Planting Date Effects on Heliothine Larval Numbers, Fruit Damage, and Yield of Transgenic *B.t.* Cotton in North Carolina[1]

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Abstract The susceptibility of Bollgard® cotton and non-*B.t.* cotton to bollworm, *Helicoverpa zea* (Boddie), was examined under contrasting crop management strategies in northeastern North Carolina in 1995, 1996, and 1998. Specifically, planting date effects were observed in Edgecombe Co. in 1995 and 1996, in Martin Co. in 1996, and in Washington Co. in 1998. The bollworm was the only lepidopterous pest which occurred at damaging levels in these tests. Although no significant differences were detected in mean percent egg deposition, mean percent larval infestation, and mean percent damaged fruit between early-planted and late-planted plots in 1995 and 1996, yields were higher in early-planted cotton than in late-planted cotton. In 1998 mean percent damaged fruit was higher in early-planted cotton than in late-planted cotton, but early-planted cotton had significantly higher yields than late-planted cotton. Early planting may be an effective management strategy for Bollgard® cotton in northeastern North Carolina.

Key Words Cotton, bollworm, *Helicoverpa zea, Bacillus thuringiensis,* transgenic

The 1996 commercialization of cotton with the Bollgard® gene (Monsanto Agric. Co., St. Louis, MO) provided growers with a new tool for combating caterpillar pests. The delta-endotoxin protein (Cry1Ac) from *Bacillus thuringiensis* Berliner var. *kurstaki* (*B.t.*) expressed in transgenic cotton plants is toxic to most lepidopteran larval pests of cotton including tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie), (MacIntosh et al. 1990). Field trials with Bollgard cotton over the last 5 yrs have demonstrated excellent control of these caterpillar pests, especially tobacco budworm (e.g., Benedict et al. 1992, Jenkins et al. 1993, Jenkins and McCarty 1995, Luttrell et al. 1995, Mascarenhas et al. 1994). This technology offers great promise for control of tobacco budworm in regions where the occurrence of strains resistant to synthetic insecticides has made control practically impossible.

However, laboratory studies (Stone and Sims 1993) have shown the bollworm to be much less susceptible to the Cry1Ac endotoxin than tobacco budworm. This was confirmed in field trials in North Carolina where bollworm larvae were able to 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. However, these experiments were conducted under conditions known to promote high bollworm larval populations in con-

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ventional cotton, including disruption of natural enemies through foliar application of a broad-spectrum insecticide and late planting of cotton. While much research has been conducted on the effects of such crop production practices in conventional cotton varieties (e.g., Ihrig et al. 1995), few studies have examined transgenic *B.t.* cotton crop management. 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.

Field trials reported herein were conducted from 1995 to 1998 to examine the efficacy of Bollgard® cotton under contrasting crop management tactics. Specifically, experiments examined the effects of planting date 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. The planting date studies in 1995 and 1996 were components of a larger overall field study examining arthropod natural enemy conservation versus disruption and supplemental pyrethroid applications. Only planting date effects will be reported and discussed herein.

Materials and Methods

A test was conducted at the Upper Coastal Plain Research Station (UCPRS) in Edgecombe Co. near Rocky Mount, NC in 1995. This planting date study was part of a larger field trial which also examined the effects of arthropod natural enemy conservation versus disruption and supplemental pyrethroid applications. The experiment was a split-split plot design with main plots, subplots, and sub-subplots as natural enemy conservation, planting date, and cotton treatment, respectively, replicated four times. The planting dates were 5 May 1995 and 22 May 1995. The cotton treatments consisted of: (1) *B.t.* cotton treated as needed for caterpillar control; (2) *B.t.* cotton untreated; (3) non-*B.t.* cotton untreated. The *B.t.* cotton was 'NuCOTN 33B', and non-*B.t.* cotton was the recurrent parent, 'DP5415.' Each sub-subplot was four rows wide by 12.2 m long with 91.4 cm row-spacing. Lambda-cyhalothrin (Karate® 1EC, Zeneca, Inc., Wilmington, DE) was applied at 44.8 g ai/ha to all treated plots when the North Carolina State University Extension Service threshold of 10 bollworm eggs per 100 terminals was met or exceeded in non-*B.t.* cotton plots. This necessitated two pyrethroid applications in 1995 on 27 July and 7 August.

In 1996, tests were conducted at two locations in eastern North Carolina-the UCPRS in Edgecombe Co. and the C. A. Martin Farm in Martin Co. Each test was a split-split block design with four replicates. As in 1995, both tests had main plots as natural enemy conservation/disruption, subplots as planting date, and sub-subplots (4 rows wide × 12.2 m long with 91.4 cm row-spacing) as cotton 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. and on 15 May 1996 at Martin Co. The four cotton treatments were (1) *B.t.* cotton treated as needed; (2) *B.t.* cotton untreated; (3) non-*B.t.* cotton treated as needed; and (4) non-*B.t.* cotton untreated. The *B.t.* treatments were 'NuCOTN 33B' and non-*B.t.* treatments were 'DP5415.' Treated plots were protected with three applications of the same rate of lambda-cyhalothrin used in 1995.

The study was conducted at the Tidewater Research Station in Washington Co., in 1998. The test was a randomized complete block design replicated four times with main plots as planting date and subplots as cotton treatment. Early-, mid-, and late-planting dates were 27 April, 14 May, and 27 May, respectively. Cotton treatments consisted of *B.t.* cotton (NuCOTN 33B) untreated or treated as needed for supplemental bollworm control. Lambda-cyhalothrin (44.8 gai/ha) was applied to treated plots on 29 July and 11 August.

Aldicarb (Temik® 15G, Rhone-Poulenc Ag Company, Research Triangle Park, NC) was applied at 0.84 kg ai/ha in-furrow for early season thrips control each year. Fertility, weed control, plant growth regulation, and defoliation were standard production practices for maximum cotton yields in North Carolina (North Carolina State Univ. 1994). All cotton plots in Edgecombe Co. were irrigated as necessary each year. All planting dates used were well within the normal planting period for cotton in northeastern North Carolina.

Cotton plants were sampled on six dates in 1995 for percent larval infestation and percent damaged fruit per plot. Egg deposition was measured on the first sampling date, 31 July 1995, as the number of heliothine eggs per 25 terminals in the *B.t.* and non-*B.t.* plots only. The number of live larvae per 25 (31 July and 3 and 31 August) or 50 (7, 14, and 21 August) 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 heliothines.

The numbers of heliothine larvae and damaged fruit were recorded for all plots at each test site on four sampling dates in 1996. The same procedures used in 1995 for determining percent egg deposition, percent larval infestation, and percent damaged fruit were used in 1996. The terminals of 25 cotton plants in each sub-subplot were examined for bollworm eggs, larvae, and damaged squares 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-subplot were examined for heliothine larvae and damaged fruit. Bolls (50 per sub-subplot) on random plants were observed at each test site on 19 and 25 August for larvae and damaged fruit. The procedures described for data collection in 1996 were utilized in 1998 on three sampling dates. Bolls were examined on 10, 19, 24 August 1998 for live larvae and damaged fruit.

Each year 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 field plots each year and transported to the laboratory for species identification using methods described by Neunzig (1969). Finally, the center two rows of each plot were harvested mechanically at the end of each season. Harvest dates were 19 October 1995; 24 October 1996 (Edgecombe Co.); 13 November 1996 (Martin Co.); and 19 October 1998 (Washington Co.).

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

Results

The bollworm was the only lepidopterous pest which occurred at damaging levels in these tests. Samples collected from *B.t.* plots in 1995 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 non-*B.t.* plots in 1995. Bollworm comprised the majority of the larval pest population in 1996. Of samples collected from both *B.t.* and non-*B.t.* plots on two dates in 1996, 99% (n = 198) were identified as bollworm and 1% (n = 2) were tobacco budworm. Bollworm comprised 100% of the larval heliothine population in *B.t.* plots (n = 60) and 100% in adjacent conventional cotton fields (n = 75) in 1998. Other pest populations of European corn borer, armyworms, plant bugs, and stink bugs were too low at test sites each year to affect yield.

No significant differences in heliothine egg deposition were detected between early-planted and late-planted cotton in 1995 (F = 3.73; df = 1,6; P = 0.1018): When early- and late-planted cotton are separated into *B.t.* or non-*B.t.* cotton (Table 1), no significant differences in percent damaged fruit were detected between early- and late-planted cottons. However, early-planted cotton had significantly higher yields than late-planted cotton for all three treatments.

No significant differences were detected in mean percent heliothine egg deposition (Edgecombe Co.: F = 0.17; df = 1,6; P = 0.6957; Martin Co.: F = 1.36; df = 1,6; P = 0.2885), mean percent larval infestation (Edgecombe Co.: F = 1.90; df = 1,6; P = 0.2169; Martin Co.: F = 0.17; df = 1,6; P = 0.6972), and mean percent damaged fruit (Edgecombe Co.: F = 1.78; df = 1,6; P = 0.2307; Martin Co.: F = 1.62 df = 1,6; P = 0.2498) between early- and late-planted plots at either test site in 1996. Mean percent damaged fruit in early- and late-planted cotton plots at Edgecombe Co., and Martin Co., are listed in Table 2. Yields were generally higher in early-planted cotton than in late-planted cotton; specifically, yields were significantly higher in early-planted plots of *B.t.* cotton untreated, non-*B.t.* cotton treated, and non-*B.t.* cotton untreated in Edgecombe Co. and early-planted non-*B.t.* cotton untreated in Martin Co. (Table 3).

Although no significant differences in larval numbers were detected among different planting dates of *B.t.* cotton (F = 0.35, df = 2,6; P = 0.7211) in 1998, significant differences in damaged fruit were detected (F= 13.31; df = 2,6; P= 0.0062). Early and mid-planted cotton had significantly higher percentage damaged fruit than late-planted *B.t.* cotton on the first sampling date in 1998 (Table 4). By the second sampling date (19 August 1998), cotton planted on the middle planting date had significantly higher numbers of damaged fruit than early-and late-planted cotton (Table 4). Again, earlier plantings of cotton had significantly higher yields than cotton that was planted in late-May (Table 5).

Table 1. Mean percent (%) Heliothine damaged fruit and mean yield (kg seed cotton/ha) in early- and late-planted cotton treatments, Edgecombe Co., NC, 1995*

Cotton treatment**	Percent damaged fruit		Yield (kg seed cotton/ha)	
	Early	Late	Early	Late
B.t.-TAN	5.83 a ± 1.07	3.75 a ± 0.794	3812 a ± 107	3165 b ± 60
B.t.	12.3 a ± 1.43	8.15 a ± 0.948	3233 a ± 99	2694 b ± 100
Non-B.t.	29.3 a ± 2.62	27.3 a ± 2.50	2325 a ± 169	1455 b ± 171

* Means followed by the same letter within each row for percent damaged fruit and yield are not significantly different according to LSMEANS procedure ($P \leq 0.05$).

** TAN = treated as needed with lambda-cyhalothrin for heliothine control.

Table 2. Mean percent Heliothine damaged fruit for each cotton treatment in early-planted and late-planted cotton at Edgecombe Co., NC, and Martin Co., NC, 1996*

Cotton treatment**	Mean percent damaged fruit			
	Edgecombe County		Martin County	
	Early	Late	Early	Late
B.t.-TAN	0.63 a ± 0.329	1.0 a ± 0.336	1.6 a ± 0.638	1.1 a ± 0.381
B.t.	5.0 a ± 1.74	4.6 a ± 0.935	8.1 a ± 1.05	10.2 a ± 1.38
Non-B.t.-TAN	4.4 a ± 0.970	4.1 a ± 0.808	5.3 a ± 1.15	5.7 a ± 1.05
Non-B.t.	27.1 a ± 3.21	30.9 a ± 3.16	47.3 a ± 4.19	50.9 a ± 4.52

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

** TAN = treated as needed with lambda-cyhalothrin for heliothine control.

Table 3. Mean yield (kg seed cotton/ha) for each cotton treatment in early-planted and late-planted cotton at Edgecombe Co., NC, and Martin Co., NC, 1996*

Cotton treatment**	Mean yield (kg seed cotton/ha)			
	Edgecombe County		Martin County	
	Early	Late	Early	Late
B.t.-TAN	4477 a ± 55	4203 a ± 84	3074 a ± 117	2962 a ± 131
B.t.	4156 a ± 122	3603 b ± 124	2457 a ± 52	2207 a ± 178
Non-B.t.-TAN	3977 a ± 74	3489 b ± 79	2942 a ± 135	2769 a ± 169
Non-B.t.	2348 a ± 199	1625 b ± 225	773 a ± 97	435 b ± 79

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

** TAN = treated as needed with lambda-cyhalothrin for heliothine control.

Discussion

Previous studies in North Carolina demonstrated that planting cotton early typically minimizes economic loss by reducing crop attractiveness and boll susceptibility to late-season lepidopteran pests as well as crop susceptibility to late-season weather-related stresses (Ihrig et al. 1995). This research of planting date effects in conventional cotton indicated that the impact of bolls damaged by insects is greater with a delay in planting date. Later-planted cotton (mid- to late-May) had significantly higher numbers of susceptible bolls during the moth flights each year than cotton that was planted at the end of April. Therefore, higher numbers of damaged bolls were detected in plots planted in mid- to late-May each year. In addition, early-planted cotton

Table 4. Mean percent Heliothine damaged fruit for each cotton treatment on two sampling dates in early-, mid-, and late-planted cotton in Washington Co., NC, 1998*

Cotton treatment**	Mean percent damaged fruit					
	10 August 1998			19 August 1998		
	Early	Mid	Late	Early	Mid	Late
B.t.-TAN	1.5 a ± 1.5	0.5 a ± 0.5	0.0 a	2.0 a ± 0.82	0.0 a	1.0 a ± 0.58
B.t.	30.0 a ± 4.7	25.0 a ± 3.1	16.0 b ± 2.6	22.0 b ± 2.2	35.5 a ± 3.2	19.5 b ± 3.0

* Means followed by the same letter within a row for each sampling date are not significantly different according to LSMEANS procedure ($P \leq 0.05$).
** TAN = treated as needed with lambda-cyhalothrin for heliothine control.

Table 5. Mean yield (kg seed cotton/ha) for each cotton treatment in early-, mid-, and late-planted cotton in Washington County, NC, 1998*

Cotton treatment**	Yield (kg seed cotton/ha)		
	Early	Mid	Late
B.t.-TAN	3683 a ± 70	3501 a ± 282	2773 b ± 114
B.t.	2752 a ± 160	2677 a ± 155	2216 b ± 127

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

** TAN = treated as needed with lambda-cyhalothrin for heliothine control.

had higher yields than later planted cotton in conventional cotton studies. Ihrig et al. (1995) concluded that the maximum utilization of available seasonal heat units through early planting of cotton resulted in increased boll loads which were more mature and less susceptible to late-season bollworm infestations.

Yield differences in 1995 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. 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.

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 probably was not sufficient in 1996 to allow late-planted cotton to reach a maximum yield potential.

Extremely high bollworm populations in 1998 (Bacheler and Mott 1998) contributed to significant differences in numbers of damaged fruit among *B.t.* cotton planted from late April to late May. Late-planted cotton suffered less damage from lepidopteran pest populations than earlier-planted cotton. This is in direct contrast to results reported by Ihrig et al. (1995) where late-planted cotton plots had higher numbers of larvae and damaged fruit. Boll susceptibility in *B.t.* cotton production settings is influenced by additional factors such as delta-endotoxin protein levels. Adamczyk et al. (2000) reported a decline in delta-endotoxin levels in *B.t.* cotton plants throughout the growing season. Studies by Greenplate et al. (1998) reported that average Cry1Ac insecticidal protein levels in primary fruiting positions of *B.t.* cotton dropped below 5µ g/g by 80 to 90 days after planting. Early-planted cotton (27 April) in 1998 was approximately 90 d old at the initiation of the third generation bollworm moth flight (27 July), and late-planted cotton (27 May) was approximately 60 d old. Therefore, earlier-planted *B.t.* cotton was more susceptible to bollworm larval establishment than late-planted cotton by 10 August. As earlier planted cotton matured, boll age had a greater effect on boll susceptibility to bollworm, and fruit in mid- to late-planted plots was more susceptible as the levels of Cry1Ac declined.

Although a greater percentage of damaged fruit may be observed in early-planted *B.t.* cotton in years of high bollworm pest populations, early planting of cotton resulted in increased yields in all cases. These findings are consistent with those with conventional cotton varieties where earlier planted cotton can fully utilize available seasonal heat units for maximum yields and may be more immune to late season weather related stressors (Ihrig et al. 1995). Early planting will very likely be an effective management tactic for transgenic *B.t.* cotton varieties in North Carolina.

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