18 and 36 m (5, 10, 20 and 40 paces, respectively) (**Figure 2**), and left exposed for 45-60 minutes (17 food lures were used in total per plot), before the number of worker ants on each food lure was estimated (0-100). In addition, colony counts were conducted where all active ant mounds within each of the 50 m radius circular plots was recorded. Assessment of ant activity was conducted three times during 2009 (March 1, 2, 3, 4, 9, 11 – April 13, 14, 21, June 12, 15, 16, 17, 18, 19, 22 and September 21, 22, 23, 24, 25 – October 27, 28), four times during 2010 (March 2, 3, 5, 9, 31 – April 5, 6, 7, 8, 9, 12, 13, May 3, 4, 5, 10, 11, 12, 13, 20, 21, September 7, 8, 9, 13, 14, 15, 16, 28 and October 6, 7, 11, 12, 13, 19, 20, 21) and four times during 2011 (February 28 – March 1, 2, 7, 9, 10, 12, 14, April 6, 7, 17, 19, 20, 25, 27, 18, May 13, 17, 18, 19, 23, 24, 26, 27 and September 6, 7, 8, 9, 12, 13, 14, 15). Colony densities were recorded twice during 2009 (June 4, 5, 8, 9, 10, 12 and September 21, 22, 23, 24, 29 – October 27, 28), five times during 2010 (March 1, 2, 3, 4, 5, 9, 11, 31, April 5, 6, 7, 8, 9, 12, 13, May 3, 4, 5,

10, 11, 12, 13, 20, 21, September 7, 8, 9, 13, 14, 15, 16, 28 and October 6, 7, 11, 12, 13, 19, 20, 21) and four times during 2011 (February 28 –March 1, 2, 7, 9, 10, 12, 14, April 6, 7, 19, 20, 25, 27, 28, May 13, 17, 18, 19, 23, 24, 26, 27 and September 6, 7, 8, 9, 12, 13, 14, 15).

Natural enemies detection and co-occurring ant species monitoring. Three groups of organisms were included in the detection and monitoring program:

1) Phorid flies (*Pseudacteon spp*.). These parasitoids have been introduced mainly in the southeastern U.S. to help provide sustainable control of *S. invicta*. Species released have been demonstrated to be specific for *S. invicta* (Porter and Alonso 1999). At present, four species have been released and have been successfully established in Texas (*P. tricuspis*, *P. curvatus*, *P. obtusus* and *P. nocens*) (Gilbert et al. 2008). We used sticky traps (PTS) (Puckett et al. 2007) for the detection of these organisms, deploying ten traps twice a year (April 20 and October 27 2009, May 10 and October 19 2010, April 8 and September 14) in a single location in Camp Bullis. Traps consisted of a plastic "pizza-tri-stand", sticky tape and a lure (hot dog slice) to attract ant workers (**Figure 6**). The flies respond to the presence of workers and are attracted to the traps. In the process of finding a host or finding a perch, the sticky tape traps the flies. Traps were deployed for 24 hr periods, and then they were collected and returned to the lab for their inspection. We recorded the number of flies and species collected with these traps.

2) The microsporidian, *Kneallhezia solenopsae*. This natural disease found in *S. invicta* was screened for in the area using established DNA extraction and genetic methods (Polymerase Chain Reaction or PCR) protocols. We collected *S. invicta* workers from the food lures used around the caves included in the study to determining if the ants at Camp Bullis were infected with this microsporidian (Valles et al. 2002). In addition, we determined the predominant social form (monogyne or single queen form, and polygyne or multiple queen form) present at the study site (Valles and Porter 2003, Oi et. al 2009). We collected *S. invicta* specimens from food lures in Ziploc bags when they were present from field plots at the time when *S. invicta* population levels were assessed.

DNA extraction of *S. invicta* was done using a lysis buffer containing 50mM Tris, 4% SDS and 5% BME. The sample of ants was put into 1.5mL tubes with 150µl of lysis buffer and then crushed with a plastic pestle for 10 sec. and boiled for 15 min before placement on ice for 1 min. Thereafter, 200µl of a 1:1 solution of phenol:chloroform was added to allow the nucleic acids to rise to the top and the other organic material to fall to the bottom. Once the aqueous layer was removed to another tube, it was rinsed twice with chloroform to ensure no transfer of phenol occurred. DNA was precipitated out using 100% ethyl alcohol and placed on ice for 1 to 2 hours and later is spun to the bottom of the tube in a centrifuge at 14,000 RPM for 10 min. The liquid was removed and allowed to dry completely, usually overnight. The next day, the DNA was rehydrated in 40µl of sterile water. PCR to detect Gp-9 was accomplished using multiplex PCR in a 50µl reaction that contained 1X Taq buffer, 200µM dNTP mix, 2mM $MgCl_2$, 0.4µM of each primer, 1U/µl of Taq, and 1µl of DNA with a concentration of 50-500ng (Valles and Porter 2003). PCR to detect *K. solenopsae* was accomplished in a

50µl reaction that contained 1X Taq buffer, 200µM dNTP mix, 2mM $MgCl_2$, 0.4µM of each primer, 1U/µl of Taq, and 1µl of DNA with a concentration of 50-500ng (Valles et al 2002, Oi et al 2009).

The Gp-9^B sense primer code is CT CGCCGATTCTAACGAAGGA and the antisense primer code is ATGTATACTTTAAAGCAT TCCTAATATTTTGTC. The Gp-9^b sense primer code is TGGAGCTGATTATGATGAAGAGA AAATA and the antisense primer code is GCTGTTTTTAATTGCATTTCTTATGCAG. The *K. solenopsae* sense primer code is CGAAGCATGAAAGCGGAGC and the antisense code is CAGCATGTATATGCACTACTGGAGC. DNA was inserted in a thermocycler (1 cycle at 94°C for 2 min, then 35 cycles at 94°C for 15 sec, 55°C for 30 sec and 68°C for 1 min followed by an elongation step at 68°C for 5 min) before 15µl of PCR product was added to 1% agarose and stained with ethidium bromide for visualization. The homozygous monogyne colonies produce a band at 517bp and heterozygous polygyne colonies produce bands at 517bp and 423bp. Colonies positive for *K. solenopsae* produce a band at 318bp while negative colonies will not produce bands (**Figure 7**).

3) <u>Monitoring for native and exotic competitor ant species</u>. Many ant species are known to compete with *S. invicta*, they can pose a risk for incipient *S. invicta* colonies and are currently part of sustainable management of *S. invicta*. Several studies have indicated that native ant communities are restored followed the reduction of *S. invicta* numbers by using broadcast applied insecticide bait products which is a strong indicator of the compatibility of native ants with insecticide baiting programs (Calixto et al. 2007a, Calixto et al. 2007b, Calixto 2008). To assess treatment effects (i.e., insecticide bait or boiling water injection treatments where *S. invicta* was reduced compared to untreated control plots) on competitor ant numbers, 2 gr slices of Bar-S hot dog food lures were deployed on the ground: one in the plot center, and the along 4 transects (North, South, East and West) radiating from the central cave formation at intervals of 4.5, 9, 18 and 36 m (5, 10, 20 and 40 paces, respectively) (**Figure 2**), and left exposed for 45-60 minutes (17 food lures were used in total per 0.8 ha plot), before the number of worker ants on each food lure was estimated (0-100). Assessment of co-occurring ant activity was conducted at the same time when *S. invicta* population levels using lures was monitored. The relative number and species of ants was recorded, and specimens were collected on lures for further identification or identified in the field.

<u>Effects of *S. invicta* and insecticide baits on lab colonies of Cave Crickets (*Ceuthophilus spp.*)</u>. Cave crickets, *Ceuthophilus spp*., were collected from cave formation openings at Camp Bullis, Bexar Co., TX, during June 6-10, 2011 and delivered to the laboratory at Texas A&M University in College Station on June 17, 2011. Cricket housing units used were made from 5-gallon plastic (30 cm tall, 38 cm tall) buckets (**Figure 8**) with the inner surface dusted with baby powder to prevent escape. A lid was prepared for each bucket with a 5.5 cm diameter hole drilled in the center covered with a 2 mm mesh window screen taped to the top, also to prevent escape. Inside, a plastic pot saucer (25 cm diam., 3 cm tall) was placed on the bottom filled with 200 ml tap water in which an inverted 8 inch clay pot saucer (21 cm diam., 3 cm tall) was positioned. A 8.5 inch clay planting pot (22 cm dam., 15 cm tall) was placed upside down so that it rested on the

rim of the clay saucer. This provided a simulated high humidity “cave” environment to house the crickets. On top or beside this structure were placed condiment cups (3.5 cm diam., 4 cm tall) containing cricket diets (a: Fluker’s Hich-Calcium Cricket Diet; and b: Fluker’s Orange Cube Complete Cricket Diet; Flucker Farms, Port Allen, LA, www.flukerfarms.com) and water soaked in a cotton ball. Crickets were immobilized with CO^2 to separate, count and sex them for experimental purposes.

S. invicta predation on Ceuthophilus cave crickets. To assess *S. invicta* predation on cave crickets, two types of exposure units were used: 1) 8.8 cm diam., 1.5 cm tall Petri dishes with 2 mm hole melted into one side and bottom covered with 70 mm moistened filter paper and containing one late instar to adult cricket; 2) tall cylindrical plastic containers (6.5 cm diam., 8 cm tall) with 2 mm hole melted close to the bottom, containing a wire 1/5 mm mesh 15.5 cm long by 2 cm wide screen standing on end, a water soaked cotton ball and one late instar to adult cricket. Five of each chamber were prepared and placed on a plastic tray supported by more tall plastic containers over a conventional red imported fire ant laboratory colony (**Figure 9**). Similar sets of these ten exposure units was prepared and placed beside the ant colony to serve as unexposed controls.

To obtain ant colonies, each mound of red imported fire ants was shoveled into 5-gallon plastic bucket with inner surfaces dusted with talcum powder to prevent ant escape. Water was then dripped into the buckets slowly, causing ant colonies to float on the surface. Ants are removed and placed in plastic trays measuring 27 by 37 cm and 9 cm tall. One Petri dish (14 cm diameter and 2.5 cm tall) containing set Castone® moistened with water and covered with lids containing holes melted into them to allow ants to enter and exit was placed in each colony tray to house the queen, brood (eggs, larvae and pupae) and worker ants. Each colony was provided with distilled water and a standard laboratory diet of dead insects (crickets or mealworms) and diluted honey water daily. The trial was initiated 9:15 am, June 21, 2011 and observations were made periodically until all crickets were dead.

Ant Perimeter bait station access. Ability of cave crickets to enter B&G Perimeter insect bait stations was evaluated by placing crickets of various sizes and life stages immobilized with CO^2 into one of the devices along with a water soaked cotton ball and cricket diets (**Figure 10**). The trial was initiated June 21, 2011 at 9:00 a.m. The closed station was placed in a clear plastic box (31.5 long, 17 cm wide, 9.5 cm tall) with similar provisions on the outside and closed with a lid. Crickets appearing outside the bait station thereafter were collected, frozen and measured. The trial was terminated June 23, 2011.

Cave cricket consumption of ant bait. To verify the consumption of conventional ant bait formulations comprised of de-fatted corn grit soaked with soybean oil in which the active ingredient is dissolved, three crickets (late instar to adult) were placed in a plastic box (31.5 long, 17 cm wide, 9.5 cm tall) along with a water soaked cotton ball and cricket diets. Two such boxes were prepared and in one box was placed 10 particles of fresh ant bait (Advion® containing 0.045% indoxacarb, http://www2.dupont.com/Professional_Products/en_US/assets/downloads/pdfs/SL-1409.pdf) at 4:00 p.m., July 26, 2011. Presence of bait particles was documented the following morning (8:30 a.m.). Thereafter, additional bait was provided and observations were made periodically to document any toxic effects from bait consumption.

Esteem® Ant Bait exposure trial. Six housing buckets were prepared as described above, using smaller sized potting elements: 20 cm diam., 3 cm tall plastic pot saucer, 6 inch clay pot saucer (15.5 cm diam., 2.5 cm tall) and 6.5 inch clay pot (17 cm diam., 12 cm tall). Nymphal stages (small to medium sizes not having noticeable ovipositors) of crickets were separated into 5-gallon plastic buckets containing 15 crickets each. An additional 16 crickets of similar sizes were frozen and measured (from front of head to end of abdomen) and pinned for further assessment to document stage of exposure. In addition to all housing buckets being provisioned with cricket diets and water, three of the buckets were treated with a volume of 270 ml (about 2 fl oz) Esteem® Ant Bait in a plastic cup (**Figure 11**). The ant bait was replaced with fresh bait (about 1 oz or 30 ml) periodically over the course of exposure. The trial was initiated on the afternoon of June 20, 2011 and crickets were monitored periodically to replace food and water. On July 25, 2011, after 5 weeks in the laboratory, crickets in each bucket were immobilized with CO^2 counted and sexed. After a similar assessment 8:30 a.m., Aug. 26, 2011, 10 weeks of development, upon development of adult females (with fully developed ovipositors), crickets were dissected to observe ovary and egg development (4:00 p.m.).

Data Analysis. Data obtained from the different sampling methods were analyzed using Mixed Model - Repeated Measures (Type III sum of squares and diagonal repeated covariance) comparing the impact of the different treatment regimens on the response variable (*S. invicta* and native ant relative abundance and behavioral dominance). Individual Analysis of Variance (ANOVA) and Post hoc analyses were also conducted for every sampling date to determine differences among groups. The statistics software, SPSS 19.0 (SPSS Inc. 2011), was used to perform these analyses (values sig. dif. $P < 0.05$).

Results and Discussion

Labor requirements for plot establishment and treatments. The table below summarizes estimated man hours to establish, treat and maintain LBS and boiling water injection plots. Set up time and monitoring required would be similar for both treatment programs. The LSB approach was initially more labor intensive, but ongoing LSB baiting was similar to boiling water. Equipment cost (Hotsy, liquid dishwashing detergent, propane, burner, pick ax and trailer for boiling water injections; bait stations and bait product cost for LSB) was not included.

Activity	Number Plots	Total Days Needed*	Total Man Hours	Average Individuals/ Day	Average Man Hours/Plot
Set Up	18	7	183	5.5	10.16
LSB Initial Baiting	6	4	45	4.5	7.5
LSB Initial Bait Pick Up	6	3	36	2.6	6
LSB Ongoing Baiting	6	3	12-18	1-2**	2-3
Monitoring	18	5-6	3.75-4.5	1+	.75
Boiling Water	76	20	224	2	2.9

* Days needed is dependent on weather, temperatures, and available maneuver areas.
** No more than 2 individuals recommended; three or more would result in same amount of time, but increased man hours.
+ Only one person needed; additional people would increase man hours.

LBS Set Up – included finding karst features, figuring transect directions, pruning, etc. Required 3-4 individuals for this task; any additional results in increased man hours with little different in time spent in field.

LSB Initial Baiting – included additional pruning on bruch canopy covered plots, if needed, putting hot dog food lures along the grid system (89 station locations), and replacing hotdogs with bait stations. This was the first baiting performed, and has the process since has significantly reduced in time required. Once the plot areas become familiar to personnel involved, the man hours tend to decrease drastically.

LSB Ongoing Baiting **–** note the decrease in required man hours and time spent per plot. We recommend that 1 or 2 individuals perform this work. Any additional personnel may only increase man hours, but does not decrease the time spent in each plot. The range listed in the table below indicates time spent in an area without many fire ants (e.g., few bait stations needed to be placed out) versus a plot that needs to have bait stations placed at every station location.

Boiling Water Technique - Time needed to inject with boiling water was based on size of mound: 100 = 3-5 min; 500 = 5-7 min; 1000 = 7-10 min; 5000 = 10-15 min. The plot treatment man hour estimates in table below were calculated from time estimates taken from the treatment of 76 total sites treated but 2 individuals (Marco Jones and Chris Thibidaux). Treatment took 28 hours per week and 4 weeks to complete 76 plots (totaling 112 hours per person of 224 man hours), thereby, requiring approximately 2.9 man hours per plot.

Treatments assessment of cave formation plots.

2009 Season. S. invicta relative abundance pre-treatment counts using food lures were conducted April 2009. No significant differences were found across karst formation centered circular 0.8 ha treatment plot means for *S. invicta* worker ant abundance (**Table 1, Fig. 12**). The first LSB treatment was applied at the end of June. The total amount of bait deployed on the first application was approximately 1,824.5gr. Stations were collected 24 hours later but weight measurement was not made because the rain either soaked or washed out some of the material from the traps. At this point we did not collect information on how much bait the ants removed. *S. invicta* relative abundance post-treatment counts using food lures were conducted during June. We found significant differences among treatments, with baited treated plots having significantly lower number of ants compared to boiling water injection treatment (still untreated) and reference untreated plot means. Mean numbers in LSB plots were below 10-ant worker ants average across the 17 lures compared to 15 in boiling water and 25 in untreated plots. The second LSB treatment was applied at the end of June, leaving bait stations exposed for 48 hrs for this and subsequent applications. Plots were re-treated again in October to provide maximum control. Zara did not make any hot water treatments in Camp Bullis in the spring. At this point we were unable to observe methods used or equipment needed, labor required or time needed. We found using colony surveys (numbers of active ant mounds per 0.8 hectare or 2-acre plot) conducted in June and September - October, and 6 month post-treatment, respectively a significant reduction in LSB plots, while densities in boiling water injection treatment plots remained similar to those observed in untreated plots (**Figure 13** and **Table 2**).

2010 season. We conducted the first round of assessments to determine action levels between March and April and observed that ant workers plot means for both LSB and boiling water were still around 10 ants average across the lures (**Figure 12, Table 1**). We treated the LSB plots again in March. Post-treatment data in May showed a slight reduction of foraging worker ant number means in LSB plots and slight increases for boiling water injection plots. LSB plot means remained significantly lower compared to those from boiling water injection and untreated plots throughout the rest of the season (less than 10 worker ants average/17 hot dog slices across the LSB plot lures). Two more LSB treatments were deployed given the increase in ant activity around the plot center perimeters, one in May and another in October. Ant activity significantly decreased following the May treatment and the numbers were close to those observed for the previous year (ant workers numbers below 10 per hot dog). Colony surveys (numbers of active ant mounds per plot) conducted for this year (March, April, May, September and October) showed that LSB plots were significantly lower in colony densities compared to untreated and boiling plots. The latter remained similar to untreated plots but showed a slight reduction following the treatments during the spring. However, this reduction was not sustained throughout the fall, unlike LSB plots (**Figure 13** and **Table 2**).

2011 season. We conducted pre-assessments between February-March and found that mean foraging worker ant numbers on food lures in LSB plots was slightly higher than 10, and those numbers in boiling water injection plots were statistically similar to those in untreated plots (**Figure 12, Table 1**)**.** We deployed one treatment beginning at the end of March and a second treatment at the end of May to maximize control. Numbers in both LSB and boiling water injection plots significantly declined, the latter was observed to increase by May reaching a mean level similar to that of untreated plots. We suspect that a combination of low foraging, the unusual high temperatures and low moisture in the soil observed for this year prevented the ants to build colonies making detecting active ant mounds difficult for the boiling water injection treatments. Mean numbers in LSB plots remained lower throughout the rest of the season. However, the boiling water and untreated plots numbers also declined when warmer temperatures were reached, approaching numbers observed in LSB plots. Colony surveys (active ant mounds) conducted February-March, April, May and September showed that LSB plots and boiling water injection plot numbers were similar throughout the year. This could be explained due the difficulty in detecting active ant mounds due to the warm weather that caused *S. invicta* colonies become more subterranean and less conspicuous. However, mean colony numbers in these treatment plots remained significantly lower than untreated plots until September (**Figure 13** and **Table 2)**.

Number of stations and insecticide bait deployed and retrieved. Weight data were not collected for 2009 but we recorded the number of stations deployed for the initial treatment (June 28-July 1) and found nearly 20% to be empty after 24 hours of exposure (**Table 3**). Thereafter, we recorded the number of stations deployed (which represents the actual number of food lures with *S. invicta* workers), retrieved and weight of the bait remaining on the station after 48 hours of exposure was recorded (**Figure 14 and 15**). It is important to note that very few bait stations were lost after deployment.

For the 2010 season, the number of stations needing to be deployed due to the presence of *S. invicta* foragers on food lures was reduced nearly 50% and from those that were initially deployed. We also found that the ants emptied all stations in one plot. The amount of insecticide bait taken was also reduced (i.e., more product left on the stations) throughout 2010 (**Table 4**). For 2011 we observed a similar pattern, however, more stations were deployed as a result of the increased *S. invicta* activity in plots but only a few stations were found empty, the others were found with significant amounts of product left (**Table 5**). Overall, we noticed that as we continued with the LSB treatments, the amount of bait taken by the ants were less which is explained by the reduction of *S. invicta* abundance by previous treatments (**Figure 12 and 13**). These results shows that the LSB method provided sustainable control, required less labor and used less product over time, making it more efficient and environmentally friendly.

<u>Social form</u>*. S. invicta* worker ants collected from food lures we determined to be predominantly the multiple queen form or polygyne social form. However, across Texas, *S. invicta* populations consist of a mix of both social forms (Porter et al. 1991). In Camp Bullis karst cave formation centered plots, nearly 85% of the ants collected and genetically analyzed were polygyne while the remaining 15% were monogyne. Over time, the incidence of the monogyne form increased on LSB plots as more treatments were delivered and population levels were reduced, and accounted for almost 55% of the samples collected following the regime of insecticide bait applications. This is an important component that needs to be accounted for in *S. invicta* management practices because the monogyne form is territorial. Thus at low population levels fewer colonies may discover and dominate the food resources, in our case, the insecticide baits, resulting in less optimal control (Calixto et al 2011).

Natural enemies detection and monitoring of co-occurring ant species.

Kneallhezia solenopsae. This microsporidian was found present in all ants collected around the karst formation centered plots, and was particularly abundant in plots not treated with insecticidal baits or boiling water. Findings suggest no further inoculations are needed as the infection level appears well established in Camp Bullis' *S. invicta* colonies. However, we encourage continued monitoring of this microsporidian.

Phorid flies. One species of the phorid fly previously released in another localities in Texas was detected. The species, *Pseudacteon curvatus*, was the second phorid fly species introduced and established in Texas (Gilbert et al. 2008) and appears more suitable to the polygyne or multiple queen form of *S. invicta* ants found in the state. We suspect other phorid species established in Texas (e.g., *P. tricuspis*) will find their way into Camp Bullis and we believe no further releases would be required. Again, we suggest continued monitoring for phorid fly population levels in Camp Bullis.

Native ants. As we observed significant reductions of *S. invicta* workers and colonies in karst formation centered plots resulting from the LSB treatment regime, we observed corresponding significant increases in diversity and abundance of other ant species on food lures (**Figure 16**). Numbers of native ants increase following initial LSB treatments and were significantly higher when compared to those in boiling water injection and untreated plots for the 3 year study (**Figure17, Table 6**). As a consequence of the removal *S. invicta*, we observed significant increases of the following nine ant species: *Crematogaster laeviuscula* Mayr, *Forelius pruinosus* Roger, *Monomorium minimum* (Buckley), *Solenopsis* (*Diplorhoptrum*) *molesta* (Say), *Brachymyrmex sp.*, *Pheidole sp*. *Dorymyrmex flavus* McCook and *Tapinoma sessile* Say.

Effects of *S. invicta* and insecticide baits on Cave crickets under laboratory conditions.

S. invicta worker ants prey heavily on exposed cave cricket nymphal and adult stages (**Figure 18**). After roughly 3 hrs (12 noon) of exposure to foraging imported fire ant workers, two of the cave crickets in the Petri dishes had been attacked and after about 6 hrs (3:30 p.m.) three of five of the Petri dishes contained ants attacking crickets, with two crickets already dead. No ants were in the tall cylindrical plastic exposure units. The next morning after about 24 hrs of exposure (10:30 a.m., June 22), all crickets in Petri dishes were dead and three of five tall cylindrical plastic containers were dead. After 48 hrs all RIFA exposed cave crickets were dead and all unexposed control crickets were alive (by June 28, after 7 days in chambers with only water three of the five crickets in tall cylindrical plastic containers were still alive, although all in Petri dishes had died). Ants consumed all soft tissues over the next few days, leaving thin translucent piles of cuticle debris. This trial confirmed previously published observations. However, by comparing the two different exposure units, data support that crickets with access to perches or other means of escape survive longer than confined crickets. Conversely, the ants took longer to inflict their first stings on crickets with more mobility and in more complex environments. This finding bodes well for free ranging crickets. However, once bitten and stung, cricket behavior changes to reacting to the irritation and venom.

Ant Perimeter baits station access. Crickets of all stages and sizes were able to escape from the enclosed Ant Perimeter bait station (nymphs from 4 to 9 mm and an adult female, 14 mm long). Their ability to squeeze through small cracks had been observed in the cricket housing containers as they were found to crawl between the plastic and clay saucer and pot arrangement, sometimes drowning in the process.

Cave cricket consumption of ant bait. All ten Advion® indoxacarb-containing bait particles were gone within 12 hours of exposure to cave crickets. Crickets were not observed to feed during daylight hours. Also, it was noted that crickets have problems walking on plastic surfaces, so paper needed to be added as a walking substrate and height of containers housing fool and water lowered to 0.5 cm or less allowed for better access to diet and bait particles. Additional bait was provided July 27 and by the next morning (8:00 a.m., July 28), the three crickets in the treated container were observed to be slow moving and lethargic. By the afternoon (4:40 p.m.), they were moribund (lying down and unable to walk normally). This condition persisted the next day (July 29) and after 4 days following exposure (August 1) only two of the three treated crickets were dead and the third being moribund, versus only one untreated cricket, with the other two untreated crickets still healthy, walking and hopping normally. Indoxacarb is a faster-acting imported fire ant bait insecticide that can eliminate 80-90 percent of broadcast-treated ant colonies in 7 to 10 days following application. Obviously, cave crickets consuming this ant bait formulation can be affected as well if they consume enough of a dose. Because the crickets are solitary and do not seem to recruit to bait as do social insects like ants, impact of populations in the field using discrete doses applied in a grid-spot pattern only where ants first recruit to food lures seems unlikely, but remains undocumented. Presumably, any crickets that did consume enough ant bait could be affected, and moribund crickets would be easy prey for foraging ants.

Esteem exposure trial. The 16 crickets similar to those exposed to pyriproxyfen were 12.6 $\pm$ 1.6 mm in length. On July 25, 2011, after 5 weeks in the laboratory, numbers of crickets in buckets had declined and only a few females (crickets with ovipositors, **Figure 19**) were evident (**Table 7)**. Ovipositors on females were rather small, and seemed to represent a late instar stage of development rather than an adult (**Figure 20**), so more time was allowed prior to dissections. An adult female, not exposed to an insecticide treatment was dissected, July 25, 2011, to reveal the structure of a “healthy” ovary (**Figure 21**). No evidence of egg formation was seen in this specimen, which was evidently not mated. By Aug. 26, 2011, after 10 weeks of development, numbers of live crickets had further declined, but three females in pyriproxyfen exposed and unexposed buckets remained and were dissected and photographed (**Figure 22**). Ovaries of both exposed and unexposed females appeared to be healthy, with one female seeming to have produced eggs. Thus, exposure to the IGR insecticide, Esteem® Ant Bait containing pyriproxyfen, was not documented to affect development of ovaries. This trial should probably be duplicated with greater survival of females to allow for statistical evaluation and assuring bait consumption occurred, as described above for indoxacarb, before accepting the conclusion that exposure to the IGR has no effect of the crickets.

Conclusions:

1) In the field study we demonstrate the use of behavioral features of *S. invicta* to achieve significant, sustainable, economic and environmental-friendly control of this invasive around cave formations in Camp Bullis. This approach achieved more than 90% control and allowed co-occurring native ants to resurge, a strong

indication that the LSB treatment is highly specific for *S. invicta*. These results are consistent with other studies and demonstrate that grid-targeted treatments achieved significant control and that suppression of *S. invicta* allows for the resurgence of several native ant species.

2) The amount of insecticide bait delivered and later retrieved in the bait stations that resulted in the decimation of *S. invicta* workers and colonies provided a strong indication that the LSB approach does not affect co-occurring arthropods such as other ant species in the field.
3) The boiling water injection treatment did achieve some significant control, but it did not provide the level and sustainable control presented by the LSB. Colonies that are not eliminated by these treatments, in part because they are more difficult to detect in hot, dry weather conditions, could potentially pose as a treat for co-occurring arthropods, including endangered and species of concern. The lack of native ant species resurgence following initiation of the boiling water injection treatment regime documented in this field study supports this possibility.
4) The LSB treatment was observed to be more cost-effective and less-labor intensive than boiling water injection treatments. It is assumed, however, that insecticide baits could pose a threat to the environment. The amount of toxic (active ingredient) delivered was minimal and amount required for periodic reapplication declined after *S. invicta* population levels were suppressed. The combination of boiling water along with commercial dish soap used could also be considered to be a chemical treatment and affects microbial organisms present in the soil at injection sites.
5) In the laboratory trial, cave crickets, *Ceuthophilus sp.*, were found attracted and affected by insecticide ant baits and capable of entering (and exiting) B&G Perimeter insect bait stations, suggesting using a different design that excludes crickets for future applications. However, the results failed to show that the female reproductive system was affected by exposure to pyriproxyfen, an IGR ant bait. This laboratory trial that should be repeated to increase numbers of developing female cave crickets exposed because a previous study using field crickets did document that ovaries were affected. Regardless, these findings are difficult to extrapolate to field populations where crickets must compete with *S. invicta* and other co-occurring species for access to the insecticide bait placed in stations only where *S. invicta* have already recruited to a food lure. Crickets are non-social insects that do not recruit to a food source and LSB treatments are applied in discrete (48) hr exposure periods. We concede that under natural conditions, crickets consuming the IGR ant bait could be affected by the LSB treatments; but that the percentage of the population affected would likely be minimal relative to the threat from *S. invicta* predation using no or insufficient suppression methods.
6) We found nine co-occurring native competitor ant species and two natural enemies of *S. invicta* around karst formation centered plots. We encourage continued monitoring for these natural enemies as they are indicators of environmental restoration and habitat quality, and can also help provide some level of sustainable suppression of *S. invicta* (Oi et al. 2008). Our results indicate that boiling water injection treatments would not benefit other ant competitors as

the level of suppression achieved does not allowing other ants to increase and outcompete with *S. invicta*, the exotic invasive pest ant present.

Management Recommendations:

We recommend that Camp Bullis management personnel consider the adoption of the LSB management approach over boiling water injection treatment regime. This study shows that this approach is 1) threshold driven; 2) highly specific for *S. invicta*; 3) uses less than 900 gr (2 pounds) per 0.4 ha (one acre) insecticide IGR bait and that the amount of insecticide bait needed to maintain sustainable is reduced after initial treatments suppress *S. invicta* population levels; 4) uses less labor after the initial treatment, requiring one person for monitoring and deploying the bait stations and only taking a single day to treat several 0.8 hectare plots; and, 5) allows for monitoring competitor ant abundance and activity by using food lures while simultaneously assessing *S. invicta*. If this approach were to be adopted, we encourage continued use of food lures (hot dogs) to monitor ants and where *S. invicta* are recruited apply a bait station containing an IGR granular ant bait product.

Future considerations:

The next phase of evaluating the LSB approach is to conduct field trials to assess the possible impact of cave crickets accessing and being affected by placements of IGR-containing bait stations in the field using dispensers better able to exclude crickets than the B&G Perimeter stations used in the trials reported herein. These trials would best be conducted around karst cave formations harboring the crickets with no known arthropods of concern and monitoring crickets leaving and entering the caves.

We also encourage adjusting and maintaining an action level or treatment or re-treatments threshold using food lures as described herein to more than the current level of one live *S. invicta* ant. We suspect that less than 10 foraging worker ants on 10 hot dog slice food lures probably fails to justify treatment actions because *S. invicta* may be so low that they are outcompeted by other ant species for surface dominance. In this case, their threat to cave crickets could be nominal. One of the research issues previously discussed was the concept of *S. invicta* detectability and methodology to allow a confident prediction of existing *S. invicta* densities. Such study would require a more rigorous design and time for the development of models that would be capable of determining the density of sampling units and their required distribution. We provide in **Appendix II** preliminary concepts in relation to the detectability of *S. invicta* in ecologically sensitive environments such as around karst cave formations. As mentioned, this could be an entire new project that would require planning and effort from all the parties involved.

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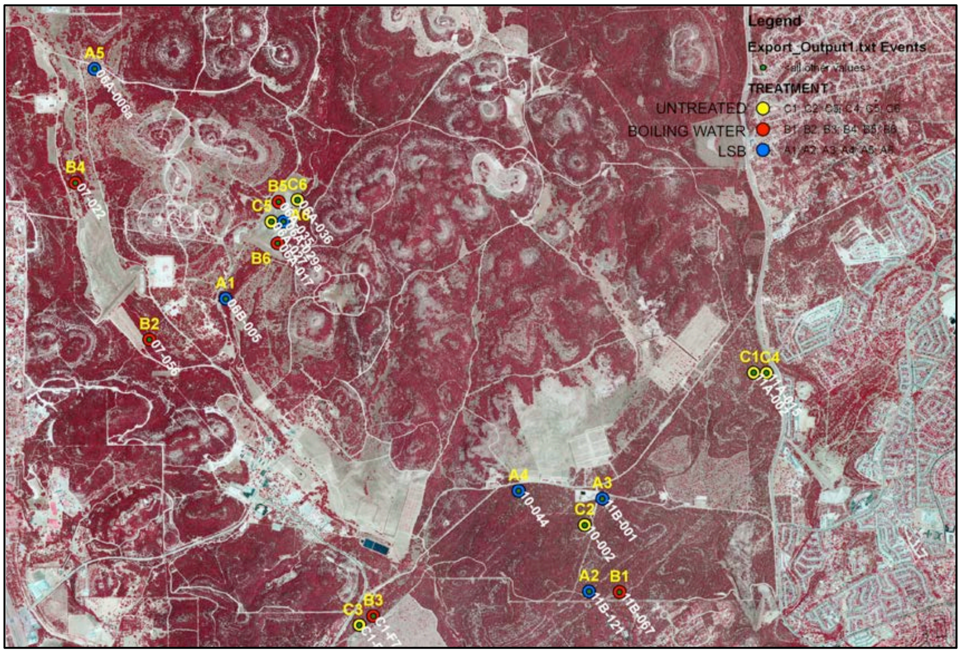


Figure 1. Location of 18 karst formation centered 0.8 ha (2 acre) plots indicating locations of possible caves not known to harbor arthropod species of concern used for the field experiment, Camp Bullis, Bexar Co., TX, 2009-2011.

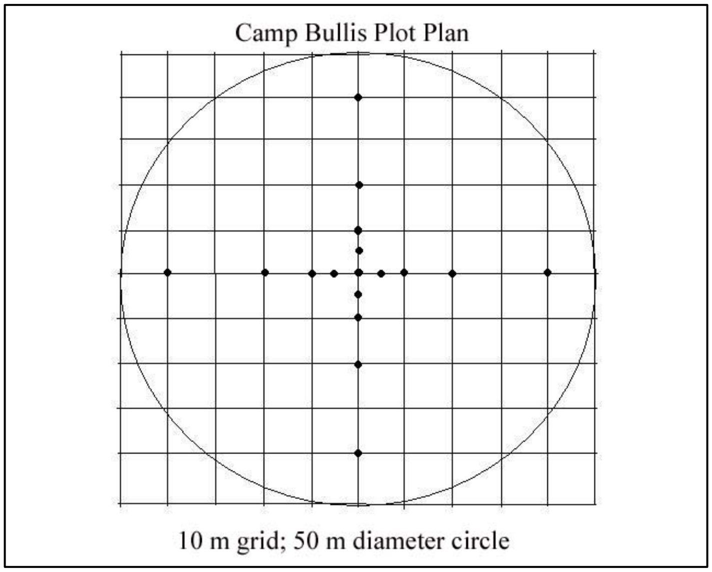


Figure 2. Food lure (hot dog) layout around caves formations, with a total of 17 units deployed on the ground for monitoring ant foraging periodically in all plots(bold dots) and 10 by 10 m grid (89 stations) used to implement the Lust- Switch-Bait (LSB) treatment regime, Camp Bullis, Bexar Co., TX 2009-2011.

Figure 3. Boiling water and liquid dishwashing detergent injection treatment applied by Zara Environmental LLC. to *S. invicta* colonies, Camp Bullis, Bexar Co., TX, 2009.

Figure 4. Food lure (hot dog slice) used as a decision-making tool to evaluate *S. invicta* relative density and determine treatment triggers.

Figure 5. B&G Perimeter insect bait station containing Esteem® Ant Bait with the insect growth regulator active ingredient, pyriproxyfen, open (left) and closed (right).

Figure 6. Traps (PTS) used for phorid fly monitoring in Camp Bullis, Bexar Co., TX.

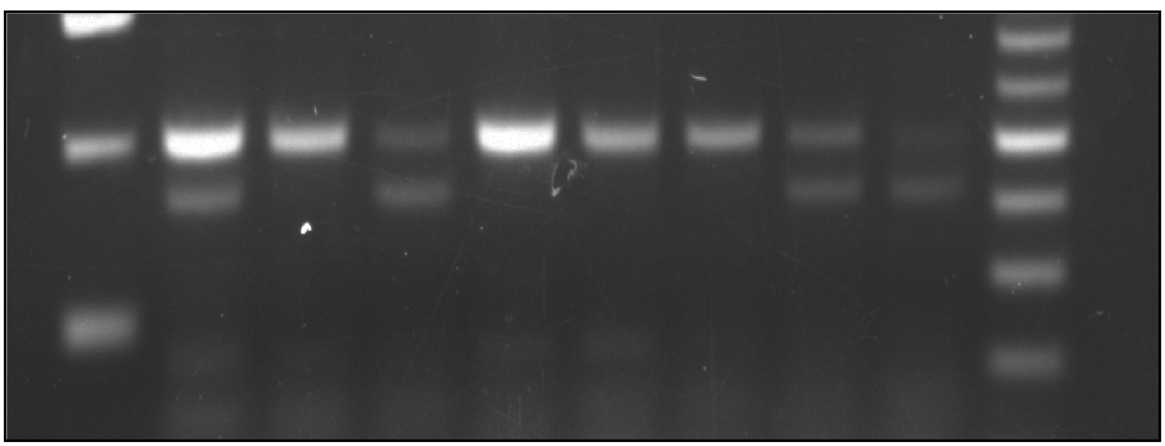


Figure 7. PCR gel product for genetic analysis of *S. invicta* social form using a Qiagen DNeasy kit for worker ants collected at Camp Bullis, Bexar Co., TX.

Figure 8. Five-gallon plastic bucket cave cricket housing units used for maintenance in the laboratory and for insecticide exposure trials, Texas A&M University, College Station, TX 2011.

Figure 9. Apparatus used to expose cave crickets to *S. invicta* workers exposure, Texas A&M University, College Station, TX 2011.

Figure 10. Ant Perimeter bait station (open) showing lid (left), bottom (right) provisioned with cricket diets and water in cotton ball, and crickets, Texas A&M University, College Station, TX 2011.

Figure 11. Housing buckets provisioned with cricket diets and water, with three of six buckets treated with a volume of 270 ml (about 2 fl oz) Esteem® Ant Bait (pyriproxyfen) in a plastic cup, Texas A&M University, College Station, TX 2011.

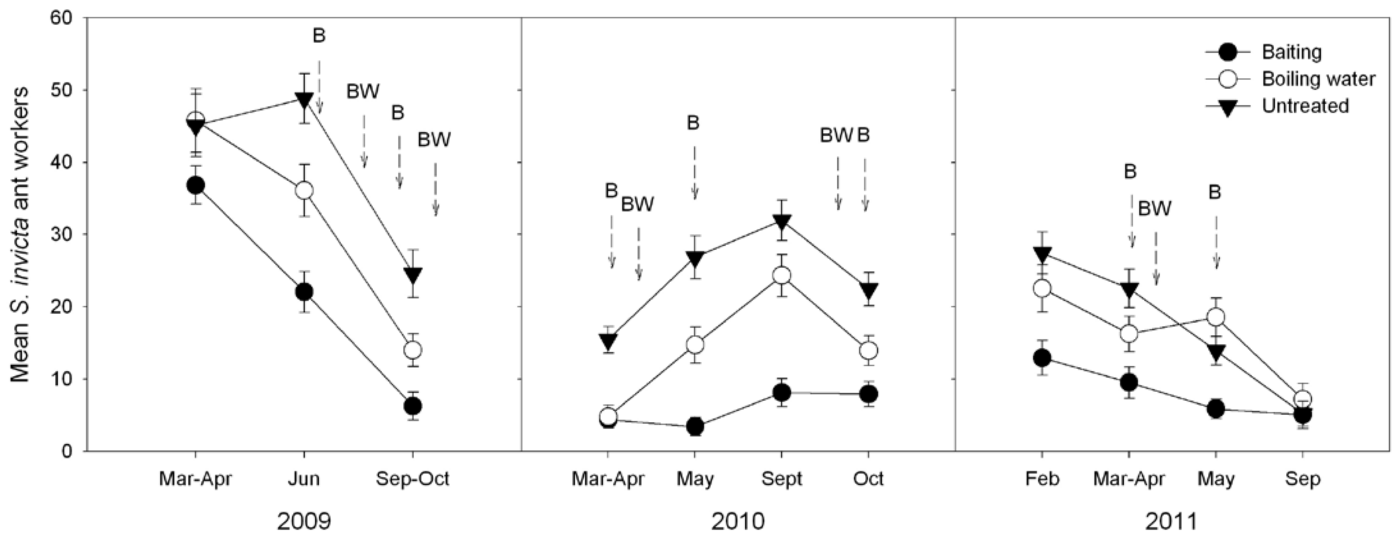


Figure 12. Mean number of *S. invicta* foraging worker ants attracted to a slice of hot dog food lures of 17 placed in transects of karst formation centered circular 0.8 ha (2 acre) treated (baiting or LSB shown as "B" and hot water injection shown as "BW" regimes) and untreated plots replicated six times, before and after initial May 20-28, 2009 LSB treatments, Camp Bullis, Bexar Co., TX, FY 2009-2011. (From **Table 1**).

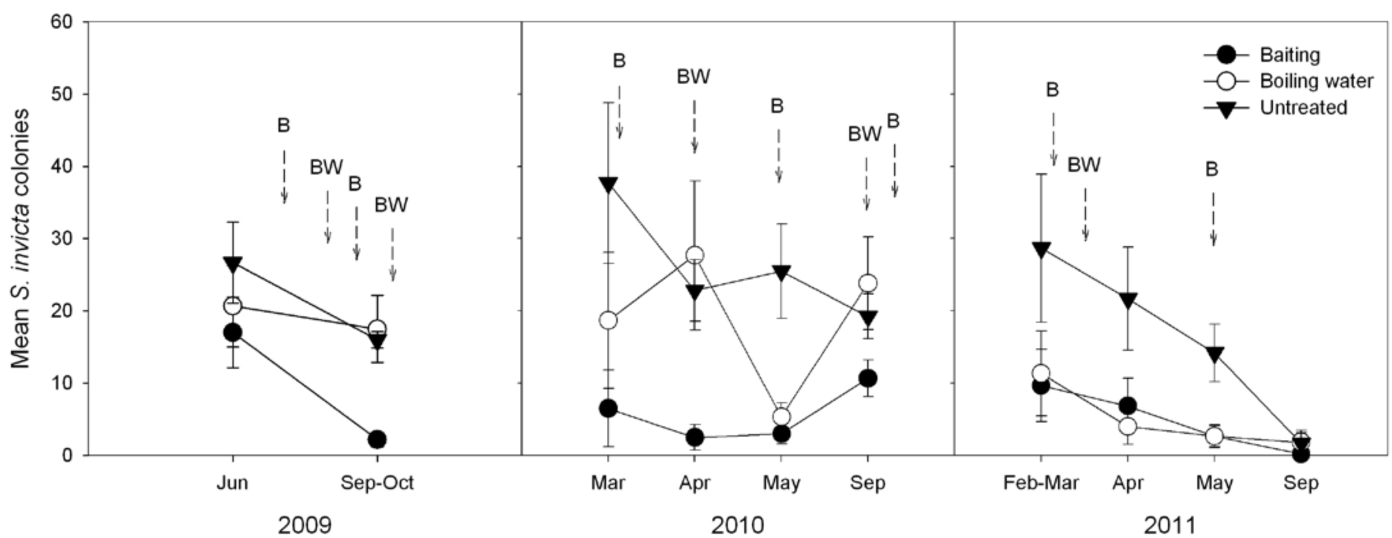


Figure 13. Mean number of *S. invicta* colonies or active mound numbers in transects of karst formation centered circular 0.8 ha (2 acre) treated (baiting or LSB shown as "B" and hot water injection shown as "BW" regimes) and untreated plots replicated six times, before and after initial May 20-28, 2009 LSB the treatments, Camp Bullis, Bexar Co., TX, FY 2009-2011. (From **Table 1**).

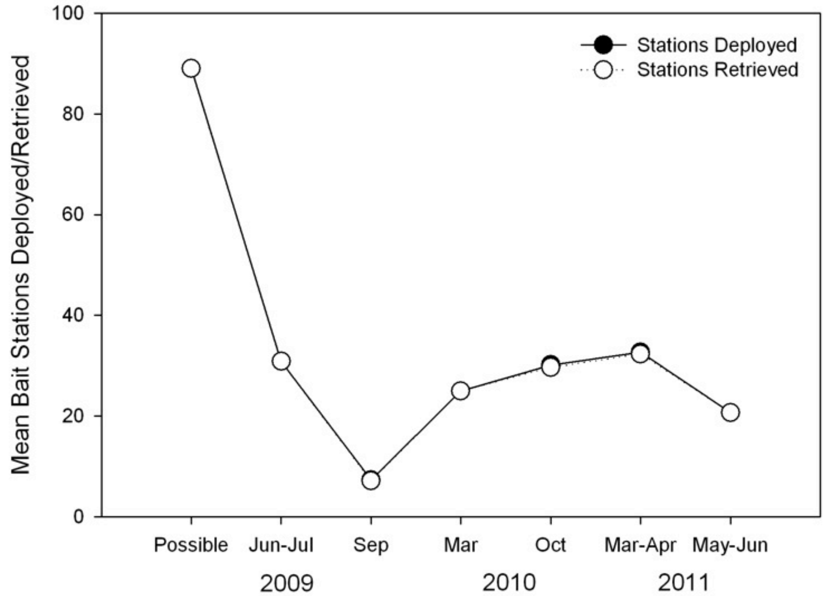


Figure 14. Mean number of B&G Perimeter insect bait stations deployed and retrieved in circular 0.8 ha (2 acres) Lure-Switch-Bait (LSB) plots on a 10 by 10 m grid pattern (89 station sites) where *S. invicta* foraging workers were attracted first to hot dog slice food lures, replicated 6 times, Camp Bullis, Bexar Co., TX. FY 2009-2011 (from **Table 3, 4 and 5**).

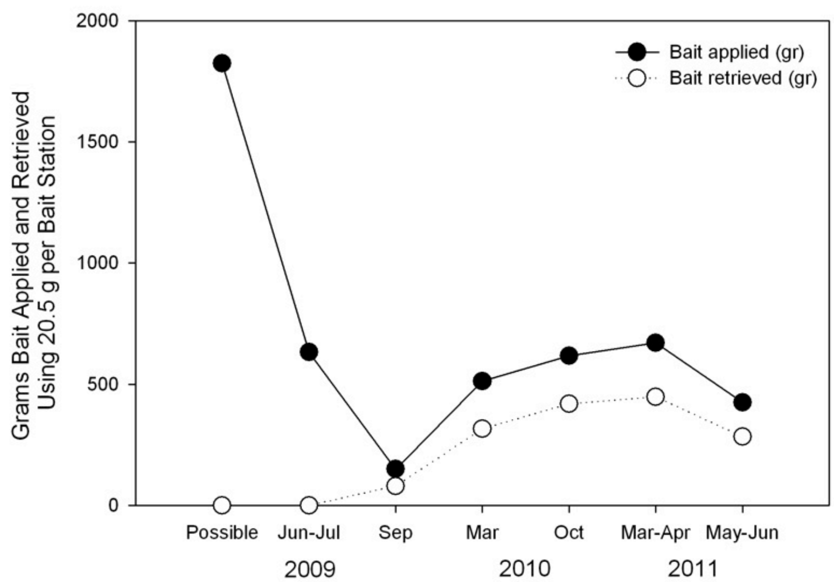


Figure 15. Amount (grams) of Esteem® Ant Bait (pyriproxyfen) provided (20.5 g per bait station with a maximum amount of bait per 0.4 ha allowed being 907 g) and retrieved

over a 48 hours exposure period, Camp Bullis, Bexar Co., TX, 2009-2011 (from **Table 3, 4 and 5**).

Figure 16. Native ants (*Pheidole sp.*) observed on food lures, Camp Bullis, Bexar Co., TX.

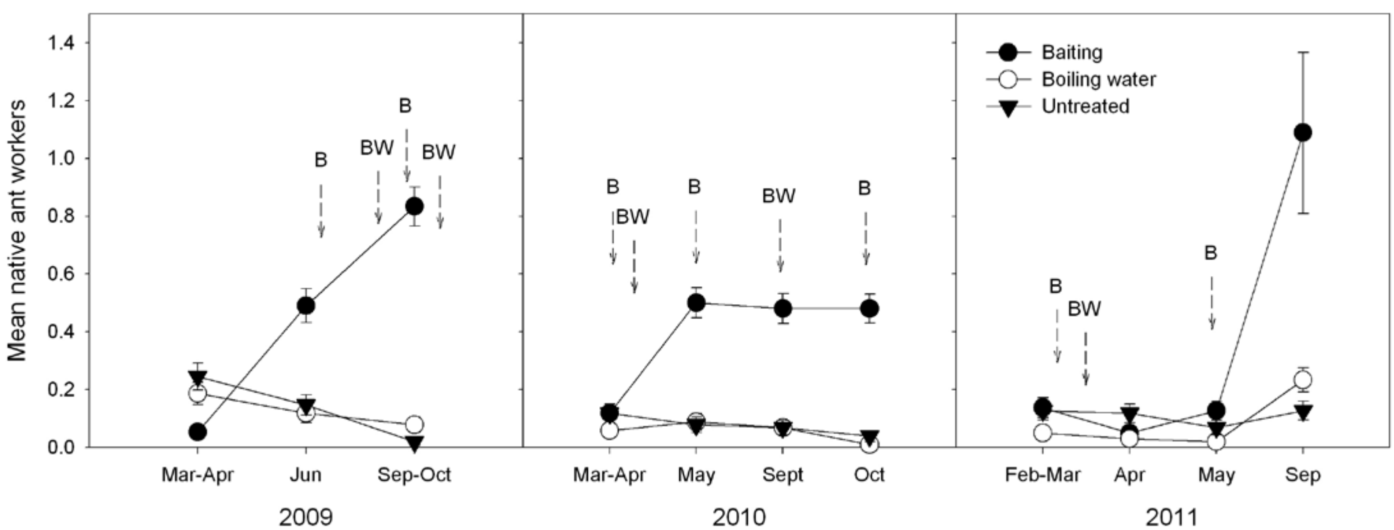


Figure 17. Mean number of native *S. invicta* competitor ants attracted to 17 hot dog slice food lures exposed for 45 to 60 minutes per baiting or Bait-Switch-Lure (LSB, shown as “B”), boiling water injection treatment (shown as “BW”) regime and untreated plots replicated six times before and after the initial May 20-28, 2009 treatments, FY 2009-2011, Camp Bullis, Bexar Co., TX (from **Table 6**).

Figure 18. *S. invicta* predation on a *Ceuthophilus* cave cricket adult female.

Figure 19. Female (left, with ovipositor) versus male late instar nymphal *Ceuthophilus* cave crickets.

Figure 20. *Ceuthophilus* cave cricket developmental stages (note a: 4 mm early instar nymph top left; b. presence of short ovipositor in nymphal stage, center).

Figure 21. Dissected *Ceuthophilus* cave cricket female ovipositor and ovaries.

Figure 22. Dissected *Ceuthophilus* cave cricket female ovipositor and ovaries after 10 weeks development unexposed (top) and exposed to pyriproxyfen (Esteem® Ant Bait)(bottom), Texas AgriLife Research, Texas A&M System, College Station, TX, 2011.

Table 1. Assessment of red imported fire ant (*Solenopsis invicta*) population levels using food lures (17 hot dog slices) placed in a transact pattern in 0.8 ha (2 acre) circular karst-centered circular plots before ant following initial May 20-28, 2009 implementation of treatments using the Lure-Switch-Bait (LSB treatment was applied on June 28 and October 15 in 2009; March 23, May 24 and October 3-7 in 2010; and on March 28-April 14, May 31-June 4 in 2011) and boiling water injection treatment regimens compared to untreated plots replicated six times, Camp Bullis, Bexar Co., TX.

	2009*			**2010****				**2011*****			
	Mean *S. invicta* workers foraging on food lures per 0.8 ha plot ± Standard Error or SE										
	Month										
	0	**3**	**6**	**12**	**14**	**18**	**19**	**23**	**25**	**26**	**30**
Treatment	**Mar-Apr**	**Jun**	**Sep-Oct**	**Mar-Apr**	**May**	**Sep**	**Oct**	**Feb-Mar**	**Apr**	**May**	**Sep**
Untreated	45.1±4.34	48.82±3.45	24.61±3.29	15.44±1.83	26.86±2.99	31.96±2.82	22.45±2.29	27.45±2.87	22.55±2.65	13.92±2.01	5.20±1.73
Boiling Water	45.78±4.38	36.08±3.61	14.02±2.29	4.80±1.55	14.71±2.47	24.31±2.88	13.92±2.07	22.55±3.25	16.27±2.43	18.57±2.66	7.18±2.23
LSB	36.84±2.62	22.06±2.82	6.27±1.92	4.41±1.24	3.43±1.21	8.14±1.94	7.94±1.73	12.94±2.42	9.51±2.18	5.88±1.33	5.10±1.97
P- value	0.1	**<0.000**	**<0.000**	**<0.000**	**<0.000**	**<0.000**	**<0.000**	**0.002**	**0.001**	**<0.000**	0.705
F	2.26	16.33	12.84	13.49	24.87	22.04	12.72	6.6	7.18	9.61	0.351
MSE	1,674.12	1,110.33	672.51	443.75	563.15	684.67	426.35	840.89	603.95	435.57	405.85
df	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2

, ** and ***.** Linear Mixed Model (*P*< 0.000, *F*= 12.70, MSE= 1351.84, *df*= 1,2;** *****P*< 0.000, *F*= 55.74, MSE= 551.37, *df*= 1,2;** ******P*< 0.000, *F*= 15.85, MSE= 605.38, *df*= 1,2**) and Analysis of Variance (ANOVA) table in which significant difference occurred when P<0.05, Tukey Post hoc test: **bold means±se** indicates significant differences compared to untreated Control (SPSS 18.0).

Table 2. Assessment of red imported fire ant (*Solenopsis invicta*) population levels by counting all active colonies or visible ant mounds within 0.8 ha (2 acre) circular karst-centered circular plots before ant following initial May 20-28, 2009 implementation of treatments using the Lure-Switch-Bait (LSB) and boiling water injection treatment regimens compared to untreated plots replicated six times, Camp Bullis, Bexar Co., TX.

	2009*		**2010****					**2011*****			
	Mean *S. invicta* active colonies per 0.8 ha circular plot ± Standard Error or SE										
	Month										
	3	**6**	**12**	**13**	**14**	**18**	**18**	**23**	**25**	**26**	**30**
Treatment	**Jun**	**Sep-Oct**	**Mar**	**Apr**	**May**	**Sep**	**Oct**	**Feb-Mar**	**Apr**	**May**	**Sep**
Untreated	26.67±5.61	37.67±11.1	37.67±11.02	22.83±4.26	25.5±6.52	23.17±5.33	19.33±4.16	28.67±5.02	21.67±7.14	14.17±3.97	1.67±1.47
Boiling Water	20.67±5.63	18.67±9.4	18.67±9.4	27.67±10.29	5.33±1.94	23.83±6.41	10.83±2.48	11.33±5.85	4±2.46	2.67±1.6	1.83±1.64
LSB	17±4.87	6.5±5.33	6.5±5.33	2.50±1.76	3±1.36	10.67±2.52	8±2.19	9.67±5.02	6.83±3.84	2.67±1.45	0.17±0.16
P- value	0.459	**0.003**	**0.076**	**0.036**	**0.002**	0.149	**0.05**	0.168	**0.048**	**0.01**	0.607
F	0.821	9.07	3.08	4.2	9.52	2.17	3.68	2.01	3.75	6.44	0.51
MSE	174.04	47.22	480.41	254.51	96.45	152.06	56.67	329.46	143.87	41.03	9.8
df	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2

, ** and ***.** Linear Mixed Model (*P*= 0.4, *F*= 3.54, MSE= 131.83, *df*= 1,2;** (*****P*< 0.00, *F*= 13.16, MSE= 219.51, *df*= 1,2;** (******P*= 0.002, *F*= 6.85, MSE= 158.35, *df*= 1,2**) and Analysis of Variance (ANOVA) table (Mean ± Standard Error or SE) in which significant differences occurred when P<0.05, Tukey Post hoc test: **bold means±se** indicates significant differences compared to untreated Control (SPSS 19.0).

Table 3. Number of B&G Perimeter insecticide bait stations containing 20.5 g Esteem® Ant Bait (pyriproxyfen) deployed and retrieved to circular 0.8 ha or 2 acre Lure-Switch-Bait (LSB) plots with a maximum allocations of 907 g/0.4 ha or 2 lbs/acre using 89 bait stations per plot, and amount of weight remaining and the number of empty stations after 24 hrs of exposure, Camp Bullis, Bexar Co. TX, FY 2009.

	2009											
	6/28/09	6/28/09	6/29/09	6/29/09	7/1/09	7/1/09	Fall 2009	Fall 2009	Fall 2009	Fall 2009	Fall 2009	Fall 2009
Site	1	2	3	4	5	6	1	2	3	4	5	6
Cave	6A-17	6A-35	7-022	7-056	11B-67	C1-F7	6A-17	6A-35	7-022	7-056	11B-67	C1-F7
Stations Deployed	56	34	32	27	16	20	No Data	No Data	No Data	No Data	No Data	No Data
Stations Retrieved	56	34	32	27	16	20	No Data	No Data	No Data	No Data	No Data	No Data
Bait Weight	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
# of stations empty	13	2	7	6	0	0	No Data	No Data	No Data	No Data	No Data	No Data

Table 4. Number of B&G Perimeter insecticide bait stations containing 20.5 g Esteem® Ant Bait (pyriproxyfen) deployed and retrieved to circular 0.8 ha or 2 acre Lure-Switch-Bait (LSB) plots with a maximum allocations of 907 g/0.4 ha or 2 lbs/acre using 89 bait stations per plot, and amount of weight remaining and the number of empty stations after 48 hrs of exposure, Camp Bullis, Bexar Co. TX, FY 2010.

	2010																	
	3/23/10	3/23/10	3/23/10	3/23/10	3/24/10	3/24/10	5/24/10	5/24/10	5/26/10	5/26/10	5/25/10	5/25/10	10/3/10	10/3/10	10/2/10	10/2/10	10/4/10	10/7/10
Site	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Cave	6A-17	6A-35	7-022	7-056	11B-67	C1-F7	6A-17	6A-35	7-022	7-056	11B-67	C1-F7	6A-17	6A-35	7-022	7-056	11B-67	C1-F7
Stations Deployed	25	1	5	7	1	5	77	10	26	18	10	9	34	50	52	21	15	9
Stations Retrieved	25	0	5	7	1	5	77	10	26	18	10	9	34	50	52	18	15	9
Bait Weight	311	0	28.34	28.34	28	85.04	963	113	311	226	141	141	538	708	736	255	226	56
# of stations empty	2	0	0	0	0	0	3	2	0	1	0	0	0	0	0	0	0	0

Table 5. Number of B&G Perimeter insecticide bait stations containing 20.5 g Esteem® Ant Bait (pyriproxyfen) deployed and retrieved to circular 0.8 ha or 2 acre Lure-Switch-Bait (LSB) plots with a maximum allocations of 907 g/0.4 ha or 2 lbs/acre using 89 bait stations per plot, and amount of weight remaining and the number of empty stations after 24 hrs of exposure, Camp Bullis, Bexar Co. TX, FY 2011.

	2011											
	4/14/11	4/10/11	3/27/11	3/27/11	3/28/11	3/28/11	6/5/11	6/5/11	5/30/11	5/30/11	5/31/11	5/31/11
Site	1	2	3	4	5	6	1	2	3	4	5	6
Cave	6A-17	6A-35	7-022	7-056	11B-067	C1-F7	6A-17	6A-35	7-022	7-056	11B-067	C1-F7
Stations Deployed	6	82	40	29	13	26	9	36	14	24	28	13
Stations Retrieved	6	82	40	27	13	26	9	36	14	24	28	13
Bait Weight	28	1077	623	368	226	368	28	566	198	368	368	170
# of stations empty	1	6	0	0	0	0	4	0	0	0	2	0

Table 6. Assessment of native and imported fire ant (*Solenopsis invicta*) competitor ant species (*Crematogaster laeviuscula* Mayr, *Forelius pruinosus* Roger, *Monomorium minimum* (Buckley), *Solenopsis* (*Diplorhoptrum*) *molesta* (Say), *Brachymyrmex sp.*, *Pheidole sp*. *Dorymyrmex flavus* McCook and *Tapinoma sessile* Say) using food lures (17 hot dog slices) placed in a transact pattern in 0.8 ha (2 acre) circular karst-centered circular plots before ant following initial May 20-28, 2009 implementation of treatments using the Lure-Switch-Bait (LSB treatment was applied on June 28 and October 15 in 2009; March 23, May 24 and October 3-7 in 2010; and on March 28-April 14, May 31-June 4 in 2011) and boiling water injection treatment regimens compared to untreated plots replicated six times, Camp Bullis, Bexar Co., TX.

	2009*			**2010****				**2011*****			
	Week										
Treatment	**Mar-Apr**	**Jun**	**Sep-Oct**	**Mar-Apr**	**May**	**Sep**	**Oct**	**Feb-Mar**	**Apr**	**May**	**Sep**
Untreated Control	0.25±0.04	0.15±0.03	0.02±0.01	0.12±0.02	0.08±0.02	0.07±0.02	0.04±0.01	0.13±0.033	0.12±0.03	0.07±0.02	0.13±0.03
Boiling Water	0.19±0.03	0.12±0.03	0.08±0.02	0.06±0.02	0.09±0.02	0.07±0.02	0.01±0.01	0.05±0.02	0.03±0.17	0.02±0.01	0.23±0.04
LSB	0.05±0.016	0.49±0.05	0.83±0.06	0.12±0.03	0.5±0.05	0.48±0.05	0.48±0.05	0.14±0.034	0.05±0.02	0.13±0.03	1.09±0.278
P- value	**<0.000**	**<0.000**	**<0.000**	0.24	**<0.000**	**<0.000**	**<0.000**	0.07	**0.028**	**0.012**	**0.012**
F	11.823	22.52	114.66	1.43	41.536	43.129	70.896	2.56	3.63	4.49	4.46
MSE	0.120	0.194	0.183	0.092	0.142	0.134	0.1	0.093	0.06	0.06	0.05
df	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2

, ** and ***.** Linear Mixed Model (*P*< 0.000, *F*= 30.17, MSE= 0,20, *df*= 1,2;** *****P*< 0.000, *F*= 120.43, MSE= 0.12, *df*= 1,2;** ******P*< 0.000, *F*= 11.08, MSE= 0.79, *df*= 1,2**) and Analysis of Variance (ANOVA) table (Mean ± Standard Error or SE) with significant differences occurring when P<0.05, Tukey Post hoc test: **bold means±se** indicates significant differences compared to untreated Control (SPSS 0.18).

Table 7. Number of cave crickets, *Ceuthophilus sp.*, in buckets after pyriproxyfen exposure under laboratory conditions, Texas AgriLife Research, Brazos Co., TX, 2011.

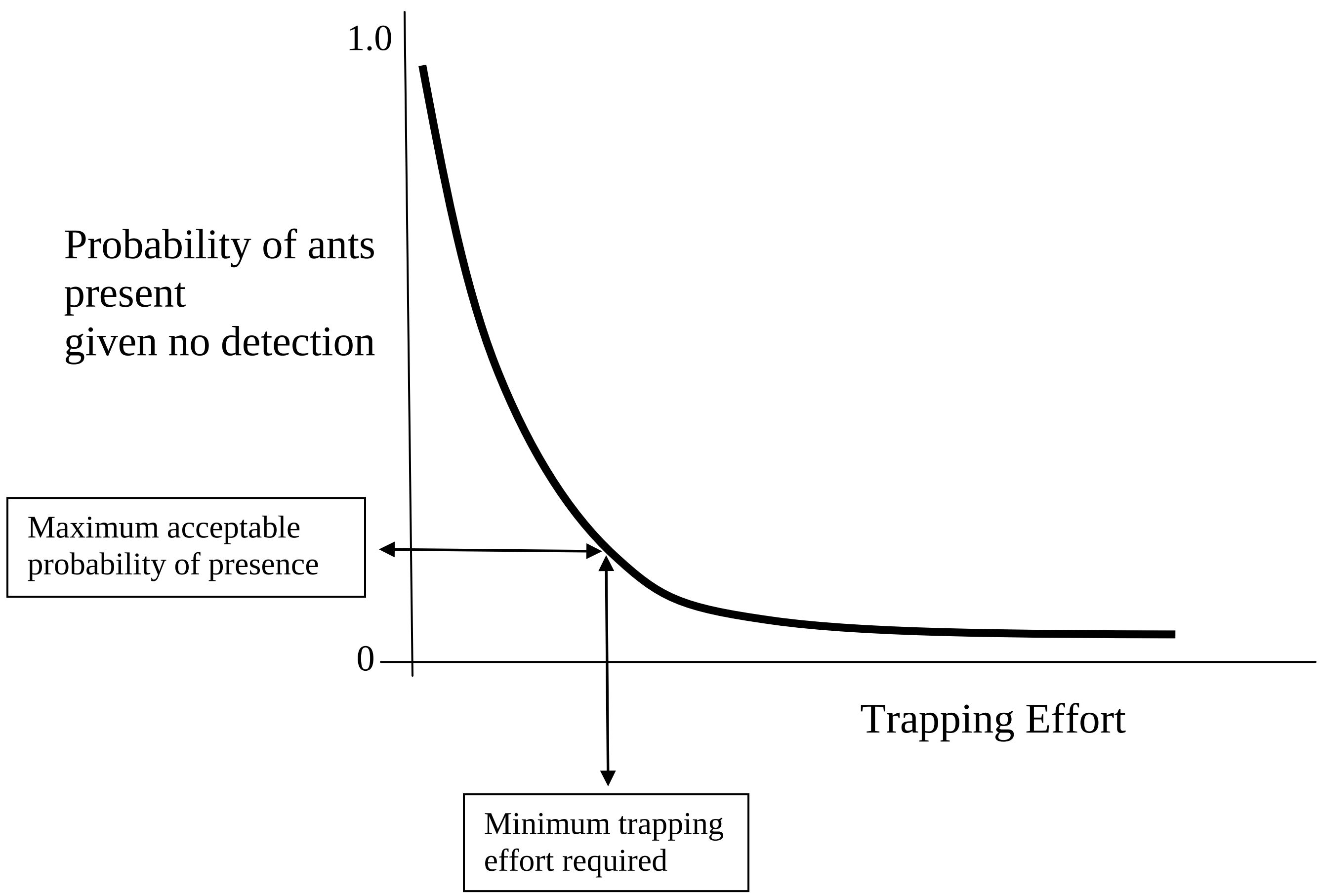
Bucket	**20-Jun**	**25-Jul**	**(No. females)**	**26-Aug**	**(No. females)**
1 Treatment	15	5	1	3	1
2 Treatment	15	7	1	6	1
3 Treatment	15	6	1	3	1
4 Control	15	10	2	8	3
5 Control	15	5	0	5	1
6 Control	15	6	2	3	1

Appendix 1. Pre-treatment assessment of red imported fire ants, plot numbers (Karst) arrayed into replicate blocks and treatment assignments, Camp Bullis, Bexar Co., TX, 2009.

Rep		Karst	Mean	Treatment A	Treatment B	Treatment C	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
		08B-F1	3.529				2.09010	-.9014	7.9602
		10-084	5.882				4.77885	-4.2483	16.0131
		08B-F3	6.471				2.83637	.4578	12.4834
		7-019	8.235				4.94345	-2.2444	18.7149
	1								
A		06B-005	12.940	12.940			5.54200	1.1900	24.6900
C		11A-002	14.706			14.706	5.88603	2.2281	27.1837
B		11B-067	16.471		16.471		6.23918	3.2441	29.6971
	2								
C		10-002	22.500			22.500	8.29156	4.8270	40.1730
A		11B-121	24.118	24.118			9.51233	3.9524	44.2829
B		7-056	25.294		24.118		8.04937	8.2302	42.3580
	3								
B		C1-F7	30.588		30.588		7.14712	15.4370	45.7395
A		11B-001	34.118	34.118			10.18429	12.5279	55.7074
C		C1-F4	34.118			34.118	10.11183	12.6815	55.5538
	4								
A		10-044	35.294	35.294			10.88434	12.2203	58.3679
C		11A-015	35.882			35.882	11.85261	10.7559	61.0088
B		7-022	50.588		50.588		8.93072	31.6560	69.5205
	5								
B		6A-35	71.176		71.176		6.79978	56.7616	85.5914
A		6A-086A	72.353	72.353			8.38106	54.5859	90.1200
C		6A-027	80.588			80.588	7.44937	64.7963	96.3802
	6								
C		6A-036	84.118			84.118	6.36274	70.6292	97.6060
B		6A-17	90.000		90.000		6.18347	76.8916	103.1084
A		6A-029A	95.882	95.882			1.23038	93.2741	98.4906

Appendix II. Consideration for future research: *S. invicta* detection around cave formations – Camp Bullis, Bexar Co., TX.

This figure below depicts what Camp Bullis environmental monitoring personnel need in order to improve red imported fire ant (*S. invicta*) management around karst cave formations housing arthropods of concern. When there is suspicion of imported red fire ants in an area but when population levels are low, how confident can managers be that the ants are not present following some food luring or pitfall trapping effort in which no ants were found? Because there was an initial suspicion of ants, the probability of presence will never be zero but it will approach zero as luring/trapping effort becomes overly labor intensive and time consuming. New field research could provide Camp Bullis' fire ant management with an objective measure of the probability of presence of ants across a range of luring/trapping effort. It will then be up to Camp Bullis personnel to decide at what point the probability is so low that there is no longer a need to continue monitoring efforts.



To quantify the probability of *S. invicta* presence given no positive detection across a range of luring/trapping efforts, we would require data that quantifies the number of individuals caught (for a given food lure density) and how many were available to be caught. The number of ants attracted to each food lure or pitfall trap and the location (GPS coordinates) of each monitoring station needs to be recorded. The number of ants available to be attracted/caught will be a function of how many colonies are in the area and their size (to approximate the total number of workers likely to be out foraging). Can we obtain a reasonable measure of the number of foragers that emanate from a given nest? Is there an established allometric relationship between some field measurement of nest size and the number of foragers that move away from the nest? This is critical information for our current modeling approach and necessary to assure confidence in sampling for *S. invicta* be the Camp Bullis – Environmental group. If we cannot get reasonable estimates of "available" ants, then we need to think creatively about another approach. However, use of hot dog slice food lures shows promise in helping to provide us with some of this information.

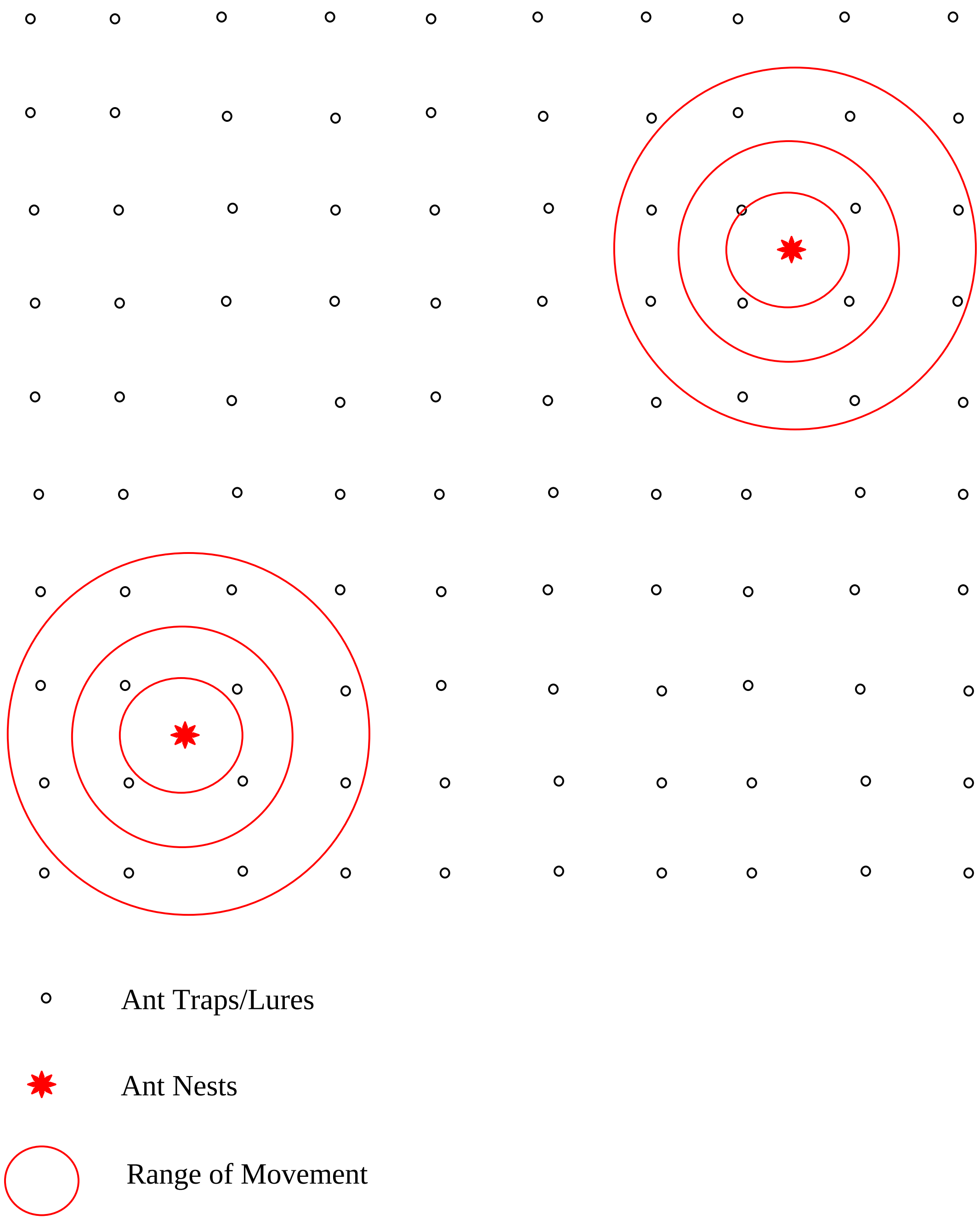
Our current thinking is focused on detecting individual ants because this is what Camp Bullis managers will do in case of a suspected *S. invicta* presence near environmentally sensitive environments such as karst cave or in golden cheek warbler habitats: they will put out traps to detect foraging worker ants. While the detection of an individual usually (perhaps always) signals the presence of a colony, only individual ants are being detected on the lures.

There are two principal components of the scale of trapping effort that we need to explore in relation to detection probabilities: extent and grain or resolution. Extent is the spatial area over which we set out our monitoring stations. Grain or resolution is the distance between stations. If there is a suspected presence of ants, Camp Bullis managers will need to deploy the most effective combination of extent and grain to detect ants (**see Figures below**). Over-sampling will deplete resources, and under-sampling will not detect ants that are present. We recommend setting up the sampling grid so that we are sure to detect ants and so that we can sub-sample the data to determine effective and ineffective grains and extents. The grain size should be determined by the movement biology of the ants. As a starting point for discussion, we could decide to make the grain size about half the distance that ants tend to wander from the nest. Therefore, if we were to have a nest located within our sampling grid, we should easily detect the ants.

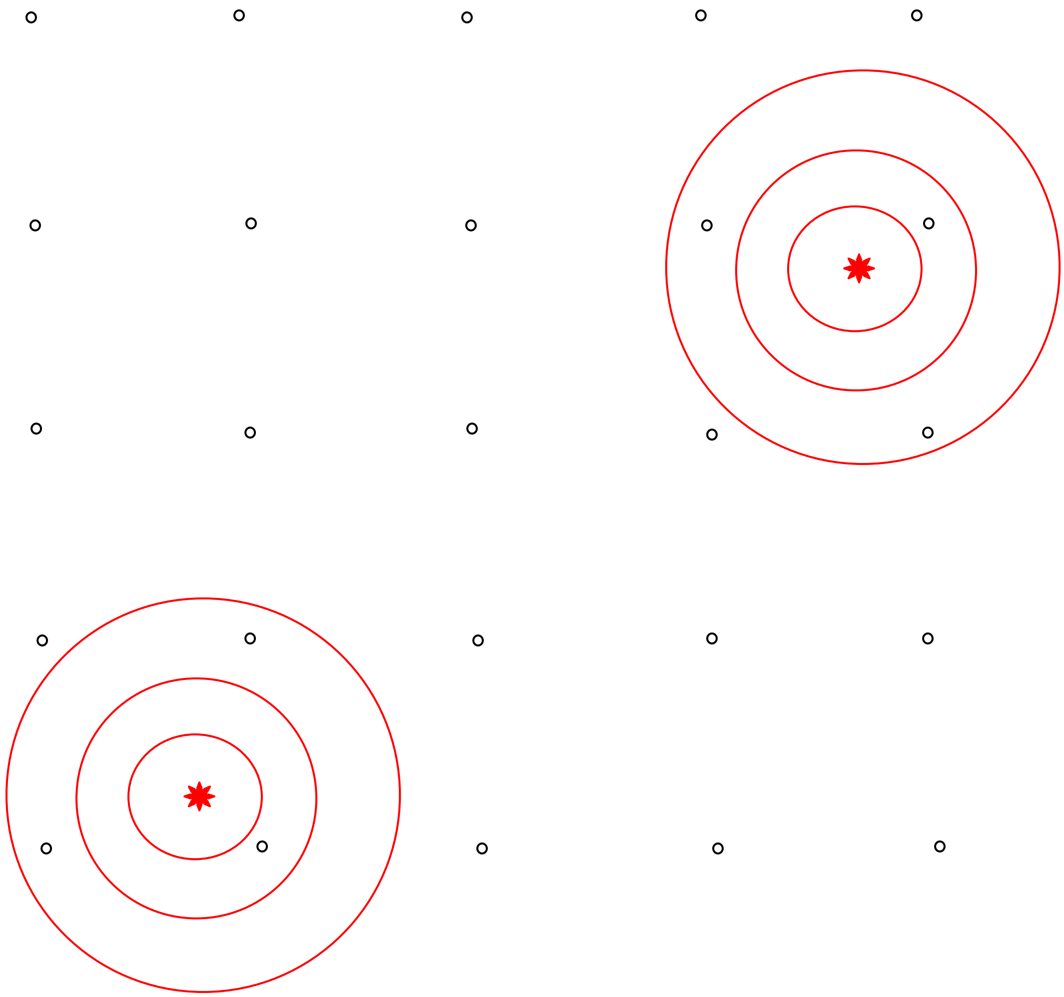
Some preliminary study design questions are:

1) How far do ants move from their nests?
2) How many traps do we want in a grid or X (say 10 x 10 = 100)?
3) What are the grain and extent?
4) How many replicate grids?
5) How long do we collect data on trap captures?
6) How soon we need to do the fieldwork, do we want reading on detection probabilities on spring, summer and fall?

Large Extent, Fine Grain

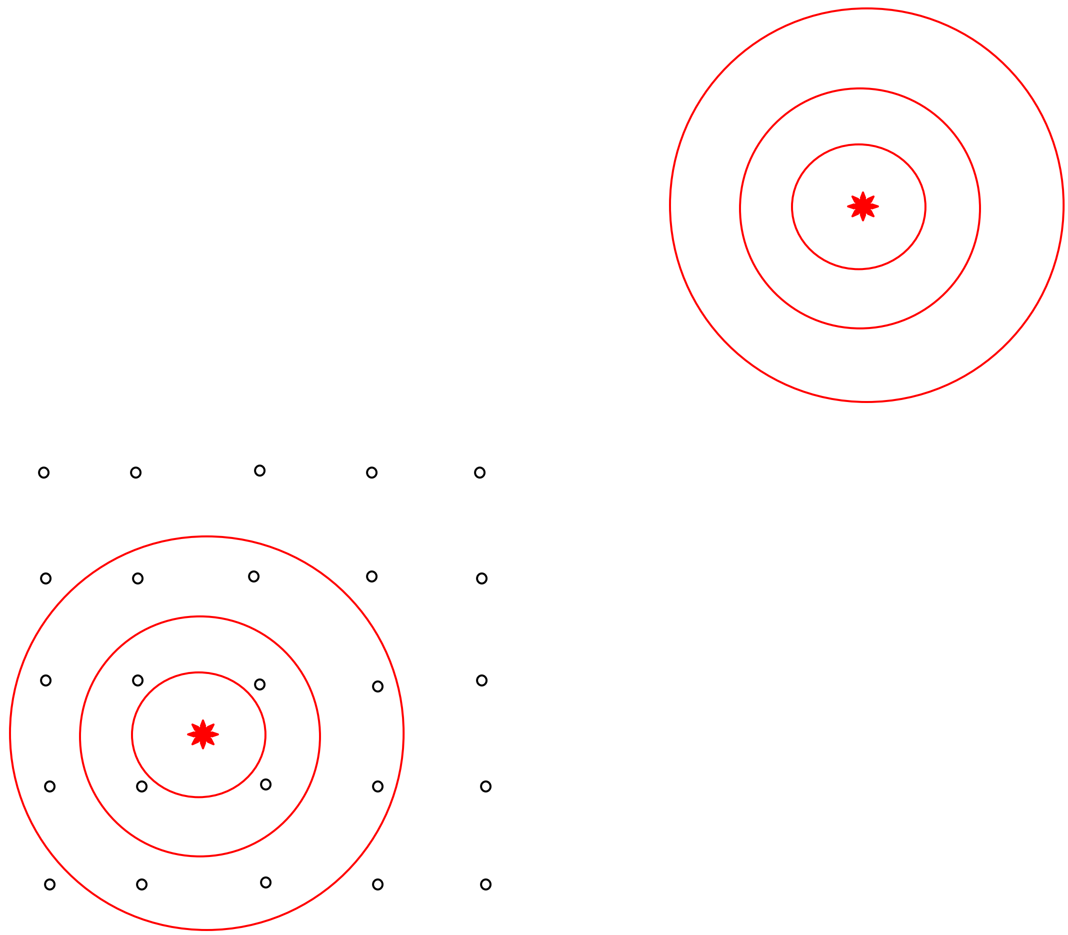


Large Extent, Coarse Grain



This result would suggest to Camp Bullis to have relatively large extents and coarse grain sizes to maximize the area covered by the trapping grid.

Small Extent, Fine Grain



Appendix III. List of presentations, proceedings, extension publications and press releases resulted from the project.

<u>Presentations</u>.

Keck, M., B. Drees, A. Calixto and N. Cervantes. 2011. Target-specific lure and switch grid spot treatment approach for fire ants: Camp Bullis Karst Caves Project. Annual Red Imported Fire Ant Research Conference. Galveston, TX.

Cervantes, N., M. Keck, B. Drees, and A. Calixto. 2010. Resource discovery and dominance as a management tool for fire ant management. Entomological Society of America Annual Meeting, San Diego, CA.

Calixto, A., B. Drees, M. Keck and N. Cervantes. 2010. Reclutamiento y dominancia de recursos como herramienta para el manejo de hormigas invasoras y conservacion de especies nativas. XXXIVII Congreso Sociedad Colombiana de Entomología. Bogota, Colombia.

Calixto, A., B. Drees, M. Keck and N. Cervantes. 2010. Resource discovery and dominance as a management tool for fire ant management. International Invasive Ant Management Workshop. Darwin, Australia.

<u>Proceedings.</u>

Keck, M., B. Drees, A. Calixto and N. Cervantes. 2011. Target-specific lure and switch grid spot treatment approach for fire ants: Camp Bullis Karst Caves Project. PP. 60-62. *In* proceedings Annual Red Imported Fire Ant Research Conference. Galveston, TX.

Calixto, A., M. Keck and N. Cervantes. 2010. Reclutamiento y dominancia de recursos como herramienta para el manejo de hormigas invasoras y conservacion de especies nativas. *In* proceedings XXXIVII Congreso Sociedad Colombiana de Entomología. Bogota, Colombia.

<u>Extension publications.</u>

B. M. Drees, A. Calixto, M. Keck and N. Cervantes. 2009. Progress Report: Evaluation of Red Imported Fire Ant Treatment Programs to Preserve Federally Endangered Species of Concern and Endemic Cave Adapted Arthropod Species at Camp Bullis, Bexar County, Texas. Pp. 107-114. *In* Urban IPM Program Summary Report 2009, Texas AgriLife Extension Service. (http://fireant.tamu.edu/research)

<u>Press releases</u>.

Keck, M. Fire ants and cave crickets. 2009. Dead rat press.
http://lylerosdahl.com/blog/?p=244

Result Demonstration at San Antonio Botanical Garden for Treatment of Red Imported Fire Ants Using Insect Growth Regulator Bait in Vegetable Gardens

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David Rodriguez, Extension County Agent – Horticulture
Lauren Lewis, IPM Intern
Texas AgriLife Extension Service, Bexar County

The San Antonio Botanical Garden is a 33 acre public park in the city of San Antonio, TX with a mission to inspire people to connect with the plant world to understand the importance of plants in our lives. The garden reaches this mission through demonstration gardens as well as public outreach educational programs. One of these educational programs is the Children's Vegetable Garden Program, which was developed and is currently supported and run by Texas AgriLife Extension Service in Bexar County. Through the Children's Vegetable Garden Program, Texas AgriLife Extension Service and the San Antonio Botanical Garden provide an opportunity for children ages 8-13 to learn about gardening by growing their own vegetables under the mentoring of Bexar County Master Gardener volunteers. Each child is allotted a 3.5'x 28' plot in which they grow various seeds, herbs, vegetables, and ornamental annual flowers. Children meet weekly on Saturdays to tend to their garden and receive educational gardening presentations. During the week volunteers water the garden and help remove weeds.

Approximately 120 children participate each year during spring and fall programs. Due to the presence of children in the garden, constant watering, and various attractants such as food, okra, and other plants, red imported fire ant management is necessary. However, fire ant treatment must be done when children are not present, and the least toxic methods are preferred by the City of San Antonio.

During the summer of 2011, Texas AgriLife Extension performed a broadcast bait treatment in the Children's Vegetable Garden area. During treatment, no children were present, and no vegetable plants had yet been planted.

Materials and Methods

Prior to baiting, red imported fire ant foraging counts were taken, using a food lure (hot dog slices). Hot dog slices were placed around the 2 acre garden in 10 locations. They were left out for at least 45 minutes, and the species of ant present on the hot dog lures was observed and recorded. Observations occurred during the morning at approximately 7:30am.

After food lures positively identified that red imported fire ants were present, a broadcast application of Esteem® fire ant bait (insect growth regular, active ingredient pyriproxifen) was applied using a hand held spreader at a rate of 1.5 pounds per acre. The entire 2 acre garden and the immediate area outside the garden fence were treated with Esteem®. Esteem® was chosen because it is labeled for use in edible crops.

Seven weeks after the bait application, post treatment ant foraging activity was evaluated. Food lures (hot dog slices) were placed in 15 locations around the garden and left for at least 45 minutes, upon which slices were observed for ant species present.

Results and Discussion

One hundred percent (10 of 10) food lures placed out pre-treatment had red imported fire ants activity. Seven weeks after baiting, 13% (2 of 15) of the hot dogs had fire ants foraging activity, a reduction of 87% in the bait treated area (Table 1). Native ants also increased by 40% after fire ant treatment. No native ants were found on hot dog lures before treatment, but 6 of 15 (40%) had native ant species activity after treatment for imported fire ants Table 2.

Due to extreme drought conditions, no imported fire ant mounds were discovered pre or post treatment. However, approximately one week after treatment, the area did receive approximately 1 inch of rain. Upon this, many mounds were visible.

Table 1. Food lure results of red imported fire ants pre and post treatment.

	Food Lures Placed Out	Food Lures with Imported Fire Ants	Percent of Food Lures with Imported Fire Ants
Pre Treatment	10	10	100%
Post Treatment	15	2	13%

Table 2. Food lure results of native ants pre and post treatment.

	Food Lures Placed Out	Food Lures with Imported Fire Ants	Percent of Food Lures with Imported Fire Ants
Pre Treatment	10	0	0%
Post Treatment	15	6	40%

Broadcast baiting is an effective management practice for red imported fire ants at the Children's Vegetable Garden Program. By broadcasting fire ant bait in mid-summer, when all plants had been removed and preparations for the fall plantings were not yet underway, time was allotted for the bait to be effective in managing imported fire ants before the garden area became a heavy traffic area for children and adults. Based on the results of this demonstration, the Children's Garden Program will be applying a broadcast bait to the area between garden programs (late fall and early summer).

Soil Temperature Monitoring of a Green Roof in Houston Texas 2010-2011 with Notes on Implications for Pest Management

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Green roofs are a subject of growing interest due to their many documented benefits. Reduced urban heat-island effects and lowered cooling costs for buildings are commonly cited benefits (USEPA 2003). Green roofs have the potential to mitigate storm water runoff by retaining a portion of rainfall and reducing peak runoff surge following a storm event. Another reported benefit is that green roofs have the potential to provide wildlife habitat (Thompson and Sovig 2000). Throughout the world, most green roofs are located in temperate climates, such as northern parts of Europe and the United States. Information about the design and use of green roofs in subtropical regions of the United States is limited.

Very little information has been published about the urban pest management implications of green roofs in the coastal areas of Texas although plant species showing potential for such sites have been recently evaluated (Harp and Pulatie, 2008; Harp and Suttle, 2009; Camerino, 2011). Extremes in soil temperatures, consistent winds and high humidity are common on green roofs along the coastal areas of Texas (personal observations). The vegetation, growth media, regular irrigation, and underlying air gap (needed for excess water drainage) associated with green roofs provides a potential habitable niche for pest insects. The authors have personally witnessed a range of insects on green roofs including crickets, red imported fire ants, rover ants, butterflies, fungus gnats, and cockroaches.

Insects require food, water and harborage in order to survive and develop. Host plant selection can influence insects attracted to the site. Trash, soil and landscape elements can serve as harborage areas where insects can hide when not feeding. Temperature and moisture also influence insect survival and development. Very high or very low temperature can limit survival, while optimal temperatures and humidity can increase developmental rates of these cold-blooded arthropods. Extreme temperature ranges on rooftops can affect insect pests such as imported fire ants with have a temperature range from 10 to 50°C (50 to 122°F) for worker ant foraging activity and survival (Drees et al. 2007).

Materials and Methods

In an effort to gain a better understanding of soil temperature variation on green roofs in coastal Texas, ECH_2O soil temperature sensors (Decagon Devices, Pullman, WA, USA) were placed at two depths in a green roof growing media located in southeast Houston. A total of eight soil temperature sensors were installed (four at each depth). The green roof was located on a 3-story commercial office building in an urbanized area along interstate highway 45. Probes were installed at the bottom of the growth media (about 8 inches deep) and just below the growth media surface (about 1.5 inches deep) on an area with no vegetation. The media consisted of 60% expanded shale, 30% leaf mold compost, and 10% loam. Data collection began on October 1st, 2010 and continued until September 30th, 2011.

Results and Discussion

Data loggers recorded temperatures (degrees Fahrenheit) at 6 a.m. and 6 p.m. from Oct. 1 through Nov. 5, 2011 (**Figures 1** and **2**). Each data point was the average recorded temperature of the four temperature sensors. Typically, the early morning and late evening time periods were when the soil temperatures where the lowest and highest respectively.

The highest and lowest temperatures were recorded by the temperature sensors placed near the soil surface. The surface temperature sensors had the greatest variation in soil temperature between morning and evenings. The greatest diurnal temperature change at a particular soil depth within a day occurred in the months of July and September. The highest recorded average temperature was 39.7 °C (103.4°F) at 6 pm on June 2, 2011 and the lowest recorded average temperature was -0.3°C (31.5°F) at 6 am on February 5, 2011. Both of these temperature extremes were recorded by the surface soil temperature sensors. The lowest recorded average temperature for the temperature sensors located at the bottom of the soil was 0.7°C (33.4 °F).

Figure 1. Average temperature (degrees F) at the bottom the green roof growing media, Harris Co., TX.

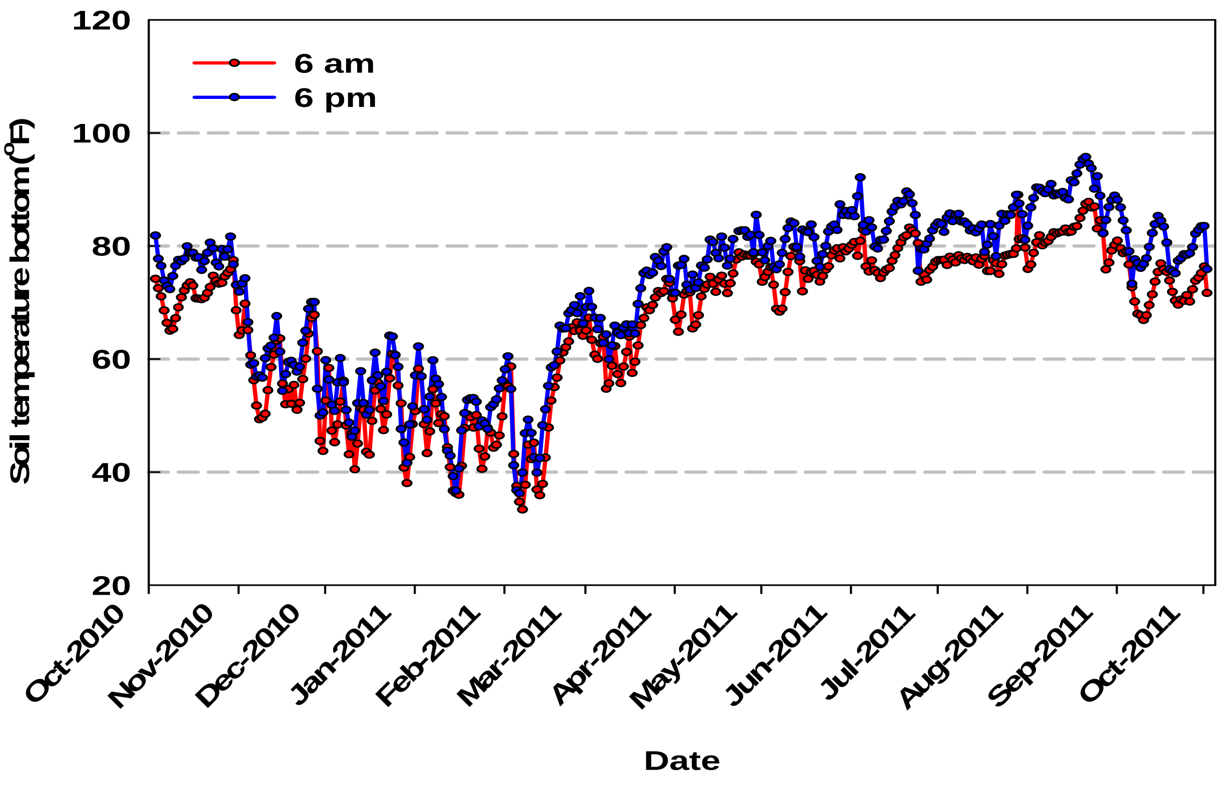
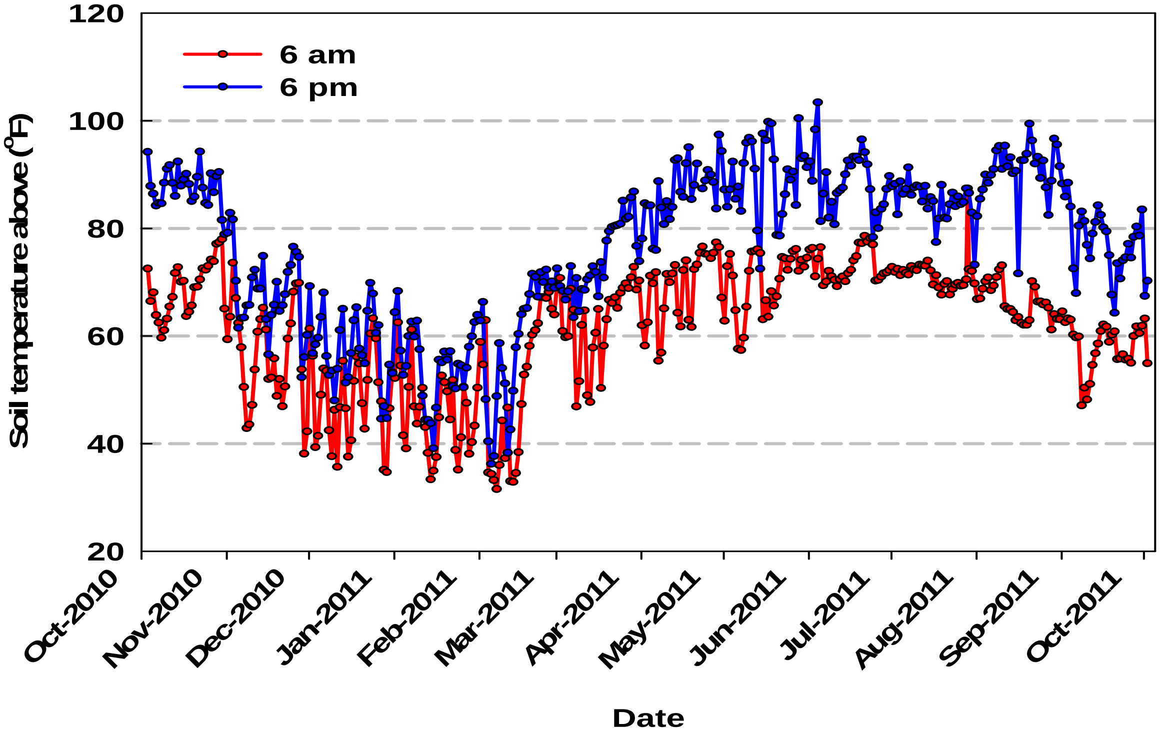


Figure 2. Average temperature in degrees F near the surface of the green roof growing media, Harris Co., TX.



Literature Cited

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Drees, B. M., B. Summerlin, and S. B. Vinson. 2007. Foraging activity and temperature relationship for the red imported fire ant. Southwestern Entomologist 32(3):149-156.

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United States Environmental Protection Agency. 2003. Cooling summertime temperatures: strategies to reduce urban heat islands. Publication No. 430-F-03-014.

Survey of Arthropods at Randolph Air Force Base Airfield through Pitfall Traps and Bird Crop Necropsies

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Michael Pacheco, USDA
Lauren Lewis, IPM Intern, Texas A&M University
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Randolph Air Force Base (AFB), San Antonio, TX is an air education and training command of the United States Air Force. Randolph AFB has two runways, located on the east and west sides of the base. Surrounding Randolph AFB are residential areas to the north and field crops to the south. Migratory birds such as white winged doves and insectivorous birds such as mockingbirds, starlings, scissortails and loggerhead shrikes are found in fields along the runways and surrounding areas.

Bird strikes, in which a bird hits a plane as it lands or takes off from the runway, is an issue that Randolph AFB's Bird Aircraft Strike Hazard (BASH) Team must address and try to mitigate to prevent economic loss and injuries to pilots. BASH uses a variety of techniques such as pyrotechnics, loud noises and raptor bird calls to discourage nuisance birds from occupying the runway areas.

Texas AgriLife Extension was contacted by the BASH Team to assist in creating a management plan to reduce insects in the fields directly adjacent to the runways. It is believed that insectivorous birds, the main air strike hazard, are attracted to the insects that inhabit the fields. A survey was performed to determine the arthropod fauna in the runway fields in an effort to develop a specific management plan for the insects that are a potential food source for the birds.

Materials and Methods

Pitfall traps (model 2838A) acquired from BioQuip Products (www.bioquip.com) were purchased and placed in four locations from end to end along the fields adjacent to the runways (Figure 1). A total of 8 pitfall traps were deployed, with four traps placed along each runway area. Pitfall traps were placed in the soil with the top flush with the ground and a cover was staked into the ground to encourage insects to move into the pitfall traps (Figures 2 & 3). Pitfall traps were installed on July 22nd, 2011. Pitfall trap fluid consisted of a soapy water and Listerine® mixture (approximately ½ cup of soap per gallon of water and 1/8 cup of mouthwash). Listerine® served to prevent the solution from growing fungus or mold and destroying the collected insects while in the field.

Traps were checked, any captured arthropods emptied from the traps and fluid replaced two to three times per week for a total of four weeks. The fluid solution was collected in jars, labeled with collecting and trap information and stored until sorting.

Birds found flying or foraging on and near the airfields were shot when possible and collected. Salvageable birds involved in strikes with planes were also collected. All birds were dissected, and guts removed. The crops and stomachs were examined for insects and insects were identified when possible.

Figure 1. Aerial view of Randolph Air Force Base. Locations of pitfall traps marked by "X".

Figures 2 & 3. Pitfall Trap used during survey (covered trap on left, uncovered trap on right).

Results and Discussion

Arthropods collected in the field from pitfall traps were sorted, counted and identified to the highest taxonomic rank possible. All arthropods were identified to at least family level.

The arthropods and insects collected from pitfall traps, deployed for four weeks were red imported fire ants (*Solenopsis invicta*), Field crickets, earwigs, spiders, cockroaches, beetles, spider wasps, bee, and hover fly (Table 1).

Table 1. Insect and Arthropods identified in pitfall traps.

Order	Family	Further Description	Number
Blatteria	Undetermined	Field cockroach	23
Orthoptera	Gryllidae	Not the common field cricket	49
Dermaptera	Carcinophoridae Labiidae		34
Coleoptera	Tenebrionidae Carabidae		9 1
Diptera		Hover fly	1
Hymenoptera	Formicidae Pompilidae Apidae	Solenopsis invicta (red imported fire ant) Unknown Formicidae species Bee	>1,000 1 3 1
Aranae	Lycosidae Unknown	Wolf Spiders Unknown Spiders	6 22

A total of 14 birds were collected from the field and dissected. Four swallows, one shore bird, five meadowlarks, three scissortail flycatchers, and one American Kestrel. All bird crops were empty, so stomachs were removed and dissected with the result of the contents in Table 2. It is not known where the birds ingested the identified insects, but by knowing what their preferred foods are, and correlating that information with the insect populations from the pitfall traps, we can determine if the birds may be attracted to the airfields for foraging.

Of all birds, swallows had the most insects, numerically, and the greatest variety of species in their stomachs. There was very little to no plant material in this group of birds. Swallows also ingested very small insects, less than 1 mm.. Meadowlarks had the most plant material in their stomachs, which compromised an estimated average of 85% of the gut material. Meadowlarks ingested small insects (less than 1 mm), however three insects were whole enough to identify to family or species: red imported fire ant (*Solenopsis invicta*), spotted cucumber beetle (*Diabrotica undecimpunctata*), and burrowing bugs (Cydnidae). Scissortail flycatchers had ingested the largest insects (on average, greater than ½ cm). In each of the three scissortail flycatchers at least one whole American Shorthorned Grasshopper was identified, along with many insect pieces such as mandibles, legs, and wing parts. The shore bird had the least amount of insects found in the gut; only beetle (Coleoptera) wings were identifiable. The American Kestrel only had small amounts of cellulose material in its stomach.

Table 2. Insects identified from bird stomachs.

Bird	**Gut Contents**	**Comments**
Swallows (4)	Hymenoptera Ant alates (2) Alate wings (9) Worker Ants (6) Dermaptera, Earwig (1) Orthoptera Unidentifiable Coleoptera	 5 legs Wings, head, thoraxes
Shore Bird	Coleoptera Seeds (2) Plant material	Wings and body parts
Meadowlarks (5)	Hemiptera Cydnidae (3) Hymenoptera *Solenopsis invicta* Coleoptera Unidentifiable *Diabrotica undecimpunctata* Snails Seeds Plant material Flower Petals Rocks	 Whole ant Body parts, wings etc. Cucumber beetle wing Average 85% of gut content
Scissortail Flycatcher (3)	Orthoptera Acrididae Unidentifiable Coleoptera Cerambycidae Unidentifiable insects	 American Shorthorned Grasshopper, whole insects. Mandible and other body parts
American Kestrel	No insects	

The purpose of this study was to determine if a specific management plan could be implemented based on what insects were found in the fields adjacent to the airfield and in the birds' guts. Because the range of insects found in the guts was so wide, I do not believe management of one specific insect would reduce birds from entering the airfield runway areas. However, because fire ants were identified in bird guts and abundant in the pitfall traps, utilizing fire ant baits, twice a year (fall and spring) would reduce fire ants and fire ant alates, and potentially reduce bird activity after a rain storm. In addition, grasshoppers were found in both pitfall traps and bird guts, and an early summer application of an insect growth regulator may reduce the grasshopper populations in the airfields.

Coleoptera, Dermaptera, Diptera, and Aranae (spider) populations cannot necessarily be managed, and these orders contain species that are considered beneficial predators and may be helping to keep pest insect populations at a lower threshhold.

Continued cultural control practices such as maintaining grass at heights of 2-3 inches may reduce harborage. This practice and the drought experienced during this study is likely why grasshoppers were not found as abundantly as expected. Low grass heights near and around the airfield are important, especially since most insects found in the guts of the birds were terrestrial insects, likely ingested while insects foraging along the ground.

Based on the arthropod fauna found in the fields and in bird guts, cultural control practices are likely the best management plan to use for bird abatement on the airfields.