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BOLLWORM POPULATION PRODUCTION AND ASSOCIATED DAMAGE IN BOLLGARD AND BOLLGARD II COTTONS UNDER INSECTICIDE-TREATED AND NON-TREATED CONDITIONS

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Abstract

Transgenic cottons expressing either one or two *Bacillus thuringiensis* (*B. t.*) Berliner proteins, along with the conventional sister line, were evaluated in four 2002 field experiments with regard to impact on bollworm production in North Carolina. The relative numbers of bollworms that were capable of successfully completing development on conventional, Bollgard®, and Bollgard II® cottons under insecticide-sprayed and unsprayed conditions were estimated. Bollgard and Bollgard II genotypes reduced bollworm larvae, pupae, and damaged bolls produced per acre as compared to the conventional variety; the Bollgard II genotype further reduced the numbers of bollworm larvae, pupae, and damaged bolls per acre below that of the commercial Bollgard variety. Utilization of an insecticide application for bollworm control also significantly reduced numbers of bollworm larvae, pupae, and damaged bolls produced per acre as compared to untreated genotypes. With regard to per acre adult production, insecticide-treated Bollgard and Bollgard II cottons, along with untreated Bollgard II cottons, successfully reduced numbers of adults when compared to pyrethroid-treated and untreated conventional cotton and untreated Bollgard cottons. However, application of insecticides to Bollgard II cottons offers the most effective *B. t.* resistance management strategy for bollworm.

Introduction

Bollgard II® (Monsanto Agric. Co., St. Louis, MO) cottons produce two insecticidal proteins, Cry1Ac and Cry2Ab, as compared to one insecticidal protein exhibited by the commercial Bollgard varieties. Bollgard II cottons have been demonstrated to be much more active against bollworm under field conditions than Bollgard (Jackson et al. 2000, 2001, 2002). Bollgard II cottons produce approximately the same level of the Cry1Ac endotoxin as Bollgard cottons, but are further protected 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 U. S. Environmental Protection Agency has demanded insect resistance management requirements for Bollgard cotton such as change in refuge size, structure, and deployment as a means of achieving the goal of producing 500 susceptible adult bollworms from refuge cottons as potential mates for each bollworm adult produced by Bollgard cottons (Matten 2001). Jackson et al. (2002) reported that production of bollworm adults from unsprayed conventional cottons to be 33X and 40X that produced in untreated and pyrethroid-treated Bollgard cottons and 64X that produced in unsprayed Bollgard II cottons in North Carolina field studies. Pyrethroid-treated Bollgard II cottons produced no bollworms that successfully emerged as adults. Thus, 2001 field estimates of adult bollworm production from conventional and Bollgard cottons suggest that the goal of producing the 500:1 ratio for resistance management may be obtained only through plantings of Bollgard II cottons in combination with insecticides active against heliothines, or through the use of alternate hosts as supplemental refuges for these Bollgard cottons.

The objectives of the study reported herein were to confirm field production of bollworm adults from conventional, Bollgard and Bollgard II cottons under non-sprayed and insecticide-sprayed conditions.

Materials and Methods

A field study was conducted in North Carolina during 2002 at the following locations: the Upper Coastal Plain Research Station in Edgecombe Co., the C. A. Martin Farm in Martin Co., the Tidewater Research Station and Albemarle Beach Farms in Washington Co. in 2002. 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 20 rows by 45 feet in length. Subplots consisted of 16 untreated rows and 4 rows that were treated with a pyrethroid as required for supplemental bollworm control.

Conventional, Bollgard, and Bollgard II cotton genotypes were planted on 14 May in Edgecombe Co. and one site in Washington Co., NC. The second Washington Co. site was planted on 15 May and the Martin Co. site was planted on 16 May. 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 in combination with spinosad (Tracer 4 SC, Dow AgroSciences LLC, Indianapolis, IN) at 0.089 lb. a.i./acre were made to appropriate subplots for supplemental bollworm control on 23 July and 1 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, 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 four sample dates. 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 pupation medium. Numbers of successfully emerged bollworm adults from each genotype were counted and converted to a per acre basis prior to analysis as were the total numbers of bollworm-damaged bolls, live fourth-to-fifth instar larvae, and pupae. These data provided an estimation of bollworm production from the respective cotton genotypes.

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.

Results

Differences in numbers of damaged bolls per acre were characterized by main plot effects of cotton genotype and subplot effects of insecticide regime. The conventional cotton variety had an estimated 109,724 damaged bolls per acre averaged across insecticide regimes, which was significantly higher than the 45,769 and 5,217 damaged bolls per acre in the Bollgard and Bollgard II cottons, respectively (Table 1). The dual-gene construct of the Bollgard II genotype significantly reduced the number of damaged bolls per acre below that of the single-gene Bollgard variety. Subplot effects of insecticide regime were also evident as the insecticide-treated subplots had significantly fewer numbers of damaged bolls per acre (24,015) than the untreated subplots (81,863) when averaged across genotypes.

The 2002 North Carolina field season was characterized by extremely high bollworm populations as indicated by the numbers of large larvae produced per acre in the untreated conventional variety (Table 2). Main plot as well as subplot effects were apparent for differences in numbers of bollworm larvae produced per acre. Averaged across insecticide regimes, the conventional variety produced 24,021 large bollworm larvae per acre, as compared to 14,816 by Bollgard and 1,236 by Bollgard II. The Bollgard II genotype significantly reduced the number of large bollworm larvae per acre below that of the Bollgard genotype. The application of insecticides to the various cotton genotypes significantly reduced the numbers of large larvae produced per acre (5,266) below that of untreated genotypes (21,209) when averaged across cotton genotypes.

Numbers of bollworm pupae produced on a per acre basis were depicted by main plot and subplot effects. Bollgard and Bollgard II cotton genotypes produced 12,546 and 1,083 bollworm pupae per acre averaged across insecticide regimes, which were significantly lower than the 18,805 produced by the conventional genotype (Table 3). As with larval production, the Bollgard II genotype produced significantly fewer numbers of bollworm pupae per acre as compared to the Bollgard variety. Averaged across cotton genotypes, insecticide-treated cottons significantly lowered pupal numbers by producing 3,162 bollworm pupae per acre as compared to 18,281 by the untreated cottons.

Interaction effects of genotype* insecticide regime were evident for numbers of bollworm adults produced on a per acre basis. Genotype*insecticide regime treatment combinations revealed that untreated conventional cotton produced the highest numbers of adults per acre (26,172), but did not differ statistically from production by the untreated Bollgard genotype (15,777) (Table 4). Insecticide-treated conventional cotton significantly reduced the numbers of adults per acre (5,714) below that of the untreated conventional variety but did not statistically differ from the untreated Bollgard variety. Untreated Bollgard II cottons produced 1,067 bollworm adults per acre, which was significantly less than that produced by the untreated Bollgard and the insecticide-treated conventional varieties. No significant differences with respect to numbers of adults produced per acre were apparent among untreated Bollgard II cottons and insecticide-treated Bollgard and Bollgard II cottons, which produced 999 and 0 bollworm adults per acre, respectively.

Discussion

Mean numbers of large bollworm larvae and bollworm-damaged bolls per acre depicted similar trends, since numbers of bollworm-damaged bolls were directly affected by numbers of bollworm larvae. Numbers of damaged bolls were reduced by both *B. t.* genotypes as compared to the conventional variety, with the Bollgard II genotype producing fewer damaged bolls than the Bollgard variety. These data confirm previous production estimates (Jackson et al. 2002) where untreated Bollgard II cottons sustained lower numbers of damaged bolls as compared to the untreated Bollgard variety. Larval production was significantly reduced by Bollgard II cottons as compared to the Bollgard variety during the 2002 field season due to extremely high populations of bollworm larvae at the test sites. Under the moderate bollworm populations encountered during the 2001 field season, larval production did not differ among these two cotton genotypes (Jackson et al. 2002). Therefore, based on production estimates of large bollworm larvae and bollworm-damaged bolls, Bollgard II cottons have much more profit potential during seasons with high bollworm numbers. Bollworm pupal numbers followed the same trend as larval numbers, since the effects of the *B. t.* toxins are most pronounced in the early larval stages. The addition of insecticide applications when averaged across cotton genotypes significantly reduced bollworm larval and pupal production, as well as the numbers of bollworm-damaged bolls below that of untreated cottons.

A genotype*insecticide interaction demonstrated that high bollworm populations reduced the effectiveness of the untreated Bollgard cottons with respect to adult production as compared to 2001 production estimates (Jackson et al. 2002). Under moderate bollworm pressure in 2001, untreated Bollgard cottons performed better than insecticide-treated conventional varieties with respect to bollworm adult production; however, under high level bollworm infestations in 2002, untreated Bollgard cottons produced numerically higher numbers of bollworm adults compared to the insecticide-treated conventional variety. The addition of insecticide applications to Bollgard cottons made adult production comparable to that of untreated and insecticide-treated Bollgard II cottons. Again, this differed from estimates of Jackson et al. (2002) where under moderate bollworm populations, untreated Bollgard cottons performed similarly to insecticide-treated Bollgard and Bollgard II cottons and the untreated Bollgard II genotype with respect to adult production.

Bollworm larval and adult production estimates demonstrate that Bollgard II cottons add increased benefits over commercial Bollgard varieties from both bollworm efficacy and resistance management standpoints. For example, per acre production of bollworm adults on untreated conventional cotton versus untreated and insecticide-treated Bollgard cottons was 2:1 and 26:1, respectively; production ratios from untreated conventional cottons versus untreated Bollgard II cottons was 25:1. The addition of insecticide applications to the Bollgard II genotype resulted in no bollworms surviving to adult emergence. Results from the 2002 field season confirm those from 2001 in that the addition of an heliothine-active insecticide to Bollgard II cottons offers the best alternative for bollworm resistance management. This combination is very likely since bug pests often reach threshold and are treated with broad-spectrum insecticides coincident with the onset of the bollworm flight into cotton in North Carolina. Thus, with Bollgard II cottons management emphasis will shift from heliothines to “bug” pests; however, incidental control of bollworm larvae would likely give a resistance management benefit.

The results reported herein represent the final year of a three-year study documenting *H. zea* moth production in conventional, Bollgard and Bollgard II cottons in North Carolina. The three-year study confirmed that moth production did not approach the 500:1 ratio desired to substantially delay resistance development; however, since Bollgard cottons do not offer a “high dose” for bollworm, the 500:1 ratio is an unrealistic objective. It is obvious that Bollgard II cottons oversprayed with heliothine-active insecticides present the best option for cotton production and for *B. t.* resistance management. Also, our results show no field level *B. t.* resistance development in bollworm and suggest that unknown fitness costs for development on Bollgard cotton, bollworm moth production on alternate crop hosts is much more substantial than anticipated, or other resistance mitigating phenomenon are active in nature. It is apparent that alternate crop hosts should be given more consideration for their value in supplementing the *B. t.* cotton refuge, particularly in diverse agroecosystems characteristic of the southeast U. S. cottonbelt. These data remain essential as inputs for computer models that predict changes in the genetic makeup of bollworm populations over time as they relate to resistance development and present important implications for future resistance management strategies for bollworm.

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Table 1. Estimated mean (SE) numbers of bollworm-damaged bolls per acre produced under extremely high bollworm populations by conventional, Bollgard, and Bollgard II cotton genotypes under insecticide-treated and untreated conditions averaged across four sample dates and four sites in North Carolina, 2002.

	Insecticide Regime		
Genotype	**Insecticide-treated**	**Untreated**	**Mean**
Conventional	57,796 (5,092)	161,653 (7,103)	109,724 (6,510) a
Bollgard	14,379 (2,767)	77,158 (7,384)	45,769 (4,868) b
Bollgard II	997 (390)	9,436 (1,826)	5,217 (1,007) c
Mean	24,015 (2,624) b	81,863 (5,792) 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 2. Estimated mean (SE) numbers of live bollworm larvae (L4-L5) per acre produced under extremely high bollworm populations by conventional, Bollgard, and Bollgard II cotton genotypes under insecticide-treated and untreated conditions averaged across four sample dates and four sites in North Carolina, 2002.

	Insecticide Regime		
Genotype	**Insecticide-treated**	**Untreated**	**Mean**
Conventional	13,208 (1,980)	34,833 (5,408)	24,021 (3,039) a
Bollgard	2,856 (746)	26,775 (3,925)	14,816 (2,271) b
Bollgard II	0 (0)	2,471 (760)	1,236 (395) c
Mean	5,266 (809) b	21,209 (2,436) 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 bollworm pupae per acre produced under extremely high bollworm populations b y conventional, Bollgard, and Bollgard II cotton genotypes under insecticide-treated and untreated conditions averaged across four sample dates and four sites in North Carolina, 2002.

	Insecticide Regime		
Genotype	**Insecticide-treated**	**Untreated**	**Mean**
Conventional	7,814 (1,302)	29,796 (4,671)	18,805 (2,623) a
Bollgard	1,827 (478)	23,264 (3,396)	12,546 (1,970) b
Bollgard II	0 (0)	2,167 (639)	1,083 (333) c
Mean	3,162 (516) b	8,281 (2,102) 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 4. Estimated mean (SE) numbers of successfully emerged bollworm adults per acre produced under extremely high bollworm populations by conventional, Bollgard, and Bollgard II cotton genotypes under insecticide-treated and untreated conditions averaged across three sample dates and four sites in North Carolina, 2002.

Genotype	**Insecticide Regime**	**Numbers of Adults Per Acre**
Conventional	Untreated	26,172 (4,245) a
Bollgard	Untreated	15,777 (2,504) ab
Conventional	Treated	5,714 (1,073) b
Bollgard II	Untreated	1,067 (435) c
Bollgard	Treated	999 (359) c
Bollgard II	Treated	0 (0) c

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