FIELD EVALUATION OF AERIAL APPLICATIONS OF HYDRAMETHYLNON AND METAFLUMIZONE TO CONTROL THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA* (BUREN), AND RELATED ANT SPECIES (HYMENOPTERA: FORMICIDAE)

A Thesis

by

AARON NEAL THOMPSON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

December 2008

Major Subject: Entomology

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Approved by:

Chair of Committee,	Roger E. Gold
Committee Members,	Julio S. Bernal
	Leon H. Russell
	James W. Austin
Head of Department,	Kevin M. Heinz

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ABSTRACT

Field Evaluation of Aerial Applications of Hydramethylnon and Metaflumizone to Control the Red Imported Fire Ant, *Solenopsis invicta* (Buren), and Related Ant Species (Hymenoptera: Formicidae). (December 2008)

Aaron Neal Thompson, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Roger E. Gold

The red imported fire ant (RIFA) was introduced to the United States from South America over 75 years ago, and has become a pest in wildlife settings. Hydramethylnon fire ant bait has been the industry standard for controlling the red imported fire ant. It can be compared to novel baits, and used to evaluate different aerial application techniques, such as the "skip swath" method. Two baits, hydramethylnon and metaflumizone, and a skipped-swath method were evaluated through observations of the activity levels of RIFA mounds and abundance**.** The effects of RIFA on other ant species were determined by eliminating RIFA with insecticides, and then sampling for all remaining ant species. Measurements of RIFA mound activity was done by recording their response to the vibration of wire flags located in active mounds. This method indicated control of RIFA with hydramethylnon and metaflumizone from 61 to 180 d post-treatment. Additional monitoring for RIFA activity, in the same plots, was done with baited vials. These results indicated that complete control of RIFA was never achieved with either hydramethylnon or metaflumizone within 180 d post-treatment; however, there were significant reductions in RIFA population as a result of both chemical baits from 3-92 d post-treatment. A

reduction of RIFA populations occurred with both baits, as determined through scheduled sampling of all ant species using baited vials. Native ants, such as *Dorymyrmex* spp., were found in higher numbers once RIFA populations were reduced, indicating that the two ant species compete for resources such as food and space. *Dorymyrmex* spp. numbers were suppressed by RIFA populations, while other ants, such as *Paratrechina* spp. were unaffected.

DEDICATION

This thesis is dedicated to my parents Tommy and Elaine Thompson, to my sister Andrea, to my brother Travis, and my fiancée Edie Boyle. Thanks for your support and for being a huge part of my life. I would also like to thank David and Anne Boyle for the permission to marry their daughter.

ACKNOWLEDGEMENTS

I would like to thank Dr. Roger Gold, my committee chairman, for accepting me as a graduate student and his guidance in setting up, completing, and helping with experiments used for this thesis. I would also like to thank my committee member, Dr. James Austin, for being available to answer questions concerning data analyses and writing. Thanks also to my committee members, Dr. Julio Bernal and Dr. Leon Russell for their ideas and suggestions concerning design of experiments and data analysis. I would also like to thank Bill Summerlin for helping me to identify ants collected and all the other graduate students including Chris Keefer, Jason Meyers, Tara McGuigan and Jennifer Pechal for their help and encouragement. I am also thankful for the Robert Jenkins, Sr. Memorial Endowed Scholarship that helped me financially while in graduate school.

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1. INTRODUCTION

The red imported fire ant (RIFA), *Solenopsis invicta* (Buren), was introduced into the United States through the port of Mobile, Alabama, in the 1930s (Buren 1972). Since then, RIFA has spread to other states, including North and South Carolina, Florida, Georgia, Alabama, Tennessee, Mississippi, Louisiana, Arkansas, Oklahoma, Texas, New Mexico, Arizona, and California (Drees and Gold 2003). The success of RIFA in new habitats can be attributed to their aggressive foraging behavior, high reproductive capability, absence of predators, and strong competitiveness with other ant species (Allen et al. 2004). Colonies in the United States may contain multiple queens, which result in larger numbers of mounds with more ants (Vinson and Sorenson 1986). There are some habitats where RIFA are not able to live, including densely wooded areas where sunlight does not reach the ground.

RIFA cause painful stings, unsightly mounds, and economic losses to agricultural crops. Constant irritation from RIFA stings affect the foraging behavior of cattle, causing them to avoid areas with high densities of RIFA. In addition to being a human and livestock pest, RIFA adversely affects wildlife, such as ground nesting birds, amphibians, reptiles, and deer (Allen et al. 2004). Ground nesting birds, for example, are often attacked and killed by RIFA soon after they hatch (Drees 2002). Recent studies in Texas showed that Northern Bobwhite Quail *Colinus virginianus* (L.) populations were reduced as a result of the spread of RIFA (Allen 1995). In addition, RIFA may cause deer fawns

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This thesis follows the style of the Journal of Economic Entomology.

to increase their movement, making them more vulnerable to coyote attacks (Mueller et al. 2001). Recent experiments examined the impact of RIFA in an environment by removing ants with baits and observing changes in the behavior and abundance of native ant species (Calixto 2007a). Those experiments showed that the use of a poison bait for *S. invicta* management benefited numerous resident species in the ant assemblage and shifted dominance by *S. invicta* over the native pyramid ant, *Dorymyrmex flavus* McCook.

The RIFA distributes food throughout the colony and its castes via trophallaxis (Lofgren et al. 1975, Cassill and Tschinkel 1995, Vinson 1983, 1997). Solid foods are carried by foragers to nurse ants that deliver the food to fourth instar larvae. Fourth instar larvae are the only members of a colony that can digest solid foods. Through trophallaxis, the larvae feed nurses that are then able to feed workers and the queen. The active ingredients in ant baits, such as hydramethylnon, act slowly so that the toxins can be transported throughout a colony and kill the queens.

Attempts to control the RIFA in the United States have relied on many methods, including residual chemical insecticides such as mirex, dieldrin, and heptachlor (Drees et al. 1996). The use of these chemicals resulted in the death of many non-target organisms, and remained in the environment for long periods of time thus were banned. Baits are a desirable method of control because they take advantage of the aggressive foraging behavior of RIFA (Allen 2004). Highly attractive baits are quickly carried back to the colonies, which often limit the bait's availability to non-target ant species. Application techniques such as the skipped swath method would decrease the amount of active ingredient applied to an area by one half by applying bait to alternating swaths (Flanders et al. 2004).

Mirex was one of the first widely distributed baits, and consisted of a corn cob grit treated with an active ingredient. Mirex bait provided +99% control in early studies, and was used to treat large areas (Lofgren et al. 1964). It was a chlorinated hydrocarbon that was applied by aircraft on hundreds of thousands of acres of land. Mirex was banned in 1977 due to its persistence and biological magnification (Johnson 1976).

Amdro® is a RIFA bait that utilizes the active ingredient hydramethylnon (Tetrahydro-5,5-dimethyl-2(1H)-pyrimidinone [3-[4 -(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl)ethenyl]-2-propenylidene]hydrazone), which is dissolved in soybean oil and applied to defatted corn grit. Amdro® was conditionally registered in 1980 for use against RIFA. Currently, Amdro Pro® (BASF Corp., 26 Davis Dr., P.O. Box 13528, Research Triangle Park, NC) is registered for use on pastures, range grasses, lawns, turfs, and non-agricultural land (Meister 2008). No published results have been done to evaluate the effectiveness of aerial applications of Amdro Pro®.

Siesta® (BASF Corp., 26 Davis Dr., P.O. Box 13528, Research Triangle Park, NC) is a RIFA bait containing the active ingredient metaflumizone (EZ-2-[2-(4-cyanophenyl)-1-(a,a,a-trifluoro-m-toly)ethylidene]-4-(trifluoromethoxy)carbanilohydrazide (IUPAC); 2-[2-(4-cyanophenyl)-1-[3-(trifluoromethyl)phenyl]ethylidine]-N-[4-(trifluoromethoxy)phen-yl]hydrazinecarboxamide), which is impregnated onto defatted corn grit (Meister 2008). Siesta® is currently an experimental use product for RIFA control, and is labeled only for research and investigational use.

The objective of this study was to evaluate the effectiveness of two baits, hydramethylnon 0.73% (Amdro Pro®) and metaflumizone 0.063% (Siesta®), for RIFA control applied aerially with the skipped swath method to a rangeland in South Texas. Additional evaluations were done to determine the impacts of those two baits on non-target ant species.

2. MATERIALS AND METHODS

Experiments were conducted in four different fields including; Kansas, Nevada, New Mexico, and Delaware on the Rollins Ranch (N28°05`79`` W98°05`75``), 400 Highway 281, George West, Texas. The ranch is managed for quail and deer, and consists of ~4,046 ha. The experimental fields were mowed one week prior to treatment with granular baits. Granular baits included 0.73% hydramethylnon (Amdro Pro®) and 0.06% metaflumizone (Siesta®) applied at a rate of 1.68 kg/ha (1.5 lb/acre). Hydramethylnon bait granules were used to treat 1,012 ha, and metaflumizone bait was applied to 2 ha. Siesta® was available only in small quantities given its limited production as an experimental active ingredient for RIFA control.

Plots were established with a minimum of three replications. Each plot consisted of either a treated swath or a skipped swath (control plot) (Figure 1). The control was bordered on either side by treated plots. Each plot was 18.3 m wide X 60 m long. Wooden stakes with colored ribbons were placed at the four corners of each plot. Stakes with orange ribbons marked the borders of treated plots, while yellow ribbons marked the control plots.

Within the plots, active RIFA mounds were identified by first placing a 50 cm long wire, with a 1.6 X 2 cm orange florescent flag into the center of each mound. The metal wire was then vibrated to determine the activity level of the colony based on the number of RIFA responding within 10 sec. An "ordered-category item", commonly referred to as a Likert scale (Likert 1932, Uebersax 2006) was used to quantify the level of activity of RIFA mounds in the test plots. This method has routinely been used in

RIFA research to assign numbers from 0-3 that categorize the number of responding ants, 10 sec after a metal wire was vibrated in the RIFA mound (Gold et al. 1996a, 1996b). In this study, responses were rated as follows: 0 = inactive (no ants responding), 1 = minor activity (1-50 ants responding), 2 = moderate activity (51-100 ants responding), and 3 = fully active (more than 100 ants responding). Only mounds producing a response of 3 were used in this study. Plots contained varying numbers of mounds due to the uneven distribution of RIFA in test plots.

The application rate of the baits was monitored closely. The initial calibration was performed in the laboratory to determine the number of granules that made up 100 mg of each bait. Five 100 mg samples of each bait were weighed, and the mean number of granule in each sample were determined. Calculations were then made to determine the number of granules that should be applied per m^2 at the rate of 1.65 kg/ha (1.5 lbs/acre).

In the field, 45.72 X 45.72 cm adhesive calibration boards were placed on the ground in all treated and control plots. The top sides of the calibration boards were covered with an adhesive (Con-Tact Brand Kittrich Corporation, 14555 Alondra Blvd., La Mirada, CA). The designated plots were treated with granular baits using a Robinson R22 Betta helicopter, traveling at 12 m above the ground at 96 km/hr. Following application, counts from the calibration boards were used to determine the amount of bait actually applied with the helicopter based on the number of granules per m^2.

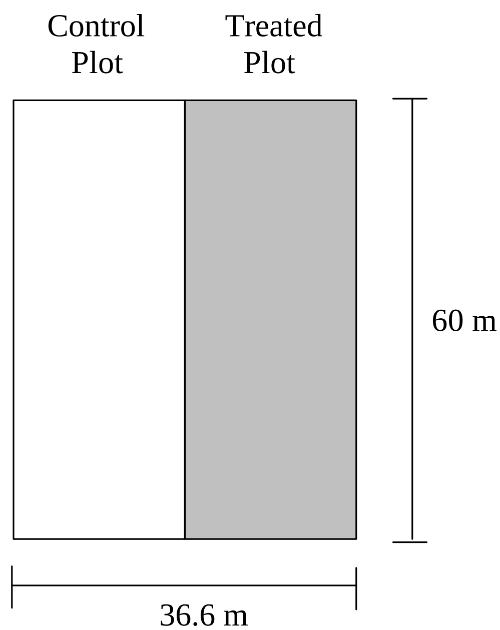

Figure 1. Diagrammatic representation of plots used in experiments.

2.1 Mound activity experiment: Part 1. The first part of this experiment tested the effectiveness of hydramethylnon on RIFA mounds, and included hydramethylnon treated and control plots. There were a total of three replications with each replication consisting of two plots. The activity level of mounds, which had been marked with numbered flags, were evaluated and data were recorded at 0 d, and then at 1, 3, 7, 10, 17, 21, 28, 61, 92, 123, 154, and 180 d post-treatment in the Kansas, Nevada and New Mexico Fields.

2.2 Mound activity experiment: Part 2. The second part of this experiment tested the effect of metaflumizone on RIFA populations. Part 2 of the experiment was set up identically to Part 1 except metaflumizone was used instead of hydramethylnon, and all of the metaflumizone plots were located in the Delaware field. By comparing the results of Part 1 and 2 of the experiment, it was possible to determine the effectiveness of the two baits on RIFA control and the skipped swath method.

There were two different applications of hydramethylnon in this study. The first was done on April 11, 2007; however, because of low mortality of RIFA within 10 d, the second treatments were made on May 14, 2007. The first applications were considered ineffective, so new plots were established. Metaflumizone was added to the experimental design and the second set of applications was made.

2.3 Sampling for ant species diversity and abundance. Ant species diversity and abundance was determined by sampling the plots before and after application of the hydramethylnon and metaflumizone baits. The treated plots and control plots were sampled at -12 d (pre-treatment), and then at 1, 3, 7, 10, 17, 21, 28, 61, 92, 123, 154, and 180 d post-treatment. Two 17 mm X 60 mm threaded glass 8 ml vials (Fisher Scientific

International Inc., Hampton NH) were taped together with opposing ends 180° apart. One vial in the set was baited with a cotton swab soaked in 50% honey water solution, and the other with a protein-rich food source (Vienna Sausage, Pinnacle Foods Corporation, Cherry Hill, NJ). Each vial set was assigned a number that corresponded to a location within a plot. A total of 10 vial sets were placed in each plot at designated sites. The vial sets were placed every 3 m in a row, and the locations of the vials were marked with a flagged wire. The vial sets were placed in the plots at approximately 8:00 am and collected at 10:00 am on sampling days. The vial sets were quickly sealed with a cap as they were collected. The vial sets were then stored in a freezer until the ants could be identified and counted.

2.4 Statistical analysis. SAS software (SAS 2006) and SPSS software (SPSS 2005) were used to conduct all statistical analyses. Statistical analysis was performed on the number of granules that were applied to calibration boards. A one-way ANOVA was first performed to determine if there was a significant difference between treatments followed by The Waller-Duncan k-ratio t test. Kruskal-Wallis one-way analysis of variance (Kruskal and Wallis 1952) was performed on Likert values associated with the different treatments and dates throughout this study. The Kruskal-Wallis one-way analysis of variance by ranks is a non-parametric method for testing equality of population medians among groups. Intuitively, it is identical to a one-way analysis of variance with the data replaced by their ranks; It is an extension of the Mann-Whitney U test (Mann and Whitney 1947) to 3 or more groups. In this study we had 4 different treatments that were considered and are the basis for its use. Since it is a non-parametric method, the Kruskal-Wallis test does not assume a normal population, unlike the analogous one-way analysis of variance. However, the test does assume an identically-shaped distribution for each group, except for any difference in medians. Post hoc analyses applying Tukey's HSD were applied to RIFA mound Likert scale values to determine significant differences and means were separated at the a = 0.05 level. Both the general linear model (PROC GLM) and a repeated measures analysis (SPSS 2005) were applied to ant data to evaluate ants collected during sampling with vial sets. Ant count means evaluated by PROC GLM were evaluated using Tukey's Studentized Range Test and *Post hoc* tests applying Mauchly's sphericity test (Mauchly 1940) and Multivariate analyses (Wilk's Lambda) were considered to evaluate within-subjects main effects and between-subjects main effects. The multivariate output is considered if the sphericity assumption is not met (SAS 2006). The null hypothesis is that the mean RIFA numbers do not change across different times. RIFA sampled from vial sets were evaluated by ANOVA followed by paired evaluations. All possible comparisons were made between hydramethylnon and metaflumizone treated plots and their respective (adjacent) control plots.

3. RESULTS

3.1 Comparison of targeted versus actual application rates. In the laboratory it was determined that the mean numbers of granules in 100 mg of hydramethylnon and metaflumizone were 56.6 and 65.8, respectively. Thus, for the targeted application rate of 1.65 kg/ha, hydramethylnon should have been applied at a rate of 98.5 granules/m^2 (1.5 lb/acre), while metaflumizone should have been applied at a rate of 110.6 granules/m^2(1.5 lb/acre) (Table 1). However, results indicate that both baits were under-applied. The hydramethylnon treatment calibration boards had a mean of 16.3 granules per board, indicating a mean application rate of 77.9 granules/m^2, 79% of the desired amount of bait applied per unit area. Metaflumizone had a mean of 13.7 granules per calibration board, indicating a mean application rate of 65.6 granules/m^2, 59% of the desired amount of bait applied per unit area (Table 1). Metaflumizone bait was lighter than hydramethylnon, and was apparently more difficult to apply accurately with the helicopter. The Waller-Duncan groupings demonstrated that the mean granule counts collected on calibration boards were significantly higher ($P < 0.05$) for hydramethylnon treated plots than all other treatments (Table 1). Metaflumizone treated plots had a higher mean number of granules than control plots of both hydramethylnon and metaflumizone, but were not significantly different.

Table 1. Comparison of ant bait granules collected on calibration boards from aerial applications of hydramethylnon and metaflumizone fire ant baits using a helicopter at ~ 96 km/h and 12 m above designated treatment plots.

Mean[3]	N	Label Rate (Granules/m^2)	Actual Rate (Granules/m^2)	Bait/Method	Grouping Method	
					Waller[1]	Duncan[2]
16.3	7	98.5	77.9	Hydramethylnon Treated Plots	A	A
13.7	7	110.6	65.6	Metaflumizone Treated Plots	AB	AB
1.0	3	--	4.8	Hydramethylnon Control Plots	B	B
0.0	3	--	0.0	Metaflumizone Control Plots	B	B

[1]Waller parameters include: *K* ratio = 100, df = 16, Error Mean Square = 83.55, F = 3.62, Critical Value of *t* = 2.28, Minimum Significant Difference = 14.39, Harmonic Mean of Cell Sizes = 4.2. Note that cell sizes are not equal.

[2]Duncan multiple range test parameters are identical to Waller groupings. The critical range(s) were 13.37, 14.02, and 14.43 for 2, 3 and 4 means, respectively. Note that this test controls the Type I comparisonwise error rate, not the experimentwise error rate.

[3]values are actual granule means from cards.

3.2 Effectiveness of granular baits on RIFA p[opulations. All of the RIFA mounds within the plots had an initial Likert scale value of 3.0 at the pre-treatment sampling date (Figure 2). Likert scale values decreased through time indicating diminished activity of RIFA in the mounds being monitored.

Results for RIFA mound activity grouped by Likert Scaled values show significant differences due to time (Kruskal-Wallis H = 467.371; df = 3; P = 0.000), but failed to show significant differences due to treatment (Kruskal-Wallis H = 3.566; df = 3; P = 0.312). As there were no differences observed among treatments, groups were evaluated collectively to observe the general decline of RIFA populations. Results showed that fully treated swaths had sufficient bait to suppress or arrest RIFA in skipped (control) swaths, which were adjacent to fully treated swaths.

A repeated measures ANOVA (SPSS 2005) was applied to evaluate RIFA decline due to time and the significant time*treatment interaction. Mauchly's sphericity test (Mauchly 1940*)* and epsilon adjustment values demonstrate that assumptions of sphericity were indeed violated (i.e., the Chi-square approximation has an associated p-value less than the alpha level, 0.05; x^2 approximation = 2406.279; df = 77; $P > x^2$ = 0.0000); therefore, the multivariate analyses were most appropriate to evaluate these differences (SAS 2006). The within subject tests indicate that there was a significant time effect for RIFA counts (Wilks' Lambda = 0.363; F = 21.092; df = 12, 144; P = 0.000), and the interaction of RIFA counts*treatment (Wilks' Lambda = 0.395; F = 4.371; df = 36, 426.192; P = 0.000). In other words, RIFA activity based on mound evaluations changed over time. The between groups test indicates that there was no significant differences among treatments. We therefore reject the null hypothesis, and conclude that RIFA numbers change with time in the population from which the samples were drawn. This fact was also supported by applying the general linear model to ranked groups for the dates mounds were monitored (F = 332.39, df = 11, P = < 0.0001), and the interaction effect for treatment*date (F = 2.46, df =33, P < 0.0001). Similarly, there was no significant difference between the metaflumizone treatment and metaflumizone control plots (Table 2). Metaflumizone treated plots resulted with lower activity than all other treatments (mean = 0.51, n = 228), but were not significantly different from hydramethylnon treated plots (mean = 0.59, n = 252). Metaflumizone treated plots had the lowest mean number of active RIFA mounds (Likert scale), followed by hydramethylnon treated, metaflumizone control, and hydramethylnon control plots (Table 2).

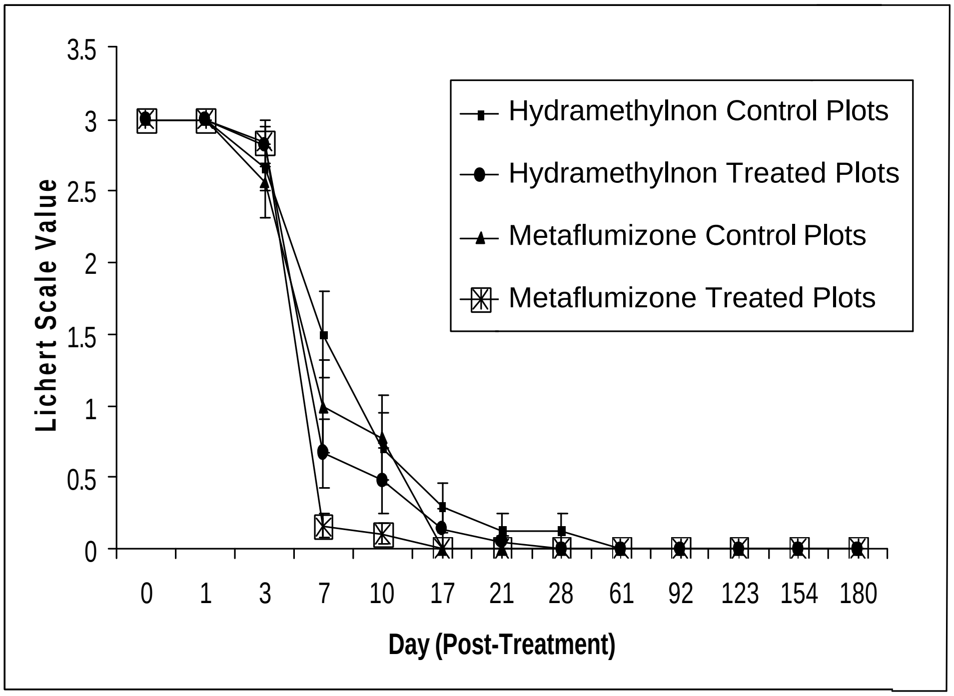

Figure 2. Temporal changes in RIFA mound activity (Likert Scale values) in hydramethylnon and metaflumizone plots in S. Texas in 2007.

Table 2. ANOVA of mean Likert scale values on mound activity in hydramethylnon and metaflumizone plots (± MSE) over 180 d (F = 5.62; df = 3; P = 0.0008).

Treatment[1]	Number of mounds	Mean[2]
Hydramethylnon Control	288	0.7 ± 0.07a
Metaflumizone Control	216	0.6 ± 0.08ab
Hydramethylnon Treated	252	0.6 ± 0.07ab
Metaflumizone Treated	228	0.5 ± 0.07b

[1]Hydramethylnon at 0.73%, Metaflumizone at 0.06%, Hydramethylnon control was an untreated area with similar width between treated plots, and Metaflumizone control was an untreated area with similar width between treated plots

[2] Means followed by the same letter are not significantly different (P < 0.05) by Tukey's Test.

3.3 Sampling with vials. Applying the General Linear Model (Proc GLM, SAS) to all ant species collected in vials observed significant differences ($P < 0.05$) for; the number of ants collected on different days ($P < 0.0001$), species ($P < 0.0001$), treatments ($P < 0.0001$), sampling methods ($P < 0.0001$), and locations (field where applied) ($P = 0.016$). The species and numbers of ants sampled on different days (Figure 3), and the relative proportions of ant species collected are in Figure 4. The mean number of ants collected for different treatments, sampling methods, and locations are in Tables 3 - 5.

A One Way ANOVA was applied to RIFA counts from sampling vial sets in all the treatments and found no significant differences. Furthermore, several paired evaluations were considered to determine if any other differences could be found. The only paired comparison resulting with differences in the numbers of RIFA collected were between control plots of hydramethylnon and metaflumizone. Hydramethylnon control plots recovered three times as many RIFA as metaflumizone plots.

3.4 Hydramethylnon plots. Figures 5 and 6 show the effects of hydramethylnon on the number of foraging RIFA. Hydramethylnon treated and control plots had reduced populations of RIFA within 1 d post-treatment. Baits applied in the treated plots caused slightly higher levels of control than control plots. RIFA populations in both treated and control plots had consistently lower levels through 90 d, after which populations began to increase. RIFA populations continued to increase with each sampling period through 154 d as shown in Figures 5 and 6.

3.5 Metaflumizone treated plots. Metaflumizone had similar effects to hydramethylnon on populations of RIFA in treated and control plots (Figures 7 and 8). Both metaflumizone treated and control plots had reduced numbers of RIFA collected by

3 d post-treatment. RIFA populations were maintained at low numbers until 92 d post-treatment, after which the number of RIFA increased in metaflumizone control plots. Metaflumizone treated plots maintained low numbers of RIFA through 123 d post-treatment (Figure 7).

All ant species had reduced populations by 1 d post-treatment in metaflumizone treated and control plots, based on the use of vial sets to sample populations (Figure 9).

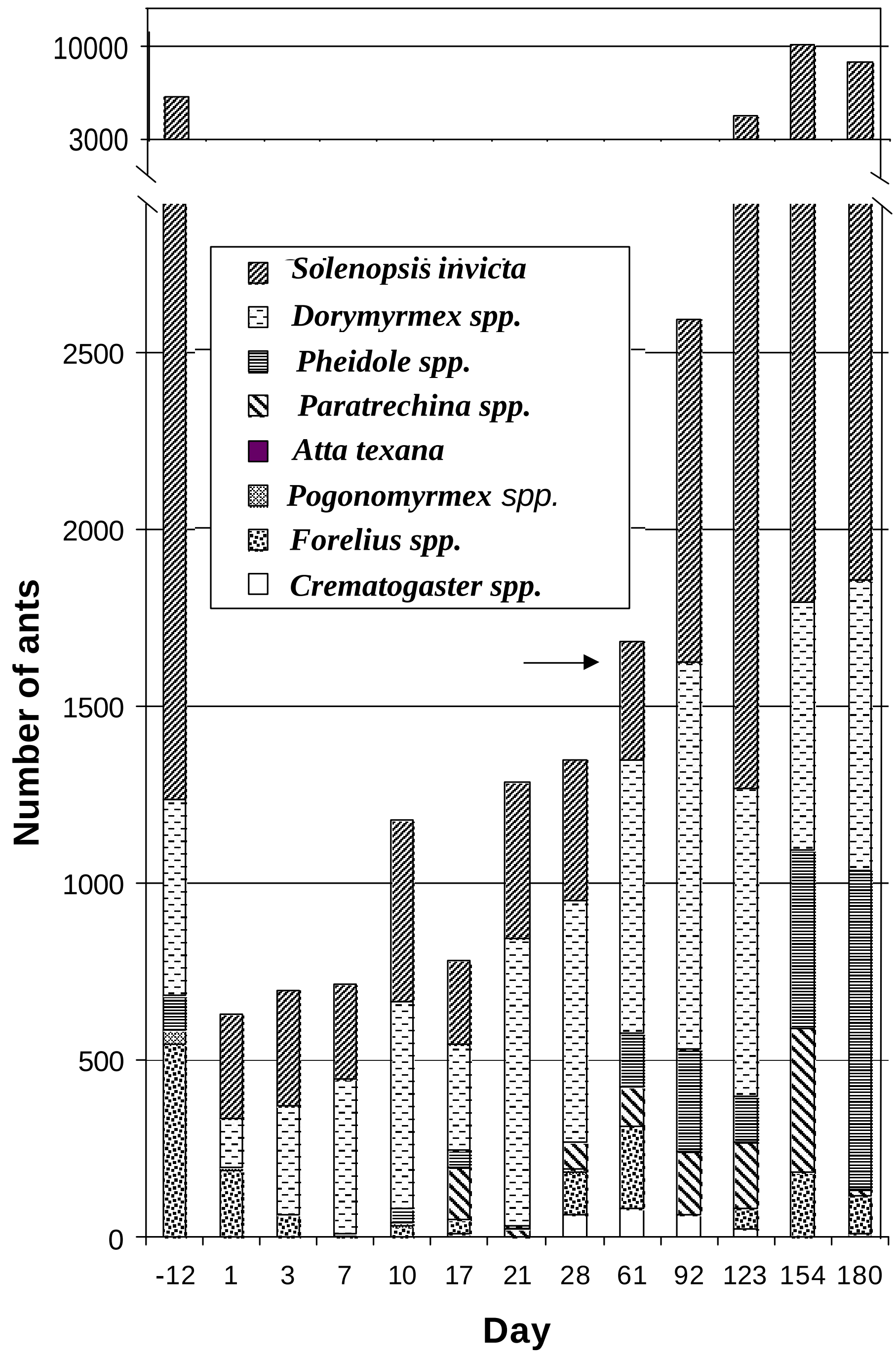

Figure 3. Ant diversity and abundance in hydramethylnon and metaflumizone treatments through time. Day 1 = May 15, 2007 (1 d post-treatment). Arrow indicates date where a single *Atta texana* specimen was recovered in baited ant counts.

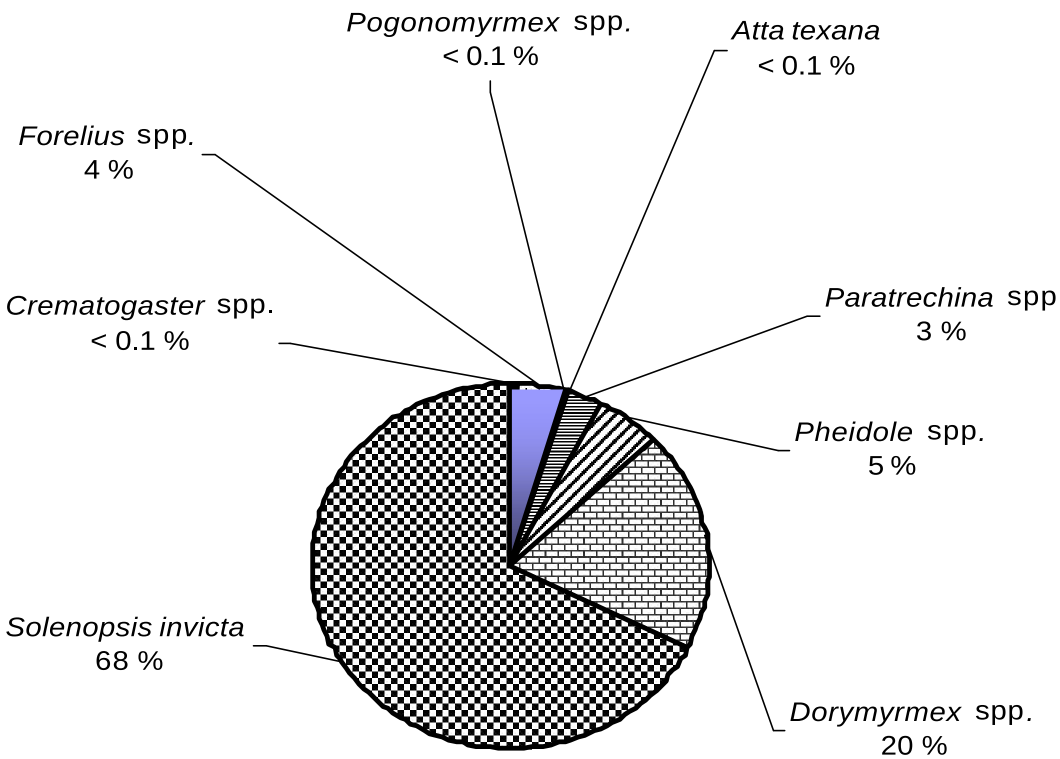

Figure 4. Proportions of all ants collected during sampling.

Table 3. Analysis of ant counts, regardless of species, in treatments (± SEM).

Treatment	Number of sampling vials	Mean number of ants/ sample vial
Metaflumizone Treated	506	20.4 ± 1.64a[1]
Hydramethylnon Control	747	16.5 ± 1.37b
Hydramethylnon Treated	754	15.1 ± 1.46b
Metaflumizone Control	513	13.3 ± 1.64b

[1] Means followed by the same letter are not significantly different ($P < 0.05$) by Tukey's Test.

Table 4. Analysis of ant counts in vials containing sausage and honey water (±SEM).

Sampling Method	Number of Sampling Vials	Mean number of ants/vial
Sausage	1254	25.9 ± 1.29a[1]
Honey Water	1266	6.6 ± 0.54b

[1]Means followed by the same letter are not significantly different ($P < 0.05$) by Tukey's Test.

Table 5. Analysis comparing mean ant counts in different locations (± SEM). Hydramethylnon plots were within Kansas, Nevada and New Mexico fields and metaflumizone plots were located in the Delaware field on the Rollins Ranch in S. Texas.

Field	Number of Sampling vials	Mean number of ants/ Sampling vial
Kansas	506	21.5 ± 2.1a[1]
Nevada	747	17.5 ± 1.8b
Delaware	754	16.8 ± 1.0b
New Mexico	513	8.4 ± 1.1c

[1] Means followed by the same letter are not significantly different ($P < 0.05$) by Tukey's Test

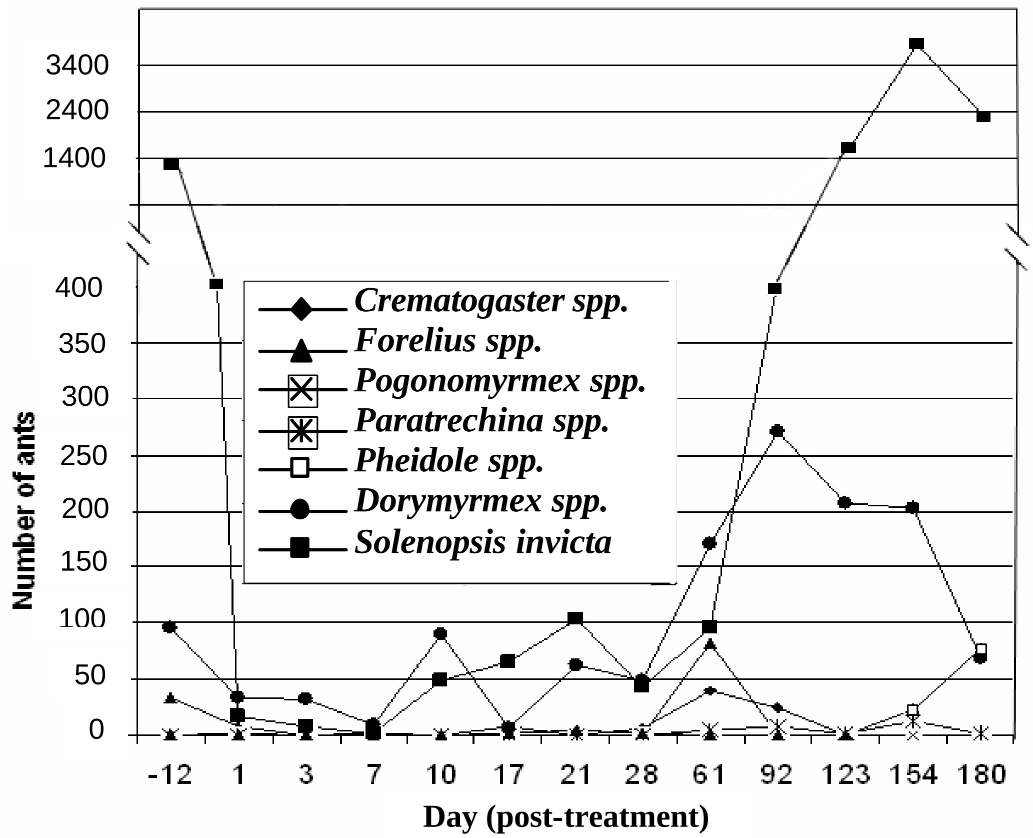

Figure 5. Ant diversity and abundance in hydramethylnon treated plots through time.

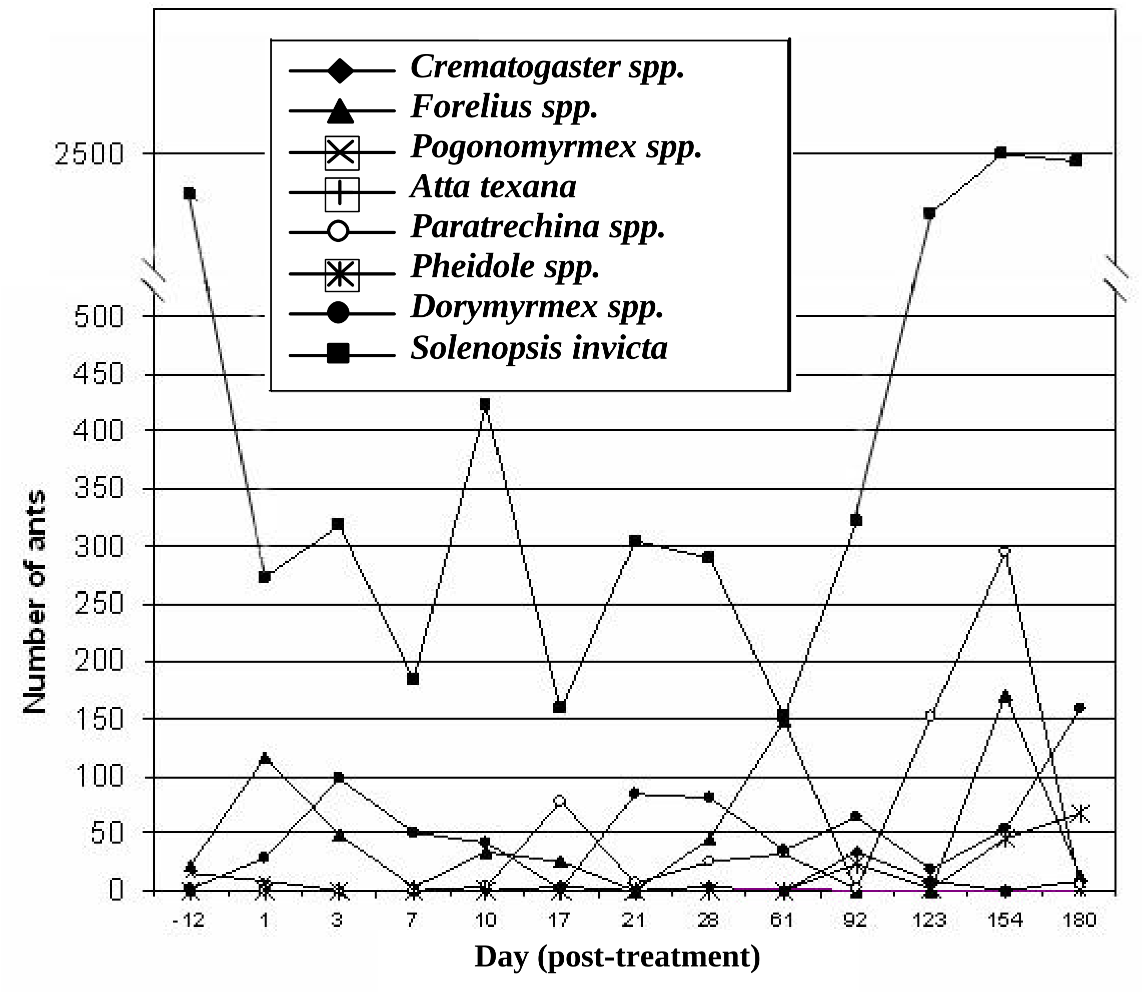

Figure 6. Ant diversity and abundance in hydramethylnon control plots through time.

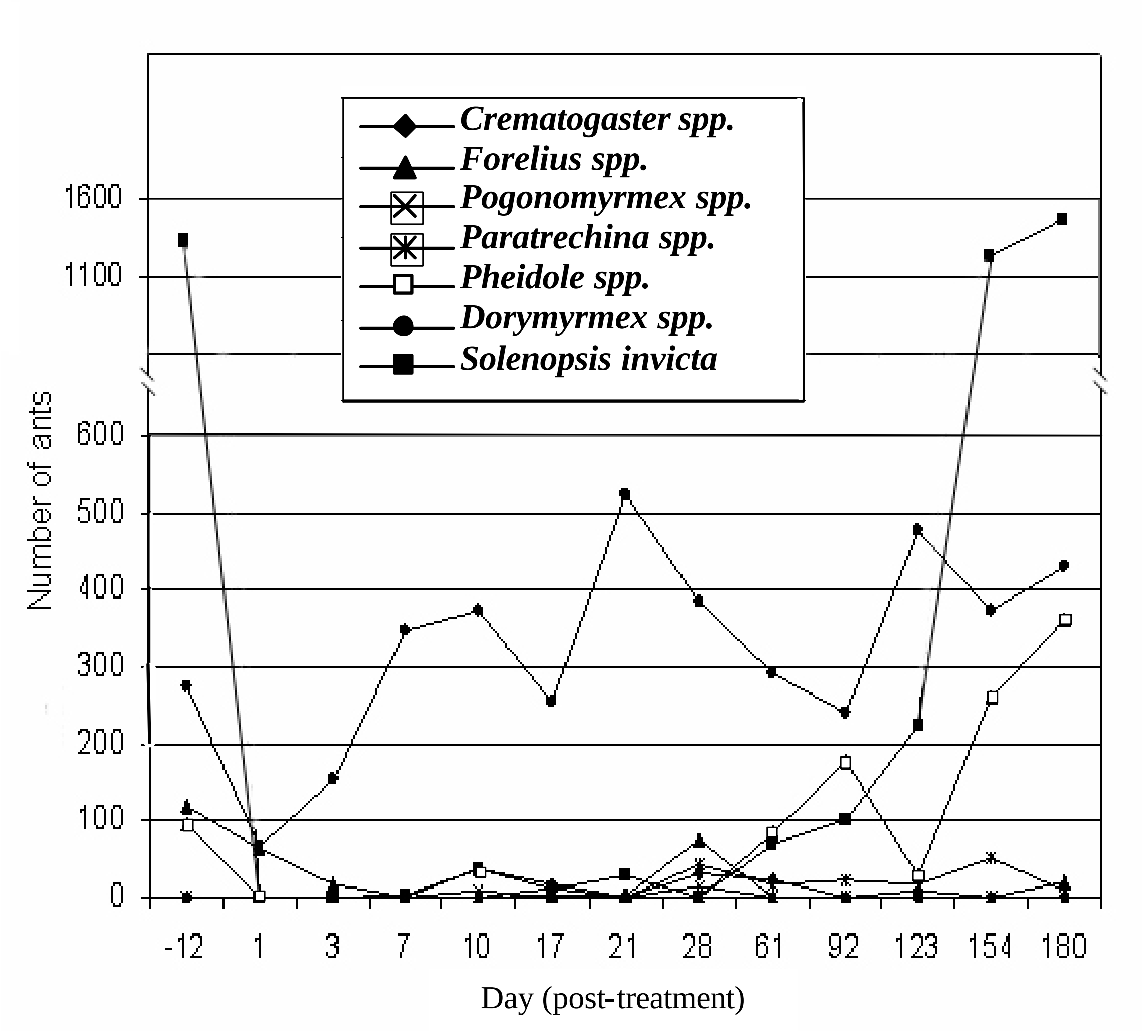

Figure 7. Ant diversity and abundance in metaflumizone treated plots through time.

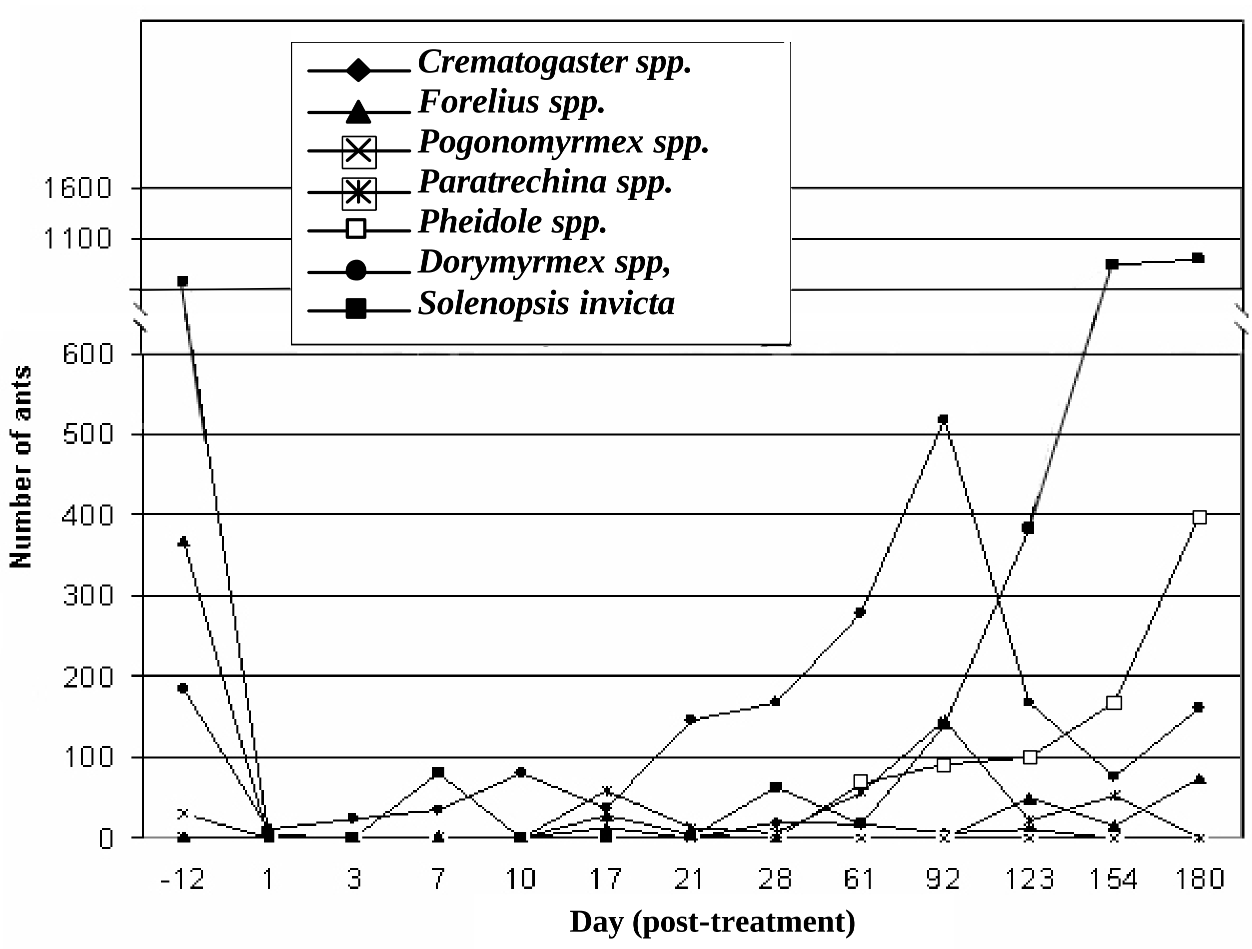

Figure 8. Ant diversity and abundance in metaflumizone control plots through time.

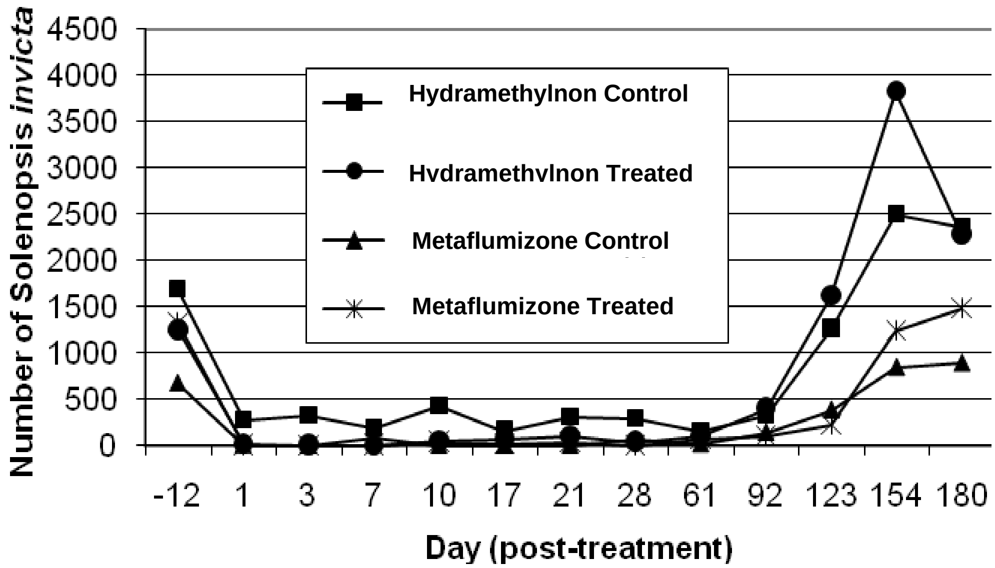

Figure 9. Number of *Solenopsis invicta* collected within treatments during sampling days. Day 1 = May 15, 2007 (1 d post-treatment)

4. DISCUSSION

4.1 First application. The importance of using fresh bait was evident in early attempts to perform this work. Plots had been established and bait was applied via helicopter on April 11, 2007. According to the ranch manager, the bait did not achieve adequate control of RIFA within 10 d, therefore, the experiment was terminated. The reason for the poor performance of the bait was undetermined; however, based on the lot numbers on the bait bags, the bait may have been too old or unpalatable to ants. The soybean oil in the bait may have become rancid and unattractive to RIFA. Most RIFA bait manufacturers claim that their products have a shelf life of 2 to 3 years, if the packages remain unopened (Barr 2005); however, the bait would still need to be stored in a cool, dry area. Distributors frequently retain inventory beyond expiration dates recommended by manufacturers, and care should be given to determine production dates to avoid this problem. All further discussions of this work are based on the second application series initiated on May 14, 2007.

4.2 Calibration. When evaluating the number of aerially applied granules which were captured by calibration boards for RIFA control, significant differences (P = 0.0362) were detected by ANOVA (SAS Institute 2006). The Waller-Duncan *k*-ratio *t* test, a multiple range test, was applied to means because of its ability to compare both Type I and Type II error rates based on Bayesian principles (Steel et al. 1997). The Waller-Duncan groupings demonstrated that the mean granular counts were significantly higher ($P < 0.05$) in hydramethylnon treated swaths as compared to the untreated plots for both chemicals (Table 1); however metaflumizone treated plots were not significantly different than hydramethylnon and metaflumizone untreated plots. This was likely because of the calibration boards, located within the metaflumizone treated plots, that did not record any granules (Table 1). Similar results were observed applying Duncan's Multiple Range Test (Table 1). This test controls the Type I comparisonwise error rate, not the experimentwise error rate (SAS Institute 2006). The calibration boards, used to determine the amount of bait that was actually applied to each plot in the field, demonstrated that both baits were under applied**.** The low mean number of granules applied to metaflumizone treated plots was likely due, in part, to two calibration boards located in the corner of a plot that received no bait granules, thus skewing the recovery data. The rest of the metaflumizone treated plots received much higher application rates. Weights of metaflumizone and hydramethylnon baits may have contributed to the overall delivery distribution on applied fields. The metaflumizone was lighter than the hydramethylnon bait, and was difficult to apply with a helicopter**.** In the laboratory, it was determined that the mean numbers of granules in 0.1 g of hydramethylnon and metaflumizone were 56.6 and 65.8, respectively**.** While the baits may not have all been deposited in the treated plots, they remained effective in controlling ants on the ranch. The weights of baits are particularly important given that aerial applications for another invasive fire ant species, little fire ant, *Wasmannia auropunctata* (Dennis) were targeted more effectively by adjusting bait weights (Causton et al. 2005), and because the capability to distribute granular baits aerially has improved. Multiple examples of systems that demand aerial delivery of baits are known (Farry et al. 1995, Campbell et al. 2006). On Christmas Island, for example, aerial application of the ant bait Presto® was used to effectively manage *Anoplolepis gracilipes* (Green 2002). Historical perspectives on the use of baits for RIFA have already been documented (Summerlin et al. 1977).

In one instance, three granules of bait were found on a calibration board present in a hydramethylnon control plot. Wind gusts may have caused the granules to drift into the control plot while bait was being applied to treated plots in adjacent areas. Calibration boards present in the metaflumizone control plots did not receive any granules of bait. Loss of baits into unplanned areas may have profound consequences for controlling RIFA when granules land in areas that negatively impact the integrity of baits, whether altering their size, oil content, moisture content or availability of active ingredient. The occurrence of baits and their compositions are important in sustaining palatability and attractiveness for sustained RIFA control. Furman et al. (2006) demonstrated that both grit size and concentration of active ingredient can affect RIFA foraging and control which may be important when time considerations are a factor. These findings were also supported by the work of Barr (2003).

4.3 Monitoring mounds. One of the principle goals of this research was to determine if there are any differences in the observed control of RIFA in pastureland, via aerial application by helicopter, between an industry standard such as hydramethylnon 0.73% (Amdro Pro®) and and the experimental active ingredient metaflumizone 0.06% (Siesta®). The General Linear Model (PROC GLM) of SAS (SAS Institute 2006) was used to evaluate the impact of granular applications on the activity of RIFA mounds. Four levels of treatment and 13 levels of time were evaluated from May 14, 2007 through November 12, 2007. Over this 6 month period, there were significant differences detected for treatments (P = 0.0008), and for the dates that mounds were monitored (P =

< 0.0001); There was also a significant interaction effect for treatment*date (P < 0.0001). Although metaflumizone treated plots had the lowest mean activity, they were not significantly different from hydramethylnon plots. Although evaluating scaled values is not generally preferred due to statistical considerations, logistically they are more practical when dealing with RIFA under field conditions. Evaluation of date (days after application) demonstrated that significant differences were observed as early as 2-days post treatment, with significant differences recorded in mound activity occurring throughout the month of May (last date sampled in May was May 31, 2007).

4.4 Sampling with baited vial sets. The effectiveness of hydramethylnon and metaflumizone bait products were determined through sampling in treated and untreated plots (control plots). It has been previously reported that broadcast treatments for RIFA control needn't be continuous to elicit the desired level of control due to RIFA foraging (Drees et al. 1993). This is particularly important since larger volumes of bait, more time and needless expense would be required to broadcast continuously over large areas, equating to significantly higher investment costs for RIFA management. All possible comparisons were made between hydramethylnon and metaflumizone treated plots and their respective (adjacent) control plots. ANOVA demonstrated that there were no significant differences among all treatments. Furthermore, several paired evaluations were considered to determine if any other differences could be found. The only paired comparison resulting with differences in the numbers of RIFA collected were in control plots adjacent to hydramethylnon and metaflumizone treated plots, respectively;.Significant differences ($P = 0.0429$) were found between hydramethylnon and metaflumizone controls (skipped swaths), with three times as many RIFA recovered from baited vials located in hydramethylnon control plots. Results of these comparisons suggests that both bait products were equally effective at controlling RIFA for prolonged periods of time. There was no significant difference between either treatment and their respective controls (control plots). This implies that continuous broadcast treatments would not be more effective at controlling RIFA than alternating swaths (skipped swaths).

The decrease in RIFA populations affected the abundance of other ant species in the hydramethylnon treated and control plots. *Paratrechina* spp. had not been collected in the hydramethylnon treated or control plots before RIFA populations were reduced with baits. At 10 d post-treatment, *Paratrechina* spp. were collected for the first time in hydramethylnon control plots (Figure 6). Similarly, *Paratrechina* spp. were collected in hydramethylnon treated plots for the first time at 61 d post-treatment, and then during all sampling periods through 180 d (Figure 5). *Dorymyrmex* spp. and RIFA were collected in hydramethylnon treated and control plots throughout the experiment. Populations of *Dorymyrmex* spp. increased as RIFA populations decreased in hydramethylnon control plots (Figure 5). This inverse relationship provides evidence that RIFA populations suppress *Dorymyrmex* spp.

There was an interaction between populations of RIFA and *Dorymyrmex* spp. in the metaflumizone control plots; Within metaflumizone control plots, there was an increase in the populations of *Dorymyrmex* spp. while RIFA populations were reduced by RIFA baiting. Then, as RIFA populations recovered, populations of *Dorymyrmex* spp. decreased sharply (Figure 3). Similar trends were recorded for *Paratrechina* spp. in the metaflumizone control plots. *Paratrechina* spp. populations increased as RIFA populations decreased, then decreased sharply as RIFA populations increased. This interaction was not seen in metaflumizone treated plots, although populations of *Dorymyrmex* spp. increased while RIFA populations were reduced. Again, these results support the concept that RIFA decrease the populations of other ant species.

There appears to be a slight difference in the rate at which ant populations were reduced, with metaflumizone being slightly faster than hydramethylnon in RIFA colony control. All marked mounds present in metaflumizone control and treated plots had a Likert scale value of zero, while hydramethylnon treated and control plots still had active mounds 17 d post-treatment. Metaflumizone treated plots had the lowest post-treatment Likert scale values, but both metaflumizone and hydramethylnon had diminished ant populations by 7 d post-treatment (Figure 2)**.**

RIFA are omnivores that feed on carbohydrates, proteins, and lipids. Their diet is dictated by the nutritional requirements of the colony. A colony that is producing new offspring, for example, will require larger amounts of protein. Research done in Texas indicates that RIFA prefer protein-rich food sources when temperatures are warm, but carbohydrates are selected during cooler months when colonies have lower reproductive rates (Stein et. al 1990). During sampling intervals with vials sets containing Vienna sausage and honey water, there was a clear preference for the protein rich food (Table 4).

Monitoring mound activity with Likert scale values and sampling with vials are two methods commonly used to measure the abundance of RIFA in the field. In recent studies, baited vials were used to measure ant diversity in pecan orchards following treatment with RIFA baits (Calixto et al. 2007a). In the present study, results from the Likert scale values, used to measure mound activity, are presented in Figure 2, while the actual number of RIFA collected in vial sets during sampling is shown in Figure 9 Sampling with vials appears to be more accurate in estimating RIFA abundance than monitoring mounds with Likert scale values. During the experiment, RIFA mounds that were observed in all the swaths decreased in activity until 61 d post-treatment, at which time, all mounds became completely inactive. The mounds did not recovered once they became inactive, as shown in Figure 2, although RIFA had become re-established as indicated by the sampling vials. This is an artifact of emigration events of RIFA from bordering areas. Aerial applications of discontinuous swaths of bait revealed that RIFA can be reduced with hydramethylnon or metaflumizone, though they subsequently recover (Figure 9); however, reestablishment of RIFA is more likely due to high densities in adjacent untreated areas. The number of RIFA collected in sampling vials seemed to decrease sharply following the application of hydramethylnon and metaflumizone (Figure 9). Metaflumizone plots seemed to have a higher level of RIFA control than hydramethylnon plots, and control lasted longer in metaflumizone plots as shown in Figure 9. Sampling with vials determined that RIFA populations were controlled in hydramethylnon plots until 92 d post-treatment, while RIFA populations were controlled in metaflumizone plots until 123 d post-treatment.

5. CONCLUSIONS

This research examined the effects of both hydramethylnon, and metaflumizone on RIFA applied with the skipped swath method. Monitoring the application rates of the two baits with calibration boards determined that both baits were under-applied, or that wind caused the bait to drift from areas designated as treatment swaths. The effects of the two pesticides on RIFA populations were determined by sampling for ants in addition to monitoring the activity level of marked mounds through time in treated and untreated swaths. Observations of mound activity and sampling with vial sets determined that the skipped swath method is effective in controlling RIFA populations for 92 d post treatment, with metaflumizone achieving slightly higher levels of control than hydramethylnon. This is particularly important when considering large-scale area-wide management of RIFA. It has been demonstrated that broadcast baiting is both faster and overall more effective than individual mound treatments, resulting in significant financial savings compared to virtually all other known application methods (Barr 2005). This is important when aerial applications of baits are employed, given the obvious higher costs of delivery, distribution, and operational considerations. The skipped swath method achieves comparable levels of control to complete coverage applications at a savings of approximately one half the total costs for both bait and application. In this study, a total of 1,014 hectares were treated at a cost of $27,000. This equates to a total cost of $26.6/hectare.

It appeared that sampling with baited vials was far more accurate in determining RIFA abundance within plots than monitoring activity of mounds. Monitoring mound activity determined when RIFA populations were reduced with bait, but did not indicate when RIFA re-infested an area. Also, sampling with baited vials monitored native ants in the plots through time, and gave insights into the interactions that RIFA have with other ant species. Native ants such as *Dorymyrmex* spp. were found in higher numbers once RIFA populations had been reduced. In a recent study, *Dorymyrmex flavus* McCook was demonstrated to defend and defeat RIFA in attacks both in the laboratory and field in central Texas (Warriner et al. 2008). Calixto et al. (2007a) observed *D. flavus'* ability to sustain higher densities for extended periods (2 yr) after cessation of bait treatments, and an ability to resist reinvasion of the treated area by RIFA. Furthermore, Calixto et al. (2007a) suggested that *D. flavus* may delay domination of the ant assemblage by S. *invicta*. These findings have important implications for area-wide management of RIFA because indigenous ants may pose less threat to humans, companion animals, or wildlife. Additional studies are justified regarding strategies to enhance the role of *D. flavus* in effected ecosystems. In the present study, results indicate that RIFA and *Dorymyrmex* spp. compete for resources such as food, and that *Dorymyrmex* spp. numbers may be restricted by RIFA populations.

In a study by Calixto et al. (2006b), a combination of pitfall traps, baited vials, and direct collection (by aspiration) were used to evaluate RIFA control by granular baits. Pitfall traps yielded the greatest catch numbers (in terms of diversity) of all sampling methods. In the present study, pitfall traps were not suitable for the rugged terrain and the treatment areas (the ranch) evaluated; most of the ants collected during sampling were RIFA and a clear preference for sausage over honey water in baited vials was evident. Future studies that investigate the impact of area wide management of RIFA should consider some combination of pitfall and baited vials to gain a better perspective of ant assemblages, when terrain and time permit. Additional calibration boards should be used in order to determine how far bait granules drift, and to more accurately determine the number of granules applied to swaths. In this study, control plots (skipped swaths) were situated immediately adjacent to the treated swaths because of the high application cost of placebo bait and the limited area which could be set aside and used for control plots. A large tract of land, away from treated areas, would have been needed for a control plot as a result of the highly mobile foraging behavior of RIFA. Future experiments should incorporate an isolated control plot.

It was determined that both hydramethylnon (Amdro Pro®) and metaflumizone (Siesta®) were effective in controlling RIFA using aerial applications with a skipped swath method. At this time, metaflumizone is being considered for registration against the RIFA. This chemical had activity on RIFA mounds and provided complete control by 17 d post-treatment with an active ingredient concentration of only 0.06% applied at 1.00 kg/ha. Siesta® has less than 10% of the active ingredient metaflumizone (0.06%) than Amdro Pro® has of hydramethylnon (0.73%). With the concerns for reduced pesticide usage, metaflumizone shows great promise for RIFA population management.

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