

**INTERACTIONS OF HELICOVERPA ZEA
AND BT COTTON IN NORTH CAROLINA**
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Abstract

The efficacies of Bollgard™ cotton and non-*B.t.* cotton were examined under contrasting crop management strategies at two locations in eastern North Carolina in 1996. The effects of natural enemy conservation/disruption, early/late planting of cotton, and application of pyrethroids as needed for supplemental insect control were observed. Results suggest that disruption of natural enemies with insecticides during midseason did not negatively affect yields in pyrethroid-treated and untreated BT plots and treated NBT plots because mean yields (lbs seed cotton/acre) were significantly higher in the conserved plots of only the untreated NBT cotton plots. Although no significant differences were detected in mean % egg deposition, mean % larval infestation, and mean % damaged fruit between early-planted and late-planted plots at either test site in 1996, yields were higher in early-planted cotton than in late-planted cotton. Pyrethroid-treated BT plots had significantly lower % bollworm larval infestation and % damaged fruit and significantly higher yields than untreated BT cotton at both test sites in 1996. Yields in BT plots were increased by 11% (Edgecombe Co.) and 23% (Martin Co.) when a pyrethroid was applied for bollworm control.

Introduction

The 1996 commercialization of Monsanto's Bollgard™ (Monsanto Agric. Co., St. Louis, MO) cotton provided growers with a new tool for combatting caterpillar pests in cotton. The delta-endotoxin proteins from *Bacillus thuringiensis* Berliner var. *kurstaki* (*B.t.*) expressed in the transgenic cotton plants are toxic to most lepidopteran larval pests of cotton including tobacco budworm [*Heliothis virescens* (F.)] and bollworm [*Helicoverpa zea* (Boddie)] (MacIntosh et al. 1990). Numerous field trials with Bollgard™ across the cottonbelt in the last five years demonstrated excellent control of these caterpillar pests, especially of tobacco budworm (e.g., Benedict et al. 1992, Jenkins et al. 1993, Jenkins and McCarty 1995, Luttrell et al. 1995, Mascarenhas et al. 1994). However, field trials in North Carolina where bollworm constitutes the majority of the bollworm/budworm complex on cotton each year indicated that a proportion of the bollworm larvae could survive, feed, and damage squares and/or bolls on these transgenic plants.

Mahaffey et al. (1994, 1995) observed bollworm larval feeding that resulted in boll damage levels as high as 32% and significant yield reductions when field experiments were conducted under conditions known to promote high bollworm larval populations. These field trials were expanded in 1995 by Lambert et al. (1996) to examine the efficacy of transgenic *B.t.* cotton under contrasting crop management tactics of natural enemy conservation and disruption and early and late planting. These studies also showed that transgenic *B.t.* cotton alone would not provide sufficient control of bollworm to prevent economic yield loss in areas where bollworm populations occur at high levels. This was further evidenced across the cottonbelt in 1996 when Bollgard™'s protection had to be supplemented with one or two applications of pyrethroids when bollworm populations reached unusually high levels (Soybean Digest 1996).

Field trials examining the efficacy of Bollgard™ cotton under contrasting crop management tactics were repeated in 1996. Again, the experiment examined the effects of arthropod natural enemy conservation versus disruption and early versus late planting dates on bollworm larval population development, fruit damage, and yield in *B.t.* and non-*B.t.* cotton that was either untreated or treated with a pyrethroid to minimize the impact of bollworm on yield in selected plots.

Materials and Methods

Tests were conducted at two locations in eastern North Carolina - the Upper Coastal Plain Research Station in Edgecombe Co., NC, and the C. A. Martin Farm in Martin Co., NC. Each test was a randomized complete split-split block design with four replicates. Both tests had main plots as natural enemy conservation/disruption, sub-plots as early/late planting date, and sub-sub-plots (4 rows wide X 40 ft long with 36-inch row-spacing) as cotton seed treatment. Early -planted plots were planted on 29 April 1996 at both test sites. Late-planted plots were planted on 14 May 1996 at Edgecombe Co., NC, and on 15 May 1996 at Martin Co., NC. The late-planted dates were well within the normal planting period for cotton in northeastern North Carolina. The four seed treatments included 1) BT-TAN; 2) BT; 3) NBT-TAN; and 4) NBT. The BT seed treatments were NuCOTN 33B and NBT seed treatments were DP5415. TAN plots were treated as needed with Karate™ for caterpillar control.

Arthropod natural enemies were disrupted in selected plots in 1996 as in 1995 (Lambert et al. 1996) with single insecticide applications of aldicarb (sidedress incorporated) and acephate (broadcast foliar) during midseason. All plots at both test sites were sampled with a sweepnet for quantification of natural enemy populations before and after insecticide applications designed for disruption. Lambda cyhalothrin (Karate™ 1EC, Zeneca, Inc., Wilmington, DE)

was applied initially to TAN plots at each test site when 10 or more bollworm eggs per 100 terminals were observed (= 3 total applications). Other cotton production practices performed in 1995 as recommended by North Carolina State University (1994) were repeated in 1996. Cotton plots in Edgecombe Co. were irrigated with 1" H₂O/A twice in July 1996.

Cotton plots at each test site were monitored in 1996 for eggs, larvae, and damaged fruit throughout the period of bollworm infestation. The numbers of bollworm larvae and damaged fruit were recorded for all plots at each test site on four sampling dates. The same procedures used in 1995 (Lambert et al. 1996) for determining % egg deposition, % larval infestation, and % damaged fruit were used in 1996. The terminals of 25 cotton plants in each sub-sub-plot were examined for bollworm eggs, larvae, and damaged fruit on 6 August and 8 August in Martin Co. and Edgecombe Co., respectively. On 12 August (Martin Co.) and 13 August (Edgecombe Co.), 50 squares from each sub-sub-plot were examined for bollworm larvae and damaged fruit. Bolls (50 per sub-sub-plot) on random plants were observed at each test site on 19 and 25 August for larvae and damaged fruit. Larvae were collected and identified (Neunzig 1969) twice during the season. A mechanical harvester was used to harvest the center two rows of each four-row sub-sub-plot.

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

Results

Bollworm comprised the majority of the larval pest population in 1996. Of samples collected from both BT and NBT plots on two dates, 99% (n=198) were identified as bollworm and 1% (n=2) were tobacco budworm. Other pest populations of European corn borer, armyworms, plant bugs, and stink bugs were sufficiently low at both test sites in 1996 not to have an effect on yield in any of the cotton seed treatments.

Sweepnet samples of arthropod natural enemies before and after insecticide applications designed for disruption indicated that conservation/disruption was accomplished in selected plots. Prior to application of aldicarb and acephate, no significant differences in predators/parasitoids were detected in Martin Co. and plots selected for disruption in Edgecombe Co. had significantly higher predator/parasitoid counts. After the application of acephate, selected conserved plots had significantly higher natural enemy counts than plots where insecticides had disrupted arthropod natural enemies. Mean % bollworm larval infestation and mean % damaged fruit were significantly higher in

disrupted plots of BT and NBT cotton at the Edgecombe Co. site; disruption of bollworm natural enemies had no effect on larval infestation at the Martin Co. site (Tables 1 and 2). Mean % bollworm damaged fruit was significantly higher in NBT cotton only at Martin Co., NC. Mean yield (lbs seed cotton/A) was significantly lower in disrupted plots of NBT cotton only at both test sites (Table 3).

No significant differences were detected in mean % egg deposition, mean % larval infestation, and mean % damaged fruit between early- and late-planted plots at either test site in 1996 (Table 4). However, yields were generally higher in early-planted cotton than in late-planted cotton; specifically, yields were significantly higher in early-planted BT, NBT-TAN, and NBT plots in Edgecombe Co., NC, and NBT cotton in Martin Co., NC (Table 5).

BT-TAN cotton had significantly lower % bollworm larval infestation than BT and NBT cotton at both test sites in 1996 (Table 6). Percent damaged fruit in BT-TAN plots was also significantly lower than BT, NBT-TAN, and NBT cotton at the Edgecombe Co. test site and significantly lower than BT and NBT cotton at Martin Co., NC (Table 7). BT-TAN cotton yielded significantly higher than all other cotton seed treatments at Edgecombe Co. and significantly higher than BT and NBT at Martin Co., NC (Table 8). Yields in BT-TAN plots were increased by 11% and 23% at the Edgecombe Co. and Martin Co. sites, respectively, when pyrethroids were applied as needed for bollworm control.

Discussion

The results of this study were almost identical to those obtained in 1995 field trials with a few minor exceptions. Stink bug and plant bug populations in 1996 field trials were minimal compared to those populations in 1995; therefore, yield reductions from insects observed in 1996 can be attributed solely to bollworm. Tests in 1995 and 1996 were designed to examine the effects of natural enemy conservation primarily because of the concern that disruption of these beneficial populations in previous tests (Mahaffey et al. 1994, 1995) was a prerequisite for bollworm larval populations to develop to levels that would negatively impact yields of *B.t.* cotton. Tests in both years suggested that disruption of arthropod natural enemies did not negatively affect yields in BT-TAN, BT, or NBT-TAN cotton plots. Disruption did however have a pronounced negative impact on untreated NBT plots. One reason for this is that beneficial predator/parasitoid populations were low in BT cotton plots prior to insecticide applications designed for disruption. The lack of a sufficient food source (i.e., caterpillars) in these plots was due to the mortality factor associated with the *B.t.* toxin in the transgenic plants. It should also be noted that significantly lower numbers of bollworm larvae and damage were observed in conserved BT and NBT plots that were treated with a pyrethroid at the Edgecombe Co. test site indicating

that application of pyrethroids was a stronger compensatory mortality factor than the *B.t.* toxin alone.

Studying the effects of conserving/disrupting natural enemy populations in cotton integrated pest management settings is necessary in understanding the potential for insect adaptation/resistance to transgenic *B.t.* plants. Studies by Gould et al. (1991) support previous arguments that the rate of pre-adaptation to host-plant defenses would be increased or decreased by the occurrence of mortality due to natural enemies. Furthermore, it is imperative that we examine these transgenic *B.t.* cottons under the conventional cotton production management schemes where an array of insecticides may be applied to the crop throughout the season. Insecticide applications for control of non-caterpillar pests in cotton often disrupt the natural enemy populations within the system and thereby increase larval pest populations which may result in significant economic yield loss.

Early-planted cotton again yielded higher than late-planted cotton in 1996, although no significant differences were detected in numbers of larvae and damaged fruit between early- and late-planted cotton. This may be explained by weather-related stresses associated with cooler temperatures during the latter part of the growing season. Accumulation of heat units was not sufficient in 1996 to allow late-planted cotton to reach a maximum yield potential. In fact, studies by Ihrig et al. (1995) showed that planting cotton early may minimize economic loss by reducing crop susceptibility to these weather-related stresses.

The Edgecombe Co. test site was irrigated two times early in the 1996 season while the Martin Co. test site represented dryland cotton production systems. Although irrigating cotton plots may increase caterpillar survival and establishment beyond expected levels (Bacheler 1996), we observed greater differences in larval infestation and therefore damaged fruit and yields between untreated cotton plots and cotton plots treated with a pyrethroid for supplemental insect control in the dryland cotton production setting. Pyrethroid-treated BT plots yielded 23% higher than untreated BT plots in Martin Co. and 11% higher in Edgecombe Co. Similarly, NBT plots treated with a pyrethroid yielded 79% higher than untreated NBT plots in Martin Co. and 47% higher in Edgecombe Co. These differences were observed because bollworm larval populations were highest at the Martin Co. test site, and application of pyrethroids served as a compensatory mortality factor in the pyrethroid-treated plots. In all cases, across natural enemy conservation/disruption and planting date, cotton plots treated with a pyrethroid for supplemental insect control had significantly lower numbers of larvae and damaged fruit and yielded significantly higher than their respective untreated plots.

In summary, results indicated that disruption of arthropod natural enemies was a yield-reducing factor only in

untreated NBT cotton, did not negatively affect BT cotton, and had no effect on systems where supplemental insect control was used. Early-planting of cotton resulted in increased yields in all cotton plots and may be an effective management strategy for transgenic *B.t.* cottons in North Carolina. Finally, application of insecticides as needed for caterpillar control decreased bollworm larval populations and fruit damage and increased yields in all cotton plots and may thereby be necessary for reducing yield loss in BT cotton when bollworm populations reach high levels.

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Table 1. Mean percent (%) bollworm larval infestation in disrupted and conserved plots for each cotton seed treatment at Edgecombe Co., NC, and Martin Co., NC, 1996.

Cotton Seed Treatment	Mean % Larval Infestation ^a			
	Edgecombe County		Martin County	
	Disrupt	Conserve	Disrupt	Conserve
BT-TAN	0.88 a	0.50 a	0.63 a	0.19 a
BT	4.75 a	1.63 b	8.00a	4.69 a
NBT-TAN	1.56 a	1.06 a	1.81 a	2.44 a
NBT	17.00 a	11.00 b	22.63 a	21.88 a

^aMeans followed by the same letter within a row for each county are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 2. Mean percent (%) bollworm damaged fruit in disrupted and conserved plots for each cotton seed treatment at Edgecombe Co., NC, and Martin Co., NC, 1996.

Cotton Seed Treatment	Mean % Damaged Fruit ^a			
	Edgecombe County		Martin County	
	Disrupt	Conserve	Disrupt	Conserve
BT-TAN	0.69 a	0.94 a	1.00 a	1.69 a
BT	7.44 a	2.19 b	12.19 a	6.13 a
NBT-TAN	4.69 a	3.88 a	4.97 a	6.00 a
NBT	35.31 a	22.75 b	53.19 a	45.06 b

^aSquares and/or bolls were sampled as fruit depending on cotton plant physiology at time of sampling.

^bMeans followed by the same letter within a row for each county are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 3. Mean yield (lbs seed cotton / acre) in disrupted and conserved plots for each cotton seed treatment at Edgecombe Co., NC, and Martin Co., NC, 1996.

Cotton Seed Treatment	Mean Yield (lbs seed cotton / acre) ^a			
	Edgecombe County		Martin County	
	Disrupt	Conserve	Disrupt	Conserve
BT-TAN	3909.5 a	3836.9 a	2713.4 a	2673.5 a
BT	3368.6 a	3553.8 a	2023.7 a	2138.1 a
NBT-TAN	3336.0 a	3326.9 a	2577.3 a	2519.2 a
NBT	1515.5 b	2029.2 a	399.3 b	678.8 a

^aMeans followed by the same letter within a row for each county are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 4. Mean % bollworm egg deposition, larval infestation, and damaged fruit and yield (lbs seed cotton/acre) for early-planted and late-planted cotton at Edgecombe Co., NC, and Martin Co., NC, 1996.

Sample Collected	Planting Date ^a			
	Edgecombe County		Martin County	
	Early	Late	Early	Late
% Egg Deposition	63.4 a	65.4 a	45.31 a	52.38 a
% Larval Infestation	4.4 a	5.2 a	7.92 a	7.64 a
% Damaged Fruit	9.3 a	10.2 a	15.59 a	16.96 a
Yield (lbs seed cotton/acre)	3336.0 a	2882.2 b	2061.8 a	1867.6 a

^aMeans followed by the same letter within a row for each county are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 5. Mean yield (lbs seed cotton / acre) for each cotton seed treatment in early-planted and late-planted cotton at Edgecombe Co., NC, and Martin Co., NC, 1996.

Cotton Seed Treatment	Mean Yield (lbs seed cotton / acre) ^a			
	Edgecombe County		Martin County	
	Early	Late	Early	Late
BT-TAN	3994.8 a	3749.8 a	2742.5 a	2642.6 a
BT	3708.0 a	3214.4 b	2192.5 a	1969.3 a
NBT-TAN	3548.3 a	3112.7 b	2624.5 a	2470.2 a
NBT	2094.5 a	1450.2 b	689.7 a	388.4 b

^aMeans followed by the same letter within a row for each county are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 6. Mean % bollworm larval infestation for each cotton seed treatment at Edgecombe Co., NC, and Martin Co., NC, 1996.

Cotton Seed Treatment	% Larval Infestation ^a	
	Edgecombe County	Martin County
BT-TAN	0.69 c	0.41 c
BT	3.19 b	6.34 b
NBT-TAN	1.31 bc	2.13 c
NBT	14.00 a	22.25 a

^aMeans followed by the same letter within each column are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 7. Mean % bollworm damaged fruit for each cotton seed treatment at Edgecombe Co., NC, and Martin Co., NC, 1996.

Cotton Seed Treatment	% Damaged Fruit ^a	
	Edgecombe County	Martin County
BT-TAN	0.81 c	1.34 c
BT	4.81 b	9.16 b
NBT-TAN	4.28 b	5.48 bc
NBT	29.03 a	49.13 a

^aMeans followed by the same letter within each column are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

Table 8. Mean yield (lbs seed cotton / acre) for each cotton seed treatment at Edgecombe Co., NC, and Martin Co., NC, 1996.

Cotton Seed Treatment	Mean Yield (lbs seed cotton / acre) ^a	
	Edgecombe County	Martin County
BT-TAN	3873.2 a	2693.4 a
BT	3461.2 b	2080.0 b
NBT-TAN	3330.5 b	2548.3 a
NBT	1771.4 c	539.1 c

^aMeans followed by the same letter within each column are not significantly different according to LSMEANS procedure ($P \leq 0.05$).