

# **EVALUATION OF RED IMPORTED FIRE ANT BAITS CONTAINING METHOPRENE**

1992-1996

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## I. METHOPRENE BAIT PREFERENCE TESTS

Methoprene is an insect growth regulator and an active ingredient in insecticides such as Precore® (Zoecon) marketed for flea control. This active ingredient mimics juvenile hormones in insects and is sometimes called a "juvenoid". In flea biology, exposure of developmental stages to methoprene breaks the life cycle by preventing successful development to adults. Precore applied to areas infested with flea larvae prevent successful development for 7 months in indoor areas. Application of the methoprene-containing product, Petcor™ Flea Spray (Zoecon) to animals prevents flea eggs from hatching for 30 days. However, exposure to sunlight is thought to rapidly break down the methoprene molecule.

This series of trials was conducted to develop methoprene as an ingredient in a conventional bait formulation for acceptance by red imported fire ant, *Solenopsis invicta* Buren, foraging worker ants in laboratory colonies. Two baits (Corn grit and pre-gel, defatted, corn cob grit, were evaluated. Baits, coated with soybean oil containing concentrations of the active ingredient, were prepared by the manufacturer for evaluation. In addition, concentrations of technical methoprene in soybean oil were prepared and evaluated for attractiveness to foraging ants.

### Materials and Methods

Trial 1. Four methoprene corn grit bait (FA Bait 320-928) formulations were evaluated: blank (320-928), 0.5, 1.0 and 2.0 percent methoprene. Six red imported fire ant colonies were dug from the field and placed in 5-gallon plastic buckets. The soil was watered and allowed to sit for two days while the ants rebuilt their mound structure. One half gram of each bait formulation was placed in plastic weighing boats, arranged on a piece of cardboard and placed in each of the ant colonies for a total of six replications. The ants were allowed access to the bait until one of the baits was completely removed from the weighing boat in each colony. Results of mean bait weight removal were analyzed using Analysis of Variance (ANOVA) at  $P \leq 0.05$  and separated using the Least Significant Difference (LSD) test.

Due to inconclusive results in laboratory and field efficacy studies using the corn cob grit bait carrier, new formulations were supplied using the more common pre-gel, defatted, corn cob grit found in other fire ant bait products. Additionally, some technical methoprene was supplied.

Trial 2. Bait formulations were tested by weighing out 0.25g of bait into small, plastic weighing boats. To conserve ant colonies, all three replications were placed in the same colony box containing very aggressive ants. The three formulations evaluated were: 0.5, 1.0 and 2.0 percent methoprene. The weighing boats were allowed to remain in the colony until one was completely emptied. All boats were then removed and the ants allowed to move out of the boats. The samples were then re-weighed and the amount of bait remaining recorded. Results of mean oil formulation volume removed were analyzed using Analysis of Variance (ANOVA) at  $P \leq 0.05$  and separated using Tukey's Studentized Range test.

Trial 3. The technical material was tested by dissolving the methoprene in soybean oil to make 0.0, 0.1, 1.0 and 5.0 percent concentrations. The three concentrations and untreated soybean oil control were drawn into glass capillary tubes. The quantity of oil in each tube was measured and recorded in millimeters. The tubes were then sandwiched between two glass slides to secure them and raise them above the surface to prevent material seepage. The tube arrangements were then placed on Petri dish lids and placed in a single, active fire ant colony. When one of the tubes had been emptied, all tubes were removed and remeasured. Subtraction yielded the amount of oil consumed. Results of mean oil formulation volume removed were analyzed using Analysis of Variance (ANOVA) at  $P \leq 0.05$  and separated using Tukey's Studentized Range test. Regression analysis was also performed on results of this trial.

## **Results and Discussion**

Trail 1. The blank numbered compound, FA bait 320-928, was much preferred by fire ants in these laboratory colonies (**Table 1**). Numerically, the lowest concentration (0.5 percent methoprene) was more attractive to foraging ants than the higher concentrations, although differences in weight of bait removal were not significantly different between the 1.0 and 2.0 percent methoprene formulations.

Trial 2. There was, again, an obvious rate response to the attractiveness of methoprene concentrations in this more conventional fire ant bait formulation, with the 0.5 percent methoprene bait being chosen for the laboratory mode of action test (**Table 2**).

Trial 3. Attractiveness of soybean oil was reduced as concentration of methoprene increased (**Table 3**). Although the statistical test (Tukey's) used to analyze these data did not show differences between mean formulation removal between treatments, regression analysis provided an  $r$  value of 0.967, significant at  $P \geq 0.01$ . These results further support use of 1.0 percent or less in further testing.

**Table 1.** Attractiveness of corn grit plus soybean oil formulations of methoprene concentrations to worker red imported fire ants in laboratory colonies (Trial 1), 1992.

Compound	Grams of bait remaining						Mean*
	Laboratory fire ant colony number						
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	
blank (320-928)	0.00	0.00	0.00	0.00	0.00	0.10	0.02 c
0.5%	0.35	0.25	0.40	0.40	0.10	0.00	0.25 ab
1.0%	0.45	0.35	0.40	0.50	0.10	0.35	0.36 ab
2.0%	0.35	0.50	0.35	0.30	0.30	0.40	0.37 a
Exposure Time	1:15	0:50	0:40	1:15	1:00	1:25	—

\* Means in column followed by the same letter are not significantly different using Analysis of Variance (ANOVA) at  $P \leq 0.05$  and separated using the Least Significant Difference (LSD) test (LSD, 5% = 0.136;  $F$ -ratio = 13.151;  $P = 0.0$ ).

**Table 2.** Attractiveness of corn cob grit defatted corn grit plus soybean oil formulations of methoprene concentrations to worker red imported fire ants in laboratory colonies (Trial 2), 1992.

Treatment	Grams of bait remaining			Mean* (g)
	Laboratory ant colony number			
Processed corn grit	<u>1</u>	<u>2</u>	<u>3</u>	
2.0% methoprene	0.25	0.25	0.25	0.25 a
1.0% methoprene	0.25	0.15	0.20	0.20 a
0.5% methoprene	0.00	0.05	0.05	0.03 b

\* Means in column followed by the same letter are not significantly different using Analysis of Variance (ANOVA) at  $P \leq 0.05$  and separated using the Tukey's Studentized Range test ( $F = 12.73$ ;  $P > 0.0151$ ; MSD = 0.1137; CV = 5.040).

**Table 3.** Attractiveness of soybean oil formulations of methoprene concentrations to worker red imported fire ants in laboratory colonies (Trial 3), 1992.

Treatment in soybean oil	Length of oil column (mm) in capillary tube containing formulation before/after exposure				Total*
	1	2	3	4	
0%	34/19=15	36/20=16	31/0=31	33/18=15	77 a
0.1%	34/22=12	35/20=15	34/17=17	32/16=16	79 a
1.0%	35/19=16	35/7=28	30/17=13	39/32=7	64 a
5.0%	35/25=10	35/22=13	35/18=17	35/30=5	45 a

\* Means in column followed by the same letter are not significantly different using Analysis of Variance (ANOVA) at  $P \leq 0.05$  and separated using the Tukey's Studentized Range test (no significant differences)

## II. METHOPRENE MODE OF ACTION STUDY

Based on previous bait-acceptability trials, 1.0 percent formulations of methoprene were used to treat and monitor red imported fire ant colonies to document the effects of treatment of production of brood (eggs, larvae and pupae) and caste (worker ants, winged (alate) reproductive ants) and colony vigor.

### Materials and Methods

This trial was conducted in two parts. The initial trial involved only one replication. Once the methodology was confirmed to be successful, the experiment was repeated using 3 replications of treated and untreated fire ant colonies. Methoprene treated colonies were given unlimited access to 1.0 percent methoprene bait. All methoprene-treated fire ant "colonoids" (described below) were derived from a single original colony. Results are presented with all four replications.

Tall Petri dishes were coated with Fluon® to prevent ant escape. Glass collection vials were partially filled, horizontally, with plaster of Paris to provide a water retention medium for the colony nesting chamber. Approximately ½ teaspoon of ants from the efficacy study colonies were placed in the petri dishes along with plastic vial caps for food and water. Queens were placed in the nesting vials and placed in the dishes also. The number of queens per dish of untreated control colonoids varied from one to three depending on availability. Three methoprene-treated queens were placed in the vial for the first experiment and two queens each for the second. Colonoids were observed periodically (2, 7, 14, 21 and 29 days).

## **Results and Discussion**

A few eggs were present in all methoprene-treatment colonoids at the beginning of the test (**Table 4**). They gradually disappeared and were replaced by "clutches" of a few, very small larvae, which also disappeared after 1 to 2 weeks. These results indicate that egg production continues for some time after methoprene exposure, though at a greatly reduced rate. Those eggs that do hatch fail to develop to the pupal stage and probably not into third or fourth larval stages. These observations are similar to the observed mode of action of another "juvenoid" insect growth regulator, fenoxycarb (Logic® and Award® by Ciba-Geigy), although no reproductive larvae and pupae developed in treated colonies as is sometimes the case when colonies with larvae present are treated with fenoxycarb.

**Table 4.** Status of red imported fire ant colonies (colonoids) following unlimited exposure to 1.0 percent methoprene bait (e/E = few eggs/many eggs; l/L = small larvae/small (worker) and large (reproductive) larvae; p/P = small (worker) pupae only/small plus large (reproductive) pupae; NB = no brood), 1992.

Treatment & colony	<u>2-days</u>	<u>1-week</u>	<u>2-week</u>	<u>3-week</u>	<u>1-month*</u>
untreated					
1	E,L	E,L	E,L,p	E,L,P	dead
2	E,L,P	E,L,P	E,L,P	E,L,P	E,L,P
3	E,L,P	E,L,P	E,L,P	E,L,P	E,L,P
4	E,L,P	E,L,P	E,L,P	E,L,P	E,L,P
methoprene treated					
1	e	l	NB	NB	dead
2	e	e	NB	NB	NB
3	e	NB	l	l	NB
4	e	e	l	l	l**

\* colonies frozen and ants examined outside of colony dishes

\*\* single clutch of 6 small larvae

### III. METHOPRENE FIELD TEST - LAKE CONROE DAM, 1992

The first field trial was conducted using the corn grit experimental formulations of methoprene, using concentrations evaluated in preference trials in laboratory colonies. Another juvenile hormone analog ("juvenoid"), Logic® (fenoxycarb) by Ciba-Geigy, was used as a "standard" treatment.

#### Materials and Methods

The test was located on the land between the spillways of the Lake Conroe dam, in Montgomery County, Texas. The area is controlled by the San Jacinto River Authority, mowed semi-yearly and access is strictly limited to authorized personnel. The site itself is gently sloping to flat, with a few scattered clumps of trees. Vegetation is dominated by Bahia Grass, but with a considerable variety of other grasses, broad-leaved weeds and spots of tall sedges. Due to heavy spring rains and flooding, ant densities were somewhat clumped, concentrating on the higher patches of ground within the test plots.

The test consisted of 24 half-acre square plots, 150 feet on a side, arranged in a four-plot by six-plot rectangular block. Plot corners were flagged and the centers were marked with 3 ft. wooden

stakes and 6 by 8 inch metal plates nailed to the ground. The number of active mounds were determined by disturbing the mounds within a 59-foot radius circular subplot (approx. 1/4 acre) in the center of the plots. A mound was considered active if ants came to surface within about 15 seconds of disturbance.

Pre-counts were taken for all plots on June 22, 1992. Treatments were then assigned by first ranking the plots from highest to lowest in active mound numbers. The highest six were designated the first replication, the next highest six the second replication, and so on to make four replications. The treatments were numbered from one through six and, using a random number table, they were then assigned to plots within each replication.

Treatments were as follows:

- 1) Untreated control
- 2) 0.5% methoprene (corn grit formulation)
- 3) 1.0% methoprene (corn grit formulation)
- 4) 2.0% methoprene (corn grit formulation)
- 5) numbered compound, blank (?) (corn grit formulation)
- 6) Logic® (fenoxycarb) "standard"

Application of the treatments were made on June 23 from 3:30 p.m. to 8:30 p.m. The baits were applied using a Cyclone 1C1 hand-held seeder. Eight swaths were applied across each plot giving an application rate of 1.5 lbs per acre for all treatments. The methoprene and numbered compound carrier granules were quite small and required that the seeder gate be set at its smallest opening. The material was distributed quite consistently and evenly, however.

Subplots were monitored as described above periodically (3, 8 weeks, 3, 6, 12 and 18 months) following application. Resulting mound number per plot data were analyzed using Analysis of Variance (ANOVA) at  $P \leq 0.95$  and separated using Tukey's Studentized Range test.

## Results and Discussion

No differences between treatments was observed until 3 months following application (**Table 5**). From that date until 12 months after treatment, Logic® (fenoxycarb) treated plots contained significantly fewer red imported fire ant mounds than did untreated or blank (Numbered compound) treated plots. Methoprene formulations produced significant reductions in numbers of ant-active mounds beginning 6 months after application, and from that date performed statistically similar to the Logic "standard", with the 0.5 and 2.0 percent methoprene treatments continuing to provide significant reduction of ant mound numbers for 18 mounds after application relative to ant mound numbers found in untreated control and blank (Numbered compound) treated plots.

**Table 5.** Number of active red imported fire ant mounds before and following application of bait-formulated insecticides, Lake Conroe Dam, Montgomery County, Texas, applied June 22, 1992.

12 Months	Mean no. fire ant mounds/0.25 acres*							
	Pre-count 18 Months		3 Weeks		8 Weeks		3 Months	6 Months
	22 June	7 July	13 Aug.	25 Sept.	13 Jan.	3 June	3 Jan.	
untreated		41.25a	31.50a	21.50a	33.25a	34.75a	37.25a	50.25a
methoprene								
0.5%	38.25a	26.75a	20.75a	23.00ab	9.75b	10.75b	20.00b	
1.0%	39.25a	32.75a	21.50a	24.00ab	10.25b	13.75b	29.75ab	
2.0%	42.50a	29.25a	19.50a	17.50a	7.50b	12.50b	23.50b	
blank								
Numbered		36.50a	25.75a	16.50a	32.50a	35.75a	32.75a	48.75a
Logic®	42.00a	35.25a	16.75a	6.50b	4.75b	8.00b	29.75ab	
<i>F</i>	15.08	2.54	0.92	3.38	7.76	13.57	5.45	
<i>P</i>	0.0001	0.0532	0.5296	0.0201	0.0004	0.0001	0.0024	
M.S.F.	23.8528	59.8194	71.1111	90.9750	80.1528	30.5556	102.8667	
Crit. Val. =	4.595							

\* Means followed by the same letter(s) are not significantly different using analysis of variance and the Tukey's Studentized Range Test ( $P \leq 0.05$ ).

#### IV. METHOPRENE FIELD TEST - GRANGER LAKE, 1993-94

This test was conducted to replicate results of the 1992-1993 trial conducted at Lake Conroe (Montgomery County), but using a conventional fire ant bait formulation containing processed corn cob grit defatted plus soybean oil formulations of methoprene concentrations.

#### Materials and Methods

This test was established behind the earthen dam of Granger Lake, Williamson County, Texas on June 29, 1993. Twenty ½ acre square plots were arranged consisted of 2 rows of 10. Pre-count active red imported fire ant mound numbers, obtained by monitoring 1/4 acre circular subplot areas in the centers of each plot, were arrayed from highest to lowest then grouped into 4 replications of 5 plots each. Treatments, consisting of 0.25, 0.5 and 2.0 percent formulations of methoprene on corn cob grit defatted plus soybean oil, a Logic® (fenoxycarb) "standard" treatment, and an untreated control, were randomly assigned within replications. All evaluations were conducted using the minimal disturbance technique in which a mound was considered active in numerous ants emerged from a disturbed mound within 15 seconds. Treatments were applied

the evening of July 26, 1993 using a hand-carried Cyclone 1C1 seeder at a rate of 1.5 lbs. formulation or product per acre.

Subplots were monitored as described above periodically (2, 8, 12 and 20 months) following application. Resulting mound number per plot data were analyzed using Analysis of Variance (ANOVA) at  $P \leq 0.05$  and separated using Tukey's Studentized Range test.

## **Results and Discussion**

The area did not receive rain from the last week of June through the last week of September. Post-treatment counts were postponed until the first rain so that active mounds would be more easily visible. The over 50 percent drop in mound numbers in the untreated plots is illustrative of the effects of dry, hot weather on fire ant activity. Due to an early freeze and the onset of cold weather in early October, further evaluations were postponed until spring when untreated plot mound numbers were found to have actually risen above pre-count levels.

Significant reductions in active fire ant mound numbers in treatment plots did not occur until 8 months following application (**Table 6**). Thereafter, Logic® and methoprene treated plots contained significantly fewer active ant mounds than did untreated control plots through the 12 month post-treatment evaluation, although no statistical differences were documented between the effects of these chemical treatments or methoprene formulations.

**Table 6.** Number of active red imported fire ant mounds before and following application of bait-formulated insecticides, Granger Lake, Williamson County, Texas, treated 29 June 1993.

<u>Treatment</u>	<u>pre-count</u>	<u>Mean number of active mounds*</u>			
		<u>2 months</u>	<u>8 months</u>	<u>12 months</u>	<u>20 months</u>
Untreated	36.5 a	17.0 a	44.5 a	42.00 a	82.75 a
Logic® methoprene	40.7 a	7.0 a	2.5 b	6.25 b	56.25 ab
2.0%	35.5 a	13.8 a	2.0 b	8.25 b	66.00 ab
0.5%	36.7 a	10.0 a	0.5 b	11.25 b	64.00 ab
0.25%	36.5 a	23.8 a	1.0 b	7.75 b	52.25 b
<i>F</i>	11.95*	1.59	5.31	5.53	8.14
<i>P</i>	0.0001	0.2291	0.0059	0.0050	0.0009
MSE	91.7083	71.775	170.167	124.300	180.142
d.f. = 12					
Crit. value = 4.508					

\* Means followed by the same letter(s) are not significantly different using analysis of variance and the Tukey's Studentized Range Test ( $P \leq 0.05$ ).

## V. SULFLURAMID/METHOPRENE TEST - GRANGER LAKE, 1995

Previous work has shown that the combination of bait formulations containing a "juvenoid"-type insect growth regulator (*e.g.*, fenoxycarb) plus a metabolic inhibitor (*e.g.* hydramethylnon) suppressed active red imported fire ant mound numbers more quickly than the insect growth regulator (IGR) and for longer than the metabolic inhibitor alone (Drees *et al.* 1993, 1995). This trial was conducted to determine if a similar effect could be achieved using methoprene (IGR) plus sulfluramid (metabolic inhibitor). Because earlier trials failed to document consistent or significant differences in the performance between 0.25, 0.5, 1.0 and 2.0 percent methoprene formulations, only the 0.5 percent conventional bait formulation was used in this trial. A potential new insect growth regulator formulation containing V-71639 0.5% BA (Nylar™ Fire Ant Bait 2629) from Valent U.S.A. was also evaluated.

### Materials and Methods

Thirty two 1 acre rectangular plots were established on land behind the earthen dam behind Granger Lake in Williamson County, Texas. Prior to treatment, the number of active red imported fire ant mounds in each 1/3 acre subplot sampling area was counted. Plots were arrayed in order from highest to lowest active fire ant mound numbers and divided in to four blocks (replicates) of eight plots each. The following treatments were assigned at random to each of the blocks:

<u>Treatments</u>	<u>Rate(s)</u>	<u>Plots</u>
1. sulfluramid <sup>1</sup>	1.5 lb/acre)	5,11,17,25
2. methoprene <sup>2</sup>	1.5 lb/acre)	10,14,26,28
3. sulfuramid-methoprene	0.75 + 0.75 lb./acre)	1,4,6,20
4. Amdro® <sup>3</sup> /Award® <sup>4</sup>	0.75 + 0.75 lb/acre)	19,21,22,32
5. Nylar™ <sup>5</sup>	1.5 lb/acre)	12,15,27,30
6. untreated control	---	2,8,9,24
7. Award	1.5 lb/acre	3,13,23,29
8. methoprene + sulfluramid skip swath	(0.25 + 0.25/acre)	7,16,18,31

<sup>1</sup> Finitron® Fire Ant Bait (0.55% sulfuramid) Lot # 300-95; Griffin Corp., Valdosta, GA

<sup>2</sup> Fire Ant Bait with 0.5% S-methoprene; Lot # 411-131; Sandoz Corp. (pre-gel, defatted corn grit plus soybean oil bait)

<sup>3</sup> Amdro® Granular Insecticide 24567-41 12/92 (Unopened jugs); American Cyanamid

<sup>4</sup> Award Brand of Logic Fire Ant Bait; CGA119L2A 032; Ciba-Geigy Corp.

<sup>5</sup> V-71639 0.5% BA 3.624 kg. Batch VS-1813-97; 5/10/95; GLP#NA; SR#:V01996 (2481); Lot #:V050495 JNF Ext. 5/4/0; Valent U.S.A. (Nylar)

Treatments were applied, 11 July 1995 on a clear, hot day (Treated 10:00 am - 1:09 pm & 4:01 - 7:30 pm. Max temp, 4:50 = 102.4 degrees F.) using a tractor mounted Herd Model GT-77 seeder using 10 swaths/plot (7-9 paces apart; 10 min./plot).

## Results and Discussion

No significant differences in mean number of red imported fire ant mounds occurred prior to treatment (**Table 7**). Metabolic inhibitors or mixtures with these compounds (sulfuramid, Amdro®, sulfluramid + methoprene, Amdro® + Award®) produced significant reductions of active ant mounds within 3 weeks after application. "Juvenoid" insect growth regulators or IGRs (methoprene, Nylar™, Award®) did not produce significant reductions in mound numbers relative to untreated check plots until 8 weeks (Nylar) or 12 weeks (methoprene, Award) after treatment. Methoprene bait performed similar to the Award® "standard" throughout this trial, providing a 84.3 and 94.2 percent level of suppression relative to untreated control plots at 12 weeks and 6 months after treatment, respectively. Although sulfluramid reduced mound numbers only for less than 12 weeks, the mixture of metabolic inhibitor plus "juvenoid" IGR treatments suppressed them for the 6 month duration of this trial. These results confirm earlier studies of an enhanced product performance "profile" realized with the application of these product mixtures.

The "skip swath" application of the mixture of methoprene + sulfluramid was accidentally applied at less than effective rates, and did not reduce mound numbers relative to untreated areas in this trial. If applied as a 0.37 + 0.37 lb. mixture, this treatment might have performed better. This trial was terminated after only 6 months of treatment because of poor weather conditions. No rain was received in this test location from May through late August, reducing ant mounding activity to almost undetectable levels.

## Citations

- Drees, B. M., C. L. Barr, M. E. Heimer and R. Leps. 1995. Reducing treatment costs for fire ant suppression in Texas cattle production systems. *in* Proceedings of the Fifth International Pest Ant Symposia and the 1995 Annual Imported Fire Ant Conference (ed. S. B. Vinson and B. M. Drees), San Antonio, Texas. pp.146-154.
- Drees, B. M., C. L. Barr and M. E. Heimer. 1993. Skip-swath application of Amdro® and Logic® broadcast baits for the suppression of the red imported fire ant *in* Proceedings of the 1993 Imported Fire Ant Conference (ed. J. P. Ellis) Charleston, South Carolina.

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**Table 7.** Number of active red imported fire ant mounds before and following application of bait-formulated insecticides, Granger Lake, Williamson County, Texas, treated 11 July 1995.

<u>Treatments</u>	<u>Precount</u>	<b>Mean number of active mounds*</b>			
		<u>3 weeks</u>	<u>8 - week</u>	<u>12 - week</u>	<u>6 month</u>
untreated control	29.5	25.0a	9.75 a	12.75 a	43.50 a
<b>Metabolic inhibitors:</b>					
sulfluramid 1.5 lb/acre	28.3	6.3bc	1.50 bc	6.75 abc	23.75 ab
<b>"Juvenoid" IGRs:</b>					
methoprene 1.5 lb/acre	28.0	21.8a	6.75 ab	2.00 c	2.50 b
Nylar™ Award®	27.5	15.5abc	1.25 bc	2.00 c	4.00 b
1.5 lb/acre	29.3	17.3abc	7.25 a	1.50 c	3.25 b
<b>Mixtures:</b>					
sulfluramid + methoprene 0.75 + 0.75 lb./acre	27.5	3.3c	0.50 c	3.00 bc	10.25 b
Amdro®/Award® 0.75 + 0.75 lb/acre	28.3	4.8bc	0.00 c	1.75 c	4.50 b
methoprene + sulfluramid skip swath 0.25 + 0.25/acre	27.3	18.8ab	8.25 a	11.25 ab	41.00 a
<i>F</i> =	NS	5.44	8.31	4.16	6.76
<i>P</i> =	NS	0.0005	0.0001	0.0029	0.0001
MSD =	NS	14.747	5.7202	9.009	26.956
d.f. = 21					
Critical value. = 4.743					

\* Means followed by the same letter(s) are not significantly different using analysis of variance (PC SAS PROC ANOVA) and the Tukey's Studentized Range Test ( $P \leq 0.05$ ).