FIELD AND GREENHOUSE PERFORMANCE OF BOLLWORM ON BOLLGARD II COTTON GENOTYPES

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Abstract

The performance of four cotton genotypes was evaluated against *Helicoverpa zea* (Boddie) in field and greenhouse studies in North Carolina in 1999. The field studies were designed to determine if two Bollgard II™ lines exhibited an increased efficacy against bollworm compared to the current commercial Bollgard™ variety. The impact on yield of supplemental bollworm control with pyrethroids was evaluated. Two greenhouse studies were designed to compare the Bollgard II lines with the commercial Bollgard variety with respect to efficacy against a field strain of bollworm only in one study and against a field strain and a strain which had been selected in the laboratory for thirteen generations for tolerance to the Cry1A(c) toxin in the second study. Results from the field studies indicated that Bollgard II lines (15813 and 15985) sustained significantly lower terminal, square, and boll damage compared to the commercial Bollgard variety (DP50B) and a conventional cotton variety (DP50). The yield response of Bollgard genotypes to pyrethroid oversprays varied among test sites as expected under modest bollworm population levels. In greenhouse studies, Bollgard II lines significantly reduced penetration of fruiting structures for the field and laboratory-selected strains of bollworm compared to the Bollgard variety. However, larval survival and fruit damage by the laboratory-selected bollworm strain were higher than that of the field-collected, non-selected strain.

Introduction

The availability of Bollgard™ (Monsanto Agric. Co., St. Louis, MO) cottons has provided growers an excellent means for management of lepidopteran pests. However, Stone and Sims (1993) reported that the bollworm, *Helicoverpa zea* (Boddie), the major insect pest of cotton in North Carolina, was much less susceptible to *B.t.* endotoxins than the tobacco budworm, *Heliothis virescens* (Fab.). Reports from field trials in North Carolina demonstrated that Bollgard cottons could sustain significant amounts of damage from that portion of the bollworm population that survives on the transgenic plants (Burd et al. 1999; Lambert et al. 1996, 1997; Mahaffey et al. 1994, 1995). Because yield loss is almost certain, Bollgard cottons in North Carolina frequently require supplemental bollworm control with insecticides.

The major bollworm flight to cotton occurs in North Carolina in late July to early August. The invasion period coincides with the observed drop in average levels of Cry1A(c) in cotton fruit (Greenplate 1999; Greenplate et al. 1998). With the need for supplemental bollworm control due to the decrease in average levels of Cry1A(c) in cotton fruit, new technologies with increased activity against the bollworm are imperative for cotton producers. Also, high efficacy *B.t.* cotton would better suit resistance management needs than current Bollgard varieties.

Results from field and greenhouse trials evaluating the efficacy of Bollgard II™ cotton lines versus the commercial Bollgard variety and a conventional variety by measuring bollworm numbers, fruit damage, and yields under pyrethroid-treated and untreated regimes are reported herein.

Materials and Methods

Field Study

The tests were conducted at the Tidewater Research Station, Washington Co., NC, the Upper Coastal Plain Research Station, Edgecombe Co., NC, and at C. A. Martin Farm, Martin Co., NC, in 1999. Each test site consisted of a randomized complete split-plot design with four replicates. Whole plots were 12 rows by 40 ft. at two locations and 12 rows by 45 ft. at C. A. Martin Farm. Subplots consisted of 8 untreated rows and 4 rows that were sprayed with a pyrethroid as needed for supplemental bollworm control. Yield differences between pyrethroid-treated and untreated subplots were determined for each site.

Cotton genotypes DP50, DP50B, and Bollgard II lines 15813 and 15985 were planted on 20 May in Martin Co., 21 May in Washington Co., and 24 May in Edgecombe Co. Aldicarb (Temik™ 15G, Rhone-Poulenc Ag Company, Research Triangle Park, NC) was applied in-furrow at planting at 0.75 lb. a. i./acre for control of early season insect pests. Acephate (Orthene™ 75S, Valent USA Corp., Walnut Creek, CA) was applied at 1.0 lb. a. i./acre in Washington Co. and at 0.75 lb. a. i./acre at the other sites as a mid-season broadcast spray to eliminate stink bugs and plant bugs and to reduce arthropod natural enemies. Supplemental bollworm control within appropriate subplots was accomplished by applications of cypermethrin (Ammo™ 2.5 EC, FMC Corp., Philadelphia, PA), lambda cyhalothrin (Karate™ 1.0 EC, Zeneca Inc., Wilmington, DE), and cyfluthrin (Baythroid 2™ 2.0 EC, Bayer Corp., Kansas City, MO). Two pyrethroid applications consisting of Ammo™ 2.5 EC @ 0.1 lb. a. i./acre and Karate™ 1.0 EC @ 0.04 lb. a. i./acre were sprayed at Martin Co. (4 August and 16 August, respectively) and at Washington Co. (6 August and 19 August, respectively) for

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bollworm control. Only one application of Baythroid 2™ 2.0 EC @ 0.05 lb. a. i./acre was used at Edgecombe Co. (5 August) for bollworm control. Other cotton production practices such as fertilization, plant growth regulation, weed control, and defoliation were achieved as recommended by North Carolina State University.

Assessment of bollworm eggs, live larvae, and damage were made in the terminal region of cotton plants on 4 and 10 August. Square evaluations were made on 10 August for live larvae and damage. Boll assessments for live larvae and damage were made on 10, 17, and 25 August. Fifty terminals, squares, or bolls per treatment replicate were examined on the respective sample dates. Bollworm egg, live larvae, and damage numbers per plot were converted to percentages prior to analysis. Yields were determined by picking the entire lengths of the two middle rows of each subplot using a mechanical cotton harvester on 1 November at Edgecombe Co., 5 November at Washington Co., and 12 November at Martin Co. Yields were expressed as lb. seed cotton /acre.

Greenhouse Study 1

Tests were conducted in two different greenhouse chambers at North Carolina State University, Wake Co., Raleigh, NC. Each test was a randomized complete block design with five replicates. Each replicate consisted of 8 plants, two plants per cotton genotype. Distance between plants within blocks was 2 ft. Blocks were separated by a 3 ft. space on tables.

Cotton genotypes DP50, DP50B, Bollgard II lines 15813 and 15985 were planted in 3 gallon pots at one plant per plot on 29 June. Aldicarb was applied to soil at planting and as a side-dress one week prior to bollworm infestation to achieve a rate of 0.75 lb. a. i./acre for the reduction of arthropod natural enemies and the elimination of aphids and whiteflies. Fertilization was performed as required for cotton plants per greenhouse conditions.

Bollworm adults were collected in light traps and held in the laboratory for egg collection. Neonate larvae from these eggs were used in the experiment. Five neonate larvae were infested onto fruiting structures on each plant using a fine-haired artist paint brush when plants reached approximately 80 days. Assessments of surviving larvae and fruit damage were made by examining whole plants at seven days after infestation. Each fruit on each cotton plant was given a damage rating of no damage, surface damage, or fruit penetration.

Greenhouse Study 2

Tests were arranged and conducted as in the previous study. Each replicate consisted of 16 plants, two plants per treatment combination of cotton genotype and bollworm strain. Aldicarb and fertilizer was applied to plants as in the previous study.

The field strain of bollworm was collected as above. The laboratory bollworm strain was originally collected from *B.t.* sweet corn and selected for tolerance to the Cry1A(c) toxin (MVP) in artificial diet for thirteen generations (Burd et. al in prep.). Neonate larvae from each strain were infested as above when cotton plants reached approximately 100 days. Both larval strains were infested onto each of the four cotton genotypes. Assessments of live larvae and fruit damage were made as above.

Data Analysis

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

Results

Field Study

Bollworm egg distribution in cotton terminals was consistent across fields at each location with no differences found among the four cotton genotypes with respect to egg percentages averaged across locations and dates (Table 1). Bollgard II lines were not significantly different from the DP50B variety with respect to percent live larvae in the terminal region, but contained a significantly lower percentage of live larvae compared to the conventional variety (Table 1). Table 1 also shows that Bollgard II lines sustained significantly less terminal damage than the DP50B variety, which had significantly less terminal damage than the conventional variety. Bollgard cottons contained significantly lower percentages of live bollworm larvae in squares than the conventional variety (Table 2). Only line 15985 contained fewer live larvae than the DP50B. However, both Bollgard II lines sustained significantly lower square damage compared to the DP50B variety and the conventional variety (Table 2). Percent live bollworm larvae and percent boll damage in Bollgard II lines was significantly less than that in the DP50B variety and the conventional variety (Table 2).

Yield differences in lb. seed cotton per acre between treated and untreated subplots varied among test sites. Pyrethroid-treated subplot yields averaged across cotton genotypes were significantly higher than the untreated subplots at 1149 and 1025 lb. seed cotton per acre, respectively, at the Washington Co. test site (Table 3). No differences were observed between cotton genotypes with respect to yield at this site. At the Edgecombe Co. site, no yield differences were evident among cotton genotypes or between pyrethroid-treated and untreated subplots averaged over genotypes (Table 4). Yield differences were observed between pyrethroid-treated and

untreated subplots at 2052 and 1337 lb. seed cotton per acre for DP50 at the Martin Co. site (Table 5). No differences, however, were observed between subplots for the other genotypes.

Greenhouse Study 1

Percent live larvae from the field-collected bollworm strain was significantly reduced by Bollgard cottons (ca. 2.7% versus 47.4%) in comparison with the DP50 variety averaged across greenhouse chambers (Table 6). Among Bollgard II genotypes, only line 15985 contained a significantly lower percentage of surviving larvae than DP50B. Percent surface damaged fruit varied among greenhouse chambers. No differences in surface damage were observed among cotton genotypes in one greenhouse chamber; whereas, the Bollgard II lines contained lower percentages of surface damage than DP50B or DP50 in the other chamber (Table 7). Table 6 also shows a reduction in percent fruit penetration in the Bollgard cottons (ca. 2.3% versus 40.1%) in comparison with DP50. A significant difference in percent fruit penetration was observed among Bollgard genotypes with Bollgard II lines sustaining less fruit penetration than the DP50B variety (Table 6).

Greenhouse Study 2

Averaged across cotton genotypes, the percent live larvae was significantly higher for the laboratory strain of bollworm at 18% compared to 11% for the field strain (Table 8). Averaged across bollworm strains, Bollgard cottons significantly reduced the percent live larvae (ca. 4.8% versus 43.5%) compared to DP50. Bollgard II lines further reduced the percent live larvae below that of DP50B averaged across bollworm strains (Table 8). Averaged across genotypes, the percentage of surface damaged fruit was significantly higher for the selected strain of bollworm (Table 9) than for the field-collected strain of bollworm. No differences were observed among cotton genotypes with respect to percent surface damaged fruit when averaged across bollworm strains. Table 10 shows a significant increase in percent fruit penetration for the laboratory strain of bollworm over the field-collected strain averaged across cotton genotypes. Averaged across larval strains, Bollgard cottons significantly reduced the percent fruit penetration below that in DP50 (ca. 3.7% versus 30.1%, respectively). Among the Bollgard genotypes, Bollgard II lines sustained significantly less fruit penetration than the DP50B (Table 10).

Discussion

Field Study

Uncharacteristically low populations of bollworm were encountered in cotton fields in North Carolina in 1999. Subsequently, damage to both conventional and transgenic cotton was minimal. Bollgard II lines did not differ from the commercial Bollgard variety (DP50B) with respect to percent live larvae in cotton terminals but did reduce the amount of terminal damage below that of DP50B. Line 15985 reduced the percentage of live larvae in squares below that of DP50B, whereas, both Bollgard II lines had lower percentages of square damage compared to DP50B. Bollgard II lines again outperformed DP50B with respect to percent live bollworm larvae in bolls and percent boll damage. These results suggest an increased efficacy against bollworm larvae in Bollgard II cotton lines compared to the commercial Bollgard variety (DP50B) under low levels of bollworm.

Yield differences between pyrethroid-treated and untreated subplots varied among test sites due to low populations of bollworm and unfavorable late-season weather conditions which were encountered when a series of hurricanes substantially reduced yield potential at each test site. Subplots treated with a pyrethroid insecticide had a higher mean yield than the untreated subplots at the Washington Co. test site. These results are consistent with earlier studies that indicated the potential yield benefit of spraying Bollgard cotton with pyrethroid insecticides. Unusual weather conditions and low bollworm numbers negated the benefits of treating Bollgard cottons with pyrethroids in the tests at the Martin Co. and Edgecombe Co. test sites. However, supplemental control with insecticides may be necessary to avoid yield reductions in Bollgard II cottons under more normal weather conditions and bollworm population levels typical for North Carolina.

Greenhouse Study 1

All Bollgard genotypes provided a high level of control for field-collected bollworm compared to the conventional variety. Bollgard II lines performed better than DP50B with respect to surviving larvae and fruit damage. The increased performance could be due to the drop in Cry1A(c) levels in cotton fruit in the DP50B variety at approximately 80 days as demonstrated by Greenplate et al. (1998) or the increased toxicity of stacked genes in the Bollgard II lines. The test was initiated when the cotton reached approximately 80 days to determine if the Bollgard II lines would illustrate an increased performance against bollworm since the performance of DP50B was known to decrease significantly at this point. As hoped, Bollgard II lines, especially line 15985, provided excellent control of bollworm at this stage of growth in the cotton plant.

Greenhouse Study 2

Averaged across bollworm strains, Bollgard II lines demonstrated an increased efficacy against bollworm compared to DP50B. The observed increase in performance of Bollgard II lines was partially due to the increased larval survival and damage caused to DP50B by the laboratory-selected strain of bollworm. Nevertheless, Bollgard II lines provided increased control of both larval strains over the DP50B. These results suggest that Bollgard II lines may be

effective in the management of bollworm populations that have demonstrated a trend toward adaptation for tolerance to Cry1A(c).

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Table 1. Mean (SE) percent bollworm egg, live bollworm larvae, and damage in the terminal region of four cotton genotypes averaged over three locations and two sample dates, August 4 and 10, 1999, in North Carolina.

Genotype	Percent Egg	Percent Larvae	Percent Damage
DP50	4.7 (1.1) a	6.1 (1.3) a	29.2 (2.7) a
DP50B	3.3 (0.9) a	3.3 (0.9) ab	12.0 (1.7) b
15813	3.3 (1.0) a	1.4 (0.5) b	7.8 (1.3) c
15985	5.3 (1.5) a	1.6 (0.6) b	6.8 (1.0) 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, live bollworm larvae in bolls, and boll damage of four cotton genotypes averaged over three locations and the respective sample dates in North Carolina, 1999.

	Square Observations		Boll Observations	
Genotype	% Larvae	% Damage	% Larvae	% Damage
DP50	6.0 (1.1) a	13.2 (2.4) a	7.8 (1.3) a	24.1 (2.3) a
DP50B	1.0 (0.5) b	3.3 (0.9) b	1.5 (0.4) b	4.0 (0.8) b
15813	0.2 (0.2) bc	0.9 (0.3) c	0.3 (0.1) c	1.1 (0.3) c
15985	0.0 (0.0) c	0.8 (0.3) c	0.4 (0.3) c	1.3 (0.8) c

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) yield in pounds of seed cotton per acre for pyrethroid-treated and untreated subplots of four cotton genotypes at the Tidewater Research Station, Washington Co., NC, taken on 5 November, 1999.

Genotype	Treated	Untreated	Mean
DP50	1165 (20.3)	980 (98.3)	1073 (58.1) a
DP50B	1146 (62.1)	1124 (76.0)	1135 (45.6) a
15813	1154 (46.9)	1008 (70.1)	1081 (47.8) a
15985	1131 (62.6)	989 (115.1)	1060 (66.3) a
Mean	1149 (23.0) a	1025 (43.6) 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 4. Mean (SE) yield in pounds of seed cotton per acre for pyrethroid-treated and untreated subplots of four cotton genotypes at the Upper Coastal Plain Research Station, Edgecombe Co., NC, taken on 1 November, 1999.

Genotype	Treated	Untreated	Mean
DP50	1861 (78.3)	1922 (230.0)	1892 (113.0) a
DP50B	1668 (80.0)	1539 (218.7)	1603 (110.5) a
15813	1754 (146.1)	1922 (153.5)	1838 (103.1) a
15985	1731 (168.9)	1999 (95.3)	1884 (98.0) a
Mean	1755 (55.8) a	1845 (93.9) 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 5. Mean (SE) yield in pounds of seed cotton per acre for pyrethroid-treated and untreated subplots of four cotton genotypes at C. A. Martin Farm, Martin Co., Jamesville, NC, taken on 12 November, 1999.

Genotype	Treated	Untreated
DP50	2052 (172.3) a	1337 (89.2) b
DP50B	2178 (118.8) a	1998 (160.4) a
15813	2014 (98.1) a	2037 (137.5) a
15985	2071 (176.8) a	1987 (352.6) a

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

Table 6. Mean (SE) percent live bollworm larvae and fruit penetration at seven days after infestation averaged over two greenhouse chambers for four cotton genotypes at approximately 80 days after planting, 1999.

Genotype	% Live Larvae	% Fruit Penetration
DP50	47.4 (6.9) a	40.1 (6.3) a
DP50B	6.0 (2.1) b	5.1 (1.5) b
15813	2.0 (1.4) bc	1.7 (1.2) c
15985	0.0 (0.0) c	0.0 (0.0) c

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

Table 7. Mean (SE) percent surface damaged fruit at seven days after bollworm infestation of four cotton genotypes at approximately 80 days after planting in two greenhouse chambers at North Carolina State University, Wake Co., NC, 1999.

Genotype	Chamber 1	Chamber 2
DP50	9.4 (2.6) a	19.3 (6.3) a
DP50B	3.5 (1.5) a	9.7 (1.4) a
15813	4.9 (1.8) a	0.8 (0.8) b
15985	3.5 (2.2) a	1.7 (1.1) b

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

Table 8. Mean (SE) percent surviving larvae at seven days after infestation of four cotton genotypes with a field strain and a laboratory strain of bollworm selected for tolerance to Cry1A(c) at approximately 100 days after planting in the greenhouse at North Carolina State University, Wake Co., NC, 1999.

Genotype	Field Strain	Lab Strain	Means
DP50	40.0 (5.6)	47.0 (5.5)	43.5 (3.9) a
DP50B	4.0 (1.8)	19.0 (3.7)	11.5 (2.4) b
15813	0.0 (0.0)	1.0 (1.0)	0.5 (0.5) c
15985	0.0 (0.0)	5.0 (2.5)	2.5 (1.3) c
Means	11.0 (2.4) b	18.0 (2.7) 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 9. Mean (SE) percent surface damaged fruit at seven days after infestation of four cotton genotypes with a field strain and a laboratory strain of bollworm selected for tolerance to Cry1A(c) at approximately 100 days after planting in the greenhouse at North Carolina State University, Wake Co., NC, 1999.

Genotype	Field Strain	Lab Strain	Means
DP50	4.5 (2.4)	6.1 (2.2)	5.3 (1.6) a
DP50B	2.3 (0.9)	4.9 (1.8)	3.6 (1.0) a
15813	0.3 (0.3)	2.5 (1.2)	1.4 (0.7) a
15985	1.2 (0.6)	5.5 (1.5)	3.3 (0.9) a
Means	2.1 (0.7) b	4.7 (0.9) 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 10. Mean (SE) percent penetrated fruit at seven days after infestation of four cotton genotypes with a field strain and a laboratory strain of bollworm selected for tolerance to Cry1A(c) at approximately 100 days after planting in the greenhouse at North Carolina State University, Wake Co., NC, 1999.

Genotype	Field Strain	Lab Strain	Means
DP50	27.6 (3.9)	32.6 (2.9)	30.1 (2.4) a
DP50B	6.9 (2.0)	11.7 (2.0)	9.3 (1.5) b
15813	0.0 (0.0)	1.8 (2.8)	0.9 (0.4) c
15985	0.0 (0.0)	1.7 (0.8)	0.8 (0.4) c
Means	8.6 (1.7) b	11.9 (1.7) a	

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