

D. Wojcik

# PROCEEDINGS OF THE 1991 IMPORTED FIRE ANT CONFERENCE

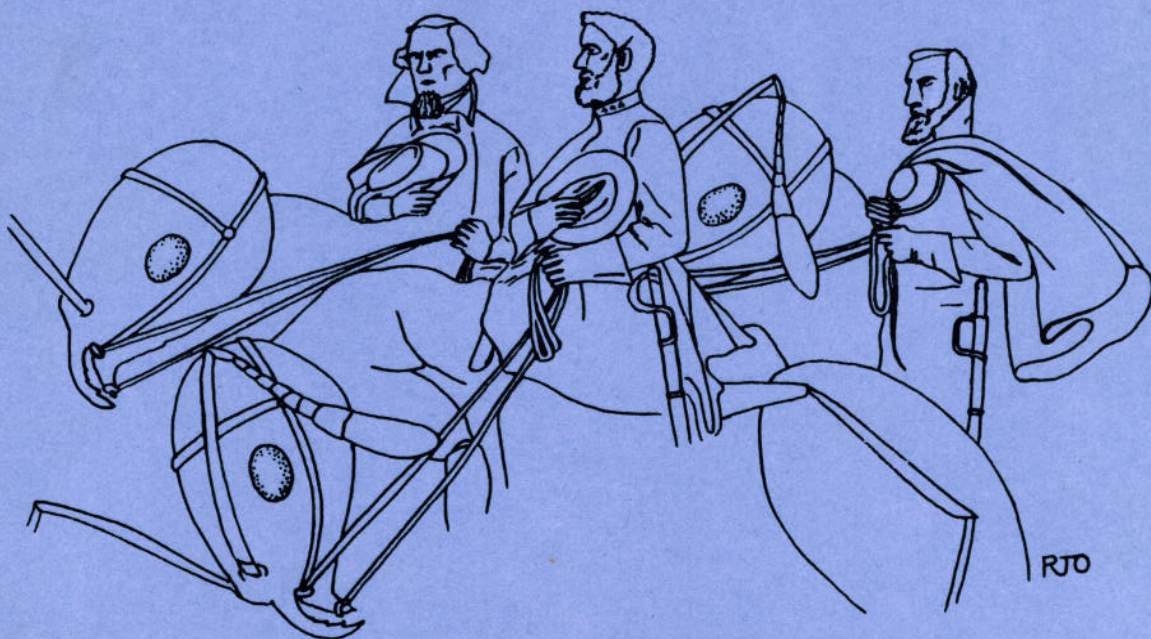
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*Department of Entomology  
The University of Georgia*

*Stone Mountain Memorial Park  
Atlanta, Georgia*

*March 19-20, 1991*



*Compiled and Edited by: Michael E. Mispagel, Ph.D.*

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1991 IMPORTED FIRE ANT CONFERENCE**

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March 19-20, 1991

Stone Mountain Inn  
Stone Mountain Memorial Park  
Stone Mountain, Georgia

*Compiled and Edited by:*

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(NS = Not Submitted)

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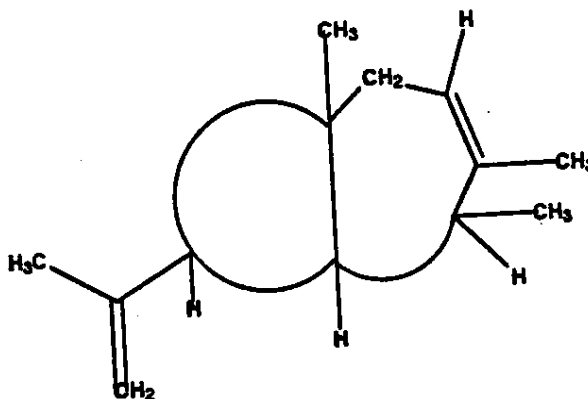
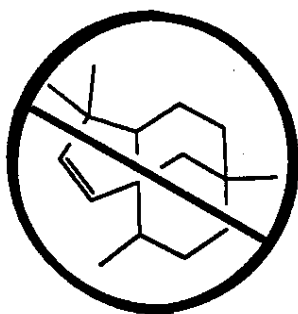
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In Search of the Holy Trail; the recruitment component of the IFA trail pheromone system. Pedro E. Hernández, Medical and Veterinary Entomology Research Laboratory, United States Department of Agriculture, Agricultural Research Service, Gainesville, Florida 32604

**Abstract-** During the course of the synthesis of the recruitment component of the IFA Trail pheromone discrepancies between the spectral data of late synthetic intermediates and that of the naturally occurring pheromone prompted a revision and reassignment of the target structure. This recruitment component of the trail pheromone referred in the literature as "C-1/C-2" and previously described as two isomeric "tricyclic homosesquiterpene monoene" is actually one bicyclic homosesquiterpene diene. Revision of the original data, new spectral data and derivatization establishes "C1" as having a bicyclic fused skeleton with a bridge methyl, an isopropene appendage and a methyl  $\alpha$  to a trisubstituted ring olefin: a C-16 homosesquiterpene.



## SMALL MAMMAL COMMUNITY RESPONSE TO RED IMPORTED FIRE ANT REMOVAL

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The introduction and subsequent spread of the red imported fire ant (*Solenopsis invicta* Buren) throughout the southern United States is well documented (Summerlin and Green, 1977; Hung and Vinson, 1978; Lofgren, 1986; Cokendolpher and Phillips, 1989), yet little is known about the effects of this invasion on the native fauna. Decreased species diversity has been observed in arthropod communities following *S. invicta* invasion (Summerlin et al., 1977; Porter and Savignano, 1990), but the effects on vertebrate communities are unknown.

A recent study found resident northern pygmy mice (*Baiomys taylori*) were captured less frequently in areas of high *S. invicta* mound density than were non-residents (Smith et al., 1990). The value of using mound density alone to assess the effects of fire ants on rodent habitat use is questionable however, as small mammals are threatened by foraging workers, not mounds. In addition, correlations between habitat use and mound density can be misleading because small mammal capture location is static and predetermined by trap placement, and may not accurately reflect habitat use. When mapped, small mammal captures appear as discrete and regular points overlaying an irregular and shifting mosaic of fire ant densities. A removal approach, in which ants are removed from selected areas, provides an unambiguous definition of high and low ant density areas, provides greater confidence that capture location accurately reflects habitat use, and allows simple statistical comparisons between areas of different ant densities.

### METHODS

The project was conducted at the Welder Wildlife Foundation, about 12 km northeast of Sinton, Texas, beginning in June 1989 and ending in November, 1990. Two sampling grids, each 110 m by 130 m including a 15 m wide buffer strip, were established in an ungrazed area of relatively homogeneous vegetation. Two treatment areas of equal size and shape were delineated on each grid. The boundaries between treatment areas were located so as to intersect as many small mammal home ranges as possible. After five months of baseline data collection, all *S. invicta* mounds on one-half of each grid were treated with an insecticide (Accudose Aerosol<sup>®</sup>, Cessco Corp., active ingredient: 0.7% pyrethrin) injected directly into the mound; *S. invicta* colonies on the second half of each grid were not treated or disturbed in any way. Treatments were maintained through monthly use of Amdro<sup>®</sup> (American Cyanamid Corp., active ingredient: 0.88% amidinohydrazone) and bimonthly use of the aerosol insecticide. The grids were

adjacent and separated by a small fence to discourage small mammal movement between grids. Ant removal areas were arranged on each grid so that they too, were adjacent. This arrangement created a contiguous removal area of about 1.4 hectares, reducing problems associated with *S. invicta* workers from colonies outside the study area foraging into treatment areas.

*S. invicta* Sampling.-- Ant foraging activity at each trap station was measured each sampling period by placing a 4 cm by 4 cm plastic weigh boat, baited with a small amount of canned cat food, directly on the ground near the trap; after 20 minutes the number of ants on each card was recorded by species and the cards were removed. Locations of *S. invicta* colonies were mapped to aid in treatment application and to calculate mound density on each grid. Head width of workers from 10 randomly selected colonies on each grid were measured to determine whether the population was monogyne or polygyne (Greenberg et al., 1985).

Small Mammal Sampling.-- Each grid contained 108 trapping stations arranged in a 9 by 12 grid with 10 m spacing between rows and columns (Grant 1982). One Sherman live trap (7.5 cm by 7.5 cm by 25 cm) was placed at each station, baited with scratch grains (a mix of millet, wheat, and corn), and left open four consecutive nights each month. A small amount of commercial fire ant killer in granular form (various manufacturers, active ingredient: 5% diazinon) was placed underneath each trap at the beginning of each sampling period to discourage bait predation by ants (Chabreck et al., 1986), and to reduce fire ant predation on rodents confined to traps (Masser and Grant, 1986). Each animal captured was individually marked and identification number, trap location, species, and sex was recorded. Animals were released at the point of capture. In each trapping period a few small mammals were marked with fluorescent pigment and tracked with a hand held ultraviolet lamp (Lemen and Freeman, 1985) to locate burrows. Additional burrows were found by following animals after release from the trap. When small mammal burrows were found, 15 bait cards were arranged in a grid around the burrow entrance, and the time required to recruit five *S. invicta* foragers to each card was recorded. The procedure was then repeated around a randomly chosen point located within 20 m of the burrow to minimize differences in vegetation between the two points. In the post-treatment period only burrows in untreated areas were used.

Statistical Analyses.-- The Mann-Whitney procedure (test statistic = U) was used to compare mean number of foraging ants between treatment areas of each grid. Frequency of occurrence of small mammals in ant removal areas was examined using the log-likelihood ratio (test statistic = G). Mean *S. invicta* recruitment time around burrows and random points was compared by t-test. Small mammal species richness and evenness in each treatment area were measured with Shannon's diversity index (Margalef, 1958) and compared by t-test (Zar, 1984).

## RESULTS AND DISCUSSION

*S. invicta* Removal.-- The imported fire ant population on the study site was assumed to be monogyne based on worker head width ( $0.89 \text{ mm} \pm 0.052$ , mean  $\pm 1$  SE) and mound density (109/ha). There was no difference in *S. invicta* foraging activity between treatment areas before mounds were removed at the end of October, 1990 (Table 1). Following treatment application, the mean number of

workers recruited to bait cards was lower in treated areas than in untreated areas, though differences did not become significant until February, 1990 on grid B and March, 1990 on grid A. Mean number of workers recruited to bait cards was significantly lower ( $P < 0.05$ ) in treated areas than in untreated areas each month thereafter, except for November 1990 on grid A (Table 1). Lack of significance in the first few months following application can be attributed in part to low winter temperatures suppressing *S. invicta* foraging activity, and in part to the partial effectiveness of the initial application of insecticides.

TABLE 1. Mean *S. invicta* foragers recruited to bait cards after 20 minutes. Dashed line indicates treatment application.

MONTH	GRID A		GRID B	
	TREATED (N=54)	UNTREATED (N=54)	TREATED (N=54)	UNTREATED (N=54)
1989				
June	20.6	22.6	16.7	24.9
July	21.9	23.9	19.2	18.7
August	24.1	20.1	16.3	16.1
September	16.4	14.5	14.3	12.9
October	19.2	22.0	22.8	22.2
November	7.4	6.8	5.7	8.7
December	†	†	†	†
1990				
January	4.7	5.0	4.3	4.6
February	4.5	5.1	4.5	6.5 *
March	1.9	11.2 *	2.1	7.6 *
April	0.6	2.9 *	0.3	2.5 *
May	3.2	11.9 *	3.2	12.2 *
June	7.1	21.1 *	5.8	20.3 *
July	8.7	18.5 *	11.5	17.5 *
August	7.8	14.2 *	9.1	14.2 *
September	4.4	9.5 *	2.6	6.2 *
October	6.2	18.1 *	3.9	16.4 *
November	6.1	8.4	4.5	10.8 *

\* value is significantly larger (Mann-Whitney U,  $P < 0.05$ ).

† temperatures below *S. invicta* foraging threshold.

**Small Mammals.**— A total of 1017 small mammal captures were made, the majority (93%) of which were northern pygmy mice. Capture location of pygmy mice was independent of treatment area before the treatments were applied ( $G=5.2$   $df=4$   $.25 < P < 0.5$ ). Only one other species, the fulvous harvest mouse (*Reithrodontomys fulvescens*), was captured prior to treatment application; the number of captures was insufficient to test. In the thirteen month post-treatment period pygmy mice were captured more frequently in the fire ant removal areas than in the untreated areas ( $G=21.2$   $df=12$   $P < 0.05$ ). In the post-treatment period there

were a total of six small mammal species captured: the northern pygmy mouse, the fulvous harvest mouse, the white-footed mouse (*Peromyscus leucopus*), the hispid cotton rat (*Sigmodon hispidus*), the marsh rice rat (*Oryzomys palustris*), and the house mouse (*Mus musculus*). Except for pygmy mice, the number of captures of any individual species was too low to be tested, though in each case, more animals were caught in treated than in untreated areas. Analysis of the combined data for these five species indicates a greater number were captured in removal areas than would be expected under the null hypothesis that capture location and treatment were independent ( $G=4.4$   $df=1$   $P<0.05$ ).

A total of 42 pygmy mice burrows were found using fluorescent pigments and by visually tracking released animals. One fulvous harvest mouse burrow was located. Fire ants were recruited faster to bait cards placed around random points than to cards placed around pygmy mice burrows ( $3.0 \pm 0.34$  and  $5.5 \pm 0.88$  minutes respectively, mean  $\pm$  SE,  $P < 0.025$ ). Recruitment to cards placed around the fulvous harvest mouse burrow was considerably slower than recruitment around the randomly chosen point ( $24.7 \pm 10.3$  and  $9.2 \pm 1.6$  minutes respectively, mean  $\pm$  SE,  $0.25 < P < 0.5$ ), but was not significant due to the small sample size.

Small mammal species diversity indices were calculated separately for treated and untreated areas each month, and for all months pooled into pre-treatment and post-treatment periods. The pooled data indicate post-treatment species diversity in the removal areas increased relative to pre-treatment diversity ( $t=5.03$   $v=222$   $P<0.001$ ) (Table 2). There was also an increase in diversity in the untreated areas ( $t=3.34$   $v=225$   $P=0.001$ ), thus the increase cannot be attributed solely to fire ant removal, but diversity was higher in the removal areas than in the untreated areas ( $t=3.02$   $v=241$   $P<0.005$ ). There was no difference in diversity between areas in the pre-treatment period. When the data are ungrouped and examined month by month, the increasing trend in species diversity following treatment application is still apparent (Figure 1).

Preliminary results of this project suggest red imported fire ants have a significant effect upon small mammal communities in terms of species composition and habitat use by members of individual species. Because small mammals serve important functional roles in most ecosystem types and are prey for a number of species, fire ant induced changes in small mammal communities could ultimately result in widespread decreases in vertebrate biodiversity, similar to that observed in arthropod communities.

#### ACKNOWLEDGEMENTS

We thank the Rob and Bessie Welder Wildlife Foundation for financial and scientific support of this project.

TABLE 2. Shannon's diversity index ( $H'$ ) for small mammal species. An asterisk between pairs in either rows or columns indicates a significant difference ( $P < .005$ ).

AREA	PERIOD	
	PRE-TREATMENT	POST-TREATMENT
Removal	0.06	0.27*
Nonremoval	0.03	0.14*

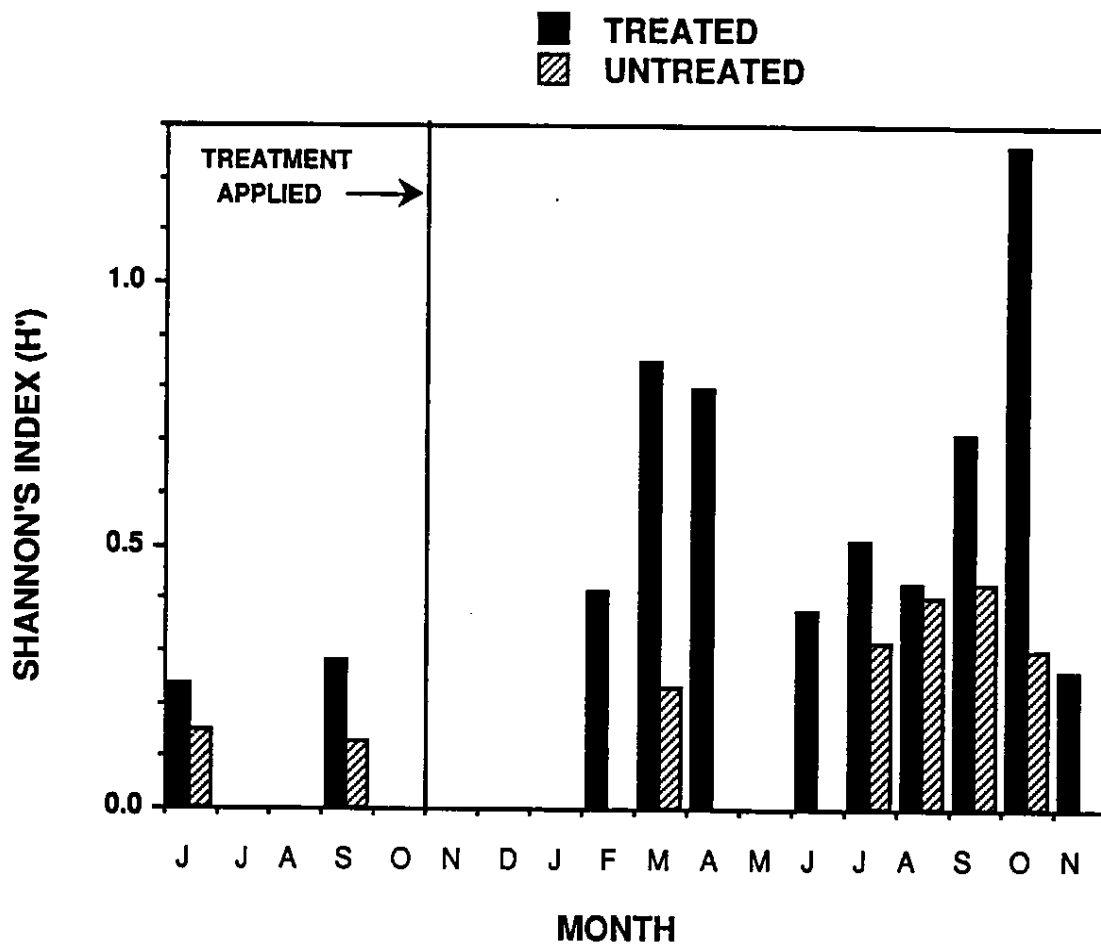


FIGURE 1. Monthly small mammal species diversity.

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POPULATION DENSITIES OF THE ANT FAUNA INCLUDING Solenopsis  
invicta IN PASTURES IN BRAZIL

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## ABSTRACT

A 2-year study of the population densities of the ant fauna was conducted on 3 farms in Brazil. Also, mound densities of the fire ant, Solenopsis invicta, on the farms were compared to densities on improved pastures in the U.S. There were 116 ant species identified. Nests counts of S. invicta varied depending on the farm with the lowest counts averaging 20 nests/hectare (8/acre) while the highest averaged 100 nests/hectare (41/acre). S. invicta populations on these farms were not unlike those seen on farms in many locations in the southeastern U.S. The mean S. invicta nests/hectare on the Brazil farm with the highest fire ant populations was not significantly different from the mean S. invicta nests/hectare in the U.S. The most recently developed farm, 4 years old at the beginning of the study, had the greatest number of S. invicta nests. The farm with the lowest number of S. invicta nests/hectare had the highest number of total ant species while the farm with the highest number of S. invicta nests/hectare had the lowest number of ant species. Pheidole diligens had the highest mean frequency of occurrence of all ant species. In pitfall traps, the percent P. diligens was lower than the percent S. invicta at the newest farm and higher than the percent S. invicta at the oldest farm. As in the U.S., large populations of S. invicta in Brazil were associated with disturbed habitats. Rainfall and temperature had no noticeable effect on populations of S. invicta.

## INTRODUCTION

Some investigators have implied that populations of the fire ant, Solenopsis invicta never reach densities in its homeland in the state of Mato Grosso, Brazil that it does in the southern United States (Buren et al 1978; Buren 1980, Whitcomb 1980, Porter, personal communication). Contrary, Banks et al. 1985 and Wojcik 1983, 1986, Trager (personal communication), indicated that fire ant populations in certain habitats were as abundant as those in the U.S.

It is well known that fire ants are an insect that thrives in disturbed habitats (Buren et al. 1978 and Tschinkel 1987). Therefore, we undertook a 2-year study of the ant fauna in improved pastures, which are disturbed habitats that do not differ very much between the United States and Brazil. The primary objectives of the study were to : (1) compare the mean number of ant species on the three farms, (2) determine the population levels of S. invicta, during the study period, (3) compare mound densities of S. invicta on the 3 farms with those in improved pastures in the U.S., and finally, (4) determine if temperature or precipitation had any effect on the S. invicta populations.

## MATERIALS AND METHODS

The three farms were all located near the town of Caceres in the state of Mato Grosso in the southwestern corner of Brazil. The following is a description of each farm:

I--Piraputanga farm: 1) ca. 10 years old

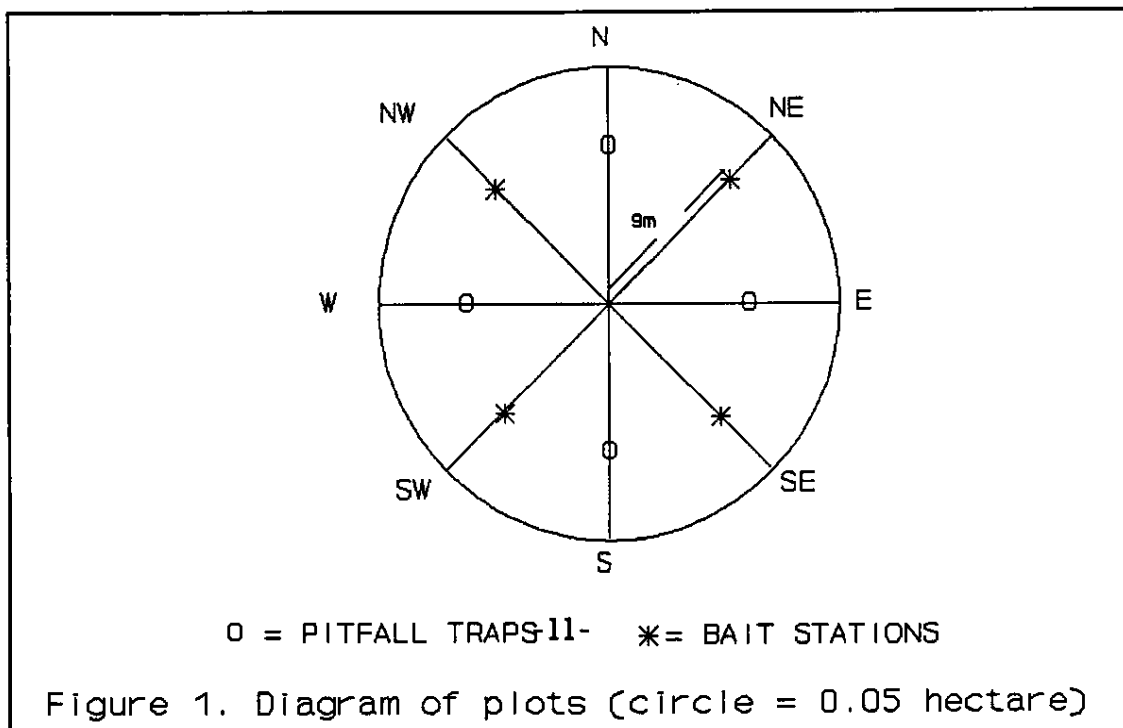
- 2) ca. 193 hectares in size
- 3) ca. 10-15 cattle
- 4) Grass height was 5-26 cm.

II--Facao farm :

- 1) ca. 100 years old
- 2) ca. 11037 hectares in size
- 3) ca. 1000 cattle
- 4) Grass height was 6-36 cm.

III--Assis farm :

- 1) ca. 4 years old
- 2) ca. 124 hectares in size
- 3) ca. 50 cattle
- 4) Grass height was 5-29 cm.



Three circular plots, 0.05 hectares each (Figure 1), were established on each farm and populations of ants were sampled on a monthly basis for the 1st year and bimonthly for the 2nd year. The 3 methods of sampling used were:

- a) pitfall traps (4/plot operated 1 week per month)
- b) mound densities in each plot (Solenopsis invicta only). Mounds were rated by modifying the population index method described by Harlan et al. (Table 1).

Table 1. Population index method.

ABNORMAL NESTS (WITHOUT BROOD)		NORMAL NESTS (WITH BROOD)	
INDEX	NO. OF WORKERS	INDEX	NO. OF WORKERS
1	<10,000	5	<10,000
2	10 - 50,000	10	10 - 50,000
3	>50,000	15	>50,000

- c) bait stations (4 stations/plot, 3 baits/station).

NOTE: The bait station samples have not been sorted and therefore, this report does not include this data.

Mound densities in the U.S were obtained from control plots of monogyne Solenopsis invicta populations used in previous surveys for field test sites. Since these sites were used for field tests, they were always moderate-heavily infested with IFA, and therefore, this data was biased towards higher densities in the U.S.

## RESULTS AND DISCUSSION

During the 2 years, 624 pitfall samples were collected. There were 116 ant species identified from the pitfall samples. Nests counts varied depending on the farm with the Piraputanga farm the lowest, averaging 20 nests per hectare (range 0-54/ha) while Assis farm was the highest averaging 100 per hectare (range 60-133/ha). Facao farm was in the middle with an average of 62 nest per hectare (range 20-120/ha).

The nest counts of S. invicta on these farms were similar to those we have made in many locations in the southeastern U.S. Assis, which had the highest densities of S. invicta was the most recently developed farm, about 4 years prior to our study. As the habitat at this farm stabilizes, the populations of S. invicta will probably stabilize and may actually decline to a lower level depending on how much disturbance or competition from other ants occurs in the future. In fact, at Assis farm, the populations of S. invicta appear to be declining while the populations of Pheidole diligens, a very abundant ant found at all 3 farms, are increasing. We hope to conduct additional monitoring of this occurrence on future trips to Brazil. A farm comparison of the mean number of S. invicta nests/hectare and the mean number of ant species on each farm is shown in Table 2. Piraputanga farm had a mean of 21.9 (SE 3.5) S. invicta nests/hectare or ca. 8 per acre with a mean of 9.6 (SE 0.4) ant species. Facao farm had a mean of 60 (SE 5.9) S. invicta nests/hectare or ca. 25 per acre with a mean of 8 (SE 0.3) ant species. Assis farm had a mean of 97.4 (SE 5.1) S. invicta nests/hectare or ca. 41 per acre with a mean of 6.5 (SE 0.3) ant

species. The population index which is representative of the total numbers of S. invicta per hectare was greatest at Assis.

The farm with the lowest mean number of S. invicta nests per hectare had the highest average number of species of other ants. The most recently developed farm (newest) had the greatest number of S. invicta nests. The large populations of S. invicta were associated with disturbed habitats. Pheidole diligens had the highest mean frequency of occurrence of all ant species at each farm. Rainfall and temperature had no consistent effect on populations of S. invicta.

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Table 2. Farm comparison of S. invicta mounds and number of ant species.

FARM	MEAN <u>S. invicta</u> MOUNDS/HA *	MEAN NUMBER OF SPECIES *
PIRAPUTANGA	21.9(3.5)c	9.6(0.4)a
FACAO	60.0(5.9)b	8.0(0.3)b
ASSIS	97.4(5.1)a	6.5(0.3)c

\*Means (SE) followed by the same letter are not significantly different using Tukey's studentized range test (P<0.05)

---

Although there is still discussion on whether the densities of fire ant populations in the U. S. are different than in Brazil, these studies indicate that densities can be equal in similar habitats and under similar conditions. As always, there is the

problem of comparing habitats between two countries such as Brazil and the U.S. which can be difficult at best, especially because of the differences between the land use (the U.S. is more developed in the fire ant infested area) and the general ecology (Wojcik 1986). However, this is true for any such comparisons and based on the strong similarities between improved pastures on farms in the two countries, the results shown here indicate, at least in these types of disturbed habitats, population densities of the monogyne form of S. invicta are similar in the U.S. and Brazil.

#### ACKNOWLEDGMENT

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**Bifenthrin: A Promising New Imported Fire Ant  
Quarantine Treatment for Containerized Nursery Plants**

by:

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and Lee McAnally**

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## INTRODUCTION:

Because of the extremely limited number of chemicals labeled for imported fire ant (IFA) quarantine use, our laboratory continuously screens candidate insecticides for possible quarantine use. Bifenthrin, a synthetic pyrethroid insecticide/miticide produced by FMC Corporation, is one candidate which has shown great promise as an IFA quarantine program insecticide.

Bifenthrin has a wide spectrum of activity against insects and mites of field crops, fruit crops, nut crops, and ornamentals. Formulations include 2EC, 10WP, 0.2G and a flowable. Chemically, the compound is known as cyclopropanecarboxylic acid, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl(2-methyl[1,1-biphenyl]3-yl)methyl ester.

A 2EC formulation marketed as Capture® 2EC is registered for foliar use on cotton for numerous pests including boll weevil, bollworms, plant bugs, armyworms, spider mites and pink bollworm. Application rates range from 0.02 to 0.1 lbs AI/acre.

More importantly, a 10WP formulation (Talstar® 10WP) is registered for foliar use on ornamental trees, shrubs, plants, flowers, and non-bearing fruit and nut trees for numerous pests including aphids, scales, mites, lacebugs, caterpillars, leafminers, Japanese beetles, etc. Rates of application range from 0.004 to 0.02 lbs AI/10 gallons of finished spray.

More recently, Talstar Flowable (0.66 lb AI/gal flowable formulation) was registered for similar ornamental uses, pest spectrum, and rate range. The 0.2G bifenthrin formulation is not currently registered, but is being tested and evaluated for a number of uses.

Bifenthrin is stable in sterile water under acidic, neutral, and basic pH conditions. Its stability, particularly at high pH, is due to the low water solubility of the compound, which is less than 0.1 ppb. Bifenthrin is tightly bound to most agricultural soils. It possesses low mobility in sand and is immobile in sandy loam, silt loam, and clay loam soils. Under laboratory conditions, bifenthrin has a half-life of 2-4 months in soil depending on soil type. The potential for crop uptake or translocation is expected to be negligible due to its limited water solubility (Knabke 1985).

Due to registration status of some formulations, most of the IFA trials summarized in this report are based on Talstar 10WP. The first trial with bifenthrin against IFA was initiated at Gulfport, MS on December 14, 1988. In the two years since that first trial, numerous other evaluations of dose rates, formulations, application techniques, etc., have been initiated and most are ongoing at this time. Most trials were conducted under local environmental conditions. However, several other sites including Miami, Florida; Whiteville, North Carolina; El Campo, Texas; Tifton, Georgia; Jackson, Mississippi; Mobile, Alabama; and Monticello, Florida have also been included. The trials summarized in

this report evaluate bifenthrin as a possible new IFA quarantine insecticide for containerized plants. Once efficacy is established for a candidate insecticide, residual activity is examined. The length of residual activity desired for IFA quarantine insecticides used for treatment of containerized nursery stock is dependent on use pattern:

Use Pattern	Desired Residual Activity
Incorporated	18-24 months
Drench	6 months
Topical	6-12 months

This report includes evaluations of all of these use patterns. We expect to present data at next year's IFA research conference on other use patterns including grass sod and possibly balled-and-burlapped plant material.

#### MATERIALS AND METHODS:

##### Incorporation Trials

Incorporation of insecticides into potting media is the most common method of application utilized by the IFA quarantine due to cost and simplicity for the grower. Both granular (G) and wettable powder (WP) formulations of bifenthrin can be utilized in this manner. In the trials reported here, a predetermined quantity of insecticide (based on amount of soil to be treated, bulk density of soil and insecticide formulation)

was incorporated into potting media using a 2 cu. ft. portable cement mixer. The mixer was operated for at least one hour per load to insure a homogenous blend. Two-liter nursery pots were then filled with treated soil and weathered outdoors under natural conditions. In most trials, irrigation in addition to rainfall, was applied to simulate normal nursery conditions.

One trial evaluated the effects of various amounts of irrigation on Talstar 10WP incorporated into Strong-Lite® potting media at a rate of 50 ppm. Treated soil was placed in nursery pots and divided into 3 groups. Each group received either 1", 2" or 4" irrigation per week in addition to natural rainfall.

At monthly intervals, soil from 2-3 pots of each treatment group was composited. This soil was then subjected to standard laboratory bioassay using IFA alate queens which is a slight modification of the procedure of Banks et al. (1964). The test chamber consists of a 3"x3" square plastic nursery pot equipped with a Labstone bottom. The Labstone prevents the queens from escaping through the drain holes and also absorbs moisture from an underlying bed of wet peat moss (prevents dessication of ants). An inverted plastic petri dish on top of the test chamber prevents escape over the top of the pot. Each treatment to be evaluated was divided into 4 replicates; one test chamber per replicate. A 20-50 cc. subsample of treated soil was placed in each test chamber and 5 IFA alate queens then introduced into each pot. After a 7 day exposure period, percent queen

mortality was determined and recorded.

### Drench Trials

Another method of application utilized in the IFA quarantine program is a drench treatment. Drenches can be formulated using emulsifiable concentrate (EC) or wettable powder (WP) formulations of bifenthrin. Nursery pots were filled with soil and drenched using various rates of bifenthrin solution (based on amount of soil to be treated, bulk density of soil and insecticide formulation). The amount of finished solution used was 1/5 the volume of the container. For example, a 6"x6" plastic nursery pot has an approximate volume of 2 liters. Therefore, each pot filled with soil received about 400 ml. drenching solution. These trials were weathered outdoors and received a minimum of 1" of water per week through rainfall and/or irrigation. Monthly bioassays as previously described were performed.

### Topical Trials

IFA quarantine treatments normally utilize either the incorporation or drench method of insecticide application. Another possible method of application is an "over-the-top" or topical application directly to the surface of the media in lieu of traditional methods. To evaluate this procedure, nursery pots were filled with potting media and treated topically with various rates of Talstar 10WP (based on amount of soil to

be treated, bulk density and insecticide formulation). Pots were irrigated immediately after treatment, and maintained under normal environmental conditions, receiving a minimum of 2" of water per week through rainfall and/or irrigation. Standard laboratory bioassays using IFA alate queens were performed monthly as previously described.

Types of Potting Media Used

Initial trials, and a number of subsequent trials to evaluate bifenthrin, were conducted using Strong-Lite Potting Media. Other potting media were also used. The following is a list of the various potting media used in the bifenthrin trials:

Potting Media	Producer	Composition
Strong-Lite®	Strong-Lite Products Corp., P.O. Box 8029, Pinebluff AR	a blend of composted peat moss, pine bark and vermiculite
Baccto®	Michigan Peat Co., Houston TX	unknown
Dodds	George Dodd Nursery Supply, P.O. Box 86, Semmes AL	1:1 pine bark to peat moss, 1 cu ft/cu yd airo-lite, 8 lbs lime/cu yd, and 1.5 lbs micromax/cu yd (micronutrients)
Lab mix	IFA laboratory mix, Gulfport MS	1:1:1 sand to pine bark to peat moss
Greenleaf mix	Greenleaf Nursery mix, El Campo TX	5:2:1 pine bark to sand to rice hulls

## RESULTS:

### Incorporation Trials

The various trials summarized in Table 1 are arranged more or less in chronological order. Five of the six trials are ongoing. The longest residual activity to date is 25 months and was achieved with the 0.3G formulation of bifenthrin. The 0.2G formulation has achieved 15 months residual activity at 100 ppm, 12 months with dose rates as low as 25 ppm, and 10 months with a rate of 12.5 ppm.

The excellent results achieved by bifenthrin when applied at dose rates of 25 ppm and greater prompted the evaluation of the insecticide at lower rates (Table 2). Bifenthrin 0.2G was incorporated into Strong-Lite potting media at rates of 2.5, 5.0 and 10.0 ppm. The 10.0 ppm rate achieved 100% efficacy at 3 months post-treatment (PT) and has maintained that level of efficacy through 8 months PT. Lower rates produced extremely variable results. A possible explanation for this is uneven blending caused by the small amount of material used to achieve these low dose rates. For example, 12.04 g. of bifenthrin 0.2G blended into 1.5 cu. ft. of Strong-Lite potting media results in a rate of 2.5 ppm.

Results of the irrigation trial are shown in Table 3. At an initial dose rate of 50 ppm, Talstar 10WP has been 100% effective against IFA alate queens for 4 months while receiving in excess of 90 inches of water.



This average is more than 5 inches of water per week. From observations made at nurseries, an average of ca. 2 inches of water per week is the norm, depending on type of plant and environmental conditions such as rainfall, drought, temperature, etc.

#### Drench Trials

The initial drench trial (April 1989) showed 100% efficacy up to 6 months PT (Table 4). At that time the test was terminated. The second trial (June 1990), using the 2EC and the 10WP formulations of bifenthrin applied at two initial dose rates (100 and 200 ppm), is ongoing. Results to date show 7 months residual activity for both formulations at both application rates.

#### Topical Trials

Two topical application trials have been initiated recently using Talstar 10WP. An "off-station" cooperative study was conducted with FMC Corp. and various state cooperators in GA, AL, FL and MS. Cooperators topically treated a potting media of their choice with Talstar 10WP at rates of 5, 10, 25, 50 and 75 ppm. Monthly soil samples were sent to our lab for bioassay analysis. The MS cooperator, R. Mitchell (FMC Corp.) conducted bioassays for that site. Rates of 25 ppm and higher have generally shown excellent control for 6 months (Table 5). The Mobile AL site has shown some unexplained varying results at the higher rates,

especially at 3 months PT. While we do not know the direct cause of these results, it may be due to application error, uneven composition of bioassay soil, etc.

The second trial was conducted in Gulfport, MS, to determine the impact of bulk density and components of potting media on the residual activity of Talstar 10WP applied topically. Rates of application were 0.01625 g AI/pot, which is equivalent to 25 ppm in Strong-Lite. Due to differences in bulk densities of the 4 types of media used, initial dose rates for each media varied. At 4 months PT, all media are exhibiting excellent control of IFA alate queens.

#### SUMMARY

Bifenthrin has been shown to be a highly effective and residual treatment for control of IFA in containerized nursery stock. A variety of use patterns including preplant incorporated, drench and topical applications have been evaluated for treatment of containerized plants. Due to registration status, as well as efficacy, a recommendation has been made that Talstar 10WP be adopted for IFA quarantine program use on containerized plants as soon as possible. Consideration is being made by PPQ, with input from the federal IFA Technical Work Group, on how best to accomplish this. Eventually either a FIFRA Section 3 label or a 24c (state and local needs) label is anticipated.

Recommended use patterns, dose rates and certification periods are as follows:

Use Pattern	Formulation	Dose Rate	Certification Period
Incorporated	10WP	50 ppm	1 year
Drench	10WP	25 ppm	6 months
Topical	10WP	25 ppm	6 months

Following completion of studies now in progress and others, it should be possible to offer additional certification periods based on dose rates and use patterns. Studies are also underway evaluating the efficacy of bifenthrin for use on grass sod or balled and burlapped nursery stock.

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Table 1. Residual Activity of Various Bifenthrin Formulations Incorporated into Potting Media.

Trial No.	Date Initiated	Trial Location	Formulation	Initial Theoretical Dose (ppm)	Potting Media	Residual Activity* in Months
I	Nov. '88	Gulfport MS	0.3G	72.6	Strong-Lite	25
II	Nov. '89	Gulfport MS Miami FL Whiteville NC	0.2G	100	Strong-Lite	15 15 15
III	Dec. '89	Gulfport MS	10WP	12.5 25 50 100	Strong-Lite	12**, *** 12 12 12
IV	Jan. '90	E1 Campo TX	0.2G	25 50	Greenleaf Nursery mix	12 12
V	Mar. '90	Gulfport MS	0.2G	12.5 25 50 75 100	Strong-Lite	10 10 10 10 10
VI	Sept. '90	Gulfport MS	10WP	25 16.6 30.1 8.9	Strong-Lite Baccto Dodds' lab mix	4 4 4 4

\* number of months showing 100% queen mortality in standard laboratory bioassay

\*\* all results in trial are final

\*\*\*slight decrease in efficacy (90-95% mortality) in indicated dose rate at 8, 9 and 10 months PT

Table 2. Activity of Selected Pyrethroid Insecticides at Low Rates of Application, May 1990.

Insecticide	Initial Theoretical Dose (ppm)	Average % Mortality to Alate IFA Queens at Indicated Months Post Treatment							
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Bifenthrin .2G	2.5	10	35	100	60	70	30	95	95
	5.0	10	70	100	20	100	85	100	100
	10.0	40	60	100	100	100	100	100	100
Cypermethrin .75G	2.5	5	35	90	5	15	0	60	70
	5.0	10	15	60	5	15	15	90	70
	10.0	25	40	95	45	10	5	95	100
Force 1.5G	2.5	20	55	65	55	10	0	15	55
	5.0	85	100	100	15	95	0	20	15
	10.0	100	100	100	100	100	100	100	100
Check	0	5	5	5	0	0	5	10	5

Table 3. Influence of Irrigation on Residual Activity of Talstar 10WP Incorporated into Strong-Lite® Potting Media at a Rate of 50 ppm, Sept. 1990.

Irrigation Schedule (inches/wk)	1 Month		2 Months		3 Months		4 Months	
	% Mort	Cumul. H <sub>2</sub> O (inches)	% Mort	Cumul. H <sub>2</sub> O (inches)	% Mort	Cumul. H <sub>2</sub> O (inches)	% Mort	Cumul. H <sub>2</sub> O (inches)
1	100	6.35	100	13.75	100	20.70	100	+39.14 <sup>2/</sup>
2	100	11.35	100	22.75	100	33.70	100	+59.14
4	100	21.35	100	40.75	100	59.70	100	+90.14
Check	10	6.35	0	10.75	15	14.70	15	+31.14

1/ check received approximately 1 inch of irrigation per week in addition to minimal rainfall or only rainfall when totaling 1" or more per week

2/ rainfall during the week of 1/6/91 was in excess of 11.25 inches (rain gauge overflowed one night, therefore +6.00 inches fell); additional irrigation was not added this week; all totals from this interval will be recorded as "+ inches"

Table 4. Residual Activity of Various Bifenthrin Drench Formulations in Strong-Lite Potting Media.

Trial	Initiated	Formulation	Initial Theoretical Dose (ppm)	Trial Location	Residual <sup>1</sup> Activity in Months
I	April '89	2EC	230	Gulfport MS	6 <sup>2</sup>
II	June '90	2EC	100 200	Gulfport MS	7 7
		10WP	100 200		7 7

<sup>1</sup> number of months showing 100% queen mortality in standard laboratory bioassay

<sup>2</sup> Trial was only planned for 6 months based on no. of treated pots. At termination of trial, bifenthrin was still active.



Table 5. Residual Activity of Talstar 10WP Applied Topically at Various Rates and Aged in Various Geographical Locations, June 1990.

Treatment Location/ Cooperator	Initial Theoretical Dose (ppm)	% Mortality to Alate IFA Queens at Indicated Months Post-Treatment <sup>1/</sup>					
		(1)	(2)	(3)	(4)	(5)	(6)
Jackson, MS	5	100	100	85	100	85	
R. Mitchell	10	100	100	100	55	100	
FMC Corp.	25	100	100	100	100	100	
	50	100	100	100	100	100	
	75	100	100	100	100	100	
	Check	5	5	15	15	5	
Tifton, GA	5	100	45	100	100	100	100
B. Sparks	10	100	100	100	100	100	100
Ga. Ext. Ser.	25	100	100	100	100	100	100
	50	100	100	100	100	100	100
	75	100	100	100	100	100	100
	Check	0	25	5	10	45	35
Monticello, FL	5	100	100	100	100	85	100
R. Mizell	10	85	85	100	100	100	100
Agric. Res. & Educ. Center	25	100	100	100	100	100	100
	50	100	100	100	100	100	100
	75	100	100	100	100	100	100
	Check	0	25	50 <sup>2/</sup>	0	45 <sup>2/</sup>	5
Mobile, AL	5	80	100	95	100	90	50
J. Stephenson	10	80	90	100	80	70	100
Auburn Univ.	25	100	100	60	100	100	95
Hort. Sta.	50	100	100	75	100	100	100
	75	100	100	100	100	100	100
	Check	25	15	0	15	5	0

<sup>1/</sup> Standard laboratory bioassay using IFA alate queens

<sup>2/</sup> High check mortality unexplained

Table 6. Residual Activity of Talstar 10WP Following Topical Application onto Various Potting Media, Sept. 1990.

Potting Media	Treatment	Initial Theoretical Dose (ppm)	% Mortality <sup>1</sup> at Indicated Months Post Treatment			
			(1)	(2)	(3)	(4)
Strong-Lite	treated	25	100	100	100	100
	check	0	10	0	15	15
Baccto	treated	14.1	100	100	100	100
	check	0	0	0	0	15
Dodds	treated	23.2	100	95	100	100
	check	0	0	5	5	10
Lab mix	treated	9.8	100	100	80	100
	check	0	10	5	0	5

<sup>1</sup> standard laboratory bioassay using IFA alate queens

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**Seasonal Effectiveness of Amdro® and Logic® for RIFA Control**

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#### ABSTRACT

Amdro® and Logic® baits applied monthly (June 1989 - May 1990), resulted in definite seasonal trends in rates of activity and levels of control that were attributable to the time of bait application. Spring (March-May) application with both Amdro and Logic resulted in rapid declines in pretreatment population indices. Control peaked 3-4 months after application, followed by reinfestation about six months after treatment. Summer treatments were similar to spring, except reinfestation was not as rapid. Fall applications with Logic were characterized by a slow gradual decrease in population index, attaining a maximum level of control eight months after treatment. Winter applications with Amdro produced highly erratic results. Logic applied in winter resulted in slowly declining population that attained maximum decrease 4-6 months after application. Reinfestation began 8-9 months after application. Therefore, if rapid population suppression is the main objective of a control effort, applications would be best applied in the spring. However, summer and fall treatments generally result in gradual population suppression over a more extended period of time.

## INTRODUCTION:

Successful control of red imported fire ants, Solenopsis invicta Buren (RIFA), with bait toxicants is based on a complex set of poorly understood variables. It is assumed that all fire ant bait toxicants must be ingested by foraging workers soon after bait application in order to prevent photolysis or other forms of bait degradation. Weather conditions such as rain, dew, soil moisture, and temperature impact foraging behavior of the ant as well as bait palatability. Lofgren et al. (1964) noted that pickup (harvest) of bait by poikilothermic ants is dependent on their being warm and active enough to forage. Markin et al. (1974) reported that, with the exception of the very coldest weeks in northern Mississippi and South Carolina, some foraging was recorded throughout the winter at six different study sites throughout the range of the RIFA. Lofgren et al. (1964) found that mirex bait applied in the cooler part of the year (November-April) eventually gave good control (96-100%). However, fast and complete kill was dependent upon warm weather. Markin et al. (1975) showed that winter applications of mirex in south Mississippi resulted in 84-94% kill, but required 4-6 months to occur. An additional study by Summerlin et al. (1976) showed that temperature at the time of treatment seemed to be the main factor affecting mortality and rate of kill with mirex bait.

Harlan et al. (1981) reported that large scale field tests with American Cyanamid AC-217,300 (Amdro®) provided 90% control 22 weeks after a spring application. Banks et al. (1988) showed that baits containing fenoxycarb

(Logic<sup>®</sup>) eliminated 60% of the RIFA colonies and reduced the population indices by 67-99% within 12-13 weeks in a series of spring/summer treatments. Collins (1986) reported that maximum colony mortality and reduction in pre-treatment population indices were obtained 42 weeks after fall applications of both Amdro and Logic.

It is generally desirable to achieve maximum RIFA control as soon as possible after bait application. Although all RIFA baits must provide delayed toxicity in order to be effective, homeowners often demand rapid control. Since longer delays in kill are usually associated with cool weather applications, it was assumed that a "window of opportunity" exists for maximum control in the shortest period of time. A study to better define that "window of opportunity" was initiated in June 1989.

#### METHODS AND MATERIALS:

Test plots infested with a moderate RIFA population (average of 77.6 totally monogynous colonies per acre) were located in non-grazed permanent pastures in Harrison County, Mississippi. Treatments were applied at monthly intervals from June 1989, through May 1990. Each month (except December 1989, in which no treatment was applied), three 1-acre test plots (replicates) were treated with Amdro while three adjacent plots received Logic bait. All treatments were made with a shop built granular applicator mounted on a farm tractor (Collins 1987 unpublished). Effects of the treatments were evaluated in all test plots and 3 adjacent untreated control plots for 12 months or until reinfestation occurred. Reinfestation of test plots was evident when the

appearance of many new or incipient colonies were observed during any post-treatment rating interval. The population index method (Harlan et al. 1981), as modified by Lofgren and Williams (1982), as well as colony mortality, was used to rate all test plots. Soil temperature at the 1" depth and air temperature was recorded immediately prior to each application. Cumulative rainfall data for the month preceding the first bait application and for each month of application was obtained from a U.S. Forest Service fire tower approximately 1.0 mile from the test plots.

#### RESULTS:

This study has not been completed; therefore all results should be considered preliminary.

#### Environmental Conditions

Soil temperatures at the time of application ranged from a low of 46° (January 1990), to a high of 90° in July 1989, and soil moisture ranged from dry to moist (Table 1). Therefore, a wide spectrum of environmental conditions were encountered during this trial. Rain did not occur within 24 hours of any application. Although lower levels of control were generally associated with soil temperature extremes, careful analysis of the data indicated that soil temperature and maximum control obtained with either bait were not correlated (Figure 1).

### Rates and Levels of Control

#### Amdro:

Treatment effects are summarized in Table 2. One month after treatment, control with Amdro ranged from a high of 91.3% (February 1990) to a low of 42.3% (October 1989). The highest level of control with Amdro occurred 3 months after the October application. When averaged over the entire year, RIFA control following Amdro applications generally peaked about three and a half months after treatment, versus about 6 months for Logic (Table 3). Amdro treatments maintained population suppression  $\geq 75\%$  of pretreatment levels for 1 to 10 months, averaging 6 months.

#### Logic:

Logic reduced the pretreatment population by 89.0% 1 month after a May 1990 application, but only a 6.7% reduction was achieved 30 days after treatment in October (Table 2). One-hundred percent (100%) control was observed 9 and 10 months following both October and November applications with Logic. Thus very distinct differences in the levels of population suppression at various treatment intervals were apparent. However, with only few exceptions (January, 3 months post, February, 6 months post and August, 12 months post), treatment means for any given month and post-treat interval were not significantly different. Population suppression  $\geq 75\%$  of pretreat levels with Logic ranged from 2 to over 12 months, with an average of 8.3 months.



Differences in the length of control afforded by each bait were not statistically significant (Table 3).

### Seasonal Trends

By pooling treatment means into seasonal categories and graphically presenting the pooled data, definite trends in the seasonal effectiveness of both baits becomes apparent. Seasonal categories were arbitrarily established into the following groups: spring (March, April, and May); summer (June, July, and August); fall (September, October, and November); and winter (January and February).

#### Logic:

As shown in Figure 2, spring treatments with Logic rapidly reduced the pre-treatment population indices achieving maximum reduction 4 months after treatment. Resurgence was initiated 6 months after application. Summer applications rapidly reduced pretreatment population indices and maintained good control for about 11 months at which time resurgence was noted. Confirming previous results (Collins 1986), fall treatments were much slower in activity, achieving maximum reduction 8-10 months after application with resurgence beginning in the 12th month. Winter treatments were similar to fall, except that maximum control was achieved sooner (4-6 months post-treat), with population resurgence 9 months post-treatment. These seasonal reinfestation differences may be due to the heavy influx of new queens from

the spring peak in mating flights (Markin and Dillier 1971; Morrill 1974) i.e., control following winter treatments peaked March-July resulting in very little competition to founding queens and incipient colonies resulting from spring flights. Control resulting from fall applications peaked June-September (after the maximum mating flight period). Thus, surviving colony remnants (colony classes 1-4 on the population index rating scale) that were present during the maximum mating flight period may have defended their territory against reinvasion by founding queens. Another possibility would be that surviving major workers (repletes as reported by Glancey et al. 1973), may have served as a reservoir to introduce fenoxycarb to founding queens. Either mechanism, (territorial defense or retention of fenoxycarb in the replete caste with subsequent transfer to founding queens) could account for the delay in reinfestation associated with fall treatments.

#### Amdro

Control with spring Amdro treatments was most effective 2-5 months after application, followed by relatively rapid resurgence of incipient colonies (Figure 3). Summer applications, though slightly less effective, maintained population suppression for about 11 months. The prolonged delay in activity of fall treatments was readily apparent. Winter treatments produced highly erratic results which peaked one month after treatment. Heavy reinfestation was recorded 7-9 months after application.

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Table 1. Environmental Conditions During Monthly Applications of Amdro and Logic Baits.

Month of Application	Air Temp. ( °F ) <u>1/</u>	Soil Temp. ( °F ) <u>2/</u>	Rainfall (inches) <u>3/</u>
May 1989	--	--	10.8
June 1989	85	85	8.3
July 1989	90	90	11.4
August 1989	85	82	1.4
September 1989	86	86	6.1
October 1989	74	68	1.7
November 1989	72	66	10.2
January 1990	65	46	6.7
February 1990	76	52	11.7
March 1990	80	70	6.7
April 1990	78	60	3.0
May 1990	82	72	6.6

1/ 4' from soil surface, indirect sun at time of bait application.

2/ 1" depth, indirect sun at time of bait application.

3/ Monthly cumulative total.

Table 2. Mean % ± (SEM) Reduction in Pretreat IFA Population Indices at Various Post-Treatment Intervals Following Monthly Applications of Andro and Logic Baits.

Month of Application	Percent Reduction in Pretreat Population Index for each Indicated 1/ 2/ Bait and Post-Treatment Interval (Months)							
	Pretreat 1/ Pop. Index		(1)		(2)		(3)	
	Andro	Logic	Andro	Logic	Andro	Logic	Andro	Logic
January	401.6 ± (25.2)	421.7 ± (64.1)	81.6 ± (3.4)a	79.3 ± (.7)a	70.3 ± (2.7)a	78.0 ± (.6)a	15.3 ± (2.9)a	78.3 ± (1.2)b
February	266.7 ± (33.2)	308.3 ± (35.3)	91.3 ± (4.2)a	71.3 ± (5.6)a	66.7 ± (3.2)a	80.0 ± (1.1)a	87.7 ± (1.7)a	93.7 ± (2.9)a
March	400.0 ± (16.0)	403.3 ± (10.9)	62.6 ± (4.4)a	76.3 ± (1.7)a	81.0 ± (2.1)a	77.7 ± (4.0)a	93.6 ± (.9)a	89.0 ± (2.6)a
April	390.0 ± (72.9)	481.6 ± (66.2)	75.7 ± (6.6)a	80.0 ± (2.0)a	94.7 ± (1.8)a	91.3 ± (.9)a	97.0 ± (1.5)a	98.3 ± (.6)a
May	421.6 ± (19.7)	473.3 ± (68.0)	85.3 ± (4.9)a	89.0 ± (2.0)a	98.0 ± (1.0)a	94.0 ± (1.0)a	98.3 ± (1.5)a	99.9 ± (.1)a
June	227.6 ± (26.5)	236.6 ± (60.0)	74.3 ± (10.6)a	87.0 ± (6.0)a	95.0 ± (1.7)a	93.7 ± (2.4)a	97.0 ± (1.5)a	80.7 ± (11.9)a
July	145.0 ± (10.0)	170.0 ± (38.2)	-----	-----	86.0 ± (3.8)a	95.3 ± (1.4)a	88.0 ± (2.3)a	78.0 ± (8.4)a
August	283.3 ± (37.2)	303.3 ± (55.3)	88.7 ± (3.3)a	80.3 ± (4.2)a	91.3 ± (.3)a	73.0 ± (7.6)a	90.3 ± (4.2)a	78.6 ± (2.4)a
September	226.3 ± (40.2)	261.6 ± (15.9)	70.3 ± (19.3)a	68.3 ± (3.3)a	88.6 ± (4.3)a	76.3 ± (1.4)a	96.6 ± (3.2)a	76.0 ± (2.6)a
October	466.7 ± (34.2)	274.0 ± (65.6)	42.3 ± (6.3)a	6.7 ± (14.1)a	75.0 ± (9.0)a	86.0 ± (2.0)a	78.7 ± (.9)a	76.3 ± (4.0)a
November	341.3 ± (36.6)	381.7 ± (46.7)	-----	-----	-----	-----	88.0 ± (3.6)a	80.0 ± (2.1)a

1/ Mean based on 3 replicates per treatment ± (SEM).

2/ Treatment means for any given month and post-treat interval followed by the same letter are not significantly different (P > 0.05) according to a "t" test.

Table 2. (Continued)

Month of Application	Percent Reduction in Pretreat Population Index for each Indicated 1/ 2/											
	(6)		(8)		(9)		(10)		(11)		(12)	
	Amdro	Logic	Amdro	Logic	Amdro	Logic	Amdro	Logic	Amdro	Logic	Amdro	Logic
January	72.3 ± (8.9)a	68.0 ± (7.4)a	---	87.5 ± (4.7)	---	85.3 ± (4.2)	---	79.4 ± (2.7)	---	---	---	---
February	96.7 ± (2.0)a	72.3 ± (3.3)b	66.9 ± (9.9)a	64.9 ± (14.8)a	9.4 ± (30.4)a	38.0 ± (24.5)a	---	29.7 ± (30.2)	---	---	---	---
March	84.0 ± (2.9)a	88.0 ± (3.6)a	65.0 ± (4.9)a	63.1 ± (2.1)a	60.7 ± (3.9)a	50.3 ± (8.3)a	---	---	---	---	---	---
April	79.6 ± (3.3)a	94.0 ± (1.1)a	77.0 ± (6.0)a	78.3 ± (3.8)a	---	---	---	---	---	---	---	---
May	49.6 ± (27.8)a	88.3 ± (1.8)a	---	94.3 ± (2.2)	---	---	---	---	---	---	---	---
June	82.7 ± (3.7)a	94.7 ± (2.0)a	82.6 ± (3.9)	94.5 ± (2.1)	95.2 ± (.7)a	90.4 ± (6.3)a	57.8 ± (4.6)a	77.6 ± (11.2)a	51.3 ± (13.0)a	50.8 ± (19.9)a	---	---
July	61.3 ± (16.5)a	70.6 ± (7.4)a	74.9 ± (7.9)a	70.7 ± (.6)a	66.4 ± (17.0)a	36.4 ± (25.6)a	---	---	---	---	---	---
August	92.3 ± (1.8)a	85.6 ± (.9)a	88.3 ± (2.5)a	88.6 ± (2.6)a	---	---	---	97.6 ± (1.0)a	74.0 ± (3.3)a	97.8 ± (2.2)b	---	---
September	92.0 ± (4.0)a	75.3 ± (2.3)a	83.0 ± (8.6)a	79.3 ± (5.7)a	93.0 ± (1.9)a	95.3 ± (2.3)a	93.0 ± (1.9)a	95.3 ± (2.3)a	50.6 ± (8.8)a	81.8 ± (6.5)a	---	---
October	80.7 ± (3.8)a	92.0 ± (3.8)a	80.7 ± (3.7)a	92.1 ± (3.8)	93.1 ± (1.3)a	100 ± (0)a	88.6 ± (3.8)	100 ± (0)	88.9 ± (3.2)a	97.5 ± (2.5)a	---	---
November	79.3 ± (5.9)a	91.6 ± (1.8)a	95.3 ± (1.2)a	99.6 ± (.2)a	89.0 ± (1.6)a	100 ± (0)a	61.4 ± (6.8)a	100 ± (0)b	74.8 ± (3.8)a	61.7 ± (4.7)a	---	---

1/ Mean based on 3 replicates per treatment ± (SEM).

2/ Treatment means for any given month and post-treat interval followed by the same letter are not significantly different (P > 0.05) according to a "t" test.

Table 3. Time Required to Achieve Maximum Control and Maintain Population Suppression  $\geq$  75% of Pretreatment Levels Following Monthly Applications of Amdro and Logic Baits.

Application Period	Months required to achieve maximum reduction in pretreatment population index		Months population suppression $\geq$ 75% of pretreatment levels	
	Amdro	Logic	Amdro	Logic
January	1	8	1	10 <sup>+</sup>
February	6	3	6	2
March	3	3	4	6
April	3	3	8 <sup>+</sup>	8 <sup>+</sup>
May	3	3	3	8 <sup>+</sup>
June	3	6	5	10
July	3	2	3	3
August	6	12	10	12 <sup>+</sup>
September	3	10	8	12 <sup>+</sup>
October	6	9	10 <sup>+</sup>	10 <sup>+</sup>
November	3	6	9	10
$\bar{x} \pm$ (SEM)	3.6 $\pm$ (0.5)a	5.9 $\pm$ (1.0)a	6.0 $\pm$ (0.9)	8.3 $\pm$ (1.0)

1/ Means followed by same letter are not significantly different (P>0.05) according to a "t" test.



Figure 1. Scattergram of Soil Temperature and IFA Colony Mortality with Amdro and Logic

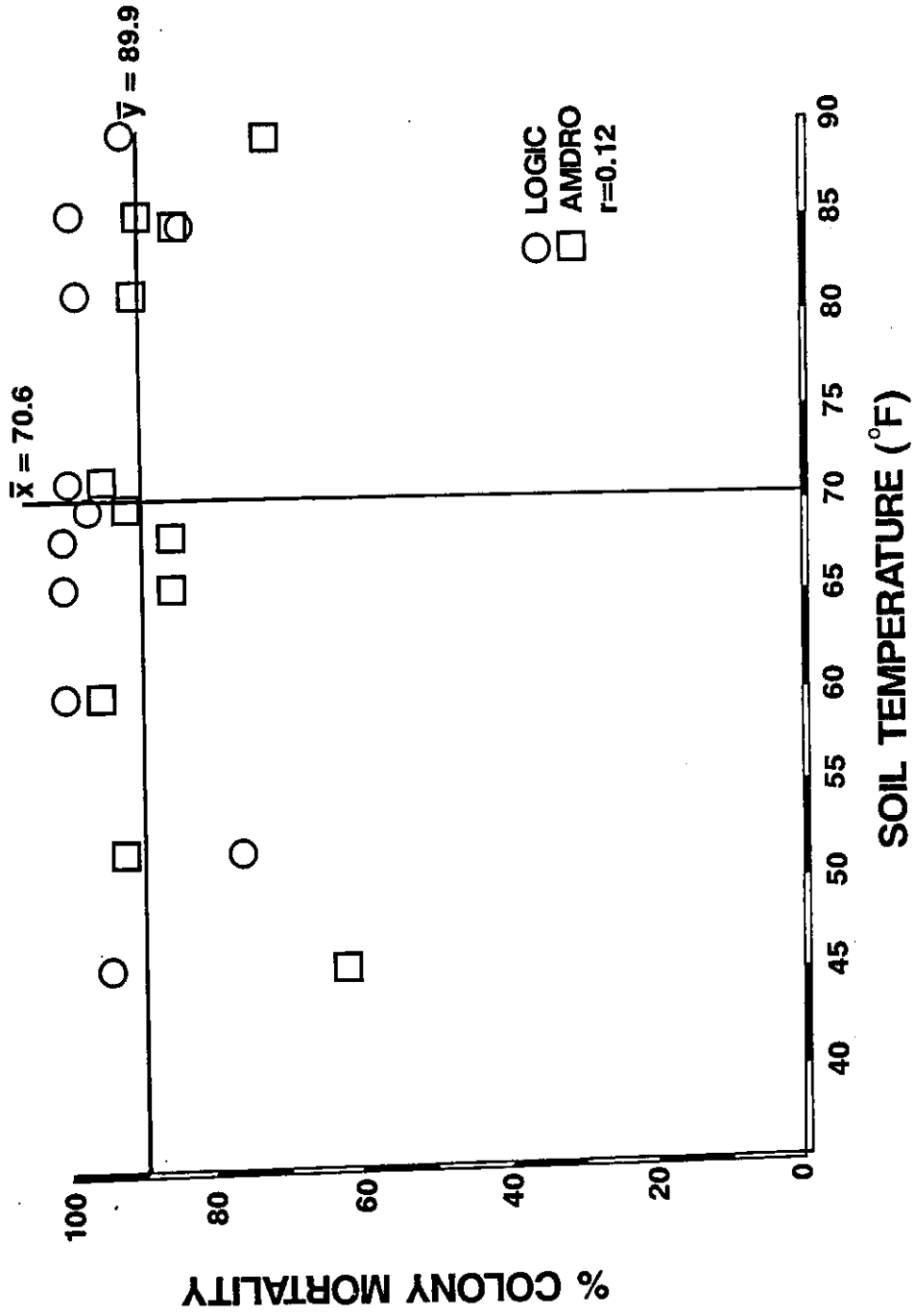


FIGURE 2

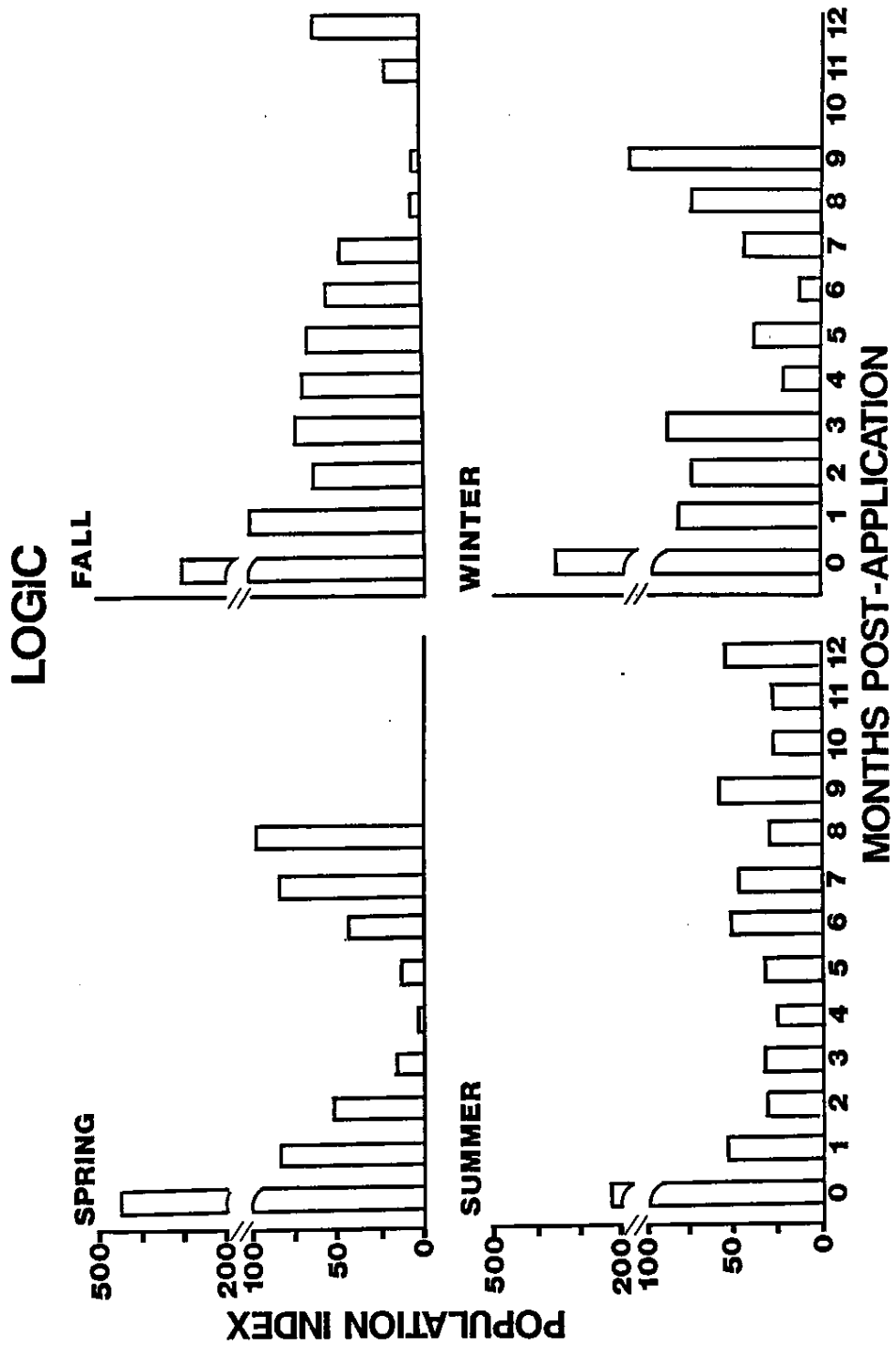
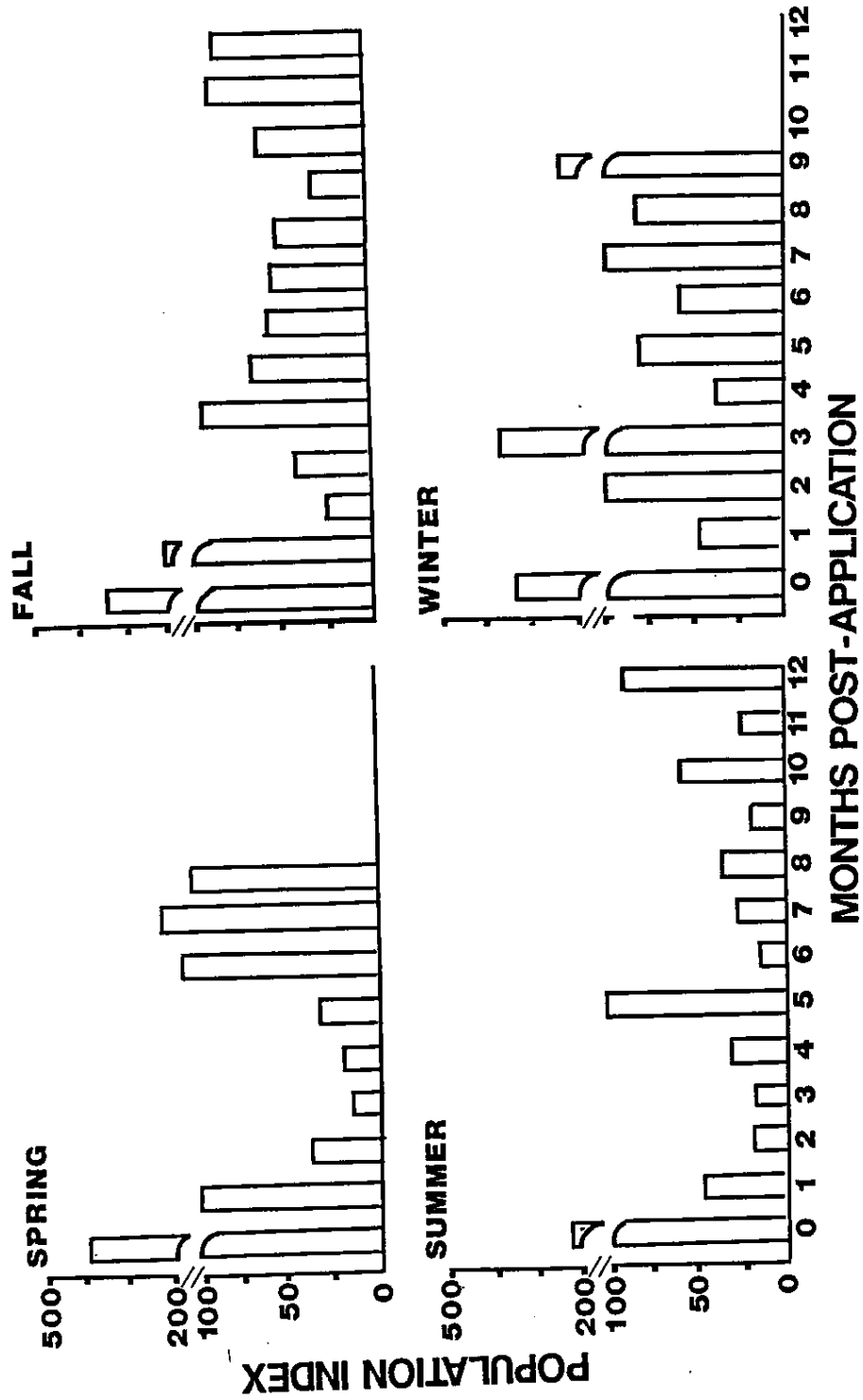
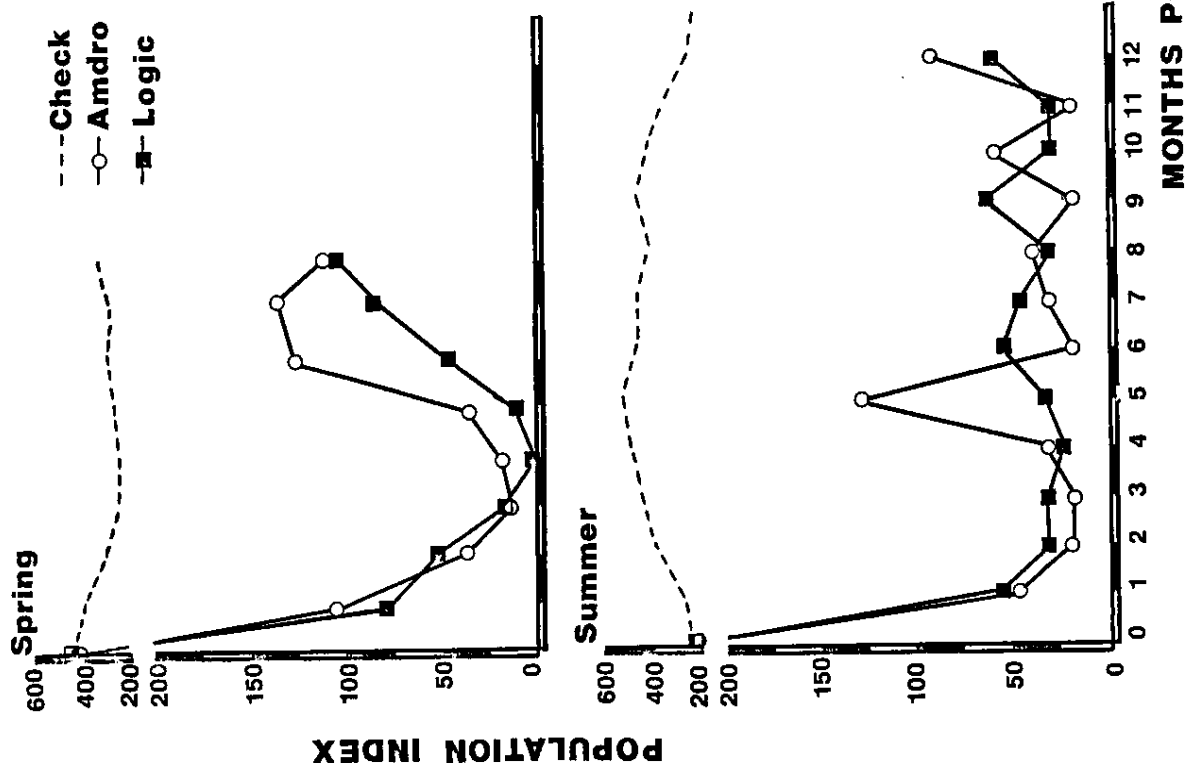
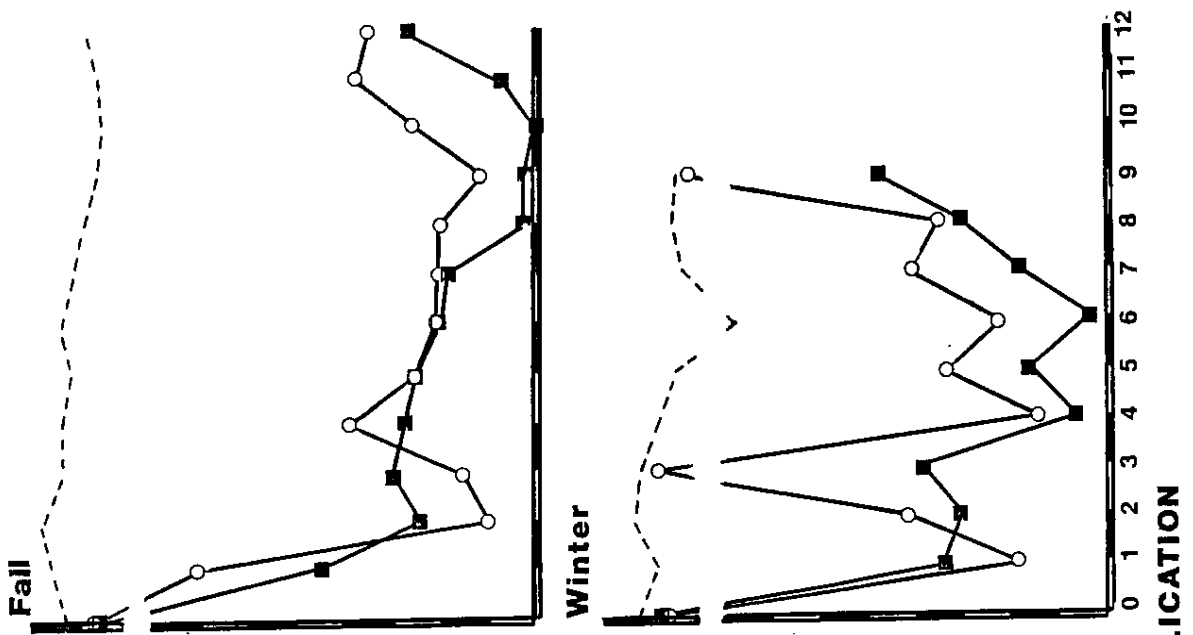


FIGURE 3

# AMDRO





--- Check  
 ○ Amdro  
 ■ Logic

POPULATION INDEX

MONTHS POST-APPLICATION

**EFFECTS OF SPOT TREATMENTS OF LOGIC® (FENOXYCARB) ON  
POLYGYNOUS RED IMPORTED FIRE ANTS:  
AN INDICATION OF RESOURCE SHARING?**

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and Associate Professor  
C. L. Barr, Research Assistant  
S. Bradleigh Vinson, Professor

Food exchange between adjacent nests of the red imported fire ant, *Solenopsis invicta* Buren, was elucidated by Summerlin et al. (1975) using dye-impregnated soybean oil. Bhatkar and Vinson, (1987, 1987a, and 1989) demonstrated foraging pattern differences between monogyn and polygyn red imported fire ants by marking foraging ants with a non-toxic paint, finding that worker ants moved freely between mounds of the polygynous form.

Logic® Fire Ant Bait contains the active ingredient, fenoxycarb, which acts as an insect growth regulator. Ingestion of fenoxycarb by the brood redirects larval development toward production of winged reproductive castes, only. Queen ant ovaries are also affected (Glancey 1987) and egg production is severely reduced or eliminated. In the absence of worker brood production, colonies treated with Logic decline slowly as worker ants are not replaced. This process can take up to several months. During this period, affected colonies can be detected because of the absence of worker brood and prevalence of reproductive brood (large larvae and pupae with wing pads).

Individual spot Logic applications were used to determine whether or not, and to what extent neighboring red imported fire ant mounds were affected by the treatment.

**Materials and Methods**

Native pasture at the edge of an abandoned pecan orchard in Burelson County was used to establish test plots. Logic® Fire Ant Bait (fenoxycarb) applied either to 1) the top of individual fire ant mounds or 2) placed randomly in the pasture at a rate of 3 tablespoons per spot on 21 September 1990. Treatment spots and mounds were established along transect lines and were separated by a minimum of 70 ft. Treatments were replicated six times and marked with plot flags. An additional set of six spots were marked to serve as an untreated check. These plots were randomly selected from an area adjacent to the treatment plots but at least 150 feet away from any flag. The entire area appeared uniform in terrain, soil type, and vegetation. Adequate moisture was present throughout the test period with temperatures varying greatly, but staying well above 70°F.

Five weeks following treatment, all active mounds within a 30 ft. radius were mapped and inspected for presence of worker and reproductive brood. The total number of fire ant

mounds and mounds affected by the Logic treatment, as indicated by the lack of worker brood and the presence of reproductive brood, was determined within 0-5, 5-10, 10-15, 15-20, 20-25 and 25-30 ft. radius from the location of the spot application for each treatment. These values were converted to percent affected mounds and these values were correlated to the distance from treatment location.

## Results

Average mound density was 24.78 mounds per plot or 381 mounds per acre, indicating the prevalence of polygynous form of the the red imported fire ant. Significant negative correlations ( $P \geq 0.01$ ) between the number of Logic<sup>®</sup>-affected mounds and the distance from the spot application (Table 1). Linear regression equations [Y, percent affected mounds = (Y intercept) + (slope) X, distance from treatment spot] were:

$$\text{Treated central mound: } Y = 93.7 + -3.66X \quad (r = -0.8993)$$

$$\text{Random treatment: } Y = 105.10 + -4.113X \quad (r = -0.8706)$$

Relationship between treatment location and affected mounds were very similar, regardless of placement of bait on mounds or randomly within an infested area (Fig. 1). A calculated range, for the distance from the spot application to where 50 percent of the mounds were affected by the treatment was 11.9 to 13.4 ft. for bait placed on a mound versus randomly, respectively. The maximum treatment spot to affected mound distance was 23 ft.

## Discussion

Clearly, more than a single fire ant mound was affected by the spot applications of Logic applied. In areas infested by the polygyn form, these mounds may represent a single colony comprised of numerous mounds. Results of this study, however, can not be used to conclusively argue that food exchange occurred between individuals from adjacent mounds. Conceivably, foraging workers from adjacent mounds or colonies shared a single resource (a spot application of bait) over time, although fire ant baits readily decompose in the environment. Regardless, results support the findings of previous studies (Summerlin et al. 1975, Bhatkar, A. P and S. B. Vinson, 1987, 1987a, and 1989).

Other aspects of these results may have implications to research methodology and fire ant management. The distance between bait treatment location and affected mounds reported here would conceivable differ with varying densities of fire ants within the study area and the dose. However, during evaluations of individual mound or broadcast bait applications, the researcher must be aware of the possibility of the exchange and/or sharing of the toxicant-laden bait between neighboring colonies. In establishing broadcast application plots, buffer zones of at least 60 ft. between treatments may be necessary to completely eliminate the possibility of treatments affecting adjacent plots.

In fire ant management, the food exchange and/or resource sharing of a toxicant bait can be beneficial in a number of ways. The knowledge that bait applications in restricted small spaces, such as home lawns can affect mounds in neighboring areas for roughly 20 feet from the edge of the treatment can be beneficial and help justify the urban use of slow-acting pesticides such as Logic. Furthermore, documented resource sharing between mounds could help justify the use of bait stations for fire ant management. And finally, resource sharing among the polygyn form make them more vulnerable to being capable of transmitting natural enemy agents, particularly pathogens, from mound to mound and perhaps between colonies.

#### Literature Cited

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**Table 1.** Number of red imported fire ant mounds affected by a spot treatment of Logic (fenoxycarb) Fire Ant Bait five weeks following application (21 Sept.), Burleson County, Texas, 1990.

Radius	No. of Mounds with reproductive brood only/total mounds (percent in parentheses)		
	Treated mound	Random treatment	Untreated
0-5	5/6 (83)	1/1 (100)	0/11 (0.0)
6-10	10/14 (71)	6/6 (100)	0/17 (0.0)
11-15	4/26 (15)	3/24 (12.5)	0/41 (0.0)
16-20	2/45 (4)	0/18 (0.0)	0/36 (0.0)
21-25	1/38 (3)	1/23 (4.3)	0/34 (0.0)
25-30	0/44 (0)	0/31 (0.0)	0/31 (0.0)

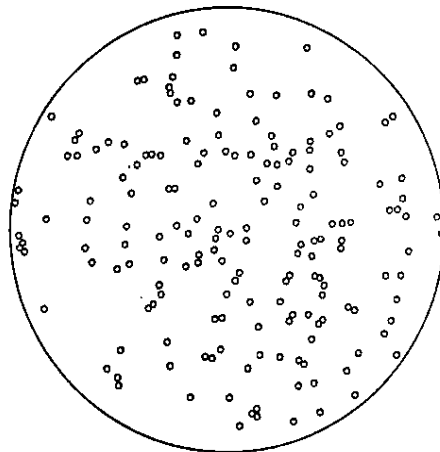
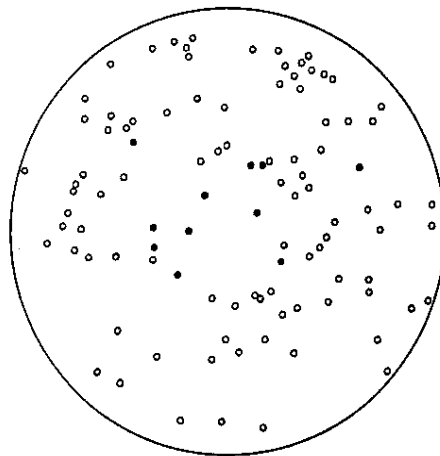
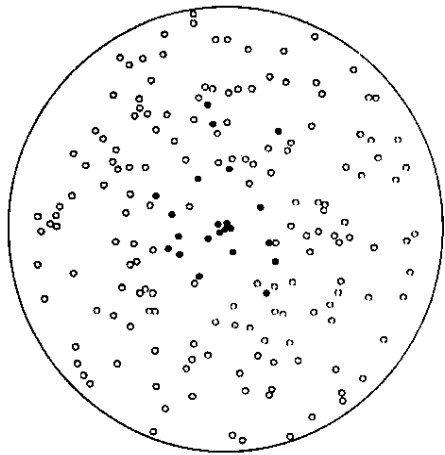
r                      -0.90\*                      -0.87\*

\* Indicates significant correlation (df = 11, Corr. Coef. = 0.708, P = 0.01)



**Fig. 1.** Composite plot maps from 6 replicates of 3 treatments: a) spot applications of Logic to central mounds, b) spot Logic applications at random central locations, and c) untreated. Open circles represent active unaffected red imported fire ant mounds while black dots indicate affected mounds containing reproductive brood, only. Burleson County, Texas, 1991.

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## FIRE ANT SPECIES-SPECIFIC BAITS AND REPELLENTS

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There are at least two methods to utilize pheromones - A) as a behavior disruptant and B) incorporated into a bait to enhance that bait's species-specificity and efficacy. The USDA has opted to pursue bait enhancement. This means that we must incorporate a species-specific pheromone into the currently used bait.

A fire ant bait is composed of: A) soybean oil - a solvent for the insecticide and very importantly an excellent phagostimulant that induces the worker ants to ingest both it and the dissolved insecticide; B) an inert carrier - pregeled defatted corn grit; and C) the insecticide - of which there are three commercially available and registered for limited fire ant use.

Our working hypothesis is that the use of an attractant pheromone will decrease the time it takes for *S. invicta* workers to discover a bait particle. If this can be achieved, then the bait will be more species-specific and more efficient. In addition, if fire ants can be removed from an area while leaving native ant species, then the re-infestation rate will decrease through native ant predation on the vulnerable newly mated fire ant queens.

### WHAT PHEROMONE SYSTEMS ARE AVAILABLE?

The glands associated with the sting apparatus are the source of the queen and recruitment pheromones. In the queen the poison gland produces chemicals that elicit a wide variety of behaviors and likewise the Dufour's gland elicits several worker behaviors. In both cases our interests lie with the worker attraction elicited by each of these systems.

**QUEEN PHEROMONE.** Three components (A, B, C) were isolated and identified from whole queen extracts. The bioassays used in the isolation measured several different behaviors. Consequently an olfactometer was used to investigate the relationship between the three compounds and the specific behavior - attraction. Components B and C are optically active, thus the possible test materials are A, +B, -B, ±B, +C, -C, and ±C. Of the nine possible three component mixtures, several had significant attraction (>65%) and one of these, A, ±B, ±C, did not require optically pure isomers. This is desirable in terms of potential commercialization because optically pure compounds cost significantly more to synthesize. On the negative side incorporation of three synthetic compounds into a bait system still represents cost problems.

Of the 15 two component possibilities there only 3 mixtures gave

statistically significant olfactometer results. The results for two active mixtures had undesirable high standard deviations. However, the third mixture (A,  $\pm$ B) looks good in all respects - significant attraction; small standard deviation; no optically pure isomers are required. From a commercial perspective this movement in the right direction. No single components were active in the olfactometer bioassay at physiological levels.

An important factor for the successful utilization of a pheromone in a bait is the range of concentration at which it maintains significant activity. The active two component system (A,  $\pm$ B) is active over almost two orders of magnitude. Interestingly, Racemic B at greater than physiological concentrations gives significant attraction over 1.5 orders of magnitude, thus we have the possibility of incorporating a drastically simplified attractive pheromone into a bait.

Continuation of this research has been hampered by the loss of biological support. Fortunately, American Cyanamid expressed interest in this work throughout the olfactometer evaluations. I am pleased that we have now entered a Cooperative Research and Development Agreement with American Cyanamid that provides funds for the materials and technical support necessary to carry this work to fruition.

**RECRUITMENT PHEROMONE.** Much behavioral and chemical work has centered on this pheromone. Three distinct behavioral sub-categories have been defined: A) attraction, B) orientation induction, and C) orientation.

For *S. invicta* the chemistry associated with these sub-categories is complex. The complete orientation response is achieved with Z,E-alpha-farnesene, whereas attraction requires a combination of Z,E-alpha-farnesene and an as yet unidentified minor component, C-1 (ca. 75pg/gland). The chemistry of orientation induction is complex and thus far it appears that to get 100% of Dufour's gland activity the its multi-component chemical profile will have to be duplicated.

The Dufour's gland of *Solenopsis richteri* contains "C-1" as its major component (ca. 4ng/gland). We have recently isolated about 2 mg from *S. richteri* for structural determination. For *S. richteri* C-1 is responsible for 100% of both orientation and attraction, however, only 80% of orientation induction can be explained with C-1 alone.

Cross-species quantitative bioassays and chemical analyses have allowed us to understand and rationalize why *S. invicta* responds to *S. richteri*'s recruitment pheromone, whereas *S. richteri* workers respond to *S. invicta*'s recruitment pheromone only in the orientation bioassay.

We have demonstrated that C-1 at greater than physiological levels is a good attractant for *S. invicta* workers. As with the queen pheromone this material has potential for enhancement of fire ant baits. We anticipate that its structure will be fully elucidated and that synthetic material will be available soon.

## REPELLENTS

Al Banks and I collaborated on the following experiments designed to evaluate the efficacy of a fire ant repellent in keeping fire ants out of plant

nurseries. There are several fire ant sources for contamination of nursery stock, e.g. colony founding queens from mating flights dropping onto nursery grounds, and movement of incipient and/or mature colonies into nursery stock.

**INCIPIENT/MATURE COLONIES.** In the laboratory small queenright colonies were set up in petri dish cells, in which they are content as long as they do not have soil as an alternative. Within 30 minutes after introduction of a peat pot and soil worker fire ants discover the pot and move the entire colony into the soil.

In the course of screening for attractants using the olfactometer we discovered a very good fire ant repellent. Application of this repellent to peat pots at increasing concentrations gave the expected increase in repellent longevity. Such that at the highest concentration used the colonies were kept at bay for almost 2 months.

Peat pots treated at high repellent concentrations were aged outside for up to 56 days, and periodically brought into the laboratory to measure the time of fire ant invasion. The highest concentration maintained 100% repellent activity through 4 weeks of aging outdoors.

Another experiment used plastic pots filled with soil. There were two treatments repellent painted on the outside; and repellent mixed with lacquer and painted on the outside of the pot. Ants moved into the controls within 30 minutes. The repellent treated pots kept the ants out for over 24 hours; but significantly the lacquer/repellent combination enhanced the length of activity by a factor of five! This is important because it illustrates that altering the release rate of the repellent extends its active life. Controlled release technology may be able to significantly improve the results shown here.

**NEWLY MATED QUEENS.** A newly mated queen - colony foundation choice test consisted of a large colony tray into which 4 smaller trays were countersunk so that when the small trays were filled with soil, a flat surface was formed across the bottom of the large tray. Two soil controls and two repellent treatments of different concentrations were used. Newly mated queens collected the afternoon of their mating flight were brought back to the laboratory and immediately distributed equally on the surface of the controls and treatments. The soil was excavated and searched for queens 2 to 3 days later.

The results show that there was no significant difference between the two controls and that only one out of 48 queens chose repellent treated soil at the lowest repellent concentration and no queens choose the higher concentration. The repellent successfully discouraged newly mated queens from founding colonies in treated soil.

This particular repellent is highly suitable for controlled release formulation, which should increase its efficacy for excluding fire ants from areas where they are not wanted. Preparation of a patent application is in progress.

**STUDIES OF BAIT TOXICANTS ON RED IMPORTED FIRE ANTS AND NON-TARGET ANTS IN SOUTH FLORIDA, 1989-1991<sup>1</sup>**

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1989-1990 tests

Bait Tests:

Logic<sup>R</sup> and Amdro<sup>R</sup> baits were applied as broadcast and single-mound treatments for control of imported fire ants in Caloosa park in Palm Beach County, Florida. Both baits were applied as a broadcast treatment using a tractor-mounted applicator at the label-recommended rate of 1.7 kg bait/ha (1 lb/acre). Both baits were also applied as a single-mound treatment by hand by 8 workers at the label-recommended rates of 1 to 5 tablespoons of bait per mound (amount applied depended on mound size). All blocks were treated on 15 February 1989. Each block was 1.6 to 2.4-ha (4 to 6 acres) and all were bounded by physical barriers (canals, woods, RR right-of-way, insecticide treated ball fields, or paved areas) which precluded immigration of fire ant colonies. An untreated check block was set up in Lake Ida Park, Delray Beach, ca. 2 km south of Caloosa Park.

<sup>1</sup> This article represents the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by the USDA.

Evaluation was made by examination of all colonies within 5 circular subplots established in each treatment block. The circular plots had a radius of 17.8 m (58 ft 6 inches) yielding a 0.1 ha (1/4 acre) plot. The plots were checked by 3 or more persons moving along with a measuring tape, opening each fire ant mound with a shovel, and examining the colony for worker ant brood. The number of ants in the mound was estimated in the field and the population index calculated (Banks et al. 1988). Pretreatment counts were made in December, 1988. Posttreatment counts were made at the following weekly intervals: 10 (April 1989), 14 (May), 18 (June), 26 (August), 30 (September), 38 (November), and 46 (January 1990).

The data were analyzed statistically using SAS-GLM on mean ranks of the population indexes by time. This is a conservative parametric test using ranked transformation as recommended by SAS Institute (1988). Only one small study using Amdro<sup>R</sup> (Reinert 1982) presented data on fire ant control measures from southern Florida.

Both materials gave the expected levels of control of fire ant populations (Banks et al. 1981, 1988; Phillips and Thorvilson 1989). Differences between the broadcast and mound treatments were negligible; probably because the large number of workers used to apply the baits in the single-mound treatments resulted in good coverage of the blocks. With 1 exception, all of the indexes were not significantly different from the check or were significantly lower than the check. Logic broadcast, at 46 weeks posttreatment, was the only treatment significantly higher than

the check. The seasonal drought which occurred during the 14 week counts, accounted for the low check index. By 38 weeks posttreatment, reinfestation was well under way and the fire ant populations were rebounding as expected from previous studies given the small size of the treatment blocks (Lofgren & Williams 1985). At 10 weeks posttreatment, Logic<sup>R</sup> broadcast and single-mound treatments gave 71.5% and 87.7% and Amdro<sup>R</sup> broadcast and single-mound treatments gave 89.7% and 79.4% reductions in population index (Fig. 1 & 2). Fire ant populations were suppressed through the summer and by 46 weeks posttreatment, had gradually returned to or above pretreatment levels. Costs of the utilized control strategies was determined by labor costs.

#### Non-target Ant Test:

Logic<sup>R</sup> and Amdro<sup>R</sup> baits were applied for control of imported fire ants in a park in Palm Beach County, Florida. The effects of the treatments on non-target ants were monitored with pitfall traps. Each trap consisted of a 12.8 cm length sleeve (5 inch) of 7.5 cm diameter (3-inch) PVC pipe, a plastic 414 ml (14-oz) beverage cup, approximately 350 ml (12-oz) of preservative (1/4-ethylene glycol, 1/4-water, 1/2-isopropanol), and a rain-cover (23 cm (9-inch) square of plywood with a nail in each corner). The traps were filled with preservative and left open for 1 week. The contents of the traps were removed and the ants were separated, identified, and counted.

The pitfall traps were placed in groups of 10 at each pitfall collection site; with 4 pitfall collection sites in each treatment block. Pretreatment collections were made in December,

1988. Posttreatment collections were made at weekly intervals: 10 (April), 14 (May), 18 (June), 22 (July), 26 (August), 30 (September), 38 (November 1989), and 46 (January 1990).

The data were subjected to statistical analysis using SAS. Each group of 10 pitfall traps were treated as one replicate; for 4 replicates per treatment. The data were analyzed using SAS-ANOVA on square roots of the means from 10 pitfall traps +1. Waller-Duncan test was used to separate the means.

The following 26 species of ants in 18 genera were collected in the pitfall traps: Aphaenogaster ashmeadi (Emery), A. near rudis, Brachymyrmex obscurior (Forel), Camponotus floridanus (Buckley), Cardiocondyla emeryi Forel, Conomyrma bureni Trager, Crematogaster clara Mayr, Cyphomyrmex rimosus (Spinola), Formica archboldi Smith, Hypoponera opaciceps (Mayr), Neivamyrmex opacithorax (Emery), Odontomachus ruginodis Wheeler, Paratrechina boubonica (Forel), P. faissonensis (Forel), P. longicornis (Latreille), Pheidole dentata Mayr, P. metallescens Emery, P. moerens Wheeler, P. morrиси Forel, Pseudomyrmex mexicanus Roger, Solenopsis geminata (F.), S. invicta Buren, Solenopsis (Diplorhoptrum) sp., Strumigenys louisianae Roger, Tapinoma melanocephalum (F.), Tetramorium caldarium (Roger). Non-target ants were not adversely affected (Fig. 3, 4, 5, 6) by the termination of the test at 46 weeks posttreatment.

#### 1990-1991 TESTS

Logic<sup>R</sup> bait was applied for control of imported fire ants before and after mating flights in a park in Palm Beach County,



Florida, using the plots and methods described in the previous tests. By 49-54 weeks posttreatment, fire ant populations had returned to or above pretreatment levels (Fig. 7) and non-target ants were not adversely affected (Fig. 8). The indications are that the pre-mating flight treatment resulted in higher RIFA populations by the termination of the test. However, the test needs to be repeated to verify the results.

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Figure 1. Solenopsis invicta population index after treatment with Amdro<sup>R</sup> and Logic<sup>R</sup> broadcast (=BC) and single mound treatment (=MD) in Palm Beach County, Florida.

Figure 2. Percent change from expected in the Solenopsis invicta population index after treatment with Amdro<sup>R</sup> and Logic<sup>R</sup> broadcast (=BC) and single mound treatment (=MD) in Palm Beach County, Florida.

Figure 3. Numbers of Conomyrma bureni collected in pitfall traps after treatment with Amdro<sup>R</sup> and Logic<sup>R</sup> broadcast (=BC) and single mound treatment (=MD) in Palm Beach County, Florida.

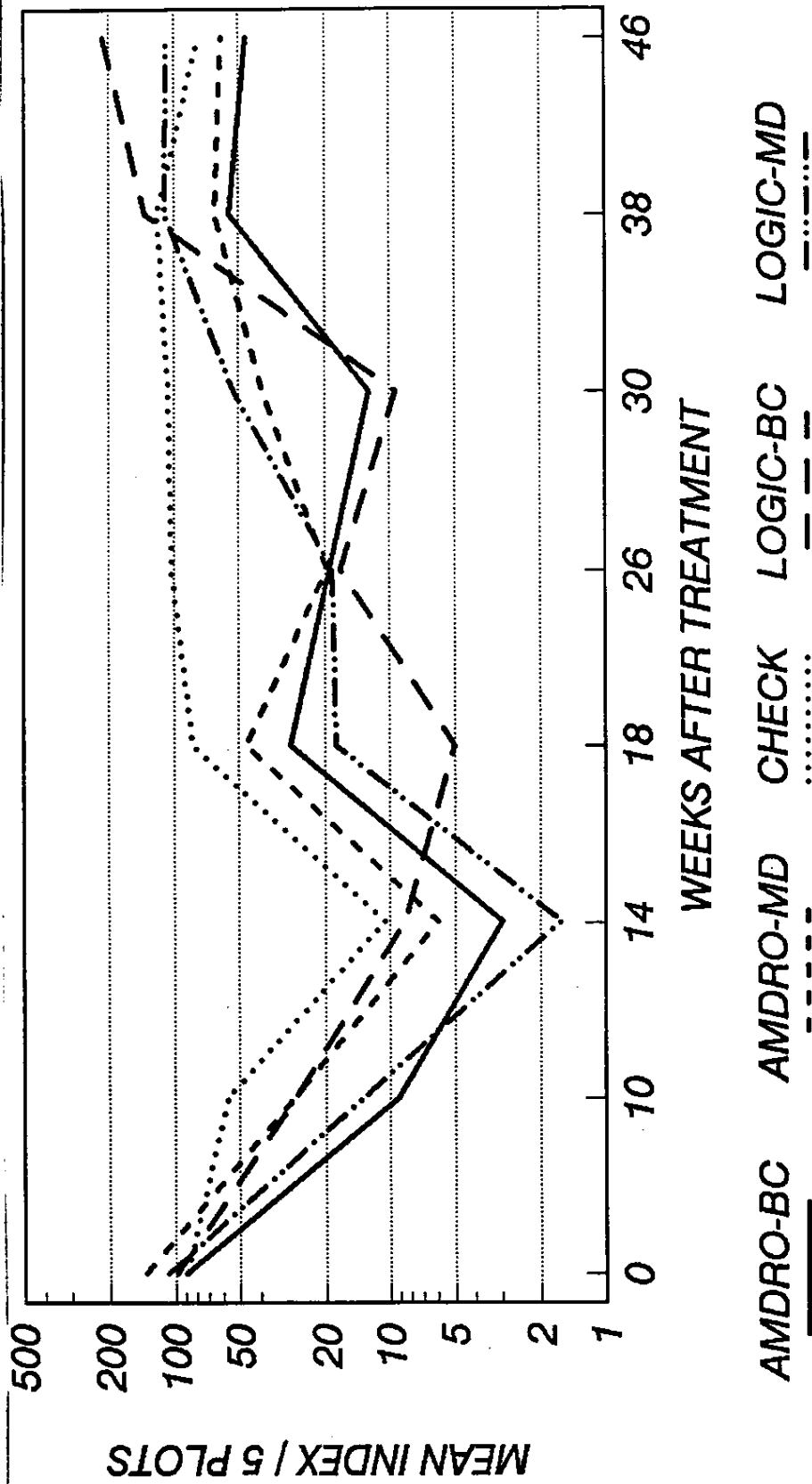
Figure 4. Numbers of Brachymyrmex obscurior collected in pitfall traps after treatment with Amdro<sup>R</sup> and Logic<sup>R</sup> broadcast (=BC) and single mound treatment (=MD) in Palm Beach County, Florida.

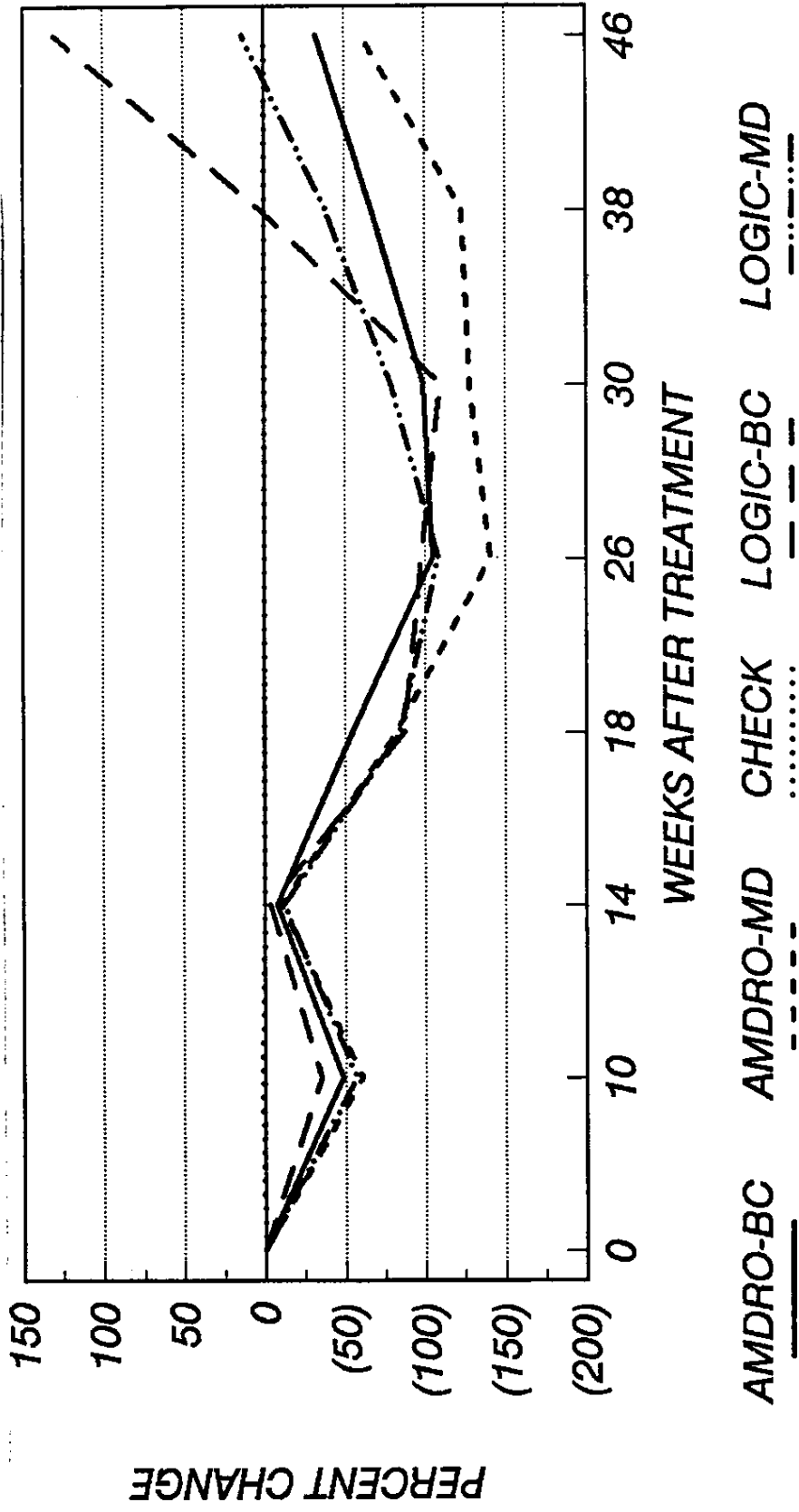
Figure 5. Numbers of Camponotus floridanus collected in pitfall traps after treatment with Amdro<sup>R</sup> and Logic<sup>R</sup> broadcast (=BC) and single mound treatment (=MD) in Palm Beach County, Florida.

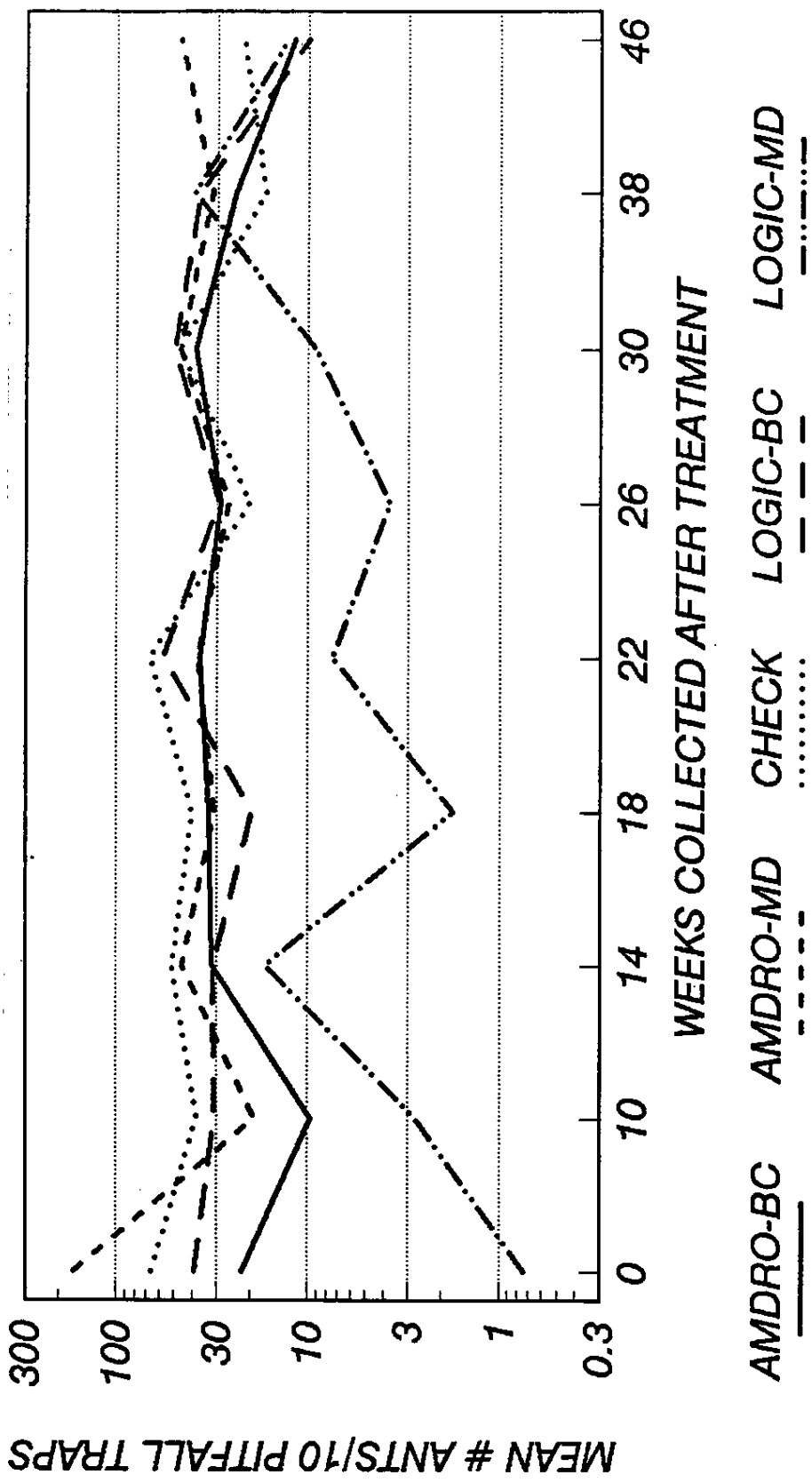
Figure 6. Numbers of Miscellaneous ants collected in pitfall traps after treatment with Amdro<sup>R</sup> and Logic<sup>R</sup> broadcast (=BC) and single mound treatment (=MD) in Palm Beach County, Florida.

Figure 7. Percent change in the Solenopsis invicta population index after pre- or post-mating flights treatment with Logic<sup>R</sup> broadcast treatment in Palm Beach County, Florida.

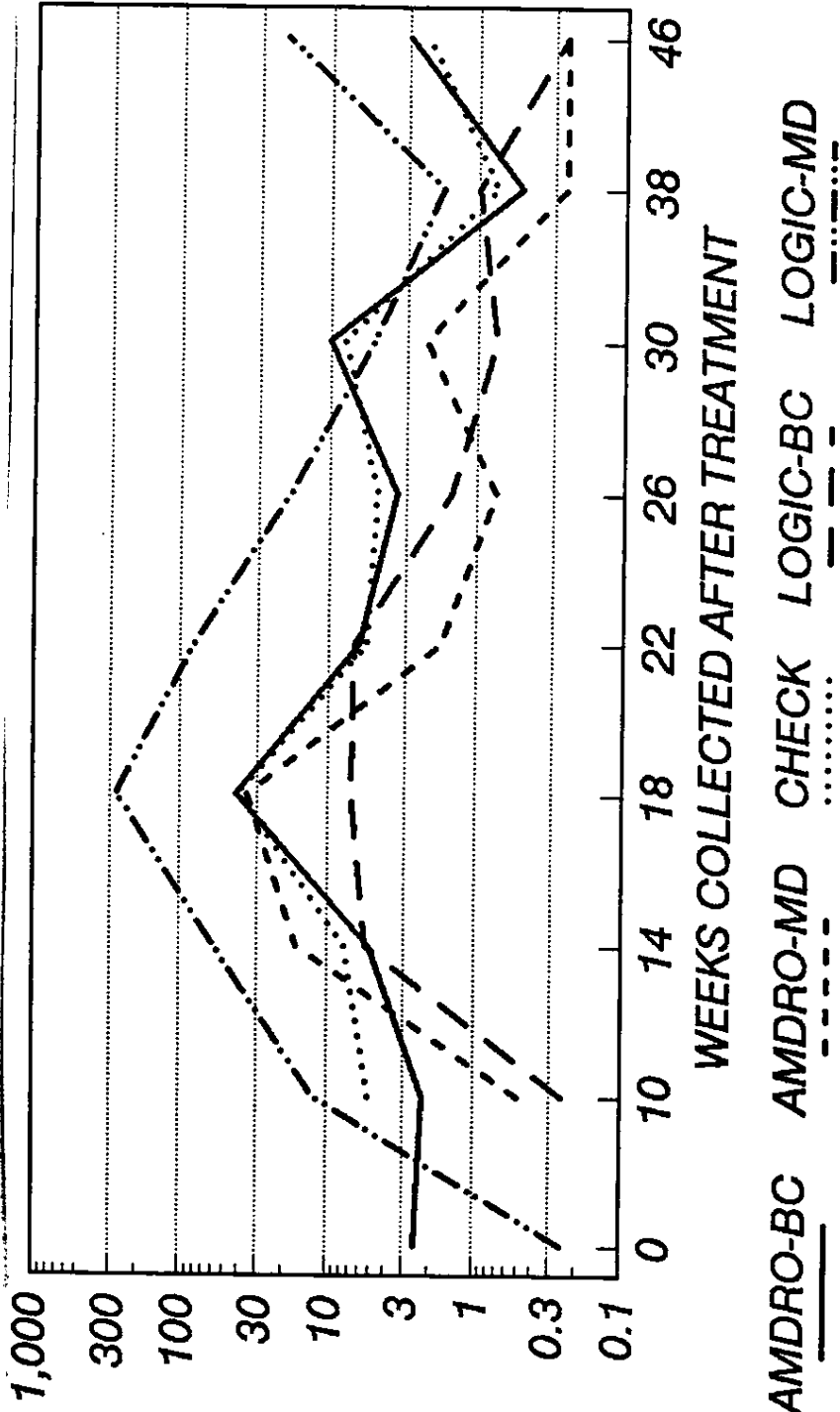
Figure 8. Numbers of non-target ants collected in pitfall traps after pre- or post-mating flights treatment with Logic<sup>R</sup> broadcast treatment in Palm Beach County, Florida.

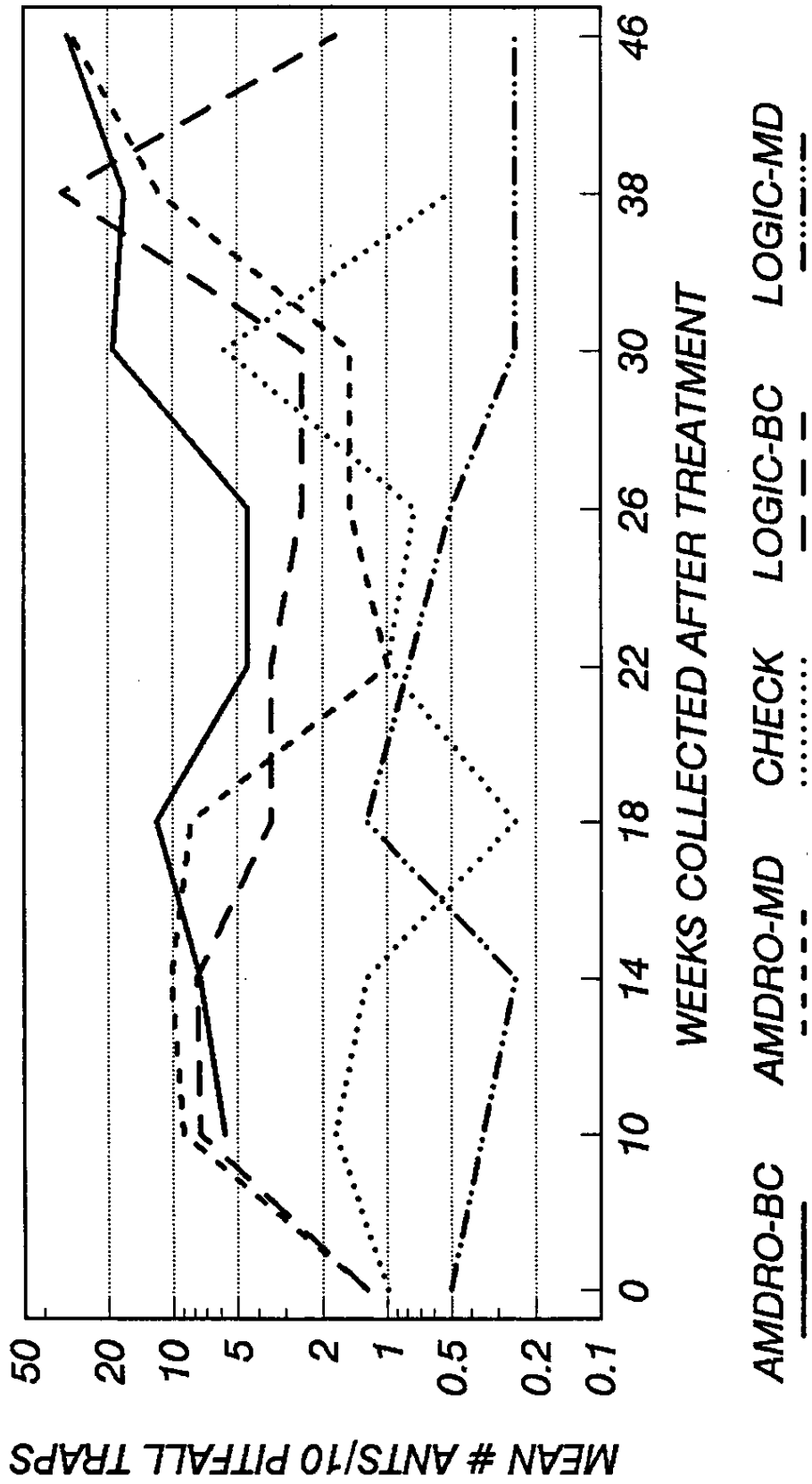


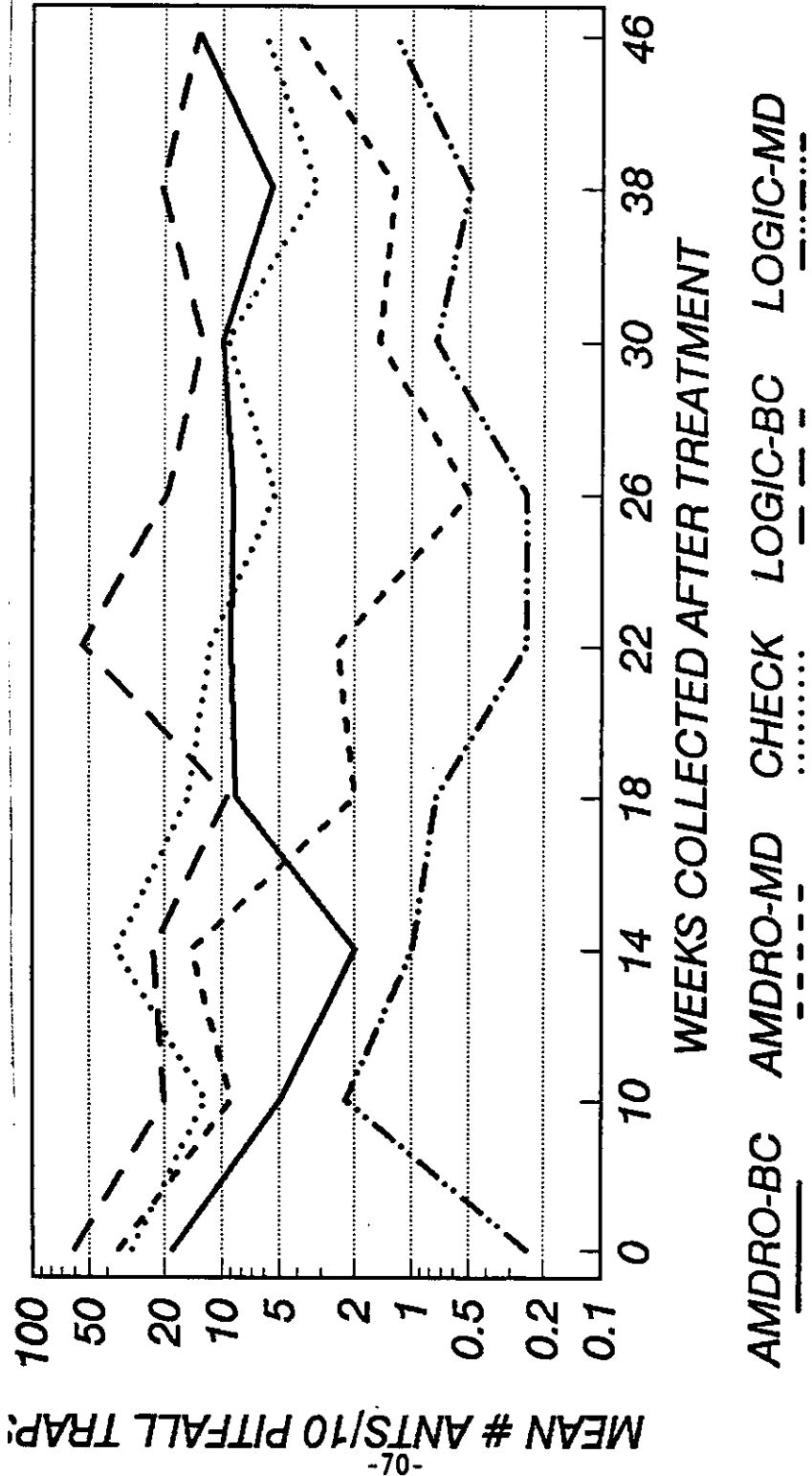




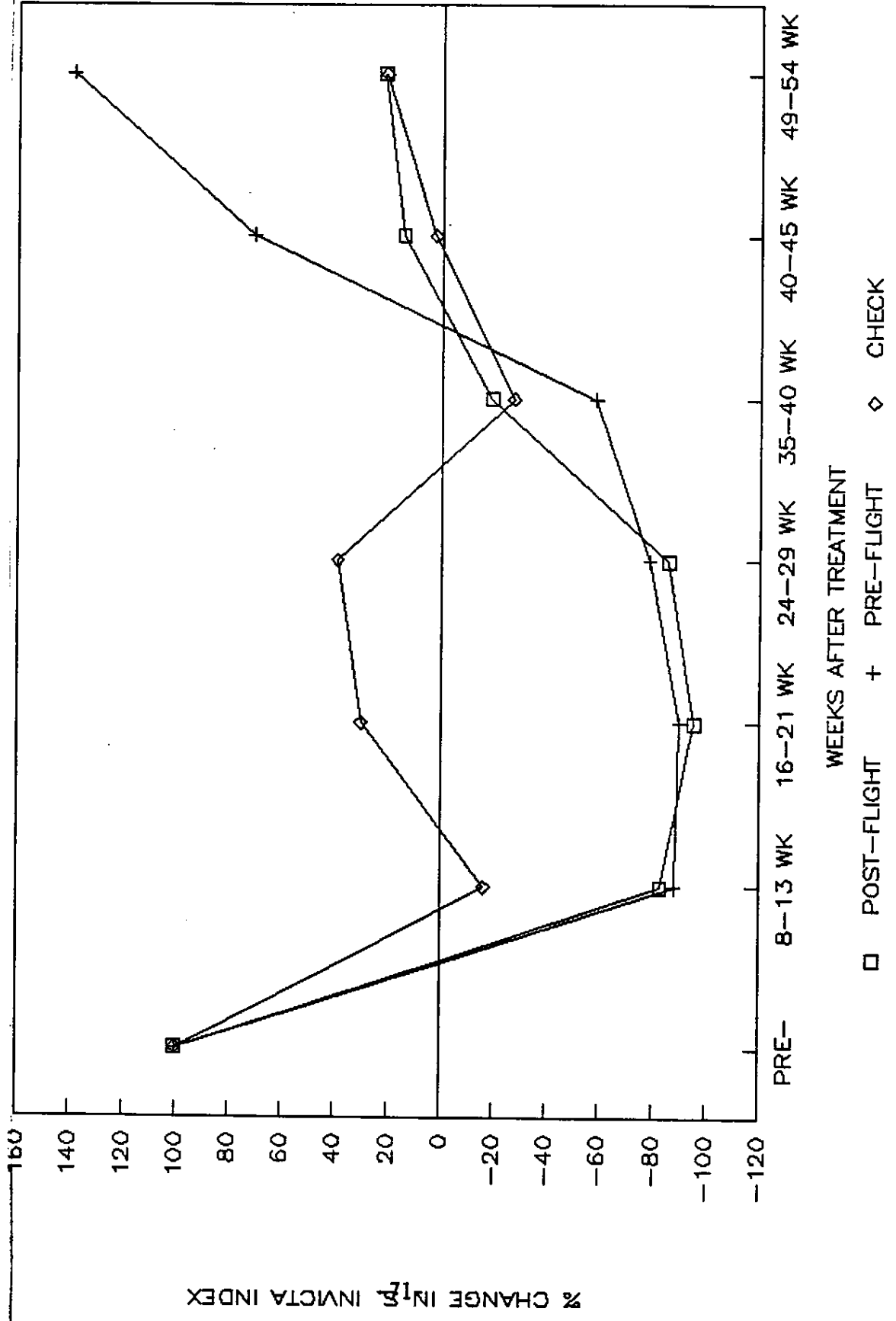
MEAN # ANTS/10 PITFALL TRAPS

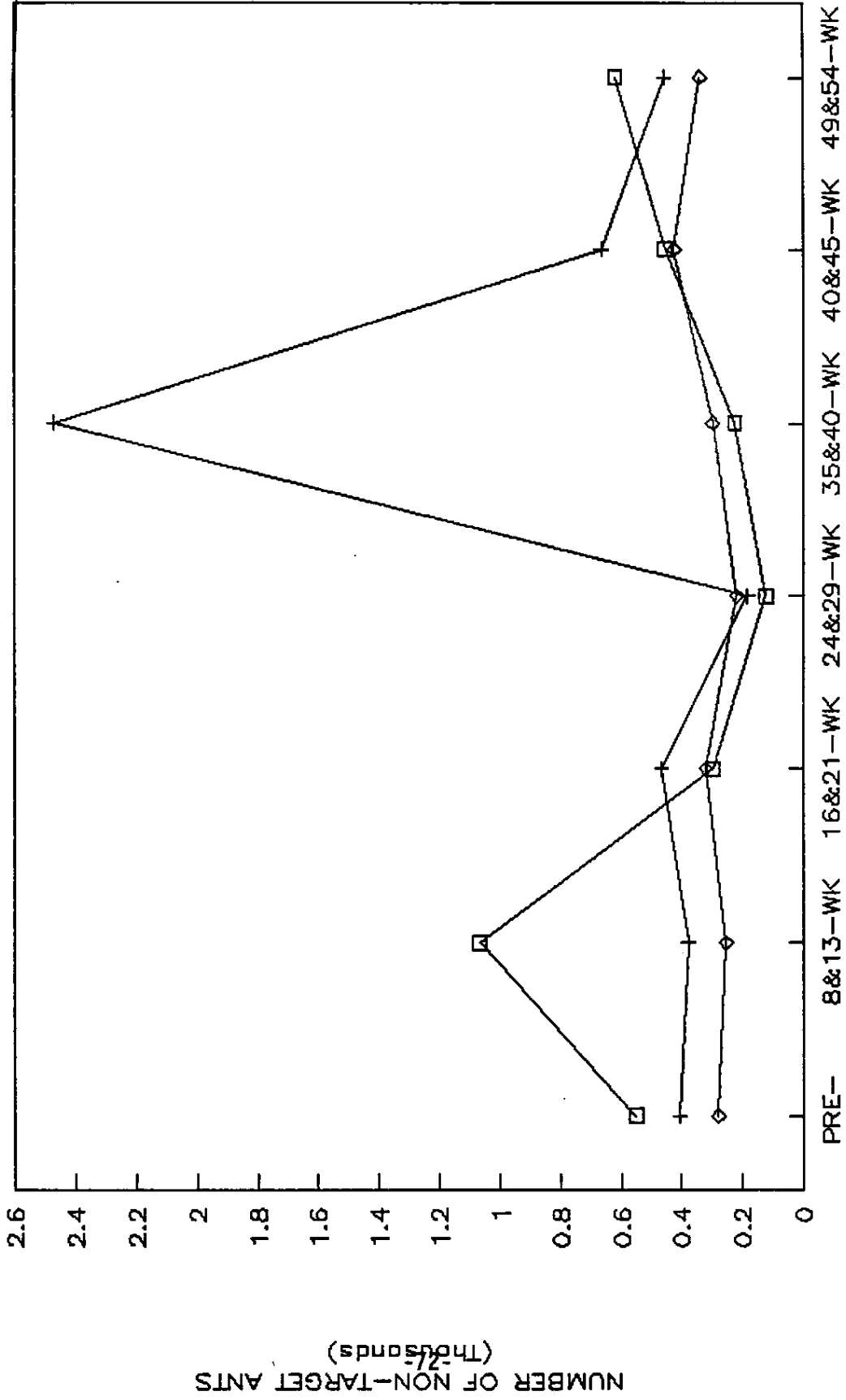












NUMBER OF NON-TARGET ANTS  
(Thousands)

COLLECTION INTERVALS IN WEEKS

**Nematode parasites of fire ants from South America.**

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The search for biological control agents of fire ant in South America has resulted in the discovery or re-discovery of many organisms associated with fire ants in South America (Wojcik 1988, 1990). Among these are three previously unknown nematodes. One, Tetradonema solenopsis, has subsequently been described, but the other two remain undescribed. The information known about these three nematodes is summarized here to document their discoveries.

Thelohania solenopsae:

Tetradonema solenopsis Nickle & Jouvenaz was described from material found in Solenopsis invicta Buren workers, collected along the roadside of BR-070, Km-616, between Cáceres and Cuiabá, Mato Grosso, Brazil (Nickle & Jouvenaz 1987). Subsequent collections have extended the range of the nematode over large areas of Mato Grosso and Mato Grosso do Sul, in central-western Brazil. Over 2250 fire ant colonies have been examined for this nematode from the states of Amazonas, Rondônia, Mato Grosso, and Mato Grosso do Sul. T. solenopsis was found in 67 or 2.9% of the fire ant colonies.

Eggs, juveniles, males, and egg-laying adult females were found in S. invicta workers and males. The large sausage-shaped female is essentially an enlarged uterus filled with developing eggs; juveniles occur free in the hemolymph. Large worker ants contained 17 to 35 females. At least 1,000 eggs and juveniles (total) were present in large workers (Jouvenaz et al. 1988).

Living ants parasitized by these nematodes may be recognized by their slightly enlarged gasters, the dorsal plates of which have a scalloped appearance. There are no other morphological signs of infection, nor are there any observable changes in behavior. T. solenopsae stresses its host severely. Under the additional stresses of collection, separation from the soil, and laboratory culture, infected ants die rapidly, making it difficult to maintain the parasite in laboratory colonies. Attempts to transmit the infection to Floridan S. invicta in the laboratory were not successful.

#### Mermithid:

In 1987, during a survey for natural enemies of fire ants in Argentina, a nematode was collected in 2 colonies of the black imported fire ant, S. richteri Forel. The first colony was on the premises of the Instituto Nacional de Tecnologia Agropecuaria, Hurlingham, Buenos Aires Province, and the second on the roadside of RN-12 at Sagastume, Entre Rios Province (Fig. 2). The nematodes were identified as belonging to the Mermithoidea, based on the morphological modification of the hosts, their large size, and the presence of one worm per host.

Larval mermithids are unidentifiable to genus (W.R. Nickle, USDA, ARS, Beltsville, MD). These colonies were hand-carried (under USDA, APHIS permits) to our laboratory in Gainesville, FL, where they were maintained in local soil.

The ants were parasitized by one large, ca. 15 mm long, larval nematode per host. The intracolony infection rates were substantially less than one per cent. The parasitized workers were diagnosed by their enlarged gaster and highly modified thorax (Fig. 3). The heads and thoraces of the parasitized major workers were modified to resemble minor workers. However, these S. richteri workers did not have rudimentary ocelli, as has been reported in other cases of mermithid parasitism (Wheeler 1910). Several parasitized workers were removed from the colonies and held in groups of 3-5 on moist sand in plastic petri dishes. Although nematodes emerged from several of these ants, they did not survive in the sand. Three nematodes were observed during emergence; they exited their hosts via the anus. After emergence by the nematodes, the host ants died. We were unable to propagate the nematode in the laboratory.

We have examined 427 fire ant colonies for pathogens and inquiline in detail from Argentina, including 22 colonies from the field in which the first infected colony was found. Only the 2 colonies collected on the first trip were found to be parasitized by mermithid nematodes. We have not observed nematodes of this type in our extensive survey for natural enemies of fire ants in Brazil.

New nematode:

In February, 1990, samples from 600 fire ant colonies were collected in Argentina, southern Brazil, and Uruguay. The samples were preserved in the field in a pathology sample preservative: 3% formalin and 2% glycerine in water. In the United States, the samples were macerated with a tissue grinder, and examined by phase-contrast microscopy. Nematode juveniles and eggs were found in 26 samples (4.3%) from all 3 countries (Fig. 2). The developing juvenile nematode could be seen in some of the eggs. This nematode is not the same as the other 2 nematodes known from fire ants in South America.

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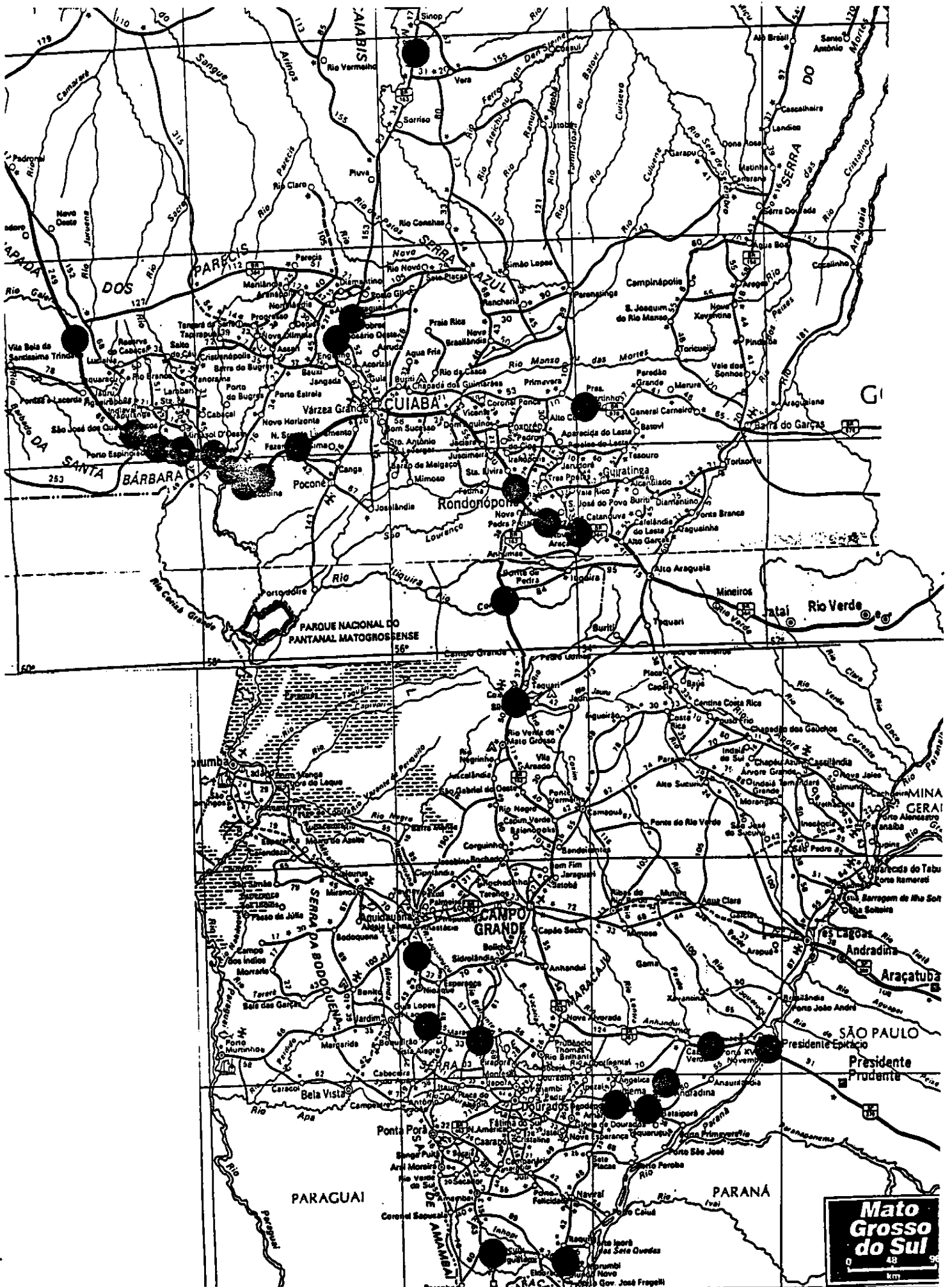
Wojcik, D.P. 1990. Behavioral interactions of fire ants and their parasites, predators and inquilines, p. 329-44. In R.K. Vander Meer, K. Jaffe, A. Cedeno (ed.), Applied myrmecology, a world perspective. Westview Press, Boulder, CO, 741 p.

Figure 1. Known distribution of Tetradonema solenopsis in the States of Mato Grosso and Mato Grosso do Sul in central-western Brazil.

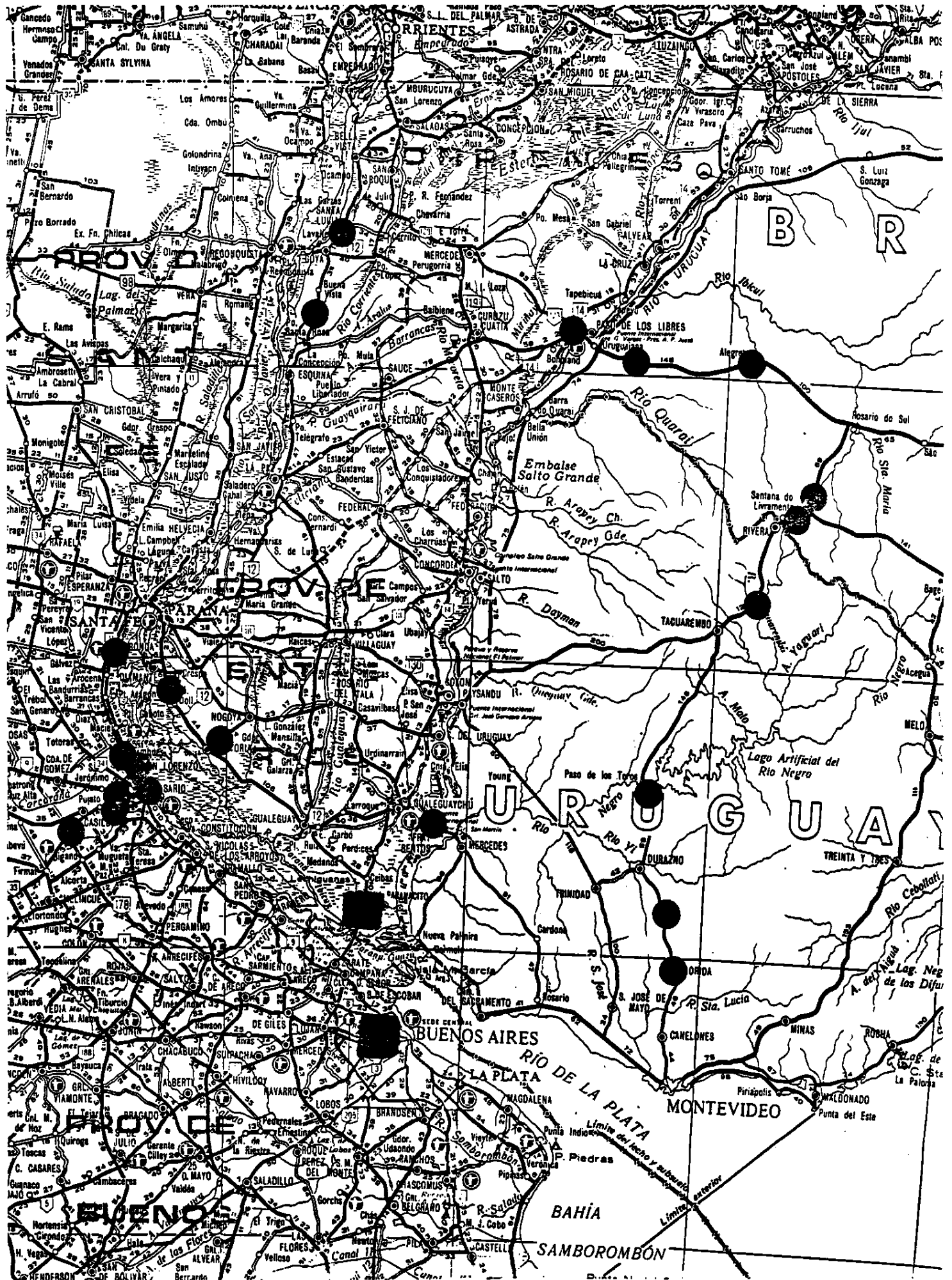
Figure 2. Known distribution of unidentified mermithid nematode (squares) and new unidentified nematode (dots) in central Argentina, southern Brazil, and Uruguay.

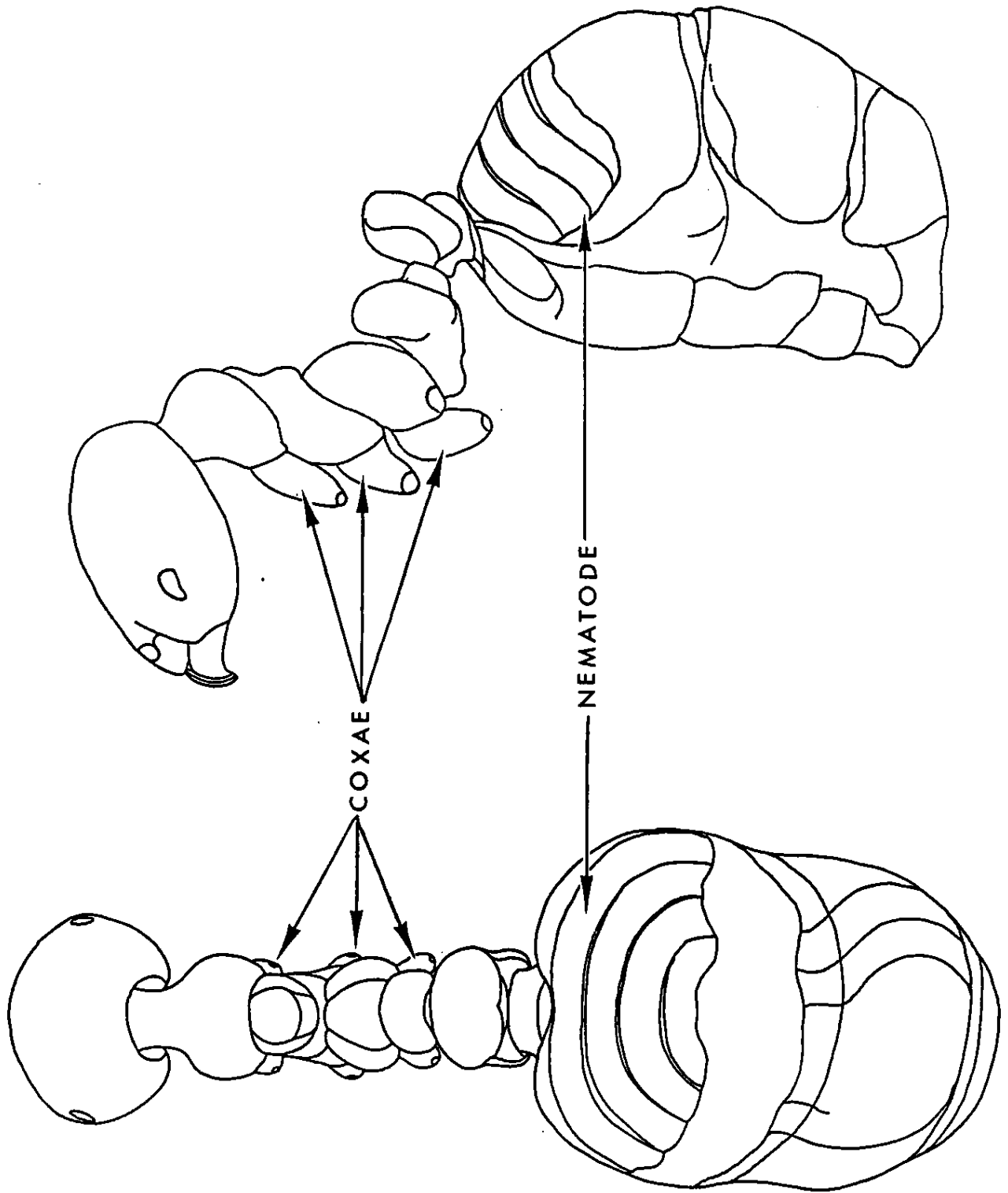
Figure 3. Solenopsis richteri worker parasitized by a mermithid nematode, which is visible through the cuticle of the enlarged gaster. The morphology of the head and thorax is modified, as is common in ants parasitized by mermithids.





**Mato Grosso do Sul**  
48  
km





**THE IMPORTED FIRE ANT: A MAJOR URBAN PEST**

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Although the two species of imported fire ant, Solenopsis invicta and Solenopsis richteri are not considered major urban or agricultural pests in their native habitat in South America, they certainly have become a pest of monumental proportions since their arrival in the United States. Solenopsis richteri, the black imported fire ant, arrived in the early 1900's in Mobile, Alabama (Creighton 1930). By 1937 according to Smith 1949 they had spread to several thousand acres. A federal eradication program was started in 1937, unfortunately it was discontinued ca 4 years later because of World War II. By the time interest in fire ants was generated again, another more aggressive species Solenopsis invicta, the red imported fire ant had been imported into Alabama from Brazil and it rapidly spread throughout the southeast. In 1949 it was found in three states: Alabama, Mississippi and Florida (Bruce et al. 1949). Within 10 years, the red imported fire ant S. invicta had become firmly established as the dominant species in 10 southeastern states. While the black imported fire ant S. richteri was found only in a small area of northeastern Mississippi and northwestern Alabama (Buren et al. 1974). These two species are capable of interspecies mating and producing a fertile hybrid or progeny.

In the last forty years, fire ants have spread into the 12 southeastern states. Small infestations have also been found in California, New Mexico and Arizona, these have been subsequently destroyed once they were detected. They have the potential to spread into Hawaii and up along the Pacific coast if they become established on the West Coast. At present, state and federal quarantine efforts have prevented the establishment of fire ants into the western Pacific states. In the east the IFA is probably at its ecological limits (Lofgren 1986) and will not spread much more unless a more cold tolerant strain is evolved. There have been incidences of fire ant populations being found in Maryland, Pennsylvania, Delaware, Washington D.C. and New Jersey, but these have always been in association with contaminated nursery stock that came out of the fire ant infested states. These small infestations were quickly destroyed. However, it is very conceivable that fire ants could become established in any of the Northern states inside greenhouses, the heated malls and atriums where large numbers of plants are maintained.

Fire ants are a disturbed habitat species, by this we mean that they are only found in areas where the natural habitat has been disturbed, such as urban yards, playgrounds, parks, road sides, and agricultural areas. They are not found in high numbers, in dense woody areas, or in high grass, but are abundant in well maintained lawns, golf courses, gardens, and playgrounds. Their mounds are often very numerous and literally thousands of foraging ants may be active in a typical urban yard (Banks et al. 1985).

The spread of the polygyne form of S. invicta in the United States has increased the risk of fire ants and humans interactions because of the greater number of ants present at any one site. One polygynous fire ant colony may be spread over several urban yards containing hundreds of thousands of ants. As the indigenous food supply for the ants is diminished, they will start to forge inside homes and are attracted to food sources not normally associated with fire ants (Glancey et al. 1973).

The imported fire ant, especially the red imported fire ant, Solenopsis invicta Buren is a problem because of five factors: 1) This is one ant species that is capable of inflicting on its

enemies a very painful and possible fatal sting. It is also a very aggressive ant and each colony contains thousands of foraging workers or its biological reproductive capacity is great. 2) As the ant forges indoors or in hospitals, they frequently contaminate food and sterile areas. 3) They are capable of doing structural damage to buildings, roads, etc. when they establish their colonies and forage. 4) They cause injury and can kill plants and animals thus inflicting a severe economic loss to homeowners or farmers. 5) The fire ants when numerous, literally destroy arthropod and other invertebrate fauna of the areas.

Adams and Lofgren (1981) state that ca a third of the human population living in the fire ant infested states are stung by these insects one or more times each year. For most people, except for the painful sting, subsequent red welt and small white pustule, there is little after effect. However, for the small percentage of people who are hypersensitive to fire ant stings, a single sting can mean death. Like many insect related deaths caused by allergic reactions only a small number are ever documented. Antibodies indicating a reaction to insect venom was found in 23% of humans which had died of unknown causes, whereas the general public exhibits less than one percent of the population has these antibodies (Schwartz 1987). In a recent case in Palm Beach, Florida a 47-year old golfer stepped into a fire ant mound and was dead within an hour, although initially, his death was tentatively listed as allergic reaction to fire ant venom, it was later changed to heart failure. However, the patient exhibited no signs of stress or heart problems until he stepped into the ant mound and was severely stung on his legs (Williams 1991 Personal Communication). A more common scenario is running over a fire ant mound when one is mowing the lawn and throwing the ants up on ones ankles and legs and/or stepping in a disturbed mound. Just this past month in Florida a healthy 57 year old man did this exact thing with his lawn mower and within 24 hours he was dead. He had never exhibited any allergic reactions to insect venoms in the past, however, his death was listed as an allergic reaction to fire ant stings (Annon. 1991). As macabre as it may seem, cemeteries are an ideal habitat for fire ants. The grass is short and the ants appear to like the protection of the tomb stones for their nests or colonies. A similar situation exists in parks and playgrounds where the ants will build their mounds in protected areas near or under recreation equipment such as play houses, jungle gyms, exercise stations, swings, etc.

Bieman and Wojcik 1990 did a survey of pest control operators in Florida to determine the major ant problems. In North Florida the imported fire ant, *S. invicta* was listed as the main indoor ant problem. In South Florida the imported fire ant was listed as the second most important indoor ant pest. The reason for this is fire ant populations are usually not as heavy in South Florida as in the Northern part of the state.

When fire ants invade a home, they normally do so through a small opening or crack in the wall or under a door or window frame. The foraging ants set up a pheromone trail and will continue to follow it until the food supply is exhausted. The ants will often carry into the house fresh dirt. These small piles of soil are often an indicator of fire ant foraging activity. Some of this soil carrying is probably a precursor of establishing the colony inside. If the soil is allowed to accumulate often the entire colony, queens, brood and workers, will be moved inside in a protected site such as inside cabinets, housings of motors, etc. Sometimes entire colonies will be established under rugs, furniture, etc. and the worker ants will forage throughout the apartment for food. However, in most cases the main colony is outside and the ants only forage

inside. What is deceiving is that fire ants may forage long distances from their main colony. In one incidence, which I was personally involved, residents of a seven story apartment house were being stung frequently by fire ants. Only the residents on the fourth, fifth and sixth floors were being troubled and only in one wing of the building. Careful examination of the roof indicated no fire ants were present. However, ca 15 feet from the building was a very large fire ant mound. Upon careful examination the worker ants were observed crossing beneath a four foot wide cement sidewalk, then crossing a 10 feet garden and foraging up the walls of the apartment building. For some reason the ants did not forage on the first three floors, but all the residents on the fourth, fifth and sixth floors which faced this large mound had some ant problems. When these apartment were treated with insecticides the ant problems subsided, but did not cease. In fact within a week after the treatments the complaints were almost to the level prior to the treatment. However, when the one colony was treated, the problem ceased to exist and no more complaints were voiced on residents being stung in their apartments. For some reason the ants did not forage on the first three stories of this large apartment building. Since that time, residents on the ground floor have complained of fire ant problems, but the ants were invading the apartments beneath the wall air conditioner units. Again when the few fire ant mounds in the garden were treated and destroyed the ant problem ceased. Therefore, one must seek and find the source of the ants if a permanent ant solution is going to be achieved. Often small fire ant colonies are carried inside in infested potted plants or potted plants on balconies or terraces may become infested by newly mated queens and colonies established which then send foraging workers indoors to seek a food source. Infested potted plants can be treated quickly and easily with a number of contact drenches.

Fire ants have been reported in malls and atriums where large numbers of potted plants are kept. In almost all cases the ants have been carried in with the plants. These infestations are easily eliminated by contact drenches of pesticides before the ants have a chance to establish themselves in a more inaccessible site in the mall. Since plants are continually being changed in these malls and atriums, the soil should be treated with a pesticide that has good residual life. Some chemicals will last up to a year or more in potting soil.

One problem that has become more prevalent in recent years is fire ants cutting holes in the rubber type roofing material placed on flat top roofs before gravel and tar is laid down. At present there must be a half dozen or more law suits in the southeast going on to determine if the ants cut the holes in the material to seek a suitable nest site or to seek moisture because the material was not installed properly. Some of these suits involve hundreds of thousands of dollars because of the water damage attributed to leaking roofs. There is one law firm in Florida that specializes in insect damage, etc. to flat roof construction in malls, offices, etc. they claim that in the last ten years more and more of their business has involved the presence of fire ants.

Roads, especially highways, are another area that fire ants are a problem that one does not often realize. Fire ants will build their nests under the road because of the protection and warmth. However, as Banks et al. 1988 reported the ants will chew their way through the sealant between the cement sections. Then moisture will get under the highway bed and cause breaking of the concrete with heavy traffic flow. The Department of Transportation for the state of Florida spends thousands of dollars annually repairing the interstate roads from fire ant

damage. Along secondary roads the ants will build their nest under the edges of the roads and because the ground is soft, the shoulder where the ants are present will break under the weight of cars and trucks and soon a deep pot hole will emerge which has to be filled in, otherwise it will continue to grow.

In Florida, as in Texas and other areas, fire ants are attracted to electrical currents. They are commonly found in traffic light switch boxes, telephone switch boxes, etc. Here the ants do damage by chewing on the wires, but mainly by getting soil and debris in the relay switches as reported by MacKay and Vinson 1990.

Fire ants may be a very serious problem to the home owners in Florida, but economically they do not rank even close to the major arthropod pests such as cockroaches, termites, flies and mosquitoes. Still, the general public places the imported fire ant near the top of the list of major pests because of its abundance in urban yards and its painful sting. Because of this perception the fire ant has become a politically important insect; the urban taxpayers want them eliminated from his or her environment and the residents of the non-infected states do not want them there. Eradication attempts were tried by the Federal Government in the past, but for a number of reasons they were never carried out to completion. After the last attempt of using mirex was discontinued almost 25 years ago no future eradication programs will be forthcoming unless some revolutionary control technique is developed or fire ants become a much more serious economic problem than they currently are.

There is still a lot of debate about the polygyne form of this insect and how it will spread throughout the region and devastate the indigenous invertebrate and small vertebrate fauna. Studies are underway and preliminary research findings indicate that the establishment of the polygyne form of the imported fire ant can have a long term influence on the local fauna. There is still a lot that must be learned concerning the proliferation of the polygyne or multiqueen form in fire ants. In South America it does not seem to be present in large areas, although polygyne has been reported in isolated cases from Brazil in Solenopsis invicta.

The biggest problem for the state and federal regulatory people is the movement of fire ants, especially in nursery stock from infested states to uninfested areas. The profit margin in nursery products is such that many of the currently available pesticide compounds for fire ant control will not assure freedom from any ant infestation for a year or more. Chlordane did do this at a very low cost, but currently registered compounds will not do it. Therefore, to assure fire ant free nursery stock, a more comprehensive plan of control must be developed. This will entail bait treatments of the nursery property to assure elimination of residual fire ant colonies on the property. Also mixing a good residual material of low cost in the potting media so that newly mated queens will not establish themselves in the nursery stock whether it be in potted or wrapped in burlap.

One of the biggest jobs that federal and state people must do is educate the inspectors of where to look for ant colonies and that worker ant presence does not necessarily mean that numerous fire ant colonies are present, but that some might be present. Also we must develop a baiting system that will draw ants from fair distances so that an infestation in a trunk can be noted. There are excellent control techniques available, but they often are not used properly.



The public must be educated as to the materials available and where they can be purchased and how to use them.

The imported fire ant, Solenopsis invicta is here to stay. In the southeast it has probably spread to its ecological limit because of climatic conditions. Small pockets of ants may establish themselves in protected areas in the northern states such as in malls and atriums in small numbers. There will be the continued threat of establishment in the Western Pacific states and Hawaii. Fire ants will continue to increase to be urban pest as more and more land is cleared for housing developments and upsetting the balance of nature making conditions ideal for the fire ant. If the polygynous form becomes the dominate one then fire ants will become a more serious household pest as they will continue to seek food inside structures and establish colonies. Currently we do have excellent control technologies that have been developed and better ones are being developed to keep these ants in line so that they will not ravish our indigenous fauna and make outdoor living impossible for the urban dweller.

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## Microbial Formicides: Current USDA Research

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ARS biocontrol research has emphasized the study of the specific natural enemies of fire ants, with the goal of establishing a complex of pathogens and arthropods in the United States to effect a permanent amelioration of the fire ant problem. A secondary goal has been to develop a biological formicide. In this report, two investigations we have recently completed in the latter area are presented.

### Ingestion of Entomopathogenic Bacteria.

Effective formicides destroy the reproductive capacity (kill or sterilize the queen or queens) of ant colonies. Toxicants which act rapidly kill only worker ants; they reduce the size of colonies temporarily, but do not effectively eliminate them. Therefore, toxicants, including bacterial toxins, must exhibit slow or delayed action to insure distribution through the colony to the queen(s) before the workers begin to die. Furthermore, delayed-action toxicants must remain effective over at least a 10-fold, and preferably greater range of concentrations, for serial dilution occurs through trophallaxis (Banks 1990). These properties are not common to bacterial toxins. Thus, unless cells or spores are actually ingested by the queens, elimination of colonies by non-host specific bacteria is very improbable.

Fire ant queens are fed only highly filtered, regurgitated liquids and (possibly) glandular secretions; bacteria are absent or highly restricted in the gut. Glancey et al. (1981) found that the pharyngeal filters of worker fire ants remove latex microspheres  $\geq 0.88 \pm 0.02$   $\mu$ m in diameter. Most bacteria, therefore, should be removed from food during trophallaxis.

Several species of bacteria have been tested as formicides against worker ants, but no large-scale screening to identify strains especially virulent for fire ants has been attempted. Moreover, the vulnerability of fire ant queens to bacteria has not been investigated. The purpose of this study was to determine whether selected bacteria are ingested by fire ants, and thereby determine the feasibility and parameters such as size and flagellation for screening strains as microbial formicides.

We created seven small, homogeneous colonies, each composed of 12 queens, approximately 5,000 workers, and 1.5 g of brood, by partitioning a laboratory colony of polygyne *S. invicta*. Daily for 14 days, duplicate colonies were fed either spores of *Bacillus thuringiensis*, spores of *Bacillus sphaericus*, or cells of *Serratia marcescens* mixed with boiled egg yolk to a paste consistency. Daily for the last 10 days of the test, each colony was also fed

one large, living corn earworm larva which had been injected with 0.25 ml of the suspension of bacteria. The ants readily consumed the diet containing bacterial cells or spores, as we observed daily and confirmed by isolating *S. marcescens* from the gut of fourth instar larvae. The seventh colony served as a control.

Ten queens from each colony were surface-sterilized, decapitated, and dissected aseptically in Grace's insect cell culture medium. First, the venom glands were removed, for the venom is antibacterial (Jouvenaz, 1972). The thoraces and gasters were dissected, homogenized and plated separately on differential media (see Appendix for details). All plates were incubated at 28° C and examined at 24 and 48 h. After completing the ingestion tests using queens, we extended this study to include workers that had fed to repletion on egg yolk containing bacteria.

*Serratia marcescens* was not recovered from the 20 queens or 10 workers fed this bacterium. Cells of *S. marcescens* measure about 0.5 x 0.5-1.0  $\mu$ m and have four peritrichous flagella. Since the smaller cells are below the 0.88  $\mu$ m filtration limit of fire ant workers, we assume the long flagella become entangled in the filter.

*Bacillus sphaericus* was recovered from the gaster of only one of 20 queens from two colonies fed spores of this species. The pharyngeal crop of this specimen was sterile; the gaster yielded 10 colonies of *B. sphaericus*. Spores of *B. sphaericus* measure 0.7-1.2  $\mu$ m in diameter. Thus, the smaller spores, if naked, should pass the pharyngeal filter; however, the sporangium adhered strongly to the spores in this preparation, effectively increasing their size. All 10 workers exposed to *B. sphaericus* yielded sterile plates.

*Bacillus thuringiensis* was not recovered from 20 queens from colonies fed this bacterium. Seven of the workers yielded sterile plates; the remaining three yielded four, six, and 14 colonies of bacteria, all of which were *B. thuringiensis*. The spores of *B. thuringiensis* measure 1.0-1.5  $\mu$ m in diameter and are too large to pass the pharyngeal filters of worker fire ants except rarely.

The suggestion is frequently made that if a large number of strains of *B. thuringiensis* or other entomopathogenic bacteria were screened, a microbial formicide might be found. Indeed, Miller and Brown (1983) reported significant mortality in fire ants fed *S. marcescens*, *Pseudomonas aeruginosa* and two *Enterobacter* spp. under laboratory conditions. However, only workers were used in these tests and death was due to intoxication, for essentially equal mortalities resulted whether the ants ingested food containing living cells or culture medium with cells removed. Fire ant queens are seldom killed by fast-acting toxins. Miller and Brown concluded that fire ants readily ingest bacteria; however, their methods did not differentiate between bacteria from the infrabuccal cavity and those from the gut. In light of the known filtration ability of worker fire ants and our data, we conclude that the

bacteria detected in workers by Miller and Brown were from the infrabuccal cavity.

Only nonflagellated organisms small enough to pass pharyngeal filtration during trophallaxis are candidates for screening as microbial insecticides. Recently we isolated a tiny, slow-growing bacterium (a symbiont?) from the hindgut of apparently healthy fire ant queens. There is no indication that this bacterium is pathogenic; however, biotechnology offers the exciting possibility of making commensals virulent. Other possible candidates for genetic manipulation are Mollicutes (spiroplasmas, mycoplasmas, achleplasmas). Arthropods, including Hymenoptera, are proving to be a rich source of these microorganisms and potential fire ant-mollicute associations appear worthy of investigation.

#### Evaluation of nematodes for protection of nursery stock.

Early research by Poole (1976) and Quattlebaum (1980) indicated a potential of steinernematid nematodes for control of fire ants. However, Jouvenaz et al. (1990) did not achieve significant control of fire ants in field trials using nematodes produced by Biosys. Considerable relocation of fire ant nests occurred in our tests. Had we not taken mound movement into consideration, our apparent control in one test would have been 78%. (Poole and Quattlebaum scored all mounds uninhabited after treatment as dead). We subsequently confirmed the aversion of fire ants to nematodes by consistently driving small laboratory colonies from their nests in sandy soil in containers.

The readiness with which fire ants relocated their nests in the field and vacated soil in containers to avoid nematodes prompted us to investigate the potential of these parasites to eliminate fire ants from nursery stock. The early spread of fire ants in the southeastern United States was greatly facilitated by the shipment of infested nursery stock (Lofgren 1986).

Nursery and greenhouse crops ranked third nationally among farm crops in 1990, with approximately 7.6 billion dollars in cash receipts. The states within the Southern Plant Board account for nearly 30% of total U. S. nursery production. In addition to the direct costs (material and labor) of compliance with quarantine regulations, nurseries may also face loss of market opportunities and reduced worker productivity due to fire ants. Currently, federal certification of nursery stock for shipment through quarantine is based solely on incorporation of chlorpyrifos. Granular chlorpyrifos is not as effective as previously believed, drenching poses problems of worker exposure, and both treatments are expensive. According to Mr. Craig Regglebrugge of the American Association of Nurserymen, the improvement of methods for control of fire ants is a critical need of the nursery industry.

We conducted three sequential tests using Biosys nematodes against fire ants nesting in 1 gallon nursery pots containing *Pittosporum* shrubs. The test colonies consisted of five queens, 3,000-5000 workers and about 1.5 g brood. In the first test, 20 pots in separate trays were drenched with an aqueous suspension of 30,000 nematodes (about 1,000 per square inch of soil surface), 20 pots were drenched with 300,000 nematodes, and the remaining 10

pots received water only. An identical container of watered potting soil (but without a plant) was placed in the tray to provide the ants with untreated soil in which to relocate their nests. Without an "escape pot", ants evacuating the treated soil would have clustered around the base of the container, making evaluation difficult. Also, ants would be free to relocate in untreated soil in a nursery. Half of the pots were examined three days, and half 14 days after treatment, by spreading the soil on a white plastic cloth.

The second test was conducted using replanted shrubs, new ant colonies, a new batch of nematodes, and a different brand of potting soil. The same procedures were employed except that the nematodes were sprayed rather than poured onto the soil surface. The third test consisted of 30 pots infested with new colonies, of which 20 were sprayed with a single dose of 75,000 nematodes (about 2,500 nematodes per square inch of soil surface). These pots were arranged and treated in a random pattern on the ground, which was covered with short grass and partially shaded by two oak trees. Thus, the ants were free to relocate in field soil. This test was evaluated four days after treatment.

Our results were, in a word, negative. Of the 80 pots treated in the first two tests, fire ants were not eliminated from a single pot. In the first and second tests, queens and brood remained in all of the treated and control pots; neither was found in the escape pots. The numbers of workers found in the escape pots were generally fewer than 100, and were probably just foragers. In the third test, queens, brood and workers remained in 11 (55%) of the 20 treated colonies and five (50%) of the 10 control colonies. We do not plan further work with steinernematid nematodes.

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#### APPENDIX

The extracts of queens from colonies fed *S. marcescens* were plated on mannitol-salt agar, on which this bacterium produces the red pigment prodigiosin in abundance, permitting identification. The extracts of queens from colonies fed bacilli were plated on nutrient yeast salt agar, which stimulates sporulation and thereby aids identification of *Bacillus* spp. The bacterial colonies which grew on the *Bacillus* spp. ingestion test plates were compared with reference colonies of *B. sphaericus* and *B. thuringiensis*. Cells from colonies having morphology similar to those of the reference colonies were examined by phase-contrast microscopy. *Bacillus sphaericus* was tentatively identified by the characteristic swollen, terminal spore, and confirmed by bioassay against highly susceptible, insectary-reared, *Culex quinquefasciatus* mosquitoes. Cells from colonies similar to those of *Bacillus thuringiensis* were examined for the presence of parasporal bodies.

**THE ENTOMOPATHOGENIC FUNGUS Beauveria bassiana  
FOR FIRE ANT MANAGEMENT IN NURSERY STOCK**

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Fungi have been studied as potential biocontrol agents of red imported fire ants (Solenopsis invicta Buren) (Jouvenaz 1986, Stimac et al. 1987); however, until recently, all research has used conidial preparations directly applied to ants or to the ants' environment. Dry myceliar formulations that have been developed recently for biological control of insect pests (McCabe and Soper 1985, Rombach et al. 1986a, 1986b, Roberts et al. 1987) have several advantages over spore formulations, including easier production and storage (Roberts and Wraight 1986). We wanted to test dry mycelium of Beauveria bassiana (Balsamo) Vuillemin (Hyphomycetes: Moniliales) as a potential biocontrol agent of red imported fire ant (RIFA) founding queens in small containers.

#### MATERIAL AND METHODS

The Beauveria bassiana strain was originally isolated from workers of the Mexican leaf-cutting ant, Atta mexicana (F. Smith) collected on the Pacific plain of the Mexican state of Sinaloa, near El Fuerte, on December 1986 (Sánchez-Peña 1990). The fungus was isolated on Sabouraud dextrose agar plus 1% yeast extract (SDAY) and successively passed five times through harvester ant (Pogonomyrmex sp.) adults. After the fifth passage, the fungus was reisolated on SDAY and used for inoculation of Sabouraud dextrose broth plus 1% yeast extract in 300-ml flasks with 150 ml of broth on a rotary shaker (150 strokes/ minute) at 25 °C. The method was similar to that described by Roberts et al. (1987) and was based on the process described by McCabe and Soper (1985) for entomophthoralean fungi. The resultant myceliar preparation was dried and stored in non-sterile conditions; therefore, presumably, many air-borne contaminant spores were in contact with the mycelium immediately during and after its harvest and subsequent drying. The dry mycelium was fragmented with a blade into 2.0-0.3 mm pieces and stored at 4°C for four days before its use in the experiments.

##### a) B. bassiana mixed with vermiculite

Fungal fragments were incorporated into sterile vermiculite: different percents of mycelium (on a weight basis) which constituted different treatments, 0.015, 0.15, 0.75, and 1.5% were added to and mixed with the vermiculite, and a control with no fungus was included. Each treatment consisted of 100 g of vermiculite plus mycelium. Five hundred ml of sterile deionized water were added to the fungus-vermiculite mixtures until saturation, and excess water was drained away. About 15 ml of the mixtures were dispensed into 30-ml transparent plastic cups, and two RIFA founding queens were placed in each cup. Four replicates per treatment were prepared with twenty cups per replicate. The cups were covered with a cardboard lid and incubated in the dark under high humidity conditions at 25°C. Mortality was recorded each 24 h for 21 days. Mean accumulative mortality values for the 13th day post-inoculation were analyzed for significant differences using the Mann-Whitney test (Sokal and Rohlf 1981). Probit analysis (SAS Institute 1985) was performed on the total mortality data.

To test the survival and infectivity of the conidia produced in cups, 30 RIFA workers were placed in separate treatment cups at various intervals of time and mortality was recorded. Cups were used only once and then discarded.

We wanted to test the stability of the dry mycelium (its capacity to resume growth and subsequent sporulation) after storage in refrigeration and at room temperature. At various time intervals, samples of dry mycelial pieces were taken from both storage conditions, placed in moist chambers, and incubated at room temperature. Mycelial growth and sporulation were noted.

**b) B. bassiana mixed with a commercial potting soil mix**

Different amounts of Beauveria dry mycelium were added to commercial potting soil (Baccto-Lite, Michigan Peat Co., Houston, TX.): 0.005, 0.01, 0.05, 0.1 and 0.4% (percents are expressed on a weight basis). A control soil mix with no fungus was included. Approximately 45 g of the fungus-soil mixes were separately dispensed into 355 ml styrofoam cups, 20 ml of non-sterile demineralized water were added to the cups with soil, and two alate

RIFA females were added to each cup. A plastic lid was used to prevent ant escape. The cups were incubated at 25-27°C.

**c) Effects of Beauveria mycelium on plant growth.**

Although artificial distribution of Beauveria in agroecosystems has been reported, the effects on plants in soil containing relatively high levels of the fungus (such as the levels used in our tests) have not been described. A monocot, bermudagrass (Cynodon dactylon L.), and a dicot, pinto beans (Phaseolus vulgaris L.), were used in our trials. For the first test, we used fungus-commercial potting soil mixes in which the fungus had sporulated six weeks before; therefore, the infective stage (conidia) was present in the pots. The fungus had been added to soil at 0.005, 0.01, 0.05, 0.1 and 0.4% (w/w). Ten seeds of bermudagrass and three seeds of pinto beans were added to the surface of 355 ml cups containing about 45 g of soil-fungus mix. Twenty ml of non-sterile, demineralized water were added to each cup. The cups with seeds and fungus were incubated at 25-27°C and 100% R.H for two weeks. These conditions would probably lead to infection in case the fungus is a potential plant pathogen.

For the second test, 0.5 and 1.0 % of dry mycelium were added to commercial potting soil, and this mix was dispensed into 355 ml cups. Five pinto bean seeds were added to each cup, and incubation conditions were as described for the first test with plants. Number of leaves, leaf area, dry aerial biomass, and percent seedling establishment were evaluated.

## RESULTS

**a) B. bassiana mixed with vermiculite**

The progression of queen mortality is shown in Table 1. The greatest mycelium concentration (1.5%) produced 100% mortality after 13 days. There was a direct relationship between the amount of mycelium present in the treatment and the slope of the mortality lines (Fig 1). Lethal dose values are shown in Table 2. In addition, S. invicta workers added to the treatment cups after 206 days of

storage at room temperature showed 100% mortality in all the treatments after six days.

When placed in humid conditions, 100% of the fungal particles stored at room temperature for 403 days became reactivated and sporulated after 72-96 h. Particles stored at 4°C for 206 days also showed 100% viability, but started to grow 24 hours later than did the particles stored for 206 days at room temperature.

**b) B. bassiana in commercial potting soil mix**

Mortality data are shown in table 3 and figure 2. In this case there was also a direct correlation between the amount of fungus in soil and mortality.

Fungus treatments ranging from 0.05 to 0.4% caused 92-100% mortality after 13 days in potting soil. As little as 0.005% of Beauveria mycelium added to the soil mix caused 63.3% mortality after 19 days.

**c) Effects of Beauveria on plant growth**

There were no significant differences in plant development for both species due to the fungal treatments. Bermudagrass leaf area was not measured due to the small size of the seedlings; however, no apparent differences in germination and plant establishment among the control and treatments were detected (table 4). This same trend was apparent for pinto beans. There were no major differences in the number of pinto bean leaves among treatments and control (table 4). The leaf area for P. vulgaris plants grown in soil containing different amounts of dry mycelium was not significantly affected (table 5), and aerial dry biomass for these same plants is shown in table 6. No major differences were noticeable among treatments and control.

## DISCUSSION

If a dry myceliar preparation for control of S. invicta founding queens in potted plant soil is to be effective, an adequate density and homogeneous spatial distribution of the myceliar particles in the soil are essential. This would increase the probability of a

queen ant becoming contaminated with conidia produced by the mycelial particles. The size of the particles should also affect its viability and distribution.

Our Beauveria dry mycelial preparation shows good potential as a soil amendment for potted plants and other nursery stock. This is supported by the reports of the natural occurrence of Beauveria as a soil insect pathogen in cultivated soils (Vanninen et al. 1989). Some desirable characteristics of Beauveria as a biocontrol agent include ease of production using conventional fermentative technology, resistance to air-borne contaminants that undoubtedly were present through its preparation (harvest, handling and storage), and viability for at least 403 days of storage at room temperature. Also, conidia produced by the dry mycelium remain infective in the vermiculite under non-sterile conditions for at least 206 days at room temperature in high humidity.

Entomopathogenic fungi, Beauveria spp. in particular, are regarded as safe in comparison to chemical control methods, and environmentally sound and compatible with other control strategies (Roberts and Humber 1984). Despite the intensive research conducted worldwide on Beauveria, this fungus has never been found to be a plant pathogen.

The artificially warm and humid environment of commercial, potted-plant soil appears to be an ideal environment for the pathogenic action of dry Beauveria mycelium on S. invicta founding queens. Given that regulations affecting transportation of containerized plants from quarantined areas is expected to become even more rigorous in the future, further research on this potential biocontrol procedure is needed, particularly under commercial production conditions.

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Table 1.- Mean percent mortality of fire ant founding queens after incubation in vermiculite mixed with different percents of *Beauveria bassiana* dry mycelium (W/W)

Treatment <sup>a</sup>	Days post-inoculation												
	1	2	3	4	5	6	7	8	9	10	11	12	13
0.0	0	0	0	0	0	1.2	1.2	1.2	1.2	1.2	1.2	1.2	3 a
0.015	0	0	0	0	0	1.2	1.2	1.2	6.2	9.0	10	15	27 b
0.15	0	0	0	0	0	1.2	2.5	3.7	3.7	7.8	19	33	44 b,c
0.75	0	0	0	2.5	2.5	5.0	5.0	7.5	15	19	32	58	72 c,d
1.5	0	0	0	0	0	0	1.2	5.0	11	16	49	80	100 d

<sup>a</sup> Treatments followed by the same lower case letter are not significantly different (P < 0.05, multiple comparison using the Mann-Whitney test [Sokal and Rohlf 1981])

Table 2. LD values for *S. invicta* queens in vermiculite mixed with dry mycelium (Probit analysis; SAS Institute, 1985)

LD 20 = 0.008%  
 LD 50 = 0.053%  
 LD 90 = 0.956%



Figure 1. Founding queens mortality, dry mycelium in vermiculite bioassay

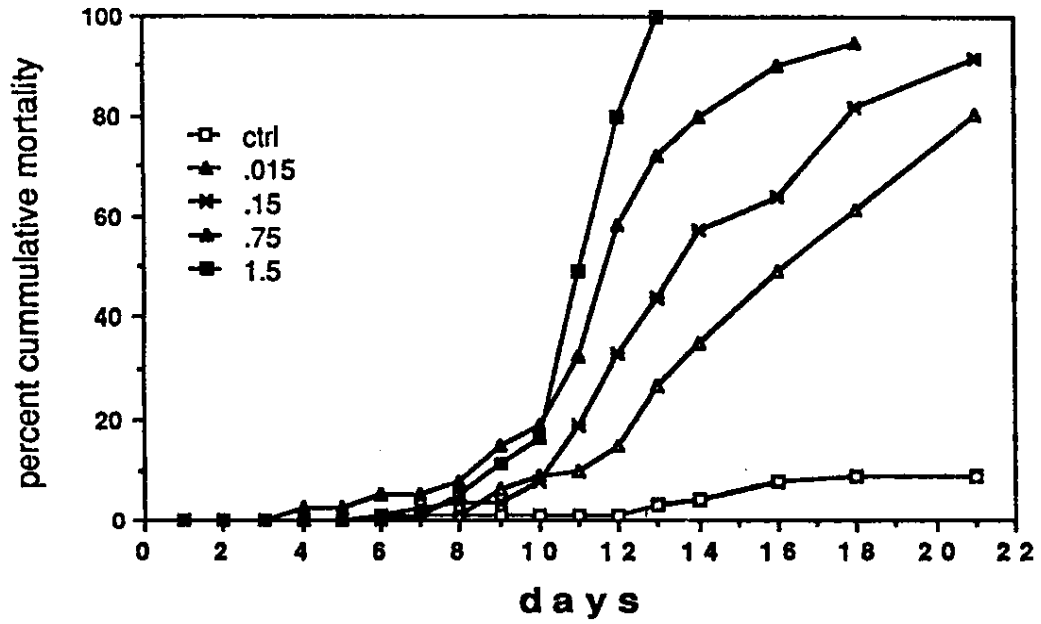


Figure 2. Founding queens mortality, dry mycelium in potting soil bioassay

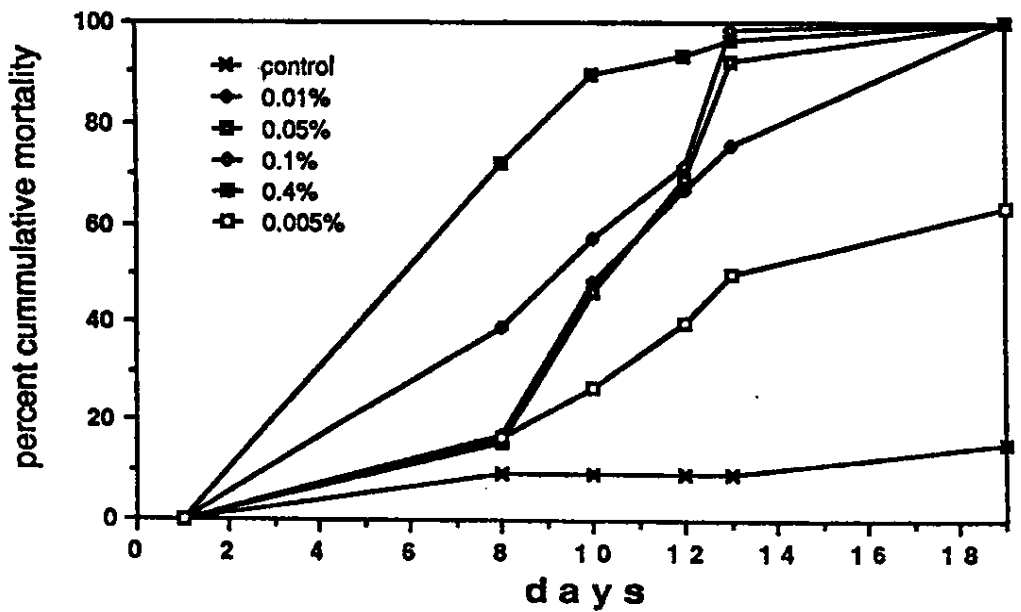


Table 3. Cummulative percent mortality of fire ant founding queens exposed to potting soil/*Beauveria* dry mycelium mixtures.

Treatment	days post-inoculation					
	0	8	10	12	13 *	19
0.005	0	16.6	26.6	40.0	50.0a	63.3
0.01	0	17.2	48.2	67.0	75.9b	100.0
0.05	0	15.4	46.1	69.2	92.3c	100.0
0.1	0	39.3	57.1	71.4	93.7c	100.0
0.4	0	72.4	89.6	93.1	100.0d	100.0
control	0	9.4	9.4	9.4	9.4e	15.6

\* Treatments within the column and followed by the same letter are not significantly different (Mann-Whitney test,  $P > 0.05$ ).

Table 4. Number of *C. dactylon* and *P. vulgaris* seedlings (percent plant establishment), and total number of bean leaves for different percents (w/w) of *Beauveria* mycelium added to potting soil

Treatment (%)	bermudagrass	pinto beans	total no. leaves
0.005	11 (0.13)	17 (0.71)	42
0.01	10 (0.12)	16 (0.66)	39
0.05	15 (0.18)	16 (0.66)	47
0.1	21 (0.26)	13 (0.54)	35
0.4	19 (0.23)	15 (0.62)	44
Control	6 (0.20)	16 (0.66)	43

Table 5. Mean ( $\pm$ SEM) leaf area of beans (*Phaseolus vulgaris*) grown in potting soil with different concentrations of *Beauveria* dry mycelium.

Treatment	r e p l i c a t e s				$\bar{X}$ (+ SEM) *
	a	b	c	d	
0.5	354.4	375.6	419.6	478.0	406.9 (54.6)a
1.0	346.1	322.0	392.8	438.3	374.8 (51.5)a
control	343.8	341.5	415.0	418.4	379.7 (42.8)a

\*Means followed by the same letter are not significantly different according to Student's *t* test ( $P < 0.05$ ).

Table 6. Aerial biomass dry weight (grams) of *Phaseolus vulgaris* plants grown in potting soil with different amounts of *Beauveria* mycelium

Treatments *	r e p l i c a t e s				$\bar{X}$
	a	b	c	d	
0.5% a	2.760	2.362	2.779	2.226	2.531
1.0% a	2.636	2.245	2.468	2.805	2.538
control a	2.346	2.574	2.258	2.230	2.352

\*Treatments followed by the same letter are not significantly different (Mann-Whitney test,  $P < 0.05$ )

**Ground Penetrating Radar:**

**A Look into Fire Ant Mounds Underground  
and their Possible Effects on Groundwater**

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The *Solenopsis* hybrid has existed in several northwestern Georgia counties since 1980. Currently, it can be found near the Tennessee border. Supercooling studies have been conducted comparing the hybrid with *S. invicta* and *S. richteri*, the two parental lines (Diffie and Sheppard 1989). Overwinter survival has been monitored since 1985 and compared to survival of *S. invicta* in other parts of the state. Neither of these studies have produced any evidence suggesting the hybrid is any more tolerant of cold weather than the parental lines. Several possibilities exist explaining the success of the hybrid in north Georgia. There has not been any sustained cold weather (i.e. ground frozen for a week) in this area during the past ten years. This factor probably has done more for the survival of the fire ant than any other factor. If the queen is able to establish a colony for a year without appreciable mortality due to cold weather, the colony stands a good chance of surviving. A second explanation to the survivability of the hybrid could be the mound's construction. Little information is available concerning the underground portion of the fire ant mound. Casts have been poured and

mounds have been excavated. The labor and time involved have limited replicated work concerning the depth and width of the colony underground. The problem of surveying the mound underground was brought to the attention of Drs. Vellidis and Smith. They were coincidentally interested in fire ant mounds because of work they had been conducting concerning groundwater contamination. They suggested a cooperative study to look at mound construction and the influence on groundwater. The USDA-ARS Southeast Watershed Research Laboratory has a ground penetrating radar unit which was made available for use during this study.

The GPR is a by-product of the Vietnam War. It was invented to detect enemy tunnels during the war. Figure 1 is an example which shows a tunnel constructed by a gopher tortoise. Private industry has adopted the methodology to conduct geological surveys and search for buried pipes and drums among other things. The UGA CPES Ag Engineering Dept. is using the radar to study the flow of water as it moves through the soil (Vellidis et al. 1991, Smith et al. 1990). This will help in understanding the fate and transport of pesticides applied to the soil.

GPR works by radiating shortwave electromagnetic pulses into the soil. Reflected signals from interfaces between materials having different dielectric constants produce an image on a chart or on magnetic tape. The natural image of soil can be enhanced by adding water to dry soil thus creating a greater contrast in the dielectric constants of soil particles. However, when the soil is near saturation, the addition of water confuses the interfaces rather than contrast them.

Work conducted thus far has led to the formulation of several hypotheses. The galleries of the fire ant mound definitely enhance the flow

of water through the soil. The amount of water necessary to produce a flow through the mound is probably more than the amount present in broadcast applications of pesticides in normal situations. The closest example to the necessary amount of water would be the application of drenches. Drenches work by pouring two gallons of the desired solution on the mound. Under most situations, two gallons of solution are probably still not enough to flow directly into the groundwater. However, the caution should be noted that if the ground is already near saturation the solution may be more inclined to flow directly through the mound. Saturated soil could also mean the water table is closer to the surface than normal. Another consideration is the possibility of rain. If rain occurs immediately after application it may push the pesticide further down toward the groundwater. In a previous study at the Coastal Plain Station, a bromide tracer that was sprayed over a fire ant mound prior to a rainfall event was detected in shallow groundwater near the mound on the following day (Stone et al. 1989). This contrasts with uninfested areas where it usually takes ten days for the tracer to reach groundwater.

In our other objective, determination of the depth and width of fire ant mounds has been moderately successful. With the addition of several gallons of water directly on the mound, the radar clearly picks up breakage in the soil four feet deep. From four to six feet the readings are not quite as clear. Further work with the radar should allow fine tuning of the system to depths of six to eight feet. A study by Hubbard and Cunningham (1976) showed fire ant mounds above ground tend to orient north-south. In our initial observations, the mounds tend to break up the soil underground in an East-West orientation. The underground mound also appears to cover an area about twice the diameter of the above ground portion. The wet season experienced thus far

in 1991 has prohibited our work with the radar. The soil has been saturated therefore limiting the contrast normally produced by the galleries.

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Figure 1. Radar view of a gopher tortoise burrow.



Observations of the Red Imported  
Fire Ant, *Solenopsis invicta* Buren  
in Turkey Grow Out Houses and  
Preliminary Control Investigations  
Jefferson, SC 1990

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A study was initiated in August 1990 to investigate the impact the red imported fire ant (RIFA) *Solenopsis invicta* Buren has in and around turkey houses. Development of an IPM strategy for turkey producers using products labeled for use on or around birds was also investigated. The primary problem found with RIFA in the turkey houses studied was RIFA build their mounds outside the houses and forage on dead birds inside the houses. This creates a problem for the workers that collect dead birds and make daily dead bird counts. Preliminary screening of registered insecticides at four weeks post treatment, reduced RIFA colonies around the perimeter (within 50 ft.) of turkey houses as follows: Sevin 80 S (100%), Malathion 50 WP (100%), Dursban 50 WP (100%), Rabon 50 WP (100%), Co-Ral 25 WP (95%), Orthene 75 S (95%) and Dursban 0.5 G (100%). Amdro® was applied on 30 November 1990 broadcast at the label rate around the perimeter of these houses to control any migration of RIFA colonies into the area. Final data will be collected in the Spring of 1991 to assess the bait application.

## Red Imported Fire in Urban Environments Current Research Status

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The red imported fire ant *Solenopsis invicta* Buren is the most extensively distributed medically and economically important social insect pest in urban environments. It is distributed nearly through 130 counties of the State of Texas and occurs in single and multiple queen forms.

The 1988-89 county-wide survey, undertaken together with the Texas Department of Agriculture (through Roger Mulder and Dan Clair, IPM Specialists) and the University of Texas (Sanford Porter, now at the USDA, Gainesville), showed that 76 of 126 (60%), counties sampled were infested with the multiple queen form, in addition to the original single queen form (Porter et al. 1991). It is estimated that rest 40% still have the single queen form.

In 1990, an Urban Survey was initiated under the auspices of the Center for Urban and Public Health Entomology and in collaboration with the Texas Department of Agriculture's Fire Ant Inspectors through Roger Mulder, IPM Specialist.

Various urban habitats of six major cities were surveyed. The city center, intercity and suburban habitats of the cities sampled (Austin, Dallas-Fort Worth, Houston, San Antonio, Corpus Cristi and Bryan-College Station) were found to be infested with RIFA. The ant samples are being analyzed to give the relative estimates in each region.

Home owners are seriously impacted and concerned with the infestations of RIFA. The infestations occur not only in the lawns and gardens but also inside the habitations (kitchens, bath rooms, child's room, playground, laundry, electric appliances, air conditioners, pet food, etc.). Presently, RIFA may be considered as a number one urban pest by inhabitants. It is known to infest electric motors, junction boxes, telephone and cable boxes, transformers and airport runway lights, increasing liability costs.

Survey will also show the impact of RIFA on urban populous and the remedial measures taken by public. The status of RIFA as an urban structural pest needs to be evaluated. The surveys will help to re-evaluate that status so that measures can be undertaken to control RIFA in the most sensitive and threatened urban areas with priority.

An integrated pest management (IPM) approach is being developed to control RIFA in urban environments. Currently the Urban Center's research focus is on: 1. Studying the nesting, foraging and reproductive habits of the single and multiple queen forms of the fire ant. 2. Developing facilities for determining species and their pest status through periodic surveys in human habitats. 3. Screening biorational products for remedial measures. 4. Evaluating landscaping practices to make urban areas less inhabitable to fire ants. 5. Developing IPM models to implement a practical population management strategy.

However, a good deal of basic information is presently being collected through supplementary or privately funded research projects. An objective commitment through state legislated funds for positions and programs in myrmecology and social insects, to create a rigorous research-educational base, is required for IPM of fire ants and other social insect pests in the State of Texas.

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