RED IMPORTED FIRE ANT APPLIED RESEARCH AND DEMONSTRATION REPORTS 2003-2004

Compiled by: Bastiaan M. Drees, Professor and Extension Entomologist

Acknowledgments

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EVALUATION OF ESTEEM® FIRE ANT BAIT (PYRIPROXYFEN) FOR SUPPRESSION OF IMPORTED FIRE ANTS IN AGRICULTURE

Bastiaan M. Drees and Elizabeth "Wizzie" Brown
Field assistance provided by: Paul Nester, Dale Mott, Charles Barr,
Alan Brown, Nathan Riggs, Anna Kjolen, and Kim Engler

Esteem® Fire Ant Bait (pyriproxyfen, Valent U.S.A.), also sold as Distance® Ant Bait is an insect growth regulator (IGR) that produces a slow but extended period of 80 to 90 percent reduction of mounds or nests of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). When applied in the spring, maximum control generally occurs in a month or two of application, whereas fall application does not result in maximum control until the following spring, about 6 months later. This product is being evaluated for potential registration in pastureland. Currently, the only other IGR registered for use in pastureland is Extinguish® (methoprene) ant bait.

Previous studies by Texas Cooperative Extension has documented that with the IGR, fenoxycarb (Logic®), the initial period of ant mound reduction can be shortened by blending the IGR product with a metabolic inhibitor containing hydramethylnon (e.g., Amdro®Pro registered for pastureland, Probait® or others) and applying each at half rate, thereby not increasing the cost of a treatment. Furthermore, application of an IGR in a "skip swath" pattern whereby the product is applied at full rate (1.0 to 1.5 lbs./acre) but skipping every other swath has been shown in some studies to result in control equivalent to the full broadcast application, thus reducing the chemical cost of treatment and application time roughly in half.

This trial was conducted to document efficacy of Esteem® Fire Ant Bait in comparison to no treatment (untreated control) and "standard" treatment, Extinguish®, and to assess the performance profile when using Esteem in a "hopper blend" or as a "skip swath" treatment.

Materials and Methods

This trial was established, October 2, 2003, 9:30 a.m. - 8:20 p.m. The temperature was in the high 70 degree F range. Wind was low (less than 5 mi. per hr.) but gusty, and skies were predominantly clear. Soil was very dry, with the area not having received rains recently. This trial was delayed until the area behind the earthen dam had been shredded just prior to establishing the trial. Twenty plots were established for 5 treatments replicated 4 times. Plots were 100 ft. wide and 800 ft. long (roughly 2 acres). Imported fire ant mounds were counted in an area 30 ft wide and 740 ft long (0.51 acres) by disturbing suspected nest or mound sites with a stick or shovel and counting it as active if many (dozens) of worker ants were observed to emerge. Pre-treatment ant mound numbers per sub-plot area were arrayed from lowest to highest and blocked in groups of 5 plots forming replicates. Treatments were assigned randomly to each replicate or block so that only minor numerical differences occurred for average mound per plot values. Treatments included:

1. Untreated control or check - Ck

2. Esteem (0.5% pyriproxyfen) Fire Ant Bait (Valent U.S.A.), 1.25-1.5 lb/acre (five swaths on 20-ft centers or swath widths with the center swath beginning in the center of on 100 ft side of each plot)- Py
3. "Skip swath" of Esteem (0.5% pyriproxyfen), applied at a rate of 1.25-1.5 lb/acre, but with the center swath left untreated and skipping another 20 ft before applying additional swaths along the outside of each plot (this 2 swaths per plot and an additional 2 swaths along the length of each plot- Sk
4. "Hopper Blend" - 0.75 lb each of Esteem and Probait® (0.73% hydramethylnon) Ant Bait (Wellmark International) applied at 1.25 to 1.5 lb blended product per acre - HB
5. Extinguish® (0.5% s-methoprene) Fire Ant Bait (Wellmark International) - Ex

Plots were re-evaluated 1, 3 and 7 months following treatment (Nov. 6, 2003, Jan. 14 and April 8, 2004) as described for the pre-treatment assessment of mound numbers. Data were analyzed for each pre- and post-treatment date using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test at $P \leq 0.05$ (Microstat 2.03, Ecosoft., Inc., and SPSS for Windows, Lead Technologies, Inc. Version 13).

Results and Discussion

Red imported fire ant mounds were low in number (20.15 mounds per 0.51 acre sub-plot or 39.5 ant mounds/acre), but exceeding 20 mounds per acre which serves as an "action threshold" for application of broadcast baits when this trial was initiated. Worker ants in some mounds were observed to be rather large on average, indicating that perhaps some colonies were monogyne. Ant colonies were observed to be most numerous along two parallel levees running lengthwise behind the dam to direct runoff rainwater to drains. Tops of these rises were therefore chosen as plot centers to take advantage of the topographical nature of ant nests. These conditions may have influenced results of some treatments, particularly the "skip swath" were no bait was applied to the center of the sub-plot ant mound monitoring area.

Rains had occurred the month (Nov. 6) after trial establishment (Oct. 2). Many "new" and generally very small mounds were observed and were easy to spot due to worked soil. These colonies were likely present but not noticeable at the time the trial was initiated possibly because they were beneath the surface. This change likely added variability between treatment plots that was minimized by blocking during the establishment of this trial. On January 14, soil was dry, temperatures had been moderate and were in the high 60's F, foggy and overcast skies. Overall mound numbers had declined since the November assessment, but colonies were active and near the surface, with many containing winged reproductive stages.

The "hopper blend" treatment (the Insect Growth Regulator or IGR, pyriproxyfen, plus the metabolic inhibitor, hydramethylnon) treatment resulted in the most rapid decline of active ant mounds per monitored sub-plot area (**Table 1**). The addition of hydramethylnon to the IGR product significantly improved the "earliness" of control compared to application of pyriproxyfen alone. Although not statistically significant from the untreated control treatments after 1 month of application using the Duncan's Multiple Range Test, this treatment was significantly different using less rigorous Least Significant Difference method to separate means. By 3 months after treatment, the hopper blend mean was significantly less than the untreated control plot mean, but only numerically less than full broadcast applications of IGR products. As expected, the pyriproxyfen IGR “skip swath” means were not significantly different from untreated check plot means during the first 3 months of this trial.

Seven months following fall application, mean mound numbers in Esteem® (pyriproxyfen) were finally significantly lower than those in the untreated plots (**Table 1**). However, observations of brood in mounds remaining in Esteem-treated plots indicated that more colonies were in decline, having no worker larvae (brood) and only reproductive (male and female winged sexual stage) brood. Mild, wet fall and winter conditions had delayed worker mortality allowing colonies to survive. Hotter, dryer conditions anticipated later in the spring would quickly result in elimination of the colonies. Other treatments were performing similarly, but were not significantly different from either untreated control or Esteem plot in mean mound numbers.

On June 17, 9 months following application, all treatment plots had significantly less ant mounds than those receiving no treatment although those treated with the hopper blend did not separate statistically. This reduction in level of suppression was likely caused, in part, by threatment pattern in relation to sub-plot sampling along a strip not receiving bait application. Frequent rains had occurred throughout the spring and early summer and grass had not been shredded, making mound detection somewhat challenging. By September 22, 12 months following application, no significant differences occurred between 0.51-acre sub-plot means of fire ant mound numbers.

On October 15, 2004, treatments were re-applied using a pickup truck mounted air assisted GT-77 model Herd Seeder. Plots were treated from 11:45 a.m. to 3:15 p.m. (3.5 hrs to treat 32 acres traveling at 10 mph).

Table 1. Mean number of fire ant mounds per 0.51 acre sub-plot prior to and following broadcast treatment of ant bait products, Lake Granger dam, Williamson County, Texas, treated Oct. 2, 2003.

Treatment	**Pre-treat** Oct. 2, 2003[c]	**1 Month** Nov. 6, 2003[cd]	**3 Months** Jan. 14, 2004[c]	**7 Months** Apr. 8, 2004[c]	**9 Months** June 17, 2004[c]	**12 Months** Sept. 22, 2004
untreated control	20.75	64.50 ab	40.00 a	36.25 a	20.00a	24.00
pyriproxyfen (Esteem®)	19.50	69.50 a	24.50 bc	11.25 b	8.00b	19.00
methoprene (Extinguish®)	20.00	47.50 ab	22.00 bc	20.00 ab	6.00b	15.50
"Hopper blend"[a]	20.75	17.25 b	11.00 c	22.50 ab	10.25ab	22.50
"Skip swath"[b]	19.00	51.75 ab	31.25 ab	30.50 ab	6.75b	18.00
Statistics: (d. f. = 4)						
Mean Square	2.375	1672.325	466.50	373.08	130.43	NS
F ratio	0.161	2.001	5.215	3.036	2.376	0.334
P	0.953	0.158	0.008	0.051	0.099	0.851

[a] Blend of hydramethylnon bait (ProBait®) and pyriproxyfen (Esteem®), 50:50 applied at 1.5 lb blended product per acre

[b] Pyriproxyfen bait (Esteem®) applied at a rate of 1.25-1.5 lb/acre, but with the center 20 ft. swath (sub-plot area) left untreated

[c] Means followed by similar letters are not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $P \leq 0.05$ (Microstat 2.03, Ecosoft., Inc. and SPSS for Windows 11.5)

[d] Note: In separating means using Least Significant Difference (LSD), $P \leq 0.05$, the "hopper blend" was significantly different, followed by a "c" (LSD 5%, SSD5%, range 5 = 48.542, range 2 = 44.534), from those for untreated control (ab) and pyriproxyfen (a); with methoprene (abc) and the "skip swath" (abc) not separating from this treatment.

EFFICACY OF ESTEEM® (PYRIPROXYFEN) FIRE ANT BAIT TREATMENTS COMPARED TO STANDARD TREATMENTS FOR CATTLE PASTURES

Bastiaan M. Drees, Professor and Extension Entomologist
Paul Nester, Extension Agent - Integrated Pest Management (Fire Ants)
and Mike Heimer, County Extension Agent, Montgomery County, Texas

Esteem® Fire Ant Bait (pyriproxyfen, Valent U.S.A.), also sold as Distance® Ant Bait is an insect growth regulator (IGR) that produces a slow but extended period of 80 to 90 percent reduction of mounds or nests of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). In a previous study (Evaluation of Esteem® Fire Ant Bait (pyriproxyfen) for suppression of imported fire ants in agriculture by B. M. Drees and E. Brown), initiated at Lake Granger in Williamson County, Texas, October 2, 2003, the skip swath treatment was applied to either side of the monitoring or sub-plot area, and possibly resulted in documentation of less than optimal performance of this treatment. It was also conducted in an area with no cattle. Cow dung could potentially compete with bait formulations and result in reduced dose resulting from ant foraging. These complications warranted re-assessment of treatments.

This trial was conducted to further document efficacy of Esteem® Fire Ant Bait when applied in early summer rather than the fall to compare no treatment (untreated control) and "standard" treatments, Extinguish® (methoprene) and Amdro®Pro (hydramethylnon), and to assess the performance profile when using Esteem in a "hopper blend" (pyriproxyfen plus hydramethylnon) or as a "skip swath" treatment (1.0 lb per acre applied to every other 25 ft swath).

Materials and Methods

Twenty four plots, 150 by 300 ft (4,5000 sq ft/43,560 sq ft/acre = 1.03 acre), were established in pastureland near Dobbin, Texas, June 1 and 2, 2004. Pre-counts of ant mounds were taken, June 7, 2004, by sampling two 30 ft wide by 260 ft long swaths (7,800 sq ft x 2 = 15,600 sq ft/43,560 sq ft/acre = 0.358 acre sub-plot sampling area/plot) starting and ending 20 ft from the ends of each plot. Ant mounds were considered active if dozens of ants emerged when the mound was disturbed with a shovel.

Treatments were applied, June 14, 2004 (9:38 a.m. through 3:47 p.m.), and included:

1. pyriproxyfen (Esteem® Fire Ant Bait) - 1.5 lb/acre
2. "Hopper blend" of 1.0 lb pyriproxyfen (Esteem®) plus 0.75 lb hydramethylnon (Amdro®Pro)
3. "Skip swath" of pyriproxyfen (Esteem®) - 1.0 lb bait applied to every other 25 ft swath
4. hydramethylnon (Amdro®Pro) - 1.5 lb/acre
5. methoprene (Extinguish®) - 2 lbs/acre
6. Untreated control

All products were applied using a GT-77 model Herd Spreader mounted on a utility vehicle. Although no rain occurred during application, rain showers reached the area early (approximately 6:00 a.m.) the following morning. Thus, treatments were applied roughly 12 hours before the rain event, providing plenty of time for ants to forage and collect the products applied.

All plots were re-assessed for active ant mounds periodically after treatment (July 9, September 14-16, and Dec. 17, 2004, March 8, 2005). For each pre- and post-treatment sampling dat(s) the number of active ant mound per sub-plot data for each treatment were analyzed using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $P \leq 0.05$ (SPSS for Windows 11.5).

Results and Discussion

No significant differences between means occurred prior to application of bait product treatments (**Table 1**). As expected, 1 month after application, hydramethylnon and "hopper blend" treatments significantly reduced mean active ant mound numbers per 0.36 acre sub-plot. All insecticidal bait treatments significantly reduced ant mound numbers relative to those found in untreated control plots 3 months following early summer application. As compared to the previous 2003 trial conducted at Lake Granger where treatments were applied in the fall, these results document that insect growth regulator (IGR) products (pyriproxyfen and methoprene) reduce mound numbers faster than when applied in early summer.

Seven months following application, mean mound numbers in the hydramethylnon and "hopper blend" treatment plots began to increase and were no longer significantly different from untreated plot means. This may have been due to hydramethylnon-based treatments eliminating most fire ant colonies early on, thereby leaving few colonies (treated or not treated with a slow-acting insect growth regulator) left to prevent provide "biotic resistance" to prevent re-invasion. By 9 months after application, the insect growth regulator (pyriproxyfen, methoprene) and "skip swath" (pyriproxyfen) treated plots had significantly fewer mean ant mound numbers per 0.36 acre sub-plot compared to untreated plot means. The number of mounds in untreated plots increased dramatically due to the mild, wet winter. Thus, hydramethylnon treated plot means were, once again significantly lower than untreated plot means. In this trial, however, the pyriproxyfen component (pyriproxyfen) in the "hopper blend" treatment did not provide significantly "longer-lasting" ant mound number suppression.

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Table 1. Mean number of active red imported fire ant mounds per 0.36 acre subplots within 1.03 acre treatment plots (150 by 300 ft) following June 14 treatment with broadcast application of bait-formulated insecticide products, Montgomery Co., Texas.

Treatment	**Pre-treat** June 7, 2004[c]	**1 Month** July 9, 2004[c]	**3 Months** Sept. 14-16, 2004[c]	**7 Months** Dec. 17, 2004	**9 Months** March 8, 2005
untreated control	20.25	15.25a	5.25a	12.5a	48.5a
pyriproxyfen (Esteem®)	16.50	10.50ab	2.50b	1.75cd	5.8b
"Hopper blend"[a]	15.25	0.75b	2.25b	9.3ab	22.0ab
"Skip swath"[b]	20.75	16.75a	1.75b	1.5d	7.8b
hydramethylnon (Amdro®Pro)	13.25	1.50b	0.50b	5.3ab	14.8b
methoprene (Extinguish®)	13.25	19.25a	1.75b	4.3cd	10.3b
Statistics: (d. f. = 4)					
Mean Square	179.89	70.94	3.553	11.278	214.44
F ratio	0.198	3.542	2.833	6.695	4.745

[a] "Hopper blend" of 0.75 lb hydramethylnon (Amdro®Pro) and 1.0 lb pyriproxyfen (Esteem®) baits, applied at 2.25 lb blended product per acre

[b] Pyriproxyfen bait (Esteem®) applied at a rate of 1.0 lb/acre to evey other 20 ft. swath

[c] Means followed by similar letters are not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $P \leq 0.05$ (SPSS for Windows 11.5)

EVALUATION OF SAFER® FIRE ANT KILLER AS A MOUND DRENCH TREATMENT FOR THE RED IMPORTED FIRE ANT

Kimberly M. Engler, Program Specialist-Urban IPM

An extract derived from citrus peel, d-limonene, causes abnormal activity to an insects' sensory and nervous systems. This active ingredient causes cell death (i.e., it is a cytotoxicant). After contact, this chemical compound causes the insect to twitch, convulse and become paralyzed. Several fire ant mound treatments contain d-limonene as the active ingredient, such as Safer® Fire Ant Killer. These products are applied as individual mound treatments to eliminate fire ant colonies, if applied appropriately. This trial was conducted to determine how effectively d-limonene mound treatments eliminate individual fire ant colonies, compared to Howard Garrett's fire ant drench concentrate (1/3 orange oil, 1/3 compost tea, and 1/3 molasses) and indoxacarb bait.

Materials and Methods

On October 12, 2004, 40-foot wide strips were marked on the north side of the Dallas Research and Extension Center's property. Then two surveying flags were placed on either side of the strip after locating and flagging 10 active mounds. Flags were also placed along both edges of the plots, to delineate the boundaries of each plot. Therefore, each plot was 40 feet in width, but varied in length. Also, each plot was spaced 20 feet away in all directions to adjacent plots providing a treatment buffer that prevents effects of treatments from affecting ant colonies in adjacent plots.

The plots were then divided into 5 sets, block or replications, containing 4 plots each. The total lengths of the 5 plots within each replicate were summed, so the total length for all treatment plots within each block was approximately equal. Within each replication, treatments were randomly assigned to the plots. This technique provides approximately equal area available for ant re-infestation and colony ground migration between all treatments.

Treatments were applied in the morning on October 13, 2004. Skies were clear and the temperature was in the mid-70s in the afternoon.

<u>Treatment</u>	
Water drench	1 gallon water only drench/mound
Garrett's fire ant drench	1 cup per 1 gallon water drench/mound
Safer Fire Ant Killer	4 oz. per 1 gallon water drench/mound
Indoxacarb	4 T sprinkled 3-4 ft. around undisturbed mound

Evaluations were made at 1, 2, 7, 14, 28 and 35 days post-treatment. Evaluations were conducted by lightly disturbing the treated mound with a plastic rod. A mound was considered active, if more than 25 ants came to the surface in a defensive manner within 15 seconds of disturbance. All plots were surveyed at 14 and 28 days for satellite mound formation. Results were analyzed using SPSS Analysis of Variance (ANOVA) test ($p \leq 0.05$).

Results and Discussion

The first and second days after treatment, the d-limonene treatment had significantly less active mounds than all the other treatments (**Table 1**). At seven days after treatment, plots treated individually with d-limonene or indoxacarb had significantly less active mounds than the water only control or Garrett's fire ant drench treated plots. Fourteen days after treatment, the number of active mounds in the d-limonene and indoxacarb treated plots continued to decrease and had significantly less ant mounds than the water only control and Garrett's fire ant drench treated plots. Twenty-eight days following treatment, d-limonene and indoxacarb had significantly less active mounds than the other treatments. Thirty-five days following treatment, d-limonene treatment had significantly less mounds than indoxacarb, Garrett's fire ant drench and water only control plots; the indoxacarb treated plots had significantly fewer mounds than the Garrett's fire ant drench and water only controls.

Overall, the plots containing ant mounds treated with the d-limonene drench eliminated the most fire ant mounds at the beginning of the experiment and continued to show a decrease in mound activity throughout the trial compared to the control and Garrett's fire ant drench treatments. The plots treated with indoxacarb bait were documented to be the next best at eliminating fire ant mounds after the d-limonene drench treatment. The indoxacarb plots showed control after 7 days, followed by a decrease in mound activity as time progressed. Heavy rains could have caused the fire ant mounds in the control plots to relocate to areas outside the treatment plots.

Surveys of the total number of ant mounds per plot documented possible relocation of fire ant colonies 14 and 28 days following application (**Table 2**). The amount of relocation was low and there was no statistical differences found between treatments. These data indicate that the insecticide treatments eliminated the colonies, instead of causing them to relocate. This is important since customers frequently complain that treatments are not killing the fire ant colony, but rather moving them to another location.

Since the plots were suspected of containing polygene colonies, a limiting factor of the individual mound treatments was the possibility of not killing all the queens with one application. If the queens were not exposed to the treatment, then the colony would not be eliminated. Perhaps, a follow-up treatment should be applied when using these products.

In conclusion, d-limonene was the most effective product at eliminating fire ant colonies in this test. However, cost as well as time of application needs to be considered. Even though the colonies were eliminated, this product is expensive, more labor intensive and time consuming compared to applying other treatments such as dust or bait formulated products to individual fire ant mounds.

Table 1. Mean number of ten active red imported fire ant mounds following individual mound treatments, Dallas Co., Texas, treated 19 October 2004.

Treatment	Day 1 Oct. 20 2004	Day 2 Oct 21 2004	Day 7 Oct 28 2004	Day 14 Nov 4 2004	Day 28 Nov 11 2004	Day 35 Nov 18 2004
Control	8.60a	9.00a	7.80a	6.60a	5.80a	5.40a
Garrett Fire Ant Drench	8.80a	9.20a	7.40a	7.80a	6.20a	6.40a
D-Limonene	6.00b	5.00b	4.20b	2.80b	1.80b	0.80b
Indoxacarb	9.40a	9.20a	4.40b	3.00b	2.60b	2.80c
Statistics (d.f. = 4)						
Mean Square	11.33	21.40	18.32	32.05	24.73	32.18
F Ratio	6.21	10.19	4.31	9.71	7.98	16.09
P Value	0.005	0.001	0.021	0.001	0.002	0.000

[a] Means followed by similar letters are not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $P \leq 0.05$ (SPSS for Windows 11.5)

Table 2. Mean number of red imported fire ant mounds that relocated in each treated plot, Dallas Co., Texas, treated 19 October 2004.

Treatment	Day 14 Oct. 20, 2004	Day 28 Nov. 11, 2004
Control	0.00a	0.20a.
Garrett Fire Ant Drench	0.20a	0.40a
D-Limonene	0.20a	0.20a
Indoxacarb	0.00a	0.00a
Statistics (d.f.=4)		
Mean Square	0.07	0.18
F Ratio	0.67	0.92
P Value	0.59	0.46

[a] Means followed by similar letters are not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $P \leq 0.05$ (SPSS for Windows 11.5)

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EFFECTS OF THE ORGANIC TEXAS TWO STEP METHOD COMPARED TO INDIVIDUAL MOUND TREATMENTS WITH SPINOSAD FIRE ANT BAIT
dllas/Fort Worth Airport, Tarrant County, Texas-2004

Kimberly Engler, Program Specialist-Urban IPM
Bastiaan M. Drees, Professor and Extension Entomologist
Margie Barton, Entomology Lab Technician

The Texas Two Step Method for controlling the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera:Formicidae) is the most cost-effective and environmentally- friendly way to control fire ants in moderate (0.5 acre) to larger size areas (see http://fireant.tamu.edu). In past years, consumers have relied on synthetic chemicals to treat their yards. However, beginning in 2004, there are several products on the market certified by the Organic Materials Review Institute (OMRI) that constitute the elements of an organic Texas Two Step. Safer® Brand Fire Ant Bait and Green Light® with Conserve are fire ant baits that contain a natural, fast acting insecticide called spinosad. Spinosad is a byproduct from the fermentation of a soil bacterium, *Saccharopolyspora spinosa*. Spinosad targets an insects' nervous system to cause rapid excitation, paralysis, and then death. In previous studies, spinosad was proven to be a fast-acting organic product that caused death to red imported fire ant workers within 24 hours after application and elimination of colonies in 3 to 14 days using the individual mound treatment rate. In contrast, when these products are applied as a broadcast treatment over an area, elimination of red imported fire ant colonies may take longer than 14 days. As a broadcast-applied bait, spinosad formulations can constitute the first step in the organic Texas Two Step program.

Safer® Brand Fire Ant Killer containing d-limonene is an OMRI certified individual mound treatment. D-limonene is an extract of the oil from citrus peel. This active ingredient degrades the insect's cuticle to allow penetration inside the insect causing cell death. This individual mound treatment is the second step in the organic Texas Two Step Method and its application is recommended after a spinosad bait has been broadcast over the entire area. This treatment should be reserved for nuisance mounds, only, to provide faster elimination of remaining colonies.

This trial was conducted to demonstrate the effect of a single broadcast of spinosad fire ant bait followed by an individual mound treatment with d-limonene compared to plots treated with spinosad fire ant bait on individual mounds and plots that contained no treatment (untreated controls).

Materials and Methods

On June 16, 2004, an area approximately 5 acres was divided into nine 0.25 acre plots with a 15 ft. buffer zone in between plots. The center of the plot was then determined to create a 41 foot radius circular (0.125 acre) sub-plot for sampling ant mound numbers within a 104.1 X 104.1 foot square treatment plot. Red imported fire ant mounds were counted within each plot by disturbing suspected mound sites with a stick to determine activity. Mounds were considered active if many (dozens of) worker ants were observed within 15 seconds. All active fire ant mounds within the plot were counted and recorded. Pretreatment ant mound numbers per sub-plot area were arrayed from lowest to highest and blocked into groups of 3 plots which formed the replicates. Treatments were assigned randomly to each block so that only minor numerical differences occurred for average mound per plot values.

Treatments included:

1. Untreated control or check (CK) received no treatment
2. Spinosad (0.015% spinosad) Fire Ant Bait (Dow) 4 lbs./acre (broadcast bait on ¼ acre plots); after 28 days remaining active individual red imported fire ant mound treatments were treated using Safer® Brand Fire Ant Killer containing 78.2% d-limonene at a use rate of 4 ounces mixed with 1.0 gallon of water per ant mound
3. Spinosad (0.015% spinosad) Fire Ant Bait (Dow) 4 tbs./mound to each active red imported fire ant mound found in the 41 foot radius circle

The test site was located at Dallas/Fort Worth Airport, adjacent to Bear Creek Golf Course. This location had clay soil with a vegetative cover of native grasses and shrubs. The area was mowed only by request, so the native vegetation was extremely tall when the treatments were placed into their respective plots. The entire area was mowed 3 days after treatment was applied and was left un-mowed for the duration of the trial.

On June 18, 2004, from 8:00 a.m. - 12:00 p.m., the treatments were applied in their respective plots. The temperature was around 80° F, the wind was low, less than 5 miles per hour, and skies were cloudy. Plots were reevaluated 7, 14, 28, and 56 days following treatment as described for the pre-treatment assessment of the mound numbers. Data was analyzed for each pre- and post- treatment date using Analysis of Variance (ANOVA) with means separated using Duncan's Multiple Range Test at $P \leq 0.05$ (SPSS for Windows, Lead Technologies, Version 11.5).

Results and Discussion

The red imported fire ant mounds were low in number, but exceeded 20 mounds per acre, which serves as an action threshold for application of broadcast baits when this trial was initiated. After one week, the mound numbers had declined somewhat in treatment plots since the June 15th pretreatment assessment. This may be due to the hot weather that causes ant colonies to be less active near the soil surface. After the first week of treatment, the broadcast treatment of spinosad mean showed the most rapid decline of active fire ant mounds per monitored sub-plot area (**Table 1**). Two and four weeks after treatment, the average (mean) number of active fire ant mounds in the broadcast treatment plots was significantly less than the individual mound treatment mean and the untreated control plot mean. Also, the individual mound treatment mean was numerically but not significantly less than the untreated control mean. After 8 weeks, the individual mound treatment mean had numerically less mounds than the broadcast treatment and the untreated control, although the means were not significantly different. However in this trial, there was not an additional dramatic reduction in the number of mounds where d-limonene drench was applied to individual mounds 28 days after broadcast-applying the spinosad fire ant bait.

Frequent rains during the course of monitoring this trail could have interfered with an accurate evaluation of the number of fire ant mounds in the experimental area. Many "new" mounds were easily observed in the area after a rain, since the soil was recently moved to the surface, particularly during hot, dry weather that characterized conditions when this trial was established. These colonies could have been present prior to treatment, but were not detected since fire ant colonies may have occurred beneath the soil surface. This possibly added variability between treatment plots that was initially minimized at the establishment of the trial by blocking, but thereafter prevented statistical separation of means between treatments.

The Texas Two Step Method is a cost effective and time efficient method to control red imported fire ants. There is a difference in cost as well as time of application when broadcasting a bait versus treating individual mounds. To broadcast a pound of bait over a quarter acre, it took one person 7 minutes of time and product cost of around $10. When compared to individual mound treatments, it took 2 people around 17 minutes (or 34 minutes for one person) to treat all of the mounds in a quarter acre plot and product cost of around $15. Based on the information found in this experiment, broadcasting a fire ant bait saves time and money and it provides the best control. In this trial, treating individual mound treatment using spinosad bait cost more, took more time to apply and did not perform better than the broadcast application.

Table 1. Mean number of active fire ant mounds per 0.25 acre sub-plot prior to and following treatment with red imported fire ant products, Dallas Fort Worth Airport, Tarrant County, Texas.

Treatment	**Pre-treat** June 18, 2004[a]	**1 Week** June 25, 2004[a]	**2 Weeks** July 2, 2004[a]	**4 Weeks** July 16, 2004[a]	**8 Weeks** August 13, 2004[a]
Untreated control	25.33	28.33 a	15.67 a	16.33 a	20.33 a
Broadcast (spinosad) and mound treatment (d-limonene)	24.00	16.00 a	7.00 b	5.33 b	11.67 a
Mound treatment (spinosad)	22.33	18.33 a	9.67 ab	9.67 ab	9.67 a
Statistics: (d.f.= 2)					
Mean Square	6.78	128.79	59.11	92.11	96.44
F ratio	0.028	1.201	4.972	4.848	1.942
P	0.828	0.208	0.053	0.056	0.124

[a] Means followed by similar letters are not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $P \leq 0.05$ (SPSS for Windows 11.5)

RELEASES OF PARASITIC PHORID FLIES TO ESTABLISH NATURAL ENEMIES OF RED IMPORTED FIRE ANT IN DENTON AND POLK COUNTIES, TEXAS

Bastiaan M. Drees, Professor and Extension Entomologist,
Alexandro Calixto, Extension Assistant,
John N. Cooper, Mark Currie and Chad Gulley, Count Extension Agents,
Kim Engler, and Charles L. Barr, Extension Program Specialists

Master Gardening Volunteers: Sandy Hiatte, Jan R. Hudson and Alex Williams in Denton County; Maurice and Nancy Petersen

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is a serious economically important pest of urban and agricultural areas in the eastern two-thirds of Texas, many other southern United States and other parts of the world. In the United States, it was accidentally introduced from an area in South America where it is not considered to be a serious pest ant. One of the likely reasons for the higher levels of populations in the United States is the lack of natural enemies that affect this species in native habitats. Increased efforts to implement classical biological control programs in Texas and elsewhere have focused on importation, mass production and field release of candidate parasites and pathogens for native habitats (Gilbert and Patrock 2002, Porter and Gilbert 2004). A number of pathogen, parasite and predator species are considered to be potential biological control agents for imported fire ants in Texas (Knutson and Drees 1998).

Parasitic flies in the genus *Pseudacteon* (Diptera: Phoridae) are associated with a number of ant species. Mated female flies live for several days during which time they seek foraging ants during daylight hours. They deposit eggs in the thorax of suitable host ants. When successful, eggs hatch into larvae that migrate into the ant's head capsule to complete development in a one to two month period. Prior to pupating, the maggots push out the mouthparts and cause the ant's head to fall off. When the flies emerge, they seek new host ants.

In South America a number of species have been identified that attack fire ants in the genus *Solenopsis*. They are very host-specific and do not affect any other ants or animals. Other species occur in Texas and attack the native fire ant species, *S. geminata* Fabricius, but not *S. invicta*. A review of biology, host specificity and field releases in the southern U. S. Can be found discussed in Porter and Gilbert 2004.

Direct rates of parasitism by parasitic *Pseudacteon* phorid flies are extremely low (e.g., 1 to 3 percent of worker ants in a colony), so these parasitic flies are not expected to affect colonies directly. The egg laying behavior of female flies, however, causes disruption in foraging ant behavior as they avoid attack. Ants detecting the presence of the flies, stop foraging and hide. This behavioral shift is believed to help native ant species, enabling them to find food, build nests and better compete with the exotic imported fire ants, thereby, resulting in sustainable suppression of *S. invicta* (Porter 1998).

One phorid fly species, *Pseudacteon tricuspis* Borgmeier, was the first species to be released at over 20 locations in Texas (Gilbert and Patrock 2002). At about 6 of locations, the flies have survived and have begun to spread. In Florida, an early release has spread to over 500 square miles. Several years may be required for the fly populations to build up enough to have a suppressive effect on the fire ant. Several other organisms, including a disease of fire ants called

Thelohania, and several other species of phorid flies are currently being investigated. A number of organisms will need to be released and established in order to duplicate the situation in South America, where the fire ant is not considered to be a major pest (Williams and deShazo 2004).

The USDA's Animal and Plant Health Inspection Service (APHIS), in cooperation with Texas Cooperative Extension, have made releases of the first species imported, *P. tricuspis*. This species imported from South America and mass-produced by the Florida Department of Agriculture. Results of two of these releases are reported here.

Materials and methods

The protocols used in these two trials were provided by Anne-Marie Callcott, USDA-APHIS (3505 25th Ave. Bldg. 16, Gulfport, MS 39501, Phone: 228-822-3100, Fax: 228-822-3102, Email: Anne-Marie.A.Callcott@aphis.usda.gov. Flies were provided by Debbie Roberts, Bio Sci Lab Tech (Insects), USDA-APHIS Plant Protection and Quarantine (1913 SW 34th St., Gainesville, FL 32608, 352/372-3505, robertd1@doacs.state.fl.us. For each shipment, roughly 6,000 parasitized imported fire ant head capsules were sent by mail in special rearing chambers. After arrival, flies were allowed to emerge. Rate of emergence is reportedly 50 to 98%. During releases, roughly 40 flies were collected using an aspirator into vials. One vial of flies was emptied on each imported fire ant mound. Mounds were disturbed by digging a hole in the top and a cattle prod was used to shock worker ants to make them emit chemicals (pheromones) used by the flies to find the ants (Morrison and King 2004, Vander Meer et al. 2002). Releases were continued every two to thee days until all of the flies had emerged from parasitized ant heads sent.

In each area, four permanent plots were established to assess the number and size of imported fire ants. These were 1/8 acre circular lots marked with a center stake, with a 42 ft radius. Ant mounds within the monitoring plot were assessed to determine population rating per mound based on USDA rating scale:

Colony type	Rating factor	No. workers present
1	5	<100
2	10	100-1000
3	15	1000-10000
4	20	10000-50000
5	25	>50000

Periodically following initial release, sites were monitored for adult phorid fly activity by disturbing ant colonies in the release area, usually assisted with electrical stimulation provided by a cattle prod.

Denton County: The release site was located on the M. T. Cole Ranch in Denton County , Texas, near Ponder (33˚11'27.698 N; 97˚12'21.268 W). This study was initiated in October 2002 by Beth Hickman, Extension Agent - IPM (Fire Ants) at that time, and A. Calixto, Extension Assistant. On the day of release, weather was cold, foggy and rainy. Resleases were made towards the front of the ranch where there were scattered trees, but in a generally open area. Release dates and number of flies released were:

Date	No. Flies released	No. Flies observed over mounds
24 Oct. 2002	200	0
25 Oct. 2002	120	0
26 Oct. 2002	120	0
27 Oct. 2002	160	0
28 Oct. 2002	160	0
29 Oct. 2002	120	0
30 Oct. 2002	120	0

The second attempt to release and establish *P. tricuspis* began on May 2, 2003 in an area towards the back of the farm near a tree line and creek bed. This release was conducted by Dr. Charles Barr, Extension Program Specialist-III, A. Calixto, John Cooper, County Extension Agent, and Texas Master Gardeners Lonnie Smith and Sandy Haitte and Texas Master Naturalist Jan Hudson. Of 6,000 parasitized *S. invicta* head capsules shipped, a total of 2,570 flies emerged (42.83%). Approximately 2,280 flies were released on 38 active imported fire ant mounds on May 7, 8, 10, 12, 14, 16, 18, 2003. Emergence of adult phorid flies and release occurred over the following 16 days:

Date	No. emerged and released	% emerged (of total)
May 3, 2003	80-80*	1.33%
May 5, 2003	280-280*	4.66
May 6, 2003	410-410*	6.83
May 7, 2003	360-360 into 9 mounds	6
May 8, 2003	120-120 into 3 mounds	2
May 10, 2003	360-360 into nine mounds	6
May 12, 2003	400-400 into tem mounds	6.66
May 14, 2003	280-280 into seven mounds	4.66
May 16, 2003	240-240 into six mounds	4
May 18, 2003	40-40	0.66

*These flies were released in Burleson Co. Near Caldwell, Texas

The site was monitored May 2 and June 23, 2003 and April 30 and September 13, 2004.

<u>Polk County</u>: On October 16, 2003, Debbie Roberts of USDA APHIS sent an estimated 6,000 parasitized heads of *Pseudacteon tricuspis*, expected to start emerging on October 22, 2003. Shipment arrived in College Station, Friday, Oct. 17, and were observed to be emerging in the shipping container on Oct. 20. Training was provided by A. Calixto to County Extension Agents, Mark Currie and Chad Gulley and Master Gardening Volunteers, Maurice and Nancy Petersen on the first day of release, October 22, 2003. Subsequent release were made by the Petersens:

Date	**Approximate Number Released**
Oct. 22	120
Oct. 25	100
Oct. 27	50
Oct. 30	100
Nov. 1	100
Nov. 3	100
Nov. 5	80

The number of flies reportedly release was low. This resulted either from poor emergence or under estimation of emerging fly numbers. The site was monitored Nov. 11, 2003 (Drees), when ant mound monitoring plots were established, and again on May 19 (Drees).

Results and Discussion

<u>Denton County</u>: No adult phorid fly activity was detected following the winter months after the October 2002 release. After the second release attempt beginning May 2, 2003, establishment was assessed on June 23, 2003 (**Table 1**) and active imported fire ant mound numbers were counted in four 1/8 acre circle monitoring plots (**Table 2**). Ant mounds were generally large and low in numbers per acre (16 to 64 mounds per acre), indicative of the single queen or monogyne form of the red imported fire ant. Four ant mounds were inspected and flies were observed at each mound, with an average of 2.75 observed at each mound.

In 2004, a visit to the release site on April 30 (Drees, Calixto, Engler, Cooper and Master Gardener volunteers, Sandy Hiatte, Jan R. Hodson and Alex Williams) during which about 10 ant mounds were disturbed and ants shocked with a cattle prod, and again on Sept. 13 (Engler) failed to document phorid fly activity. Fire ant mound numbers in monitoring plots had fluctuated (**Table 2**), but these changes from June 23, 2003 were attributed to weather condition differences.

Table 1. Adult *Pseudacteon tricuspis* phorid fly activity at disturbed red imported fire ant mounds June 23, 2003, following the May 2003 release, Denton Co., TX.

Mound No.	No. flies observed over 30 minute period
1	5
2	2
3	2
4	2

Table 2. Number of active red imported fire ant mounds per 1/8 acre (42 ft radius) monitoring plot, Denton Co., Texas.

June 23, 2003.

Evaluation Area No.	No. mounds within each colony type (as defined below) per evaluation area					No. colonies per acre
	Col type 1	Col type 2	Col type 3	Col type 4	Col type 5	
1	0	0	0	2	6	64
2	0	0	0	2	0	16
3	0	0	1	1	0	16
4	0	0	0	1	6	56

April 30, 2004

Evaluation Area No.	No. mounds within each colony type (as defined below) per evaluation area					No. colonies per acre
	Col type 1	Col type 2	Col type 3	Col type 4	Col type 5	
1	1	0	1	1	2	40
2	0	1	3	3	0	56
3	1	0	1	0	3	40
4	4	0	1	0	4	71

Polk County: Observations made during the release period by Maurice and Nancy Petersen (**Table 3**) indicate phorid fly activity over imported fire ant mounds during the release period.

Egg laying adults were expected to deposit eggs in worker fire ants that would emerge the following spring as the weather warmed.

Table 3. Phorid fly establishment data: number of flies observed over 30 minute period, Polk Co., 2003.

Mound no.	Oct. 25	Oct. 27	Oct. 30	Nov. 1	Nov. 3*	Nov. 5
1	3	8	10	4	1	5
2	12	2	5	5	5	12
3	10	-	10	10	10	10

* windy day

On November 14, 2003, four 1/8 acre plots were established and mounds rated (**Table 4**). Ant mounds were generally small and numerous (168 to 552 mounds per acre), indicative of the multiple queen or polygyne form of the red imported fire ant.

Table 4. Number of active red imported fire ant mounds per 1/8 acre (42 ft radius) monitoring plot, Polk Co., Texas.

Nov. 14, 2003.

Evaluation Area No.	No. mounds within each colony type (as defined below) per evaluation area					No. colonies per acre
	Col type 1	Col type 2	Col type 3	Col type 4	Col type 5	
1	0	9	10	2	0	168
2	1	10	22	10	1	528
3	0	7	22	13	6	552
4	0	2	19	10	1	336

During a visit to this site on May 19, 2004, no phorid fly activity was observed during a 3 hour period during which about 5 ant mounds were disturbed and ants shocked with a cattle prod.

Discussion

Pseudacteon tricuspis releases have been made at a number of sites by Texas Cooperative Extension faculty and staff in collaboration with the University of Texas (UT), United States Department of Agriculture's Agricultural Research Service (USDA-ARS), in cooperation with agencies such as the Texas Army National Guard and Attwater Prairie Chicken National Wildlife Refuge (See *Fire Ant Trails* issues 4(1) and 6(2) posted on http://fireant.tamu.edu). In addition to failures to detect the phorid flies after release at the two sites reported here, releases near Elgin (J. Cook), Dobbin in Montgomery Co. (J. Cook, B. Drees , and L. Gilbert), Attwater Prairie Chicken National Wildlife Refuge in Colorado County (R. Patrock, M. Morrow, J. Hoskins, and B. Drees), Hempstead in Waller County (C. Barr, L. Gilbert) have not been successful to date. Releases made near Vidor in Orange County (C. Barr and L. Gilbert) and Caldwell in Burleson County (C. Barr, A. Calixto, B. Drees) have been successful and this parasitic fly species has begun to spread. *P. tricuspis* was detected 2 to 3 miles from the release site near Vidor (pers. Com., C. Barr, Aug. 16, 2004). The fly has spread 4 to 9 miles around the release site near Caldwell (see Fire Ant Update 1(2) posted on http://fireant.tamu.edu). Releases in the Austin area, since 1997, have expanded 15 miles around the Breckenridge Field Laboratory (pers. Com. L. Gilbert, Jan. 2005). Releases in other areas of the southeastern U.S. (Particularly in Florida and Alabama), that have not experienced recent serious drought seasons, have resulted in even more impressive spreads.

Factors responsible for success or failure of *P. tricuspis* releases are poorly understood. Attempts in open dry areas or during extreme weather seem to have less probability for success than those made in areas near tree lines and surface water. Once established, extreme winter weather can presumably eliminate then, which seems to be the case in the site near Ponder, Texas. As a species, *P. tricuspis* is a fairly large phorid fly species, and the sex ration is dependent upon the average head capsule size of the worker ants parasitized. On average, worker ants of the multiple queen or polygyne form of the red imported fire ant are smaller than those of the single queen or monogyne form. Thus, success has been limited in sites where the polygyne form is predominant because the first generation produced primarily male flies. This is a possible explanation for the lack of success in the Polk County site. Once established and after some spread, the fly seems to persist even if eliminated from small areas within its range by soil disturbance, floods or other environmental factors within patches of the landscape.

Work to establish phorid flies continued in both locations in 2004. In Denton Co. a new site was selected where a second species of phorid fly, *Pseudacteon curvatis*, was released over the summer. In Polk Co., *P. tricuspis* was released in a more favorable site, with monogyne fire ants near a farm pond, was selected for a fall release.

Acknowledgements

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FORAGING ACTIVITY AND TEMPERATURE RELATIONSHIP FOR THE RED IMPORTED FIRE ANT

Bastiaan "Bart" M. Drees, Professor and Extension Entomologist, Department of Entomology, Texas A&M University and Bill Summerlin, Technician II, Center for Urban & Structural Entomology

Much of the damage done by imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicide) occurs when worker ants forage for food and water. The success of suppressing fire ant populations using bait-formulated insecticide products depends on applying these materials during periods when the ants are actively foraging. Defining constraints to ant foraging can be used to prevent ant damage and improve timing of insecticidal baits. Temperature inside the nest or mound as well as temperature in subterranean foraging tunnels and on the ground surface may each impact foraging behavior and intensity. This series of trials was conducted to document the impact of the temperature range in laboratory foraging tunnels on ant foraging on target baits.

Methods and Materials

Establishment of laboratory colonies. Red imported fire ant colonies were shoveled into 5 gal. plastic buckets with inner surfaces dusted with talcum powder to prevent ant escape. Six colonies were removed from the field in Brazos Co., Texas (Texas A&M Riverside Campus) on Nov. 21, 2003. Water was then dripped into the buckets slowly, causing ant colonies to float on the surface. They were 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 with lids in which holes were melted to allow ants to enter and exit was placed in each colony tray to house the queen, brood and worker ants. Each colony was provided with distilled water.

Foraging structure. A plastic tray measuring 27 by 37 cm and 9.5 cm tall was used to make a temperature-controlled foraging tunnel arena that accommodated tubes from six laboratory colonies (**Fig. 1**). Six 165 cm long clear plastic hoses (50 ft (15 m) model Clean and Fill No Spill® Aquarium Maintenance System, Python® Products, Inc. 7000 W. Marcia Rd. Milwaukee, Wisconsin 53223 414/335-7000 - "ll FDA non-toxic materials"), 2 mm thick with an 8 mm inside diameter, were threaded through holes drilled on both short sides of the tray and sealed using silicone glue. On one end, the tube emerging on the outside of the tray was sealed using a cap from a Corning 50 ml Centrifuge Tube (Corning Incorporated, Corning, NY 14831, Item #430290, 27 mm inside diameter11.3 cm long), with a hole drilled in the center so that the centrifuge tube could be screwed to accommodate a "target bait" food substance such as peanut butter (Fig. 1, right). From the other end, 125 cm of the hose was allowed to dangle into a fire ant laboratory colony onto a stand housing a dowel rod to allow ants to crawl into the hose. The outside of the long end of the hose was painted with fluon to prevent ants from crawling up the outside.

Fig. Foraging structure (left) and target bait in centrifuge tube.

<u>Temperature control</u>. The foraging tunnel arena was filled with tap water maintained at 7 to 8 cm deep so that hoses would be submerged. A NESLAB Refrigerated Circulating Bath (NESLAB Instruments, Inc., Portsmouth, NH 03801, Part No. 01115-23) was used to heat and cool water circulating through the tray housing foraging tunnels.

<u>Assay technique</u>. Each day, water was heated to 50^{N}C to remove ants from the foraging arena tunnels and ants were brushed out of the cap of the centrifuge tube. Clean centrifuge tubes housing **5 cm-long** piece of clear soda straw (Glad® Flexible Straws - See Thru...For Fun, 0.6 cm inside diam.) in which a **4 mm** diameter “bead” of Jif® Creamy Peanut Butter (The J. M. Smucker Co., Orrville, OH 44667 containing roasted peanuts, sugar, 2% or less of molasses, partly hyrdogenated vegetable oil (soybean oil), fully hydrogenated vegetable oil (rapeseed and soybean), mono- and diglycerides and salt) weighing approximately 0.3 g was applied using a plastic squeeze bottle.

Temperature was adjusted each day so that the foraging tunnel arena could be maintained at a specified temperature each 6 hour-long trial period (10:00 a.m. to 4:00 p.m., beginning Dec. 1, 2003). Over successive trial periods (days), temperature settings were randomized to eliminate potential effects of laboratory colony age, food saturation and other possible variables associated with conducting trials in a temperature gradient sequence.

After 6 hours of exposure to specified foraging tunnel temperature ants, the amount of peanut butter remaining (estimated as percent remaining and converted to grams) and number of ants associated with the peanut butter was estimated. Mean and standard deviation were calculated for data from each temperature trial. Mean and standard deviation were calculated for data from each temperature trial.

<u>Foraging worker crawling speed</u>. Using a Sony Camcorder, video tapes were taken of foraging ants crawling throug hoses at increasingly higher temperatures beginning at 2^{N}C through 50^{N}C. Usiong the editor function of I-Mac movie software, speed at which worker ants crawled was documented (cm/min). A linear regression was performed on data using SPSS for Windows, Lead Technologies, Inc. Version 11.5.

Results

Six field-collected colonies used for these assays were examined for estimated worker number, brood (eggs, larvae and pupae) status and presence of queens and winged (alate) adult reproductives (Dec. 1, 2003):

Colony	No. Workers	Brood (ml)	No. Queens	Alates
1	20,000	2 ml	none*	none
2	20,000	trace	2	none
3	30,000	1.0	none*	none
4	30,000	2.0	none*	none
5	30,000	5.0	none*	none
6	50,000	7.0	none*	none

* lack of queens does not necessarily mean there were none, just that none were observed. Presence of brood is an indication that queen ants were present but not observed.

Notes: On Dec. 2, during cooling water from 50^{N}C, worker ants were observed beginning to enter hoses at 47 to 48^{N}C (116.6-118.4^{N}F). On Dec. 4, when heating water from 2^{N}C, ants were observed entering the hoses at 7-8^{N}C (44.6-46.4^{N}F).

Table 1 documents peanut butter removal and foraging ant numbers associated with the bait at various temperatures. Maximum and minimum temperature extremes were 2 to 50^{N}C, at which no peanut butter was removed and except for one foraging ant observed at 50^{N}C, which may have crossed when the temperature dropped to 47^{N}C degrees for a short period of time, no ants were observed crossing hoses to the peanut butter bait in the centrifuge tube. Numerically, maximum peanut butter removal occurred at 27^{N}C and foraging ant numbers at 35^{N}C, but significant differences did not appear in the temperature range from 14 through 45^{N}C due to high variability between colony activity. Bait exposure for 6 hrs. resulted in recruitment that may have overcome effects of temperature on initial food discovery and initial rate of foraging recruitment to a food source. **Figure 3** depicts mean bait remaining and foraging worker ants associated with bait, illustrating maximum ant foraging and bait removal occurring in a temperature range from 25 to 35^{N}C.

Between the temperature extremes of 10 and 49^{N}C, foraging speed varied for individual ants ranged from 0.21 cm/sec (12.6 cm/min or 5.04 in/min) at 10^{N}C to 3.46 cm/sec (207.60 cm/min or 83.04 in/min) at 48^{N}C. Ants would not enter the tubes submerged in water at lower or higher temperatures. The linear regression between speed (dependent variable) and temperature (independent variable) was significant ($R = 0.71$) with an equation for the line being: speed = -019 + 0.06 temp (**Fig. 4**).

Discussion

For use of bait-formulated insecticide product, results from this trial suggest foraging temperature for *Solenopsis invicta* range from extremes of about 10 to 50^{N}C (50 to 122^{N}F), with optimum activity for application occurring from 25 to 35^{N}C (77 to 95^{N}C). For use of surface temperatures to control ant foraging activities, extreme foraging temperatures can be used as a method of control. For products, such as treatments in utility housings, protection from products must be assessed for the range of temperatures that the ants will forage. Finally, speed of foraging is important to understand when considering how fast ants can explore areas for resources or recruit rapidly, as in the case of hatching birds or new born animals.

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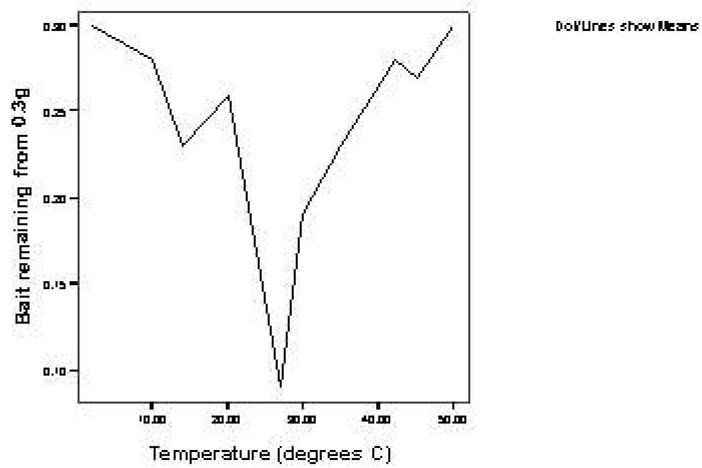
Table 1. Temperature (tube temperature/room temperature) and estimated amount of peanut butter remaining of 0.3 grams and number of worker ants associated with bait after 6 hours (10:00 a.m. through 4:00 p.m. daily as noted).

Temperatures (^{N}C) and date	**Bait Remaining/0.3g**	**Mean No. Foraging Ants**
50/24* (Dec. 1)	0.30 ± 0.00 S.D.	0.2 ± 0.4 S.D.
45/22 (Dec. 8)	0.27 ± 0.60	163 ± 99
42/24 (Dec. 3)	0.28 ± 0.01	551 ± 355
35/23 (Dec. 12)	0.23 ± 0.11	1,283 ± 1,048
30/29 (Dec. 9)	0.19 ± 0.10	395 ± 351
27/24 (Dec. 2)	0.09 ± 0.09	463 ± 448
20/26 (Dec. 15)	0.26 ± 0.05	570 ± 473
14/22 (Dec. 5)	0.23 ± 0.09	163 ± 95
10/24 (Dec. 10); 10/25 (Dec. 16)	0.28 ± 0.01; 0.29 + 0.01	558 ± 415; 583 + 779
2/26 (Dec. 4)	0.30 ± 0.00	0 ± 0

* temperature of water in foraging arena/room temperature

Figure 3. Mean weight of peanut butter of 0.3 g after 6 hrs of exposure to imported fire ant colonies foraging through submerged plastic foraging tubing soaking in water at selected various temperatures.



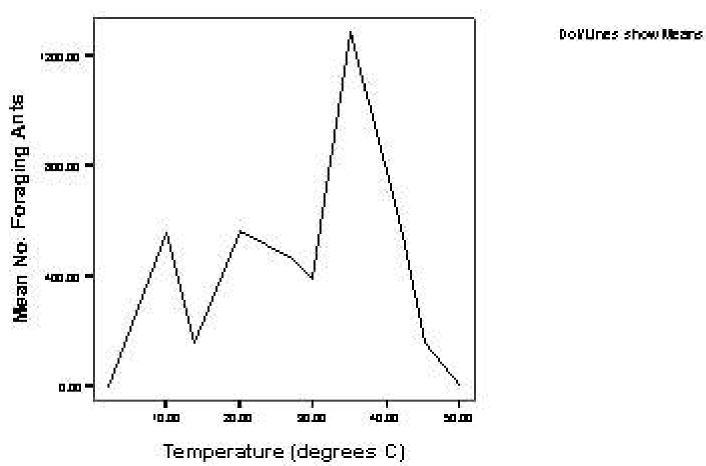
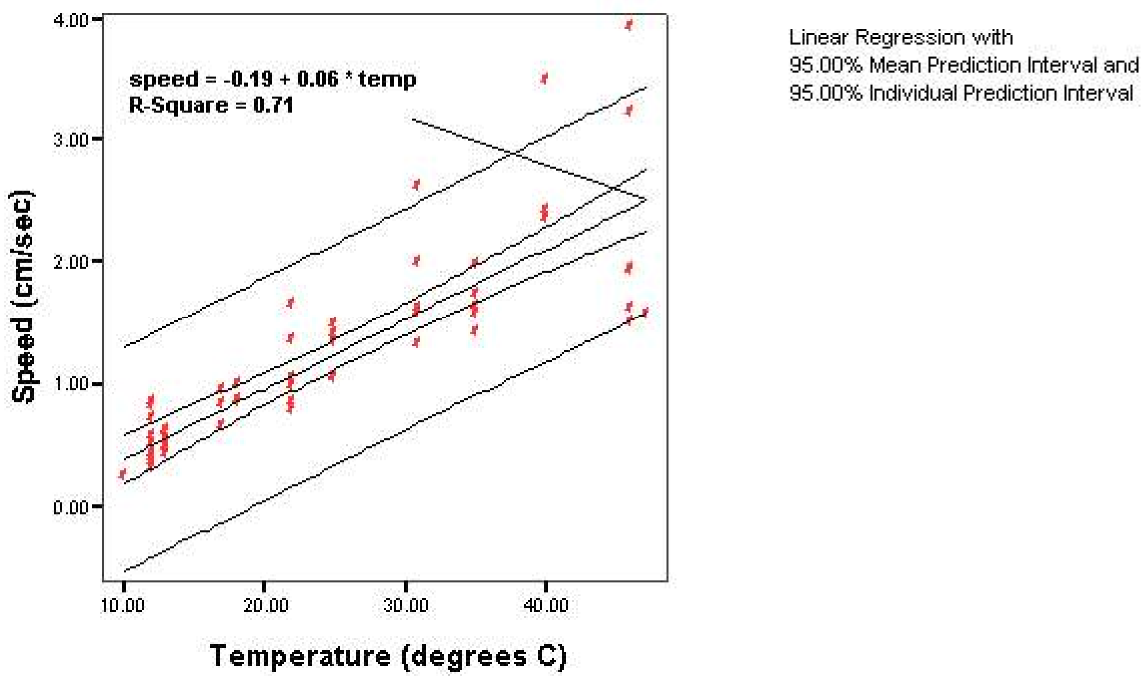


Figure 3. Foraging imported fire ant worker crawling speed (cm/sec) at various temperatures (degrees C).



ASSESSMENT OF PERMETHRIN-IMPREGNATED NYLON CYLINDERS TO REPEL FORAGING RED IMPORTED FIRE ANT WORKERS

Bastiaan "Bart" M. Drees, Professor and Extension Entomologist and
Bill Summerlin, Technician II, Center for Urban & Structural Entomology,
Department of Entomology, Texas A&M University

Nix of America (Metro Plaza, 181 Metro Dr., Suite 590, San Jose, CA 95110; 408/971-3115; Fax: 408/971-3305; nikkonix@ix.netcom.com) has developed a process to formulate permethrin-impregnated nylon plastic parts. Previously, this company developed a 9% permethrin impregnated nylon-type part for Nissan automobiles (SG Tube 1725W) to repel spiders from internal combustion engines for an estimated 10 year period. Permethrin-impregnated nylon cylinders were formulated and aged in order to conduct a series of laboratory trials to assess repellent effects on foraging worker ants of the red imported fire ant, *Solenopsis invicta* (Buren) (Hymenoptera: Formicidae).

Materials and Methods

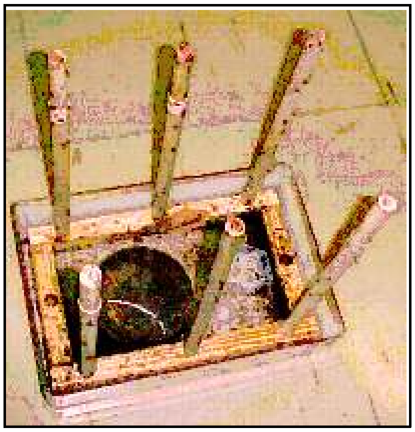
The cylindrical parts (measuring 22 mm in diameter and 15 mm long) were screened in the following trials using red imported fire ant laboratory colonies. Three samples each of cylinders containing 0%, 2.3%, 3.2%, 4.2%, 5.4% and 7.8% injection molded into cylinders of nylon of 0.8 and 1.2 mm thickness (inside diameters 19.5 and 20.5 mm, respectively) were prepared. Samples were then aged using heat treatment to simulate aging to 1, 3 and 5 years, resulting in a total of 32 treatment options.

Samples were aged by Nix of America using a heating process. The higher the temperature, the faster a given chemical reaction will proceed. By using the Arrhenius Equation, k=A*exp(-Ea/R*T), the following aging procedure was calculated: 5 years at 30°C corresponds to 57.03 days at 80°C.

Establishment of laboratory colonies. Red imported fire ant colonies were shoveled into 5 gal. plastic buckets with inner surfaces dusted with talcum powder to prevent ant escape. Six colonies were removed from the field in Brazos Co., Texas (Texas A&M Riverside Campus) on October 23, 2003. Water was then dripped into the buckets slowly, causing ant colonies to float on the surface. They were 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 with lids in which holes were melted to allow ants to enter and exit was placed in each colony tray to house the queen, brood and worker ants. Each colony was provided with distilled water.

Foraging structure. Rectangular bases were constructed of 3 by 3 cm pine and measuring 38 by 24 cm. Six 19 mm (3/4 inch) diameter and 39.5 cm tall dowel rods were placed in holes drilled into the base (**Fig. 1**). Each rod had a nail 9.5 cm from the top so that test cylinders could be fixed with the top edge 7 cm from the top of the rod. One foraging structure was placed in each laboratory colony, allowing six replications to be conducted of up to six treatments for each trial. Because only three cylinders of each treatment were provided for testing, each trial was conducted in two parts with two trials conducted simultaneously. The first three replicates placed in three of the six laboratory fire ant colonies. Treatments (cylinders) were then switched to rods in the other three laboratory colonies to provide results for a total of six replicates. Either new rods or rods washed in soapy water were used for each trial to avoid possible pesticide contamination of dowel rods between trials.

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 by permethrin-impregnated nylon cylinders placed part way to the top of the rods.



Assay technique. Nylon cylinders were placed on dowel rod nails. On top of each rod housing a cylinder, a 7 mm (1/4 inch) long, 4 mm diameter "bead" of Jif® Creamy Peanut Butter (The J. M. Smucker Co., Orrville, OH 44667 containing roasted peanuts, sugar, 2% or less of molasses, partly hyrdogenated vegetable oil (soybean oil), fully hydrogenated vegetable oil (rapeseed and soybean), mono- and diglycerides and salt) weighing approximately 0.3 g was applied using a plastic squeeze bottle. After 6 and 24 hours of exposure to foraging ants, the amount of peanut butter remaining (estimated as percent remaining and converted to grams) and number of ants associated with the peanut butter was estimated. Results were analyzed for each trial using analysis of variance (ANOVA) and means were separated using Duncan's Multiple Range Test at $P \leq 0.05$.

Trials 1 and 2. The first set of trials used only 4 treatments: untreated control, and cylinders formulated with 0% and 2.3% permethrin and aged 1, 3 and 5 years. Trial 1 used 0.8 mm thick cylinders and trial 2 used the 1.2 mm thick cylinders. Part 1 (first 3 replicates) was initiated Oct. 2 at 75^{N}F, and Part 2 (second 3 replicates) was initiated Oct. 6 at 78^{N}F.

Trial 3 and 4. The second set of trials used only 4 treatments: untreated control, and cylinders formulated with 0% , 2.3%, 3.2%, 4.2%, 5.4% and 7.8% permethrin and aged 1 year. Trial 3 used 0.8 mm thick cylinders and trial 4 used the 1.2 mm thick cylinders. Part 1 (first 3 replicates) was initiated Oct. 7 at 79^{N}F, and Part 2 (second 3 replicates) was initiated Oct. 9 at 78-79^{N}F.

Trial 5 and 6. The third set of trials used 8 treatments each: Trial 5 used untreated control, and all 7.8% permethrin cylinders of both 0.8 and 1.2 mm thickness aged 0, 1, 3 and 5 years. Trial 6 used an untreated check and a dowel with no cylinder, plus all 5.4% permethrin cylinders of both 0.8 and 1.2 mm thickness aged 1, 3 and 5 years. No effort was made to restrict the gap between the rods and cylinders for the 0.8 mm thickness parts. Part 1 (first 3 replicates) was initiated Oct. 13.

Results

Six field-collected colonies used for these assays were examined for estimated worker number, brood (eggs, larvae and pupae) status and presence of queens and winged (alate) adult reproductives:

Colony	No. Workers	Brood (ml)	No. Queens	Alates
1	45,000	22.5	none*	none
2	30,000	0.6	6	males & females
3	10,000	2.5	none*	none
4	20,000	0.6	none*	males & females
5	30,000	5.0	none*	males & females
6	10,000	0.6	1	females

* lack of queens does not necessarily mean there were none, just that none were observed. Presence of brood is an indication that queen ants were present but not observed.

Colony size, brood status and vigor can have an effect of the intensity of foraging behavior by worker ants, although starvation even in smaller colonies can also have an effect.

Trials 1 and 2. Results of this pair of trials documented some protection of the peanut butter bait target by the low-dose (2.3%) permethrin-impregnated cylinders. Although the 1-year aged cylinders offered the most protection, all treatments showed some foraging acts accessing the target (**Tables 1 and 2**). The 1 year aged 2.3% permethrin cylinders (the lowest impregnated rate provided for evaluation) of both thicknesses (0.8 and 1.2 mm) significantly reduced the amount of peanut butter bait ants removed after 24 hrs of exposure. Although, one can not compare results between trials statistically, the thicker (1.2 mm) cylinders appeared to offer greater protection from foraging ants. This difference is thought to be due to the different inside diameters of these cylinders, leaving a gap of about 1 mm between the rod and the cylinder for the 0.8 mm thick cylinders that allows ants to crawl underneath them.

Trial 3 and 4. The two high concentration treatments, 5.4% and 7.8% permethrin aged 1 year, prevented removal of the peanut butter target bait, although a few ants were found in association with the peanut butter throughout the trial period. Results were similar for both 0.8 and 1.2 mm cylinder parts (**Tables 3 and 4**). Although all treatments significantly reduced the amount of peanut butter removed by ants after 24 hrs, only the higher rates of both cylinders (0.8 and 1.2 mm) provided total protection from ant foraging. Ant foraging on peanut butter was significantly reduced in the trial assessing 1.2 mm diameter cylinders at both 6 and 24 hrs (**Table 4**), but not eliminated entirely. On dowel rods where peanut butter had been completely removed by the ants, foraging was also often reduced . Thus, the mechanism for "repellency" did not appear to result as much from ants avoiding contact with the cylinders, but rather that contact with the permethrin-impregnated cylinders was toxic and reduced or eliminated foraging and recruitment of additional foragers beyond the insecticide cylinder barriers. This mode of action appears to be similar to that described by Drees et al. (1992) for seed-protecting insecticides.

Trial 5 and 6. The two high concentration treatments, 5.4% and 7.8% permethrin aged 0, 1, 3 and 5 years, generally prevented removal of peanut butter (**Tables 5 and 6**). However, only the 7.8% cylinder of both thicknesses (0.8 ans 1.2 mm) significantly reduced peanut butter removal consistently for all treatment cylinder types and ages evaluated. The 5 year aged 5.8% 1.2 mm cylinder provided less protectin and the amount of peanut butter remaining was statistically similar to that remaining on untreated rods (with untreated or no cylinder). Although foraging ant numbers were reduced, often significantly compared to untreated controls, it was not eliminated. Only, foraging ants from one very vigorous colony (colony 5) overcame 5 year-aged treatments and to remove part or all of the peanut butter.

Discussion: These trials documented that the permethrin-impregnated nylon cylinders can significantly reduce foraging ant activity and provide protection of targets such as peanut butter bait from ant foraging when applied as a barrier. Contact with treated cylinders is toxic to the ants, thereby reducing foraging activities. As a "controlled release device," when properly installed these elements may be useful for protecting electronic and utility devices in small contained areas such as utility boxes and relay switch boxes. The capability of higher concentration formulations 5.4 and 7.8%) to provide protection for 5 years would be a dramatic improvement over currently available products which must be re-applied annually.

Literature cited

Drees, B. M., R. Cavazos, L. A. Berger and S. B. Vinson. 1992. Impact of seed-protecting insecticides on sorghum and corn seed feeding by the red imported fire ants (Hymenoptera: Formicidae). J. Econ. Entomol. 85(3): 993-997.

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prepared by Bastiaan M. Drees, Professor and Extension Entomologist, Texas A&M University, Department of Entomology, College Station, TX 77843-2475; 979/845-5878; Fax: 979/845-7029; b-drees@tamu.edu for Gene A. Ozawa, President and CEO, gakira@ix.netcom.com

Table 1. Amount of peanut butter (grams) remaining and number of ants associated with the target bait after 6 and 24 hrs of exposure to foraging red imported fire ants using 2.3% permethrin-impregnated, **0.8 mm** cylinder barrier treatments **aged 1, 2 and 5 years** between the laboratory ant colony and the target bait (Trial 1).

Treatment	**6 hrs*** Peanut butter (g)	**6 hrs** No. foragers	**24 hrs*** Peanut butter (g)	**24 hrs*** No. foragers
Untreated	0.01 ab	14.5	0.00 b	3.17 ab
1 year	0.22 a	7.8	0.14 a	5.83 a
3 years	0.08 b	14.8	0.05ab	3.00 ab
5 years	0.11 b	14.0	0.00 b	2.00 b
d.f. = 3				
Mean square	0.044	62.556	0.024	16.111
***F* ratio**	7.696	0.428	4.051	2.977
P	0.0007	0.3713	0.0083	0.209

***** Means followed by the same letter are not significantly different using analysis of variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Table 2. Amount of peanut butter (grams) remaining and number of ants associated with the target bait after 6 and 24 hrs of exposure to foraging red imported fire ants using 2.3% permethrin-impregnated, **1.2 mm** cylinder barrier treatments **aged 1, 2 and 5 years** between the laboratory ant colony and the target bait (Trial 2).

Treatment	**6 hrs*** Peanut butter (g)	**6 hrs*** No. foragers	**24 hrs*** Peanut butter (g)	**24 hrs*** No. foragers
Untreated	0.00 b	19.0 ab	0.00 b	4.83
1 year	0.29 a	5.5 c	0.22 a	6.17
3 years	0.25 a	20.7 ab	0.04 b	5.67
5 years	0.05 b	21.33 ab	0.00 b	2.17
d.f. = 3				
Mean square	0.121	335.819	19.042	19.042
***F* ratio**	25.566	3.695	1.081	1.081
P	0.0000	0.0111	0.1536	0.1536

***** Means followed by the same letter are not significantly different using analysis of variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Table 3. Amount of peanut butter (grams) remaining and number of ants associated with the target bait after 6 and 24 hrs of exposure to foraging red imported fire ants using 2.3%, 3.2%, 4.2%, 5.4% and 7.8% permethrin-impregnated, **0.8 mm** cylinder barrier treatments **aged 1 year** between the laboratory ant colony and the target bait (Trial 3).

Treatment	**6 hrs*** Peanut butter (g)	**6 hrs*** No. foragers	**24 hrs*** Peanut butter (g)	**24 hrs*** No. foragers
Untreated	0.12 d	39.0 ab	0.00 d	5.67 b
2.3%	0.27 abc	4.33 b	0.16 bc	6.83 b
3.2%	0.30 ab	14.33 b	0.15 bc	14.17 a
4.2%	0.30 ab	8.17 b	0.24 ab	4.50 b
5.4%	0.30 a	1.83 b	0.30 a	2.00 b
7.8%	0.30 a	1.33 b	0.30 a	0.33 b
d.f. = 5				
Mean square	0.121	1228.267	0.079	140.183
***F* ratio**	25.566	8.891	9.915	4.946
P	0.0000	0.0000	0.0000	0.0005

***** Means followed by the same letter are not significantly different using analysis of variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Table 4. Amount of peanut butter (grams) remaining and number of ants associated with the target bait after 6 and 24 hrs of exposure to foraging red imported fire ants using 2.3%,3.2%, 4.2%, 5.4% and 7.8% permethrin-impregnated, **1.2 mm** cylinder barrier treatments **aged 1 year** between the laboratory ant colony and the target bait (Trial 4).

Treatment	**6 hrs*** Peanut butter (g)	**6 hrs*** No. foragers	**24 hrs*** Peanut butter (g)	**24 hrs*** No. foragers
Untreated	0.22 b	19.5 a	0.00 d	9.00 a
2.3%	0.26 ab	6.5 b	0.20 c	3.33 b
3.2%	0.30 ab	3.3 b	0.27 abc	2.83 b
4.2%	0.30 a	1.0 b	0.30 ab	2.17 b
5.4%	0.30 a	1.0 b	0.30 ab	2.00 b
7.8%	0.30 a	0.67 b	0.30 a	0.33 b
d.f. = 5				
Mean square	0.006	318.467	0.083	53.378
***F* ratio**	1.872	3.130	21.917	8.082
P	0.0262	0.0045	0.0000	0.0000

***** Means followed by the same letter are not significantly different using analysis of variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Table 5. Amount of peanut butter (grams) remaining and number of ants associated with the target bait after 6 and 24 hrs of exposure to foraging red imported fire ants using **7.8%** permethrin-impregnated, **0.8 and 1.2 mm** cylinder barrier treatments **aged 0, 1, 3 and 5 year** between the laboratory ant colony and the target bait (Trial 5).

Treatment	**6 hrs*** Peanut butter (g)	**6 hrs*** No. foragers	**24 hrs*** Peanut butter (g)	**24 hrs*** No. foragers
Untreated	0.10 b	35.83 a	0.00 b	8.33 a
1.2 mm 0 yr.	0.30 a	0.33 b	0.30 a	1.00 bcd
0.8 mm 1 yr.	0.30 a	0.17 b	0.30 a	1.17 bcd
1.2 mm 1 yr.	0.30 a	0.67 b	0.30 a	0.50 d
0.8 mm 3 yr.	0.30 a	0.17 b	0.30 a	4.33 bcd
1.2 mm 3 yr.	0.30 a	0.67 b	0.27 a	3.33 bcd
0.8 mm 5 yr.	0.30 a	2.67 b	0.25 a	4.83 abc
1.2 mm 5 yr.	0.30 a	1.00 b	0.28 a	5.00 ab
d.f. = 7				
Mean square	0.031	923.926	0.083	41.807
***F* ratio**	17.164	29.595	21.917	4.424
P	0.0000	0.0000	0.0000	0.0002

***** Means followed by the same letter are not significantly different using analysis of variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Table 6. Amount of peanut butter (grams) remaining and number of ants associated with the target bait after 6 and 24 hrs of exposure to foraging red imported fire ants using **5.4%** permethrin-impregnated, **0.8 and 1.2 mm** cylinder barrier treatments **aged 0, 1, 3 and 5 year** between the laboratory ant colony and the target bait (Trial 6).

Treatment	**6 hrs*** Peanut butter (g)	**6 hrs*** No. foragers	**24 hrs*** Peanut butter (g)	**24 hrs*** No. foragers
Untreated	0.09 b	36.50 a	0.00 c	3.8 abc
No cylinder	0.14 b	27.83 ab	0.10 bc	7.50 a
0.8 mm 1 yr.	0.30 a	0.00 d	0.30 a	0.70 c
1.2 mm 1 yr.	0.30 a	0.33 d	0.30 a	0.30 c
0.8 mm 3 yr.	0.30 a	5.00 cd	0.27 a	4.5 abc
1.2 mm 3 yr.	0.30 a	3.00 cd	0.26 a	5.30 abc
0.8 mm 5 yr.	0.30 a	5.67 cd	0.21 a	6.00 ab
1.2 mm 5 yr.	0.25 a	18.00 bc	0.20 ab	1.70 bc
d.f. = 7				
Mean square	0.042	1146.131	0.067	40.902
***F* ratio**	7.727	7.806	10.157	2.985
P	0.0000	0.0000	0.0000	0.0020

***** Means followed by the same letter are not significantly different using analysis of variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

ASSESSMENT OF PERMETHRIN-IMPREGNATED NYLON PROTOTYPES TO REPEL FORAGING RED IMPORTED FIRE ANT WORKERS

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Nix of America (Metro Plaza, 181 Metro Dr., Suite 590, San Jose, CA 95110; 408/971-3115; Fax: 408/971-3305; nikkonix@ix.netcom.com) has developed a process to formulate permethrin-impregnated nylon plastic parts. Previously, this company developed a 9% permethrin impregnated nylon-type part for Nissan automobiles (SG Tube 1725W) to repel spiders from internal combustion engines for an estimated 10 year period. This part, plus other prototype devices were provided to be evaluated under laboratory conditions to document mortality or repellent effects on foraging worker ants of the red imported fire ant, *Solenopsis invicta* (Buren) (Hymenoptera: Formicidae).

Materials and Methods

Trial 1. This trial evaluated all prototype materials provided by Nix of America **(Fig. 1)**.

a b c d e f

Fig. 1. Prototype permethrin-impregnated nylon parts: a. Original prototype part, 1999; b. SG Tube 1725W; c. Cylinder 7.8% permethrin aged 1 year; d. Untreated cylinder; e. SG Spiral tube NS-20; f. Cylinder 7.8% permethrin aged 0 year.

a. Original Nissan auto part, SG Tube 1725W ; 9% permethrin) (16 mm long, 16 mm outside diam., 2 mm thick) - affixed to top of rods around the bottom of a 138 mm long, 11 mm diam dowel rod attached to the top;
b. Original prototype part, 1999 (3 cm long, 25 mm outside diam, 2 mm thick);
c. SG Spiral tube NS-20, T - 1, 9.5% permethrin (15 cm long, 19 mm outside diam, 1 mm thick);
d. Untreated check (cylinder (measuring 22 mm in diameter and 15 mm long);
e. Nylon cylinder (measuring 22 mm in diameter and 15 mm long, 1.2 mm thinck) formulated with 7.8% permethrin not aged (0 years);
f. Nylon cylinder (measuring 22 mm in diameter and 15 mm long, 1.2 mm thick) formulated with 7.8% permethrin aged 1 year.

<u>Trial 2</u>. This trial assessed various lengths of a prototype Spiral SG tube (NS-15) developed as a "cable wrap" for electrical housing units and utility boxes (Fig. 2).

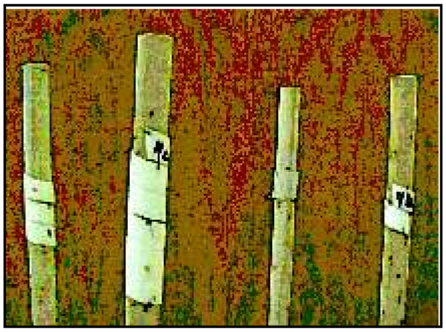

Fig. 2. Spiral SG tube, NS-15, assessed in 4.5, 9.5 and 15.5 cm lengths.

a b c d

a. Spiral SG tube, NS-15 (15 x 70) t =- 1 mm, 9.5% permethrin (10 mm diam spiral, ____ mm thick) - 4.5 cm;
b. As above, but 9.5 cm long section;
c. 1 As above, but 15.5 cm long section;
d. Untreated check (cylinder measuring 22 mm in diameter and 15 mm long, 0.8 mm thick)

<u>Procedure</u>. These trials were conducted in a manner similar to that described by the authors in a previous report (I. Assessment of Permethrin-impregnated Cylinders to Repel Foraging Red Imported Fire Ant Workers) using six laboratory colonies of red imported fire ants. Nylon cylinders were placed on dowel rod nails. On top of each rod housing a cylinder, a 7 mm (1/4 inch) long, 4 mm diameter "bead" of Jif® Creamy Peanut Butter (The J. M. Smucker Co., Orrville, OH 44667 containing roasted peanuts, sugar, 2% or less of molasses, partly hyrdogenated vegetable oil (soybean oil), fully hydrogenated vegetable oil (rapeseed and soybean), mono- and diglycerides and salt) weighing approximately 0.3 g was applied using a plastic squeeze bottle. After 6 and 24 hours of exposure to foraging ants, the amount of peanut butter remaining (estimated as percent remaining and converted to grams) and number of ants associated with the peanut butter was estimated. Where appropriate, results were analyzed for each trial using analysis of variance (ANOVA) and means were separated using Duncan's Multiple Range Test at $P \leq 0.05$. Three replicates of Trials 1 and 3 were left for 13 days to observe any forager recovery or peanut butter bait removal over a longer period of time. However, results were not statistically analyzed.

<u>Additional trials</u>. Due to high foraging worker ant mortality in trials 1 and 2, six fresh colonies were obtained from the field and two more trials were conducted using fewer treatments and initiating trials immediately after treatment devices were placed on dowel rods in colony trays. Treatments for **Trial 3** included: 1) untreated control (1.2 mm thick); 2) 9.5 cm length of Spiral SG tube NS-15 (9% permethrin); and, 3) 1999 Prototype (9% permethrin). **Trial 4** treatments included: 1) untreated control (0.8 mm thick cylinder); 2) 4.5 cm length of Spiral SG tube NS-15 (9% permethrin); and 3) 7.8% permethrin, 0 year aged cylinder. Procedure used was similar to that used in trials 1 and 2. Results were not statistically analyzed.

Results and Discussion

For Trials 1 and 2, six field-collected colonies used for these assays were examined for estimated worker number, brood (eggs, larvae and pupae) status and presence of queens and winged (alate) adult reproductives:

Colony	No. Workers	Brood (ml)	No. Queens	Alates
1	45,000	22.5	none*	none
2	30,000	0.6	6	males & females
3	10,000	2.5	none*	none
4	20,000	0.6	none*	males & females
5	30,000	5.0	none*	males & females
6	10,000	0.6	1	females

* lack of queens does not necessarily mean there were none, just that none were observed. Presence of brood is an indication that queen ants were present but not observed.

Devices were placed on dowel rods October 16 at 11:30 a.m. By Oct. 17, colonies were observed to have many dead worker ants that were presumably foragers. The trials were initiated Oct. 20 at 9:30 a.m. At the end of these trials, colonies had declined dramatically. Colony status prior to trials:

Colony	No. Workers	Brood (ml)	No. Queens	Alates
1	45,000	22.5	none*	none
2	30,000	0.6	6	males & females
3	10,000	2.5	none*	none
4	20,000	0.6	none*	males & females
5	30,000	5.0	none*	males & females
6	10,000	0.6	1	females

* lack of queens does not necessarily mean there were none, just that none were observed. Presence of brood is an indication that queen ants were present but not observed.

Colony status after conducting trials (Nov. 3, 2003):

Colony	No. Workers	Brood (ml)	No. Queens	Alates
1	35,000	3.0	none*	none
2	25,000	0.0	4	males & females
3	9,000	0.0	none*	none
4	18,000	trace	none*	males & females
5	25,000	0.2	1	males & females
6	8,000	0.0	none*	females

* lack of queens does not necessarily mean there were none, just that none were observed. Presence of brood is an indication that queen ants were present but not observed.

Elimination of foraging ants prior to and during this evaluation resulted in little, if any, removal of peanut butter even on some untreated control (check) dowel rods (Tables 1 and 2. Three replicates of each of these trials were maintained and observed for 13 days without providing additional food sources to the ant colonies with little change in results from permethrin treated nylon parts the 24 hour observation.

The 9% permethrin impregnated nylon prototype parts evaluated prevented ants from reaching the bait "target" throughout the trial. The mechanism of protection appeared to result from elimination of foraging ants from colonies through contact with the insecticide surfaces of the parts. Varying lengths of Spiral SG tube, NS-15 , did not approve protection. Mass forager elimination reduced or eliminated all foraging in some colonies to the point that even peanut butter on top of untreated check cylinder dowel rods was not removed.

<u>Additional trials</u>. Six "fresh" colonies collected from the field and extracted from soil to establish laboratory colonies were examined, Nov. 11, 2003, for estimated worker number, brood (eggs, larvae and pupae) status and presence of queens and winged (alate) adult reproductives:

Colony	No. Workers	Brood (ml)	No. Queens	Alates
1	20,000	none	none*	none
2	35,000	trace	3	females
3	20,000	trace	9	females
4	35,000	trace	none*	none
5	20,000	trace	1	males & females
6	50,000	6 mls	4	none

* lack of queens does not necessarily mean there were none, just that none were observed. Presence of brood is an indication that queen ants were present but not observed.

As in trials 1 and 2, a lot of foraging worker ant mortality was observed during the course of this second effort to show protection by the 9% permethrin-impregnated nylon devices, the highest concentrations provided for evaluation. Although some peanut butter target bait was removed by foraging ants on top of the dowel rods housing untreated control cylinders, and fewer foraging ants were associated with the target bait on top of dowels housing permethrin-treated parts, differences were very small (**Tables 3 and 4**).

These trials, using recently-collected fire ant colonies, confirmed findings in trials 1 and 2, and illustrated a limitation of this within-colony treatment assay method: When ant foragers are exposed to (or contact) higher concentrations of permethrin-impregnated nylon (e.g., 9%) in these confined laboratory colonies, the number of foragers is reduced to such an extent that target bait even on dowel rods housing untreated nylon cylinders is not removed. Thus, major differences between treatments and untreated controls become less dramatic. However, implications of this effect in field situations, such as in utility box housings, could be that properly installed devices can offer protection of the units' components that are not merely beyond a nylon barrier, providing broader protection of components in the box because foraging ant populations are reduced overall from colonies in the vicinity of the fixture.

Table 1. Average amount of peanut butter (grams) remaining and average number of ants associated with the target bait (in parentheses) after 6 and 24 hrs of exposure to foraging red imported fire ants using (Trial 1 initiated Oct. 20,2003, 9:30 a.m.).

Treatment	6 hrs. Peanut butter (g)	6 hrs. No. foraging ants	24 hrs. Peanut butter (g)	24 hrs No. foraging ants	13 days Peanut butter (g)	13 days No. foraging ants
Untreated control	0.28	8.5	0.25	0.5	0	0.33
SG Tube 1725W	0.30	0	0.30	0	0.30	0
Prototype 1999	0.30	0	0.30	0	0.30	0
SG Spiral NS-20	0.30	0	0.30	0	0.30	0
7.8% 0 yr. Cylinder	0.30	0	0.30	0	0.30	0
7.8% 1 yr. Cylinder	0.30	0	0.30	0	0.30	0
d.f = 5					d.f = 2	d.f = 2
Mean square	0.001	72.250	--	--	--	--
F ratio	1.00	1.048	--	--	--	--
P	0.096	0.0922	--	--	--	--

Table 2. Average amount of peanut butter (grams) remaining and average number of ants associated with the target bait (in parentheses) after 6 and 24 hrs of exposure to foraging red imported fire ants using Spiral SG tube, NS-15 (Trial 2 initiated Oct. 20,2003, 9:30 a.m.).

Treatment	6 hrs. Peanut butter (g)*	6 hrs. No. foraging ants*	24 hrs. Peanut butter (g)*	24 hrs No. foraging ants*	13 days Peanut butter (g)	13 days No. foraging ants
Untreated control	0.20 b	11.5a	0.10 b	1.0 a	0	0.33
4.5 cm	0.30 a	0 b	0.30 a	0.0 b	0.30	0
9.5 cm	0.30 a	0 b	0.30 a	0.0 b	0.30	0
15.5 cm	0.30 a	0 b	0.30 a	0.0 b	0.30	0
d.f = 5					d.f = 2	d.f = 2
Mean square	0.017	198.375	0.060	1.5	--	--
F ratio	4.20	4.156	10.0	3.75	--	--
P	0.0074	0.0036	0.0002	0.0106	--	--

***** Means followed by the same letter are not significantly different using analysis of variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Table 3 Average amount of peanut butter (grams) remaining and number of ants associated with the target bait (in parentheses) after 6 and 24 hrs of exposure to foraging red imported fire ants using 1) untreated control (1.2 mm thick); 2) 9.5 cm length of Spiral SG tube NS-15 (9% permethrin); and, 3) 1999 Prototype (9% permethrin)(Trial 3, replicated 6 times, initiated Nov. 13,2003, 10:43 a.m., 75^{N} F).

Treatment	6 hrs. Peanut butter (g)	6 hrs. No. foraging ants	24 hrs. Peanut butter (g)	24 hrs No. foraging ants
Untreated control	0.27	2.3	0.22	2.2
9.5 cm	0.30	1.7	0.30	0.0
1999 Prototype	0.30	1.5	0.30	1.2

Table 4. Average amount of peanut butter (grams) remaining and number of average number of ants associated with the target bait (in parentheses) after 6 and 24 hrs of exposure to foraging red imported fire ants using 1) untreated control (0.8 mm thick cylinder); 2) 4.5 cm length of Spiral SG tube NS-15 (9% permethrin); and 3) 7.8% permethrin, 0 year aged cylinder (Trial 4, replicated 6 times) initiated Nov. 13,2003, 10:43 a.m., 75^{N} F).

Treatment	6 hrs. Peanut butter (g)	6 hrs. No. foraging ants	24 hrs. Peanut butter (g)	24 hrs No. foraging ants
Untreated control	0.29	5.3	0.29	7.8
4.5 cm	0.30	6.2	0.30	3.3
7.8% 0 yr.	0.30	2.7	0.30	1.5

ASSESSMENT OF VAPOR AND CONTACT INSECTICIDAL PROPERTIES OF PERMETHRIN-IMPREGNATED NYLON TO RED IMPORTED FIRE ANT WORKERS

Bastiaan "Bart" M. Drees, Professor and Extension Entomologist and
Bill Summerlin, Technician II, Center for Urban & Structural Entomology,
Department of Entomology, Texas A&M University

Nix of America (Metro Plaza, 181 Metro Dr., Suite 590, San Jose, CA 95110; 408/971-3115; Fax: 408/971-3305; nikkonix@ix.netcom.com) has developed a process to formulate permethrin-impregnated nylon plastic parts. Previously, this company developed a 9% permethrin impregnated nylon-type part for Nissan automobiles (SG Tube 1725W) to repel spiders from internal combustion engines for an estimated 10 year period. Permethrin-impregnated nylon cylinders were formulated and aged in order to conduct a series of laboratory trials to assess vapor and contact insecticide properties on worker ants of the red imported fire ant, *Solenopsis invicta* (Buren) (Hymenoptera: Formicidae).

Materials and Methods

Fig. 1. Corning 50 ml Centrifuge Tube (Corning Incorporated, Corning, NY 14831, Item #430290)

Corning 50 ml Centrifuge Tube (Corning Incorporated, Corning, NY 14831, Item #430290), 27 mm inside diameter11.3 cm long, were prepared with a nail inserted into the side at the 37.5 mm mark so that Nix of America permethrin-impregnated nylon cylinder parts could be inserted and retained at the top of the tubes. After dusting the inside surface with talcum powder to prevent ants from crawling up the tubes, groups of 10 red imported fire ant workers were collected from laboratory colonies and placed inside the centrifuge tubes before the Nix parts were inserted and caps screwed in place. Each treatment was replicated six times and worker ant mortality was assessed at 6 and 24 hrs after trial initiation. Two trials were conducted in this manner: Trial 1 was conducted with tubes placed capped end up to assess potential vapor actions on mortaliy of worker ants separated from impregnated parts by 8 cm; Trial 2 was conducted with centrifuge tubes placed capped down so that worker ants were in contact with the insecticide-impregnated parts. Treatments used in both trials included:

1) untreated control (1.2 mm thick untreated nylon cylinder)
2) 7.8% permethrin-impregnated 1.2 mm thich cylinder aged 0 year
3) 7.8% permethrin-impregnated 1.2 mm thich cylinder aged 1 year
4) 7.8% permethrin-impregnated 1.2 mm thich cylinder aged 3 year
5) 7.8% permethrin-impregnated 1.2 mm thich cylinder aged 5 year
6) prototype 9% permethrin-impregnated SG Tube 1725W

Trial 1 was replicated 6 times and data were analyzed using analysis of variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. Trial 2 was replicated 3 times and results were not analyzed.

Results and Discussion

Higher concentration permethrin-impregnated nylon (e.g., 9% 1999 prototype and 0-1 year aged 7.8% nylon cylinders) were shown to have vapor action by significantly reducing the number of live fire ant workers in centrifuge tubes, with treatment devices placed 8 cm away from the ants (**Table 1**). Effects disappeared in parts aged 3 years or more. Conversely, contact by ants with the treated nylon resulted in ants displaying uncoordinated movement (e.g., moribund) within 5 to 6 hours and increasing mortality by 24 hours (**Table 2**).

Results of this trial are useful to argue that higher-concentration permethrin-impregnated nylon parts act as controlled-release insecticide dispensers in addition to showing only contact insecticide properties, at least for some period of time after being produced. This property of these devices can add to the effectiveness of barriers such as cable wrap spirals (e.g., SG Spiral Tubes, NS-20), if installed around bundles of wires resulting in gaps through which foraging ants can crawl. In addition, this information should be of interest in the packaging, placement and handling of these insecticide-impregnated parts.

Table 1. Average number of live ants (including moribund ants) of 10 following exposure to vapors of permethrin-impregnated nylon parts.

Treatment	**5-6 hrs.***	**24 hrs.***
untreated control	10.0 a	9.3 ab
7.8% permethrin-impregnated nylon, aged 0 years	9.0 b	5.2 cd
7.8% permethrin-impregnated nylon, aged 1 year	9.8 b	6.0 cd
7.8% permethrin-impregnated nylon, aged 3 years	10.0 a	8.2 abc
7.8% permethrin-impregnated nylon, aged 5 years	10.0 a	10.0 a
9.0% permethrin-impregnated nylon, 1999 prototype	9.8 ab	0.0 e
d.f. = 5		
Mean square	0.911	80.778
F Ratio	2.181	14.244
P	0.0167	0.000

***** Means followed by the same letter are not significantly different using analysis of variance (ANOVA) with means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Table 2. Average number of live ants of 10 following contact exposure to permethrin-impregnated nylon parts (replicated 3 times).

Treatment	**5-6 hrs.**	**24 hrs.**
untreated control	9.7	9.3
7.8% permethrin-impregnated nylon, aged 0 years	5.3*	0.3*
7.8% permethrin-impregnated nylon, aged 1 year	6.3*	0.0
7.8% permethrin-impregnated nylon, aged 3 years	6.0*	0.0
7.8% permethrin-impregnated nylon, aged 5 years	6.7*	2.7*
9.0% permethrin-impregnated nylon, 1999 prototype	4.0*	1.3*

* all ants moribund

LABORATORY ASSESSMENT OF GRANULAR BIFENTHRIN AND FIPRONIL TREATMENTS ON COLONIES OF THE RED IMPORTED FIRE ANT

Bastiaan "Bart" M. Drees, Professor and Extension Entomologist, Department of Entomology, Texas A&M University and Bill Summerlin, Technician II, Center for Urban & Structural Entomology

Fipronil granular products such as Over 'N Out and TopChoice are slow-acting contact insecticides. In field trials, 90+ percent control of colonies of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) has been achieved after roughly 4 weeks after application. Bifenthrin granular products are fast-acting contact insecticides that eliminate surface ant activity for weeks to months following treatment. However, observations from previous field trials suggest that colonies can remain active deeper underground for prolonged periods unless a rain event or heavy irrigation producing saturated soils force colonies up to the treated surface. Similar effects can be expected to result following use of other pyrethroid insecticides (e.g., permethrin, cyfluthrin, cypermethrin, es-fenvalerate, deltamethrin, lambda-cyhalothrin, and others).

How fipronil eliminates colonies below the surface with a soil surface application has not been conclusively demonstrated. This laboratory trial was conducted to demonstrate the ability of a granular fipronil treatment to eliminate colonies whereas granular bifentrin treatment eliminates primarily surface activity unless soil becomes saturated following application.

Materials and Methods

Sixteen red imported fire ant colonies were collected in 5-gallon plastic buckets, Feb. 19, 2004 from the Riverside Campus of Texas A&M University, Brazos County, Texas. Inner surfaces of buckets were dusted liberally with talcum powder to prevent ant escape. Ant colonies were extracted from soil using the water drip method and placed in laboratory colony trays where colony size, presence of queens, presence of brood and winged reproductive ants was assessed and estimated visually. Efforts were made to develop colonies uniform in size and reproductive status (number of queens, brood), and they were maintained on standard laboratory water and diet regime.

On March 3, 2004, 5-gallon plastic buckets (16 x 5-gallon white buckets, 0.70 mil, Enco, $2.67 each; 11 1/4 inch diameter, 13 3/4 inch deep inside dimensions; surface area = 99.4 sq in = 0.69 sq. ft.) were filled with 5.5 to 7.0 inches of soil (**Table 1**). Ant colonies were placed in talcum powder dusted 5-gallon buckets. They were allowed to establish nest galleries for 6 days days before treatments were applied, March 9, 2004 (completed at 3:45 p.m.). Four treatments were established, each replicated four times using four 5-gallon buckets containing ant colonies in a volume of soil measured by height (5.5 to 7.0 inches deep) and weight (21 to 30 lbs.)(**Fig 1, Table 1**).

Fig. 1. Buckets containing ant colonies

Fig.2 (right). Surface tray and centrifuge tube on plastic hose inserted in soil to monitor surface and sub-surface ant foraging activity.

Treatments, applied on March 9, included:

1) untreated control;
2) fipronil granules (Over 'N Out, GardenTech, purchased at Lowe's $19.44/10 lbs or 4.54 kg, Feb. 9, 2004). Use Rate: 2.0 lbs/ 1,000 sq ft.
3) bifenthrin granules, 0.20% (Ortho® Fire Ant Killer Broadcast Granules *sold in* Ortho® Fire Ant Combo, The Ortho Group, Marysville, OH, purchased at Lowe's $19.44, Feb. 9, 2004); Use Rate: 3.5 lb or 1.5 kg (1,000 g = 1 kg) treats 1,524 sq ft; Bucket rate: 1,500 g/1,524 sq ft = x/0.69 sq ft = 0.69 g; and,
4) bifenthrin granules (as in 3, above) to be saturated, Mar. 15, as described below.

After application of granules using a "salt shaker" using rates provided on product label directions, soil surfaces were sprayed with water using a newly-purchased pump-up compression sprayer (1 gal Eliminator Promo Sprayer , $9.97) to "water in" granules with 4 mm inch (200 mls) water using the Hudson 1 gal Eliminator Promo Sprayer (1:10 minutes spray per colony bucket). On Mar. 15, six days following initial treatment, the bifenthrin treatment #4 buckets were slowly filled (from 8:00 to 10:56 a.m.) by dripping water into buckets to bring ant colonies up in contact with the treated surface. About 1 gal. water was used to saturate the soil to the surface (colonies 10 and 11) or slightly below the surface (colony 9, 2 in below surface; colony 12, 1 in. Below surface).

Ant colonies were monitored weekly (March 10, 16, 23, 30, April 6, 13 and 20, 2004) using two methods (**Fig. 2**): 1) approximately 0.3 g peanut butter placed in a small plastic dish on the soil surface away from the inner side of the bucket; 2) 0.3 g peanut butter placed into a centrifuge vial screwed to caps glued to the end of clear plastic tubing and imbedded 3.5 inches (9.0 cm) vertically into the soil, allowing a 6-hr period to time for foraging ants to recruit to and consume this food source. In this way, both surface and subsurface ant foraging behavior could be monitored over time. The number of ants associated with the peanut butter bait and amount of peanut butter remaining were estimated in each colony bucket 6 hours after placement.

Foraging ant data were analyzed using analysis of variance (ANOVA) and means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ (SPSS for Windows 13.0). Mean percent peanut butter of 0.3 g was estimated and means calculated for each treatment.

Results and Discussion

Ant colonies were maintained on standard laboratory diet once placed in plastic buckets and had built up soil while establishing subsurface galleries. Colonies were treated March 9, 2004 at 3:45 p.m. Within 30 minutes of applying bifentrin granules and "watering in" the treatment by spraying the surface with 0.4 mm water, worker ants on the surface were uncoordinated and moribund. Ants on the surface of soil in untreated (water spray only) and fipronil treated colonies displayed normal soil working behavior. Surface and subsurface monitoring was initiated March 10 (**Table 2**). Monitoring on March 10 (post-treatment day 1), surface activity of ants in bifenthrin-treated buckets was almost entirely eliminated. This condition persisted whereas activity in fipronil treated buckets remained high and similar to surface ant activity in untreated colonies throughout the 42 day monitoring period.

Subsurface activity in bifenthril colonies was significanly different from fipronil and untreated colonies, even one day following treatment (Mar 10); but it was similar between the two bifentrin treatments (to be drenched versus not to be drenched). Following soil saturation applied March 15, 6 days after initial treatment), sub-surface ant activity in the bifenthrin-treated colonies was almost totally eliminated. In fact, by Apr. 5, only one of the four saturated bifenthrin-treated colonies had live ants before being eliminated. These ants clung to the inner side of the bucket and were not in contact with any treated surface. In contrast, colonies in bifenthrin treated buckets not subsequently saturated with water, sub-surface ant activity persisted , although at a lower level than other treatments, throughout the remainder of the trial.

These results provide support to observations made during field trials using bifenthrin or other pyrethroid insecticide applied to soil surfaces as granular or liquid formulations: If treatment is followed by a soil-saturating rain that forces ants to come into contact with the treated surface soil, colonies will be eliminated. Otherwise, imported fire ant colonies can persist for weeks following a bifenthrin treatment by dwelling deeper in the soil profile.

The surprising finding from this laboratory trial was the failure to eliminate imported fire ant colonies using a soil surface application in these 5-galon buckets during the 42 day observation period (**Table 2**). All activity was expected to be eliminated in 4 to 6 weeks. On the contrary, surface and subsurface activity persisted throughout the trial (Note that on April 20, no ants were documented in the peanut butter tray at the end of the 6-hour exposure period, but that no peanut butter remained, indicating foraging has stopped after food had been consumed). Possible explanations for this failure to eliminate colonies are:

1) ants covered the treated soil layer in the bucket soon after application and were thereafter not in contact with treated soil. This explanation is one which could be used to justify why fipronil granular products are not registered of directed for use as an individual ant mound treatment.
2) The ants found "pockets" of in treated soil in which to nest and survive. This explanation is no plausible if this compound is truly non-repellent as claimed. However, the soil or "dirt ball" used in this trial was black clayey soil that tended to "shrink" as it dried out in the laboratory and left a crack between the inner side of the bucket and the soil. Ants residing this area would survive. In field use of fipronil, observations suggest that ant colonies are able to survive in treated areas by nesting next to or underneath landscape

elements such as slabs, stones, sidewalks and other objects possibly because these colonies or their foragers do not directly contact treated surfaces.

Further trials need to be conducted to find a method to simulate, under laboratory conditions, how granular fipronil products applied to the soil surface and watered in eliminate ant colonies nesting deeper in the soil profile.

Table 1. Laboratory colony characteristics, March 4, 2004.

Colony number/Treatment	Volume of ants (mls)	Height of soil (inches)	Weight of soil (lbs)
1 untreated	250	6.5	25
2 untreated	250	6	23
3 untreated	150	6	21
4 untreated	250	5.5	20
5 fipronil	300	5.5	24
6 fipronil	200	7	27
7 fipronil	200	7	30
8 fipronil	300	6.5	25
9 bifenthrin	200	5.5	20
10 bifenthrin	100	7	29
11 bifenthrin	300	5.5	26
12 bifenthrin	200	6.5	28
13 bifenthrin drench	25-	5.5	25
14 bifenthrin drench	400	6	26
15 bifenthrin drench	200	6.5	27
16 bifenthrin drench	300	6.5	24

Table 2. Mean number red imported fire ant foraging workers associated with peanut butter (Jif®) and percent peanut butter remaining of 0.3 g provided in a plastic tray on soil surface and in a centrifuge tube glued to a hose imbedded in soil to monitor subsurface ant foraging activity in 5-galon plastic buckets in which soil surface was treated and "watered in" by spraying surface with 200 mls (4mm deep in 99.4 sq in surface area) water, March 9, 2004, with fipronil granules (Over 'N Out®), bifenthrin(Ortho® Fire Ant Killer Broadcast Granules) with and without subsequently being watered in on March 15, compared to untreated control.

Treatment, Monitored on: Mar. 10, 2004 , 9:00 am-3:00pm	**Surface activity**		**Sub-surface activity**	
	No. Ants*	**% peanut butter left**	**No. Ants***	**% peanut butter left**
untreated	60.0 b	0	837.8 bc	0.0
fipronil	78.0 b	0	1240.5 c	43.8
bifenthrin	5.0 a	100	156.8 a	95.0
bifenthrin to be drenched	10.8 a	98.5	436.5 ab	56.3

Treatment, Monitored on: Mar. 16, 2004 , 9:00 am-3:00pm	**Surface activity**		**Sub-surface activity**	
	No. Ants*	**% peanut butter left**	**No. Ants***	**% peanut butter left**
untreated	49.0 b	0	671.0 b	6.3
fipronil	53.8 b	0	603.0 b	22.5
bifenthrin	2.0 a	100	98.3 a	74.8
bifenthrin** denched Mar. 15	3.5 a	100	3.3 a	100.0

* Means with different letters are significantly different using analysis of variance (ANOVA) at $P \leq 0.05$ and separated using Duncan's Multiple Rage Test (DMRT).

** Note: Apr. 5: One of four water-saturated bifenthrin treated colony buckets contained live ants before being eliminated.

Table 2., cont.

Treatment, **Monitored on: Mar. 23, 2004 , 9:00 am-3:00pm**	**Surface activity**		**Sub-surface activity**	
	No. Ants*	**% peanut butter left**	**No. Ants***	**% peanut butter left**
untreated	36.5	0.0	712.3 b	0.0
fipronil	48.8	0.0	601.0 ab	0.0
bifenthrin	14.3	0.0	43.8 a	71.3

Treatment, **Monitored on: Mar. 30, 2004 , 9:00 am-3:00pm**	**Surface activity**		**Sub-surface activity**	
	No. Ants*	**% peanut butter left**	**No. Ants***	**% peanut butter left**
untreated	49.0	0.0	434.5 b	0.0
fipronil	52.5	0.0	516.5 b	24.5
bifenthrin	13.8	0.0	28.5 a	62.0

Note: Apr. 4, 2004, 3:04 p.m.: sprayed soil surface with 310 ml water per bucket

Treatment, **Monitored on: Apr. 6, 2004 , 9:00 am-3:00pm**	**Surface activity**		**Sub-surface activity**	
	No. Ants*	**% peanut butter left**	**No. Ants***	**% peanut butter left**
untreated	74.3	0.0	350.0 b	0.0
fipronil	83.0***	0.0***	373.8 b	0.0
bifenthrin	3.0***	0.0***	48.0 a	25.0

* Means with different letters are significantly different using analysis of variance (ANOVA) at $P \leq 0.05$ and separated using Duncan's Multiple Rage Test (DMRT).

***Plastic trays placed touching inner side of buckets

Table 2., cont.

Treatment, Monitored on: Apr. 13, 2004, 10:00 am-4:00pm	**Surface activity**		**Sub-surface activity**	
	No. Ants*	**% peanut butter left**	**No. Ants***	**% peanut butter left**
untreated	5.5	0.0	286.8 ab	0.0
fipronil	12.8***	0.0***	372.3 b	0.0
bifenthrin	16.5***	22.4***	56.8 a	25.0

Treatment, Monitored on: Apr. 20, 2004, 9:00 am-3:00pm	**Surface activity**		**Sub-surface activity**	
	No. Ants*	**% peanut butter left**	**No. Ants***	**% peanut butter left**
untreated	0.0	0.0	321.8 ab	12.5
fipronil	0.0	0.0	460.8 b	32.5
bifenthrin	0.25	0.0	111.8 a	37.5

Note: Ants fed well on surface and in tubes in fipronil and bifenthrin treated colonies, but no longer on surface at end of exposure period

* Means with different letters are significantly different using analysis of variance (ANOVA) at $P \leq 0.05$ and separated using Duncan's Multiple Rage Test (DMRT).

** Note: Apr. 5: One drenched bifenthrin treated colony bucket had live ants before being eliminated.

***Plastic trays placed touching inner side of buckets

EVALUATION OF RED IMPORTED FIRE ANT FOOD PREFERENCES IN SMALL COLONIES WITH OR WITHOUT BROOD

Bastiaan M. Drees, Professor and Extension Entomologist and
Bill Summerlin, Technician II, Center for Urban & Structural Entomology,
Department of Entomology, Texas A&M University

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) is an omnivorous social insect. Sterile female worker ants leave the colony to forage for food, retrieve the food required to maintain and increase the colony. Bait-formulated insecticide products developed to control this and other ants are based on preferred food sources, with common conventional baits utilizing soybean oil as an attractant. Observation in field trials, however, suggest that under certain conditions, this oil bait may be less attractive when colonies do not contain brood consisting of developing larvae. At those times, ants reportedly recruit more to sugary food resources. This laboratory trial was conducted to assess if food preferences would shift in colonies were brood was or was not present.

Materials and Methods

Laboratory multiple queen or polygyne colonies were exposed to carbon dioxide gas to immobilize ants and combined into a larger container (5 - gallon bucket). They were then mixed thoroughly and measured into six "colonoids" containing 1/3 cup plus 1 tablespoon ants per colony (Note: 1.9 to 2.9 or 2.5 ml ants by volume = 1,000 worker ants). In three colonoids (numbers 1 through 3), 5 milliliters of brood were added while the remaining coloniods contained no brood. Each colony contained one or more dealate females assumed to be queen ants.

Small plastic trays (weigh boats) were prepared containing approximately 0.5 ml of one of three food liquid types: 1) olive oil as a lipid source; 2) a solution of 1:3 parts honey and water as a sugar or carbohydrate source; and, 3) egg white as a protein source. Each quantity was weighed using a Metler balance before and after exposure to foraging ants from colonoids. Colonies were allowed to feed on these three food sources for approximately 24 hrs before the trays were weighed. The trial was conducted twice (Jan. 22-23, 2004 and Jan. 26-27, 2004) for 24 and 30.5 hours, respectively. At the end of the exposure periods, the number of ants in contact with each food resource was counted. Results were analyzed using analysis of variance (ANOVA) and means were separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ (SPSS for Windows, Lead Technologist, Version 13.0).

Results and Discussion

Although there were some significant pre-exposure weight differences (**Table 1**), none were between pairs of colonoids with or without brood. Colonoids consumed significantly more honey water by weight compared to other substances, and consumed significantly less olive oil. Although there were no significant differences between colonoids with or without brood in regard to food type consumed or ants recruited to a resource, numerically those with brood consumed more of each food type (lipid, carbohydrate, protein) and had more worker ants recruited to each food type.

Certainly coloniod size, amount of brood present, exposure duration and possibly other factors would likely influence results. Increasing the amount of brood in each colonoid may have resulted in greater differences than those observed in this trial. These results do not conclusively explain field observations that oil-formulated baits are less attractive to fire ant colonies with no brood. The recommended practice for proper bait application include placing a small pile of bait (or similar food substance like a potato chip or tuna fish) to be used in the area to be treated to see if foraging ants recruit to the resource within about 60 minutes. If ants are not recruiting to the bait or food lure, a number of explanations may apply: 1) The bait may not be attractive either because the ants are not "hungry" for the substance or the substance has become rancid or otherwise unpalatable; 2) Environmental conditions, such as temperature extremes, are preventing foraging ants from leaving colonies.

Table 1. Response of red imported fire ant laboratory colony or "colonoid" types (1/3 cup worker ands with and without 5 mls brood) to food resources, six replicates from 6 colonies (three from 11:00 a.m. Jan. 22, 2004 to 11:30 a.m., Jan. 23 and duplicated 11:00 a.m., Jan. 26 through 5:30 p.m., Jan. 27, 2004).

Treatment	**Pre-Exposure Weight (g)***	**Post-Exposure Weight (g)***	**Difference (g)***	**No. Ants at food source***
Olive oil (0.5 ml) w/ brood	1.0059 a	0.9952 b	0.0116 c	7.5
Olive oil (0.5 ml) w/o brood	1.001 a	0.9906 b	0.0123 c	13.2
Honey:water, 1:3 w/ brood	1.1066 bc	0.6902 a	0.4098 a	5.3
Honey:water, 1:3 w/o brood	1.0801 ab	0.6012 a	0.4780 a	10.0
Egg white (0.5 ml) w/ brood	1.0979 bc	0.8522 b	0.2458 b	7.7
Egg white (0.5 ml) w/o brood	1.1678 c	0.8739 b	0.2938 b	12.3

*****Means followed by the same letter are not significantly different using analysis of variance (ANOVA) and separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

PREFERENCE OF WORKER RED IMPORTED FIRE ANTS TO COLONY CONTAINER COLOR UNDER LABORATORY CONDITIONS

Bastiaan M. Drees, Professor and Extension Entomologist and
Bill Summerlin, Technician II, Center for Urban & Structural Entomology,
Department of Entomology, Texas A&M University

Laboratory colonies of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) can be maintained as described by Drees and Ellison (1998). However, technicians and scientists at various laboratories use slightly different practices to maintain colonies fo imported fire ants. Within the tray or container housing the colony, Petri dishes are usually placed inside to attract colonies as a nesting chamber. These dishes contain moistened plaster-like Castone bottom to provide humidity. Covers of Petri dishes have hole melted in them to allow foraging worker ants to enter and leave. Lids are often covered with a paper towel or painted black to make a darker environment to make the chambers even more attractive. However, covers obscure view of the brood and queens. This trial was conducted to see if translucent or opaque color of the lid on Petri dishes can influence attractiveness of a nesting chamber. Some nocternal insects, like cockroaches, do not detect red, and will behave in red light as if they were in total darkness. If translucent red lids were as attractive to ants as black or covered lids, ant colonies might be observed in chambers attractive as "dark" nesting sights.

Materials and Methods

Red imported fire ant colonies were shoveled into 5 gal. plastic buckets with inner surfaces dusted with talcum powder to prevent ant escape. Six colonies were removed from the field in Brazos Co., Texas (Texas A&M Riverside Campus). Water was then dripped into the buckets slowly, causing ant colonies to float on the surface. _They were removed and placed in plastic trays measuring 27 by 37 cm and 9 cm tall. The inner vertical surface of the tray was coated with Fluon® (Ag Fluoropolymers, Chadds Ford, PA) to prevent ant escape. One Petri dish (14 cm diameter and 2.5 cm tall) containing set and moistened Castone® (Dentsply International Inc. York, PA) moistened with water and with lids in which holes were melted to allow ants to enter and exit was placed in each colony tray to house the queen, brood and worker ants. Each colony was provided with distilled water.

On 22 April 2004, at 3:00 p.m., a laboratory tray (27 by 37 cm and 9 cm tall) with Fluon coating the inside vertical surfaces was established containing eight 9 cm diameter and 1.5 mm tall Petri dishes. Each had set and moistened Castone covering the lower lid with a hole melted on one side. The inner surface of the lid had been coated with colors in a set of hobby "stain glass" paints (Palmer® SunArt™ Basic Stain, Item #049006) that included the colors white, blue, red, purple, green, yellow, orange and black. No clear lids were tested. Petri dishes were positioned to the entrance holes faced outward. Roughly 1 Tbsp. of worker ants were removed from a laboratory colony, placed in the middle of the Petri dish arrangement, and allowed to select the Petri dish to colonize (Note: 1.9 to 2.9 or 2.5 ml ants by volume = 1,000 worker ants). Roughly 24 hours afterwards, presence and estimated number of ants in each dish was documented. Then, ants were again placed in the middle of the tray and the Petri dish lids were placed at new locations at random. The tray was rotated occasionally to eliminate light source as a variable that might attract a “nest” site. This trial was replicated 12 times, with new ants introduced after six replicates.

Results

Data presented in **Table 1** indicate that in each trial, most worker ants remained together in a “mass” estimated to contain over 1,000 ants. Of interest is that the frequency of ants found in Petri dishes with translucent red lids was four out of twelve, with black and orange each housing the mass two limes, white, yellow and green only one time and purple and blue never housing the ant mass. Although these results are not extremely dramatic, they do support that translucent red lids appear to provide an attractive nest site for queenless groups of imported fire ant workers relative to other colors. Certainly, translucent red is as attractive or slightly more attractive than opaque black or white lids which prevent researchers for observing colonies in chambers through Petri dish lids.

Literature cited

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Table 1. Estimated of red imported fire ant workers in Petri dishes with various translucent colored lids, beginning April 22, 2004 at 3:00 p.m. (Note: "Mass" is over 1,000 ants).

Date/Time	white	black	purple	red	orange	yellow	green	blue
Apr. 24, 10:13 a.m.	1	mass	2	1	2	1	2	0
Apr. 24, 14:45	3	6	0	5	10	mass	4	4
April 25, 14:35	2	3	0	mass	2	1	2	11
April 26, 8:15 a.m.	5	8	3	mass	3	3	6	10
April 27, 8:15 am	8	16	8	mass	32	9	9	17
April 28, 9:15 a.m.	2	mass	5	1	7	36	22	0
April 30, 10:20 a.m. - May 1, 10:20 a.m.	mass	0	2	3	4	0	3	4
May 2, 10:30 a.m.	0	1	3	0	mass	0	0	1
May 3, 13:10	5	6	2	0	6	20+	0	2
May 5, 13:25	0	0	2	mass	50+	4	1	15
May 6, 14:30	1	2	6	0	mass	1	25	0
May 7, 14:30	13	3	0	0	0	27	mass	2

LABORATORY ASSESSMENT OF PYRIPROXYFEN AND SPINOSAD IMPORTED FIRE ANT BAITS AND RESPONSE TO REMOVAL OF QUEEN ANTS FROM COLONIES

Bastiaan “Bart” M. Drees, Professor and Extension Entomologist, Department of Entomology, Texas A&M University and Bill Summerlin, Technician II, Center for Urban & Structural Entomology

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is a major urban and agricultural pest in the southern United States and other parts of the world (Brisbane, Australia and parts of Taiwan). Bait-formulated products are available to effectively suppress imported fire ant populations when applied around individual nests or mounds or as broadcast treatments applied to areas. Depending upon the mode of action of the active ingredient(s), products suppress and eliminate colonies at different rates of time. Some products (e.g., hydramethylnon, fipronil, indoxacarb, spinosad) are relatively fast-acting and eliminate colonies in days or weeks when applied as nest treatments while the insect growth regulator products (e.g., pyriproxyfen, methoprene, fenoxycarb) are slower-acting and do not perform faster when applied in higher dosages. Whereas faster acting baits eliminate queen ants, larvae and worker ants, insect growth regulators do not affect worker ants present at the time of treatment. They prevent development of worker brood (larvae and pupae) within several weeks of treatment and cause a “brood shift” where only reproductive larvae and pupae are found in treated colonies 3 to 6 weeks following treatment (Drees et al. 1992). In the absense of queen ants in colonies will also eliminate further worker brood production and cause a brood shift of developing larvae to become reproductive ants. Non-mated female reproductive ants will also drop their wings, begin laving eggs that develop into reproductive (haploid) male ants even without mating. This trial was conducted to demonstrate the effects of spinosad, pyriproxyfen and queen removal on laboratory colonies.

Materials and Methods

Twelve red imported fire ant colonies were established and maintained under laboratory conditions. On 12 May 2004 and again on 23 June 2004, three colonies each received treatment as follows:

1) untreated control
2) spinosad bait (4 Tbsp in tall Petri dish)
3) pyriproxyfen bait, Distance®/Esteem® (4 Tbsp in tall Perti dish)
4) removal of all queen ants

Colonies were maintained at room temperature (73-78 degrees F) and fed a standard dietary regime consisting of frozen crickets or mealworms and water. Colonies were inspected periodically (e.g., June 23, July 14, Sept. 10, Nov. 8, 2004) to assess presence and number of dealate (wingless) queen ants, worker ants, volume (ml) of worker brood consisting of larvae or pupae, and number of reproductive brood (Note: 1/8 tsp = 0.6 ml; 1/4 tsp = 1.4 ml; ½ tsp = 2.7 ml; 1 tsp = 5.3 ml; ½ Tbsp = 9.4 ml water) . As appropriate, data were analyzed using analysis of variance (ANOVA), with means separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ (SPSS 13.0).

Results and Discussion

Within an hour, foraging worker ants were observed and videotaped removing bait from Petri dishes placed in laboratory colonies. Less foraging was seen in pyriproxyfen treated colonies. Due to lack of attractiveness of forager ants to pyriproxyfen bait and lack of a "brood shift" in treated colonies from worker to reproductive brood, it wass suspected that the Distance® product used for the initial treatments was old , stale and ineffective. For this reason, a second treatment was made on June 23, day 42 after initial treatment thereafter considered day 0 of the second treatment (**Table 2**).

Within 48 hours of the first treatment, masses of dead worker ants were observed in spinosad treated colonies. No worker death was observed for other treatments. At the time of the second treatment, active worker ant foraging was observed and videotaped in the pyriproxyfen treated colonies, but no foraging activity was seen in colonies previously treated with spinosad bait although worker ants, queen ants and brood was still present in all three colonies. Apparently, the initial treatment eliminated foraging ant numbers, which are older sterile female worker ants, and "new" foragers had not been recruited or developed. To verify lack of forager ants in spinosad treated colonies, peanut butter was placed in plastic trays in each colony. No worker ants were recruited to the peanut butter, verifying that lack of foraging on the spinosad bait was not due to some property of this bait or "behavioral resistance" among surviving ants in these colonies. These results explain some of the inconsistency observed following spinosad bait applications in field trials and propose a possible mechanism leading to colonies surviving initial treatment.

Queen ant removal from laboratory colonies occurred over a number of days, beginning on May 13 (**Table 1**). Apparently winged female reproductive or sexual ants present in these colonies dropped or removed their wings following initial removal of wingless (dealate) queen ants. Thus, any dealate female present in these colonies was suspected to be a queen and therefore removed. This treatment was included because in the absence of queen ants, colonies

Table 1. Queens and dealate females removed from three laboratory colonies.

Colony	38119	14 May	17 May	18 May	38125
1	3	41	2	0	0
2	22	42	20	5	4
3	1	2	1	0	3

Under laboratory conditions, effects on colonies from the insect growth regulator or queen removal treatments appeared more slowly than that observed under field conditions (Drees and Barr 1992), and results were highly variable between the three replicate colonies recieving similar treatments that may have prevented separation using statistical analysis. Over the course of this trial, even untreated colonies slowly declined, typical of colonies kept in the laboratory over extended periods (**Table 2**). Relative to untreated colonies, however, those treated with spinosad displayed a dramatic redduction in worker ant numbers. However, numbers of queen or dealate female ants and worker brood volume was stable, indicating a failure of this bait product to eliminate colonies. Evidently, spinosad kills foraging ants too fast for the active ingredient to pass through colony members (moving from ant to ant via a process called trophalaxis from foraging workers to nurse ants to larvae to queen ants). Thus, by day 42 after initial treatment, colonies were "insulated" from further treatment effects due to lack of forager worker force, and colonies were capable of recovering. In this trial, only one of three colonies treated with spinosad died by Sept. 10, 121 days following initial treatment.

Pyriproxyfen-treated and queenless colonies showed similar patterns of decline following the second treatment made on June 23 using fresh product (Esteem®). The volume of worker brood was significantly less, and the number of reproductive brood was significantly greated relative to untreated colonies 21 days (3 weeks, July 14, 2004) following application. This finding is similar to that reported by Drees et al. (1992) from a field trial. Queen and worker ant numbers slowly declined, but persisted throughout this 149/107 day observation period. Field observations indicate that following an insect growth regulator application, workers persist for 5 or more weeks after a spring or early summer treatment and 5 to 6 months following a fall treatment. The last members of the colony to die are major workers and, finally, queen ants. In this trial, only one of three colonies treated with pyriproxyfen died by Nov. 8, 107 days following second treatment.

Literature cited

Drees, B. M., C. L. Barr, and S. B. Vinson. 1992. Effects of spot treatments of Logic® (fenoxycarb) on polygynous red imported fire ants: an indication of resource sharing? Southwestern Entomol. 17(4):313-319.

Table 2. Mean number of queens or dealate females, estimated number of worker ants, volume (ml) worker brood (larvae and pupae), and reproductive brood of 3 laboratory colony replicates left untreated, treated on May 12, 2004 (day 0) and again on June 23, 2004 (second treatment day 0) with spinosad, pyriproxyfen or with queen ants removed.

Observation and treatment	**Day 42/0** Jun. 23	**Day 63/21*** Jul. 14	**Day 121/79** Sept. 10	**Day 149/107** Nov. 8
Mean no. queens, dealates				
untreated	present	15.7	5	18.3
spinosad	present	7	7	9.7
pyriproxyfen	present	6	5	0.3
queenless	none	0.3	1.3	1.7
Mean no. worker ants				
untreated	present	3500	4333	668.7
spinosad	present	1683.3	666.7	116.7
pyriproxyfen	present	1683.3	3000	60
queenless	present	1666.7	600	241.7
Mean volume (ml) worker larvae & pupae				
untreated	some	12.2 a	7.9	3.3
spinosad	some	3.5 ab	5	9.9
pyriproxyfen	some	1.3 b	0.3	0
queenless	some	2.5 b	0	0
Mean no. reproductive larvae & pupae				
untreated	0	0.0 a	0	0
spinosad	0	0.0 a	0	0
pyriproxyfen	0	11.7 ab	0	0
queenless	0	33.7 b	0	0

* Means in columns of observation categories followed by similar letters are not significantly different using analysis of variance (ANOVA) and separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ (SPSS 13.0).

EVALUATION OF WEEP HOLE COVER FOR EXCLUSION OF RED IMPORTED FIRE ANTS

Bastiaan M. Drees, Professor and Extension Entomologist

A plastic weep hole cover was provided, May 1, 2003, by Jerry B. Edelman (3844 Walden Way, Dallas, Texas 75287) designed to "stop termites, fire ants, cockroaches." This trial was conducted to assess the ability of the device submitted to exclude the red imported fire ant, *Solenopsis invicta* Buren.

Materials and Methods

The plastic weep hole cover was affixed to the side of a plastic container with a rectangular cut-out on the side to allow access of insects to the inside of the device (**Fig. 1**). Ants were collected from the field and placed inside the plastic container, which was then positioned on its side within a larger square plastic container that had inner vertical surfaces dusted with talcum powder to prevent escape of any ants through the holes drilled in the weep hole exclusion device. The trial was initiated May 12, 2003 at 3:00 p.m. After 24 hours the experimental parts were placed in a freezer and the volume of ants placed within and of those that escaped the device was measured.

Results and Discussion

Smaller-sized fire ant worker ants began to emerge from holes drilled into the plastic weep hole cover almost immediately (**Fig. 2**). After 24 hrs a "volume" of 2 mm of smaller ants had escaped while 8 mm of larger ants has been excluded from escape through the holes drilled in the plastic weep hole cover. Volumetrically, 20% smaller ants would represent more ants numerically.

Red imported fire ant workers vary greatly in size. Average head capsule size of worker ants has been used to indicate the form of imported fire ants as multiple queen (polygynous) or single queen (monogynous) colonies, with those of polygyne colonies being on average smaller ants (Greenberg et al. 1955). Drees (2002) has determined that some imported fire ant workers can crawl through a hole as small as 0.5 mm or a screen mesh size of 0.51 mm.

The worker ants collected for this trial appeared relatively large suggesting the colony may have been monogyne. Had smaller ants been collected, more that 20% of the ants could have escaped from the test device. Thus, the holes in this weep hole cover design were not sufficiently small to exclude all imported fire ant workers. Reducing hole diameter would improve the device, although the number of holes would have to be increased to compensate for reduced air flow. Maintaining airflow in brick veneer homes is an important consideration and the rationale for weep holes in these structure.

Although this trial assessed only the ability to exclude ants (from escaping), use of these devices in actual homes was not assessed. This effort would also need to address the sealing of the device to the brick structure, other cracks and crevices in the structure, attractiveness of the inside of the structure to foraging ants and numbers and forms of ants around the structure.

Literature Cited

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Fig. 1. Weep hole cover affixed to test chamber.

Fig. 2. Worker ants escaping weep hole cover.

Fig. 3. Experimental apparatus for assessing weep hole cover effectiveness.