NITROGEN EFFECTS ON BIOLOGICAL CONTROL IN COTTON

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Introduction

The availability of Bt-transgenic cotton varieties and other pesticide-incorporated plants (PIPs) has been a great boon for growers in managing their caterpillar pests. However, other pests have emerged in the wake of widespread adoption of insecticidal-transgenic cotton. Stink bugs have become a serious problem with the reduced use of broad-spectrum insecticides. Further, there are caterpillar pests that remain problematic in cotton, even on Bt-transgenic varieties (particularly armyworms, but also including bollworms). There is a continued need to fit biological control into management systems to help growers reduce pest management costs and to enhance sustainability of cotton production systems. However, the factors limiting effectiveness of natural enemies in cotton are still unclear. In this project we are examining one of those factors – nitrogen levels – to assess if and how it affects the activity of natural enemies in the field and laboratory.

It has become apparent that the plant plays a critical role in the efficacy of numerous biological control agents in agriculture (e.g., Boethel and Eikenbary 1986, Hare 2002, Turlings et al. 2002). An extensive body of literature asserts the importance of the plant as a source of critical signals for a number of natural enemies; however, the role of the plant's nutritional status in the effectiveness of natural enemies is poorly understood, with only a few studies having been conducted to date. For example, increased foliar nitrogen in collards was directly related to the proportion of female offspring produced by the parasitoid *Diadegma insulare* (Fox et al. 1990). Similarly, high levels of phosphorus in soybeans contributed to consistently higher populations of big-eyed bug nymphs (*Geocoris* spp.) (Funderburk et al. 1994). Fertilizer application to *Spartina* islets in salt marshes resulted in increased abundance of herbivore species, and significantly enhanced spider activity against certain groups of herbivores (Denno et al. 2003). Thus, the nutritional status of the plant may exert effects on biological control, although the mechanisms for these effects are not known.

The bollworms (tobacco budworm, *Heliothis virescens*, and corn earworm, *Helicoverpa zea*) are important pests of cotton in the southeastern US. These two species are capable of causing extensive damage to cotton, in addition to other crops in the region. Biological control has long been an important aspect of managing these pests, as it became apparent, shortly after the advent of widespread use of synthetic insecticides, that disruption of natural enemies could produce serious pest outbreaks. Transgenic crops provide good to excellent control where they are planted, but biological control can provide an additional long-term, sustainable tool for managing these and other insect pests. Among the key native parasitoids of bollworms are the braconid

parasitoids *Cardiochiles nigriceps* Viereck, *Microplitis croceipes* (Cresson), and *Cotesia marginiventris* (Cresson). *M. croceipes* can parasitize moths from the two noctuid genera *Heliothis* and *Helicoverpa*, ranging over a substantial number of host plant taxa where representatives of these two genera occur. In comparison with *C. nigriceps*, which attacks tobacco budworms almost exclusively, it is a relatively less specialized larval endoparasitoid. *Cotesia marginiventris*, in contrast, attacks a wide range of caterpillar pests, including tobacco budworms, corn earworms, soybean loopers, beet armyworms, southern armyworms, and others.

All three of these parasitoid species have been shown to respond strongly to plant- and host-related cues to locate and accept hosts in laboratory studies (e.g., Kasas et al. 1992, McCall et al. 1993, De Moraes et al. 1999). Although these cues have been demonstrated in the laboratory, their importance in the field under the varying conditions prevalent in agricultural systems has not been elucidated. Further, there is recent evidence to suggest that the nutritional status of plants can have important implications for the foraging success of parasitoids and predators, at least in the laboratory (e.g., Dicke and Sabelis, 1988; Dicke et al., 1990; Turlings et al., 1990, 1993). Thus, understanding the role of the plant's nutritional status in the effectiveness of biological control can have very important consequences for anticipating effectiveness of biological control agents in the field, and possibly devising or modifying practices to improve biological control by modifying plant health.

The objectives of this project are to elucidate the role of plant nitrogen levels in the function of natural enemies of several cotton pests in the laboratory and field.

Methods

Greenhouse Studies

Cotton plants were grown in a potting soil/peat moss blend with hydroponic solutions that were modifications of the Hoagland Solution (Hoagland and Arnon 1950) and permitted us to manipulate the nitrogen concentrations in the solutions. These plants were used for several studies of the responses of beet armyworms to various nitrogen levels in plants (not reported here and the responses of the parasitoid *Cotesia marginiventris* to beet armyworms on the plants.

Parasitoid responses to plants. To test whether parasitoids preferred plants with low or high nitrogen fertilization, we set up a choice experiment with whole plants in cages. Test cages were (LxWxH =100x60x60 cm) made of PVC pipes covered with fine mesh outside and were placed in the greenhouse (24 $\pm$ 4°C; L:D 14:10). Two nitrogen treatments were examined in the choice test: 42 ppm nitrogen in the watering solution and 196 ppm nitrogen in the watering solution. These solutions yielded plants that were somewhat yellowed and stressed (42 ppm) and plants that were dark green and visually healthy (196 ppm), and differed significantly in leaf nitrates as determined by petiole analysis (163.40 ppm N for the 42 ppm treatment, and 14,416.60 ppm N for the 196 ppm treatment). Four cotton plants (2 for each treatment) were arranged so that the two

from the respective treatments were touching each other, and the plants of the two treatments were physically separated from one another at opposite ends of the cage. Plants of different treatments were ca. 50 cm apart to permit the parasitoids the opportunity to make a choice between the two treatments, and to limit caterpillar movement between treatments. Forty 2-d-old beet armyworm larvae were introduced to each plant in the cage and allowed to feed for 24 h before the introduction of 8 female *Cotesia marginiventris*. The parasitoid females were 3-4 days old, and had had prior ovipositional experience. They were not exposed to hosts for 24 h preceding their release into the cages. A cotton ball soaked with a 10% honey:water solution was provided as parasitoid food in cage. All surviving beet armyworm larvae were recovered 24 later and returned to the laboratory, where they were placed on artificial diet and held to monitor for parasitism. The experiment was replicated 8 times, and preference was evaluated as the percentage of caterpillars successfully parasitized in the treatments..

Field Studies

Cotton seed (variety FiberMax 989, a non-Bt variety) was planted on 15 May 2006 in field plots with four levels of nitrogen: (1) no nitrogen added; (2) 40 lbs/A (1 application of 40lbs/A); (3) 80 lbs/A (two applications of 40lbs/A); and (4) 120 lbs/A (three applications of 40lbs/A). The first application was on 9 June, the second on 19 June, and the third on 29 June. Each treatment was replicated 5 times in a randomized complete block design. Each plot was 12 rows wide and 50 feet long.

We examined the plots weekly using drop cloths to sample two rows of cotton in each plot (a total of 10 row feet were sampled per plot) to quantify populations of parasites and predators. In addition, caterpillars were collected from samples and returned to the laboratory to evaluate parasitism rates among the various plant nitrogen treatments.

Assessment of predation and parasitism. In addition to collecting naturally-occurring caterpillar pests, we also placed beet armyworm larvae on plants to evaluate predation and parasitism. Laboratory produced beet armyworm eggs and caterpillars were placed in the field to evaluate the influence of N on predation and parasitism rate. About 50 neonate caterpillars were confined to small cages made of 12-ounce styrofoam soft drink cups covered with nylon stocking material. Each cage enclosed one leaf in the middle of the cotton plants. The cages were removed 24 h later and caterpillars on leaves were counted. Caterpillars were subsequently exposed to feral natural enemies for 48 h. Then all remaining caterpillars were counted again and placed on artificial diet in groups of 5 to 10 caterpillars per diet cup. Parasitism rate was calculated as number of parasitoid cocoons divided by total caterpillars recovered. Four replicates were placed in each plot. Emerged parasitoids were identified to species for feral and sentinel caterpillars collected, and levels of parasitism will be analyzed, by parasitoid species, among the nitrogen (and moisture, if possible) treatments. Caterpillars were placed on plants in two trials. The first was placed in the field on 9 August and completed on 12 August. The second was placed on 22 August and completed on 25 August

Egg predation was evaluated by placing one egg mass with ca. 40 beet armyworm eggs attached to paper tissue on one leaf in the middle of cotton plant on 2 August. Eggs

were frozen for 2 days before experimentation, so that they were not able to emerge as caterpillar but the color and shape of eggs remained. The eggs were checked twice daily (once in the morning, ca. 9 am, and once in the afternoon, ca. 4 pm) and remaining eggs counted over a 2-d period (through 4 August). The plants were located in the middle of the plots. Four replicate egg masses were placed in each plot.

Assessment of plant nitrogen. Two petioles from each of 10 randomly chosen cotton plants in each plot were pooled together in each plot in September, 2006, to assess leaf nitrate levels. Samples were oven-dried at 65°C for 2 d then sent to the Soil, Plant, and Water Laboratory of the University of Georgia for N analysis. This plant tissue nitrate-N analysis utilizes H_2O_2-H_2SO_4 mixture for digestion of plant material in the absence of heavy metals which were previously used in the plant and soil analysis (McGill and Figureiredo, 1993). At the end of the growing season, 5 plants from each of the 2 middle rows of each plot were randomly selected to evaluate plant height number of nodes (cotyledon node 0). Yields were taken from the two middle rows of each plot using a 2-row John Deere cotton picker on 13 October.

Data Analyses

The greenhouse experiments were analyzed using a paired t-test, assuming heterogeneity of variances, and using a null hypothesis of equal parasitism in both treatments (SAS Institute 1999).

Field data were analyzed using analysis of variance (PROC GLM of SAS; SAS Institute 1999), followed by a means separation using the Waller-Duncan Bayesian *k* ratio (with k = 100) when significant differences were indicated by the ANOVA. Abundance data from the shake samples were analyzed with repeated measures ANOVA.

Results and Discussion

The greenhouse trials indicated that *Cotesia marginiventris* does not have a preference, given the two nitrogen options in the cage setting. Approximately the same percentage of beet armyworm larvae was successfully parasitized in both treatments (39.3 $\pm$ 20.28% parasitism at 42 ppm N, and 42.6 $\pm$ 9.94% parasitism at 196 ppm N), although the variability in parasitism rates was higher in the lower nitrogen treatment. These data suggest that this parasitoid may not be significantly affected by nitrogen levels in the plant, which would be a positive feature in a biological control agent.

The various nitrogen treatments in the field exerted significant effects on the plants, with the highest nitrate readings, greatest plant height, and greatest number of nodes on plants in the highest nitrogen treatments (Table 1). However, there were no significant differences in plant height or number of nodes among any of the treatments receiving nitrogen. Nor was yield was significantly affected by nitrogen treatment (Table 1). These results indicate that the nitrogen treatments did have significant effects on the plants,

which, in turn, could affect the pest and beneficial species associated with them, but that these differences did not translate into statistically-significant yield effects.

Overall, pest numbers were low throughout the season, and there were very few significant differences among treatments (Table 2). Caterpillar pests were present in low numbers, and were not sufficiently abundant to permit statistical evaluation (see Table 4 for totals). Cotton aphids appeared relatively early, built up quickly, then rapidly declined (Table 2, Fig. 1). Aphid abundance was significantly affected by nitrogen treatment, with the highest aphid numbers occurring in the 40 and 120 lbs/acre treatments. Similar to caterpillars, relatively few bug pests were observed in 2006 in the plots. The dominant bug species present were the fleahoppers – the cotton fleahopper, *Pseudatomoscelis seriatus*, and garden fleahopper, *Halticus bractatus*, but there were no significant differences among treatments for abundance of any of the bug pests.

Among natural enemies observed in samples, only the big-eyed bug *Geocoris punctipes* differed significantly in abundance among nitrogen treatments (Table 3). The predator was consistently least abundant in the 0 nitrogen treatment, while abundance varied in the other treatments (Fig. 2). The low predator population in the 0-nitrogen treatment may be in response to relative prey abundance, or may be a direct response to the plant, as *Geocoris* species are omnivores that also respond to plants. Unlike 2005, spider abundance was unaffected by nitrogen level (Table 3).

The results of the sentinel beet armyworm larval trials also failed to yield any statistically-significant differences in numbers of caterpillars collected or parasitized among nitrogen treatments. Very few of the larvae placed at each location were recovered (Table 5), with no differences among treatments. Parasitism rates were somewhat low, and did not vary significantly among treatments (Table 5), but it must be noted that the larvae were only exposed for 48 hours. Longer exposure might have resulted in higher parasitism rates, but also would have resulted in higher loss of caterpillars. Loss of larvae may have been due to predation, dislodgement, or movement, so it is difficult to interpret larval loss as predation without more detailed studies. However, the egg mass study provides greater insight into predation because the egg masses remain where they are placed, and the removal or consumption via chewing or sucking is recognizable. Sentinel eggs were predated at a significantly higher rate in the 0-nitrogen treatment than in the highest nitrogen treatment, with predation in the other nitrogen treatments generally falling numerically in between the two extremes (Table 6). This result indicates that predators were probably quite active in removing beet armyworms in all of the treatments (although some of the loss is also certainly due to dislodgement from the plants), but that the predation is greatest with the least nitrogen. This may reflect differences in plant structure, since cotton plants in the 0-nitrogen treatment were smaller and less complex than those in other treatments, and may have facilitated searching by predators. Predator abundance would not account for the difference in predation rates, because there was only one species that exhibited a significant effect of nitrogen treatment (*G. punctipes*), and it was most abundant in the higher nitrogen treatments (Fig. 2). The causes of this difference in predation among treatments are unclear at present.

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Table 1. Cotton plant growth characters and seed cotton yield in response to varying N fertilization levels (Lang Farm, Tifton, GA, 2006). Petiole samples collected in September. Height and node number were evaluated at the end of the season.

Nitrogen treatment (lbs/A)	Petiole NO_3-N Mean±SEM (ppm)	Plant height Mean±SEM (m)	Node No. *Mean±SEM*	Yield (lbs seed cotton/a)
0	151.25 ± 20.83	0.96 ± 0.0045 a	22.1 ± 0.06 a	2447.09 ± 89.35
40	219.80 ± 33.60	1.14 ± 0.0033 b	24.8 ± 0.06 b	2776.13 ± 57.47
80	427.22 ± 47.52	1.16 ± 0.0052 b	24.7 ± 0.06 b	2691.91 ± 41.78
120	446.49 ± 55.96	1.15 ± 0.0052 b	24.3 ± 0.07 b	2771.74 ± 59.13

Source	**DF**	**F**	***P***	**DF**	**F**	***P***	**DF**	**F**	***P***	**DF**	**F**	**P**
Block	5	1.01	0.44	5	30.8	< .0001	5	10.36	< 0.0001	5	3.70	0.02
Treatment	3	0.58	0.64	3	11.9	< .0001	3	10.59	< 0.0001	3	1.62	0.23

Means followed by different low case letters within a column indicated significant difference. Data were analyzed with ANOVA. Means were separated with paired Bonferroni's *t*-test if overall null hypothesis was rejected ($p<0.05$).

Table 2. ANOVA results of insect pests in cotton field (Lang Farm, Tifton, GA 2006)

		***Heliothis* spp.**		**Loopers[1]**		***Lygus lineolaris***		**Fleahoppers[2]**		**Stink bugs[3]**		***Aphis gossypii***	
Source	**DF**	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Block	5	1.78	0.12	2.63	0.03	0.39	0.86	1.58	0.17	0.80	0.55	1.89	0.10
Date	9	10.80	<0.0001	1.47	0.16	8.15	<0.0001	10.60	<0.0001	1.70	0.09	73.53	<0.0001
Treatment	3	0.50	0.68	1.19	0.31	2.04	0.11	0.73	0.54	1.98	0.12	3.09	0.03
Date*Treatment	27	0.73	0.83	0.85	0.68	1.97	0.0045	0.86	0.67	1.32	0.14	1.31	0.15

[1] soybean looper *P. includens* and cabbage looper *T. ni*; [2]cotton fleahopper *P. seriatus* and garden fleahopper *H. bractatus*; [3]southern green stinkbug *N. viridula*, green stinkbug *A. hilare*, and brown stinkbug *E. servus*). Treatment: no fertilizer throughout the growing season (T1); 1 application of 40 lbs/a during the season (T2); 2 applications during the season (T3); 3 applications during the season (T4). Data were analyzed with repeated measure ANOVA.

Table 3. ANOVA results of beneficial arthropods in cotton field (Lang Farm, Tifton, GA 2006)

2005		Ants		Spiders		*Geocoris* spp.		Ladybeetles[1]		*Orius* spp.		Lacewi	
Source	**DF**	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	
Block	5	0.36	0.87	3	0.01	1.20	0.31	4.55	0.0006	3.74	0.003	3.43	0
Date	9	13.16	<0.0001	11.22	<0.0001	31.75	<0.0001	88.66	<0.0001	53.97	<0.0001	12.09	<(
Treatment	3	0.90	0.44	1.76	0.16	7.33	0.0001	0.84	0.48	0.16	0.93	2.23	
Date*Treatment	27	0.50	0.98	1.14	0.30	1.30	0.16	0.69	0.87	0.37	1.00	1.86	(

[1] 7-spotted lady beetles, *C. septempunctana*; Asian lady beetle *H. axyridis*; convergent lady beetle *H. convergens*; scymnus lady beetle *Scymnus* spp.;[2] green lacewing *Chrysoperla* spp. and *Chrysopa* spp.; brown lacewing *Hemerobius* spp. and *Micromus* spp. Treatment: no fertilizer throughout the growing season (T1); 1 application of 40 lbs/a during the season (T2); 2 applications during the season (T3); 3 applications during the season (T4). Data were analyzed with repeated measure ANOVA.

Table 4. Seasonal parasitism of caterpillars collected during weekly drop cloth sampling (Lang Farm, Tifton, GA, 2006)

Nitrogen treatment (lbs/A)	***Heliothis* spp. (%)**	**Loopers (%)**	**Others (%)**	**No. of caterpillars collected**	**Total (%)**
0	0.00	0.00	0.00	42	36.36
40	4.55	30.77	12.50	67	16.67
80	0.00	5.56	12.12	69	30.65
120	0.00	27.27	13.04	56	20.93
				Mean	25.44

Table 5. Loss and parasitism of BAW caterpillars placed in the field (mean±SEM) on 9 and 22 August and left uncovered for two days prior to collection.

Nitrogen treatment (lbs/A)	2006	
	Recovery rate	Parasitism rate
0	6.61±0.60	7.69±1.90
40	10.89±1.74	24.76±5.52
80	17.23±1.29	18.25±2.69
120	12.18±1.04	28.39±3.24
	χ^2=2.71	χ^2=2.11
	DF=3	DF=3
	P=0.45	*P*=0.55

T1: no fertilizer throughout the growing season; T2; 1 application of 45 kg/ha during the season; T3: 2 applications during the season; T4: 3 applications during the season. Data were analyzed with non-parametric Kruskal-wallis test.

Table 6. Percent of BAW eggs predated (mean %±SEM) in the field (Lang Farm, Tifton, GA 2006)

Nitrogen treatment (lbs/A)	Sampling time after placement			
	6 h	24 h	30 h	48 h
0	37.53±1.64 b	83.28±1.43 b	90.66±1.10 b	92.52±0.94 b
40	55.20±1.82 b	66.15±1.73 b	72.93±1.54 a	81.38±1.45 ab
80	43.23±1.91 b	70.18±1.82 b	75.02±1.68 a	90.48±1.10 b
120	16.71±1.11 a	36.90±1.76 a	52.00±1.86 a	61.64±1.78 a
	χ^2=10.03 DF=3 *P*=0.0183	χ^2=14.00 DF=3 *P*=0.0029	χ^2=12.25 DF=3 *P*=0.0066	χ^2=12.48 DF=3 *P*=0.0059

Sampling time: hours after setting up of experiment. Data were analyzed with non-parametric Kruskal-wallis tests.

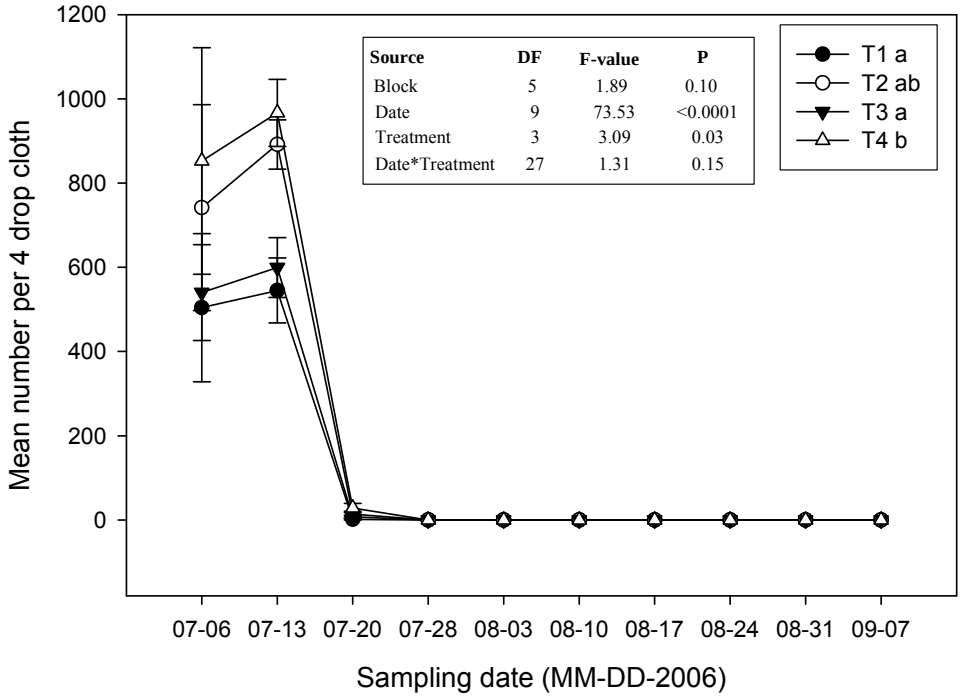

Fig 1. Seasonal dynamics of aphids in the cotton field, 2006. T1: no fertilizer throughout the growing season; T2; 1 application of 40 lbs/a during the season; T3: 2 applications during the season; T4: 3 applications during the season. Data were analyzed with repeated measures ANOVA.

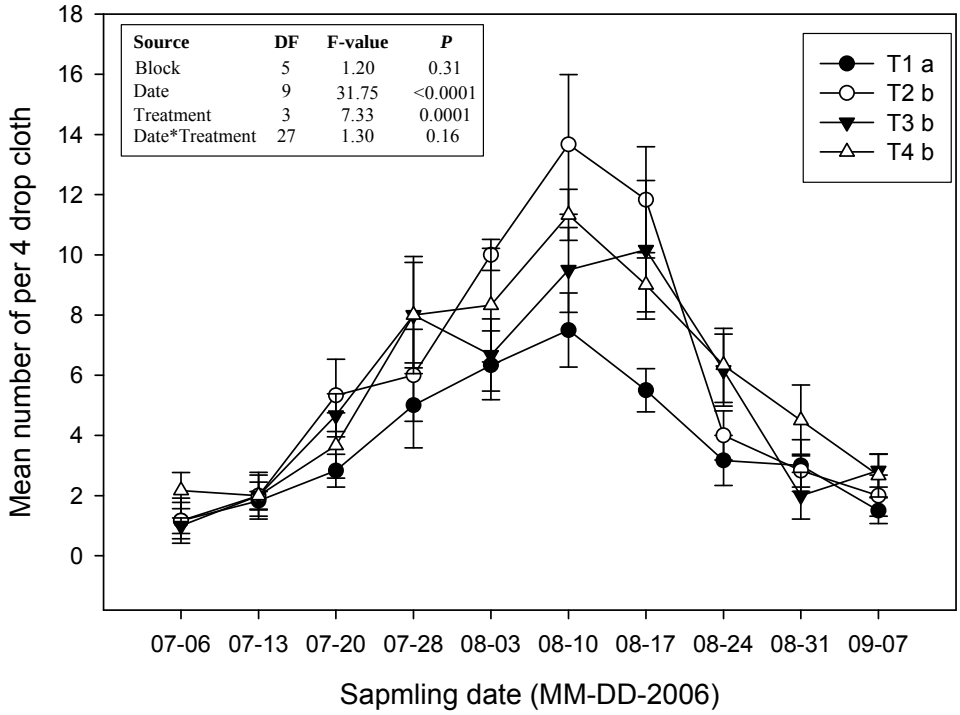

Fig 2. Seasonal dynamics of *Geocoris* spp. in the cotton field, 2006. T1: no fertilizer throughout the growing season; T2; 1 application of 40 lbs/a during the season; T3: 2 applications during the season; T4: 3 applications during the season. Data were analyzed with repeated measures ANOVA.