PROCEEDINGS of the 1989 FIRE ANT RESEARCH CONFERENCE

APRIL 18-19, 1989

BROADWATER BEACH HOTEL

BILOXI, MISSISSIPPI

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Broadwater Beach Hotel
Biloxi, Mississippi

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Dedicated To

DR. CLIFFORD S. LOFGREN

Retired from USDA/ARS April 28, 1989

Service to the USDA from 1954-1989

Conducted research on the Imported Fire Ant from 1957-1989

A TRIBUTE TO DR. CLIFFORD S. LOFGREN

"MR. FIRE ANT"

We have the privilege today to pay tribute to a gentleman who has contributed much to what we know about the imported fire ants today and has strongly influenced the direction that much of the research on these insects has taken. Dr. Cliff Lofgren has elected to retire from the USDA, effective on Friday of next week, April 28. It has been my privilege to work with Cliff, in one capacity or another, for nearly 28 years. We've lunched together, walked field plots and dug ant mounds, sat through innumerable meetings and planning sessions, traveled to such wonderfully scenic and exciting places, to name only a few, as Starkville, Miss., Ft. Meade, Fl. Americus, Ga., Weslaco, Tex., and Caceres, in Mato Grosso, Brazil.. All our shared experiences over the years have been most pleasurable for me because Cliff is the consummate gentleman, always kind and considerate. Rare have been the occasions that I've seen Cliff's demeanor change and then only with very good reason. I was asked to make a few remarks to the group assembled here today to convey the esteem that we in the Gainesville laboratory, and I am sure those of you who know him well, hold for Cliff.

Cliff was born in St. James, Minnesota, the youngest of his mother and father's seven children. I understand he came along so many years behind his siblings that his father affectionately referred to him as "The Trailer". He grew up on a farm near St. James, receiving his early schooling in a country school, built on land donated by his great-grandparents. His maternal grandparents and great-grandparents, immigrants from Sweden, were the first settlers in the St. James area. Cliff graduated from high school in St. James in 1943. Since this was at the height of World War II, and his brothers were away in service, Cliff stayed on the farm to help with the work. He later served in the Army himself, spending time in Japan.

Cliff did his undergraduate studies at Gustavas Adolphus College in St. Peter, MN, receiving a BA in Chemistry in 1950. Deciding that he didn't want to pursue a career in chemistry, he acted on some advice he was given and attended an Entomology Summer Session sponsored by the University of Minnesota at Itaska State Park. This sparked sufficient interest in entomology for him to enroll in graduate studies in entomology at the University of Minnesota, where he graduated in 1954 with an MS in entomology. It was while he was a graduate student at Minnesota that, through friends, he met Renee, the lovely lady who was to become his wife. They were married in April of 1954.

Cliff began his long and illustrious career with USDA in August of 1954, accepting a job with the Golden Nematode Project at Hicksville, Long Island. The Lofgren's first child was born on Long Island in April 1955. Three weeks later, the family moved to Florida and Cliff began work with the Insects Affecting Man & Animals Laboratory which, at that time, was located in Orlando. In Orlando, Cliff conducted research on household insect for 2.5 years,serving as project leader for the last 1.5 years. In October 1957 he was selected to organize, staff, develop and supervise a research program for the new Methods Laboratory established by the Plant Protection Division of ARS at Gulfport, MS to support the Imported Fire Ant Control Program.

That research program very quickly effected improvements and changes in the control program. Within 18 months, research showed that the application rate for heptachlor, which was used from 1957 to 1961, could be reduced from 2.0 to 1.25 lb/acre and within another year, that dosage was further reduced to two applications of 0.25 lb/acre at 3 to 6 month intervals. The most significant achievement of the team under Cliff's leadership was the development of mirex bait. It was was the first highly effective bait for control of pest ants and was acclaimed at the time as an outstanding entomological achievement and resulted in a USDA Superior Service Award for the Methods Unit. Modifications of the mirex bait formulation were widely used throughout the tropics for many years for control of leaf-cutter ants. The basic formulation concept is still in use with the fire ant baits that are presently being marketed.

In 1963, Cliff rejoined the staff of the Insects Affecting Man & Animals Laboratory, which had just relocated from Orlando to the University of Florida campus at Gainesville. He was assigned as Project Leader of the Area Control Section with responsibility for developing methods for outdoor, large-scale control of medically important insects (primarily mosquitoes). This research included chemical, biological, genetic, and sterile male techniques. In 1965 Cliff enrolled in a doctoral program at the University of Florida, receiving his Ph. D. in 1968.

When the Plant Protection Division of USDA requested in 1968 that the Entomology Research Division assist in determining the possibilities of fire ant eradication with mirex bait and made funds available for the studies, Cliff was assigned responsibility for coordinating this research also. With the ARS reorganization in 1972, he was designated as Research Leader for investigations on Mosquito Biology and Control, Insect Pathology, and Imported Fire Ants. In this capacity Cliff provided expert guidance to the Armed Forces in control of mosquitoes in Thailand and other parts of the world and assistance to the sterile male release program to control malaria mosquitoes in El Salvador.

When the Congress appropriated additional funds in 1976 for expansion of ARS fire ant research, Cliff was reassigned with responsibility only as Research Leader for this expanded work. From that time, until he surrendered direction of the unit to Dr. Patterson in October 1988, he had responsibility for directing the research activities of 5 to 6 entomologists and 1 chemist along with their support personnel.

Fire ant research under Cliff's leadership has been very productive. We have learned how to rear large numbers of fire ant colonies in the laboratory and utilize those colonies for a variety of studies of biology and behavior as well as for evaluation of chemicals for control. Considerable progress has been made toward elucidation of the trail and the queen recognition pheromones and studies are in progress to utilize these materials for improved detection of incipient infestations,and for increased efficacy and specificity of baits.

Since the withdrawal of mirex bait in 1978, four new baits have been developed and registered and are now available to the public for fire ant control. A fifth bait should be in the market place later this year or by early next year. Much information has been developed on the biology and physiology of the fire ants. The presence of and the distribution of hybrid fire ants in the United States resulting from mating of the red and black fire ants has been reported. Investigations that have shown the presence of pathogens and parasites that may prove to be effective biological controls have been and are being conducted in South America. The foregoing is only illustrative and by no means exhausts the list of accomplishments during Cliff's tenure as Research Leader.

Cliff is the author or co-author of more than 200 scientific papers; approximately 150 of these deal with imported fire ants. His accomplishments have been recognized with numerous awards, including two USDA Certificates of Merit, the USDA Sustained Outstanding Performance Award, and two Inventor's Awards. He was awarded the Plant Protection Award of Eminence by the Florida Department of Agriculture and the Outstanding 4-H Alumni Award by the State of Minnesota. His research units were twice awarded the USDA Unit Award for Superior Service. Cliff has also been recognized by American Men of Science, Who's Who in America, Personalities of the South, and Who's Who in Technology Today.

I'm not sure what Cliff has planned for his retirement years. I know he and Renee plan to do some traveling. He's an avid bowler and will no doubt continue to knock down a few pins.

I'm sure that a considerable portion of his time will be spent enjoying his grandchildren and watching and encouraging them as they grow to maturity.

Cliff, it has been a real privilege to work with you during these many years. I'm confident that I speak for the group when I say that we will miss our association with you. We're happy that you've reached this milestone in your life, we salute you, and wish for you and Renee many happy and prosperous years of retirement.

Mating Flights and Reproductive Investment: a comparison of *Solenopsis invicta* and *S. geminata* in North Florida

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The displacement of the native fire ant, *Solenopsis geminata*, by *S. invicta* in certain habitats has been documented several times in the literature (reviewed by Hung and Vinson, 1978; see also Travis, 1941). Several aspects of its ecology and life history may contribute to the success *S. invicta* has enjoyed at the expense of *S.geminata*. This study takes aim at the hypothesis that *S. invicta* gains advantage by "outreproducing" *S. geminata* in areas where the two species are sympatric. Assuming other factors equal, relatively more newly available sites will be colonized by the species with proportionally more reproductive propagules in the pool of potential recruits. That is, if *S. invicta* produces relatively more newly mated queens than *S. geminata*, then *S. invicta* would have an advantage in recruiting new colonies, assuming equal colony founding success.

Twenty *S. geminata* and sixteen *S. invicta* colonies in the high pinelands of the Apalachicola National Forest were selected for alate trapping. In this area both species are present in fairly large numbers. Starting in April 1988 the presence of alates in nearby colonies was monitored by periodically opening the mounds. When mature alates were present, traps were placed over the selected colonies.

My trap design is the same in principle as that of Morrill and Whitcomb (1972), but is intended to shade the mounds less and to collect the alates live in plastic bottles. Collecting the ants live, rather than relying on chemicals to kill them, allows accurate measurement of the weights of the trapped alates.

The traps were checked daily and any captured ants were removed after alates were no longer leaving the nest. On days when no alates were captured from a colony, the activity of the colony was determined, usually by blowing on the mound and watching for worker response. If the mound under a trap was inactive, the new colony location was usually obvious and the trap was moved. Daily rainfall was also measured at the time of collection. Collected alates were removed by aspirating, or by removing the plastic bottle, and transported to the lab. Alates were killed by freezing overnight and then oven-dried at 50C for a minimum of 36 hours. For each flight the ants from each colony were separated by sex, and the subsamples counted and weighed.

Starting in October, the size of each of the trapped colonies was estimated. To estimate the size of a colony, the entire nest was excavated, the soil/ant mixture weighed and homogenized, either by hand or with a gas-powered mortar mixer, and the number of ants in subsamples of known weight used to extrapolate the number of ants in the entire colony.

Although both *S. invicta* and *S. geminata* fly in response to rain, the timing of their mating flights is quite different. *S. geminata* typically would fly shortly after late afternoon/early evening showers. Flight activity was usually initiated an hour or two before sundown and would sometimes last until after sundown, but always ended before dark. *S. invicta* normally flew in mid-afternoon the day after a rain shower.

Seasonal differences between the two species are also apparent. The first *S. geminata* alates were collected sixteen days after the first *S. invicta* alates (Fig. I). By this time the *S. invicta* colonies had released 20% of their total alate production for the season. Also evident is the difference in magnitude of the flights; *S. invicta* colonies, on average, release about five times as many alates as *S. geminata* colonies. From June through August, the production of alates by *S. invicta* is relatively regular and slowly decreasing (Figs. I & II). Alate release by *S. geminata*, on the other hand, is distinctly bimodal, with very little production in August and September. Figure II indicates the sex ratio of released alates remains consistent. By weight, collected *S. invicta* alates were 36.2% male. Individual colony production ranged from zero to 86.0% males by weight. *S. geminata* total production was 40.7% male and individual colony production ranged from zero to 88.2% males by weight.

There is a striking difference between the two species in the way the mean alate dry weights change during the flight season (Fig. III). The weights of males of both species remain constant. *S. invicta* males average 2.21 mg and *S. geminata* males, 1.88 mg. During the flight season the mean weight of *S. invicta* females steadily declines from about 9 mg to 7 mg (overall mean = 8.07 mg). Early *S. geminata* queens average about 10 mg and this remains constant through September (mean weight of queens captured prior to 1 October = 9.72 mg). *S. geminata* queens collected in the late season burst, evident in Figures I and II, averaged only 4.47 mg, less than half that of queens captured before October.

As Figure I shows, *S. invicta* colonies produce many more alates than *S. geminata* colonies. When colony size is taken into account though, a much different picture emerges. The trapped *S. invicta* colonies had, on average, 6.6 times as many workers as the trapped *S. geminata* colonies (Fig. IV). In addition, *S. invicta* workers are larger than *S. geminata* workers (S. invicta mean dry weight = .455 mg; S. geminata, .264 mg). When adjusted for these factors, the reproductive investment, or alate production efficiency, of *S. geminata* is significantly greater than that of *S. invicta* (ANOVA, $F = 5.262$, $p = .0292$). Apparent from Figure IV is that greater alate production by *S. geminata* cannot be attributed solely to species. Due to the sizes of the colonies selected, the effects of species cannot be separated from colony size.

Looking at alate production relative to worker weight over the flight season (Fig. V), one can see that the difference in the two species is due to the burst of production in early Autumn by *S. geminata.* The ecological significance of this late-season burst is unclear. Although some of the queens I collected after mating flights in the last week of October were capable of founding colonies in the lab, it is uncertain if these queens can raise a colony large enough to survive winter in North Florida.

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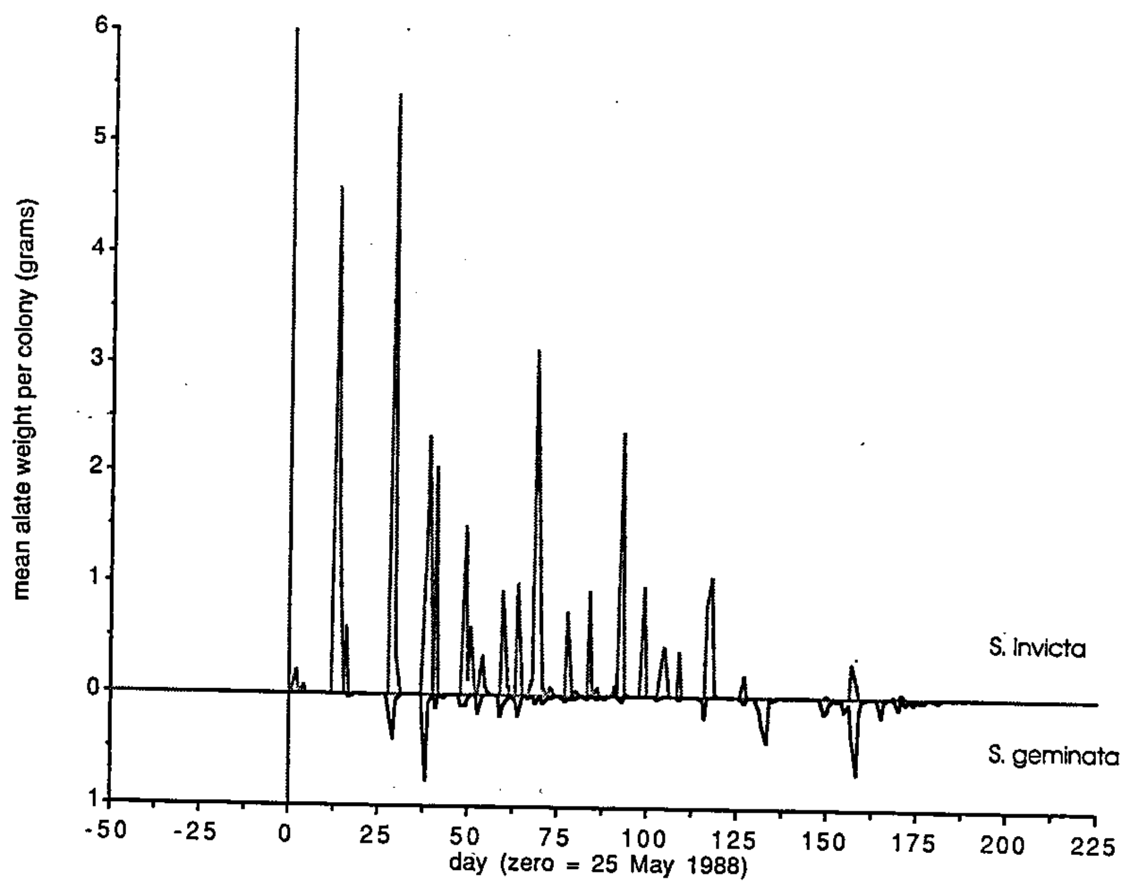
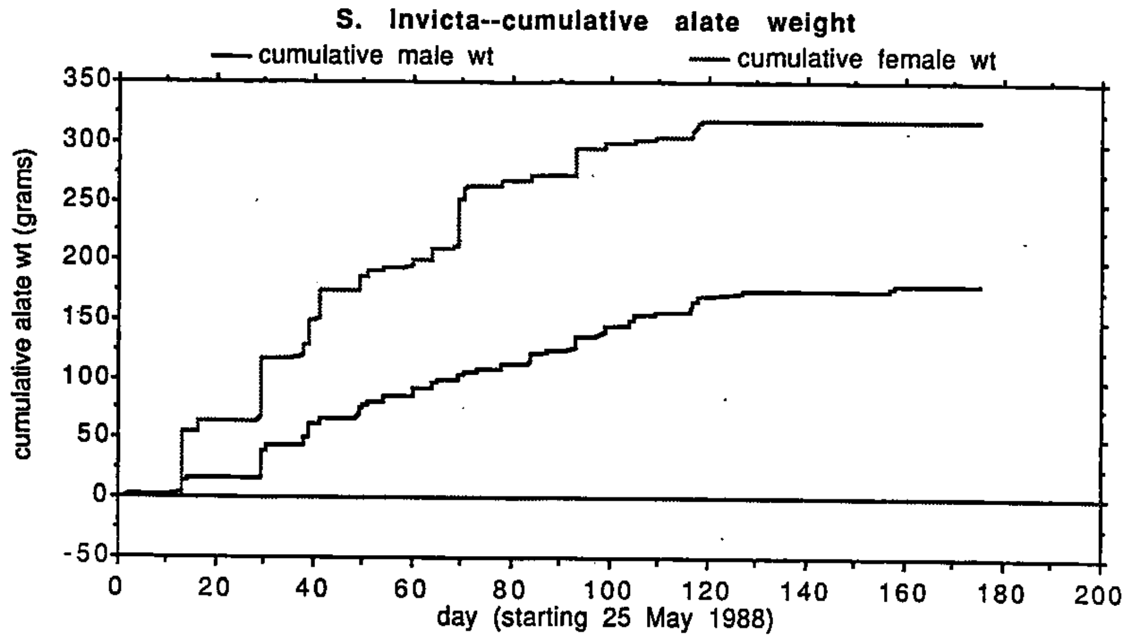


Figure I. Average alate production . The vertical axis for any particular day represents the total dry weight of collected alates divided by the number of colonies trapped.



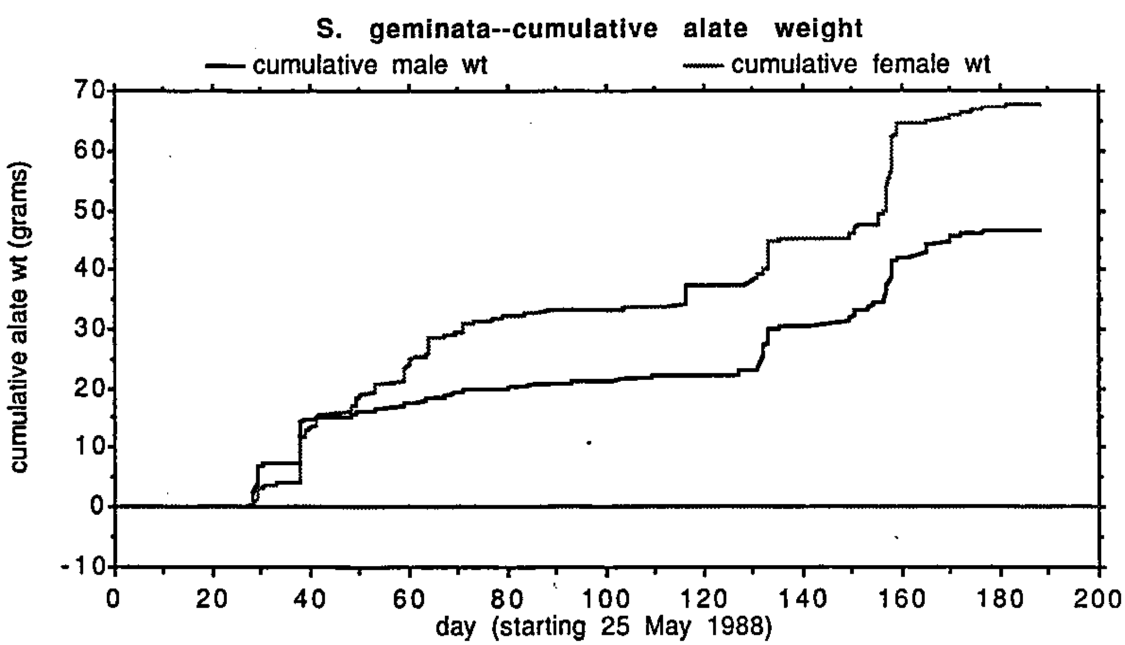


Figure II. Cumulative dry weights of collected alates from 14 S. invicta and 17 S. geminata colonies (note the vertical axes are of different scale).

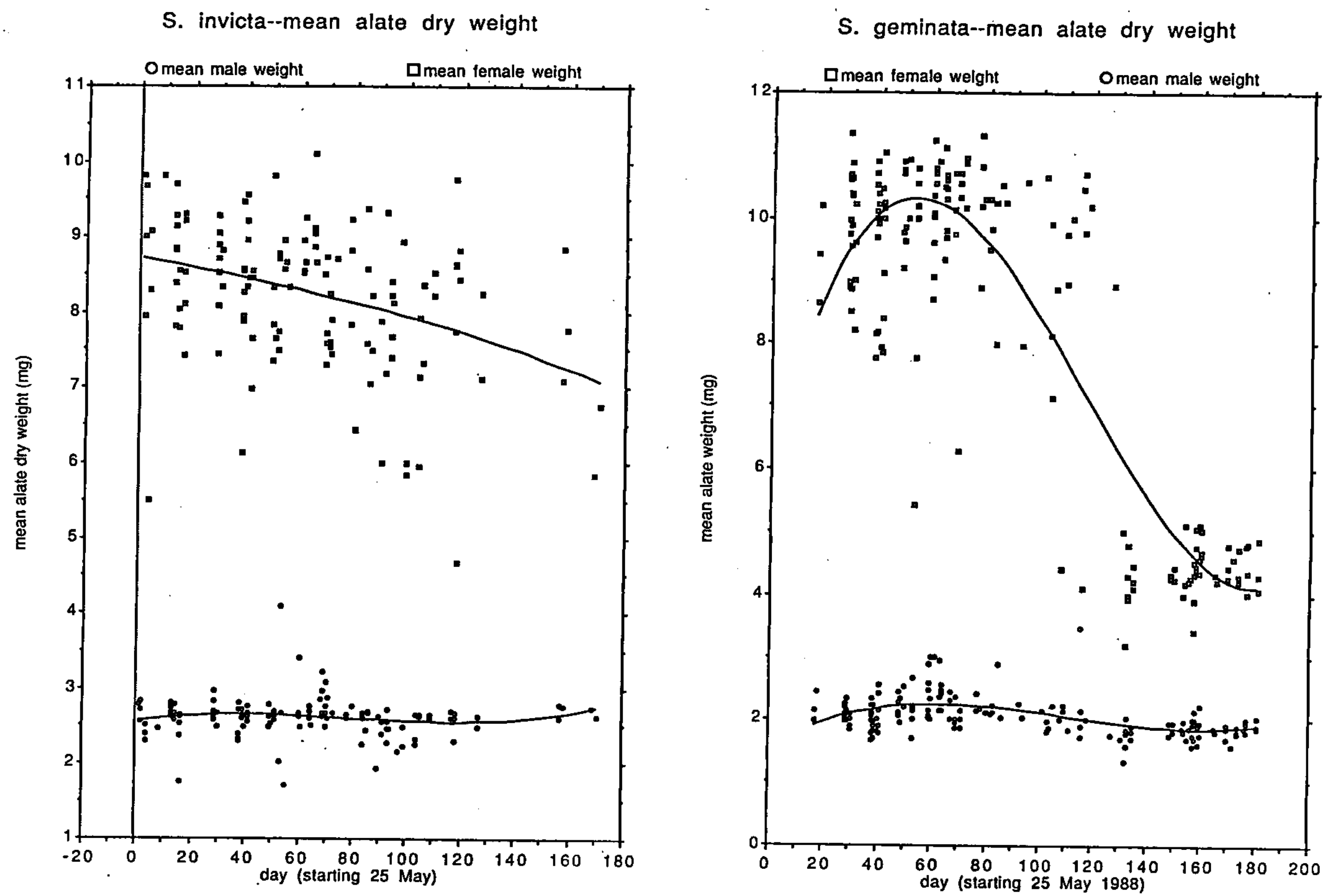


Figure III. Mean alate dry weights. The fitted curves are from third order polynomial regressions (note the vertical scales vary slightly).

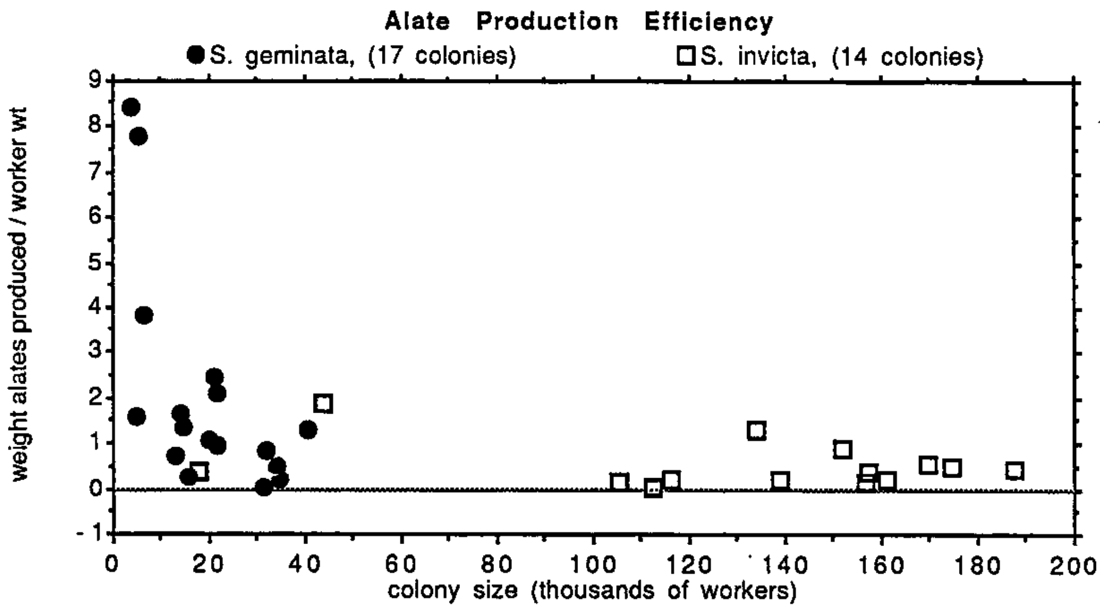


Figure IV. Each point represents the total dry weight of alates produced by a colony divided by the estimated total dry weight of workers at the time of excavation.

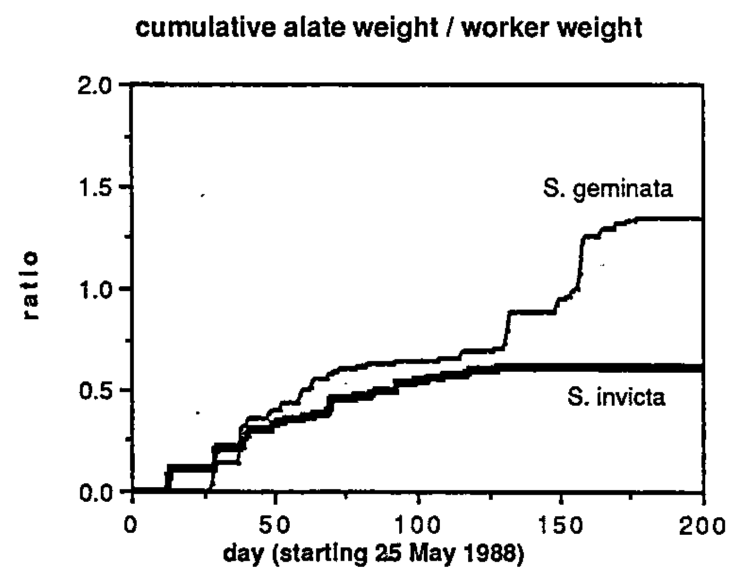


Figure V. The cumulative dry weight of collected alates divided by the estimated total dry weight of the workers of the trapped colonies.

DENSITY, DISPERSION, AND PERSISTENCE OF FIRE ANT MOUNDS ON THE TEXAS COASTAL PRAIRIE, B. D. Eshelman, G. N. Cameron, and E. N. N. Huynh; Dept. of Biology, University of Houston, Houston, Texas 77204-5513.

Abstract

An unplanned burn of an area of Texas coastal prairie in November 1987 allowed us to monitor fire ant mound density, dispersion, and persistence. Five 30 x 30 m plots (900 m^2) were established in April 1988 and monitored monthly through November 1988. All active mounds were individually flagged and mapped. Plots were checked monthly for activity of established mounds and emergence of new mounds. Ant mound dispersion was calculated monthly by testing against a Poisson distribution and by using the Hopkins method. Mounds were distributed randomly within the plots. Average density of active mounds varied among plots from 116 to 737 mounds/ha. The average length of time a mound was active was 3.6 months. Approximately 9% of the original mounds were active throughout the study.

Introduction

Comparatively little work has been done on the field ecology of populations of fire ants (*Solenopsis invicta*) on the Texas coastal prairie. Eisenberg (1972) conducted a study on spatial arrangement of these ants, but, since then, few population studies have been reported. Eisenberg's study also predated reports of multiple queen colonies, suggesting further study is necessary. Before further ecological investigations can be initiated, basic demographic information on the fire ant is essential. Our objectives were to: 1) quantify the number of mounds, 2) identify the dispersion pattern of the mounds, and 3) determine the length of time individual mounds remain active on the Texas coastal prairie.

Methods

During November 1987 an area of remnant coastal prairie on the University of Houston Coastal Center, 56 km SE of Houston, was unexpectedly burned. This burn exposed a number of fire ant mounds and allowed us to count them easily. The area is characterized by a relatively high water table with soils of the Lake Charles Series (Fine, Montmorillonitic, Thermic, Typic Pelluderts). Dominant grasses include: little bluestem grass, *Schizachyrium scoparium*; eastern gammagrass, *Tripsacum dactyloides*; and Gulf cordgrass, *Spartina spartinae*. These grasses are complimented by several dominant forbs: goldenrod, *Solidago* sp.; Marsh elder, *Iva annua*; tuber vervain, *Verbena rigida*; and sea myrtle, a shrub, *Baccharis halimifolia*.

Density and activity of fire ant mounds were monitored from April through November 1988. Five 30x30 m plots were established in randomly-selected locations in the burned area. April is the beginning of the growing season in this part of the Texas coastal prairie and is also the start of the mound establishment period. The study concluded in November 1988 because vegetation had grown so much that it was difficult to locate very small, new mounds.

All active mounds found during the April survey were flagged and mapped for future identification. Mounds were determined to be active by disturbing the top of the mound and waiting for ants to respond to the disturbance. In subsequent monthly surveys all flagged mounds were tested for activity and new mounds were identified. All active, new mounds were flagged and mapped. At the end of each survey period, maps of active and inactive mounds were produced for each plot. Density was determined monthly for all mounds on the plots and for active mounds only.

Monthly maps from all plots were used to analyze dispersion of mounds by testing their distribution against a Poisson distribution. Maps of the plots were subdivided into 9 subplots and the number of mounds in each subplot was recorded, thereby constructing a frequency distribution of observed values. This observed frequency distribution was tested against the expected Poisson distribution using a Chi-square analysis.

Temporal activity of mounds was used to categorize mounds for analysis of persistence. Mounds that were active during the entire study or mounds that became inactive and stayed that way were defined as non-switchers. Switchers were mounds that were active for a period of time, became inactive, and subsequently were identified as active mounds. Thus, these mounds alternated between periods of activity and inactivity. Persistence was determined in three ways for mounds which were non-switchers. First, the average length of time a mound remained active was calculated. Then the proportion of mounds from the April sample that were active throughout the entire study was measured. Finally, persistence was viewed as a rate of decay of mounds from the plots. This was accomplished by regressing the number of original mounds against the number of original mounds left during each month of the study. The resulting equations provide a value for the number of original mounds lost per month of the study.

Results and Discussion

Density of fire ant mounds found within each plot averaged over all months of the study was:

TABLE 1. Densities of Fire Ant Mounds Averaged Over All 8 Months of the Study for Each Plot. Other Data on Mound Densities are Included for Comparison.

PLOT	ALL MOUNDS #/ha. #/900 m^2 s.e.	ACTIVE MOUNDS #/ha. #/900 m^2 s.e.
1	287.5 25.8 1.14	143.0 12.8 1.34
2	300.0 27.0 0.96	147.2 13.2 1.75
3	205.5 18.5 0.94	116.6 10.5 1.00
4	900.0 81.0 4.71	519.4 46.7 3.40
5	1263.8 113.7 8.65	737.5 66.3 4.53
Eisenberg (Houston, Texas) 1972		125/ha
Morrill (Gadsden Co., Florida) 1974		125/ha
Porter et al. (Austin, Texas) 1988		400/ha

Density of active mounds ranged from a low of 116.6 /ha to a high of 737.5 /ha. Preliminary investigations indicate that mounds adjacent to plots 4 and 5 are of the multiple queen type. Multiple queen mounds are also found near plots 1,2 ,and 3, however, single queen mounds may also be in the area. Further sampling is currently in progress to determine the occurrence of single queen mounds.

Analyses of the distribution of active plus inactive mounds as well as the distribution of active mounds only showed that mounds were randomly dispersed. Similar findings were obtained using the Hopkins nearest neighbor technique.

The average length of time a mound remained active for each plot averaged over all months of the study was:

TABLE 2. Average Lifespan of Active Mounds Over the 8-Month Study Period for All Plots.

PLOT #	MEAN (MONTHS)	s.e.
1	3.3	0.55
2	3.5	0.46
3	3.6	0.70
4	3.9	0.31
5	3.8	0.27
	MEAN = 3.6	

Once a mound had been identified as an active mound, it remained active for an average of 3.6 months.

Another view of persistence would be to ask "of those mounds that were originally active on the plots in April, what proportion of them were active for the entire eight months of the study period?"

TABLE 3. Proportion of Original Mounds (April) That Were Active for the Entire Study Period.

QUAD	RATIO	PROPORTION
1	1:20 ORIGINAL	5.0 %
2	2:23 ORIGINAL	8.6 %
3	2:13 ORIGINAL	15.3 %
4	5:56 ORIGINAL	8.9 %
5	6:69 ORIGINAL	8.6 %
	mean =	9.3 %

Only 9.3 % of mounds originally identified as active in April remained continually active throughout the 8 months of the study.

Finally, relatively low density plots (1,2,3) from this study lost between 1.5 and 2.7 original mounds per month. Higher density plots (4 and 5) lost 7 and 8.3 mounds per month, respectively. Interestingly, the proportion of original mounds lost per month was similar for all plots.

TABLE 4. Regression Values for Loss of Ant Mounds by Plot.

PLOT	REGRESSION EQUATION	R-SQUARED	% LOST
1	Y= - 2.4x + 14.6	0.78	12.0
2	Y= - 2.7x + 18.2	0.84	11.7
3	Y= - 1.5x + 11.3	0.92	11.5
4	Y= - 7.0x + 50.9	0.95	12.5
5	Y= - 8.3x + 53.9	0.83	12.0

Conclusions

Density of fire ant mounds on the Texas coastal prairie varied from 116.6 to 737.5 mounds/hectare. These densities are relatively high compared to other studies (Table 1). The highest number of active mounds were found during the spring and early summer.

Fire ant mounds were distributed in a random pattern. This differs from earlier findings of Eisenberg (1972) who found that ant mounds were uniformly distributed prior to reports of occurrence of multiple queen mounds. Eisenberg used a different nearest neighbor technique for his analysis; we are currently reanalyzing our data with the technique he used to provide a direct comparison.

Persistence of fire ant mounds on the Texas coastal prairie was fairly short as indicated by an average lifespan of 3.6 months. Approximately 9% of the original mounds were active for the entire study and the loss rate of original mounds ranged from 1.5 to 8.3 mounds per month. Although the number of original mounds lost per month differs among plots, this does not appear to be a density effect because the proportion of original mounds on the plots remains consistent across plots Loss of established active mounds in the plots was partially compensated for by emergence of new mounds. Fewer active fire ant mounds were present on the plots in November 1988, the end of the study, than during the initial survey period in April 1988.

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Endoparasitic Fungi of Fire Ants

Donald P. Jouvenaz

At least three distinct species of endoparasitic fungi occur as budding yeasts in the haemolymph of fire ants in the United States and South America. A total of five strains (or sibling species?), representing two of these species, are in culture; the third species is known only from preserved S. invicta from Brazil. Both species in culture have been transmitted per os to healthy colonies of S. invicta; however, the rate of transmission is low (ca one percent), due to loss of invasive ability upon in vitro culture. Lepidopterous larvae become infected when inoculated by syringe, and we are attempting to preserve, and hopefully select, invasive ability by passage through corn earworm larvae. Also, cultures are being maintained in modified Grace-Yonker insect tissue culture medium. Reduction of virulence or pathogenicity is a common and difficult problem with entomopathogenic fungi.

The first report of unicellular, endoparasitic fungi in fire ants was that of Jouvenaz et al. (Fla. Entomol. 60: 275-279, 1977), who isolated a species from S. invicta in the United States. Until very recently, this was the only microorganism known to be symbiotically associated with imported fire ants in this country. Early studies of this tapered rod or "club-shaped" fungus indicated that it is only a nutritional burden to its host, producing no toxins and causing no histopathology (electron microscopy). Elevated mortality occurs in infected colonies under conditions of stress. Since the primary goal of our research is the introduction of a complex of specific natural enemies from South America, and this fungus is already in the United States, we turned our attention to other - hopefully more virulent - entomopathogens.

The rapid advances in taking place biotechnology, and our discovery of additional species, have caused us to resume studies of the endoparasitic fungi. These organisms can be mass-produced, transmitted per os, and thus far appear to be non-toxinogenous and host-specific for fire ants. Their non-toxinogenicity may ultimately prove advantageous, for it may be possible to alter them genetically to produce toxins of our choice, or juvenile hormone or other hormones, or even semiochemicals to disrupt colony organization. Ways to preserve the invasive ability of mass-cultured cells must be developed through genetic modification or cultural techniques, or both. Even without genetic alteration, however, species or strains from South America may prove valuable as stressors in a complex of introduced, specific natural enemies of fire ants.

Two strains or sibling species of club-shaped fungi are now in culture. The species reported by Jouvenaz et al. (1977) has been reisolated from S. invicta from Florida; the second strain was isolated from S. richteri from Argentina. (Interestingly, we did not encounter this parasite in over 2,000 colonies which we examined in Brazil, the presumed homeland of S. invicta). These fungi produce incompletely septate mycelia in vitro, but thus far have not sporulated. Only the unicellular phase has been observed in fire ants.

Three strains or sibling species of spherical to short, tapered rod-shaped fungi have been isolated from S. invicta from Brazil, Alabama, and Texas. The latter strain was discovered as a contaminant in fire ant tissue culture experiments at Texas Tech University by Victor Montalvo, a graduate student of Dr. Sherman Phillips. These fungi develop short chains of bacilliform cells in vitro, but not true mycelia. They also grow at higher temperatures (35°) than do the club-shaped strains.

The third distinct species of endoparasitic fungus of fire ants has been seen only in mass extracts (phase-contrast microscopy) of fire ants from Brazil which had been preserved in formalin-glycerin. (It is easy to confuse ingested organisms with true infections when examined only in this way; however, the numbers of cells present, and the numbers and distribution of positive colonies makes me confident that this organism is a true parasite of fire ants). The cells vary greatly in size and appearance; young cells are spindle-shaped, while the largest cells resemble microsporidian spores, and in fact may be confused with the free spores of Vairimorpha invictae.

The endoparasitic fungi are the only specific pathogens of fire ants which appear to have the potential for both development as a microbial formicide and for establishment in our ecosystem. Innovative, in-depth research employing sophisticated techniques will be required if their potential for inexpensive mass production, safety, species specificity, and lethality to fire ants is to be realized.

INFLUENCE OF TEMPERATURE ON COLONY GROWTH OF THE IMPORTED FIRE ANT, SOLENOPSIS INVICTA

ABSTRACT

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We have conducted studies for 8 months on the effects of constant temperatures on the growth of fire ant colonies. Newly-mated fire ant queens were set up in 6 environmental chambers maintained at 24C, 27C, 30C, 32C, 35C, or a fluctuating temp 28C - 32C. The chambers were also maintained at ca. 60% r.h. and 12hrs daylight photoperiod. The diet made available to all colonies consisted of insects, egg yolk, and honey:water agar. Test tubes containing water also were made available to each colony. As the colonies grew in size, they were moved to larger containers to compensate for their growing numbers.

Once a month, beginning with the 2nd month of the study, 5 colonies from each temperature setting were selected at random for census. Total numbers were counted for the 2nd and 3rd months, while aliquot parts were counted for the 4th through 8th month. Before each colony was terminated for census, the queen was removed and set up for a 4hr oviposition test in order to obtain data on egg-laying.

Results of 8 months data indicated that regardless of temperature, when minum workers appeared in a colony, the queen,s body weight at that point had been reduced to 1/2 her initial body weight. The weights of the newly-mated queens dropped drastically during the first 90 days and then begin to increase with the appearance of minum workers returning to slightly above or below their initial weight at 120 days. The greatest increase in the growth rate (X-fold) occurred between 30-90 days at all temperatures except 24C which occurred between 90-120 days. The slowest rate of growth occurred at 24C while the fastest occurred at 35C and the lowest queen mortality occurred at the colder temperatures while the highest occurred at the hotter temperatures. After 8 months, the largest colonies occurred in the chambers maintained at 27C averaging 242,211 totals with a range of 191,488 to 319,616. The smallest colonies were produced at 24C averaging 84,877 totals ranging from 32,896 to 134,528. After 8 months, the percent of each stage (excluding eggs) at all temperatures averaged 64% workers, 18% pupae, 6% fourth instar larvae and 12% first-third instar larvae.

Finally, the study by Markin et al. 1973 (Ann. Ent. Soc. Amer. 66: 803-808) indicated that 7-month old fire ant colonies in the field had an average number of workers of 6,576 (1,850 - 19,400) while

3 year old fully mature colonies contained approximately 50,000 workers. Although we realize our colonies were laboratory maintained and not subjected to field conditions, the sizes were enormous compared to Markin's results and we think his estimates of colony size were too low. For example, in our studies, 7-month old colonies maintained at 27C averaged 106,934 workers (86,848 - 123,648) which is more than twice as large as the 3-year old fully mature colony estimate of Markin et al. mentioned above.

Efficiency of a Biocontrol Organism *Thelohania Solenopsae* (Microspora: Microsporida) to Suppress Fire Ant Population in Argentina

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Abstract

Based on the number of active fire ant mounds or nests per hectar in Argentina in pasture situations, the density of fire ants is similar to that in the U.S.A. Over 75% of the fire ant nests were moved from their original site in a six month period - spring to fall in Argentina. The presence of the disease Thelohania solenopsae did not greatly increase the dimise of a fire ant colony. There was a 25% loss of healthy colonies over a six month period.

This is preliminary data based on six months of a year long study to determine what effect the pathogen *Thelohania solenopsae* (Knell et al. 1977) has on an indigenous fire ant population in Argentina. The species of fire ant which we are working with, based on a tentative identification, is *Solenopsis richteri*, however, some of the plots may also contain *Solenopsis quinque cuspi* (Trager, unpublished key). This study was initiated in October 1988 which is spring in Argentina and continued to March the beginning of fall. The climatic conditions in the test area are similar to that of North Central Florida and Eastern Texas.

Numerous surveys have been made of what pathogens and parasites of the fire ants are in South America (Jouvenaz 1986). Few, however, have studied the long range effect a single pathogen would have on individual fire ant colonies. In fact almost nothing is known about the effect that pathogens have on indigenous fire ant populations in South America (Jouvenaz et al. 1981). Recent studies by Dr. Jerry Stimac of the University of Florida and reported by Carruthers and Hural 1989 have shown that the fungus *Beauveria bassiana* does have a severe effect on individual fire ant colonies in Brazil rifling up to 50% of the colonies. It is currently being tested in Florida by Dr. J. Stimac for its efficiency against *Solenopsis invicta* in the Gainesville, Florida area. If it is successful, it will be a major breakthrough for the use of a biocontrol agent for the suppression of fire ants.

The object of our study was to follow a naturally-occurring infection of the microsporidian *Thelohania solenopsae* in indigenous fire ant colonies. To determine; (1) if it spread to other colonies? (2) Does it cause the infected colony to die out? (3) Does it cause the colony to move more frequently, thus eliminating the disease from the colony or (4) perhaps it has no adverse effect on the colony except to impair a few worker ants; therefore is not a viable potential biocontrol agent for importation into the U.S.A.

Methods and Materials

Thelohania solenopsae was selected because it was the most common pathogen present in the fire ant colonies surveyed in Argentina. The description of T. Solenopsae and the disease it causes was given by Knell et al. 1977; its occurrence in host ant populations has been reported by numerous scientists (Allen and Buren 1974, Allen and Gilvera -Guido 1974 and Jouvenaz et al. 1980). All attempts to transmit the disease to healthy colonies of fire ants have failed (Jouvenaz 1986). However, recent studies with Amblyosporidae in mosquitoes have shown that an intermediate host is often required before transmission back into the mosquito, (Andreadis, 1985, 1988) (Sweeney et al., 1985, 1988). There are sound biological reasons to suspect that alternate hosts are involved in intercolonial transmission of microsporidian diseases of fire ants (Jouvenaz, 1986). Since there are a limited number of arthropods which are symbiotic with fire ants (Wojcik, 1989), this is a whole new avenue of research. Early studies and observations by Knell et al. 1977 indicate that this disease did not quickly kill the colony anyway; very large, apparently healthy colonies were often observed in the field with high infection rates of this pathogen. However, we do not know the fate of these colonies, nor do we understand fire ant population dynamics in the host county.

We selected six plots in the area of Saladillo, (Buenos Aires Province) Argentina. The plots were set up in unimproved pastures containing cattle and hogs. These circular plots were 40 meters in diameter. Each active fire ant colony was plotted on a map by measuring from a central stake using the four compass quadrants. Each colony or mound was measured for its height and width. The internal temperature and moisture of the soil in the mound (the first 6 inches) were checked and recorded. The mound was examined for the number of major and minor workers present, the presence of sexual forms, and the presence or absence of brood. A small glass vial was inserted into the mound to collect ants for the presence of the disease. The vials were usually dusted inside with talc to prevent escape of the ants. The vial was left in the mound for less than 10 minutes, then capped and returned to the laboratory for microscopic examination. In the laboratory the ants were ground with water in a tissue grinder, then a small drop of material was placed on a glass slide, covered by a cover slide and examined by Phase-contrast microscopy at 400 x for the presence or absence of <u>Thelohania</u> spores. Since this sampling was a mixture of many individual ants especially major and minor workers, we could only tell the presence or absence of the disease in the colony; not how many and which castes were infected. This will be done in the next six months of this study. Jouvenaz 1986 did report that fire ant workers, sexuals and queens were all infected with this disease.

The six plots were fairly close together. Two were several kilometers away from the other four, which were almost side by side in a series of pastures. The average number of active colonies per hectare was 198 in the six plots. Three of the plots had fairly high disease rates with 35% of the colonies being infected in October. The other three plots were relatively free of the disease with only 3% of the colonies being infected.

The plots were checked monthly, the number of colonies (mounds) was checked as to the viability of the colony. If the colony moved, we wanted to know did the diseased ants move with the colony? The presence of brood, workers, and sexually, the moisture and temperature of the soil around and in the mound.

Results

The number of active fire ant colonies per hectare in Argentina in a short grass pasture habitat of the Saladillo area was almost the same as in southeastern U.S. especially in North Central Florida. Adams 1986 reported 60-150 colonies/hectare as being heavy densities of S. invicta in Florida and Georgia. There was a 25% loss of active fire ant colonies in plots from October to March (spring to early fall). This is similar to what is found with S. invicta in the Southern U.S.A. (Hayes et al. 1982).

Of the active colonies in the six plots 75% left their original nest and moved to a new location in the plot usually within a meter of the original site. This also is similar to what has been observed in the U.S. for S. invicta and S. richteri (Hayes et al. 1982). There was 10% more movement of colonies infected with Thelohania than non-infected colonies. However, statistically this may not be a valid difference.

There was greater loss of colonies with Thelohania, (45%, versus 25%) than of non-infected colonies. In the plots which average initially a 3% infection rate, all colonies were free of the disease after 6 months. However, once a colony was infected and it did not move, it remained infected. We observed no colonies losing their infection. However this is difficult to verify at this time because of colony movement and we were not always sure of the origin of each new colony. We will use oil-soluble food dyes in the near future to identify individual colonies to and follow movement.

In summary it appears that the fire ant density (colonies per acre) is similar in Argentina as that in North central Florida. There appears to be no major biocontrol agent, limiting the fire ant spread or density in indigenous country of the fire ant complex. The fire ants move their colonies frequently in Argentina over a six month period (Spring to early fall). Over 75% of the colonies had moved from their initial nest and set up a new nest. Some colonies moved almost monthly, while others have remained in the same nest site for the entire test period. Since the colonies were numerous and fairly close together it does not appear superficially that food supplies or mound disturbance are causing this movement, but more research is being planned to hopefully pin point the cause. There was a general loss of a number of viable colonies throughout the summer. In this 6 month period there was a 25% loss of healthy colonies. The presence of Thelohania solenopsae does not greatly speed up colony mortality or movement, although there was a 15% increase in die out and 10% increase in the movement. At this point, it is difficult to determine if this is a significant difference or not.

In the future we will be looking at which cast has the infection and the number of ants infected. We have brought diseased colonies into the laboratory in Argentina and will study it in more detail and in an environment from which they can't move. We also are examining diseased colonies outside the plot areas for inquilines associated with fire ants as a possible alternate host for *Thelohania solenopsae*. Hopefully upon completion of this year long study we will have a better idea of what impact this microsporidian disease has on indigenous fire ant populations in Argentina and if it has any potential as a viable biocontrol agent for importation into the United States to suppress fire ants.

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FIELD TRIAL RESULTS OF STEINERNEMA FELTIAE APPLICATIONS FOR CONTROL OF RED IMPORTED FIRE ANT IN 1988

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INTRODUCTION:

Nematode Commercialization:

Steinernematid and heterorhabditid nematodes have been extensively evaluated for pathogenicity against soil-dwelling insect pests (Kaya, 1985, Poinar, 1986). Infective juveniles (IJs) of these nematodes seek out insect hosts by crawling through thin water films on soil particles and following environmental gradients through interstitial soil pores. Once a nematode locates a host, it penetrates a body opening (mouth, anus or spiracle), burrows into the hemocoel and releases a mutualistic bacterium (Xenorhabdus) which causes a toxic septicemia in about 48 hours. Because of the small size and semi-aquatic nature of these nematodes, they are often applied as an inundative release through conventional agricultural spray and irrigation equipment. Environmental conditions conducive to successful field pathogenicity are moderate soil moistures (draining soils) and soil temperatures between 15 and 32 C (Molyneux and Bedding, 1984, Molyneux, 1985). Field persistence of a nematode application varies, but pathogenicity is often measurable for 4 - 6 weeks after application (Ishibashi and Kondo, 1986, 1987).

Large scale culture of steinernematid and heterorhabditid nematodes underwent continued progress in the past year, with successful harvests regularly occurring at the 7500 and 15,000 L fermentor levels. Harvests of several hundred billion IJs per batch have necessitated additional improvements in "downstream" processing and storage techniques. Research continues in the area of desiccation technology and shelf-life enhancement. Nematode-based products are beginning to enter consumer and agricultural marketplaces.

Previous Fire Ant Research:

Early evaluations of steinernematid and heterorhabditid nematodes for control of red imported fire ant (Solenopsis invicta Buren) were undertaken by Poole (1976) and Quattelbaum (1980). In 1986, separate evaluations by by S. B. Vinson (Texas A&M) and D. P. Jouvenaz (USDA-ARS, Gainesville) pointed to

Steinernema feltiae All as the most promising nematode candidate for field trials, ranking highest of species/strains tested in pathogenicity towards immature ants, queens and alates in laboratory cultures (Jouvenaz, et al., in press, Miller, et al., in review).

Field trials in 1987 showed that mound drenches of S. feltiae (2 - 5 million IJs per mound) had some impact on fire ant populations, but extensive colony relocation by workers limited overall reduction in colony activity to 20 - 50%.

1988 Field Trials:

Two field testing programs were conducted in 1988. A corporate demonstration program compared formulated S. feltiae drenches to aminohydrazone baits at 13 locations in 4 southern states. The formulation consisted of an interim desiccated nematode product (BioSafe). Our ongoing research program conducted various types of applications in two trials, (Texas and Mississippi). One research trial (Angleton, TX) contained both demonstration and research elements, and is reported under both programs.

METHODS:

Demonstration Program:

Trials were conducted in single queen and multiple queen sites from April - August 1988 in conjunction with a number of cooperating researchers using split plot or completely ramdomized block designs. Some evaluations were conducted with standard circle-plot techniques, while others relied on assessment of individually marked mounds and adjacent areas for satellite mound formation. Trials consisted of 1 - 3 replicates with a minimum of 20 mounds per replicate. In some cases two trials were conducted at a single site (2 replicates per "trial"). Mounds were probed for worker activity and rated active or inactive at pretreatment and again at 1, 3 and 6 weeks after initial treatment (Gulfport trial was evaluated 3 and 6 weeks after treatment only). All mounds were marked with surveyor's flags or spray paint or both. The USDA rating system was converted to an active (rating = $\geq$2) or inactive (rating = "0" or "1") status for purposes of analysis.

Sites were selected to facilitate irrigation. Irrigation was not initiated until after aminohydrazone bait had been collected. Most (but not all) sites received either rainfall or irrigation within one day after each nematode treatment. General soil moistures varied, but rainfall during many trials could be characterized as dry to droughty.

Demonstration Trial Locations | Cooperator(s):

Demonstration Trial Locations	Cooperator(s):
Sod farm, Alachua, FL	C. Lofgren, D. Jouvenaz, L. Davis
Delco-Remy property, Fitzgerald, GA	C. Sheppard. S. Diffie
Backyard, Gulfport, MS	H. Collins, T. Lockley
Sod farm, Angleton, TX	B. Drees, B. Vinson
Sod farm, Ferris, TX	B. Brewer, S. Reese
Sod farm, Wharton, TX	G. McIlveen, B. Mitchell
Golf course, Austin, TX	D. Clair, N. McClintock
City park, Denton, TX	Stewart, Hassage, Morris
Municipal utility yard, Denton, TX	" " "
Electrical station yard, Denton, TX	" " "
Golf course, Denton, TX	" " "
Coliseum, Denton, TX	" " "
Junior college campus, Fort Worth, TX	" " "

Demonstration Treatments:

Nematode: 2 million S. feltiae (rehydrated from a desiccated state) suspended in one gallon of water were poured onto active fire ant mounds; active mounds were retreated 3 weeks after initial treatment.

Aminohydrazone: 5 Tablespoons of fresh Amdro bait were applied when fire ants workers were actively foraging.

Check: Untreated (except in Angleton, TX - 1 gallon of water per mound; retreatment of active mounds after 3 weeks).

Demonstration Plot Analysis:

To facilitate analysis, frequency plots of fire ant activity assessments for all trial sites were developed. For summary presentation, plots means were considered as single data points (n=23) and analyzed with ANOVA and Newman-Keuls mean separation test (Wilkinson, 1986).

Research Program:

Angleton, TX. A single queen site on an irrigated sod farm with "Gumbo clay soil" was split up into 187 ft squares in a randomized block design (8 treatments x 3 replicates/treatment). All mounds inside a 0.25 acre circle plot inside each 187 ft square were marked with spray paint before initial treatment on 4/19/88. Treatments were applied only within 0.25 acre circle plots. Nematodes were applied as follows (million IJs/mound): 1.6, 0.8, 0.2, BioSafe 1.6. Additional nematode treatments consisted of a broadcast nematode treatment (1 billion per acre equivalent) followed by individual mound drenches of 1.6 or 0.2 million IJs per active mound. Nematodes were applied in 1 gallon of water to active mounds at trial initiation and after 3 weeks. Aminohydrazone (5T) was applied to active mounds at trial initiation. Water (1 gallon per mound) was applied as a check. Mound activity within circle plots was evaluated pretreatment, and at 1, 3 and 6 weeks after initial treatments. Fire ant activity was assessed as either active or inactive after probing 1 and 3 weeks after initial treatment. Mounds were dug to determine activity for the final assessment.

Wiggins, MS. A single queen site on an irrigated sod farm with sandy soil was split up into 1 acre squares (3 treatments x 3 replicates/treatment). Active mounds within each acre block were marked with surveyor's flags prior to treatment. Circle plots (0.25 acre) were additionally marked inside each 1 acre block. Treatments were applied to all active mounds at trial initiation (11/7/88), and again after 2 and 4 weeks. Nematodes (0.8 million IJs per mound) were applied to active mounds in 0.5 gallon of water with AgSorbent, a hygroscopic polymer (gelatinized strach polyacrylonitrile copolymer with potash). Chlorpyrifos (Dursban 2E @ 1 oz/2 gallons water) was applied at 2 gallons per mound. Water (0.5 gallon per mound) was applied as a control. Mounds were opened with shovels and rated (USDA population index) at pretreatment, and subsequently 2, 4 and 6 weeks after initial treatments.

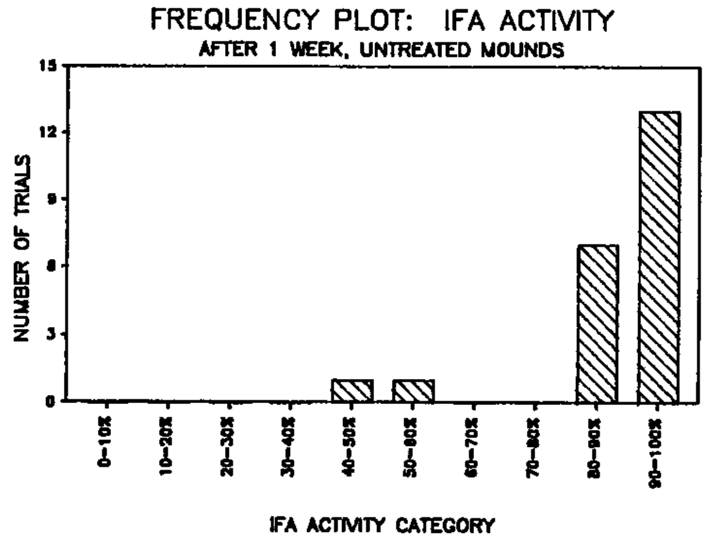
RESULTS:

Demonstration program: For summary analysis, treatment means from each trial have been analyzed as single data points.

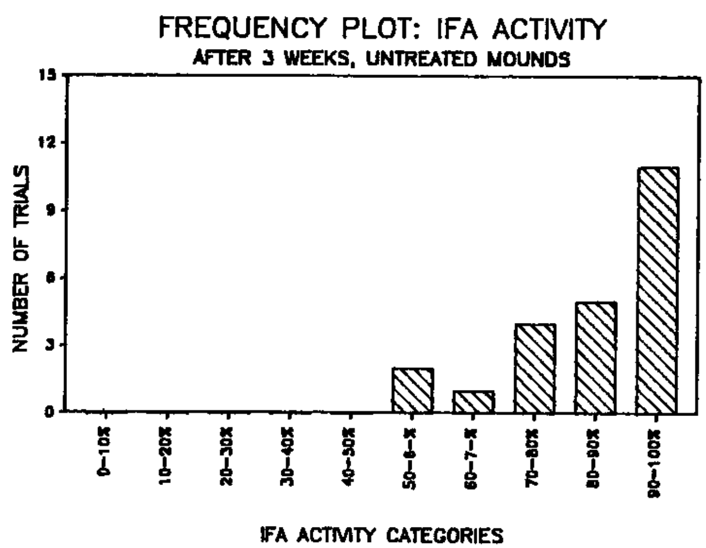
TABLE 1. Mean fire ant activity averaged over 23 demonstration trials.

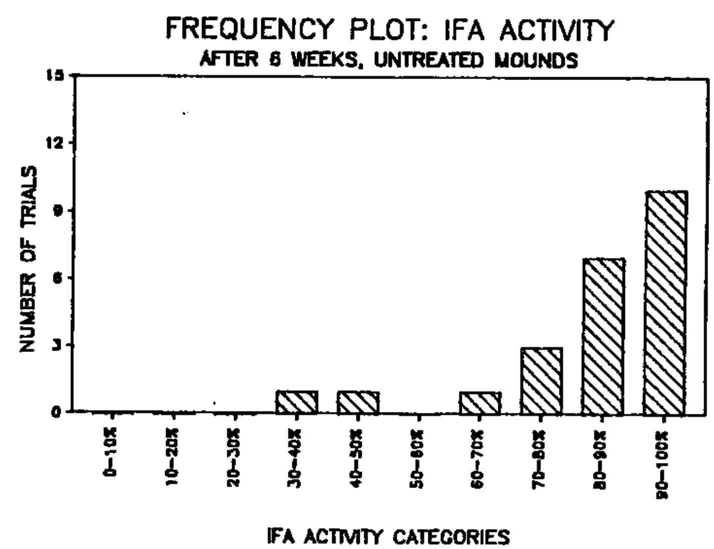
ASSESSMENT AFTER:	1 WEEK	3 WEEKS	6 WEEKS
ANOVA			
F-TEST:	29.183	28.890	32.082
PROBABILITY:	0.000	0.000	0.000
TREATMENT			
UNTREATED	0.88a*	0.85a	0.82a
BIOSAFE	0.63b	0.68b	0.54b
AMDRO	0.47c	0.45c	0.37c

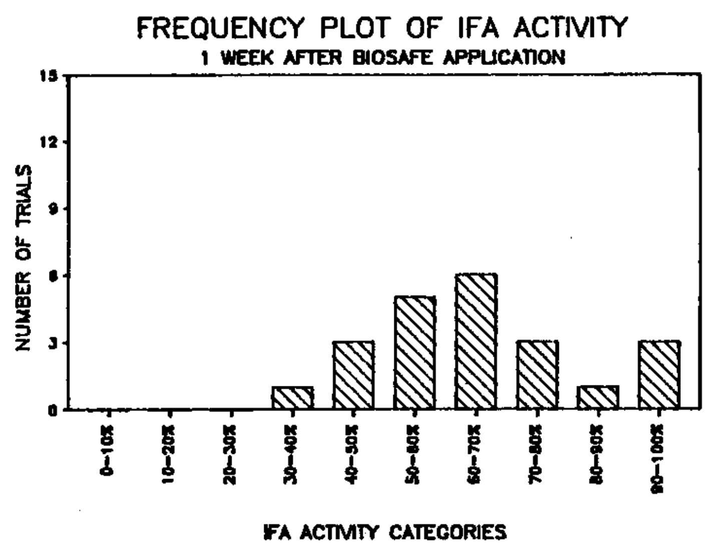
*Newman-Keuls mean separation test; means within columns followed by different letters are significantly different at $p = 0.05$.



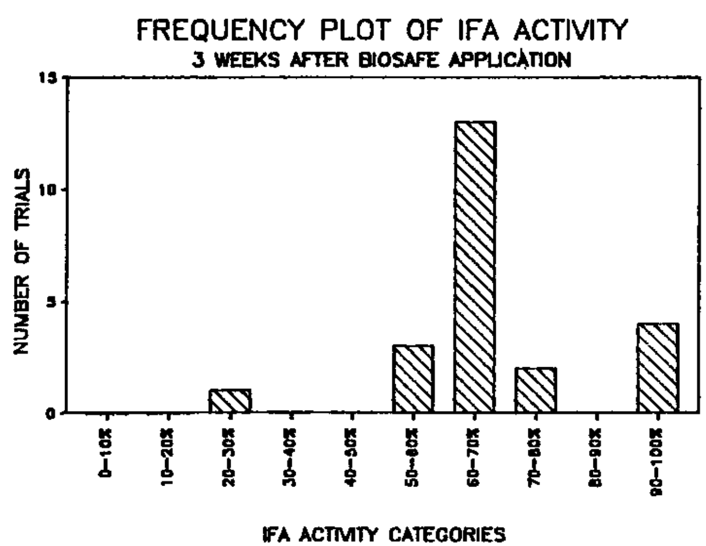
Figures 1-3. Summary plot of fire ant activity in check plots at all trials (n=22-23). Check plots were untreated, except for 1 trial which received 1 gallon water/mound.

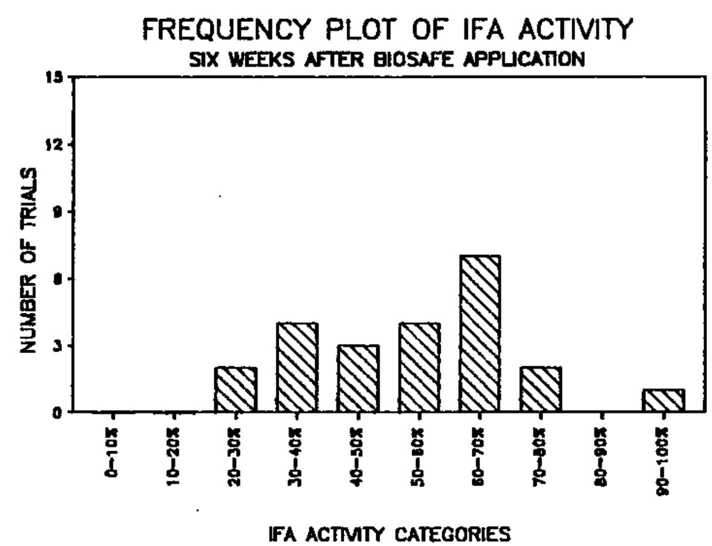


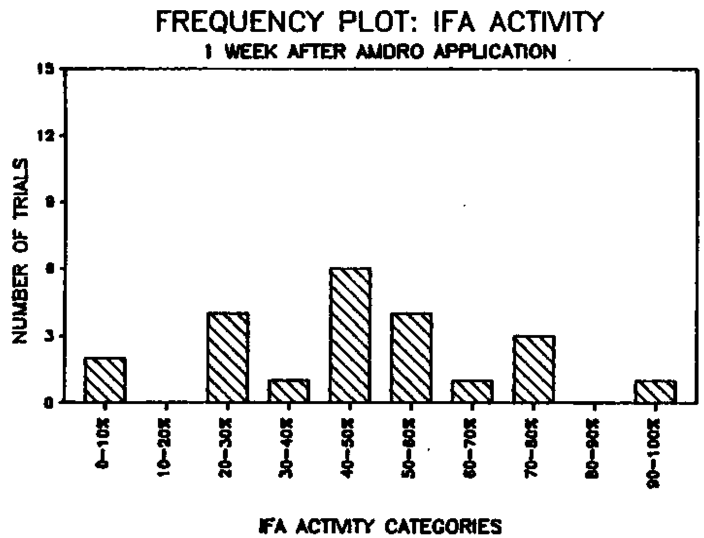




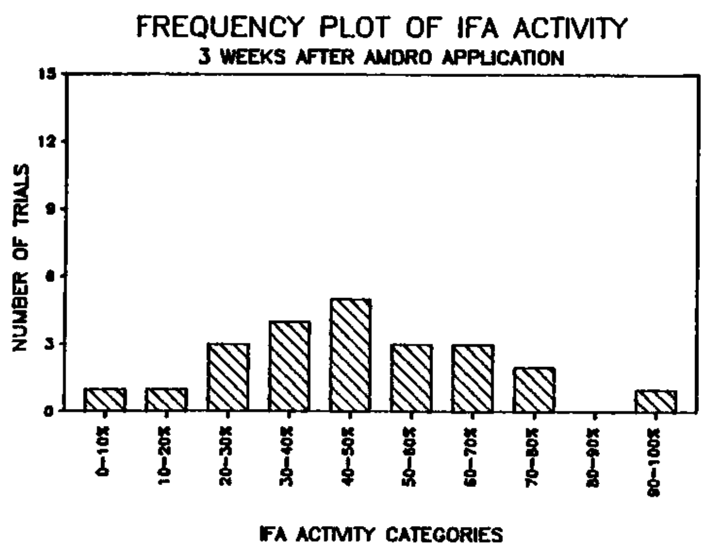
Figures 4-6. Summary plot of fire ant activity in BioSafe plots at all trials (n=22-23). Active mounds in BioSafe plots were treated at trial initiation and again after 3 weeks.

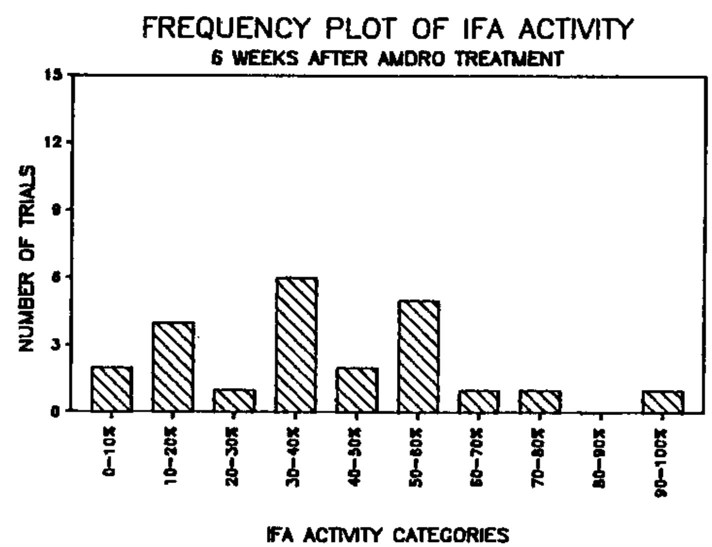


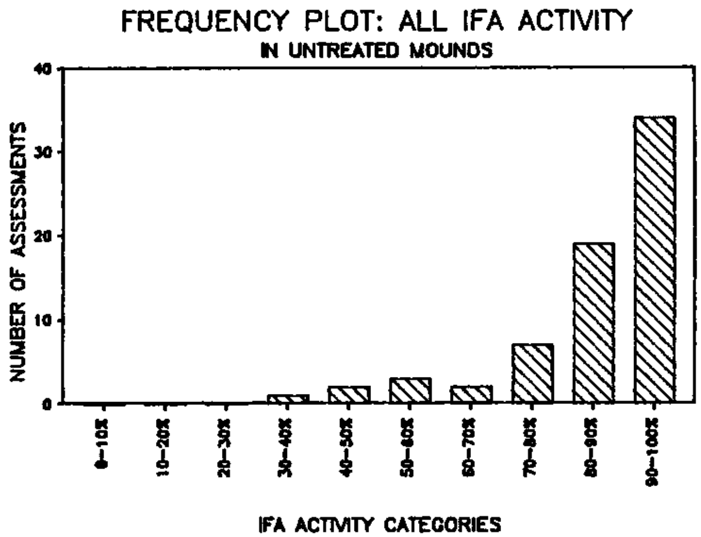




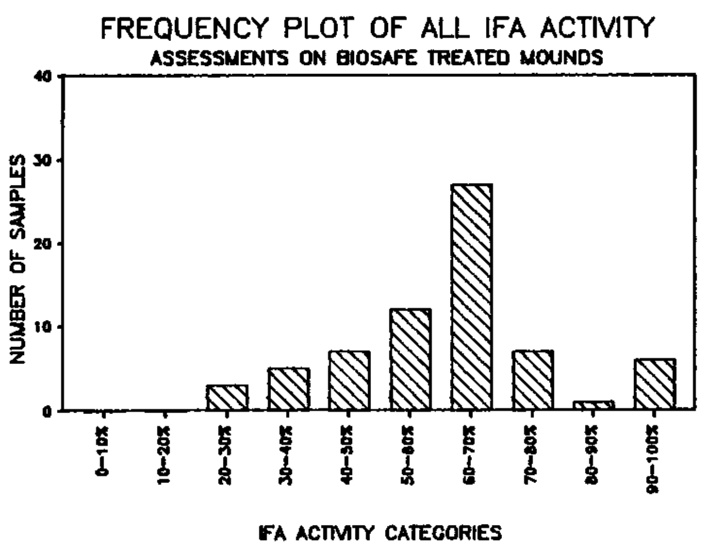
Figures 7-9. Summary plot of fire ant activity in Amdro plots at all trials (n=22-23). Active mounds in Amdro plots were treated at trial initiation only.







Figures 10-12. Summary plot of all fire ant activity for each treatment. Activity data from all three assessments (1, 3 and 6 weeks) have been combined for overview.



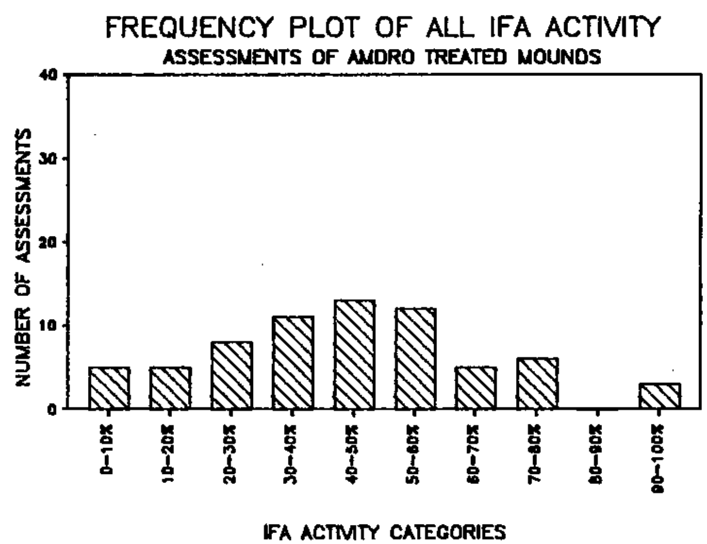
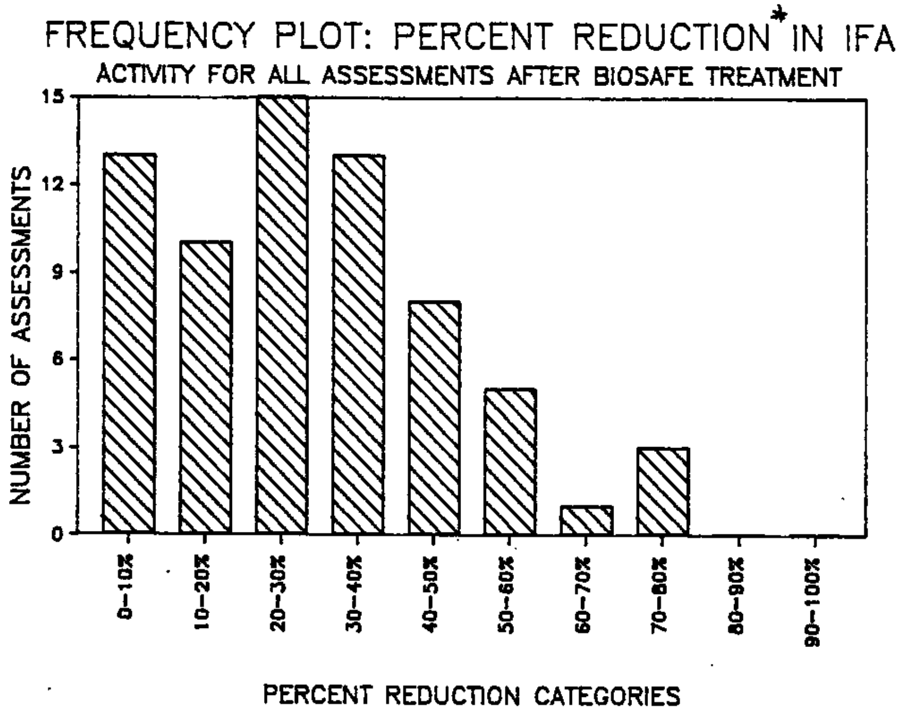
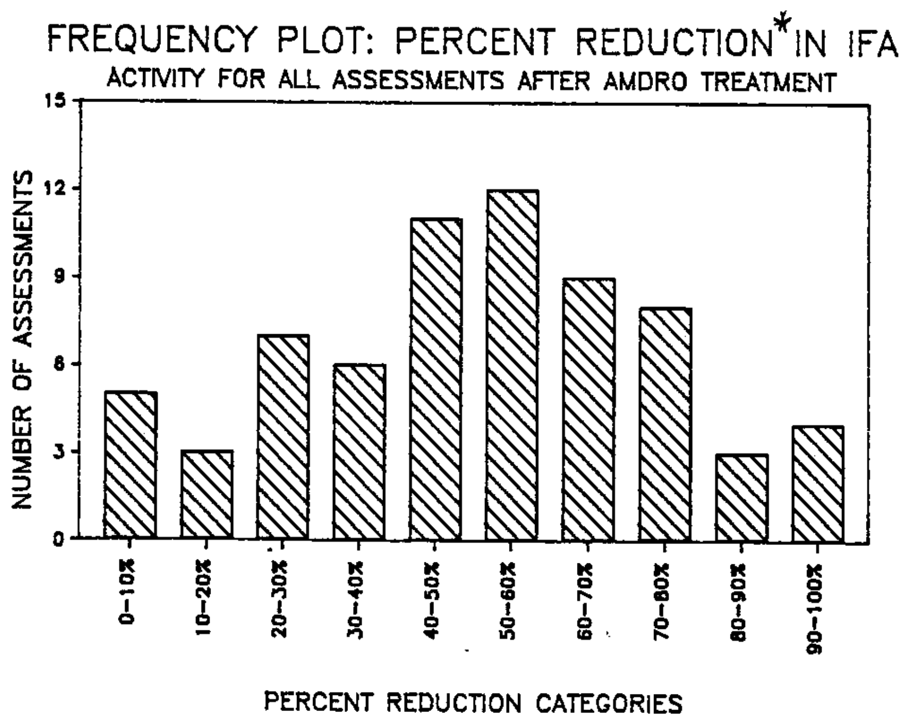


Figure 13.



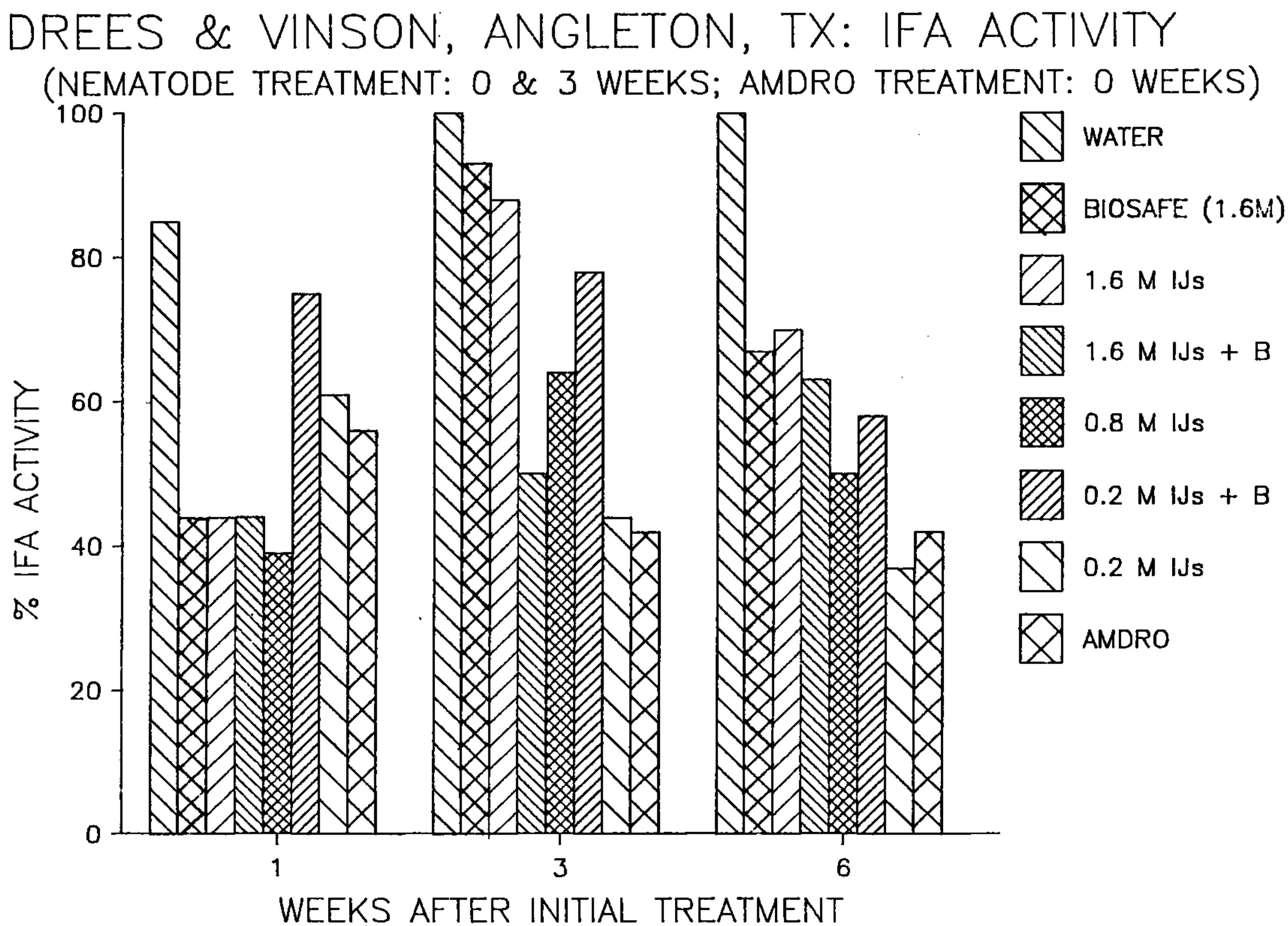
* CORRECTED FOR CONTROL REDUCTION WITH ABBOTT'S EQUATION

Figure 14.



* CORRECTED FOR CONTROL REDUCTION WITH ABBOTT'S EQUATION

Figure 15.



DREES & VINSON, ANGLETON, TX: IFA ACTIVITY
(NEMATODE TREATMENT: 0 & 3 WEEKS; AMDRO TREATMENT: 0 WEEKS)
% IFA ACTIVITY
0
20
40
60
80
100
1
3
6
WEEKS AFTER INITIAL TREATMENT
WATER
BIOSAFE (1.6M)
1.6 M IJs
1.6 M IJs + B
0.8 M IJs
0.2 M IJs + B
0.2 M IJs
AMDRO

Figure 16.

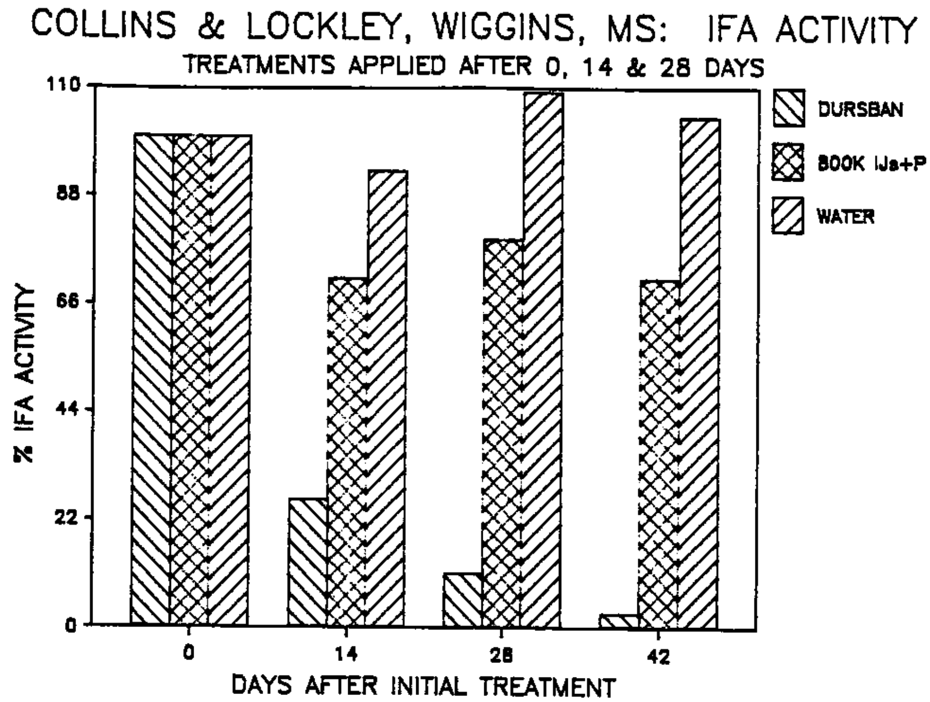
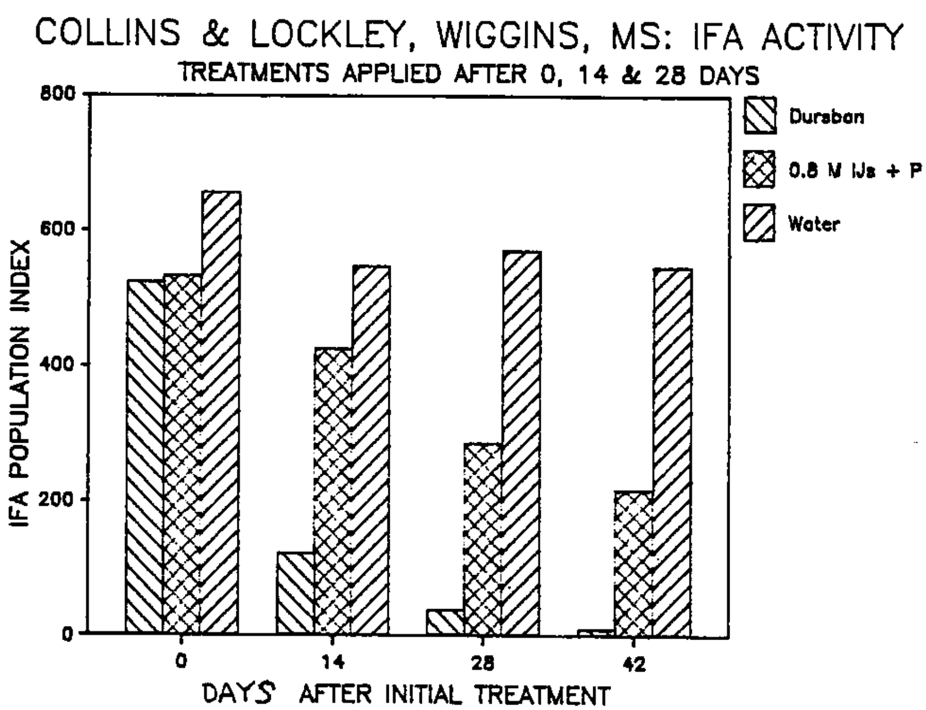


Figure 17.



DISCUSSION:

Demonstration program:

BioSafe, while not as efficacious as aminohydrazone overall, reduced fire ant activity significantly compared to untreated check plots. Worker ants often responded to nematode-drenched mounds by evacuating queen, brood and alates some distance away to a satellite location. Relocation of mounds occurred most often in sandy soils, where the energy costs of building a new mound may be lower. Trials showing low BioSafe efficacy all occurred in sandy soil situations. Nematode persistence in a number of these trials was probably also short (several weeks) because of high soil temperatures (30+ C at 6") and low soil moisture (drought conditions at some trial locations).

Research program:

Angleton, TX trial.

1. Lower rates of nematodes (0.2 or 0.8 million IJs/mound) performed as well as higher nematode rates.

2. Several lower nematode rates (0.2 or 0.8 million IJs/mound) performed comparably to aminohydrazone, although high variability between replicates prevented separation of treatment means at 3 or 6 weeks after application.

3. The BioSafe formulation (desiccated nematodes) provided fire ant reduction similar to equal rates of fresh nematodes, validating the desiccation concept.

4. Broadcast nematodes did not appear to affect fire ant activity; broadcast nematode concept should be re-examined in a separate trial.

Wiggins, MS trial.

1. Fire ant reduction in nematode treatment was comparable to other sandy soil trials (e.g., Florida, Georgia).

2. Addition of hygroscopic polymer did not appear to substantially improve effects of nematode treatment.

3. Consistent reduction in fire ant population indices in nematode treatment indicates that each nematode application reduced overall fire ant colony size relative to the check. Exact mechanism for this reduction is undetermined. Will brood mortality (vs. worker mortality) account for this type of population decline?

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Factors Affecting Sorghum and Corn Seed Feeding by th imported fire and (Hymenoptera: Formicidae) and the Impact of Seed Protectants

Bastiaan M. Drees[1/], Lori A. Berger[2/]
Robert Cavazos[2/], and S. Bradleigh Vinson[2/]

The red imported fire ant (RIFA), Solenopsis invicta Buren, has been reported to be an important pest of several agricultural crops (Lofgren, 1986). Damage to sorghum, Sorghum bicolor (L.), has remained undocumented. Damage to germinating seeds and seedlings of corn, Zea mays L., has been reported by Lyle and Fortune (1948), Wilson and Eads (1949) and Glancy et al. (1979). Lofgren (1986) listed damage to seeds and seedlings of soybean, corn, peanuts, watermelon and cabbage. Seed displacement and feeding by Solenopsis sp. has been reported by Horvitz (1981) and Vinson (1972).

In Texas, several unpublished reports of RIFA damage to sorghum have been made to the Texas Agricultural Extension Service in 1986 and 1987. In Wharton County, damage to untreated stands or stands treated with an at-plant in-furrow application of carbofuran (Furadan[R] 15G) applied for other pests such as the southern corn root worm were damaged sufficiently to warrant re-planting. In Bell County, no-till and minimum till research plots under the supervision of USDA agricultural engineer, John E. Morrison, Jr., has been reported to be extensively damaged by RIFA for several consecutive years (pers. comm.). Reports of RIFA damage to seed and seedling stages of sorghum are most frequently received during dry spring conditions.

Traditionally, the most common method of protecting corn and sorghum seeds has been the application of seed treatments (Drees and Vinson, 1988) of insecticides such as heptachlor, lindane and others. Malathion, applied to protect seed from stored product pests is not considered a seed treatment and has been observed not to protect sorghum seed planted in the field from RIFA feeding. More recently, Dow Chemicals U.S.A., has added RIFA suppression to the Lorsban[R] 15G (chlorpyrifos) label.

This research has been conducted to document characteristics of, and factors influencing RIFA feeding on corn and sorghum seed and the effects of seed protecting insecticides.

Collection and maintenance of RIFA colonies: RIFA colonies were collected using the standard bucket collection and drop method (Banks et al. 1981). Colonies were maintained in the laboratory and fed a standardized artificial RIFA diet and feeding schedule. Numbers of ants per colony were estimated based on the assumption that one small (9 cm. diameter) petri dish full of fire ants plus brood contains roughly 10,000 ants while one large petri dish can accommodate 20,000 ants (personal communication Sherri Ellison and Les Greenberg). Laboratory RIFA colonies varied in estimated size, containing from 10,000 to 50,000 ants per colony. Eighteen of the 32 colonies used in these studies were polygynous, containing more than one fertilized dealate female.

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General Description of RIFA Damage to Corn and Sorghum Seeds: Ants initially scraped the seed coat (pericarp) with their mandibles. Once the seed coat had been broken, the ants proceeded to scrape and remove the seed contents beginning with the the germ or embryo and progressing to the endosperm immediately surrounding it. In a few cases with sorghum, the entire seed was consumed. In corn, only the germ was consumed. Foraging ants would usually start moving the seeds toward the nest area upon introduction into the colony tray. Sorghum, most likely due to its smaller seed size, was moved at a greater frequency than corn. Additional observations were made that the contents of sprouted seeds were removed to a greater degree than were 24 hour water soaked (WS) seeds, and although roots and leaflets were rarely damaged, occasionally they were totally consumed by the ants. Some laboratory colonies did not respond to the seeds, seemingly ignoring their presence.

RIFA preference of germination stages of corn and sorghum seeds: Test 1: Six colonies were used either for corn and sorghum seeds, respectively. In each colony, 3 dry seeds (DS) and 3 WS seeds were exposed to colonies for 24 hours. After 2, 12, and 24 hours of exposure, the number of ants present around the seeds was recorded. **Test 2:** Ten WS sorghum seeds were and 10 DS in separate petri dishes were exposed to each of 4 RIFA colonies 48 hours, after which the number of damaged seeds was determined. **Test 3:** Sets of 10 WS sorghum seeds were exposed to 24 colonies for 24 hours. Those that fed on 100 percent of these seeds were presented DS 24 hrs. after exposure to WS seeds for an additional 24 hrs. Mean numbers of ants and damaged were subjected to a Student's t test.

RIFA workers are initially more attracted to WS seeds (**Table 1, Test 1**). Significantly more ants were in contact with WS seeds after 2 hours of exposure and for all observations for both corn and sorghum. No differences were documented during the 12 and 24 hour observations. Apparently, as WS seeds dried, their attractiveness to RIFA workers was lost relative to DS. In **Test 2,** RIFA damaged significantly more WS seeds (9.8 ± 0.5) than DS (3.8 ± 4.1)($t = 2.8963$; $P = 0.0137$). In **Test 3,** RIFA consuming WS seeds and later presented DS damaged significantly fewer DS (10.0 ± 0.0 WS seeds vs. 6.6 ± 3.5 DS; $N = 14$; $t = 3.6$; $P = 0.0006$).

Seed exposure/orientation: Petri dishes were filled with paraffin and WS seeds (sorghum) were partially imbedded in the wax, exposing certain parts of the seeds: 2 with the germ or embryo portion exposed, and 2 with germ or embryo down or unexposed per dish, placed in each of 4 colonies. Mean numbers of workers and damaged seeds imbedded with embryo exposed vs. unexposed were analyzed using the Student's t test. An additional test was conducted using 3 seeds imbedded as described above. This test was replicated 6 times and exposed to colonies 48 hours.

After 24 hours, significantly fewer seeds with unexposed embryos were damaged: 1.13 with embryo exposed vs. 0.25 with embryo unexposed in test with 2 seeds, and 2.33 vs. 0.00 in test with 3 seeds ($P \leq 0.05$). In some cases, ants had removed the pericarp from seeds with embryo unexposed.

Colony size, stress and seed feeding intensity: Laboratory RIFA colonies had been fed a standard diet on a consistent schedule regardless of colony size. Ant populations of each of 24 colonies were estimated. Colonies were then presented with 10 WS seeds for 24 hours. The number of damaged seeds was correlated with the estimated number of ants per colony. Colonies not or minimally damaging seeds were used for further study. Three of these were not offered water while the another 3 were not offered food (meal worms, artificial diet or honey water) for a 24 hours after the initial exposure period. An additional 3 colonies displaying some seed feeding activity were used as control colonies and were maintained on the normal schedule of watering and feeding. After 24 hours these 9 colonies were again offered fresh WS seeds. This process was repeated over successive three 24-hour periods. Following each exposure period, damaged seeds were tabulated and replaced with fresh WS seeds. Numbers of seeds damaged by each set of colonies were analyzed for each seed exposure period using the Least Significant Difference analysis of variance test (Ecosoft, Inc. 1981).

All but 10 of 24 RIFA colonies offered WS seeds for 24 hours damaged all sorghum seeds. A statistically significant correlation was documented between the estimated number of ants and the number of seeds consumed during the 24 hour exposure period ($r = 0.3691$, d.f. = 47; Corr. coef.(d.f. = 45, $P = 0.05$) = 0.288), supporting observations that seed damage intensity is related to colony size or vigor, as indicated by the estimated number of ants. Colonies placed on restricted (no food or no water) diets displayed a resumption of seed feeding activity relative to colonies continuing to receive the standard diet (**Table** 2). No conclusions could be made as to whether lack of food or water was the more significant stress factor. However, food or water stressed colonies increased seed feeding activity in both trials.

SEED PROTECTANTS:

Seed treatments: Seed treatments were obtained from Gustafson, Incorporated, Dallas, Texas (Terry Pitts, pers. comm.). Directions on the product labels are for ounces (1 oz. = 28 g.) of a.i./100 lbs. (45.4 kg.) of seed, mixing 75 gal. (283.88 l.) water per 1000 bu. and agitating for 3 minutes. For small lots, 0.23 kg. (0.5 lb.) seed was treated using the rates listed below:

Insecticide	Ml. formulation/0.23 kg. seed Corn	Sorghum
Lorsban 30F (chlorpyrifos)	0.424	0.424
Heptachlor 2E	0.392	0.583
Lindane 30F	0.511	0.368

The amount of formulation listed plus 2 ml. distilled water was added to the seed in a plastic bag, shaken for 3 min. and allowed to air dry prior to use.

Chlorpyrifos-treated filter paper: Approximately 0.01 g Lorsban 15% granules was applied to each standard (9 cm. diam.) petri dish to approximate the 8 oz. per 1000 ft. of row rate recommended on the product label for corn and sorghum. Where water was added, 1 ml. was used per dish.

T-band treatments with granular chlorpyrifos: A treatment rate was calculated to simulate 16 oz. of Lorsban 15G per 1000 feet of row applied as a T-Band soil treatment. The amount required to treat a 15 cm. (6 in.) furrow with an 18 cm. (7 in.) band applied over an open furrow, a method of application called a "T-band," 0.23 g. was weighed into vials. Test arenas consisted of square, 20 x 20 x 4 cm. (8 x 8 x 1 5/8 in.) aluminum trays filled with soil, having a surface area of 300 square cm. and a soil depth of 3 to 4 cm. Furrows were pressed into the soil and granules were evenly sprinkled on the soil surface.

Effect of seed treatments on foraging RIFA workers and seeds feeding: One petri dish containing treated 5 WS sorghum seeds was placed in each of 10 laboratory RIFA colonies. An additional 10 colonies received dishes containing untreated seeds. The number of ants present on the seeds was recorded at 30 minutes and 3 hours after placement. This test was conducted three times, using 1) heptachlor, 2) lindane and 3) chlorpyrifos treated seeds. Mean numbers of RIFA around each set of seeds was analyzed together for all three tests using the Least Significant Difference test. The Student's t tests was used to analyze means for each chemical treatment and corresponding untreated set.

Initially (1 hour after exposure), the number of ants around treated seeds were equal to or greater than those in association with untreated seeds (**Table** 3). Thus, the presence of a insecticide on the seed did not "repel" ants or decrease the intensity of RIFA activity. Heptachlor treated seeds were found to be associated with fewer foraging RIFA over time relative to untreated seeds after 24 hours. Lindane treated seeds showed the same trend. These results suggest that seed protection probably is due to reduced forager ant recruitment, rather than to "repellency" from treated seeds.

Seed damage and ant mortality from seed protectants: Each colony received one dish with 12 treated and one with 12 untreated sorghum seeds. Individual trials were conducted using WS seeds and DS, and included untreated vs. chlorpyrifos or heptachlor treated seeds. Ants were exposed to treatments for 17 to 24 hours. Following exposure to treatments the number of dead ants remaining in the dishes and damaged seeds were counted. The number of dead RIFA workers occurring in each dish was correlated to the number of damaged seeds per dish.

Seed treatment with either chlorpyrifos or heptachlor numerically resulted in fewer RIFA damaged seeds, although only chlorpyrifos treated WS seeds were damaged to a significantly lesser extent than corresponding untreated seeds: 3.8 untreated vs. 0.2 chlorpyrifos treated seeds ($t = 1.9918$; $df = 10$; $P = 0.0372$). The numbers of dead ants found in each sample during evaluation were consistently larger in dishes containing treated seeds, being significantly greater in two cases. A significant correlation between the estimated number of dead worker ants in dishes containing WS chlorpyrifos-treated sorghum seed ($r = 0.90863$; Corr. Coef (df 23) = 0.505; $P = 0.01$) occurred, with a linear regression equation of: $y = -68.63 + 173.92x$, where x is the number of damaged seeds and y is the number of dead ants. This equation illustrates that no damage occurs when foraging pressure (as indicated by few dead ants in the petri dishes) is low during the exposure period. Furthermore, when foraging pressure is great, the "protection" provided by seed treatments can be overcome. Between these two extremes, seed treatment offers "protection" from RIFA damage by killing sufficient numbers of worker ants to delay the onset of damage relative to untreated seeds.

Comparison of granular chlorpyrifos T-band and chlorpyrifos seed treatment for seed protection: RIFA colonies used in these tests were maintained in original soil in 5 gallon buckets in a manner similar to other laboratory RIFA colonies. Arenas, containing soil collected from the same site as soil in the colony buckets, were placed in additional 5 gallon buckets placed on opposite sides of the colony bucket. Inside surfaces of buckets were dusted with talcum powder to prevent ants from escaping. Ants from colony buckets gained access to the test arenas via 6 to 12 mm. diam., 1.25 m. long bridges connecting each colony to test arena buckets. Ten WS treated or untreated sorghum seeds were enclosed in 20 x 6 cm. aluminum screen bent into a "V" shape and inserted into the 15 cm. furrow of the each arena. Each colony was exposed to two test arenas simultaneously for approximately 24 hrs. Following exposure, the seed-containing aluminum screen was removed, and damaged seeds counted.

Seven tests, replicated 6 times each, were conducted using this procedure: 1) treated vs. untreated WS sorghum seed, in soil moistened by spraying the surface with 20 ml. of distilled water; 2) T-band treated soil vs. untreated seed, in soil moistened with 230 ml. water prior to constructing the furrow and applying treatments; 3) T-band treated soil vs. treated seed, in dry soil; 4) T-band treated soil vs. treated seed, wetting soil of arenas used in test 3 with 230 ml. water to "activate" or wash chlorpyrifos off the granules; 5) T-band treatment in soil saturated with 230 ml. water vs. T-band treatment on dry soil; 6) T-band applied 10 days prior to testing to either "wet" soil samples prepared by saturating the soil with 250 ml. water or "dry" soil and exposed to RIFA colonies after soil in the "wet" samples had dried; and 7) using the treatments of test 6, but adding 250 ml. of water to each arena. Colonies were randomly selected for treatments between tests and the locations of the arenas were changed to avoid foraging patterns established during previous tests. To document foraging activities between treatment arenas, the number of RIFA workers crossing a point on a bridge during 10 seconds 2 and 24 hours following initial exposure to arenas was recorded in tests 3, 4 and 5. Results of these tests were analyzed using the Student's t test.

Both formulations and treatment methods of chlorpyrifos resulted in significantly less damage to WS sorghum seeds than did untreated seeds (tests 1 and 2, **Table 4**). In dry soil, significantly fewer chlorpyrifos-treated seeds were damaged than were untreated seeds in the T-band treatment (test 3). However, in wet soil, the T-band provided as much protection from seed damage as did seed treatments. The poor performance of the granular chlorpyrifos treatment in dry conditions is illustrated again by the significant differences in test 5. Dry soil conditions reduced the effectiveness of the T-band treatment, even when soil was wet at the time of treatment. Efficacy was lost if soil was allowed to dry before exposure to RIFA, but regained its effectiveness if treated soil was re-moistened. In dry treated soil, chlorpyrifos apparently becomes unavailable and/or inactive (due in part to molecules binding tightly to organic material in the soil). Test 6 and 7 results indicate contrasting levels of seed protection and no statistical difference between the T-band applied either to dry or wet soil and exposed to ants after having dried or having been re-moistened. T-band applications were more effective when soil moisture was available when RIFA foraging activity occurred.

The number of RIFA workers traveling to and from the T-band treated arenas decreased significantly over time relative to ants foraging in arenas containing treated seed (**Table 5**, tests 3 and 4, 24 hour evaluations), in both the soil is wet or dry soil. Significantly fewer ants were found to be traveling across the bridge to the wet vs. dry T-band treated arenas in test 5. Results of tests 6 and 7 support findings in previous tests, and the observation that chlorpyrifos T-band treatments applied during wet or dry conditions but dry during RIFA exposure period were less effective in reducing foraging RIFA than when conditions were wet during exposure.

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Table 1. Number of foraging red imported fire ant workers on and around dry and 24 hour water-soaked corn and sorghum seeds after 2, 12 and 24 hours of exposure.

Colony number and Exposure time	Number of foraging worker ants: Corn		Sorghum	
	Wet seeds	Dry seeds	Wet seeds	Dry seeds
2 hours	23.5*	5.7*	20.0*	4.6*
12 hours	14.7	10.2	11.0	6.6
24 hours	13.0	7.9	6.8	6.8
Overall mean (2, 12 and 24 hrs.)	17.1*	7.9*	11.0*	6.0*

* Means are statistically different according to the Student's t test at $P<0.05$ (Corn, 2 hrs.: N = 6, df = 10, t = 2.2928, P = 0.0224; Sorghum, 2 hrs.: N = 5, df = 8, t = 2.6175, P = 0.0154; Corn, overall means: N = 18, df = 34, t = 2.4244, P = 0.0104; Sorghum, overall means: N = 15, df = 28, t = 2.2318, P = 0.0169).

Table 2. Number of 24 hour water-soaked sorghum seed damaged by foraging red imported fire ant workers during successive 24 hour exposure periods beginning 24 hours after initiating modified diet regimes to laboratory colonies.

	No. damaged seeds/10[1/]			
Diet type	Pre-diet	24 to 48 hrs[2/]	48 to 72 hrs	72 to 96 hrs
No water	0.0b	8.67a	6.33a	8.67a
No food	0.0b	3.33a	3.00a	3.33a
Normal	6.67a	7.67a	9.00a	7.00a
LSD (5%)	1.999	9.996	12.300	10.306

1/ Means followed by different letters are statistically different according to the Least Significant Difference analysis of variance test (N = 3; $P \leq 0.05$) (Ecosoft, Inc. 1981).

2/ Hours after initiation of diet.

Table 3. Number of foraging red imported fire ant workers in association with treated and untreated water-soaked corn seeds after 1 and 3 hours of exposure.

	Mean ± S.D. foraging worker ants associated with seeds[1/]		
Treatment	1 hr exposure	3 hrs exposure	24 hrs exposure
heptachlor	42.9a*	13.2ab	1.3*
untreated	23.4b*	20.2a	17.1*
lindane	21.5b	12.2ab	1.3
untreated	9.4b	8.0ab	3.7
chlorpyrifos	18.7b	9.3b	---
untreated	14.9b	13.2ab	---
L.S.D. (5%)	18.920	12.816	

1/ Means followed by different letters are statistically different using the Least Significant Difference analysis of variance test (P = 0.05).

* Indicates significant difference using the Student's t test ($P \leq 0.05$)(heptachlor vs untreated, 1 hr: t = 1.9681, df = 18, P = 0.0323; heptachlor vs untreated, 24 hrs: t = -2.6265, P = 0.0086).

Table 4. Number of chlorpyrifos-treated or untreated water-soaked sorghum seeds in 15 cm (6 inch) untreated or chlorpyrifos T-band treated furrows damaged by foraging red imported fire ant workers from the initial 10 seeds exposed to the ant colonies.

Test and	--------------No. damaged	sorghum seeds/10	--------------	
exposure (hrs.)	Untreated	Treated seed	T-band (wet)	T-band (dry)
1. Moist soil, 22	10.0*	1.7*	-	-
2. Wet soil, 24	10.0*	-	0.0*	-
3. Dry soil, 22.5	-	3.2*	-	7.2*
4. Wet soil, 24	-	0.2	0	-
5. Wet and dry, 24	-	-	0.3*	9.2*
			Condition at application	
6. Dry (10 days after application) 24	-	-	9.7	7.8
7. Wet (12 days after application) 24	-	-	0.0	0.0

* Significantly different means according to the Student's t test (A. t = -12.5, N = 6, df = 10, P = 0.0000; B. t = 9999.9, P = 0.0000; C. t = 2.3952, P = 0.0188; E. t = -19.4832, P = 0.0000).

Table 5. Number of foraging red imported fire ants crossing a point on a bridge leading to each of the test arenas per 10 seconds.

Test and	--------------No. foraging	foraging workers	-------------	
exposure (hrs.)	Untreated	Treated seed	T-band (wet)	T-band (dry)
3. Dry soil, 2	-	1.7*	-	6.7*
22.5	-	1.8*	-	0.2*
4. Wet soil, 2	-	5.3*	0.7*	-
24	-	3.7*	0.2*	-
5. Wet and dry, 2	-	-	3.3*	17.2*
24	-	-	0*	4.7*
			Conditions at application	
6. Dry (10 days after application) 2	-	-	20.0*	11.0*
24	-	-	2.3	2.6
7. Wet (12 days after application) 2	-	-	1.3	1.8
24	-	-	0.3	1.0

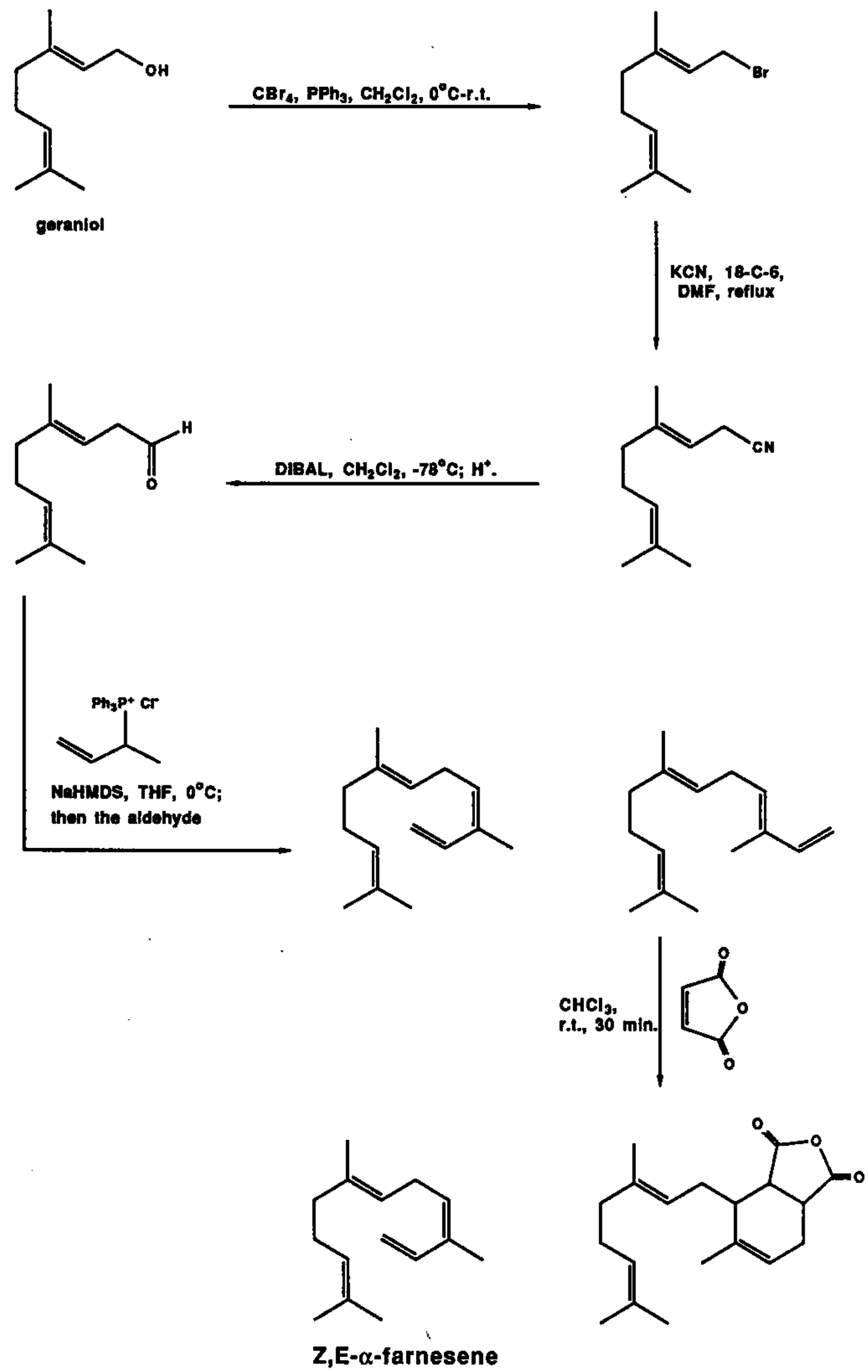
* Significantly different according to the Student's t test (C, 2 hrs. t = 2.2021, N = 6; df = 10, P = 0.0261; C, 22.5 hrs. t = -2.4693, P = 0.0166; D, 2 hrs. t = -2.2191, P = 0.0254; D, 24 hrs. t = -2.6047, P = 0.0131; E, 2 hrs. t = -4.4027, P = 0.0007; E, 24 hrs. t = -4.7194, P = 0.0004; F, 2 hrs. t = 1.9829, P = 0.0378;).

"Synthesis of the Major Component of the Trail Pheromone of the Red Imported Fire Ant"

P. E. Hernandez, USDA-ARS-IAMARL

The total synthesis of Z,E-α-farnesene (Z,E-3,4,7,11-tetramethyl-1,3,6,10-dodecatetraene) is described. The methodology employed is applicable to the synthesis of other farnesenes and homofarnesenes in the pheromone system of the IFA, solenopsis invicta.

The linear synthesis starts (see following scheme) with commercially available geraniol which is converted to the allylic bromide in >90% yield. One carbon homologation to the nitrile, followed by reduction to the aldehyde was accomplished in >60% yield for the two steps. Condensation of the aldehyde with the phosphorane ylid derived from 3-chloro-1-butene provided a mixture of Z,E-and E,E-farnesenes (50% yield). Isolation of the Z,E-α-farnesene was achieved by treatment of the crude mixture with maleic anhydride which allows the more polar Diels-Alder adduct of E,E-farnesene to be removed by flash column chromatography, to provide the desired Z,E-α-farnesene.


OH
CBr4, PPh3, CH2Cl2, 0°C-r.t.
Br
geraniol
KCN, 18-C-6,
DMF, reflux
H
O
DIBAL, CH2Cl2, -78°C; H+.
CN
Ph3P+ Cl-
NaHMDS, THF, 0°C;
then the aldehyde
CHCl3,
r.t., 30 min.
O
O
O
Z,E-α-farnesene

FIRE ANT CHEMISTRY: 1988

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SOUTH AMERICA

IN THE PAST YEAR ADDITIONAL CHEMICAL ANALYSES WERE CONDUCTED ON ANT SAMPLES COLLECTED FROM ARGENTINA, IN PARTICULAR THE AREA BETWEEN THE PREVIOUSLY KNOWN *S. INVICTA* AND *S. RICHTERI* POPULATIONS. THESE RESULTS SHOW THE S. RICHTERI CHROMATOTYPE EXTENDING ALONG THE EDGE OF AN EXTENSIVE MARSHLAND TO WITHIN 170 KM OF THE NEAREST DETECTED *S. INVICTA* POPULATION. THESE DATA SUPPORT THE HYPOTHESIS THAT IN SOUTH AMERICA THERE IS A GEOGRAPHIC BARRIER BETWEEN THE TWO SPECIES. THE GREAT EXPERIMENT OF CREATING SYMPATRIC POPULATIONS OF THE TWO FORMS HAS ALREADY BEEN DONE IN THE USA, RESULTING IN THE PRODUCTION OF VIABLE HYBRIDS. YOU DECIDE WHETHER OR NOT GEOGRAPHIC PRE-MATING ISOLATION IS ADEQUATE TO MAINTAIN THEM AS SEPARATE SPECIES.

THE PROBLEM STILL EXISTS OF THE TWO MORPHOLOGICALLY IDENTICAL POPULATIONS NAMED *S. RICHTERI*, THAT HAVE DISTINCT CHROMATOTYPES. THE USA *S. RICHTERI* CHROMATOTYPE DOES NOT OCCUR AT THE TYPE LOCATION OF BUENOS AIRES. THE IMPLICATIONS ARE 1) THE USA *S. RICHTERI* HAS A MUCH MORE LIMITED RANGE THEN PREVIOUSLY THOUGHT; 2) THE USA *S. RICHTERI* SHOULD BE RE-NAMED, SINCE IT DOES NOT CORRESPOND TO THE TYPE LOCATION OF THE SPECIES NAMED *S. RICHTERI*.

APPLICATION OF PHEROMONE ATTRACTANTS TO CONTROL SITUATIONS

BASIC PREMISE: IF *S. INVICTA* CAN BE INDUCED THROUGH THE USE OF SPECIES-SPECIFIC ATTRACTANT PHEROMONES TO GET TO A BAIT TOXICANT BEFORE OTHER ANT SPECIES, THEN THE BAIT WILL BE MORE EFFECTIVE AND AT THE SAME TIME GREATER SPECIES -SPECIFICITY WILL BE ACHIEVED.

WHAT CONTROL SITUATIONS WOULD BENEFIT FROM THE SUCCESSFUL UTILIZATION OF PHEROMONES IN FIRE ANT CONTROL?

HOMEOWNER, FARMER, NURSERY GROWER, ETC. ALL WOULD BENEFIT FOR THE FOLLOWING REASONS:

GREATER SPECIFICITY MEANS A GREATER DIVERSITY OF OTHER ANT SPECIES, WHICH IN TURN MAY, THROUGH COMPETITION, SLOW THE REINFESTATION RATE OF THE FIRE ANT.

GREATER SPECIFICITY MEANS THAT LESS ACTIVE INGREDIENT (USUALLY THE MOST EXPENSIVE BAIT COMPONENT) WOULD BE REQUIRED FOR CONTROL; THEREFORE, LOWER COSTS WOULD RESULT (PROVIDED THE PHEROMONES ARE INEXPENSIVE).

GREATER SPECIES-SPECIFICITY AND LESS ACTIVE INGREDIENT WOULD BENEFIT THE ENVIRONMENT IN GENERAL, PROMOTING SPECIES DIVERSITY AND PLACING A SMALLER INSECTICIDE LOAD ON THE ECOSPHERE.

WHAT ARE THE BAIT COMPONENTS?

A) A SPECIES -SPECIFIC ATTRACTANT

B) THE ACTIVE INGREDIENT SOLVENT (VEGETABLE OIL?)

C) CARRIER

D) ACTIVE INGREDIENT

ALL FOUR ELEMENTS HAVE TO BE EVALUATED, OPTIMIZED AND COMBINED TO PRODUCED THE MOST EFFECTIVE BAIT SYSTEM. IN THIS PAPER WE WILL ONLY DEAL WITH ELEMENT A.

SPECIES -SPECIFIC ATTRACTANTS

RECRUITMENT PHEROMONE PROBLEMS TO ADDRESS

AT PHYSIOLOGICAL LEVELS TWO COMPONENTS FROM THE DUFOUR'S GLAND ARE NECESSARY FOR ATTRACTION, Z,E-ALPHA-FARNESENE AND A TRICYCLIC HOMOSESQUITERPENE MONOENE. HOWEVER,

WHAT IS THE CONCENTRATION RANGE FOR ATTRACTANT ACTIVITY OF THE RECRUITMENT PHEROMONE?

IF THE RANGE OF ACTIVITY IS NARROW, THEN THE RECRUITMENT PHEROMONE IS NOT A GOOD CANDIDATE, BECAUSE DEVISING A NARROW TOLERANCE PHEROMONE RELEASE SYSTEM IS DIFFICULT.

AT HIGH CONCENTRATIONS WOULD ONE RECRUITMENT ATTRACTANT COMPONENT DO THE JOB?

ALTHOUGH THIS IS NOT THE NATURAL SITUATION, IT MAY PROVIDE A PRACTICAL WAY OF UTILIZING THIS PHEROMONE SYSTEM. SYNTHESIS AND FORMULATION OF A SINGLE COMPONENT WILL ALWAYS BE EASIER AND LESS EXPENSIVE THAN SYNTHESIS AND FORMULATION OF A TWO COMPONENT SYSTEM! EVIDENCE FROM SPECIES-SPECIFICITY BIOASSAYS WITH *S. INVICTA* AND *S. RICHTERI* INDICATES THAT GREATER THAN PHYSIOLOGICAL LEVELS OF THE TRICYCLIC HOMOSESQUITERPENE YIELDS GOOD ATTRACTION.

IS THE ATTRACTIVE PART OF THE RECRUITMENT PHEROMONE SPECIES-SPECIFIC?

IT IS IMPROBABLE THAT OTHER ANT SPECIES WOULD BE ATTRACTED TO *S. INVICTA* PHEROMONES. BUT PROOF IS IN THE EXPERIMENT!

QUEEN PHEROMONE PROBLEMS TO BE ADDRESSED

THERE HAVE BEEN TWO COMPOUNDS ISOLATED FROM WHOLE QUEEN EXTRACTS THAT GIVE GOOD ACTIVITY IN A DISRUPTED COLONY BIOASSAY; A THIRD COMPOUND WAS REPORTED BUT DOES NOT APPEAR TO CONTRIBUTE TO THE ACTIVITY. THE NATURAL SOURCE OF THE PHEROMONE IS THE POISON SAC.
CONCENTRATION ACTIVITY CURVES FOR SYNTHETIC PHEROMONES AND POISON SAC EXTRACTS ARE DIFFERENT, WHICH SUGGESTS THAT OTHER ACTIVE COMPONENTS MAY BE WAITING TO BE DISCOVERED IN THE POISON SAC OF THE QUEEN.

WHAT IS THE ACTIVE CONCENTRATION RANGE FOR THE SYNTHETIC QUEEN PHEROMONES AGAINST FORAGING WORKERS?

IF THE RANGE OF ACTIVITY IS NARROW, THEN THE QUEEN PHEROMONE IS NOT A GOOD CANDIDATE, BECAUSE DEVISING A NARROW TOLERANCE PHEROMONE RELEASE SYSTEM IS DIFFICULT

HOW DOES THE CONCENTRATION ACTIVITY RANGE OF SYNTHETIC COMPONENTS COMPARE TO POISON SAC EXTRACTS (AGAINST FORAGERS)?

BIOASSAY EVIDENCE WITH BROODTENDING WORKERS INDICATES THAT THERE ARE DIFFERENCES, WHICH IMPLIES THAT OTHER ACTIVE COMPONENTS MAY EXIST IN THE QUEEN POISON SAC.

ARE ALL THREE KNOWN COMPONENTS NECESSARY FOR ATTRACTION?

WHAT ARE THE EFFECTS OF A HIGH CONCENTRATION OF INDIVIDUAL COMPONENTS ON THEIR ACTIVITY?

PRESUMABLY AT PHYSIOLOGICAL LEVELS THE INDIVIDUAL COMPONENTS WILL NOT BE ACTIVE. HOWEVER, AT HIGH DOSAGES THEY MAY HAVE ACTIVITY NOT PREVIOUSLY ELICITED.

ARE THERE ADDITIONAL ACTIVITY ENHANCING COMPONENTS IN THE QUEEN'S POISON SAC?

PREVIOUS EXPERIMENTAL EVIDENCE INDICATES THAT THERE MAY BE ADDITIONAL QUEEN PHEROMONE COMPONENTS IN THE POISON SAC THAT ENHANCE THE ACTIVITY OF THE KNOWN PHEROMONES.

IS THE QUEEN PHEROMONE REALLY SPECIES-SPECIFIC?

IT IS IMPROBABLE THAT OTHER ANT SPECIES WOULD BE ATTRACTED TO *S. INVICTA* QUEEN PHEROMONES. BUT PROOF IS IN THE EXPERIMENT!

APPLICATION OF PHEROMONE ATTRACTANTS TO TRAP MONITORING

BASIC PREMISE: IF *S. INVICTA* CAN BE SPECIFICALLY ATTRACTED INTO A TRAP, THEN THAT TRAP HAS A HIGHER PROBABILITY OF DETECTING THE PRESENCE OF FIRE ANTS, AND THERE IS LESS LIKELIHOOD OF OTHER ANT SPECIES CREATING FALSE POSITIVES.

WHAT CONTROL SITUATIONS WOULD BENEFIT FROM THE SUCCESSFUL UTILIZATION OF

PHEROMONES IN FIRE ANT TRAP DETECTION?

QUARANTINE SITUATIONS WHERE THE PRESENCE OF FIRE ANTS COULD RESULT IN PESTICIDE TREATMENT, RETURN OF SHIPPED GOODS, AND/OR DESTRUCTION OF SHIPPED GOODS. FARMER, NURSERY GROWERS, SOD FARMERS, ALL SHIPPERS OF GOODS FROM FIRE ANT INFESTED AREAS. POPULATION MONITORING COULD PROVIDE REASONABLE GUIDELINES FOR THE PESTICIDE TREATMENT OF DISCOVERED POPULATIONS AND RESULT IN LOWER COSTS AND LESS INSECTICIDE IN THE ENVIRONMENT. EARLY DETECTION OF THE FIRE ANT BY USE OF AN EFFICIENT TRAP WOULD AID IN DETECTING INCIPIENT FIRE ANT COLONIES AND THEIR SUBSEQUENT ERADICATION.

WHAT ARE THE ELEMENTS OF A TRAP?

A) SPECIES-SPECIFIC ATTRACTANT

B) A HOLDING OR TRAPPING MECHANISM

C) UTILITARIAN TRAP DESIGN

SPECIES-SPECIFIC ATTRACTANT

THE SAME OBJECTIVES OUTLINED FOR THE USE OF SPECIES-SPECIFIC ATTRACTANTS FOR FIRE ANT CONTROL (BAITS) APPLY FOR FIRE ANT TRAP MONITORING!! THEREFORE, THE EXPERIMENTS DESIGNED TO ANSWER QUESTIONS REGARDING FIRE ANT PHEROMONE SYSTEMS AND TOXIC BAITS SERVE A DUEL PURPOSE. THESE EXPERIMENTS WILL NOT BE OUTLINED AGAIN HERE.

WITH A LITTLE LUCK AND TECHNICAL HELP WE SHOULD HAVE ANSWERS TO THE ABOVE QUESTIONS AROUND THIS TIME NEXT YEAR AND BE WELL ON OUR WAY TOWARD IMPROVING FIRE ANT BAITS AND DEVELOPING MONITORING TRAPS.

IMPACT OF IFA ON PECANS IN GEORGIA

W. L. Tedders, C. C. Reilly, B. W. Wood,

R. K. Morrison, and C. S. Lofgren

ABSTRACT

Pecan, Carya illinoensis (Wangenh K. Koch), orchards provide an important habitat for the red imported fire ant (IFA), Solenopsis invicta Buren, and orchards from middle to south Georgia were heavily infested. Honeydew produced by blackmargined aphids, Monellia caryella (Fitch), on pecans was an important source of carbohydrate food for IFA. IFA were found foraging at 9 m heights in trees. Mowing of orchard ground cover caused a change in the ratio of IFA on the ground to IFA in trees but was not correlated with outbreaks of M. caryella in trees. IFA was a major predator of eggs, larvae, and pupae of a green lacewing, Chrysoperla rufilabris (Burmeister), and of the pupae of Allograpta obliqua (Say) in pecans, but had little effect on the eggs of Hippodamia convergens Guerin-Meneville. A close mutualistic relationship between IFA and the mealybug Dysmicoccus morrisoni (Hollinger) was discovered. IFA promoted the development of colonies D. morrisoni on callus tissue located on the mainstem of trees by covering the colony with soil and debris, by defending the colony, and collecting honeydew.

INTRODUCTION

Reports of associations between IFA and tree inhabiting homopteran are rare. However, Wilkinson & Chellman (1979) reported IFA attending an outbreak of the pine tortoise scale, Toumeyella parvicornis (Cockerell), on 3-year-old slash pine Pinus elliottii Engelm. Mutualism between IFA and homopterans found on pecan, Carya illinoensis (Wangenh K. Koch), trees has not been reported.

For this study, we utilize the term "mutualism" as defined by Way (1963); "an association between ants and other insects without necessarily implying obligate dependence or interdependence."

IFA is best known as an opportunistic predator which simplifies the ecosystem by displacing other ant species of the same trophic level (Whitcomb et al. 1972). On cotton, it is reported to be a beneficial predator of larval boll weevil, Anthonomus grandis Boheman, (Sterling 1978) and the eggs and larvae of Heliothis virescens (F.) (McDaniel & Sterling 1979). IFA is the most important predator of sugarcane borer, Diatreae saccharalis (F.) in Louisiana (Charpentier et al. 1967). Dutcher & Sheppard (1981) reported that IFA caused a 38% reduction of pecan weevil, Curculio caryae (Horn) larvae in a caged field

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[1]Agricultural Research Service, USDA.

test in Georgia. Also, IFA is suspected of preying on certain homopterans including the pea aphid, Acyrthosiphum pisum (Harris), on alfalfa (Morrill 1978). Hays & Hays (1959) concluded that IFA though omnivorous, feeds mainly for living and dead insects. However, the full range of IFA behaviors and their consequences in varied agricultural environments remain to be explored (Lofgren 1986).

In commercial orchards of pecan, IFA mounds often interfere with orchard management, especially by damaging mowers. Growers regularly complain that IFA sting laborers hand-weeding around newly planted trees and workers grafting or topworking trees at heights up to 4.5 m. Several growers have suggested that outbreaks of aphids on pecans follow mowing operations. We suspected that IFA interfered with biological control experiments employing convergent lady beetle, Hippodamia convergens Guerin-Meneville, and a green lacewing, Chrysoperla rufilabris (Burmeister), against foliar-feeding pecan aphids (W. L. Tedders, pers. obs.). Large numbers of ants were usually present when outbreaks of blackmargined aphids, Monellia caryella (Fitch) occurred on pecan trees. Two additional honeydew producing species, yellow pecan aphid, Monelliopsis pecanis Bissell, and black pecan aphid, Melanocallis caryaefoliae (Davis), occur on pecan. Monellia caryella is the greatest honeydew producer, excreting up to 92 mg per aphid (Tedders 1978) and 9 and 13 times more glucose-equivalents per aphid than M. pecanis and M. caryaefoliae, respectively (Tedders & Wood 1987). Monellia caryella honeydew produced on pecan contains ca. 24% glucose (Tedders et al. 1982).

It is not clear what net effect IFA has on the population dynamics of the aphids on pecan so we decided to investigate. The objectives of the study were to determine: 1) the extent of IFA infestations of commercial and non-commercial pecan orchards in Georgia; 2) the height that IFA forage in pecan trees; 3) the effect of mowing on the activity of IFA in orchards; 4) possible mutualism between IFA and M. caryella, and M. pecanis; 5) possible IFA predation on M. caryella, C. rufilabris, H. convergens, and the aphidophagous syrphid, Allograpta obliqua (Say).

OBSERVATIONS

Casual Observations. From 1986 to 1988 commercial pecan orchards from middle- to south-Georgia were observed for the presence and location of IFA mounds.

Orchards in which no deliberate attempts were made to control ants were heavily infested with IFA. This species was the dominant ant present. Almost all IFA mounds were located on the southern side and at the base of trees. Mature, closely mowed orchards usually averaged about 25 mounds per ha or one mound at the base of each tree. It was evident that routine application of insecticides for various pecan pests did not eliminate IFA from pecan orchards and apparently had little effect on the numbers of colonies present.

Systematic Observations, Mature Trees. During 1986 and 1987, we studied IFA activities each month in commercial orchards of 65- to 80-year -old, sprinkler-irrigated 'Schley' and 'Stuart' trees at Albany GA., and at the Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA., and in non-irrigated, non-commercial orchards of 65-year-old 'Schley' and 'Moore' trees at Byron. The presence of worker IFA in trees were determined using 7.62 x 12.7 cm white index cards soaked in peanut oil (Dutcher & Sheppard 1981). These bait cards were fastened to the mainstem or limbs of trees with thumb tacks. Actual numbers of IFA on cards were recorded. Because of the large overlapping scales of bark commonly found on 'Schley' and 'Moore', as well as most other pecan cultivars, trails of IFA on 65- to 80-year old trees were difficult to locate irrespective of the time of the year. However, removal of scales of bark revealed IFA trails during all months of the year. These trails were easily traced from mounds on the ground up into the tree crotch where part of the colony was usually nesting. Completely hidden trails often extended several meters upwards along large scaly limbs of the trees.

Peanut-oil-soaked cards were found to be a poor method to sample IFA activity on the lower half of mature trees because of the concealed nature of trails under bark. Cards were more effective on mature trees when displayed above 6 m where large overlapping scales of bark were less prominent and trails were exposed.

In September 1986, 80-year-old trees in Albany were observed with IFA nesting among the immature nut clusters at the ends of limbs. These nests each contained several hundred workers with eggs, larvae, and pupae but no queens were observed. The ants used soil, debris, and dead insects to partially cover the nuts and nest. These nests were extensions of a large colony at the base of the tree and were located about 12 m from the mound. Ten to 20 such nests on nut clusters were seen on several trees. This phenomenon occurred during a severe drought situation.

Systematic Observations, Young Trees. During 1987 and 1988, we studied IFA activities in 6-year-old drip-irrigated 'Cheyenne' and 'Desirable' trees at Albany (commercial) and at Byron (non-commercial).

Again, the presence of worker IFA in trees or on the orchard floor were determined using index cards soaked in peanut oil. These bait cards were fastened to the ground with a nail or thumbtacked to the mainstem or limbs of trees. When the number of IFA per cards was about 25 or less, the actual numbers were recorded; larger numbers of IFA per card were estimated in 50 ant increments based upon prior knowledge of counts of IFA on cards up to 300 per card. Ants collected for identification and ants carrying insects or insect parts were aspirated into vials and stored in 70% ethanol for later study.

Peanut-oil-soaked cards were a good method for sampling IFA in young trees and on the ground. Foraging IFA were collected weekly from the mainstem of young smooth-barked trees from mid-April until the end of November. Easily-observable foraging trails on young trees were noted from the first week of May when significant aphid numbers were first noticed, until the end of October just before most foliage had fallen. The rate of movement of ants up or down these trees ranged from 1.2 ants per min. to > 30 ants per min. at temperatures ranging from 21°C to 33°C. Ant activity relative to time of day, temperature, and humidity were not of particular interest, but it was obvious that activity was adversely influenced by high temperatures, direct sunlight, and by very low humidities. Large numbers of IFA were collected on cards in the shade of the tree on the ground and on the mainstem, even during periods when the temperature was near 33°C. Foraging IFA were rarely collected from cards located on the ground in the sunshine on hot days. In commercial orchards up to 15 years old, trees were usually planted 119 trees per ha. These orchards were usually heavily fertilized and in those where ant control was not practiced, ant mound counts often approached one per tree during the spring. Here too, routine applications of insecticides applied for pecan pests had little effect on the number of IFA colonies.

FORAGING HEIGHTS

Two experiments were conducted to determine foraging by IFA in mature trees as a function of height. In the first experiment, each of 4 'Moore' trees were sampled for ants at 1.5, 3.0, 6.0, and 9.0-m heights with 10 oil-soaked cards at each height on the morning of 28 August for a period of 1.5 hrs. For the second experiment, each of 4 'Moore' trees were sampled at 9.0 m with 50 cards/tree on the morning of 2 September for a period of 1.5 hrs.

In the first experiment, after 1.5 hrs with the temperature 31°C and RH 45%, an average of 0.2 IFA workers per card were collected at 1.5 m, none were collected at 3 m, 6.3 per card at 6 m, and none at 9 m. The reason for this variation is not understood but it is probably associated with the intensity of ant foraging in the test tree at that time.

In the second experiment, after 1.5 hrs with the temperature 23°C and RH 92%, an average of 1.2 IFA per card were collected at 9 m (range 0-50/card). These two experiments demonstrated that IFA do indeed forage high within mature pecan trees, although the experiments did not indicate excessive activity at these heights. IFA activity high in trees may be limited by distance from the mound and the time required to transmit information back to the colony.

MOWING

One spring and one summer experiment were conducted to determine if mowing understory vegetation changed the relative numbers of IFA on the ground versus in trees, and whether such changes were accompanied by changes in aphid numbers. The two experiments were conducted in different parts of the same orchard in Albany. The first was conducted from 23 April until 12 June with mowings on 20 April, 12 May, and 9 June; the second from 31 July until 27 August with mowings on 12 and 18 August. The orchard was planted with 119 trees/ha and was heavily fertilized. Mowed and unmowed (control) plots were 0.3 ha square, and each contained 36 trees; the design was a randomized complete block replicated 4 times. The four center trees of each plot were sampled weekly by examining five compound leaves per tree for aphid numbers. One oil-soaked card was tacked to each of two opposing sides of tree mainstems at 1.5 m height and one card was nailed to the ground on each of two opposing sides of the tree at 1.5 m distance from the mainstem (total 32 cards per mainstems and 32 per ground per treatment per count). Cards were examined after 1 hr. The ground cover was a lush mixture of rye and bermuda grasses, various vetches and clovers and various broadleaf weeds. IFA mount counts indicated that there was approximately one per tree in the study areas. Data were analyzed by the analysis of variance.

In the spring experiment, mowing did not cause an outbreak of aphids ($P=0.93$; CV=75.2%). The aphid complex during this experiment was 95% _M. pecanis_ and 5% _M. caryella_. However, mowing significantly changed the ratio of ants in the tree to ants on the ground ($P<0.01$) (Fig. 1). The ratio of tree IFA to ground IFA in the mowed treatment was 1:1.8 and that in the control treatment was 1:5.2. The total of average numbers IFA per card on tree plus ground cards in the mowed area was 994 compared with 1090 for the same in the unmowed and these were not significantly different ($P=0.40$). Thus, mowing altered the foraging behavior of ants on the ground and caused increased foraging in trees.

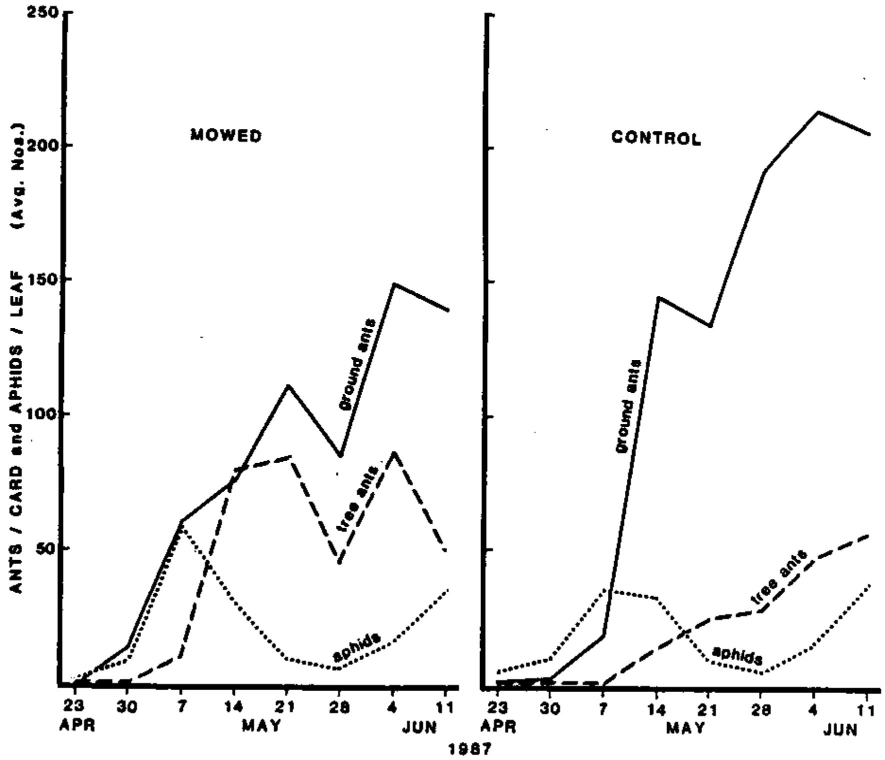

Fig. 1. Mean numbers of IFA on peanut oil-soaked index cards on trees and on the ground and mean numbers of M. pecanis on pecan foliage in mowed and control orchard habitat from 23 April to 11 June, 1987, Albany, GA.

For the summer experiment, the aphid complex was 99% M. caryella and 1% M. pecanis. Here too, we were unable to prove that mowing caused an outbreak in aphids P=0.79; CV=54.5%. Monellia caryella numbers did increase dramatically about mid-August in mowed and control treatments but as aphid numbers increased, IFA numbers on both the ground cards and tree cards decreased sharply in both treatments (Fig. 2). The total of average of numbers of IFA on cards on tree plus ground cards in the mowed treatment was 36 less than that of the control and not significantly different (P=0.48). The ratio of tree IFA to ground IFA in the mowed treatment was 1:3.1 compared to 1:4.6 in the control. Again there was a disproportionate number of IFA on the ground in the mowed as opposed to IFA on the ground in the control, but this was statistically insignificant (P=0.48). We believe that the rapid decline of IFA numbers sampled in trees and on the ground was a result of honeydew dropping to the gound where it was easily accesible and abundant honeydew rather than scarce peanut oil. The summer experiment was terminated after 4 weeks because of the rapid build-up of aphids and the need for the grower to contain the outbreak with an aphicide. We sampled 20 young trees for ants and aphids once weekly from the first of May until the end of November at Byron. One oil-soaked card was thumbtacked onto the two opposing sides of the mainstem of each tree, at ca. 1.5 m height and 1 card was nailed to the ground on two opposing sides of the tree at ca. 1.5 m distance from the mainstem of the tree (40 cards on mainstems and 40 on ground per week). Ants were counted after 1 hr. Aphids were counted on 10 compound leaves per tree. The ground cover in this orchard was common bermuda grass which was mowed frequently and closely, and resembled a lawn. The fertility level of the orchard was normal to low.

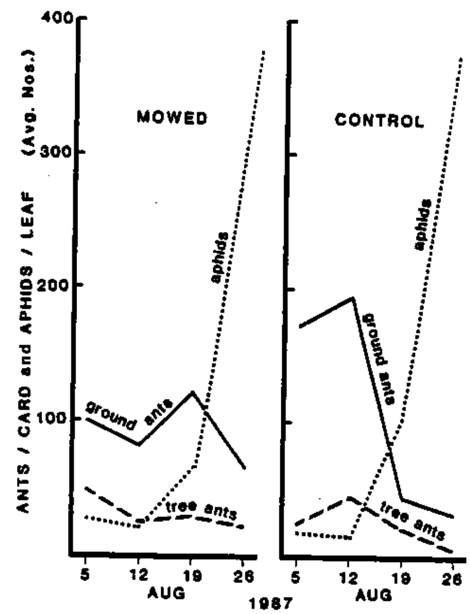

Fig. 2. Mean numbers of IFA on peanut oil-soaked index cards on trees and on the ground and mean numbers of M. caryella on pecan foliage in mowed and control orchard habitat from 5 to 26 August, 1987, Albany, GA.

Large variations were found between numbers of ants on trees and on the ground during the entire season (Fig. 3A). From 7 May until 11 June, the dominance of ants on tree cards or on ground cards varied almost weekly, but in weekly samples from 18 June and until 25 August, IFA were, on the average, twice as abundant per card on trees (92 ground:186 tree). After 25 August and until the end of the season, the reverse was true; more than twice as many ants were found per card on the ground (87 ground:33 tree). Weekly percentages of ant numbers on trees shifted significantly on 23 May from the ground to the trees and then back to the ground on about 15 September (Fig. 3B). There was a negative correlation between aphids and ants on trees ($r=-0.49875$; $P<.01$). Data from ant collections after 15 September were similar to those obtained from the mowing experiment. Copius honeydew falling onto the ground cover eliminated the need for ants to climb trees. Again, our sampling technique utilized peanut oil and the ants were probably predisposed to collect honeydew. IFA were not observed to antennate M. caryella or M. pecanis but simply gathered honeydew from leaf surfaces.

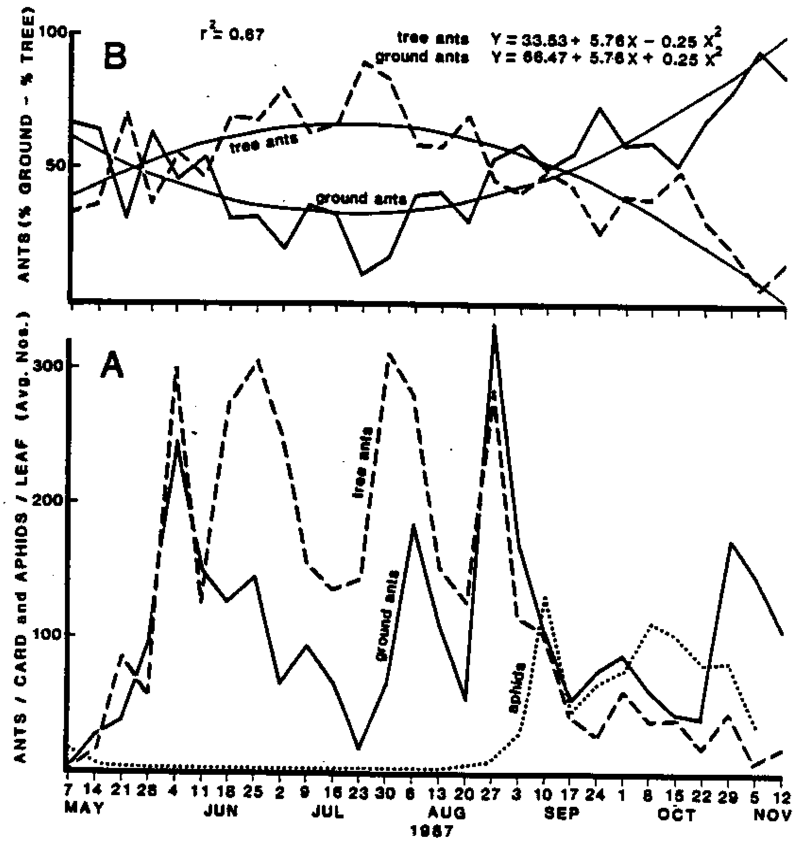

Fig. 3. (A) Mean numbers of IFA on peanut oil-soaked index cards on trees and on the ground and mean numbers of M. pecanis and M. caryella on pecan foliage in closely mowed orchard habitat from 7 May to 12 November, 1987, Byron, GA. (B) Numbers of IFA (same as Fig. 3A) converted to percentages on each date and smoothed by computer generated curves. R^2=.67;
Tree ants ($Y=33.53 + 5.76x - 0.25x^2$);
Ground ants ($Y=66.47 + 5.76x + 0.25x^2$).

In this test, IFA were never observed carrying aphids back to the mound when aphid numbers were low or increasing. Carrying aphids was common when aphid numbers were very large and the aphid population began to decline (crash). During one 30 minute collecting period, 36 IFA carrying prey back to the mound, were aspirated from a trail. Examination of the prey revealed that 33 were 1st through adult stages of M. caryella, 2 were lacewing eggs and 1 object was an unidentifiable arachnid.

MUTUALISM

Reducing Sugar in IFA. To verify that IFA workers were collecting honeydew, we aspirated workers from their trails on 12-year-old pecan, hican (intraspecific cross of pecan x unidentified Carya sp.), water hickory (Carya aquatica (Michaux f.) Nutall), and bitternut hickory (Carya cordiformis

(Wangenh.) K. Koch.), trees and analyzed them for reducing sugars. Visual observations were made of the sizes of gasters of workers relative to their movement either upward or downward in the trees. Ants moving upward and ants moving downward were aspirated separately. Afterwards, samples of ants were taken from the mounds which produced the trails on the trees by disturbing the mound with a shovel and aspirating attacking ants from the handle of the shovel. Major workers were removed from all samples and 40 similar sized workers were weighed, placed in vials with 1 ml 80% ethanol, and macerated with a glass rod. The vials were then sealed tightly and heated at 80°C for 18 hrs. Then, the ant-ethanol solutions were centrifuged in a Beckman microfuge for 1 min. and the supernatants pipetted off. Aliquots of the supernatants were placed in test tubes and dried at 80°C under streams of N_2. Next, 0.5 ml H_2O + 0.5 ml buffer (0.05 M sodium acetate, pH 4.7) was added to each sample and the procedure of Nelson (1944) was used to assay glucose content, with glucose used as the standard. Absorbance at 500 nm was determined from triplicate samples with a Bausch and Lomb Spectronic 21 spectrophotometer.

IFA were obviously carrying large amounts of unidentified substances back to the colony as indicated by the numerous ants foraging in the trees. Also, the swollen gasters of IFA traveling downwards on trees indicated feeding. IFA moving downward always weighed more than those moving upward or those taken from mounds (Table 1). Mean weights of IFA moving toward the mound ranged from 5.8 mg (C. aquatica) to 26.3 mg (C. cordiformis) more than mean weights of IFA moving upward. Mean weights of IFA from mounds ranged from about equal (pecan) to 5.7 mg heavier (hican) than mean weights of respective upward moving IFA, indicating the transfer of food material to ants in the mound. Mean amounts of reducing sugar (glucose) in IFA moving toward the mound were always greater than those moving upwards or in the mound (Table 1). Mean measurements of reducing sugar content in IFA moving toward the mound ranged from 4.25 µg (pecan) to 18.70 µg (C. cordiformis) more than the sugar content in those moving up into the trees. As was expected, slightly more reducing sugar was found in IFA from the mound than those moving away from the mound.

Table 1. Reducing sugar content of IFA from foraging trails on selected species of Carya and from the source mound.

Heading of, or source of ants	Weight/40 ants (mg)	Reducing sugar/ant[a] (µg)
	Carya illinoensis	
Upward	21.2	20.40
Downward	43.6	24.65
Mound	21.6	17.85
	Carya aquatica	
Upward	15.6	11.90
Downward	21.4	20.42
Mound	19.4	11.90
	Carya cordiformis	
Upward	17.3	15.11
Downward	43.6	34.00
Mound	21.6	21.25
	Carya illinoensis x *Carya sp.* (Hican)	
Upward	21.1	19.55
Downward	35.6	38.25
Mound	26.8	20.45

[a]Means of 3 samples per treatment.

Radiolabeled honeydew. An additional experiment utilizing ^{14}C radioactive labeling techniques was conducted to verify that honeydew excreted by M. caryella was the source of reducing sugar collected by IFA. A two-year-old pecan seedling growing in a clay pot was used for the labeling study. The foliage of the seedling was washed with tepid water to remove any traces of honeydew, aphids, and other contaminants. The seedling was held under subdued light (ca. 1,000 moles $m^{-2}s^{-2}$) sealed inside a 3 mil plastic bag also containing a glass vial with 10 μCi aqueous $NaH^{14}CO_3$ (56 mCi per m mole S.A.). Then 100 1 of 1 N HCl was added to the $NaH^{14}CO_3$ to liberate $^{14}CO_2$. The seedling was allowed to assimilate the $^{14}CO_2$ for 8 hrs after which the plastic bag was removed and allowed to "air-out" for 24 hours. Next, a 79 mesh per cm. nylon cage was placed over the seedling and infested with 300 field collected M. caryella adults. The cage with seedling and aphids was kept in a glasshouse for 6 days. After this the infested seedling was placed near a trail of foraging IFA under an open building and the cage was removed. After several hours, ants were observed on the seedling and triplicate samples of each of the following were collected: 10 ants from the ^{14}C treated seedling foliage; 10 ants from the trail on the ground that was the source of ants; 10 ants from the trail of an untreated (control) ant colony; 10 4th-instar aphids from ^{14}C treated seedling foliage; 10 4th-instar aphids from an untreated (control) seedling; 1 ml aliquots of "wash" taken from 75 mm^2 of treated leaf tissue washed with 10 ml 80% ethanol; 1.3 cm dia leaf tissue discs from treated seedling foliage with honeydew removed by washing. Samples of ants, aphids, and leaf tissue were digested separately in scintillation vials each with 1 ml of tissue solubilizer (Beckman BT5-450, Beckman Instruments, Inc., Fullerton CA.) for 24 hrs and then liquid scintillation cocktail (Beckman Rendy-Solv MP) was added to all samples. Disintegrations per minute (dpm) of ^{14}C and control samples were counted with a Beckman LS-1800 Liquid Scintillation Counter.

Many M. caryella progeny were produced in the glasshouse and these excreted copious honeydew that was deposited on the upper surfaces of leaves. Within 12 hrs, foraging IFA located the seedling, aphids, and honeydew, all of which were now radiolabeled with ^{14}C. Samples of M. caryella expressed the highest radioactivity (30,545 2,647 dpm), and honeydew measured a close second (Table 2). IFA taken from foliage were twice as radioactive as IFA from the trail and leaf tissue was roughly twice as radioactive as IFA from foliage. Control IFA and control aphids expressed only a trace of radioactivity which was background radioactivity. This experiment leaves little doubt that IFA utilized large amounts of honeydew produced by M. caryella.

Table 2. Radioactivity in 14C radiolabeled pecan seedling leaf tissue, M. caryella, honeydew, IFA, and background radioactivity in non-labeled M. caryella and IFA.

Source	Treatment	Disintegration/minute (x)±SD
Leaf tissue[a]	labeled	2,604 ± 79
Honeydew[b]	labeled	25,691 ± 4,392
M. caryella[c]	labeled	30,545 ± 2,647
IFA (foliage)[c]	labeled	1,290 ± 92
IFA (trial)[c]	labeled	552 ± 58
M. caryella[c]	control	39 ± 3
IFA[c]	control	19 ± 1

[a] - 1.4 cm diameter; [b] - 1 ml aliquot from 10 ml ethanol wash;
[c] - 10 individuals.

PREDATION

A number of experiments were conducted in the field at Byron to determine the extent of predation by IFA on M. caryella, C. rufilabris, H. convergens and A. obliqua.

Monellia caryella. In 2 experiments with M. caryella, pecan leaflets with numerous aphids on the lower side and a heavy coating of honeydew on the upper side were thumbtacked adjacent to vigorous ant trails on young trees. For each experiment, 4 leaflets were positioned with the aphid carrying side away from the tree and 4 leaflets were positioned with the honeydew carrying side away from the tree. This provided foraging IFA with a convenient choice of aphids or honeydew. The experiments were observed for one hour.

In both experiments, given a choice of M. caryella as prey or honeydew as a food source, IFA always collected honeydew and ignored the aphids. IFA preference for honeydew was also displayed by attempts of workers to collect honeydew from between the leaflets and the tree, while ignoring easily available aphids on the outer side of the same leaflet. IFA were occasionally observed carrying all 3 species of aphids down the tree, but this only occurred when aphid numbers began to decline (crash) when very high densities existed on trees. It was not determined if these aphids were utilized as food or if the ants were trying to relocate the aphid onto suitable foliage.

Chrysoperla rufilabris. Three experiments were conducted with C. rufilabris eggs. For the first experiment, fifteen field collected C. rufilabris eggs, laid on pecan leaflets, were thumbtacked adjacent to an IFA trail on a young tree and observed for ant predation after 24 hrs. In the second experiment, C. rufilabris eggs laid on strips of brown paper (1 x 55 cm) from a laboratory colony were wrapped around the trunks of trees and across the trail of foraging IFA workers and then thumbtacked in place. One strip was placed at 1.5 m above the ground on each of 5 trees. Five strips carried from 227 to 296 eggs each and strips averaged 261 eggs. IFA predation on the eggs was determined after 18 hrs. For the third experiment, C. rufilabris eggs laid on strips of paper (2 x 30 cm), taken from the laboratory colony, were wrapped around the trunk and limbs of 3 trees having trails of foraging IFA workers and around the trunk and limbs of 3 control trees without foraging IFA. IFA colonies were previously eliminated from the control trees with applications of hydramethylnon bait (Amdro). Strips were placed in each tree at intervals of 1, 2, 3, and 4 m heights. Strips averaged 85 eggs each and the strips were examined for ant predation after 18 hrs.

Two experiments were conducted with C. rufilabris larvae. In the first, second and third instar larvae were affixed with cyanoacrylate glue to each of 6-2.54 cm diam. paper discs. Discs were thumbtacked at random to the limbs of a young tree, with no consideration to the proximity of an ant trail. These were examined after 24 hrs. In the second experiment, 2.54 x 2.54 x 1.3 cm open-topped styrene plastic containers were coated around the upper-inner edge with a 2 to 3 mm band of Fluon AD-1 (Northern Products, Inc., P. O. Box 1175, Woonsocket, RI 02895). Each container served as an inescapable cage for 20-first instar, laboratory produced C. rufilabris larvae. The fluon band prevented lacewing larvae from leaving the cage, but the narrowness of the band allowed larger IFA access into and out of the containers. To reduce the incidence of cannibalism by the lacewing larvae, 0.5 ml of Sitotroga cerealella (Oliver) eggs were placed in each container as food. One container with larvae and Sitotroga eggs was affixed to each of the four cardinal sides of the mainstem of a young tree at 0.3 m intervals from the ground to a height of 3.6 m. The containers were observed after 20 and 48 hrs for the presence or absence of lacewing larvae, Sitotroga eggs, and ants.

Two experiments were conducted with laboratory produced C. rufilabris pupae within cocoons as prey for IFA. For the first, two cocoons were glued to each of 6 paper discs, 2.43 cm diam, which were in turn thumbtacked at random to the limbs of a young tree. No attempt was made to place them near an ant trail. Examinations for predation were made after 24 hrs. For the second experiment, 2 to 4 cocoons were glued onto similar paper discs and thumbtacked at random to the limbs of a young tree. A total of 42 cocoons were used and these were examined after 5 days for predation and adult lacewing emergence.

In the first experiment after 24 hrs, only one of 15 eggs of C. rufilabris was missing. Since the egg stalk was missing also, it was concluded that ants were not to be responsible for this egg loss. The results of the first test was not convincing because of the small numbers of eggs used and because of a previous observation by the senior author of IFA workers carrying eggs believed to be those of lacewings. In the second experiment, a total of 18 eggs (<2% or original number) remained on the 5 strips (range 2 to 16 eggs per strip). The stalks of the eggs were left intact as the egg had been excised from the top of each stalk. In the third experiment, after 18 hrs, on strips on the three trees with foraging IFA there was an average of 1.5% eggs remaining at 1 m, 0.8% remaining at 2 m, 0.4% at 3 m and 13.3% at 4 m. In the control trees where IFA were not present, there was an average of 44.7% of the eggs remaining at 1 m, 65.9% at 2 m, 68.2% at 3 m and 62.4% at 4 m. Thus, IFA prey heavily on C. rufilabris eggs but there are obviously other egg predators present in pecan trees where IFA are not present.

In the first experiment after 24 hrs, the lacewing larvae on two paper discs had been attacked and only pieces of the larval exoskeletons remained. IFA workers continued in their attempt to remove these remains. The larvae on 4 other discs were unmolested and ants were not present. This experiment suggests that IFA likely prey to some extent on C. rufilabris. In the second experiment, after 20 hrs all C. rufilabris and S. cerealella eggs had been removed from containers up to 1.5 m height but foraging by IFA gradually diminished up to 2.7 m (Table 3).

Table 3. Total number of C. rufilabris larvae and IFA workers found in sets of four cages placed at each of 12 heights above the ground in a pecan tree after 20 and 48 hours.

Cage Height(m)[a]	Beginning No.		After 20 Hrs.		After 48	
	C.rufilabris	IFA	C.rufilabris	IFA	C.rufilabris	IFA
0.3	80	0	0	0	-	-
0.6	80	0	0	2	-	0
0.9	80	0	0	2	-	0
1.2	80	0	0	7	-	0
1.5	80	0	0	18	-	0
1.8	80	0	1	19	0	0
2.1	80	0	22	18	0	0
2.4	80	0	45	10	0	0
2.7	80	0	50	6	0	0
3.0	80	0	35	0	1	0
3.3	80	0	80	0	0	2
3.6	80	0	80	0	0	8

[a] Sets of 4 cages were placed at 0.3 m intervals and each cage originally contained 20-first instar C. rufilabris larvae.

There was no foraging at 3.0-3.6 m after 20 hrs. After 48 hrs, IFA had removed essentially all C. rufilabris larvae and all S. cerealella eggs up to 3.6 m and had virtually abandoned all containers at all heights by this time. The combination of C. rufilabris larvae and S. cerealella eggs as prey is not ideal methodology but the experiment clearly demonstrates the expanding foraging behavior of IFA in trees, as a function of time, distance, and abundance of prey and satisfies us that C. rufilbris larvae are important prey of IFA.

After 24 hrs, the first experiment with C. rufilabris pupae in cocoons as prey revealed that IFA had preyed on the pupae on 2 of the 6 paper discs. At that time, workers were observed attempting to open 4 of the cocoons but they had succeeded only in removing the loose surrounding silk, in effect "polishing" the cocoon. Cocoons thus attacked by IFA are now easily recognized and were seen frequently during these studies.

In the second experiment after 5 days, 13 (31%) of the cocoons were missing, 22 (52%) of the cocoons had been successfully torn open by ants and the pupae were removed. The remaining 7 (17%) had been polished by ants. IFA were the predators, as they were observed attacking the cocoons prior to the end of the test. Of the 7 polished cocoons, adults later emerged from 3 while 4 pupae failed to emerge as adults. It is doubtful that IFA killed the pupae in polished cocoons; however, this question needs additional study. We concluded that after 5 days, IFA destroyed 83% of the pupae. C. rufilabris pupae require about 9 days at 27oC to develop and emerge as adults.

Hippodamia convergens. Two experiments were conducted with H. convergens. For the first, 52 clusters of laboratory produced H. convergens eggs laid on 2.54 cm diameter paper discs were thumbtacked to the mainstem and 6 limbs of a young tree during September. Egg numbers per cluster averaged 33 and ranged from 11 to 65. Egg clusters were placed at 15 cm intervals, beginning just above the ground and extending to 3.5 m along several limbs. During the experiment, the tree had an active IFA colony at the base and the foliage supported a large population of M. caryella that was excreting honeydew. The eggs were left for 48 hrs, after which the following counts were made: unchanged egg clusters; hatched egg clusters with larvae gone; hatched egg clusters with first instar-larvae still present; and destroyed or missing egg clusters.

The second experiment evaluated IFA predation on H. convergens pupae during late May. A total of 24 laboratory-produced pupae on 7 paper discs (1 to 6 pupae per disc) were thumbtacked to a tree at 1.2 m height and examined every other day for 14 days. The numbers of pupae preyed upon by IFA, the number of pupae missing, and the number of adult eclosions were recorded. An active IFA mound was located at the base of the experimental tree, but large numbers of aphids were not present.

Examination of the 52 H. convergens egg clusters after 48 hrs revealed that 18 egg clusters were unchanged, 22 egg clusters had hatched and the larvae were gone, 10 egg clusters had hatched and first instar larvae remained on the egg corions, and 2 egg clusters at 0.6 and 0.9 m height had been destroyed by an unknown predator. Foraging IFA were present in large numbers on the tree and under these conditions they obviously preferred honeydew over H. convergens eggs or first instar larvae still on the egg corions. The two missing egg clusters may have resulted from IFA attraction to one or more damaged H. convergens eggs in each cluster or could have been the work of some other egg predator. Other observations indicate that damaged coccinellid eggs may elicit predation. Thus, H. convergens eggs and larvae appear to have some resistance to IFA predation or the foraging IFA were preoccupied with the collection of honeydew or some other food source on the tree.

In the second experiment after 14 days, 5 of the original 24 pupae were missing and 19 had emerged as adults. There were no indications that IFA preyed upon the missing pupae; these could have been dislodged by other animals which also utilize pecan trees. Hippodamia convergens pupae also appear to have some natural resistance to IFA predation. IFA predation on eggs, larvae, and pupae needs additional study under variable conditions.

Allograpta obliqua. One experiment was conducted on the predation of IFA on A. obliqua puparia during September. Thirteen field-collected puparia were glued individually to 2.54 cm diam paper discs and thumbtacked adjacent to a IFA trail on a young tree. Puparia were examined after 3 days for ant predation or fly emergence. The tree supported large numbers of M. caryella that were excreting honeydew.

Upon examination of A. obliqua pupae, IFA were observed devouring one puparium. The remaining puparia had been completely removed except for a small portion of cuticle that remained glued to the paper disc. IFA obviously prey heavily on A. obliqua puparia.

ADDITIONAL OBSERVATIONS

A close mutualistic relationship was discovered between IFA and the mealy bug, Dysmicoccus morrisoni (Hollinger). Infestations of D. morrisoni were found on callus tissue associated with healing wound located on the mainstem of vigorous young trees. IFA were observed antennating these mealybugs and then collecting the excreted honeydew. Infestation sites of D. morrisoni were usually covered with a canopy of soil and other debris that was constructed by IFA. IFA were also observed moving all stages of D. morrisoni and were especially protective of D. morrisoni when the debris canopy was disturbed. Dysmicoccus morrisoni has not been previously reported as a pest on pecan and it may be limited by a shortage of proper feeding habitat. Fortuitous counts of D. morrisoni numbers were made in an orchard experiment designed to evaluate insecticidal control of IFA at Byron. The trees were young and of the same cultivar and age. The ground area of a single 1.2 ha block of trees was treated with hydramethylnon bait at the rate of 1134 g per ha on 21 and 28 April, and 18 and 28 May. The treatments eliminated IFA from the orchard during the 1987 growing season. Twenty trees were selected at random for D. morrisoni counts. An adjacent section of orchard of similar age and cultivar was left untreated as a control and 20 trees were also randomly selected for examination. Thus, there was only one replication and the trees were examined only for positive or negative infestation of D. morrisoni. Pretreatment infestation counts were not taken. Trees were examined on 11 September and again on 21 September by a different technician. On the first examination, D. morrisoni were found infesting 2 ant free trees and 14 control trees. The second technician found no mealy bug infestations on 20 ant free trees but 17 control trees were infested. These data and our previous observations strongly suggest a close mutualistic relationship between IFA and D. morrisoni. Fifteen D. morrisoni colonies were examined on 6 October. That averaged 40 mealy bugs per colony, ranging from 3 to 200.

The mainstem of young trees in orchards heavily infested with IFA at Albany were also found to be heavily infested with a non-honeydew producing armored scale Hemiberlesia diffinis (Newstead). Excessive activity by IFA on tree mainstems may affect predation by the scale feeding species Chilocorus stigma (Say) and Eurychilopterella luridula Reuter which are common on pecan.

Other species of ants were collected from trees in orchards infested with IFA but none were particularly abundant. At Albany, Paratrechina vividula Nylander, and Brachymyrmex obscurior Forel were common under the bark of mature trees. At Byron, Crematogaster ashmeadi Mayr were collected from 4.6 to 13.7 m heights in mature trees and Forelius analis (Andre) and Conomyrma bureni Trager were fairly common on young trees. The latter two species appear to utilize honeydew produced by aphids and all three co-exist in relatively low numbers with large numbers of IFA.

CONCLUSIONS

All commercial and non-commercial pecan orchards from middle to south Georgia appear to be good habitat for IFA. IFA forage as high as 9 m in the trees for honeydew and insect prey. However, they also take advantage of honeydew that drops to the ground. Mowing pecan orchards changed the ground-to-tree ratio of foraging IFA but there was no statistical evidence that IFA caused outbreaks of M. caryella or other aphids. The behavior of IFA in the presence of M. caryella or M. pecanis appears to be affected by the volume of honeydew being produced by the aphids, the number of aphids present, and the crash status of the aphid population. Thus, the strength of IFA - M. caryella mutualism is probably regulated by existing conditions and needs of the IFA colony. We now know that IFA is a major predator of the eggs, larvae and pupae of C. rufilabris and of the pupae of A. obliqua, both of which are major predators of the 3 species of foliar-feeding aphids on pecan. This, along with use of honeydew by IFA, the effect of mowing on IFA vertical distribution, probably contributes to the outbreak of aphids on pecans. Honeydew produced by M. caryella is a major source of carbohydrates for IFA. There is a close mutualistic relationship between IFA and the mealybug, D. morrisoni and possible mutualism with the scale H. diffinis. These relationships need additional study.

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A Brief Review of Some 1988 Research

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IGR Research. We have presented our original findings at the meeting in Caracas. As you know, these will form part of the book entitled "Applied Myrmecology", edited by Bob VanderMeer and Klaus Jaffe. In brief, we sought to find out: (1) what the effects were of Sumitomo S-31183 on queens of *Solenopsis invicta* (RIFA) and (2) what were the effects of fenoxycarb and Sumitomo S-31183 on the big headed ant, *Pheidole megacephala* (BHA). Both species of ant are polygynous, yet they are only distantly related.

S. invicta. Sumitomo S-31183 produces many of the same effects as fenoxycarb, e.g. disappearance of worker brood, suppression of egg production by the queen, and a shift in caste differentiation. Laboratory treated colonies died within 24 weeks. Histological examination of ovaries from queens at five and six weeks posttreatment with Sumitomo S-31183 showed the ovary to be quite large, filling the abdomen, but with most of the individual ovarioles empty, and with only a few to have very small yolked eggs present. In comparison, normal queens have 7-10 full sized eggs in each ovariole. Over 90% of the ovarioles consisted of empty structures with a thickened covering. The zone of differentiation was almost non-existent, consisting of an egg nucleus and perhaps a few nurse cells. In certain cases where cytoplasm and yolkplasm were added, it was found that resorption had occurred. In the event that a yolked egg was formed, it was about 1/3 the size of a normal egg.

P. megacephala. This portion of our work was done at the University of Hawaii at Manoa.
Fenoxycarb. It took 6 weeks before we saw any pathology to the ovaries of queens of BHA. The delay may have been caused by the repellency of the compound to the ants. By eight weeks posttreatment, histological studies showed that we were achieving effects comparable to the effects obtained with RIFA. Ovarioles were so reduced in size that the entire ovary was only about 1/4 the normal size. The individual ovarioles were highly vacuolated, nurse cells were lacking and the follicular epithelial cells lacked cytoplasm. Some ovarioles contained only egg nuclei without the supporting cytoplasm or nurse cells. Only one or two yolked eggs were found, and these were very reduced in size.

Sumitomo S-31181. Again, we obtained comparable results as with RIFA. The entire ovary was present, filling the abdomen, but all the individual ovarioles were simple tubes instead of differentiated tissues. A few of the ovarioles contained one very small yolked egg. Ovarioles were highly vacuolated, most nurse cells were underdeveloped and cytoplasm was lacking in those nurse cells that were present. The follicular epithelium was very disrupted and egg resorption was evidenced by the choria.

It appears that fenoxycarb is acting on both species as a JH overload. However, the Sumitomo compound seems to have a different mode of action. Whereas the fenoxycarb caused the ovarian tissues to shrink up and disintegrate, the Sumitomo compound caused a different effect including a dramatic shift towards egg resorption. The action of the Sumitomo S-31183 is reminiscent of the effects of Avermectin upon the queen's ovary, particularly with respect to the growth of the follicular epithelium.

Field Tests with Synthetic Queen Recognition Pheromone. In cooperation with Jim Tumlinson, we have field tested the various enantiomers of the queen recognition pheromone as made by a Japanese chemist. We have tested the various components against both the red and the black fire ants. At this time, it appears that it is the (-) enantiomer that confers recognition upon the queen, and this recognition seems to be specific. That is, the black fire ant did not respond to the various components. So we have for the first time, an indication that there is some specificity to the queen recognition pheromone.

Technique for Identifying Adopted Queen. If you wish to check and see if your multiple queen colonies are adopting newly-mated queens, we would like to share a technique with you that we learned about as we studied the adoption process. Four to five days after a mating flight, dig up the mound and rapidly separate the ants from the soil. Collect and dissect all the queens. An old established egg-laying colony queen will be inseminated, lack a fat body, lack alary muscles, have a normal, thin esophagus, and the ovary will consist of individual ovarioles, each ovariole having 2 or more vitellogenic eggs present, and the remainder of the ovariole showing many, many, eggs undergoing development. If, on the other hand, the queen is inseminated, but has a large fat body, with alary muscles present, or alary muscles undergoing hystolysis, an enlarged esophagus, and an ovary interlaced with trachea and confined to the last two abdominal segments, and with only a few ovarioles that contain a single vitellogenic egg, with no other eggs present undergoing development, then you have a newly-adopted queen.

Technique for Rapid Separation of Ants from Soil. We have used this technique over the past year and found it to be excellent in quick separation of ants from soil and with a survival rate of close to 99% of the brood, queens, and workers. A large, 10 gallon, plastic, garbage can is filled with water and epsom salts added until a saturated solution is obtained. The dug mound is dumped slowly or rapidly, whichever you prefer, into the salt solution. The mixture is stirred with a broom handle or other sutiable piece of equipment. All the colony will float to the surface. A large tea strainer is used to scoop all the ants up, and these are immediately rinsed in another container of fresh water. The rinsed ants are then dumped into a nester tray lined with absorbent paper. The colony can be immediately knocked down with carbon dioxide and the queens collected or whatever else done that you wish.

A Two Year Study on Mound Movement and Population Estimates in Polygynous and Monogynous Areas.

Two years ago, we iniated a study on a polygynous and a monogynous area to study the changes in the mound density and to obtain some estimate of the ant population in these two areas. A rectangular fields measuring 60 meters X 33 meters was set up in area containing a polygynous population of *S. invicta*. Another rectangular field was set up in an area known to contain only a monogynous population. Each field was then subdivided into 165 smaller plots of 3 X 4 meters (Figure 1). Each small rectangle was permanently marked with pieces of PVC pipe driven into the ground so that only a 2" segment remained above ground. Each meter of the 3 X 4 plot was marked with the PVC markers. All mounds within a given plot were flagged and its position in the plot recorded. A mound was defined as any raised soil which when opened was seen to contain workers, brood, and queen(s). Population estimates were done by digging a number of the mounds, and also by taking core samples using a 2" soil sampler. The mounds and the core samples were quick-floated using the technique described before. The ants were immobilized with carbon dioxide and the queens collected. The volumne of ants within the colony was recorded and a 1ml sample of ants was taken and the numbers within the sample counted. A quick multiplication gave us the total number of ants within the colony. The number of ants within a 2" core sample was multiplied by 977,392 to give us the number of ants within the total plot.

Changes in mound density--Monogynous area. Figures 2-4 show the changes in the single queen plot. Figure 5 shows a composite of the 3 observations. During July of 1988, we experienced a 6 week drought. The number of mounds had dropped by 57% due to this lack of moisture. In February of 1989, we had an overabundance of rain and the mound number increased back to its original count. Either we had re-infestation or the "missing" colonies were so deep in the soil that we missed them. The answer was a combination of both. We had re-infestation, and by multiple queen colonies. Each mound in the plot was opened and checked for numbers of queens. Five colonies were found to be polygynous and all the others monogynous. When more than one queen was found, the queens were collected and examined for insemination. Figure 6 shows the mounds that are now polygynous. The field to the north of the plot is known to be infested with multiple queen colonies. It is apparent that the polygynous form is moving into the monogynous area from this infested field.

Changes in mound density--Polygynous area. Figures 7-11 show the changes in the polygynous area. Figure 12 is a composite of 3 separate counts to show the effect of the drought and excess rainfall upon the population. During the first year (1987), the number of mounds increased from 110 to 248, a 225% increase. Three months later, there was a 7% reduction in the total number of mounds. Then, in July, with the drought, the mound number was further reduced so that only 32% of the mounds were still present. Figure 12 shows that the wet area (circle) sustained the best surviaval rate. When the rains started, the number of mounds increased, however, the ants left the "wet" area. In 2 years, we found that the total number had increased from 110 to 138, a small increase, but during the process, there had been a dramatic increase in mounds, and a small increase, but during the process, there had been a dramatic increase in mounds, and a subsequent "crash" due to the drought.

Table 1 gives the actual number of mounds recored during the sampling period.

Table 1. Mound Counts in Polygynous and Monogynous Areas.

Date	Single Queen Pasture Mound Count
3/21/88	21
7/21/88	12
2/27/89	21
	Multiple Queen Pasture
4/16/87	110
11/10/87	248
2/22/88	193
7/12/88	81
2/27/89	138

Changes in population estimate--Monogynous area. On three separate occassions, 3 mounds were dug, and 48 core samples taken of the single queen area. The results are given below.

Population Estimates--Single Queen Area

Total Mounds	Number Sampled	Cores	Ants/ac	Avg. No. Queens/md
21	3	48	1,559,206	1
12	3	48	1,399,933	1
21	3	48	1,724,627	1

Changes in population estimate--Polygynous area. As with the single queen area, three separate samples were taken over a 2 year period. The results are given below.

Population Estimates--Polygynous area

Total Mounds	Number Sampled	Cores	Ants/ac	Avg. Queens/md
193	16	48	6,876,178	9.2
81	16	48	3,781,680	1.8
138	16	48	3,975,761	4.4

It is apparent that the polygynous population is having a much more difficult time in regaining total numbers of ants. The single queen population has recovered both in number of mounds and in population. However, the multiple queen area is recovering only in mound numbers.

An important factor which we are just beginning to research, and which probably has some influence on our population estimate is the mortality rate in both populations. In a single collection, we collected all the dead ants from 16 plots in the polygynous area and from 3 plots in the monogynous area. We counted a 1ml sample and converted to total dead ants. We estimate that there were 820,165 dead ants in the polygynous area, and 300,630 in the monogynous area.

This data translates into the following: The polygynous area has 6X as many mounds as the monogynous area, it has 2.3X as many ants, and has a death rate of 2.7X of the monogynous area.

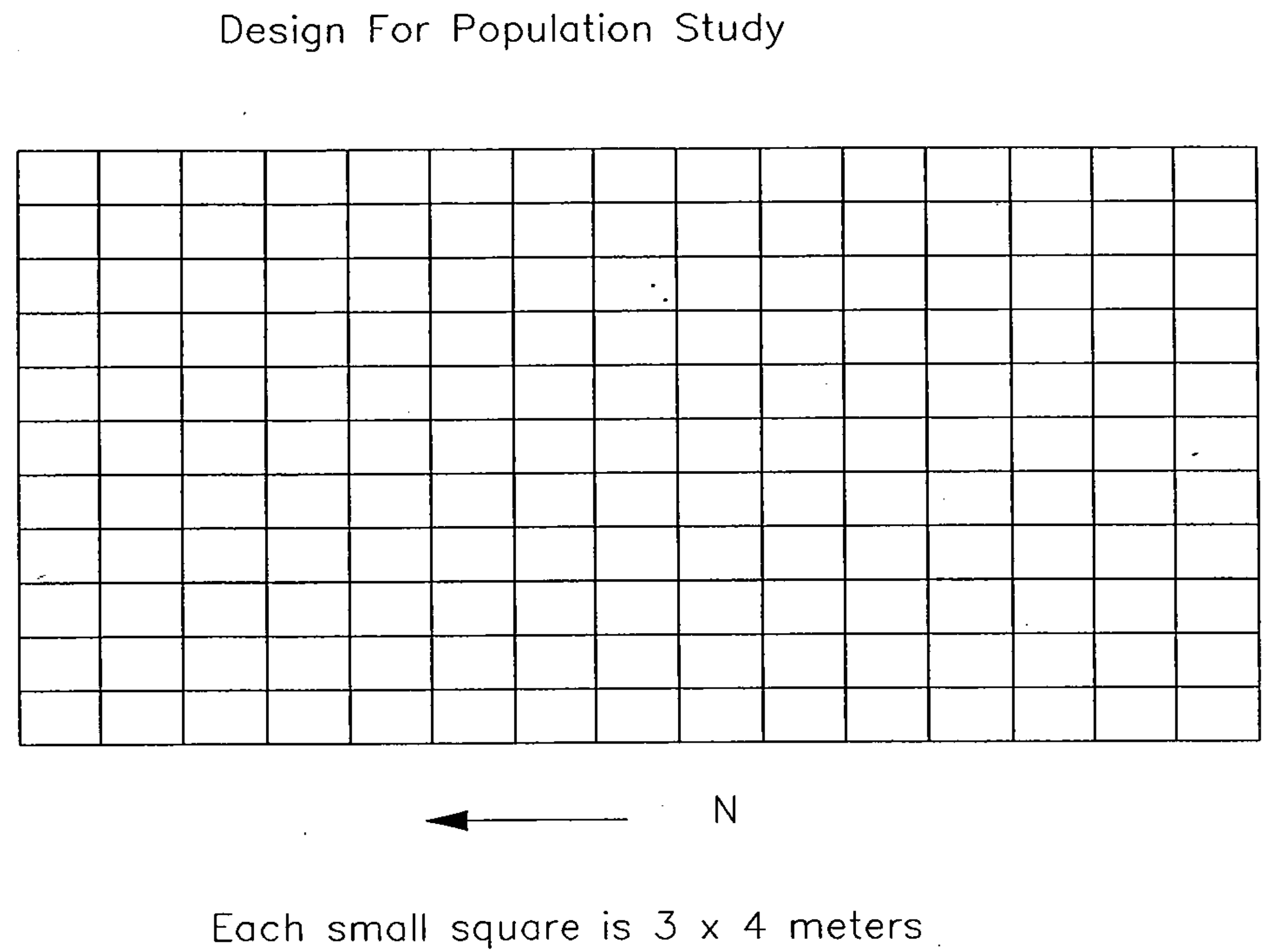

Figure 1. Design used for study of populations of monogynous and polygynous colonies. PVC pipes staked at 1 meter intervals to make permanent markers for each sub-plot. All mounds were flagged, counted, and their position recorded.

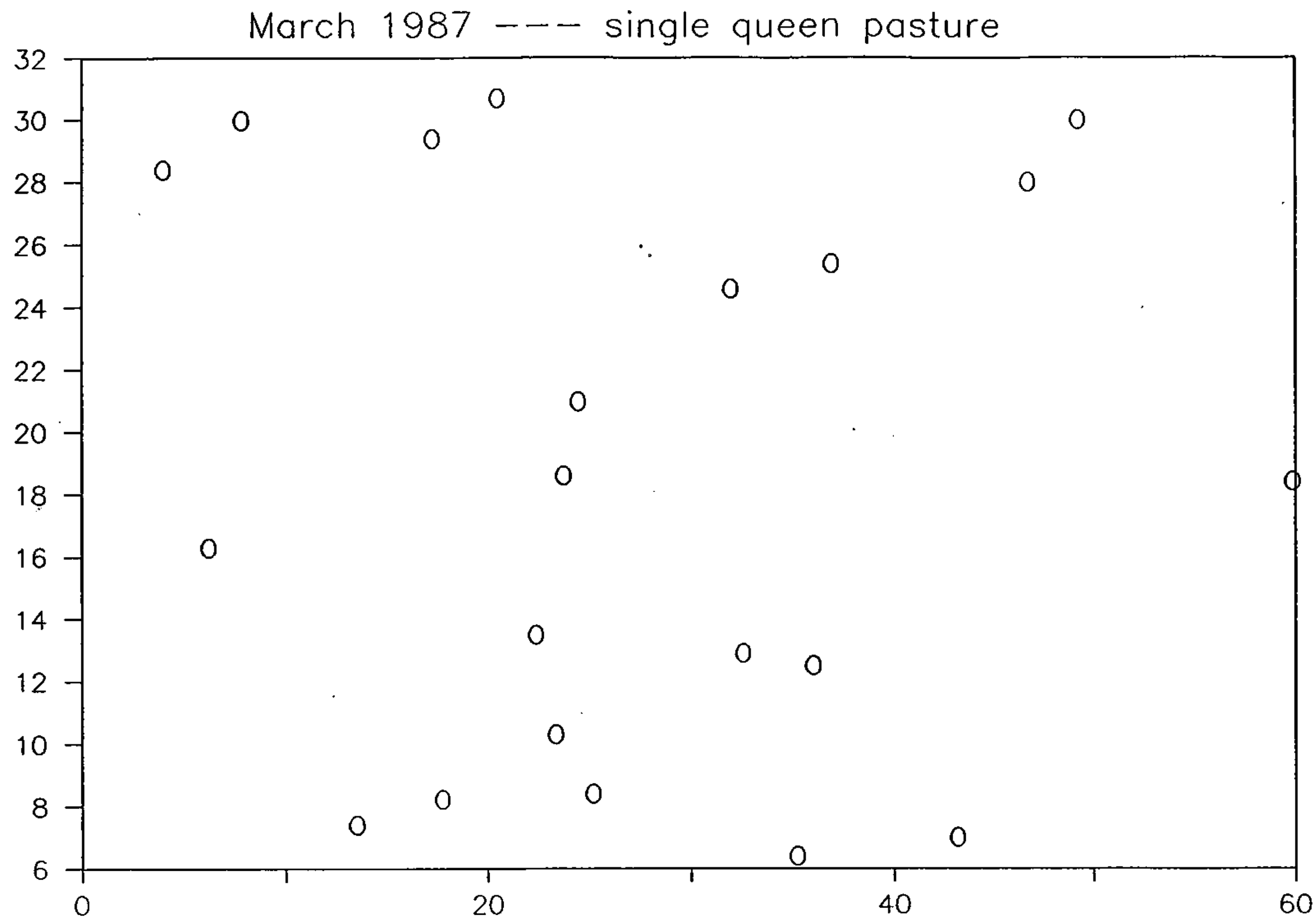

Figure 2. First count of mounds in monogynous area. N=21.

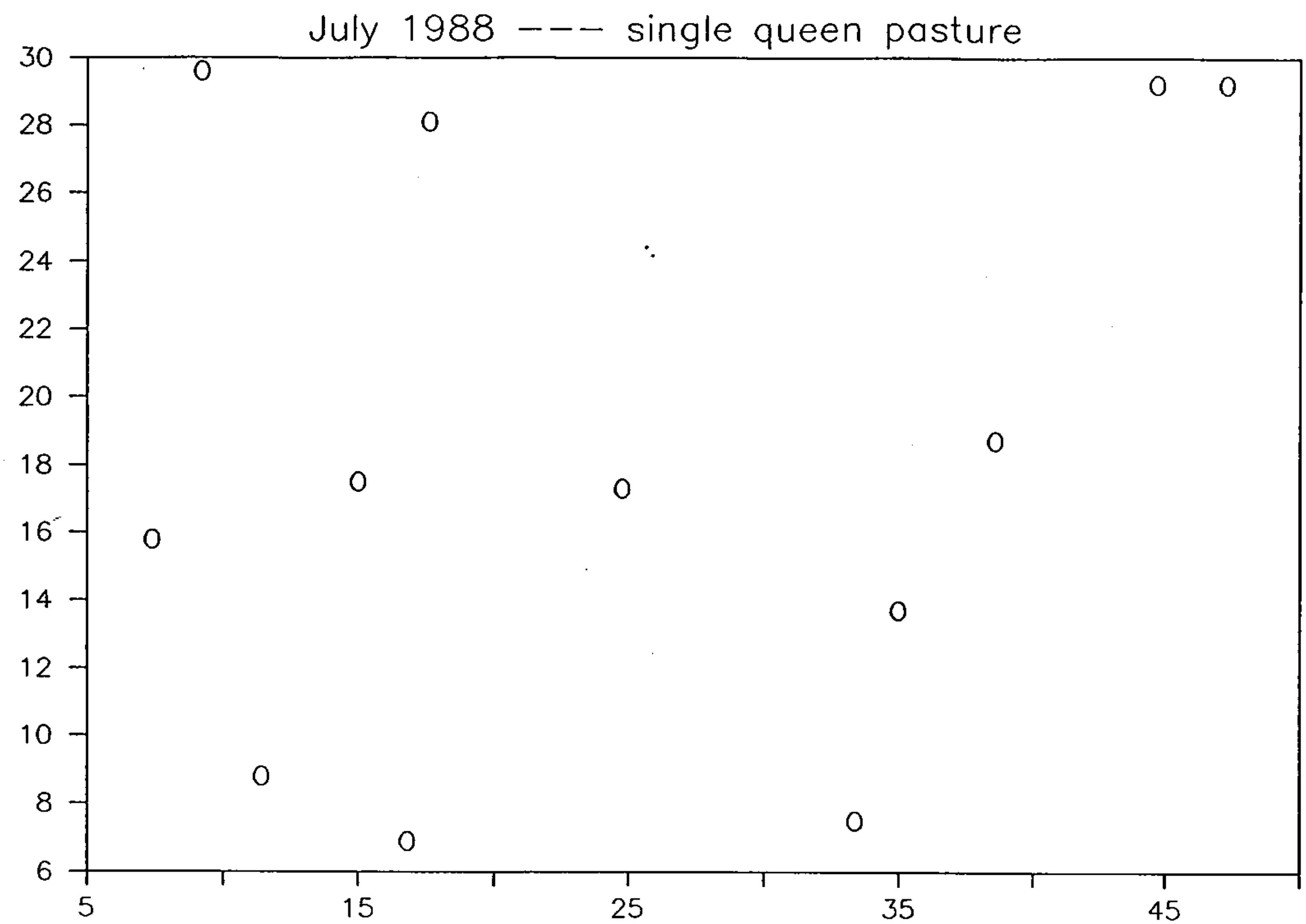

Figure 3. Second count of monogynous area. Count taken at end of 6wk drought period. N=12.

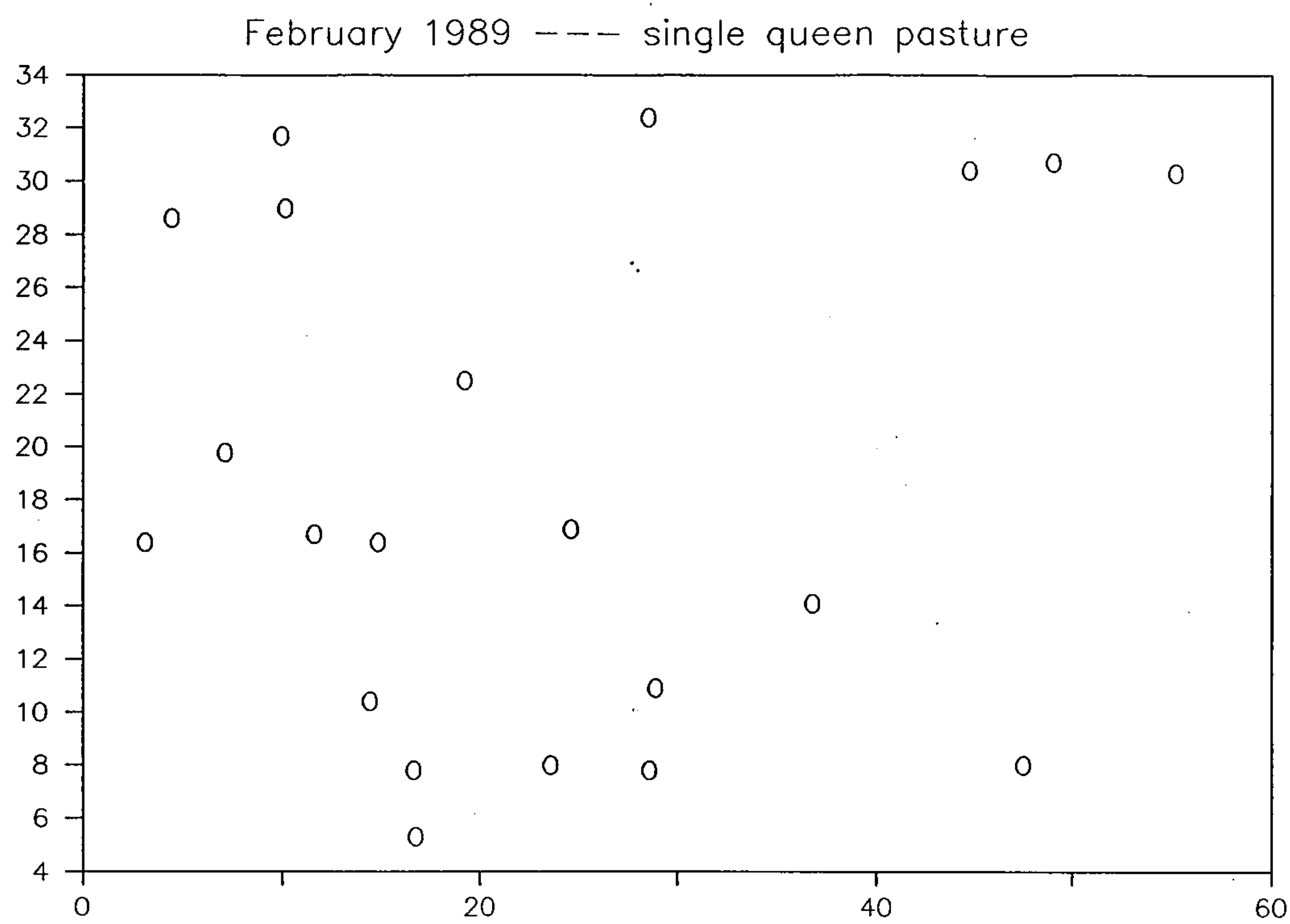

Figure 4. Third count of monogynous area. Count taken after heavy rain period. N=21.

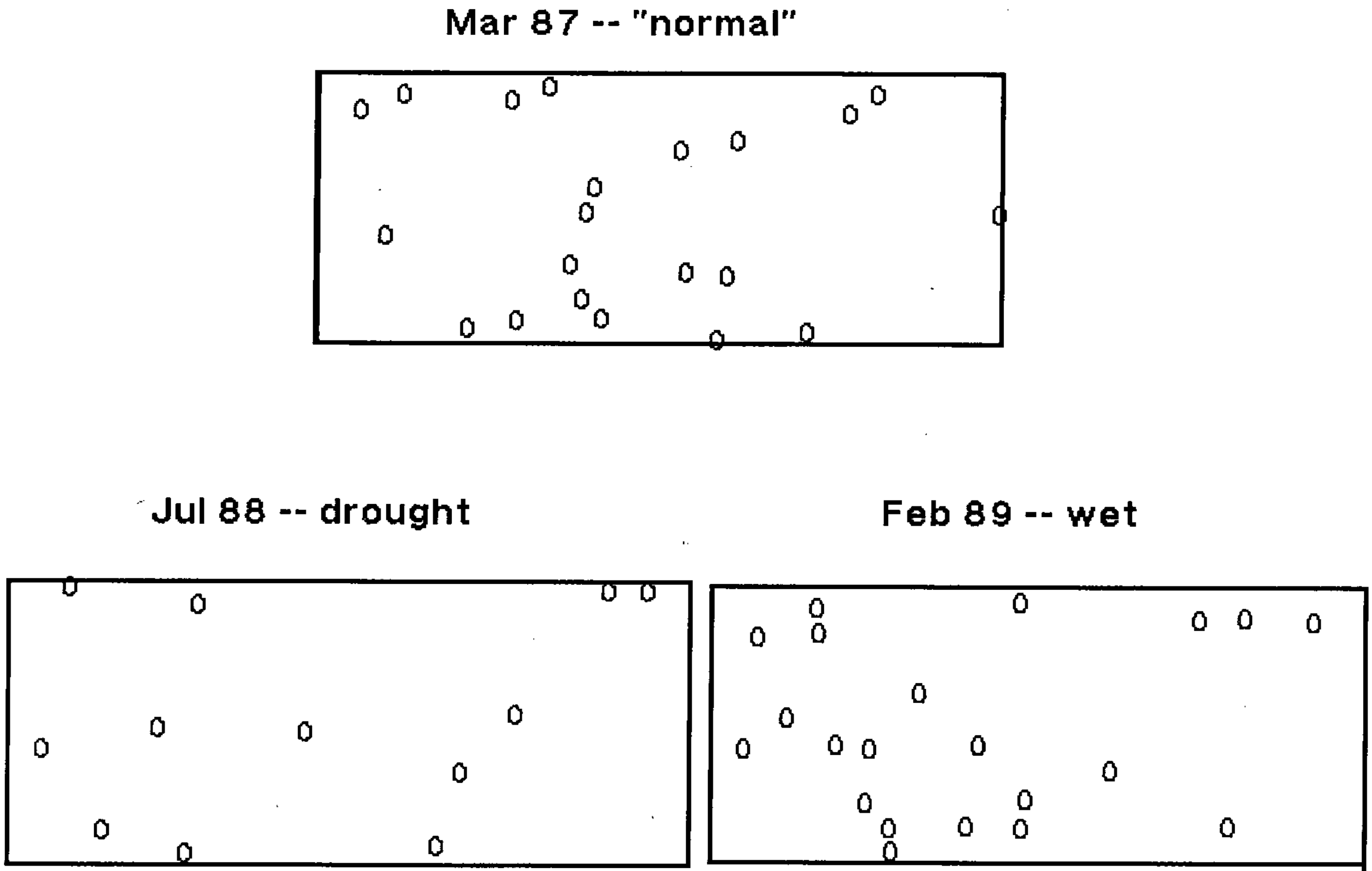

Figure 5. Composite plate showing all three counts taken of monogynous area showing weather effect.

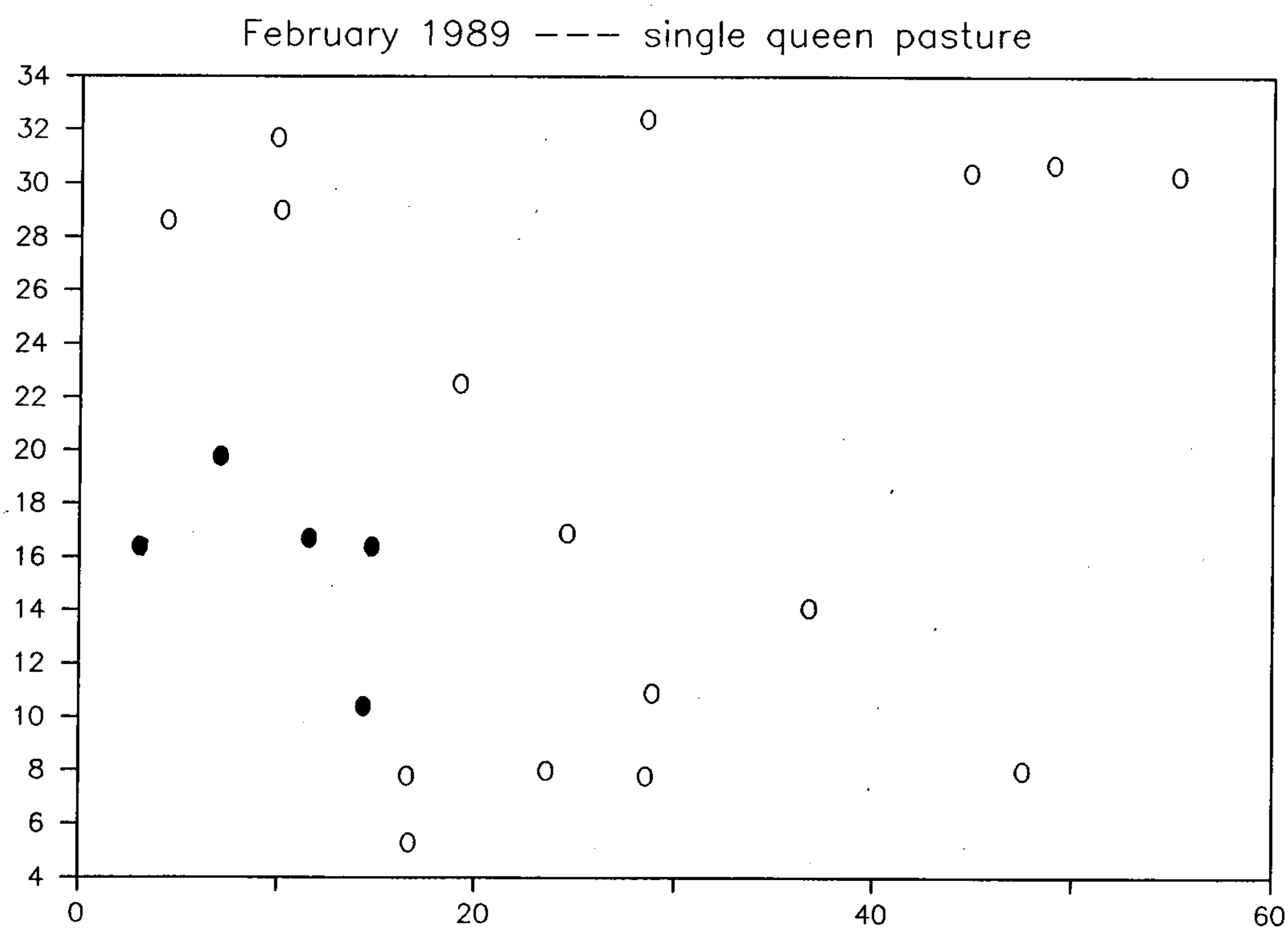

Figure 6. Invasion of single queen area by multiple queen colonies (Dark circles).

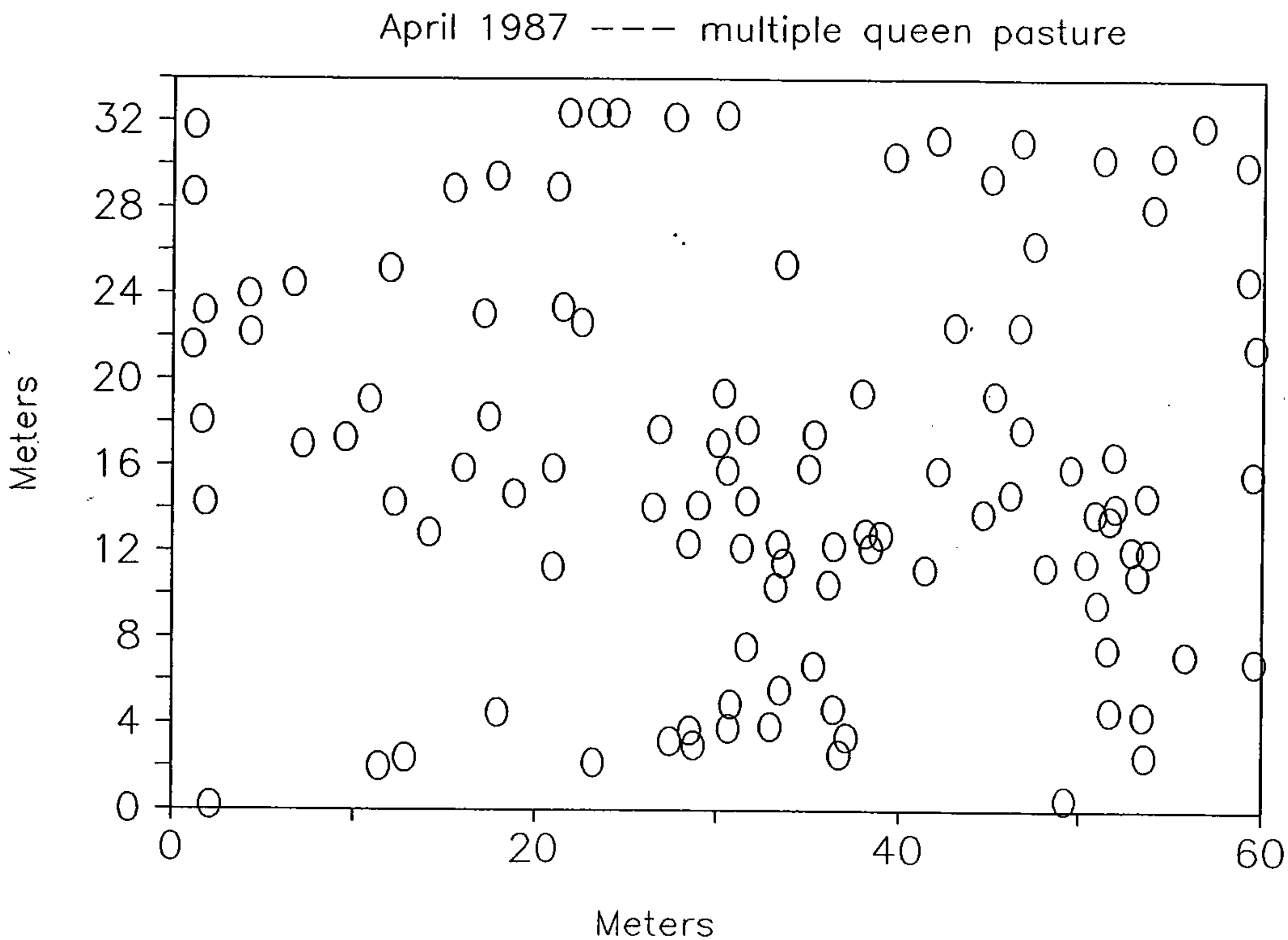

Figure 7. First count of polygynous area. N=110.

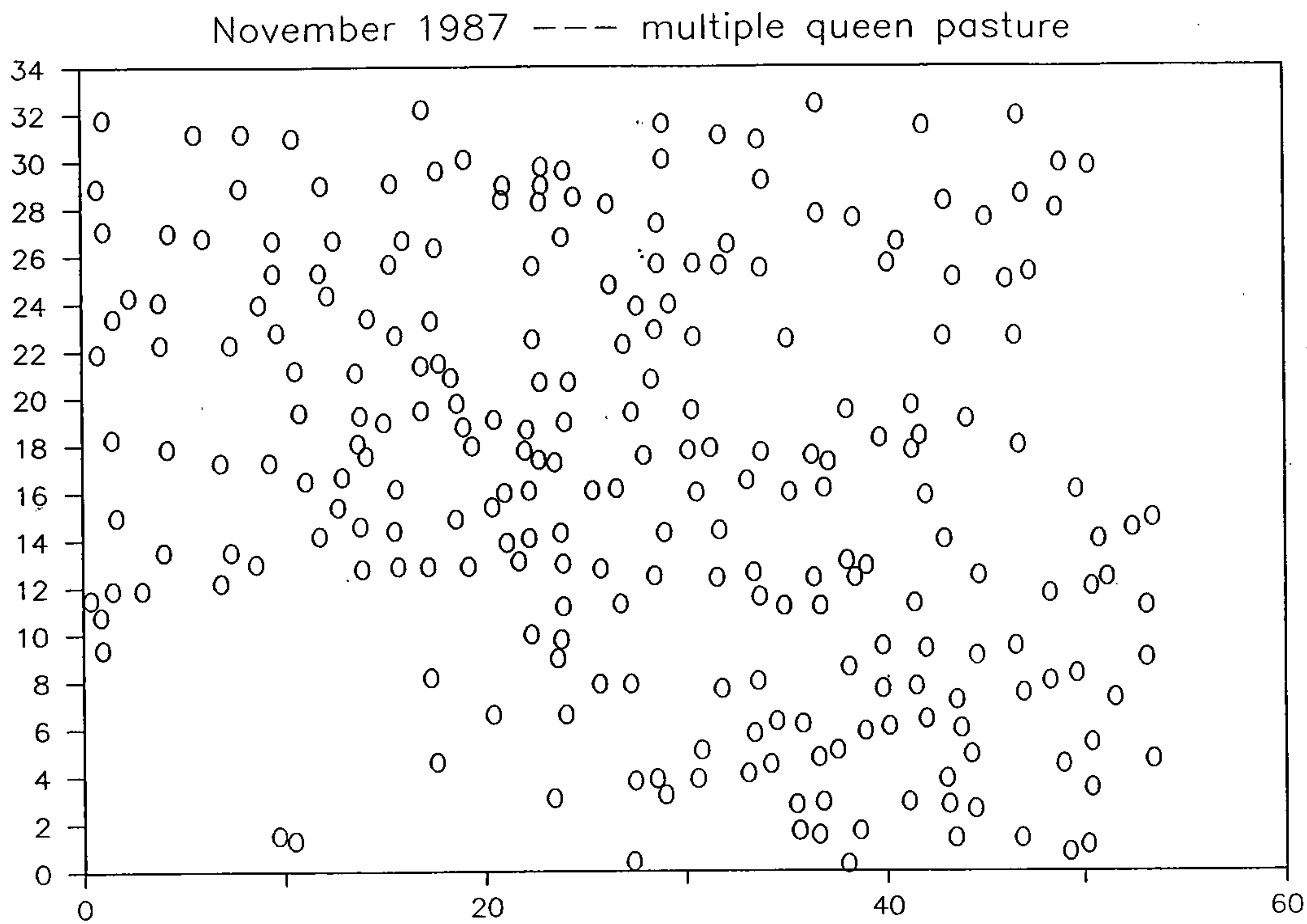

Figure 8. Second count of polygynous area. N=248.

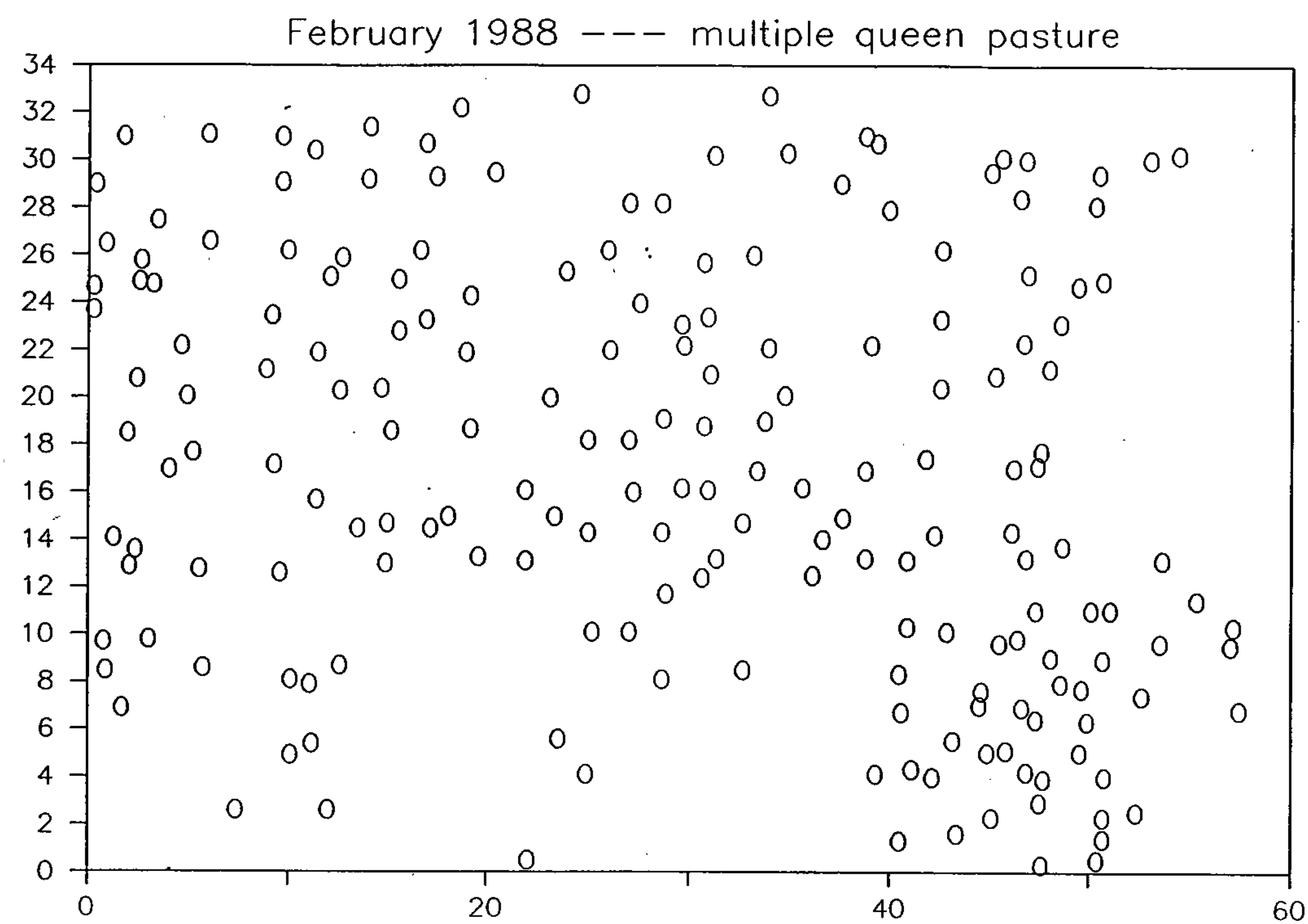

Figure 9. Third count of polygynous area. N=193.

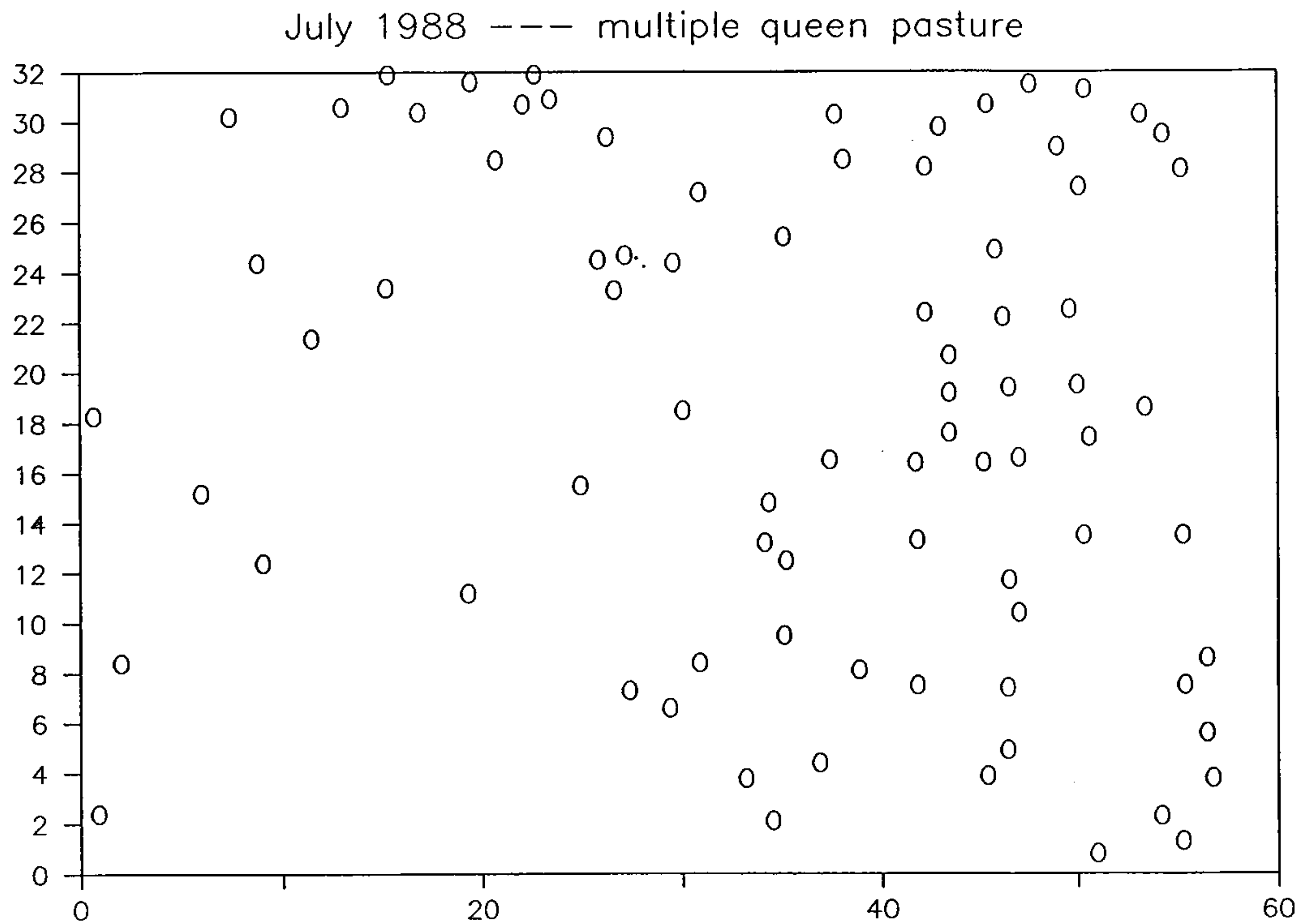

Figure 10. Fourth count of polygynous area. Count followed a 6wk drought period. N=81.

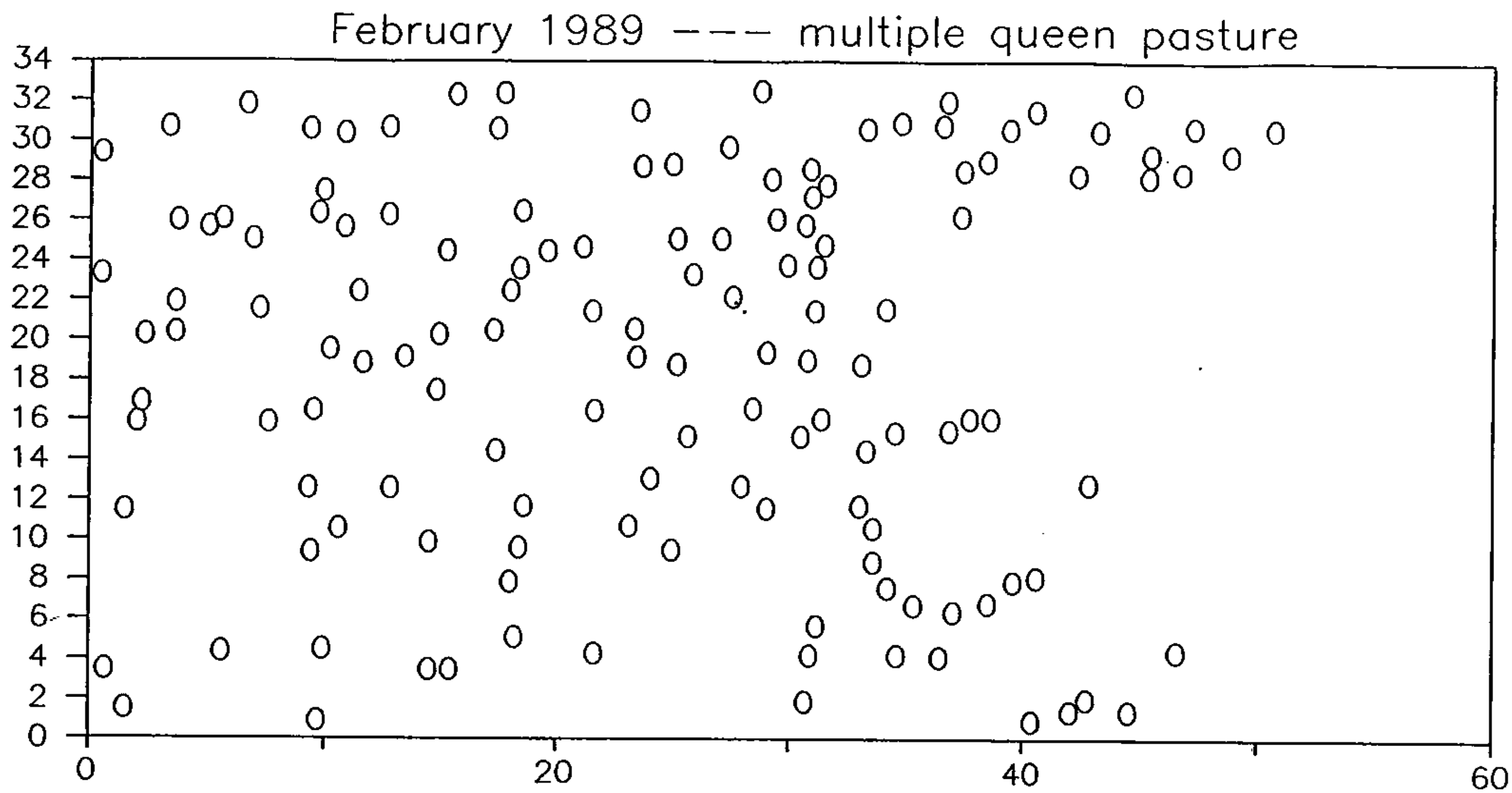

Figure 11. Fifth count of polygynous area. Count taken after heavy rain period. N=138.

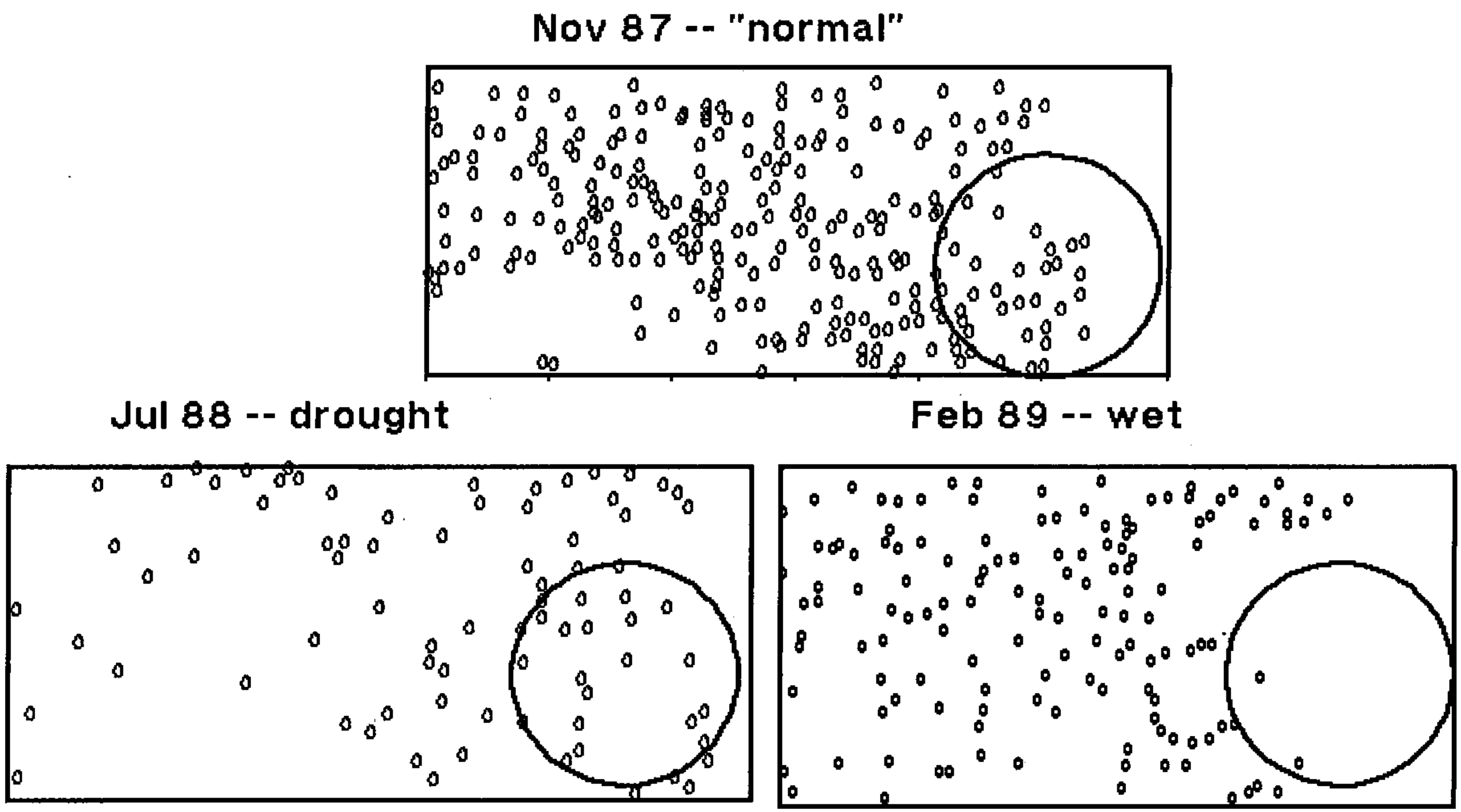

Figure 12. Composite of 3 counts of polygynous area showing effects of weather.

FIRE ANT VENOM, THEN AND NOW

Frank K. James, Jr.
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There are five species of fire ants in the continental United States, two "imported" of which *Solenopsis invicta* and *S. richteri* have successfully invaded the gulf coast area of the United States and are slowly but consistently spreading north and west. Efforts to truly control them have so far been basically unsuccessful. Severe reactions from fire ant stings have resulted in a number of human sting deaths which have now been documented. An article on the subject of human sting deaths has been accepted for publication in the allergy literature and is soon to be published.

Interest in fire ant venom has been described for over 30 years. They date back to the studies of Dr. Murray Blum at the University of Georgia. He described fire ant venom as being mainly an alkaloidal preparation. This alkaloid, since then synthesized, is 2,6 dipeperidine nucleus with a methyl group in the 2 position and an 11 to 16 member carbon alophetic side chain at the 6 position on the ring. Differences in the various structures of this alkaloid seem to reside mainly in the side chains. The number of carbons in the chain and the arrangement of the bonds in the stereo isomers account for differences between the 5 solinopsine species. It was felt by Dr. Blum and his group that the more highly evolved solinopsine species (*S. invicta and richteri*) had more highly evolved venom. We know now that this alkaloid causes pustular formation in human beings. The small protein components thought to represent less than one tenth of one percent of the venom were first evaluated by Dr. Harold Bear at the FDA. This fraction mediates the allergic (IgE) reaction.

The venom which causes toxic, hemolytic, necrotic, neurolytic, and truly allergic reactions (IgE) in human beings produces symptoms in very small amounts. Non allergic human beings are just as subject to reactions from this sting as is the general population. This is the "hooker".

Dr. Brad Vinson and Dr. Barry Paull at College Station, Texas (Texas A&M) have been studying fire ant venom sensitivity in particular comparing the whole body extract (WBE) with fire ant venom (IFV). Initially, their findings were like those found with our group in San Antonio. These both showed that the venom protein components were potent sensitizers many more times powerful than the whole body extract. They have also reported a few cases of failure of WBE immunotherapy i.e. sting reactions after using the whole body extract. I haven't had that experience treating over 50 patients with WBE in the last 20 years.

At Lackland Air Force Base, San Antonio, Texas, Dr. Richard Hyland has recently immunized patients with WBE, then sting challenged 25 patients with only moderate local response. There has also been some feeling that there are other materials in the whole body extract which are harmful to humans. Unlike the winged Hymenoptera, the whole body extract and the venom resemble each other closely save that the venom and its small protein components seem much more potent a diagnostic and therapeutic agent.

Vespa Laboaratories has perfected a method of extracting venom from the invicta workers enabling them to work on a larger pooled venom vaccine. This may be released in the near future. The possibility of using venom rather than WBE is under consideration. It will need FDA approval. Most of this work on collecting the venom in large amounts has been done by Miles Guronik at Penn State (*Vespa*).

Recent studies of Hoffman *et al.* in Greenville, North Carolina, utilized gel filtration and high performance cation chromatography have isolated four different protein peaks. These are termed, respectively, sol one, sol one two, sol one three, and sol one four. Physically and allergenic characteristics are being closely monitored.

My experience began nearly twenty years ago when a patient in Honolulu was stung by *Solinopsis geminata*. He suffered a severe systemic reaction from the sting. Later the first published study followed on the potent effects of fire ant venom on human beings. Appraising fellow physicians and other scientists that the fire ant sting was truly dangerous has been emphasized. Collecting fire ant sting death cases, mainly in Texas, has been slow coming -- averaging one sting death per year for the past 15 years. Since it is predicted that the IFA will eventually spread to and up the west coast we expect more reports of sting deaths in the future.

Work on the IFA venom has been long and hard (consider milking 4,000 IFA workers to get enough venom) but the rewards have been great and, I think, gratifying.

RELATIVE PHYTOTOXICITY OF DURSBAN 2.5G [chlorpyrifos] IN A STANDARD POTTING MEDIA TO SELECTED FOLIAGE AND WOODY LANDSCAPE PLANTS.

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In January of 1980, chlorpyrifos 5% granular (FA-5) was registered for use as a fire ant quarantine treatment for potting media. The registrant (Dow Chemical Company) elected to withdraw FA-5 from the market in 1981 when several cases of alleged phytotoxicity were alleged [1]. All of the cases reported were from central Florida and with one exception (Dwarf Yaupon, Ilex vomitoria) involved greenhouse grown foliage plants. Most of the reported injuries were linked to problems other than chlorpyrifos induced phytotoxicity.

Dursban 2.5G (chlorpyriphos) is currently the only insecticide registered for the control of the imported fire ant in commercially field grown balled and burlap and container grown plants [2, 3]. Because of the alleged phytotoxicity of Dursban, succulents must be grown "under cover" in order to meet federal quarantine requirements. This precludes large scale (outdoor) production of these plants. Trials were undertaken at the South Mississippi Branch Experiment Station at Poplarville to determine the phytotoxic potential of Dursban 2.5G against a wide variety of foliage and woody landscape plant species.

Materials and Methods

Fourteen varieties of plants were selected for evaluation of possible effects of chlorpyrifos on plant growth and phytotoxicity when incorporated into potting media. Plants were selected on the basis of availability, local popularity among commercial growers or previous history of phytotoxic response to chlorpyrifos.

The experimental media was Strong Lite Growing Medium manufactured by Stong Lite Products Corporation of Pine Bluff Arkansas. Ford's Dursban 2.5G was incorporated at two rates 1X [25.2 ppm] and 3X [75.6 ppm] the recommended rate for Dursban 2.5G. An experimental unit consisted of 2 containerized plants per plot with seven blocks per cultivar. An identical group of plants was also transplanted into pots containing untreated potting media. All plants were given a complete fertilizer [20-5-10] at the rate of 3.5 grams per container-plant on October 21 and November 16, 1988 and on January 9, 1989. Dolomitic lime was surface applied on October 31, 1988 at the rate of 8.4 grams per container plant. Foliage and woody landscape plants were placed in 3 quart containers. Each plant was coded using small white plastic stakes to aid in identification.

All plants were grown in a green house with a 62 F minimum temperature with maximum temperature approximately 20 F higher on sunny days. The plants were placed on raised benches and subjected to normal horticultural practices.

All potting media was prepared on 6 and 7 October, 1988. Planting of succulent varieties occurred on 10 October. Woody landscape plants were planted on October 18, 1988.

The following plants were evaluated in these trials:

SUCCULENTS

Antirrhinum majus
snapdragon
Begonia spp.
begonia
Brassica sp.
flowering kale
Dianthus sp.
pinks
Philodendrum oxycardium
philodendron
Saintpaulia ionantha
African viloet
Scindapsus aureus
ivy-arum

WOODY LANDSCAPE

Ilex crenata "Compacta"
Japanese holly
I. opaca "Savannah"
American holly
I. vomitoria "Straughns"
dwarf yaupon
Ligustrum lucidum
variegata
L. sinensis
variegata
Juniperus pfitzeriana
Nick's compacta
Rhaphiolepis indica
Indian hawthorn

Results

Visual examinations of each plant was made every two weeks. The phytotoxicity rating scale for these examinations is as follows:

1. Plants healthy, not different from the untreated check;
2. Slight yellowing, wilting or other mild symptoms such as marginal chlorosis;
3. Symptoms more severe, leaf drop or necrosis;
4. Severe stunting, abnormal leaf or stem structure;
5. Dead.

At the midpoint of the trial (December 9, 1988), half of the plants from each plot were sacrificed and evaluated based on a visual examination of the tops and roots as compared with measurements of the untreated check. A fresh weight measurement of each plant top (total biomass above the soil line) was carried out at the same time. No phytotoxicity symptoms were observed from any of the treatments. The woody landscape varieties *Ilex opaca* and *Rhaphiolepis indica* [Table 1] as well as the foliage varieties *Begonia*, *Philodendron oxycardium*, *Antirrhinum majus*, *Dianthus*, and *Scindapsus areus* [Table 2] each showed enhanced root structure in the treated trials. *Dianthus* particularily showed significant enhancement of root structure over the untreated check.

A final examination for phytotoxicity readings was made on February 8, 1989, (121 days after treatment) with the results given in Table 3 for foliage plants and Table 4 for woody ornamentals. The only damage noted was some stunting of *Saintpaulia ionantha*, the African violets. All other cultivars showed no relative damage. With the exceptions of the foliage plants *S. ionantha* and *S. aureus* and the woody landscape plant *L. lucidum*, all of the evaluated varieties showed enhanced root growth. It is possible that these results are due to the seasonal growth patterns of some of the varieties tested. Further tests will be made in the spring and summer to determine if these data are indeed an artifact of the experimental protocol.

Summary

Fourteen varieties of foliage and woody landscape plants were evaluated for phytotoxicity response to chlorpyrifos 2.5% granular formulation incorporated at 1X and 3X the required rate in a standard potting media. Phytotoxicity readings were made at ca. 60 and 120 days post-treatment. Only African violets showed any indication of a negative response to chlorpyrifos. Ten of the cultivars evaluated

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2. Tatum, David. 1988. Imported fire ant quarantine requirements. MS Dept. of Ag. and Comm., Div. of Pl. Ind. DPI-PLT. PEST-1S-03, 3p.
3. Imported Fire Ant Program Manual, M301.81 [Revised Aug. 1985], USDA-APHIS-PPQ, 29 p.

showed enhanced root structure in the treated tests when compared with the untreated check.

Table 1. RELATIVE EFFECTS OF CHLORPYRIFOS TO WOODY LANDSCAPE PLANTS AT DAY 60

	TOP BIOMASS			ROOT STRUCTURE		
	Check	1X	3x	Check	1X	3x
Ilex cornuta	20.0	21.4	21.3	2.00	2.00	2.00
Ilex crenata	16.3	17.2	16.7	1.57abc	2.29ab	2.00ac
Ilex opaca	18.0	15.6	14.9	2.00	2.00	2.00
Ilex vomitoria	14.3	13.7	13.3	1.86	1.86	1.86
Juniperus pfitzeriana	16.4	17.7	18.1	1.29ac	1.57	2.43ac
Ligustrum lucidum	12.9	12.4	11.9	1.86	1.86	2.00
Ligustrum sinensis	4.1ab	6.1ab	5.3	1.57ab	2.14ab	2.00
Rhaphiolepis indica	18.7	17.4	18.1	1.29ac	1.86	2.14ac

Table 2. RELATIVE EFFECTS OF CHLORPYRIFOS TO FOLIAGE PLANTS AT DAY 60

	TOP BIOMASS			ROOT STRUCTURE		
	Check	1X	3x	Check	1X	3x
Antirrhinum majus	87.6	86.7	64.2	1.67ac	2.00	2.17ac
Begonia sp.	188.3	187.5	168.7	1.00ac	1.43	2.43ac
Brassica capitatus	156.5	164.7	137.8	1.33abc	2.33ab	2.00ac
Dianthus sp.	36.0	41.8	57.2	1.00ac	1.40bc	3.001bc
Philodendron oxycardium	44.3	40.9	50.1	1.57ac	1.43bc	2.43abc
Scindapsus aureus	48.7	63.9	55.0	1.00abc	2.43ab	2.14ac

Table 3. RELATIVE EFFECTS OF CHLORPYRIFOS TO WOODY LANDSCAPE PLANTS AT DAY 120.

	TOP BIOMASS [grams]			ROOT STRUCTURE		
	Check	1X	3X	Check	1X	3X
Ilex crenata	24.6	24.0	23.3	1.71	2.00	2.29
Ilex opaca	17.9	16.0	13.7	2.00	1.86	2.43
Ilex vomitoria	21.1	21.9	20.6	1.86ac	2.00	2.43ac
Juniperus pfitzeriana	26.0	27.1	31.1	2.00	1.86	2.14
Ligustrum lucidum	12.9	13.9	14.0	2.14	2.00	2.00
Ligustrum sinensis	8.0	7.4	7.3	2.00	2.00	2.43
Rhaphiolepis indica	32.0	34.6	37.0	1.71ac	2.00bc	2.57abc

Table 4. RELATIVE EFFECTS OF CHLORPYRIFOS TO FOLIAGE PLANTS AT DAY 120.

	TOP BIOMASS [grams]			ROOT STRUCTURE		
	Check	1X	3X	Check	1X	3X
Antirrhinum majus	1674.	210.8	137.2	2.17	1.83	2.50
Begonia sp	573.5	604.9bc	507.6bc	1.63abc	2.13ab	2.38ac
Brassica capitatus	247.5	243.0	221.0	1.50ab	2.17ab	2.33
Dianthus sp.	98.3	126.3	92.2	1.00ac	1.50	2.50ac
Philodendron oxycardium	102.3	117.0	114.5	1.63	2.13	2.25
Saintpaulia Ionanatha	81.6ac	71.9	57.2ac	2.45abc	1.89ab	1.89ac
Scindapsus aureus	170.0	186.1	164.6	2.25	1.75	2.00

NOT FOR PUBLICATION

Amount of Bait taken by Red Imported Fire Ants: Effects of Alternate Food, Addition of Toxicant, and Season

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Fire Ant Research Conference
Broadwater Beach Hotel
Biloxi, MS

April 18-19, 1989

Many fire ant researchers have found that fall bait applications are less effective than spring applications. It is often assumed that this is because increased brood production increases food demand in the spring. This is certainly a factor, but another major factor might be availability of other foods. These would compete with any bait for the ants attention.

Since insects are the red imported fire ants (*Solenopsis invicta* Buren), major food, increased insect populations in the summer and fall could reduce interest in a bait.

Several people, the authors included, have noticed that "airport ants" are easier to kill with baits. The airports we have run tests on have been on very poor soil. It looked like the topsoil had been removed and plant cover was very sparse. Insect populations were very low due to the poor natural productivity of the area. RIFA mounds were much smaller and less dense than in a pasture, where other insect populations are high. So, these ants were stressed for food, eagerly took any bait offered, and were easily killed.

The study reported on today was conducted to test the theory that as insect biomass increases bait acceptance decreases. Also, toxic (Amdro®) and blank baits were offered throughout the test to determine if RIFA descriminate between the two. In many previous short term tests RIFA have always "preferred" a blank bait over an identical one containing any type of toxicant. Experimental baits containing organophosphate insecticide were usually refused altogether. Amdro® was used as the toxic bait in this study because it is well known. It has performed as well as, or better than any production toxic bait we have tested for palatability.

In this study seven areas were observed for two growing seasons, almost two years. Since it was more complete, and for the sake of brevity, only the second year (1986) is reported here. Six of the areas were in cattle pastures and one was in a fallow field. Each study area was large enough to contain about 12 50' square plots with about 50' between these plots.

Monthly measurements of bait uptake by RIFA were taken with blank bait and Amdro® in each study area. Blank bait was prepared with materials provided by American Cyanamid Company. It was formulated similar to Amdro® with 30% soybean oil-oleic acid mixture and 70% corn grit by weight, but with the toxicant ommitted. Blank bait uptake was always evaluated in the same plot within each area. This was done to reduce variability in this measurement. But uptake of Amdro® was measured in a different plot each month to avoid sampling an ant population that had been diminished by offering Amdro® earlier. To sample bait uptake in a given plot 50 grams of the appropriate bait was placed in each of 5 small paper souffle cups. These cups were distributed over the plot, placed in contact with the soil and left for 24 hours. They were then retrieved, weighed again and weight of bait taken was calculated. RIFA were the only organisms observed taking these baits.

Pitfall traps were also operated monthly in two plots per study area. Five pitfalls were distributed in the Amdro® (mobile) plot and five in the blank bait (stationary) plot. These were placed when 50 gram bait samples were put out, but were left for 72 hours, and then retrieved. Pitfall catches were sorted into RIFA and other insects. The RIFA were counted. Other insects were washed of pitfall fluid with water, air-dried for ca. 24 hours and then weighed and identified to families.

Results of all of these measurements are given in figure 1. The most apparent trend here, is that Amdro® (the representative toxic bait) is taken in lower quantities (ca 1/3) than a blank, nontoxic bait, throughout the year. Again this is not to say that Amdro® is not an effective bait. We have seen similar trends, or worse, with any toxic bait we have evaluated against a blank. Although the toxic bait was strongly discriminated against, both baits usually followed the same month to month upward or downward shift.

But the main objective of this study was to evaluate the effect of biomass of other insects (alternate food) on bait uptake by RIFA. Until May all 4 measurements were increasing, probably in a general response to warmer weather and the beginning of the growing season. In June insect biomass increased dramatically and toxic bait uptake took its first significant[1/] downturn. Blank bait uptake continued in June. From June onward the two bait trends, especially the toxic bait, appeared negatively correlated with available insect biomass. The blank bait trend did not agree with this pattern again in July and November. At all of these times insect biomass was ca. 15-20 grams per area. Perhaps when insect biomass (natural food) is at this intermediate level the blank bait is still relatively attractive, but the toxic bait is

[1/]not used as a statistical term here

discriminated against. The generally strong upward trend for all measurements through May indicates that total RIFA food supply was less than demand during that period.

Numbers of RIFA foragers caught in pitfalls also began to decline after May. From then on it seems to be negatively correlated to biomass of other insects but not as strongly as bait uptake. The decline in foraging activity after May is another indication that food supply exceeds demand in summer and fall. Overabundant natural food would require less searching to satisfy a colony's needs.

Although these results have not been analyzed statistically, the following trends are indicated:

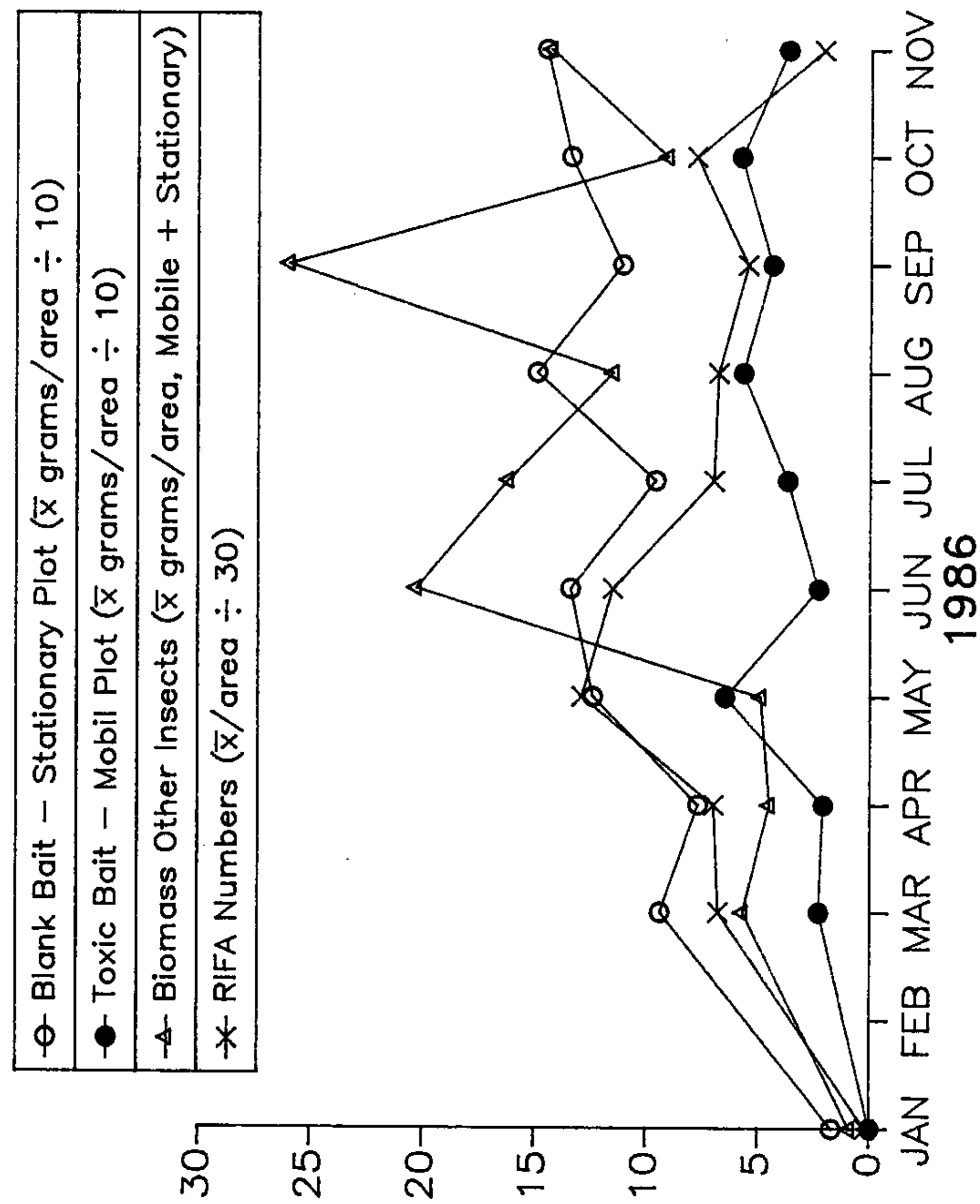
1. Toxic baits are discriminated against, especially if other foods are available.
2. When insects are abundant after "spring buildup" (May in this study) fire ants spend less time foraging and take less bait.

These trends suggest two steps to make baits more effective. These are not new ideas, but now the reason they are necessary may be more clear.

1. Apply baits as early in the year as fire ants will take them.
2. Determine the most palatable toxic formulation in a field situation with alternate foods available.

Figure Caption

Figure 1. Trends of blank and toxic oil baits uptake by RIFA, and of RIFA forager numbers and biomass of other insects caught in pitfalls; Tift and Irwin Counties, 1988.


Blank Bait – Stationary Plot ($\bar{x}$ grams/area ÷ 10)
Toxic Bait – Mobil Plot ($\bar{x}$ grams/area ÷ 10)
Biomass Other Insects ($\bar{x}$ grams/area, Mobile + Stationary)
RIFA Numbers ($\bar{x}$/area ÷ 30)
0
5
10
15
20
25
30
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV
1986

TEXAS DEPARTMENT OF AGRICULTURE

1989 IMPORTED FIRE ANT PROGRAM

BY ROGER MULDER, TDA PEST MANAGEMENT PROGRAM

The Texas Department of Agriculture's Imported Fire Ant Program is continuing much of the work initiated in recent years, as well as taking on several new projects.

TDA continues to work with USDA on Compliance Agreements with Texas nurseries shipping to non-infested areas. In the past year our inspectors have signed 90 nurseries to the Compliance Agreement, which stipulates what treatment methods they must follow before shipping any nursery stock to a non-infested area. Although the program is working fairly well, there are a few problems.

We have had two nurseries, one in Premont in South Texas and the other in Von Ormey (Aldridge) near San Antonio, caught shipping fire ants to Arizona, even though both were under the Compliance Agreement and both were following a treatment protocol. In both instances, they were shipping balled and burlapped trees which must either be dipped in a chlorpyrifos solution or drenched twice a day for three consecutive days. Both nurseries were simply spraying once before shipping.

The other nursery, Green Leaf in El Campo, Texas, southwest of Houston, was caught shipping fire ants to Memphis, Tennessee. It, too, was under the Compliance Agreement and was shipping containerized plants in pre-mixed potting soil, again using the chlorpyrifos formulation. After reviewing the situation, it would appear the 24-month certification period for chlropyrifos in the pre-mixed potting soil may be too long. Soil samples were pulled and sent both to the USDA lab in Gulfport and the TDA lab in Brenham. It should be pointed out only a handful of worker ants were found in the Green Leaf shipment, but there was one queen found by inspector Mark Trostle, as well. In both of the shipments sent to Arizona, whole colonies were being shipped inside the balled and burlapped trees.

We are currently finishing up this year's federal survey for newly infested counties. Year before last, we added about 26 counties to the quarantine, but last year we added just two. This year, it looks like several more will be added, but the rate of spread is slowing down a little, primarily, it would appear, because the ants are getting into fairly dry West Texas where they are not expected to do as well. As of today, about 130 counties covering about 60,000,000 acres in Texas are infested with fire ants.

Our inspectors also did a re-survey of currently infested counties, in order to compare data with last year's multiple queen - single queen survey. Much of Texas is currently infested with multiple queen colonies, causing significant wildlife problems due to the extremely high population densities. Because of those extremely high population densities, TDA continues to generally recommend the use of a broadcast application of one of the bait formulation products in order to knock the population densities down to tolerable levels. We recommend the baits because they're effective, because only a pound to a pound and a half per acre needs to be applied, because they do break down rapidly in the environment, and because they allow the most time between re-treating, up to a year when using the growth regulator product.

The reason we recommend a broadcast application of the baits is because there are usually mounds just starting in an area not yet visible, but which have foraging ants looking for food to the feed the queen. A mound by mound application of the baits relies on knowing where the foraging or exit tunnels are located, which is not always feasible. The only time we generally don't recommend a broadcast application of the baits is when other competing ants are still in the area, thus helping to hold back the invasion, and during the cold winter months, when little foraging activity is taking place.

We have put together a one-page handout on the do's and don'ts of applying baits.

Last summer, we successfully treated two state parks, using the bait formulation growth regulator. One of the parks, Brazos Bend State Park near Houston, contains many wildly diverse species of wildlife, including alligators, wild hogs and deer. As it says in the instructions when entering the park, when attempting to escape an alligator, back up slowly and do not stumble or fall.

Because we were able to demonstrate our success, the Texas Parks and Wildlife Department has decided to expand the model treatment program to 30 state parks this year. Again, we will be using a broadcast application of the growth regulator product. Our inspectors will be employing Herd spreaders on the back of their pick-up trucks, Herd spreaders on John Deere 5-wheel all-terrain vehicles, and Whirley Bird and Cyclone hand spreaders to treat the parks. We will be walking transects and follow up on repeat inspections to document the effectiveness of the program. We hope to start treatment later this month.

We're still working with our sod farms, although a smaller task force was sent into the Bay City area this year compared to the last two years. One reason for that is because so many of the sod producers have simply gone out of business. On the other hand, those few still left do have signficant fire ant problems and do need our constant attention. The two biggest sod producers, Milberger Turf and Triangle Turf, have both adopted our philosophy of attempting to knock their population densities down with a broadcast application of the bait formulation growth regulator. In the case of Milberger Turf, his annual chemical bill went down a total of $89,000.

Finally, I'd like to mention the Fire Ant Advisory Board, created by the 1987 Texas legislature. It's goal is to assist in the finding of natural, biological means to manage fire ants. The actual nine-member Advisory Board was appointed by the Governor and Commissioner of Agriculture, but they have relied heavily on the advice of Dr. Sanford Porter, Dr. Brad Vinson and Dr. Sherman Phillips in putting together their program.

As of today, the goal is to raise $5 million, in both public and private funds, endow the fund, take the interest and award it to those scientists submitting the best research proposals. That program specifically includes establishing additional researchers in South America as well as taking South American researchers and bringing them into the United States. This is an ambitious program and it should be noted no funds have been raised so far.

Finally, we have put together a four and a half minute music video on our philosophy of managing fire ants. We do intend to distribute this video to anyone and everyone interested in receiving a copy, and we hope you use it often.

HOW TO SAFELY AND SUCCESSFULLY MANAGE FIRE ANTS
(without resorting to the use of harsh chemicals)

Some Suggestions on a Commonsense Approach
by the Texas Department of Agriculture

The Red Imported Fire Ant can be a painful pest, but there are ways to successfully manage it without having to resort to the use of harsh chemicals. Following the Texas Department of Agriculture's management techniques, most people will be able to spend a few minutes each spring and then not have to worry about fire ants for several months or even a year.

YOU CANNOT ERADICATE THE FIRE ANT, BUT YOU CAN ERADICATE THE PROBLEM

Eradicating the fire ant is no more likely at this time, than eradicating mosquitoes, fleas or cock roaches. In some instances it's not even desirable to aim for eradication. Fire ants have proven to be immensely beneficial for cotton farmers and sugar cane growers fighting boll weevils and sugar cane borers, and many cattle producers recognize that they no longer have a tick problem thanks to fire ants.

The Department's goal is to help Texans manage their fire ant population densities, bringing them down to tolerable levels by using safe and effective methods and without having to resort to using harsh chemicals. For some individuals the tolerable population density in their back yard may be zero if they or their children are allergic to fire ant stings. For others, the tolerable densities could be fairly significant, especially if they have well-established single-queen colonies in large pastures, and the single-queen colonies are holding back an invasion of multiple-queen colonies.

Single-queen colonies are territorial and frequently maintain densities of 50 colonies per acre, or less. Multiple-queen colonies are not territorial and frequently have population densities of 300 to 600 or even 800 colonies per acre. When population densities reach the level of hundreds of colonies per acre, a dramatic reduction in the diversity of wildlife can result. Not only will other beneficial species of ants disappear, but so will other insects, lizards, snakes, rabbits, birds, deer and other forms of wildlife.

BROADCAST APPLICATION OF A BAIT

When population densities have reached an intolerable level, whether the location is your yard, a pasture, a park or wherever it may be, a broadcast application of a bait may be the best solution.

All of the baits contain soybean oil, which attracts the ants, on a corn grit base, which serves as the delivery behicle. The chemical in the bait is applied to the soybean oil. The baits are designed to be picked up by the foraging ants looking for food, taken back to the colony, passed through the food chain and fed to the queen. The only way to eliminate a fire ant colony is to kill or somehow neutralize the queen or queens. The baits have the advantage of being designed to be fed directly to the queen or queens.

A broadcast application of a bait has several other advantages over other treatment techniques. Because the recommended treatment rate is only a pound to a pound-and-a-half per acre, and because baits take longer to knock out the colonies, you're applying a minimum of pesticides and the interval before a reinfestation occurs will be much longer compared to individual mound treatments. When treating mound-by-mound, there will always be new infestations just getting started, small mounds which might be missed or mounds hidden in areas that cannot be seen. With the broadcast technique, the foraging ants will do the work for you by taking the baits back to the queens.

A one-time application in the spring, using the growth-regulator bait formulation product, should be all the treatment needed for the year. Regardless of which bait is used, a spring application is ideal because it knocks out the alates, or new queens, before they can leave on their nuptial flights and establish new colonies. When using baits other than the growth regulator chemical, a follow-up application in late summer or fall may be necessary.

BAIT LIMITATIONS

TDA has found that each of the baits is about 90 percent effective when properly used, meaning that a few mounds may be left even under the best of conditions. However, a broadcast application of the baits will get the population densities down to both tolerable and manageable levels so that follow-up treatment techniques can be applied. In some instances, simply using boiling water to drench the few remaining colonies in your yard can be effective.

HOW AND WHEN TO USE THE BAITS

When applying any of the baits, the ground must be dry, with no forecast of rain in the next few hours, and the ground temperature should be between 70 and 95 degrees Fahrenheit.

If the ground is wet, the bait material is not suitable to be picked up by the foraging ants. If the ground temperature is either too cold or too hot, the ants will not be actively foraging and little of the bait will be picked up.

If the temperature is too cold, wait for a warm day. If the termperature is too hot, wait until the cool of the evening, after the sun has gone down, and then make the application. If an application is needed July through September, it will probably have to be done in the cool of the evening. The baits rapidly degrade on hot, sunny days. A daytime applicaiton in the heat of the summer would result in the product breaking down and losing its effectiveness before the ants could pick it up.

One of the best methods of determining if it's the right time to apply a bait is simply to put out a scoop of peanut butter, tuna fish, or cheese. Check the food after 15 to 20 minutes. If it's covered with fire ants, then they are clearly out foraging and it is a good time to treat.

DRENCHES: THE MID-MORNING SNEAK ATTACK WORKS BEST

Occasionally a broadcast application of a bait is not the appropriate technique, especially if there is an ant infestation that needs an immediate solution. For example, a large fire ant colony under a picnic table which is to be used soon can be effectively treated by drenching the mound with boiling water. One problem is that the boiling water will kill the grass as well as the fire ants.

Under no circumstance shoud the mound be disturbed before treating it. Any disturbance will result in the colony immediately taking the queen to safety, either deep down in the mound or by moving to a satellite mound. You may have to sneak up on the mound to treat it.

A mid-morning drench treatment is usually best, especially if that is when the sun has started to warm the colony. The queen or queens and the brood are being moved up and down inside the colony as the temperature changes. In the morning, as the sun starts to shine on the mound the colony should be located just under the upper crust on the side of the mound facing the sun.

The colony should be drenched with enough liquid so that the mound caves in on top of itself--this usually takes from 1-3 gallons.

STRAIGHT TALK

BY MIKE MOELLER

DEPUTY COMMISSIONER
TEXAS DEPARTMENT OF AGRICULTURE

Putting Up with Fire Ants

Thoughts of revenge and retaliation cross most people's minds when they come in contact with fire ants. Numerous and painful stings have sent many picnickers, backyard gardeners, ranchers and farmers running--first for cover, then for a "knock 'em dead" solution to the problem. And this is where their frustration really begins.

Experts caution that the fire ant is here to stay. In the 40 years since its arrival in the U.S. from South America, scientists have been studying *Solenopsis invicta*, the red imported fire ant. Much has been learned about this fierce little insect, except the most important thing --how to control its spread without the heavy use of harsh chemicals.

Research has shown that the heavy chemical treatments popular until the late 1970s may have had greater detrimental effects on the environment and other wildlife than on the ant itself. Its geographic adaptability, high rate of reproduction and lack of natural predators have made this small ant a huge problem throughout all of the Southeastern states and most of Texas.

Fire ants spread from late spring to fall when new queens fly in search of nesting grounds, usually a day or two after a heavy rain. Because of these flights, fire ant infestations must be dealt with regularly and patiently.

A variety of chemical products are available for fire-ant treatment in the form of bait formulations, injectable products or mound drenches. Bait formulations contain either a growth regulator or a slow-acting poison. Baits must enter the ants' food chain and, therefore, may take several weeks or more to eliminate a mound. A broadcast application of bait in heavily infested areas allows the ants to do the work for you. The foraging ants will carry the poison or growth regulator directly to the queen.

Mound drenches work to poison the mound over a few days time, but they are more toxic than bait formulations. Some experts recommend pyrethrum-based fire ant control products. Pyrethrum is a natural toxin derived from flowers in the Chrysanthemum family. Because of its low toxicity and because it breaks down quickly in the soil, it is a good choice for treating fire ant problems in vegetable gardens.

If you choose to use chemicals, follow label directions to the letter. Use only the amounts and concentrations recommended, and avoid contact with clothing, eyes and skin. Rubber gloves are recommended. If you use a drench, don't use the container you mix it in for any other purpose.

As unusual as it may sound, there is an economical, non-chemical way to get rid of the fire ant mounds in your yard. Pour two to three gallons of boiling water into the middle of the mound. Any grass that might be damaged by the hot water usually grows back quickly. This method is particularly effective when the ants are building new mounds or when you have a minor infestation. The hot-water treatment may have to be repeated the following week if the queen was too far underground the first time.

No matter what method of treatment you choose, there are a few important facts to remember.

- **To destroy a fire ant colony, you have to kill or neutralize the queen.** Some mounds have many queens. Multiple queen mounds average 20-60 queens each.
- **To kill the queen ants, you must approach the mound very quietly.** When fire ants sense vibrations on or above the ground, they may quickly move their queens deep inside the mound for protection.
- **Time of day is important for successful treatment.** In the spring, the best time to treat is mid-morning to late afternoon, when the ants and queens stay close to the warm surface of the ground. During the summer months, the ants move deeper into the ground to escape the intense heat. So in hot weather, it's best to treat a mound during the cooler parts of the day--early morning or late evening--when the ants are nearer the surface.
- **Treatment must be on-going.** Keep a careful watch on your property. Be prepared for continued treatments as new mounds get established or old ones move to new locations because they have been disturbed.

Specially trained Texas Department of Agriculture inspectors are working with homeowners, farmers, ranchers, school and park personnel, and extension agents to lessen the impact of fire ants on the lives of all Texans.

For more information about fire ants contact your nearest TDA office.

Texas Department of Agriculture
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GROUND APPLICATION OF BAIT TOXICANTS TO TEXAS SOD FARMS

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1989 Imported Fire Ant Conference
Biloxi, MS.

ABSTRACT
The different bait products are known to eliminate colonies of imported fire ants (Solenopsis invicta) when applied under optimum conditions. The department wanted to demonstrate that the baits would aid in the reduction of fire ant colonies in sod farms and allow sod producers to ship a cleaner product to the consumer. These results indicate that LOGIC is the most suitable product for large-scale or long term control programs. The other two products, AMDRO and AFFIRM controlled the fire ants, but repeated applications would be necessary over the growing season.

I. INTRODUCTION:
Eleven treatments (four formulations of AMDRO, five formulations of LOGIC, one formulation of AFFIRM and an untreated Check) were evaluated at eight sites in the late summer and fall of 1987 series of trails. All treatments were applied in August except Plot I, Subplot 1-14 which were treated early July. The TDA field staff used Chevrolet S-10 pickups, equipped with the Herd Spreader Model 77-A.

II. EQUIPMENT SPECIFICATIONS:
1. Chevrolet one-half ton S-10 pickup.
2. Delivery system--Herd spreader, Model 77-A.
3. Eleven to twelve miles per hour operating speed of S-10 pickup.
4. Herd spreader switch at full speed.
5. Twenty-four foot working swath.
6. Operated by TDA Inspectors Tavo Garza; Lisa Alexander; Tony Dooley and assisted by Mel Clark, Robert Dixon and Mark Trostle.

III. GUIDANCE:
Vehicle guidance and swath spacing were provided by flag persons.

IV. TREATMENTS APPLIED AND TEST SITES:
Treatments were formulations of AMDRO, LOGIC and AFFIRM. Test sites were Sod Farms located in Matagorda, Wharton and Brazoria counties.

V. PROCEDURES USED TO DETERMINE EFFICACY:
A. Prior to treatment fourteen one-fourth acre efficacy plots were established within each test site. The center of each efficacy plot was marked with a wooden engineering stake with a yellow plastic lid attached by a roofing tack and a wire survey flag. Sketches showing locations and distances of each plot were done to facilitate location of the center stake during the post treatment assessment.
B. While pivoting around the center stake, a team of 8 to 9 people closely searched each plot for the presence of IFA colonies. After locating a colony, the mound was opened with a shovel and the types of relative abundance of live forms present were observed.
According to Banks et al. (1981), presence of worker brood in an ant colony is strong evidence that the queen is present and the colony is normal. Williams et al. (1979) reported that AMDRO* may cause death of the queen without total elimination of the worker force. In theory, such a colony will eventually die unless the queen is replaced by adoption of a newly mated queen. The colony classification system described by Lofgren and Williams (1982) was used to categorize all IFA colonies to compute population indices before and after treatment.
Two types of data were recorded for each subplot examined:
(1.) Total number of ant colonies present.
(2.) The relative size and type of colonies present.

Quite obviously, a fire ant population composed of predominantly Class 25 colonies with a population density of 50 colonies per acre represents a far greater threat and nuisance to man than would a population comprised of predominantly Class 2 colonies with a population density of 10 mounds per acre.

VI. RESULTS AND DISCUSSION:
Results of this test are shown in Table 1, 2 and 3. These results show AMDRO* provided far better early control at all test sites than did the other formulations due to it's mode of activity. Colony mortality with the AMDRO* averaged 88% eight weeks after application and showed a -92% change in population index. The LOGIC averaged 55% colony mortality at eight weeks and a -92% change in population index. The AFFIRM averaged 57% colony mortality at eight weeks and showed a -89% change in population index.
Applications of LOGIC and AFFIRM applied late summer to fall will result in a long delay in control of IFA colonies. Maximum results with these two products will not be achieved until mid-summer of next year. Spring applications demonstrate the most desirable results when using LOGIC and AFFIRM.

The 35 week evaluation took place during April 11 thru 12, 1988. The evaluation at 35 weeks on the various bait products yielded the following results. AMDRO* provided an average percent colony mortality of 86% and a average percent change in population index of -92%. The LOGIC* averaged 81% colony mortality and a -98% change in population index. The AFFIRM* averaged 47% colony mortality and a -87% change in population index. The CHECK averaged a 56% increase in active mounds and a plus 27% increase in population index.

The only product which showed a increase in control at 35 weeks was the LOGIC*. LOGIC* increased from a average of 55% colony mortality at 8 weeks to a average of 81% colony mortality at 35 weeks. The population index increased from -92% at 8 weeks to -98% at 35 weeks.

LOGIC*

One year and thirteen days (August 1988) after treatment, mound mortality of LOGIC ranged form 91% to 46% depending on test site locations. In general, a significant reduction of fire ants was noted still occurring in the test sites. LOGIC* had a overall average of 68% mound reduction. The average percent change in population index was -87%. The LOGIC* sites had a pretreat average population index of 12,135. The average at 1 year and 13 days was 1604.

AMDRO*

The AMDRO* mound mortality ranged form 47% to -32% depending on test site locations. In general, a re-infestation of fire ants was noted to be occurring in the test sites. AMDRO* had an overall average of 15% mound reduction. The AMDRO* sites had a pretreat average population index of 3871. The average at 1 year and 13 days was 2518. The average percent change in population was -35%.

AFFIRM*

The AFFIRM* mound mortality was 29%. The AFFIRM* had a average percent change in population index of -65%. In general, a re-infestation of fire ants was noted to be occurring in the test site.

The one year and thirteen day data was corrected for natural mortality by Abbott's formula as there was a reduction in the CHECK PLOT POPULATIONS.

SUMMARY

The Texas sod producers can reduce the imported fire ant populations by applying bait products to their sod. These baits will allow the sod to be sold to the consumer with less fire ants, assist in slowing the movement of fire ants and most importantly allow the sod producer to economically treat for fire ants over a large area.

TABLE 1.

TEST SITE	TOXICANT	PRETREAT COLONIES /SUBPLOT	AVG. COLONIES PER SUBPLOT	AVG. POP. INDEX/SUB-PLOT	AVG. % COLONY MORTALITY	AVG. % CHANGE IN POP. INDEX	NUMBER COLONIES WITH SURVIVING WORKER BROOD
I 1/	LOGIC	458	22.9	308	59	-89	14 out of 188 2/
II	AMDRO*	148	10.5	155	97	-97	3 out of 5
III	AMDRO*(85)	122	8.7	122	81	-85	20 out of 23
IV	AMDRO*	145	10.3	170	94	-96	7 out of 8
V	AMDRO*	205	14.6	236	79	-91	14 out of 44
VI	AFFIRM	178	12.7	209	57	-89	7 out of 77
VII	CHECK	343	24.5	421	+13	+12	384 out of 386
VIII	LOGIC	91	6.5	102	59	-93	0 out of 36
IX	LOGIC	132	9.4	165	59	-93	0 out of 54
X	LOGIC	145	10.3	167	39	-90	0 out of 88
XI	LOGIC	136	9.7	160	57	-93	0 out of 59

1/ Test site I composed of 20 subplots due to rain occuring in area after treatment of first 14 subplots.

2/ Twelve of these surviving colonies located in subplots which received 2" of rain within 15 minutes of application of LOGIC.

3 All treatments applied at one pound per acre.

4. All treatments applied August 10 thru 13, 1987. Treatments began on the average after 5:00 P.M. in the afternoon and lasted to midnight on the average.

5. Block I, Subplots 1 thru 14 were treated July 7, 1987.

6. Test Site III treated with AMDRO* formulated in 1985. Material slightly on rancid side.

EFFICACY TABLE #2

TEST SITE	TOXICANT	PRETREAT COLONIES /SUBPLOT	AVG. POP. INDEX/SUB-PLOT	AVG. % COLONY MORTALITY AT 8 WEEKS	AVG. % CHANGE IN POP. INDEX AT 8 WEEKS	AVG. % COLONY MORTALITY AT 35 WEEKS	AVG. % CHANGE IN POP. INDEX AT 35 WEEKS	COLONIES PER SUB-PLOT 35wk
I	LOGIC	458	6150	59	-89	80	-95	80
II	AMDRO*	148	2164	97	-97	97	-97	5
III	AMDRO* (85)	122	1707	81	-85	86	-91	17
IV	AMDRO*	145	2385	94	-96	88	-91	18
V 1/	AMDRO*	205	3297	79	-91	77	-91	*47
VI	AFFIRM	178	2926	57	-89	47	-87	94
VII	CHECK	343	5894	+13	+12	+56	+27	535
VIII	LOGIC	91	1425	59	-93	77	-96	21
IX	LOGIC	132	2315	59	-93	79	-91	28
X	LOGIC	145	2341	39	-90	72	-96	40
XI	LOGIC	136	2245	57	-93	89	-99	15

1/ At the 35 week efficacy check Plot V had lost subplots 2 thru 13 due to being plowed up. The number of colonies and population index from the 8 week check of this plot were used for a average. Subplots 1 and 14 were rated at 35 weeks.

EFFICACY TABLE # 3

TEST SITE	TOXICANT	PRETREAT COLONIES /SUBPLOT	AVG. POP. INDEX/SUB-PLOT	1 yr. & 13 day COLONIES/SUBPLOT	AVG % COLONY MORTALITY AT 1yr. & 13 days	CORRECTED	AVG. POP. INDEX/SUB-PLOT	AVG. % CHANGE IN POP. INDEX 1 yr. & 13 days
I	LOGIC	458	6150	28	94%	91%	346	-94%
II	AMDRO	148	2164	133	10%	-32%	1780	-18%
III	AMDRO	122	1707	44	64%	47%	738	-57%
IV	AMDRO	PLOWED UP						
V	AMDRO	PLOWED UP						
VI	AFFIRM	178	2926	85	52%	29%	1034	-64%
VII	CHECK	343	5894	234	-32%		3286	-44%
VII	LOGIC	91	1425	34	63%	46%	348	-76%
IX	LOGIC	132	2315	27	80%	71%	490	-79%
X	LOGIC	PLOWED UP						
XI	LOGIC	136	2245	33	76%	65%	420	-81%

Long Term Suppressive Effect of Commercial Bait Products on Red Imported Fire Ants *Solenopsis invicta* Buren Infesting Cattle Pastures

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Introduction

Several bait insecticides and IGR products are now commercially available to the public for use in controlling the imported fire ant. However, little information on long-term management of the imported fire ant using such bait products is available. Most research in the literature is short-term. This project was designed to look at long-term effects (beyond one year) of bait treatment on the red imported fire ant (RIFA) in South Carolina.

Materials and Methods

A large cattle pasture in Clarendon County, South Carolina near Lake Marion was the study area. Plots were established which were each ±3 acres to permit uniform application of baits with a commercial bait spreader. A minimum buffer strip of 100 feet between plots was established. Since buffer areas were not treated, some movement by RIFA from buffer areas was expected. All sampling of plots was by visual inspection using a 60 ft. radius tape (ca. 1/4 ac. circle). The tape was pulled from a randomly placed stake in the approximate center of the plots. All active RIFA colonies were counted (showing active defense response).

In order to suppress RIFA initially three treatments at six month intervals were planned (Fig. 1). All baits (AMDRO, AFFIRM, LOGIC, and PRO-DRONE) were applied by a HERD GT-77A electric seeder mounted on a small tractor. Following the initial treatments, subsequent applications would be applied as needed.

Results and Discussion

Figure 1 shows the population of RIFA for the first two years of the study. Very little overall reduction of RIFA was noted at 13 months but, significant reduction occurred in AMDRO, LOGIC, and AFFIRM plots by 18 months (Table 1). This was very encouraging and it was decided to delay treatment pending the Fall 1988 count. As can be seen in Figure 1 and Table 1, a tremendous influx of new colonies was detected during our 24-month inspection (October 1988). Most of these colonies were obviously from new queens and clearly demonstrated that a minimum of one treatment per year is probably necessary to maintain suppression.

All plots were retreated in October 1988 (except PRO-DRONE which is no longer commercially available). A preliminary check of plots April 12, 1989 revealed the following counts:

Treatment	x RIFA colonies/ac.
AMDRO	37.33
LOGIC	65.33
AFFIRM	92.00
CONTROL	69.33

These results are erratic and may have been influenced by heavy rain the week prior to our sample (colony relocation to avoid water). Also, IGR products are slow to reduce RIFA. A follow-up count and single application will be made in early summer (June) 1989. Hopefully, this will maximize effect on RIFA and demonstrate effective suppression with one application per year.

Figure 1. Comparison of commercially available RIFA baits applied by ground equipment after 23 months.

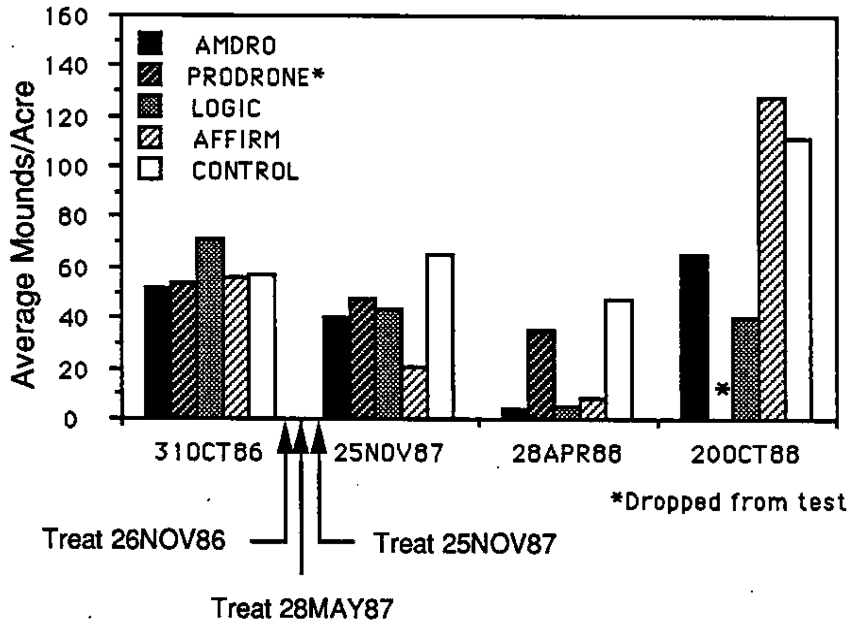

TABLE 1. LONG TERM EFFECT OF BAIT TREATMENTS ON RED IMPORTED FIRE ANT (RIFA) COLONIES. SANTEE, SC

$\overline{X}$ RIFA Colonies/AC*

	10-31-86	11-25-87	4-28-88	10-20-88
AMDRO	52.33 a	40.00 ab	4.00 b	65.33 bc
PRODRONE	54.33 a	48.00 ab	34.66 a	NO DATA
LOGIC	70.67 a	42.67 ab	5.33 b	40.00 c
AFFIRM	55.67 a	21.33 b	8.00 b	128.00 a
CONTROL	57.33 a	65.33 a	48.00 a	112.00 ab
	LSD 24.77	LSD 38.09	LSD 17.73**	LSD 60.76**

*Circular Plot 0.25 AC **SAS GLM LSD P<0.05

EFFECTIVENESS OF THE SUMITOMO INSECT GROWTH REGULATOR S-31183 AGAINST THE RED IMPORTED FIRE ANT

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The activity of insect growth regulators (IGR), primarily juvenile hormone mimics, against the red imported fire ant (RIFA) has been demonstrated in a number of studies (Banks 1986, Banks and Harlan 1982, Banks et al. 1978, 1983, 1988, Phillips et al. 1985, Vinson and Robeau 1974, Vinson et al. 1974). The most active IGR prevent worker replacement in RIFA colonies through lethality to developing immatures, degeneration of the reproductive system of the queen, and a shift in caste differentiation from worker to sexual forms. This lack of worker replacement usually results in colony death as the existing worker force ages and dies. Ingestion of 5.0-10.0 mg AI of IGR usually kills large queenright laboratory colonies in about 6 months.

Fenoxycarb is the most effective of ca. 85 IGR tested to date against RIFA. A number of field tests have shown that it produces 85-100% mortality of colonies at rates of 6-20 g active ingredient (AI) per ha (Banks 1986, Banks et al. 1983, 1988, H. L. Collins - personal communication). Fenoxycarb is currently marketed for fire ant control in the commercial bait, Logic[R].

Additional laboratory and field studies have shown that another IGR, 2-[1-Methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine (Sumitomo S-31183) is highly active against RIFA.

Materials and Methods

Laboratory Tests. Technical S-31183 was tested at 10 mg/colony in once-refined soybean oil (ORSBO) against laboratory-reared colonies of RIFA using standard procedures (Banks 1986). Effectiveness of the treatments on the colonies was determined by comparison of the colony size indices before and after treatment.

Field Tests. Granular baits composed of pregel defatted corn grits, once-refined soybean oil (ORSBO), and technical S-31183 at varying concentrations were tested against natural infestations of RIFA.

The tests were conducted in Polk Co, Fla. in 1985 and 1988, Union Co., FL. in 1986, and Brooks Co., GA. in 1987. The baits tested and rate of application for all tests are shown in Table 1. The baits were applied broadcast with a tractor-mounted granular applicator (Williams et al. 1983) to 0.3 ha plots in nongrazed permanent pasture. Each treatment was applied to 3 plots. In each test Logic bait was applied as a standard to 3 plots and 3 plots were left untreated as controls.

Efficacy of all the treatments was evaluated by comparison of the pre and posttreatment population indices. Standard methods for determination of population indices of RIFA on field plots have been established (Banks 1988).

Results And Discussion

<u>Laboratory Tests</u>. S-31183 was very effective against the laboratory colonies of RIFA (Table 1) in both tests. In test 1, effects were evident by two wk in all treated colonies; the quantity of worker brood was reduced and some sexual brood was present indicating the beginning shift in caste differentiation . The treatments had reduced the size index of all colonies by 80% after 4 wk and by about 96% after 8 wk. One treated colony died by 20 wk treatment and the other two by 24 wk after treatment. The untreated check colonies showed some fluctuation in the population indices but generally showed an increase through the test period. Effects were not evident as quickly in test 2; the size index had been reduced by only 55% after 4 wk. The size index had been reduced by ca. 98% after 8 wk and this high level of suppresssion continued through 16 wk. At 20 wk production of worker brood had resumed in one replicate, while the other two replicates had declined further.

<u>Field Tests</u>. Results of the field tests are shown in Table 2. In the first test, conducted near Lakeland in Polk Co., FL. in June 1985, 0.5 and 1.0% S-31183 baits each applied at nominal rates of 1.1 and 2.2 kg/ha were about equal in effectiveness, giving 96.0 to 98.2% reduction in the population indices after 13 wk. The Logic standard had reduced the population index by 97.7%.

In the second test, in Union Co., FL. in August 1986, 0.5 and 1.0% baits applied at the same nominal rates as in test 1 were slightly lower in overall effectiveness. Again both formulations and rates of application were about equally effective, reducing the population index 85.4 to 87.7% after 13 wk. A 90.0% population index reduction was achieved with the Logic standard. New colonies arising from summer mating flights were apparent by 20 wk posttreatment.

The third test, near Valdosta, Ga. in April 1986, evaluated only the 1.0% formulation at 1.1 and 2.2 kg/ha. As in the two previous tests, there was essentially no difference in the level of population index reduction obtained with the two rates of S-31183. The 1.1 kg rate had reduced the population index by 90.2% after 13 wk as compared to 94.8% reduction for the 2.2 kg rate and 94.8% for the Logic standard. The reduction on the 1.1 kg plots had increased slightly to 92.4% by 20 wk, however, reduction on both the 2.2 kg rate of S-31183 and the Logic standard had declined because of the appearance of small new colonies from mating flights.

The fourth test, in Polk Co., Fla. in October 1988, gave the lowest level reductions of any of the tests. Application of ca. 1.0 kg/ha of 0.75 and 1.5% baits gave only 73.6 and 76.7% reductions in the population indices after 13 wk. The Logic standard had reduced the population indices by 84.0%. By 26 wk reductions in the population indices reached 84.2 and 85.9% with the 0.75% and 1.5% formulations of S-31183, respectively, and 86.0% with the Logic standard. The lowered efficacy of S-31183 and also of Logic in test 4 can probably be attributed to the time of year that the test was applied. We have found in a number of tests over the years that toxicants or IGR applied in late fall or early winter exert their effects on the ants much more slowly and generally the overall effect is somewhat less than occurs with spring or summer treatments.

Although the data have not been statistically compared, it would appear that there are no significant differences in any given test between the various concentrations and/or rates application of S-31183 in its effect on RIFA. In all 4 tests S-31183 appeared to be equal in effectiveness to the Logic standard.

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Table 1. Effects of Sumitomo S-31183 on laboratory colonies of red imported fire ants(avg of 3 colonies, 10 mg AI per colony).

Colonies	Mean colony index before treatment	Percent reduction in colony index after indicated weeks[a]					
		4	8	12	16	20	24
			<u>Test 1</u>				
Treated	133.3	80.0	96.5	97.8	96.5	99.0	100
Control 1.3	100	11.7	- 3.3	- 3.3	5.0	-23.3	-
			<u>Test 2</u>				
Treated	141.7	55.3	97.9	98.1	98.1	96.7	
Control	66.7	-15.0	4.0	- 5.8[b]	-26.7[b]	-26.7[b]	

[a]Minus sign indicates increase in colony index.
[b]Based on 2 replicates, 1 colony escaped

Table 2. Effects of Sumitomo S-31183 baits on field populations of red imported fire ants.

IGR Conc.	Application rate		Before treatment		% reduction in population index after indicated wk[a]		
in bait	bait(kg/ha)	AI(g/ha)	No. active nests	Pop'n index	13	20	26
			Test 1				
0.5	1.05	5.26	27	650	98.2		
	2.1	10.52	25	596	97.1		
1.0	1.17	11.70	25	554	96.0		
	2.34	23.40	25	593	96.6		
Logic (std) 1.0	1.22	12.18	31	731	97.7		
untreated control			27	612	24.8		
			Test 2				
0.5	1.12	5.60	22	515	85.4	2.9	
	2.24	11.20	22	472	87.5	16.9	
1.0	1.22	12.24	24	555	87.0	25.6	
	2.44	24.48	23	494	87.7	58.9	
Logic (std) 1.0	1.69	16.95	25	538	90.0	41.3	
untreated control			27	552	-41.1	-39.7	

[a]Minus indicates increase in population index.

Table 2. Continued.

IGR Conc.	Application rate		Before treatment		% reduction in population index after indicated wk[a]		
in bait	bait(kg/ha)	AI(g/ha)	No. active nests	Pop'n index	13	20	26
			Test 3[b]				
1.0	1.10	11.00	38	806	90.2	92.4	
	2.20	22.01	35	715	94.8	93.0	
Logic (std) 1.0	1.13	11.29	41	855	97.7	91.3	
Untreated control			30	657	22.1	27.7	
			Test 4				
0.75	1.16	8.71	26	537	73.6	80.1	84.2
1.5	1.00	15.06	35	790	76.7	83.5	85.9
Logic (std) 1.0	1.00	10.04	35	765	84.0	86.4	86.0
Untreated control			32	705	-67.8	-60.4	-66.7

[a]Minus indicates increase in population index

[b]Second count in test 3 made at 17 wk rather than 20.

CONTROL OF IFA IN ELECTRICAL APPLICATIONS USING STUTTON CORPORATION'S JS-685

By Nancy Coplin Stutton Corporation

Throughout recent years, as the fire ant population has expanded through Texas and the Southern United States, many new problem areas involving the IFA are developing.

One such problem is that of the IFA'S attraction to electrical fields. We do not know why this attraction is there, we just know it does exist. This problem, however, has become a Godsend for the air conditioning repairman in many southern states. I first became aware of the problem in 1984. One of my customers with a construction company was having problems with an underground compressor in his service area. Every day in early afternoon, the ants would congregate on the contacts and short out his compressor. As a test, JS-685 was applied to the contacts and the problem was solved.

JS-685 was originally designed for control of cockroaches without resistance developing over an extended period of time. The product itself is packaged in aerosol form. JS-685 comes out of the can in a liquid form which quickly dries to a powder coating. It kills on contact and will continue to kill for up to 7 months if left undisturbed. It is also non-conductive up to 34,000 volts.

JS-685 is composed of pyrethrins, drione, silica gel and solvents which work synergistically to kill ants on contact and to act as an effective barrier to keep them out of electrical boxes and other electrical areas.

Air conditioners, switches, relays, electric meters, circuit breaker boxes, well pump motors, signs, lights, and electric gates are just a few of the electrical areas that may be shut down by fire ant infestation.

The imported fire ant has become a menace to many industries, farming, ranching, aviation, power generation and transmission and even oil and gas pipelines. We have found JS-685 to be an effective tool in reducing this menace and keeping the IFA under control.

Influence of Soil Type and Other Environmental Factors on Toxicity of Chlorpyrifos to IFA

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The fire ant quarantine, which includes 11 states and Puerto Rico, regulates the movement of certain articles capable of harboring an infestation, including nursery stock (IFA Quarantine and Regulations, Quarantine #81). The pesticide most utilized for certification of nursery stock is chlorpyrifos, applied either as a granular or drench treatment (PPQ Manual M301.81). Granular chlorpyrifos, incorporated into the potting media prior to potting, is certified for 24 months. When an EC formulation is used as drench, the certification period is 90 days.

Nursery stock shipped out of the quarantine area is inspected at state borders and/or the receiving nursery. If fire ants are detected, whether it be at colony or only a few workers, soil samples are taken and sent to Gulfport, Mississippi for chemical analysis by the National Monitoring and Residue Analysis Laboratory and for bioassay analysis by the IFA Station. More than 1 ppm of chlorpyrifos present in the soil sample has been considered as effective control against IFA (H.L. Collins memo 1987). This limit was based

on previous studies which showed the LD_{99} of Dursban to IFA workers to be .28 ppm in sandy loam topsoil (USDA, APHIS, PPQ, IFA Annual Report 1979).
In one case, out of seven samples collected from one nursery, three contianed 102 ppm of chlorpyrifos. When these samples were bioassayed using IFA workers, only 10-50% mortality was achieved. These discrepancies prompted us to re-evaluate the toxicity of chlorpyrifos to IFA in potting media.

There are many factors which can influence the toxicity of chlorpyrifos. The most prevalent of these is soil type (Whitney 1967, Harris & Svec 1968, Harris 1977, Chapman & Harris 1980, Sharom et al. 1980, Getzin 1981, 1981b, Miles et al. 1984, and Chapman & Chapman 1986). Chlorpyrifos is known to chemically bind, or to be adsorbed to particles in the soil, especially to organic matter (Freed et al. 1979, Sharom et al. 1980 and Sauer & Daniel 1987). Adsorption renders some of the chemical unavailable as a pesticide, thus more active ingredient must be present to maintain effective levels of toxicity.
This is relevant in that the process which extracts chlorpyrifos from the soil to prepare it for GLC analysis releases some of the adsorbed pesticide. More chlorpyrifos is detected in the chemical analysis than is available as pesticide in the soil.

The bulk density of the soil affects the theoretical dose rate of chlorpyrifos. As the bulk density increases, the theoretical dose rate decreases, using a 2.5G formulation applied at 1.0 lb./cu. yd. (Table 1).

Other factors that can influence the toxicity of chlorpyrifos in soil have been documented, but not in relation to IFA. These are formulation (Whitney 1967, Harris & Svec 1968, Davis & Kuhr 1976, Read 1976, Tashiro & Kuhr 1978, Getzin 1985 and Chapman & Chapman 1986), moisture (Whitney 1967, Harris & Svec 1968, Harris 1977, Tashiro & Kuhr 1978, Getzin 1981, 1981b and Miles et al. 1984), temperature (Harris 1977, Getzin 1981, 1981b and Getzin 1985), mobility - horizontal and vertical (Whitney 1967, Sharom et al. 1980, Pike & Getzin 1981 and Chapman et al. 1984), volatilization (Whitney 1967, Getzin 1981b, 1985 and Chapman & Chapman 1986), and microorganisms (Whitney 1967 and Getzin 1981).

Because of the previously mentioned discrepancies, and the fact that most toxicity studies were performed using topsoil and not potting media, we determined the influence of specific factors on the toxicity of chlorpyrifos in potting media to IFA. The factors we studied were soil type, irrigation and the time interval required for various dose rates to be 100% effective against IFA workers.

SOIL TYPE:

In the first study, the influence of soil type on the toxicity of chlorpyrifos was explored. A study by Eger & Hall (1988) showed the LD_{50} of chlorpyrifos to small IFA workers in a muck soil to be 13 times the LD_{50} in sand. Our study compares the toxicity of chlorpyrifos to major IFA workers in Strong-Lite Potting Media (a blend of composted pine bark, peat moss, vermiculite and perlite - Strong-Lite Products Corp., P.O. Box 8029, Pine

Bluff, Arkansas), and local sandy topsoil.

Procedures from other studies were modified for this test (Banks et al. 1964, Collins & Ladner 1981 and Collins et al. 1982). A stock solution of technical Dursban (99%) was prepared in acetone. Serial dilutions of the stock, to obtain various dose rates, were mixed. Additional acetone was added to bring total liquid to 80 ml. for potting media and 50 ml. for topsoil to acquire a thoroughly saturated soil mixture. The Dursban liquid was mixed into 100 g. of potting media or 150 g. of topsoil. Check soil was treated with acetone only. The solvent was evaporated by drying under a hood for 2 - 4 hours.

Bioassays were set up using 2 1/2 inch square plastic pots with a layer of Labstone® in the bottom of each pot. The Labstone® prevents the ants from escaping and retains moisture from an underlying bed of damp peat moss to prevent dessication of the ants. Each pot received 20 cc. of soil and either 5 alate queens or 20 major workers (average weight .2843g/100 workers) and four replicates per soil sample were set up. Dose rates showing 10-90% mortality were bioassayed three times and percent mortality recorded at 7 days post-treatment.

The toxicity of Dursban to alate queens in potting media showed a LD_{50} of 2.85 ppm and a LD_{99} of 4.00 ppm (Fig. 1). For major workers, the LD_{50} was 1.25 ppm and the LD_{99} was 1.98 ppm (Fig. 2).

In local sandy loam topsoil, the toxicity to alate queens showed LD_{50} of 1.39

ppm and LD_{99} of 1.75 ppm (Fig. 3). The toxicity to major workers showed a LD_{50} of .20 ppm and a LD_{99} of .35 ppm (Fig. 4).

As shown in Table 2, the toxicity of Dursban is greatly influenced by highly organic potting media. As stated earlier, the present lowest acceptable rate of Dursban in soil to insure control of IFA is 1 ppm. The influence of soil type was not taken into consideration. From this data, it is evident that the limit must be increased to insure effective IFA control.

IRRIGATION:

Another factor which, due to conflicting data in the literature, may influence the toxicity of chlorpyrifos in soil is soil moisture. In relation to IFA control and the IFA quarantine, we are more concerned with the effects of irrigation on the residual activity of Dursban. In this study, we examined the effect of irrigation on the Dursban drench to re-affirm the certification period of 90 days for containerized plants.

Six-inch standard plastic nursery pots were filled with Strong-Lite® potting media, and treated with a Dursban 2E drench per label and IFA quarantine manual (M301.81) instructions (8 fl. oz./100 gal. water). The pots were divided into four groups, each receiving a different amount of water per week through irrigation in addition to natural rainfall. Each group of pots received either 1", 2" or 4" of irrigation each week in addition to natural rainfall, with one group of pots receiving only natural rainfall. Bioassays using alate queens, were set up every 30 days using a composite of three pots from each group.

As seen in Table 3, the Dursban drench remained effective against alate queens up to 90 days post-treatment while receiving as much as 53 inches of water over the period. By 120 days post-treatment, the standard, having received 6.70 inches of rain, was still 100% effective, while those receiving irrigation plus rainfall had decreased efficacy. Thus, while irrigation does effect the residual activity of the Dursban drench, it does not nullify the presently approved 90 day certification of the Dursban drench while receiving up to 4.5 inches of water per week.

TIME VERSUS LETHAL DOSE RATES:

The final factor we investigated was that of time versus dose rate, i.e., the amount of time required for various dose rates to be lethal to IFA workers in potting media. Workers are known to forager a considerable distance from their nests (Wilson et al. 1971). Nursery stock, whether it be on the docks ready for loading or in transient, is susceptible to invasion by foragers. One to three days later, the transported stock may be inspected at a state border and/or the receiving nursery, and the workers found in the truck or on the stock. Though the stock may be certified, the load is still considered in violation of the IFA Quarantine (Quarantine #81).

Various concentrations of chlorpyrifos were prepared from a stock solution as previously described and added to 100g. of Strong-Lite potting media. The dose rates used were .3, .5, 1, 2, 4, 8, 15, 20, and 50 ppm.

Bioassays were performed using 20 major workers per replicate and four replicates per soil sample. Each dose rate was bioassayed three times and checked for mortality at 1, 4, 7, 24, 48, 72, 120, and 168 hours post ant introduction. If 100% mortality was achieved in all four replicates within 7 days, the time was recorded.

The results of this study were as expected (Table 4), the lower rates required a longer time period to prove lethal. Rates of greater than 8 ppm were lethal within 24 hours of ant introduction and the 4 ppm rate required 64 hours. In the 2 ppm dose rate, 85% mortality had been achieved in two of the trials by 7 days. Therefore, this rate was allowed to proceed until 100% mortality was achieved. The average time required for 2 ppm to be effective was 184 hours.

CONCLUSIONS:

From these studies, we see that environmental factors can influence the toxicity of chlorpyrifos to IFA. Soil type does effect toxicity in that chlorpyrifos is strongly inactivated by organic potting media. This phenomenon is also known to occur in clay soils (Whitney 1967 and Getzin 1981b). The LD_{99} to workers in potting media was shown to be about 2 ppm which is approximately 6 times the LD_{99} in topsoil. This correlates with the time study data in which more than 7 days was required for the 2 ppm dose rate to prove lethal.

Irrigation influences the residual activity of the Dursban drench, but does not nullify the certification period of 90 days. A study is presently underway to determine the influence of irrigation on the residual activity of incorporated Dursban.

In relation to the IFA quarantine, these studies show that changes must be made and that many environmental factors, or combinations of these factors, must be taken into consideration. The present lowest acceptable rate of chlorpyrifos in nursery soil of 1 ppm must be raised to at least 2 ppm or even higher to control foragers and newly mated queens. This in turn, may affect the 24 month certification period or application rate of incorporated granular Dursban, the most commonly utilized method of treatment.

Table 1. The following table gives theoretical dose rates for potting mixes varying in bulk density from 200 to 2,000 lbs./cu. yd:

Bulk Density of Potting Soil (Lbs./cu. yd.)	Theoretical Dose Rate at 1.0 lb. of 2.5% G/cu. yd. soil (PPM)
200	125.0
300	83.3
500	50.0
850	29.4
1000	25.0
1250	20.0
1500	16.6
2000	12.5

Table 2. Comparison of Toxicity of Dursban to IFA Alate Queens and Major Workers in Different Soil Types.

Bioassay Type	Soil Type	LD_{50} (ppm)	LD_{99} (ppm)
queens	topsoil	1.39	1.75
	potting media	2.85	4.00
workers	topsoil	0.20	0.35
	potting media	1.25	1.98

Table 3. Influence of Irrigation on Residual Activity of Dursban Drench.

Time Post-Treatment (Days)	Irrigation water (inches/week)	Cumulative water received (in inches)	% Mortality to Alate Queens
30	1	4.95	100
	2	8.95	100
	4	16.95	100
	rain only	.95	100
60	1	12.15	100
	2	20.15	100
	4	36.15	100
	rain only	4.15	100
90	1	17.95	100
	2	29.95	100
	4	53.95	100
	rain only	5.95	100
120	1	23.70	95
	2	38.70	50
	4	70.70	5
	rain only	6.70	100

Table 4. Time Required to Achieve 100% Mortality of Major IFA Workers at Various Dose Rates.

PPM	Time (Hours) Average of 3 Trials
50	7
20	24
15	24
8	24
4	64
2	184 *
1	**
.5	**
.3	**
Check	**

* Two trials extended past 7 days due to 85% mortality at 7 day.

** Survived 7 day test period.

Fig. 1. Dosage Mortality Curve - Toxicity of Dursban to IFA Alate Queens in Potting Media

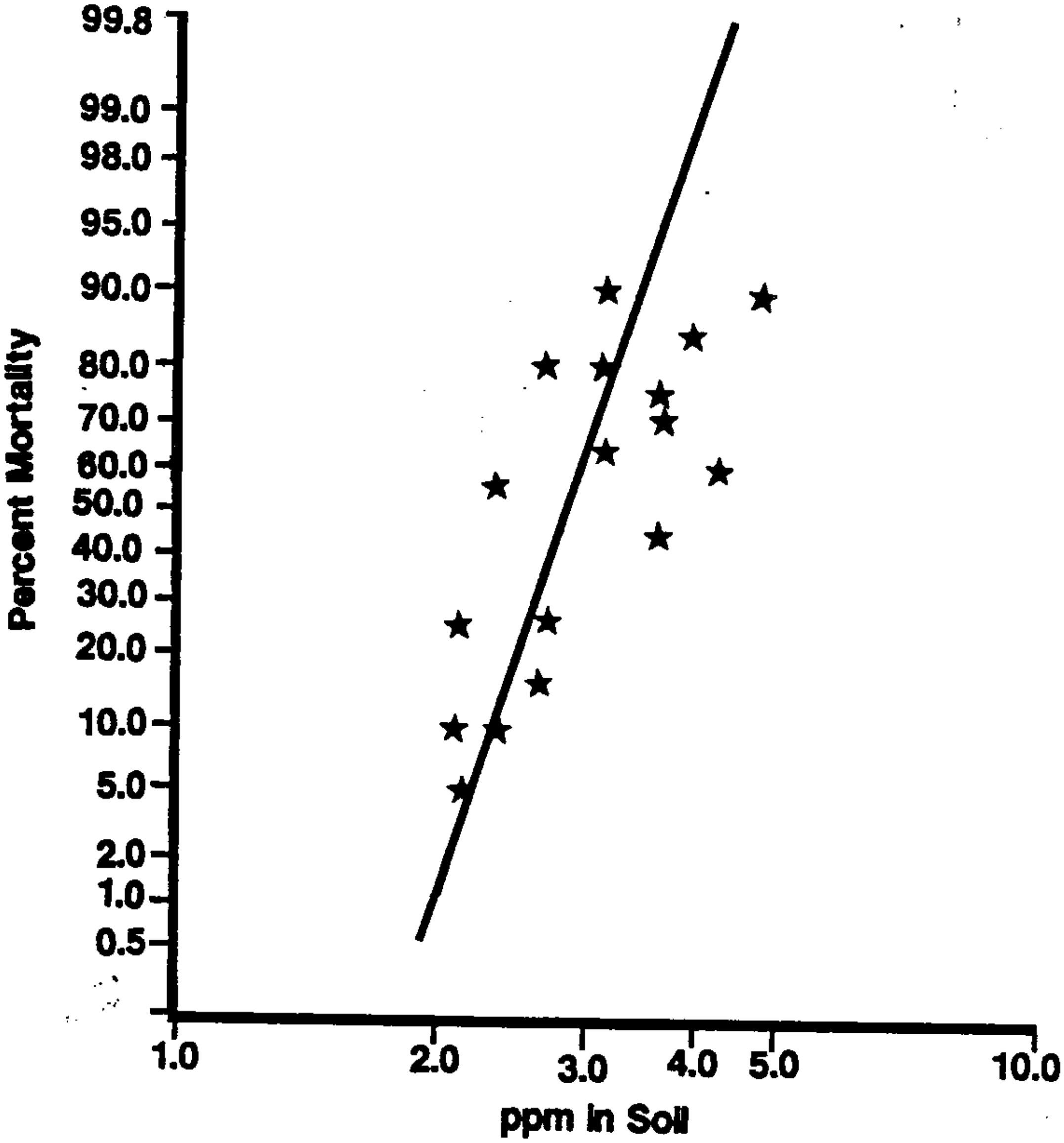


Fig. 2. Dosage Mortality Curve - Toxicity of Dursban to IFA Major Workers in Potting Media

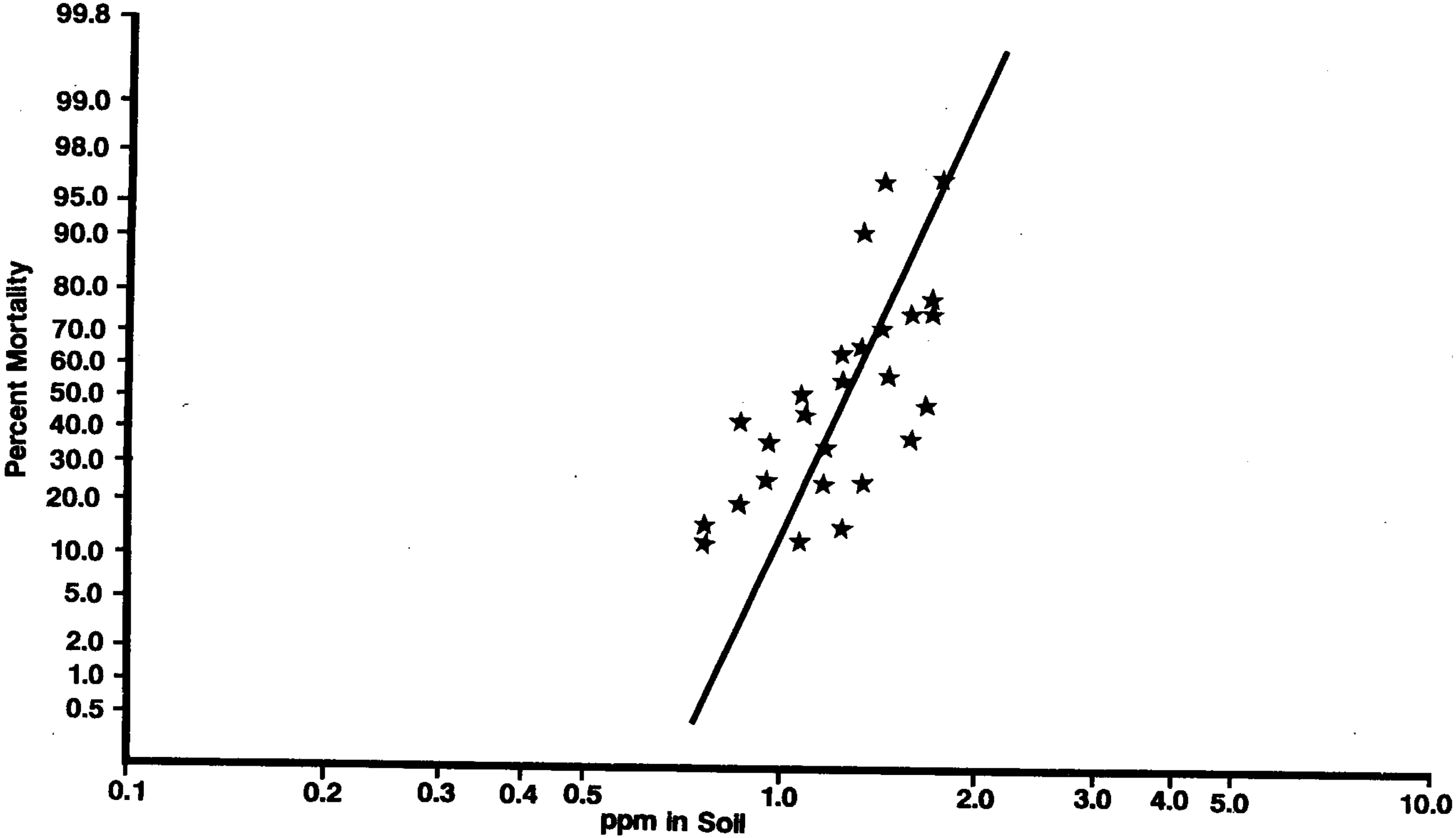


Fig. 3. Dosage Mortality Curve - Toxicity of Dursban to IFA Alate Queens in Sandy Loam Topsoil

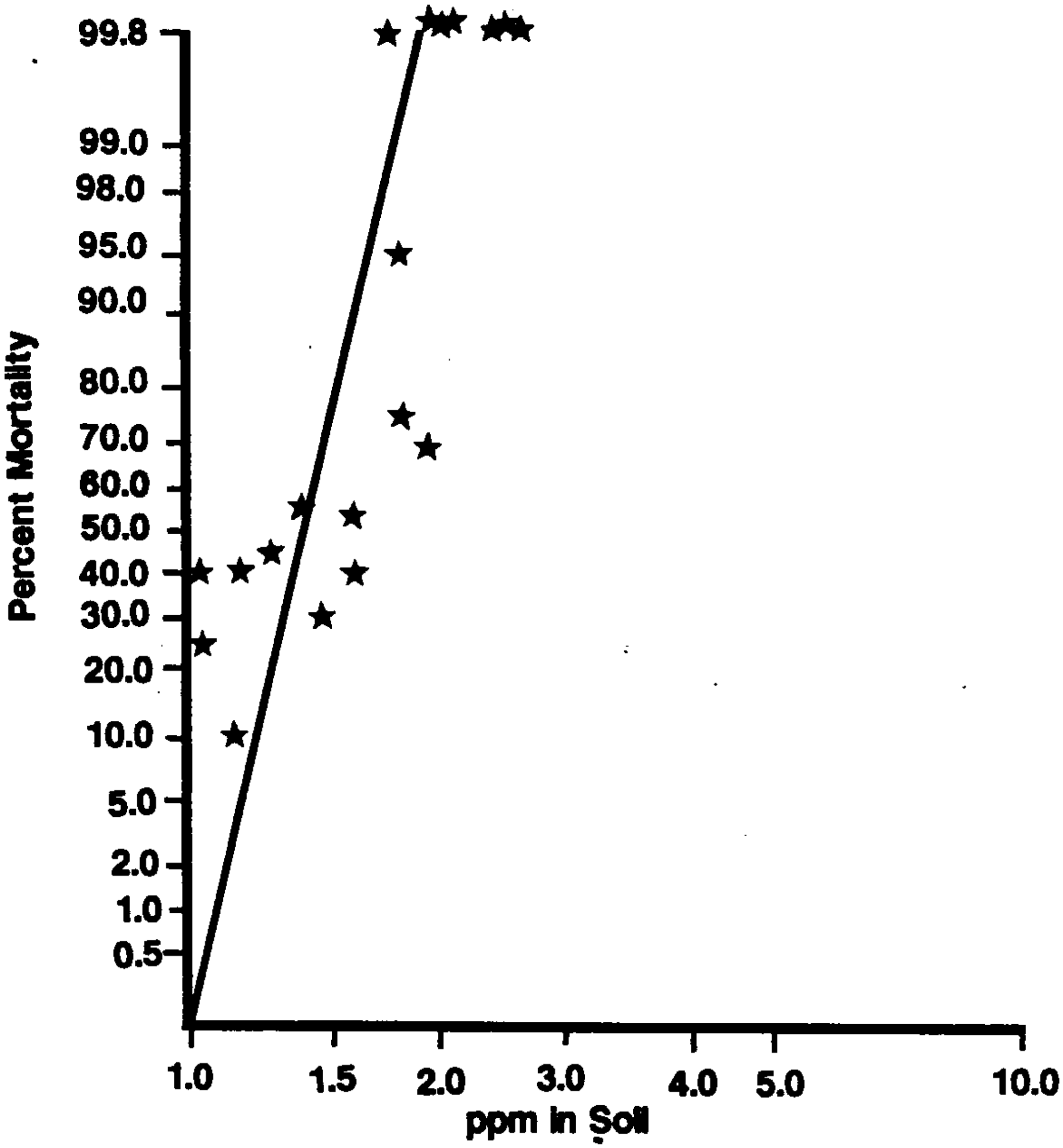
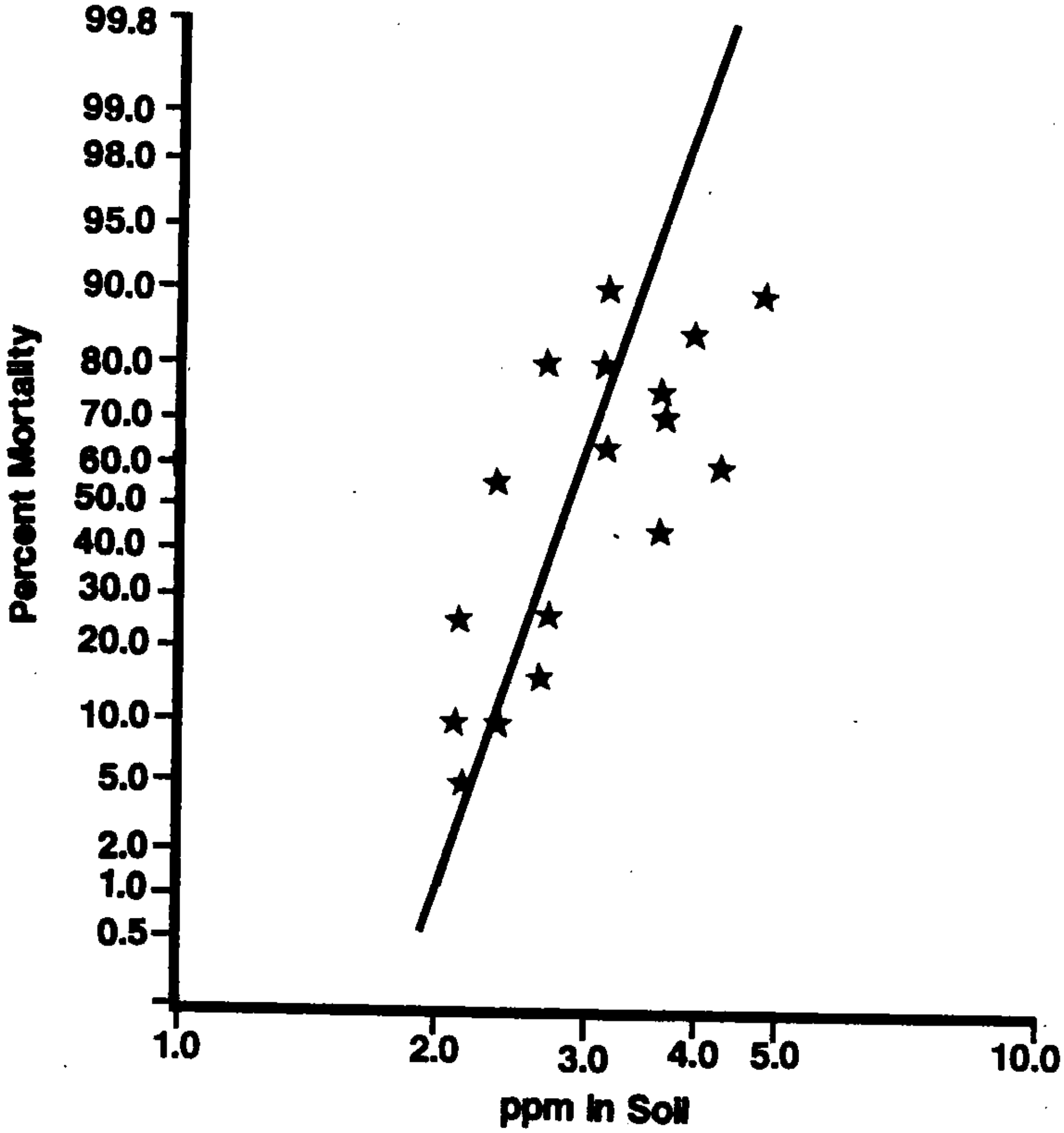


Fig. 1. Dosage Mortality Curve - Toxicity of Dursban to IFA Alate Queens in Potting Media



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Appendix 2

Tim Lockley's "Fire Ant Research Team Member" T-shirt Logo

Many of you at the Conference saw Tim proudly wearing his Fire Ant T-shirt. You, too, can have your very own T-shirt with his Fire Ant Research Team Member logo as seen on the adjoining page. Send Tim your size(s) and quantity and he will ship you your own IFA Team shirts. Each shirt should cost about $12.00 plus shipping (less with larger quantities). Send your order today to Tim Lockley, IFA Station, 3505 25th Avenue, Gulfport, MS 39501.


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