ESTIMATED PRODUCTION OF *HELICOVERPA ZEA* ADULTS FROM BOLLGARD AND BOLLGARD II COTTONS AND IMPLICATIONS FOR RESISTANCE MANAGEMENT

R.E. Jackson, J.R. Bradley, Jr. and J.W. Van Duyn
North Carolina State University
Raleigh, NC

Abstract

Transgenic cottons expressing either one or two *Bacillus thuringiensis* Berliner proteins, along with the conventional sister line, were evaluated in a 2001 field experiment with regard to impact on bollworm production in North Carolina. The relative numbers of bollworms that were capable of successfully completing development on Bollgard® II, Bollgard®, and conventional cottons under insecticide-sprayed and unsprayed conditions were estimated. Bollgard and Bollgard II genotypes were highly successful in the reduction of bollworm larvae on a per acre basis when compared to the conventional variety. Utilization of a pyrethroid insecticide for bollworm control also significantly reduced numbers of bollworm larvae per acre as compared to untreated genotypes. Pyrethroid-treated Bollgard and Bollgard II cottons, along with untreated Bollgard II cotton, significantly lowered the numbers of bollworm-damaged bolls per acre below that of other treatment combinations. With regard to pupal and adult production per acre, both Bollgard and Bollgard II cottons in combination with either insecticide regime successfully reduced numbers of pupae and adults when compared to pyrethroid-treated and untreated conventional cotton. However, utilization of pyrethroid insecticides in addition to Bollgard II cottons may present the most effective resistance management strategy for bollworm in *B. t.* cottons.

Introduction

Bollgard® (Monsanto Agric. Co., St. Louis, MO) cottons were planted to approximately 640,000 acres (67%) in North Carolina in 2001 (Bacheler, per. comm.) for control of heliothine pests. While control of tobacco budworm, *Heliothis virescens* (Fab.), has been absolute, Bollgard cottons have been less effective in controlling bollworm, *Helicoverpa zea* (Boddie). Results from North Carolina field trials confirm that supplemental insecticide oversprays are often required to achieve satisfactory bollworm control in Bollgard cotton (Burd et al. 1999; Lambert et al. 1996, 1997; Mahaffey et al. 1994, 1995). Survival of a portion of the bollworm population on Bollgard cottons may be explained by a high natural tolerance to Cry1Ac (as compared to tobacco budworm) (Stone and Sims 1993). Also, the reported drop in Cry1Ac endotoxin level during late season that is coincident with the major bollworm flight into North Carolina cotton fields (Greenplate 1999, Greenplate et al. 2001) is implicated. With a notable portion of the bollworm population surviving on Bollgard cotton, the "high dose" strategy for resistance evolution is violated.

Bollgard® II (Monsanto Agric. Co., St. Louis, MO) cottons that produce two insecticidal proteins, Cry1Ac and Cry2Ab, have been demonstrated to be much more active against bollworm under field conditions than Bollgard (Jackson et al. 2000, 2001; Rahn et al. 2001; Pitts 2001). Bollgard II cottons produce approximately the same level of the Cry1Ac endotoxin as Bollgard cottons, but are fortified by a second protein toxin, Cry2Ab (Greenplate et al. 2000, Adamczyk et al. 2001); thus, Bollgard II more closely meets the "high dose" criteria for resistance evolution.

The Scientific Advisory Panel (1998) concluded that Bollgard cotton produced only a moderate dose of the Cry1Ac delta-endotoxin with respect to the level considered necessary to delay selection for resistant strains of bollworm; thus, EPA demanded new insect resistance management requirements for 2001. Changes in refuge size, structure, and deployment were required as one means of achieving the goal of producing 500 susceptible adult bollworms in the refuge for each adult produced in the transgenic crop (Matten 2001). Jackson et al. (2001) reported production of bollworm adults from conventional cotton to be 13X that produced in Bollgard and 96X that produced in Bollgard II in North Carolina field studies that were not sprayed with insecticides. Thus, these initial field estimates of adult bollworm production from conventional and Bollgard cottons suggest the 500:1 resistance management goal will not be achieved through moths produced on cotton alone.

The objectives of the study reported herein were 1) to confirm field production of bollworm adults from conventional, Bollgard and Bollgard II cottons under non-sprayed conditions as in 2000 and 2) to quantify production of bollworm adults on the same cotton genotypes under an insecticide-spray regime. These data are essential inputs for computer models that predict changes in the genetic makeup of bollworm populations over time as they relate to resistance development.

Materials and Methods

A field study was conducted at the Upper Coastal Plain Research Station, Edgecombe Co., NC, in 2001. The experiment was designed as a randomized complete split-plot with four replicates. Whole plots consisted of cotton genotypes DP50 (conventional sister line), DP50B (Bollgard®), and DP50BX (Bollgard II®), which were 16, 20, and 24 rows, respectively, by 60 feet in length. Unequal plot sizes were established to increase the precision of the estimation of that proportion of the bollworm population completing development on each genotype. Fewer bollworms have been found on Bollgard II genotypes; therefore, larger whole plots were necessary to obtain useful numbers of bollworms for analysis. Subplots consisted of 12, 16, and 20 untreated rows for the conventional, Bollgard, and Bollgard II genotypes, respectively, and 4 rows that were treated with a pyrethroid as required for supplemental bollworm control.

Cotton genotypes DP50, DP50B, and DP50BX were planted on 2 May in Edgecombe Co., NC. Aldicarb (Temik 15G, Aventis CropScience, 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 97, Valent USA Corp., Walnut Creek, CA) was applied at 0.75 lb. a. i./acre as a mid-season overspray for control of plant bugs and stink bugs, as well as to eliminate arthropod natural enemies of bollworm. Two applications of lambda cyhalothrin (Karate Z 2.08 CS, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.04 lb. a.i./acre were made to appropriate subplots for supplemental bollworm control on 10 and 16 August. A CO_2-powered backpack sprayer fitted with one TX-12 nozzle per row delivering 12.1 gpa at 56 psi was used to apply foliar insecticides. Weed control, fertilization, plant growth regulation, and defoliation were achieved as recommended by North Carolina State University.

The total number of harvestable bolls were counted in a randomly selected area of five row feet per treatment per replicate on 23 August, which provided a means of converting numbers of larvae on a per boll basis to numbers of larvae on a per acre basis. The total numbers of bollworm-damaged bolls and large fourth-to-fifth instar larvae were counted on a predetermined number of bolls per plot on 18, 23, and 28 August. Fourth-to-fifth instar bollworm larvae were collected and placed on fresh cotton bolls from the respective genotype in individual 30-ml plastic cups and transported to the laboratory. These larvae were 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 that served as a medium to tunnel into for pupation. Numbers of successfully emerged bollworm adults from each genotype were counted and converted to a per acre basis prior to analysis along with the total numbers of harvestable bolls, bollworm-damaged bolls, live fourth-to-fifth instar larvae, and pupae. These data provided a reasonable estimation of bollworm production from respective cotton genotypes and present important implications for future resistance management strategies for bollworm.

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 Protected Least Significant Difference test or LSMEANS in SAS.

Results

A genotype*insecticide regime interaction was evident for numbers of harvestable bolls and numbers of bollworm-damaged bolls on a per acre basis (Table 1). Numbers of bolls per acre were significantly different among all treatment combinations of genotype and insecticide regime except the untreated and pyrethroid-treated Bollgard II genotype. Bollgard II genotypes produced the highest numbers of bolls per acre, followed by pyrethroid-treated and untreated Bollgard cottons and the pyrethroid-treated and untreated conventional variety. Treatment combinations of genotype and insecticide regime verified that the untreated conventional variety sustained the highest number of damaged bolls per acre (79,161) and the highest percentage (36) of damaged bolls compared to other treatment combinations. Pyrethroid-treated conventional cotton had the second highest number of damaged bolls per acre (36,337) followed by the untreated Bollgard (14,929) genotype. No significant differences with regard to number of bollworm-damaged bolls per acre were evident among the pyrethroid-treated Bollgard (4,200) or the untreated and pyrethroid-treated Bollgard II genotypes (1,685 and 1,155, respectively), even though damage levels were much lower in Bollgard II.

The bollworm infestation level at the test site was moderately high as indicated by the numbers of large larvae produced on a per acre basis in the untreated conventional variety (Table 2). Main plot effects of cotton genotype averaged across insecticide regimes were significant with the conventional variety containing greater numbers of bollworm larvae per acre (7,280) compared to lower numbers in Bollgard (1,193) and Bollgard II (117) genotypes. No significant differences were evident between Bollgard and Bollgard II cottons with regard to numbers of larvae per acre even though the Bollgard II genotype exhibited over a 10-fold reduction in larval numbers per acre compared to the Bollgard genotype. Subplot effects of insecticide regime averaged across genotypes were also significant with the pyrethroid-treated genotypes containing significantly fewer larvae per acre compared to untreated genotypes.

Interaction effects of genotype*insecticide regime were also apparent for numbers of bollworm pupae and adults produced on a per acre basis. Genotype*insecticide regime treatment combinations revealed that untreated conventional cotton produced the highest numbers of pupae and adults per acre (9,972) compared to other treatment combinations (Table 3). Pyrethroid-treated conventional cotton produced the second highest numbers of pupae and adults per acre (4,451 and 3,482, respectively) even though the two pyrethroid applications reduced numbers of bollworm pupae and adults on a per acre basis by approximately 2.5-fold as compared to the untreated conventional variety. No significant differences with respect to numbers of pupae produced per acre were evident among the remaining treatment combinations that included the untreated and pyrethroid-treated Bollgard (518 and 160, respectively) genotype and the untreated and pyrethroid-treated Bollgard II (156 and 0, respectively) genotype. Numbers of bollworm adults produced per acre also did not differ among the remaining treatment combinations that consisted of untreated and pyrethroid-treated Bollgard (298 and 87, respectively) cotton and the untreated and pyrethroid-treated Bollgard II (156 and 0, respectively) genotype.

Discussion

Mean numbers of bolls and bollworm-damaged bolls per acre depicted similar statistical separations, since numbers of bolls per acre were primarily affected by numbers of bollworm-damaged bolls per acre and secondarily by loss of squares to bollworm feeding. Untreated conventional cotton sustained approximately 36% bollworm-damaged bolls per acre, while two pyrethroid applications reduced bollworm-damaged bolls to ll.5%. Pyrethroid oversprays to Bollgard also significantly reduced boll damage (3.3% versus 0.9%); however, there was no significant reduction in boll damage from pyrethroid applications to Bollgard II cotton because of the low levels of boll damage in the non-sprayed Bollgard II (0.3%). Averaged over insecticide regimes, Bollgard produced ca. 16% as many large bollworms as the conventional genotype, whereas Bollgard II produced only ca. 1.6% as many large larvae as did the conventional genotype. Per acre production of bollworm pupae and adults followed the same trend as established with larval production. Per acre production of bollworm adults on conventional cotton versus Bollgard under untreated and pyrethroid-treated regimes was 33:1 and 115:1, respectively; unsprayed conventional cotton versus unsprayed Bollgard II was 64:1 with respect to adult production. Pyrethroid-treated Bollgard II had no bollworms surviving to adult emergence. The results obtained in this field study confirm the importance of insecticidal oversprays to Bollgard and Bollgard II cottons as a means of not only insuring against yield losses to bollworm, but also to reduce the numbers of adults produced. The use of Bollgard II cottons supplemented by insecticidal oversprays would clearly provide a more effective resistance management strategy than that presently available.

This study confirms 2000 results through documenting that moth production ratios in conventional and Bollgard or Bollgard II cottons do not approach the 500:1 ratio desired to substantially delay resistance development. Bollgard II without insecticidal oversprays produced only 1 moth for every 64 produced in the conventional genotype, but that remains far from the desired ratio. Even though no moths were produced in insecticide-treated Bollgard II, bollworm-active insecticides are not likely to be applied to Bollgard II unless the target is another insect pest. Our results suggest that it is unlikely that moth production in refuge cotton has approached the numbers desired for resistance management. This leaves us with the nagging question. Why haven't we seen adaptation in bollworm to Cry1Ac, particularly in areas where a large percentage of the overall cotton acreage has been planted to Bollgard varieties for several years? The answer is that resistance detection methods have been inadequate, there is an unknown fitness cost for development on Bollgard cotton, or moth production on alternate hosts is more substantial than originally thought.

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Table 1. Estimated mean (SE) numbers of harvestable bolls and bollworm-damaged bolls on a per acre basis produced by Bollgard II, Bollgard, and conventional cotton genotypes under insecticide-treated and untreated conditions averaged across three sample dates in Edgecombe Co., North Carolina in 2001.

Genotype	Insecticide Regime	Numbers of Bolls Per Acre	Numbers of Damaged Bolls Per Acre
Conventional	Untreated	221,430 (6,234) a	79,161 (4,295) a
Conventional	Treated	315,810 (5,516) b	36,337 (3,883) b
Bollgard	Untreated	449,394 (7,015) c	14,929 (1,678) c
Bollgard	Treated	467,544 (11,566) d	4,200 (737) d
Bollgard II	Untreated	492,954 (8,872) e	1,685 (473) d
Bollgard II	Treated	500,940 (8,679) e	1,155 (315) d

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

Table 2. Estimated mean (SE) numbers of bollworm larvae (4^{th}-5^{th} instar) per acre produced by Bollgard II, Bollgard, and conventional cotton genotypes under insecticide-treated and untreated conditions averaged across three sample dates in Edgecombe Co., North Carolina in 2001.

	Insecticide Regime		
Genotype	Insecticide-treated	Untreated	Mean
Conventional	4,587 (1,048)	9,972 (2,367)	7,280 (1,385) a
Bollgard	306 (170)	2,080 (495)	1,193 (316) b
Bollgard II	77 (77)	156 (105)	117 (64) b
Mean	1,657 (491) b	4,070 (1,063) a	

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

Table 3. Estimated mean (SE) numbers of pupae and successfully emerged bollworm adults per acre produced by Bollgard II, Bollgard, and conventional cotton genotypes under insecticide-treated and untreated conditions averaged across three sample dates in Edgecombe Co., North Carolina in 2001.

Genotype	**Insecticide Regime**	**Numbers of Pupae Per Acre**	**Numbers of Adults Per Acre**
Conventional	Untreated	9,972 (2,367) a	9,972 (2,367) a
Conventional	Treated	4,451 (1,048) b	3,482 (856) b
Bollgard	Untreated	518 (167) c	298 (128) c
Bollgard	Treated	160 (108) c	87 (87) c
Bollgard II	Untreated	156 (105) c	156 (105) c
Bollgard II	Treated	0 (0) c	0 (0) c

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