PRODUCT EVALUATIONS, FIELD RESEARCH AND NEW PRODUCTS RESULTING FROM APPLIED RESEARCH

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ABSTRACT

Three field trials conducted 1999-2000 illustrate test methodology used to evaluate product effectiveness for the control of red imported fire ants (*Solenopsis invicta* Buren). Even though test designs were different, all were arranged to minimize natural variability to help detect treatment effects. An individual mound treatment test was conducted on the product Exxant to provide efficacy data. Results showed apparent ant colony relocation followed by elimination. A broadcast treatment test was conducted on different application rates and formulations of fipronil. Results showed initial activity similar to a fast-acting broadcast bait product with long duration of control when applied as both broadcast granular and bait formulations. NouGuard, a pepper-based insect repellent was used in an ant foraging suppression study to test its effectiveness in keeping fire ants out of tree canopies. The product showed some short term ant repellence, but failed to completely exclude ants from tree canopies.

INTRODUCTION

Three tests for the control of red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera:Formicidae), are discussed here. Each had different physical designs, experimental units, products and application methods. Regardless of the type of test, we used the same method to assign treatments and group experimental units to minimize the natural, often extreme, variability in fire ant populations that could mask statistical treatment differences.

Plots were divided into blocks based on differing levels of infestation, and treatments within each block were randomized, a technique that has been used on a number of turfgrass and ornamental plant pest arthropod efficacy evaluations including the southern chinch bug (Reinert 1972), sod webworms (Reinert 1973, 1976), false oleander scale (1974), tumid spider mite (Reinert and Neel 1977), white grubs (Reinert 1979), oleander aphid (Reinert and Donselman 1981a), and bermudagrass stunt mite (Reinert and Cromroy 1981b).

Trial 1. Example of individual mound treatment evaluation: Exxant mound treatment. The individual mound treatment product Exxant® (14.2% turpentine, 0.2% ammonia, Lange Laboratories, Harrison, AR) was tested to provide efficacy data for product registration. The product was designed to appeal to the "organic" pesticide market. The test design illustrates our "railroad track" method of testing individual mound treatments (Drees and Barr 1996, 1997).

Trial 2. Example of broadcast treatment evaluation: Fipronil broadcast treatment test. This test illustrates our typical broadcast trial methodology (Barr et al. 2000, Barr et al. 1999, Drees et al. 1993) and also shows results for an important new active ingredient for fire ant control - fipronil (Aventis/Chipco Professional Products, Montvale, NJ). Previous trials showed extremely long residual activity (Collins and Callcott 1998, Sparks and Diffie 1998). This test used a low active ingredient granular formulation, as well as a bait formulation.

Trial 3. Example of evaluating repellents. Nouguard ant repellent test. Fire ants commonly forage in the canopies of trees where they are considered a nuisance and/or hazard to anyone working or standing around the tree (Tedders at al. 1990, Drees et al. 1990). Fire ants may aggravate insect problems in orchards by feeding on beneficial insects. NouGuard® (NTI International, LLC, Tequesta, FL) is a material containing 0.64% capsaicin and capsaicinoids, the "hot" in peppers, in a vegetable oil base. This product was tested to see if it would repel foraging ants and keep them out of tree canopies when applied to tree trunks.

MATERIALS AND METHODS

For all trials, experimental units were grouped into replications for both treatment and analysis purposes as follows. After pre-treatment evaluations, the resulting numbers were arrayed from lowest to highest, then divided into four equal treatment groups (replications) where one of each treatment was assigned to an experimental unit (plot) in each replication. Treatments were first randomly assigned then re-arranged within replications to equalize the total of the numbers (sum of all four replications) between treatments while still maintaining replication integrity (one of each treatment per replication). This procedure resulted in one low-density, two medium density and one high-density replication. All data were analyzed using SAS analysis of variance (ANOVA) procedures ($P \leq 0.05$) with Tukey's Studentized Range (HSD) Test for mean separations. Both treatment and replication were included in the ANOVA models.

Exxant Individual Mound Treatment. The test was established at Coulter Field, the municipal airport serving Bryan (Brazos County), Texas. The "railroad track" individual mound treatment test design, standard for our laboratory, was used. Experimental units consisted of plots of variable area containing 10-mounds each. Per-treatment areas were equalized to help detect colony relocation caused by treatments and to equalize the area available for reinvasion from outside the test boundaries.

A strip of land 10 m wide and of indeterminate length was marked with wire surveyor's flags and mowed. Beginning at one end of the strip and moving down it, flags of one color were placed just to the east side of consecutive, active fire ant mounds until 10 mounds were marked. This set of 10 active mounds constituted a plot. Flag colors were then switched for the next set of 10 and so on, alternating flag colors, until 20 plots were established. Larger flags were placed along one edge of the strip midway between the last flag of one plot and the first flag of the adjoining plot. Distances between flags were measured and recorded, thus giving a defined length to each plot. Plot lengths (areas), were then used as described above in the treatment assignment process. When diagramed, the line of plots resembles a drawing of railroad tracks, hence its informal name, "railroad track" design (Drees and Barr 1997).

Treatments included: untreated control; Exxant, 15 ml/3.8 l, applied to runoff; Exxant, 30 ml/3.8 l, applied to runoff; Exxant, 60 ml/3.8 l, applied to runoff; Organic Plus®, 60 ml/3.8 l per mound. Organic Plus® (0.02% pyrethrins, 97.9% silica dioxide, 1.1% piperonyl butoxide; Global Organic Resources, Inc., San Antonio, Texas) was included as a comparison drench-type treatment as pyrethrin plus diatomaceous earth products have shown control of treated mounds with little satellite mound formation in previous tests (Drees and Barr 1996).

Treatments were applied on 31 March 2000. Exxant solutions were mixed in watering buckets and applied to each mound until no more solution could be applied without runoff. The amount of solution applied to each plot was recorded. Organic Plus was mixed and applied similarly, but 3.8 l of solution were applied to each mound, regardless of mound size or runoff. Post-treatment evaluations of treated (marked) mounds were conducted on 1, 4, 7, 14 April and 3 May (1, 4, 7, 14 and 33 days post-treatment, respectively). Entire plots were re-surveyed at 7, 14 and 33 days to detect the presence of additional "new" (unmarked, untreated) active ant mounds.

Fipronil broadcast test. The trial was conducted at Coulter Field, the municipal airport of Bryan (Brazos County), Texas. The experimental units in this test were 0.047 ha square plots

where active mound densities ranged from 127 to700 mounds per hectare. To help reduce this five-fold difference in active mound numbers, which could easily mask treatment effects, plots were assigned so that all treatments started with almost exactly the same number of mounds, while still maintaining effective replications.

The test was established 28 April 1999. Plots were 30.5 m x 30.5 m (0.093 ha) with minimum 6.1 m untreated buffers on all sides (Drees et al. 1992). A 12.2 m radius circle (0.047 ha) in the middle of each plot was used as the sampling area. Pre-treatment counts of active mounds were conducted on 29 April with treatments applied 30 April. Granular treatments were applied with a Red Ball electric seeder mounted on a John Deere 4 x 6 Gator utility vehicle. Baits were broadcast using a hand held Cyclone 1C1 spreader. Four rates of 0.0143% active ingredient (a.i.) fipronil granular product and two rates of 0.00015% fipronil bait were tested. For clarity, treatment data from only those rates eventually used for the commercial products (Chipco® Choice granular and Firestar® Bait) are presented here. Treatments included: fipronil granular, 82.5 kg/ha (14.0g a.i./ha); fipronil bait, 1.68 kg/ha,(0.0023g a.i./ha); Talstar® 2G, (0.2% bifenthrin, FMC, Philadelphia, PA) 183.7 kg/ha (410g a.i./ha); Amdro® Fire Ant Bait, 0.73% hydramethylnon, American Cyanamid, Parsippany, NJ) 1.68 kg/ha (12g a.i./ha). Evaluations were conducted on 8 June, 21 July, 4 November 1999, and 27 April 2000 (5, 12, 17 and 52 weeks post-treatment, respectively).

Nouguard ant repellent test. The test site was a non-bearing pecan orchard in Burleson County, Texas. This trial was designed to test the effectiveness of a trunk band spray of NouGuard in keeping fire ants from moving from the ground into tree canopies. Multiple samples were included within plots to help account for expected variability between trees. Trees were also grouped into contiguous, but irregular, groups based on foraging intensity prior to treatment to compensate for this high variability.

To ensure that fire ants had to cross the treated trunk barriers, we selected trees with canopies that were not touching and cleared weeds, brush and low-hanging limbs surrounding the trunk. To detect foraging ant presence, a 2.54 cm square olive oil-soaked index card piece (bait card) (Drees 1994) was stapled to each tree trunk. After about one hour, the number of ants on each card was either counted, for very low numbers, or estimated, when many ants were present. This procedure was followed for all evaluations.

Pre-treatment counts were conducted 24 May 2000. Trees with fewer than five ants on a card were not included. Trees were grouped into plots of five adjoining trees each, giving each treatment four replications of five trees each. Treatments were assigned using the arraying procedure described above based on the sum of ants on all five bait cards in a replication.

Treatments included: NouGuard, 0.64% capsaicin and capsaicinoids; Concern® Citrus Home Pest Control (5.9% d-limonene, INTAGRA/Necessary Organics, Bloomington, MN); Lorsban® 4E, (44.9% chlorpyrifos, Dow AgroSciences LLC, Indianapolis, IN) mixed at 18 ml / 7.2 l water (label rate equivalent). NouGuard was applied by the technical representative of NTI International, Mr. Gary Higby, in two bands, each 15 - 20 cm wide and 70 - 60 cm apart using a small hand-held pressure sprayer. During evaluations, bait cards were placed in the untreated area between bands to isolate them from ants on the ground and in the canopy.

Concern, labeled as an insect repellent, was applied according to label directions using 25 - 30 pump sprays (using product container) per tree in a band approximately 15 cm wide at chest level. Lorsban, included as a standard for ant exclusion from tree canopies, was applied to the entire trunk from ground level to a height of approximately 1.5 m, sprayed to runoff using a hand pressure sprayer (Barr et al. 1991). Bait cards were placed above treatment lines on Concern and Lorsban-treated trees and at a similar height on untreated trees. Evaluations using bait cards were conducted on 1, 8, 21 June and 5 July (1, 7, 21 and 35 days post-treatment respectively). The ANOVA model included treatment effects only.

RESULTS

Exxant Individual Mound Treatment. Exxant treatments resulted in significantly ($P \leq 0.05$) fewer treated and marked active ant mounds than in untreated controls beginning at three days post-treatment and continuing for the duration of the test (Table 1). However, results suggest that these treatments may have relocated colonies initially. Though activity was eliminated in most Exxant-treated mounds by seven days, the total number of mounds (treated plus new mounds) declined only slightly. Later, these new colonies disappeared, resulting in plot-wide control similar to the Organic Plus treatment by 14 days. Significant differences ($P \leq 0.05$) in total mounds per plot disappeared by 33 days, except in Organic Plus treated plots.

TABLE 1. Mean Number of Active Marked Mounds (of 10 treated) or Marked (MD/ Total (Marked + New) Mounds Per Plot (TOT), Coulter Field, Bryan, Texas, 2000.

	Mean number mounds				
Treatment	Day 1	Day 3	Day 7	Day 14	Day 33
Untreated	9.00 a	8.75 a	8.50 a / 11.50 a	8.75 a / 11.00 a	8.25 a / 11.25 a
Exxant, 15 ml	8.25 a	5.00 b	2.25 b / 10.00 a	0.50 b / 5.50 b	2.00 b / 8.00 ab
Exxant, 30 ml	8.00 a	4.25 b	1.00 b / 9.25 a	1.25 b / 3.25 b	1.50 b / 7.00 ab
Exxant, 60 ml	6.50 a	3.25 bc	0.50 b / 7.50 ab	0.25 b / 5.00 b	0.25 b / 7.00 ab
Organic Plus	0.75 b	0.75 c	0.25 b / 3.00 b	0.25 b / 2.50 b	0.75 b / 6.50 b

Means in the same column followed by different letters are significantly different.
ANOVA statistics for treated mounds (MD) and total mounds (treated + new, MD):
Day 1: $F_{MD} = 16.84$, $P_{MD} = 0.0001$; Day 3: $F_{MD} = 9.42$, $P_{MD} = 0.0005$; Day 7:$F_{MD} = 20.54$, $P_{MD} = 0.0001$, $F_{TOT} = 4.12$, $P_{TOT} = 0.0156$; Day 14:$F_{MD} = 93.94$, $P_{MD} = 0.0001$, $F_{TOT} = 4.59$, $P_{TOT} = 0.0104$; Day 33: $F_{MD} = 31.30$, $P_{MD} = 0.0001$ $F_{TOT} = 3.82$, $P_{TOT} = 0.0204$.

Fipronil broadcast treatment test. Both formulations of fipronil and of bifenthrin performed similarly and resulted in significantly ($P \leq 0.05$) fewer active mounds versus untreated control plots over the course of the test (Table 2). Ant mound numbers in Amdro-treated plots, though still showing some control at 52 weeks post-treatment, were starting to show some reinvasion.

TABLE 2. Mean Number of Active Red Imported Fire Ant Mounds Per 0.047 ha Sample Area, for Fipronil Granular and Bait Formulations. Coulter Field, Bryan, Texas, 1999-2000.

	Mean number mounds				
Treatment	Pre-count	5 week	12 week	27 week	52 week
Untreated	15.75	14.50 a	6.75 a	9.25 a	8.25 a
0.0143% fipronil, 82.5 kg/ha	15.50	2.00 b	0.25 b	0.75 b	0.50 b
0.2% bifenthrin, 183.7 kg/ha	15.25	0.00 b	0.00 b	1.75 b	1.25 b
0.00015% fipronil bait, 1.68 kg/ha	16.00	3.25 b	0.75 b	0.75 b	1.25 b
0.73% hydramethylnon, 1.68 kg/ha	16.25	4.50 b	1.75 b	4.50 ab	5.25 ab

Means in the same column followed by different letters are significantly different.
ANOVA statistics: Pre: $F = 8.87$, $P = 0.0001$ ($F_{TRT} = 0.04$, $P_{TRT} = 1.0000$); 5 week: $F = 11.31$, $P = 0.0001$; 12 week: $F = 6.54$, $P = 0.0001$; 27 week: $F = 5.36$, $P = 0.0003$; 52 week: $F = 4.63$, $P = 0.0008$

Nouguard ant repellent test. Results in Table 3 indicate that NouGuard-treated trees had significantly ($P < 0.05$) fewer foraging ants attracted to oil-soaked cards than untreated trees at one and eight days post-treatment. However, NouGuard had significantly ($P < 0.05$) more ants than Lorsban treated trees at one day and numerically more ants than Lorsban through the remainder of the test. Concern showed little, if any, difference in ant numbers versus the untreated control trees at all post-treatment evaluations. NouGuard showed significantly ($P < 0.05$) fewer ants than Concern-treated trees at eight days post-treatment.

TABLE 3. Mean Number of Ants on 6.45 cm^2 Olive Oil-soaked Bait Cards (5 Trees X 4 Replications).

	Mean number ants				
Treatment	Pre-count	1 day	7 days	21 days	35 days
Untreated	60.00 a	32.85 a	29.85 a	29.65 ab	72.25 a
NouGuard	48.50 a	13.45 b	14.35 bc	23.80 ab	63.25 ab
Concern	45.50 a	30.25 a	28.80 ab	38.00 a	56.75 ab
Lorsban	47.00 a	0.50 c	0.40 c	13.65 b	36.30 b

Means in the same column followed by different letters are significantly different.
ANOVA statistics: Pre: $F = 0.75$, $P = 0.5260$; 1 day: $F = 19.72$, $P = 0.0001$; 7 day: $F = 11.88$, $P = 0.0001$; 21 day: $F = 5.15$, $P = 0.0027$; 35 day: $F = 4.27$, $P = 0.0077$

Though NouGuard reduced the number of ants observed on bait cards at one day and seven days post-treatment, the material did not appear to be particularly repellent to individual ants. Field notes indicated that there were ants walking across NouGuard-treated bark as early as the 24-hour evaluation and feeding trails were present across the bands by three weeks.

DISCUSSION

The method of plot assignment used in these tests was dictated by pre-treatment sampling with the same methods used to collect data throughout the tests. Factors such as soil type, water relations and vegetation that may have affected colony distribution or foraging activity were assumed to be either equal across the test or accounted for by the existing distribution. For instance, plots with high initial ant mound densities were assumed to be likely to rebound to high densities, for whatever reasons, after treatment effects declined. By accounting for natural variability before and after treatment, the effects of the treatments became much more obvious.

The technique used to define replications and assign treatments to plots based on pre-treatment levels of infestation results in a design in which treatments are randomly spread among field plots. Thus, the assumption is made that treatments do not affect population levels in neighboring plots. For individual mount treatment trials using contact insecticide treatments, effects of treatments are rapid, local and effects of treatments in one plot are unlikely to affect ant colonies in neighboring plots. However, in plots evaluating broadcast application of slower-acting bait-formulated insecticides, effects of treatments can potentially spread into neighboring plots (Drees et al. 1992). A buffer area between plots was provided to avoid any interaction between treatments, similar to buffers described for sod webworm efficacy trials (Reinert 1976).

Exxant was shown to be effective at eliminating treated colonies, though it was somewhat slow compared to other mound treatment products and appeared to relocate colonies initially. Fipronil products resulted in very good initial suppression, though slower than that of Talstar, followed by long residual activity (maximum control from 12-52 weeks).

These trials are typical examples of the type of test and methodology we used to evaluate products for the control of red imported fire ants. The designs not only tested product efficacy, but took into account other factors such as plot perimeter available for reinvasion, colony movement after treatment and natural variation in ant colony density and foraging pressure.

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