EFFICACY OF BOLLGARD AND BOLLGARD II COTTONS AGAINST BOLLWORM, *HELICOVERPA ZEA* (BODDIE) IN FIELD AND GREENHOUSE STUDIES

R. E. Jackson, J. R. Bradley, Jr., J. W. Van Duyn and A. D. Burd
North Carolina State University
Department of Entomology
Raleigh, NC

Abstract

Bollgard™ and Bollgard II™ cottons, along with the conventional sister line, were evaluated in field and greenhouse experiments with respect to efficacy against bollworm in North Carolina in 2000. Field studies were proposed to resolve the question of increased efficacy of Bollgard II over Bollgard on bollworm, as well as to determine survivorship parameters of bollworm on each cotton genotype. The impact of supplemental bollworm control on yield was evaluated at field sites. A greenhouse study was designed to determine the efficacy of three *Bacillus thuringiensis* Berliner (*B. t.*) cotton genotypes on a field-collected bollworm strain and a Cry1Ac-tolerant bollworm strain that had been selected for tolerance to the Cry1Ac toxin in the laboratory for two generations. Results from field studies indicated that although DP50BX (Bollgard II) illustrated no significant effects on larval survival beyond that of DP50B (Bollgard), a significant reduction in the level of fruit damage sustained by DP50 and DP50B was observed for the stacked-gene line. Yield response to supplemental pyrethroid oversprays varied among test sites due to differing bollworm infestation levels and moderate levels of boll rot. Greenhouse study results suggested that DP50BX was equally effective in reducing numbers of both the Cry1Ac-tolerant and susceptible bollworm larvae, as well as surface-damaged and penetrated fruit below that of DP50B.

Introduction

The commercialization of Bollgard™ (Monsanto Agric. Co., St. Louis, MO) cottons has provided growers with an excellent alternative for controlling lepidopteran pests. However, reports from Stone and Sims (1993) indicated that *B. t.* endotoxins were much less effective in controlling bollworm, *Helicoverpa zea* (Boddie), than the tobacco budworm, *Heliothis virescens* (Fab.). Results from field trials conducted in North Carolina verify that Bollgard cottons could sustain significant fruit damage and yield losses, thus creating the need for supplemental insecticide oversprays for bollworm (Burd et al. 1999; Lambert et al. 1996, 1997; Mahaffey et al. 1994, 1995). This is most likely caused by the observed drop in average levels of Cry1Ac endotoxin (Greenplate 1999; Greenplate et al. 1998) which coincides with the major bollworm flight in North Carolina. With the bollworm population receiving a sublethal dose of Cry1Ac, the "high dose strategy" for insect resistance evolution is violated by Bollgard cottons, which institutes a need for new technological developments.

Bollgard II™ cottons produce two proteins, Cry1Ac and Cry2Ab, that are active against bollworm. The novel dual-gene line illustrates approximately the same level of expression of the Cry1Ac endotoxin as the Bollgard varieties (Greenplate et al. 2000). Greenplate also reported that the Cry2Ab endotoxin is expressed at a much higher level than Cry1Ac in the Bollgard II line. The dual-gene construct, therefore, would more appropriately fit the "high dose strategy" for insect resistance evolution than would the currently utilized single-gene construct. Reports from field and greenhouse trials conducted in North Carolina demonstrated that Bollgard II genotypes significantly reduced numbers of susceptible and Cry1Ac-tolerant bollworm larvae below that of the Bollgard cottons (Jackson et al. 2000). This suggests that the implementation of Bollgard II cottons could possibly extend the time frame for bollworm resistance evolution beyond that of current Bollgard varieties.

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Results from field and greenhouse trials evaluating the efficacy of Bollgard and Bollgard II cottons and the conventional sister line by measuring bollworm numbers, fruit damage, and yield under pyrethroid-treated and untreated systems are reported herein.

Materials and Methods

Field Studies

Experiments were conducted at the Central Crops Research Station, Johnston Co., NC, the Upper Coastal Plain Research Station, Edgecombe Co., NC, and the Tidewater Research Station, Washington Co., NC, in 2000. Each test site consisted of a randomized complete split-plot design with four replicates. Whole plots were 16, 20, and 24 rows by 60 feet for DP50, DP50B, and DP50BX, respectively, at each location. Different plot sizes were chosen for determination of the survival of bollworm in each genotype through mass sampling procedures in which large numbers of bolls were examined for each genotype to estimate bollworm survival on a per acre basis for making resistance management decisions and estimating refuge requirements. Subplots consisted of 12, 16, and 20 untreated rows for DP50, DP50B, and DP50BX, respectively, and 4 rows that were treated with a pyrethroid as required for supplemental bollworm control. Yield differences between pyrethroid-treated and untreated subplots were determined for each site.

Cotton genotypes DP50, DP50B, and DP50BX were planted on 15 May in Edgecombe Co., 17 May in Johnston Co., and 18 May in Washington Co. Aldicarb (Temik 15G, Aventis CropScience, Research Triangle Park, NC) was applied in-furrow at planting at 5.0 lb. a. i./acre for control of early season insect pests. Acephate (Orthene 97, Valent USA Corp., Walnut Creek, CA) was applied at 0.75 lb. a. i./acre as a mid-season overspray for stink bugs and plant bugs, as well as to eliminate arthropod predators. Two applications of lambda cyhalothrin (Karate Z 2.08 CS, Zeneca Inc., Wilmington, DE) were made to appropriate subplots for supplemental bollworm control at Johnston and Edgecombe counties on 19 July and 7 August, as well as at Washington Co. on 27 July and 9 August. Fertilization, plant growth regulation, weed control, and defoliation were accomplished as recommended by North Carolina State University.

Terminal region assessments of bollworm eggs, live larvae, and damage were made the weeks of 30 July and 6 August by the random sampling of 25 terminals per plot. Square evaluations for live larvae and damage were made the weeks of 6 and 13 August by examination of 50 squares per treatment per replicate. Boll assessments for live larvae and damage were made the weeks of 13, 20, and 27August, as well as 3 September by examination of 50 bolls per treatment per replicate. Bollworm egg, live larvae, and damage numbers per treatment per replicate were converted to percentages prior to analysis. Yields were determined by harvesting two center rows of each subplot with a mechanical cotton picker on 30 October at Edgecombe Co. and 7 November at Johnston and Washington counties. Yields were converted to lb. seed cotton per acre prior to analysis.

The total numbers of fourth to fifth instar larvae, harvestable bolls, and damaged harvestable bolls were counted in a randomly selected area of five row feet per treatment per replicate at the Edgecombe and Washington county sites on three sample dates (24 and 31 August and 7 September). Large larvae, fourth and fifth instars, were transported to the laboratory and reared on bolls from the respective genotypes until the prepupal stage. Prepupae were then placed into 30 ml plastic cups containing non-*B. t.* artificial diet which served as a substrate to tunnel into for pupation. Numbers of successfully emerged adults from each genotype were counted and converted to a per acre basis prior to analysis along with total numbers of harvestable bolls, damaged bolls, and live larvae. These numbers provide an estimation of survival parameters of the bollworm, which is important in resistance management.

Greenhouse Study

An experiment was conducted in a greenhouse chamber at the Tidewater Research Station, Washington Co., NC. The test was a randomized complete block design with seven replicates. Each replicate consisted of 12 plants, two plants per treatment combination of cotton genotype and bollworm strain. Plants within blocks were separated by a distance of two feet, whereas, blocks were separated by a 3 foot space on tables.

Cotton genotypes DP50B, DP50X, and DP50BX were planted in three gallon pots at one plant per plot on 27 June. Arthropod natural enemies, as well as aphids and whiteflies, were eliminated by side-dressing cotton plants two weeks prior to bollworm infestation with Aldicarb to achieve a rate of 0.75 lb. a. i./acre. Fertilization and plant growth regulation were provided as recommended for greenhouse cotton plants.

Local bollworm adults were collected from light traps and held in the laboratory for egg collection. Neonate larvae from the eggs were used as the control strain in the experiment. A Cry1Ac-tolerant strain of bollworm was originally collected from light traps and selected for tolerance to the Cry1Ac toxin (MVP) in artificial diet for two generations. Infestation of five neonate larvae onto fruiting structures on respective plants from each genotype using a fine-haired artist paint brush occurred when cotton plants reached approximately 100 days. Assessments of surviving larvae, superficial fruit damage, and fruit penetration were made by whole plant examination at 14 and 21 days after infestation. Surviving larvae from the last evaluation were taken to the laboratory and placed into 30 ml plastic cups containing non-*B. t.* artificial diet which served as a medium for larvae to tunnel into for pupation. Numbers of bollworm that successfully pupated and those that successfully emerged as adults were recorded and converted to percentages.

Data Analysis

All data were subjected to ANOVA using PROC GLM (SAS Institute 1990), and means for each treatment were separated ($P<0.05$) using Fisher's Least Significant Difference test or LSMEANS in SAS.

Results

Field Studies

No differences were found among the three cotton genotypes with distribution of bollworm eggs in cotton terminals being consistent across field sites (Table 1). No significant differences were evident between DP50BX and DP50B with respect to percent live bollworm larvae in cotton terminals, and both Bollgard genotypes reduced percent bollworm larvae below that of the conventional variety (Table 1). Percent terminal damage was significantly lessened by both Bollgard genotypes compared to the conventional variety with DP50BX further reducing percent terminal damage below that of DP50B (Table 1). Square evaluations revealed that Bollgard cottons did not differ with respect to the percentage of squares containing live bollworm larvae, but both contained a significantly lower percentage of live larvae than the conventional variety (Table 2). Data in Table 2 also show a significant reduction in percent bollworm damaged squares by both Bollgard genotypes compared to the conventional variety with DP50BX further reducing square damage below that of DP50B. Boll evaluations indicated that Bollgard cottons did not differ with respect to percent live bollworm larvae in bolls but significantly lessened percent live bollworm larvae compared to the conventional variety (Table 2). However, Table 3 indicates that DP50BX sustained less boll damage than DP50B at each test site with both Bollgard cottons having less boll damage than the conventional variety.

Field evaluations for determination of total harvestable fruit on a per acre basis revealed that the conventional variety had a significantly lower number of bolls than the Bollgard cottons (Table 4). Results in Table 4 also indicate that the Bollgard cottons had significantly lower numbers of damaged bolls than the conventional variety in which greater than half the bolls were bollworm damaged. Table 5 illustrates that no differences were evident between Bollgard genotypes with respect to the number of surviving fourth to fifth instar bollworm larvae per acre. However, only DP50BX contained a significantly lower number of larvae per acre compared to the conventional variety. The number of large larvae per acre closely relates to the number of successfully emerged bollworm adults on a per acre basis which was much lower in the Bollgard genotypes than in the conventional variety (Table 5). Differences in number of successfully emerged bollworm adults were not evident among Bollgard cottons even though successful emergence was much lower numerically in DP50BX than in DP50B.

Yield differences measured in pounds of seed cotton per acre between pyrethroid-treated and untreated subplots in three cotton genotypes varied among test sites. Pyrethroid-treated and untreated subplots did not differ significantly with respect to yield in lb. seed cotton per acre at the Central Crops Research Station (Table 6). Cotton genotype also had no impact on yield at this site. Results in Table 7 reveal a genotype*insecticide interaction at the Upper Coastal Plain Research Station in which all treatment combinations were compared. Yield benefits from pyrethroid applications were only evident in the conventional variety. Both untreated Bollgard genotypes illustrated significantly improved yields compared to the untreated conventional variety. Among pyrethroid-treated subplots, DP50B produced more seed cotton than DP50BX. Neither pyrethroid-treated Bollgard variety differed from the treated conventional variety. At the Tidewater Research Station, pyrethroid-treated subplots averaged across cotton genotypes produced significantly higher yields than untreated subplots (Table 8). Yields in lb. seed cotton per acre averaged across subplots were higher in DP50BX than DP50B, which also out-yielded the conventional variety.

Greenhouse Study

The Cry1Ac-tolerant bollworm strain displayed a significantly higher percent larval survival than the susceptible bollworm strain on DP50B, but no differences among larval strains were evident for the DP50X and DP50BX genotypes (Table 9). As with larval survival, Table 10 indicates that the Cry1Ac-tolerant bollworm strain caused a significantly higher percent surface-damaged fruit than did the susceptible bollworm strain in DP50B only. Bollworm strains did not differ with respect to percent surface damaged fruit within genotypes DP50X and DP50BX. Data in Table 11 indicate that the percentage of penetrated fruit averaged across cotton genotypes was significantly higher for the Cry1Ac-tolerant strain compared to the susceptible strain. Although no differences in percent penetrated fruit existed between DP50X and DP50BX, only DP50BX significantly reduced the percentage of penetrated fruit below that of DP50B. The percentage of larvae from the Cry1Ac-tolerant bollworm strain that developed on DP50B and successfully pupated was 4.29%. No other larvae on any genotype survived until pupation. Of those that pupated, 66.7% successfully emerged as adults.

Discussion

Field Studies

Bollworm populations varied spatially in 2000, with an uncharacteristically low population at the Central Crops Research Station and moderate to high infestations at the Upper Coastal Plain and Tidewater Research Stations. In field studies, both DP50B and DP50BX provided similar levels of bollworm control with respect to percent live bollworm larvae in terminals, squares, and bolls, with both transgenic cultivars reducing larval numbers below that of the conventional variety. The DP50BX line, however, was more effective in sustaining less fruit damage than DP50B in these plant regions. Significantly less fruit damage should have equated to higher yields, but moderate levels of boll rot penalized the better treatments, rendering some possible yield differences inseparable. Both transgenic

cottons out-performed DP50 with respect to production of total and damaged harvestable bolls per acre, as well as successfully emerged bollworm adults per acre. Only DP50BX contained significantly lower numbers of large live larvae than the conventional variety. Cotton genotypes DP50B and DP50BX also performed similarly with respect to production of total and damaged harvestable bolls per acre, as well as numbers of large live larvae and successfully emerged adults per acre. However, numerical reductions made by DP50BX in numbers of bollworm that successfully completed development may extend the time frame for bollworm resistance evolution but possibly for a shorter time period than originally expected.

Greenhouse Study

The Cry1Ac-tolerant bollworm strain out-performed the susceptible strain with respect to larval survival and surface damage inflicted onto fruiting structures, which was largely due to the increased performance of the Cry1Ac-tolerant strain compared to the susceptible strain on DP50B. Averaged across genotypes, increased fruit penetration by the Cry1Ac-tolerant bollworm strain over the susceptible strain was caused by the increased success of the Cry1Ac-tolerant strain on genotypes DP50B and DP50X. Fruit penetration by the Cry1Ac-tolerant strain on DP50BX was approximately 10X less than that of DP50X and DP50B, which is likely due to some additional effect of the two-gene construct versus that of the single-gene. Averaged across bollworm strains, DP50BX sustained less fruit penetration than DP50B. These results support reports from Jackson et al. (2000) indicating the increased bollworm control provided by Bollgard II lines over the commercial Bollgard variety with respect to larval survival and fruit penetration. In addition to larval survival and fruit penetration, the percentage of the Cry1Ac-tolerant bollworm strain that successfully pupated and emerged as adults was higher in DP50B (4.29 and 2.86%, respectively) than in DP50BX (0.00%) and DP50X (0.00%). These greenhouse data and those from field studies suggest that the commercialization of the dual-gene construct (DP50BX) would reduce bollworm damage over that experienced by Bollgard varieties, as well as eliminate the need for supplemental insecticide application for bollworm control. With the incidence of field resistance to Bollgard cottons in North Carolina (Jackson et al. 2000; Burd et al. 1999; Lambert et al. 1997, 1998; Mahaffey et al. 1994, 1995), the implementation of Bollgard II lines may also be necessary to provide control for that portion of the bollworm population already expressing resistance traits to Bollgard cottons.

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Table 1. Mean (SE) percent bollworm egg, live larvae, and damage in the terminal region of three cotton genotypes averaged across three locations and two sample dates in North Carolina in 2000.

Genotype	Percent Egg	Percent Larvae	Percent Damage
DP50	8.00 (.018) a	4.25 (.006) a	15.92 (.019) a
DP50B	11.33 (.020) a	0.67 (.002) b	6.42 (.012) b
DP50BX	7.50 (.017) a	0.25 (.002) b	3.33 (.010) c

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ($P \leq 0.05$).

Table 2. Mean (SE) percent live bollworm larvae in squares, square damage, and live bollworm larvae in bolls averaged across three locations and respective sample dates in North Carolina in 2000.

	Square Evaluations		Boll Evaluations
Genotype	Percent Larvae	Percent Damage	Percent Larvae
DP50	4.92 (.005) a	16.25 (.005) a	4.92 (.009) a
DP50B	0.42 (.002) b	3.33 (.004) b	1.00 (.003) b
DP50BX	0.00 (.000) b	0.25 (.001) c	0.17 (.001) b

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ($P \leq 0.05$).

Table 3. Mean (SE) percent boll damage for each cotton genotype averaged across four sample dates for each test site in North Carolina in 2000.

Genotype	Central Crops Research Station	Upper Coastal Plain Research Station	Tidewater Research Station
DP50	12.50 (.008) a	49.25 (.030) a	44.25 (.037) a
DP50B	0.86 (.003) b	10.25 (.010) b	7.75 (.013) b
DP50BX	0.00 (.000) c	1.38 (.005) c	0.75 (.003) c

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ($P \leq 0.05$).

Table 4. Mean (SE) number of total bolls and damaged bolls per acre for each cotton genotype averaged across three sample dates and two locations in North Carolina in 2000.

Genotype	Number of Bolls Per Acre	Number of Damaged Bolls Per Acre
DP50	311,817 (23,319) b	156,695 (24,328) a
DP50B	458,832 (12,637) a	27,830 (3,185) b
DP50BX	448,668 (18,047) a	7,502 (5,008) b

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ($P \leq 0.05$).

Table 5. Mean (SE) number of live large bollworm larvae and successfully emerged adults per acre for each cotton genotype averaged across three sample dates and two locations in North Carolina in 2000.

Genotype	Number of Larvae Per Acre	Number of Emerged Adults Per Acre
DP50	6893 (1689) a	6893 (2,165) a
DP50B	3155 (816) ab	528 (197) b
DP50BX	1990 (1601) b	72 (72) b

Means within the same column or row followed by the same letter are not significantly different, Fisher's LSD ($P \leq 0.05$).

Table 6. Mean (SE) weight of seed cotton in pounds per acre for pyrethroid-treated and untreated subplots of each genotype at the Central Crops Research Station in North Carolina in 2000.

Genotype	Untreated	Treated
DP50	3,722 (193) a	3,618 (165) a
DP50B	4,107 (90) a	4,076 (84) a
DP50BX	4,055 (110) a	3,846 (135) a

Means within the same column or row followed by the same letter are not significantly different, Fisher's LSD ($P \leq 0.05$).

Table 7. Mean (SE) weight of seed cotton in pounds per acre for pyrethroid-treated and untreated subplots of each cotton genotype at the Upper Coastal Plain Research Station in North Carolina in 2000.

Genotype	Insecticide	Pounds of Seed Cotton Per Acre
DP50B	Untreated	3,902 (240) a
DP50B	Treated	3,808 (108) ab
DP50BX	Untreated	3,624 (90) abc
DP50	Treated	3,452 (258) bc
DP50BX	Treated	3,230 (166) c
DP50	Untreated	1,440 (229) d

Means within the same column followed by the same letter are not significantly different, LSMEANS ($P \leq 0.05$).

Table 8. Mean (SE) weight of seed cotton in pounds per acre for pyrethroid-treated and untreated subplots of each genotype at the Tidewater Research Station in North Carolina in 2000.

Genotype	Untreated	Treated	Mean
DP50	1,399 (27)	2,083 (193)	1,741 (157) c
DP50B	2,455 (199)	2,474 (100)	2,465 (103) b
DP50BX	2,846 (131)	3,057 (90)	2,952 (84) a
Mean	2,233 (198) b	2,537 (140) a	

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ($P \leq 0.05$).

Table 9. Mean (SE) percent surviving larvae for each bollworm strain within each cotton genotype averaged across two evaluation dates in the greenhouse in North Carolina in 2000.

Strain	DP50B	DP50X	DP50BX
Cry1Ac-tolerant Strain	5.00 (.022) a	0.71 (.007) a	0.00 (.000) a
Susceptible Strain	0.00 (.000) b	0.00 (.000) a	0.00 (.000) a

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ($P \leq 0.05$).

Table 10. Mean (SE) percent surface-damaged fruit for each bollworm strain within each cotton genotype averaged across two evaluation dates in the greenhouse in North Carolina in 2000.

Strain	DP50B	DP50X	DP50BX
Cry1Ac-tolerant Strain	4.36 (.009) a	0.65 (.005) a	1.36 (.006) a
Susceptible Strain	1.19 (.006) b	0.00 (.000) a	1.36 (.006) a

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ($P \leq 0.05$).

Table 11. Mean (SE) percent penetrated fruit for each cotton genotype and each bollworm strain averaged across two evaluation dates in the greenhouse in North Carolina in 2000.

Genotype	Cry1Ac-tolerant Strain	Susceptible Strain	Mean
DP50B	4.93 (.017)	0.85 (.005)	2.89 (.009) a
DP50X	4.10 (.020)	0.00 (.000)	2.05 (.010) ab
DP50BX	0.45 (.004)	0.00 (.000)	0.22 (.002) b
Mean	3.16 (.009) a	0.28 (.002) b	

Means within the same column or row followed by the same letter are not significantly different, Fisher's LSD ($P \leq 0.05$).