EFFECTS OF NATURAL ENEMY CONSERVATION AND PLANTING DATE ON THE SUSCEPTIBILITY OF BT COTTON TO HELICOVERPA ZEA IN NORTH CAROLINA

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Abstract

The effects of natural enemy conservation versus disruption and early versus late planting dates on the larval population development of caterpillar pests, fruit damage, and yield were examined in pure and blended genotypes of Bollgard™ *B.t.* cotton and in non-*B.t.* cotton. Percent (%) larval infestation and % damaged fruit were higher in disrupted plots than in conserved plots. Disrupted plots of 0% B.t.: 100% non-B.t. yielded significantly less than conserved plots of the same seed blend, but disrupted plots of the remaining seed blends had higher yields than their respective conserved plots. Mean % larval infestation and % damaged fruit were higher in early-planted cotton than in late-planted cotton, but early-planted cotton yielded significantly higher than late-planted cotton. As the percentage of B.t. seed in the blends decreased, numbers of live larvae and damaged fruit increased, and yields were reduced. Overspraying 100% B.t.: 0% non-B.t. cotton with lambda cyhalothrin resulted in significant reductions in % larval infestations and % damaged fruit and an increase in seed cotton yield.

Introduction

Plants expressing toxic proteins from *Bacillus thuringiensis* Berliner var. *kurstaki* (*B.t.*) will be commercially available in 1996. The delta-endotoxin proteins from this *B.t.* strain are toxic to many lepidopteran larval pests of cotton including tobacco budworm (*Heliothis virescens*), bollworm (*Helicoverpa zea*), and European corn borer (*Ostrinia nubilalis*). The tobacco budworm is particularly susceptible to the *B.t.* proteins expressed in the transgenic cottons developed jointly by Monsanto Company and Delta and Pinelands Company (e.g., Jenkins et al. 1993, Mascarenhas et al. 1994). This technology offers great promise for management of tobacco budworm in regions where the occurrence of strains resistant to synthetic insecticides has made control practically impossible. However, laboratory studies (Stone & Sims 1993) have shown the bollworm to be much less susceptible to *B.t.* endotoxins than tobacco budworm. This was confirmed in field experiments conducted in North Carolina where bollworm larval feeding resulted in boll damage levels as high as 32 percent and in significant yield reductions (Mahaffey et al. 1994, 1995). However, the North Carolina tests were conducted under conditions which are known to promote the highest bollworm larval populations (i.e., disruption of natural enemies through foliar application of a broad-spectrum insecticide and late planting of cotton). Because *H. zea* constitutes the majority of the bollworm/budworm complex on cotton each year in North Carolina (Bradley 1993, Bacheler 1995), it is essential to develop an understanding of the potential interactions of *H. zea* and *B.t.* cottons.

The study reported herein examined the efficacy of transgenic *B.t.* cotton under optimum crop management tactics. Specifically, the experiment exam-ined the effects of arthropod natural enemy conservation versus disruption and early versus late planting dates on the larval population development of caterpillar pests, fruit damage, and yield. Pure and blended genotypes of Bollgard™ (Monsanto Agric. Co., St. Louis, MO) *B.t.* cotton and non-*B.t.* cotton were used.

Materials and Methods

Test Description

The test was conducted at the Upper Coastal Plain Research Station in Edgecombe County near Rocky Mount, NC, in 1995. This study was designed to examine main treatment effects (natural enemy conservation, planting date, and seed blends) and their interactions on caterpillar populations, fruit damage, and yield. The experiment was a split-split plot with main plots, sub-plots, and sub-sub-plots as natural enemy conservation, planting date, and seed blend, respectively, arranged in a randomized complete block design replicated four times. The planting dates were 5 May 1995 and 22 May 1995. The cotton seed blends included: 1) 100% *B.t.*:0% non-*B.t.*-TAN (treated as needed with Karate™ for caterpillar control); 2) 100% *B.t.*:0% non-*B.t.*; 3) 85% *B.t.*:15% non-*B.t.* ; 4) 75% *B.t.*: 25 % non-*B.t.*; 5) 0% *B.t.*:100% non-*B.t.* Each sub-sub-plot was four rows wide by 40 feet long with 36 inch row-spacing.

Aldicarb (Temik™ 15G, Rhone-Poulenc Ag Company, Research Triangle Park, NC) was applied @ 0.75 lb. ai/A in-furrow at planting for early season thrips control. At appropriate times throughout the season, fertility, weed control, plant growth regulation, and defoliation practices as recommended by North Carolina State University (1995) for maximum cotton yields were followed.

Arthropod natural enemy disruption was accomplished in selected plots through application of insecticides during midseason. Aldicarb (Temik™ 15G, Rhone-Poulenc Ag Co., RTP, NC) was applied as a sidedress treatment @ 1.5 lb. ai/A on 12 July . These same plots were further disrupted on 21 July with a foliar application of acephate (Orthene™ 75S, Valent USA Corp., Walnut Creek, CA) @

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1.0 lb. ai/A. Lambda cyhalothrin (Karate™ 1EC, Zeneca, Inc., Wilmington, DE) was applied @ 0.04 lb. ai/A to seed blend 100% *B.t.*:0% non-*B.t.*-TAN on 27 July and 7 August when the North Carolina State University Extension Service threshold of 10 bollworm eggs per 100 terminals was met or exceeded. These applications were made to further minimize the impact of bollworms on yields in selected plots.

Data Collection

Cotton plants were sampled on six dates for percent (%) larval infestation and % damaged fruit per plot. Egg deposition was measured on the first sampling date, 31 July 1995, as the number of *H. zea*/ *H. virescens* eggs per 25 terminals in the 100% *B.t.*:0% non-*B.t.* and 0% *B.t.*:100% non-*B.t.* plots only. The number of live larvae per 25 (sampling dates 1, 2, and 6) or 50 (sampling dates 3, 4, and 5) squares and/or bolls per plot was recorded to determine percent larval infestation. Fruit damage was quantified as the number of squares and/or bolls per 25 or 50 observed which were damaged by boll-worm/budworm. Squares were considered damaged when sufficient feeding on the anthers had occurred to cause the plant to abort the square. Bolls were considered damaged when the carpel wall had been penetrated. Larvae were collected from the field plots on four sampling dates and transported to the laboratory for species identification using methods described by Neunzig (1969). In addition, sweepnet samples were taken in disrupted and non-disrupted plots four days after the acephate application to quantify arthropod natural enemy populations. Finally, the center two rows of each four-row sub-sub-plot were harvested mechanically.

Data Analysis

Numbers of eggs, live larvae, and damaged fruit per plot were converted to percentages prior to analysis. Yields are reported as pounds of seed cotton per acre. All data were subjected to ANOVA using PROC GLM (SAS Insti-tute 1990). Means for each treatment for the season and for each sampling date were separated ($P \leq 0.05$) using the LSMEANS procedure of SAS.

Results

Pest Species

The bollworm was the only lepidopterous pest which occurred at damaging levels in this test. Larval collections were made on four sampling dates from both the *B.t.* and non-*B.t.* plots. Samples collected from 100% *B.t.*: 0% non-*B.t.* plots were identified as 97.7% bollworm (n=42) and 2.3% (n=1) tobacco budworm. Bollworm and tobacco budworm larval populations were 95.2% (n=60) and 4.8% (n=3), respectively, in 0% *B.t.*:100% non-*B.t.* plots. Other caterpillar pests (e.g., European corn borer) occurred in numbers too low to impact test results.

Effects of Natural Enemy Disruption

Mean % larval infestation and mean % damaged fruit are recorded for each cotton seed blend in Table 1 and Table 2, respectively. There were higher numbers of bollworm larvae infesting fruit in disrupted plots for all seed blends; however, these differences were significant ($P \leq 0.05$) for only the 0% *B.t.*:100% non-*B.t.* treatment. As with larval infestations, percent damaged fruit was numerically higher in disrupted plots than in conserved plots, but was significantly higher in only seed blends of 85% *B.t.*:15% non-*B.t.*, 75% *B.t.*:25% non-*B.t.*, and 0% *B.t.* :100% non-*B.t.* Disrupted plots of 0% *B.t.*:100% non-*B.t.* yielded significantly less than conserved plots of the same seed blend. However, disrupted plots of the remaining seed blends had higher yields than their respective conserved plots; in fact, yields were significantly higher in disrupted plots of 100% *B.t.*:0% non-*B.t.*-TAN than in the 100% *B.t.*:0% non-*B.t.*-TAN conserved plots (Table 3).

Effects of Planting Date

When the data were averaged across seed blend treatments, there were no significant differences in egg deposition detected between early-planted and late-planted cotton. However, mean % larval infestation and mean % damaged fruit were significantly higher in the early-planted cotton plots; yet, early-planted cotton had significantly higher yields than late-planted cotton (Table 4).

Within seed blends, only the early-planted 85% *B.t.*:15% non-*B.t.*seed mixture had a significantly higher % larval infestation than the late-planted plots of the same seed mixture (5.05% and 2.70%, respectively). In addition, early-planted plots of the 85% *B.t.*:15% non-*B.t.* and 75% *B.t.*:25% non-*B.t.* seed blends had significantly higher % damaged fruit than their respective late-planted plots. Mean yield within each seed blend was significantly higher in early-planted cotton plots (Table 5).

Effects of Seed Blends

No significant differences in egg deposition were detected between the 100% *B.t.*:0% non-*B.t.* and the 0% *B.t.*: 100% non-*B.t.* seed blends. Mean % larval infestation, mean % damaged fruit, and mean yield (lbs. seed cotton/ acre) for each seed blend (across planting dates and conservation/disruption) are recorded in Table 6. Percent larval infestation and % damaged fruit were lowest in the 100% *B.t.*:0% non-*B.t.*-TAN seed blend. Numbers of larvae and fruit damage increased as the percentage of *B.t.* seed in the blends decreased. Conversely, seed cotton yields decreased as the percentage of *B.t.* seed in the blends decreased. Overspraying the 100% *B.t.* cotton with lambda cyhalothrin resulted in significant reductions in larval infestation and fruit damage and an increase in seed cotton yield.

Data for mean % damaged fruit for each seed blend on each sampling date are presented in Table 7. On 3 August and 7 August the 0% *B.t.*:100% non-*B.t.* seed blend had significantly higher % fruit damage than the remaining four blends. Significant differences in % damaged fruit were detected be-tween all seed blends on 14 August, and similar observations were made on 21 August except that there was no difference between the 100% *B.t.*:0% non-*B.t.* and 85% *B.t.*:15% non-*B.t.* seed blends on this date. On the final sampling date, 29 August 1995, seed blend 100% *B.t.*:0% non-*B.t.*-TAN had significantly less % fruit damage than all seed blends except 100% *B.t.*:0% non-*B.t.*

Discussion

The results of this field study were similar in many respects to those obtained in field studies conducted earlier in North Carolina (Mahaffey et al. 1994, 1995) where various *B.t.* seed blends (75-100% B.t. seed) were damaged by bollworm to the extent that significant yield reductions (ca.10-20 %) resulted. In previous field experiments natural control through the activity of predators and parasitoids was disrupted over the entire experimental site. Thus, the concern developed that disruption of biological control with foliar application of a broad-spectrum insecticide was a prerequisite for bollworm larval populations to develop to levels which would negatively impact yields. For that reason, the 1995 test was designed to include both disruption and conservation of biological controls. As expected, disruption of biological controls had the overall effect of increasing bollworm numbers and fruit damage; however, bollworm numbers were significantly increased only in the 0% *B.t.*:100% non-*B.t.* treatment and fruit damage was significantly higher only in the treatments which did not contain 100% *B.t.* seed. Seed cotton yields in this test suggest that disruption of biological controls with synthetic insecticides was not the factor responsible for yield reductions reported for *B.t.* cotton in earlier tests since yields were either similar or higher where biological control had been disrupted with the exception of the 0% *B.t.*:100% non-*B.t.*

Disruption of arthropod natural enemies did not negatively impact yield of *B.t.* cotton in this test for the likely reason that the synthetic insecticide applications designed to disrupt biological control provided control of stink bug and plant bug populations in disrupted plots. Thus, the yield reduction potential of these hemipteran species exceeded that of the increased bollworm larval population which resulted from disruption of biological controls. This effect only occurred in the *B.t.* cotton which has a very high inherent level of resistance to bollworm. Conversely, disruption had a very pronounced negative effect on yield in the non-*B.t.* cotton, as expected. Another reason that disruption of biological controls was not associated with decreased yields in *B.t.* cotton in this experiment is because predator/ parasitoid populations were low in the *B.t.* plots prior to the insecticide applications designed to disrupt. Perhaps the lack of a sufficient food source (e.g., caterpillars) in *B.t.* cotton plots kept beneficial arthropod populations at a minimum. Subsequent studies will further examine and quantify arthropod natural enemy populations in the field within each seed blend plot before and after insecticide applications for disruption.

Although early planting of cotton in this experiment resulted in greater % fruit damage, the early-planted cotton yielded higher than late-planted cot-ton. This may best be explained by an earlier bollworm moth flight and unusually high temperatures in the latter portion of the growing season. At the initiation of the moth flight, early-planted cotton had substantial fruit set compared to late-planted cotton and much of the fruit on the early planted cotton was mature enough to be resistant to bollworm larvae. Conversely, most of the fruit on the late-planted cotton was susceptible to bollworms. In addition, unusually high late-season temperatures caused significant square-shed in the physiologically delayed cotton plants of the late-planted plots, and this stress very likely had a negative effect on yield. Previous studies in North Carolina demonstrated that planting cotton early may minimize economic loss by reducing crop attractiveness and boll susceptibility to late-season caterpillar pests as well as crop susceptibility to late-season weather- related stresses (Ihrig et al. 1995). Early planting will very likely be an effective management tactic for transgenic *B.t.* cotton varieties in North Carolina.

Widespread adoption of *B.t.* cotton may cause lepidopteran pests to develop resistance to the *B.t.* endotoxin as they have to many synthetic insecticides. Deployment strategies are necessary to prevent, or at least delay, the onset of such resistance. McGaughey (1990) suggested alternating insecticides, using multiple toxins, providing untreated refuges, selecting appropriate doses, or treating selected plant parts. Arpaia and Ricchiuto (1993) examined alternative strategies for potato pest management, and suggested using mixtures of plants, including *B.t.* and non-*B.t.* types, as a refuge. Gould (1991) also mentions the use of untreated pest habitats offering refuge in or near the crop as a means of delaying resitance with the multiple toxin effect. One means of providing such intrafield refugia would be to mix seed of *B.t.* cotton with that of non-*B.t.* cotton. These non-*B.t.* plants may help maintain susceptible insects in the population so that the gene frequency of resistance from selected individuals is kept very low.

In this experiment the treatments incorporating blends of *B.t.* and non-*B.t.* seed (85:15 and 75:25) sustained too much fruit damage and yield loss for the blended seed concept to be practical. In areas where bollworm populations occur at high levels, lower amounts of non-*B.t.* seed in the blend will be necessary to prevent yield losses. These results are in agreement with those reported by Mahaffey et al. (1995). In addition, Mallet and Porter (1992) suggested that seed mixtures may enhance resistance development in mobile insects. Thus, it is likely that refugia will have to be accomplished through some means other than the blended seed strategy for bollworm.

In summary, our experimental results suggest that disruption of biological control does not necessarily result in a negative yield effect in *B.t.* cotton; in fact, insecticides applied to *B.t.* cotton may have a positive yield influence by controlling non-lepidopterous pests. Early planting resulted in increased yield of cotton despite higher levels of fruit injury, and blends of *B.t.* and non-*B.t.* cotton seed sustained too much fruit damage and yield reduction to be considered practical as a resistant management strategy.

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Table 1. Mean percent (%) larval infestation in disrupted and conserved plots for each cotton seed blend, Edgecombe County, NC, 1995.

Seed Blend (% B.t.: % non-B.t.)	Mean % Larval Infestation[a]	
	Disrupted	Conserved
100 : 0 (TAN)	2.04 a	1.13 a
100 : 0	3.10 a	1.60 a
85 : 15	4.95 a	2.80 a
75 : 25	6.20 a	4.00 a
0 : 100	12.83 a	7.04 b

[a/]Means followed by the same letter within each row are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 2. Mean percent (%) damaged fruit in disrupted and conserved plots for each cotton seed blend, Edgecombe County, NC, 1995.

Seed Blend (% B.t.: % non-B.t.)	Mean % Damaged Fruit[a,b]	
	Disrupted	Conserved
100 : 0 (TAN)	6.54 a	3.04 a
100 : 0	11.95 a	8.50 a
85 : 15	17.60 a	10.85 b
75 : 25	23.05 a	14.90 b
0 : 100	36.88 a	19.79 b

[a/]Squares and/or bolls were sampled as fruit depending on cotton plant physiology at time of sampling.
[b/]Means followed by the same letter within each row are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 3. Mean yield (lbs. of seed cotton/ acre) in disrupted and conserved plots for each cotton seed blend, Edgecombe County, NC, 1995.

Seed Blend (% B.t.: % non-B.t.)	Mean Yield (lbs. seed cotton/acre)[a]	
	Disrupted	Conserved
100 : 0 (TAN)	3242.04 a	2983.41 b
100 : 0	2717.96 a	2568.23 a
85 : 15	2479.74 a	2425.29 a
75 : 25	2409.41 a	2271.02 a
0 : 100	1377.13 b	1994.23 a

[a/]Means followed by the same letter within each row are not significantly different according to LSMEANS Procedure ($P \leq 0.05$).

Table 4. Mean percent (%) egg deposition, % live larvae, % damaged fruit, and yield for early-planted and late-planted cotton, Edgecombe Co., NC, 1995.

Sample Collected	Planting Date[a]	
	Early	Late
% Egg Deposition	18.25 a	23.50 a
% Larval Infestation	5.10 a	4.04 b
% Damaged Fruit	17.47 a	13.15 b
Yield	2718.87 a	2174.37 b

[a/]Means followed by the same letter within each row are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 5. Mean percent (%) damaged fruit and mean yield (lbs. seed cotton/ acre) in early- and late-planted cotton seed blends, Edgecombe Co., NC, 1995.

Seed Blend (% B.t.: % non-B.t.)	% Damaged Fruit[a]		Yield[a]	
	Early	Late	Early	Late
100 : 0 (TAN)	5.83 a	3.75 a	3401.3 a	2824.1 b
100 : 0	12.30 a	8.15 a	2884.0 a	2403.1 b
85 : 15	17.25 a	11.20 b	2639.0 a	2266.9 b
75 : 25	22.65 a	15.30 b	2597.3 a	2083.6 b
0 : 100	29.33 a	27.33 a	2074.6 a	1297.7 b

[a/]Means followed by the same letter within each row are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 6. Mean percent (%) larval infestation, mean % damaged fruit, and yield for cotton seed blends, Edgecombe Co., NC, 1995.

Seed Blend (% B.t.: % non-B.t.)	% Larval Infestation[a]	% Damaged Fruit[a]	Yield (lbs. seed cotton/acre)[a]
100 : 0 (TAN)	1.58 e	4.79 e	3112.73 e
100 : 0	2.35 cd	10.23 d	2643.09 d
85 : 15	3.88 bc	14.23 c	2452.52 bc
75 : 25	5.10 b	18.98 b	2340.22 b
0 : 100	9.94 a	28.33 a	1685.68 a

[a/]Means followed by the same letter within each column are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 7. Mean percent damaged fruit for cotton seed blends on all sampling dates in Edgecombe County, NC, 1995.

SeeBlend (% B.t.: % non-B.t.)	Sampling Date[a]				
	8-3-95	8-7-95	8-14-95	8-21-95	8-29-95
100 : 0 (TAN)	9.13 b	6.75 c	2.13 e	2.0 e	4.8 c
100 : 0	10.50 b	5.88 c	10.23 d	11.5 cd	13.0 bc
85 : 15	11.00 b	8.00 bc	15.38 c	14.8 c	22.0 ab
75 : 25	13.50 b	10.50 b	21.38 b	23.0 b	26.5 a
0 : 100	24.63 a	22.25 a	44.88 a	35.0 a	30.5 a

[a/]Means followed by the same letter within each column are not significantly different according to LSMEANS procedure ($P \leq 0.05$).