EVALUATION OF BOLLGARDII® (CV. DP50BII) IN THE MISSISSIPPI DELTA: FIELD EFFICACY AGAINST VARIOUS LEPIDOPTERA WHILE PROFILING SEASON-LONG EXPRESSION OF CRY1Ac AND CRY2Ab

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Abstract

Transgenic cotton varieties (Bollgard® and BollgardII®) were evaluated in Stoneville, MS in 2000. It was apparent that the two protein product (BollgardII®) had increased activity against a multitude of lepidopteran pests compared to Bollgard®. Studies profiling the season-long expression of both proteins in BollgardII® indicated that the new protein (Cry2Ab) was expressed at a much higher level than Cry1Ac in Bollgard® and BollgardII®.

Introduction

Since transgenic Cry1Ac *Bacillus thuringiensis* Berliner (Bt) cotton (Bollgard®, Monsanto Co., St. Louis, Mo.) became widely commercialized in the US in 1996, growers and researchers have noted that many lepidopteran pests are not controlled with this technology alone. The addition of a second Bt protein (Cry2Ab) to Bollgard® (=BollgardII®) may offer additional control of various occasional and secondary Lepidoptera in cotton. The purpose of this research was to evaluate the efficacy of experimental transgenic cotton (BollgardII®) against various lepidopteran pests of cotton as well as to profile season-long expression of both proteins to aid in developing resistance-management strategies for this forthcoming technology.

Materials and Methods

Field Plots

Three varieties (conventional, cv. DP50; transgenic Cry1Ac Bollgard®, cv. DP50B; and transgenic Cry1Ac + Cry2Ab BollgardII®, cv. DP50BII) were planted in experimental plots on 23 May 2000 near Elizabeth, MS. Plots consisted of 4 rows (1.0 m centers) x 9.15 m treatments arranged in a randomized complete block design with each variety replicated twice in each block. Only insecticides not active on Lepidoptera were applied to all plots throughout the season as dictated by local management practices. Plots were irrigated twice.

Insects

To ensure that data would be generated for occasional lepidopterous pests of cotton, two colonies of armyworms were established. Larvae (ca. 300) of the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), were collected from whorl-stage field corn near Stoneville, MS in April 2000 and reared for one complete generation in the laboratory as described by Adamczyk et al. (1998). Adult male beet armyworms (BAW), *Spodoptera exigua* (Hübner), were collected using pheromone traps located near Nepanee, MS in December 2000 and mated with a laboratory colony of females to establish a new colony suitable for field inoculations.

Inoculations of beet and fall armyworm egg masses to plants for all varieties were conducted in late July 2000. In brief, egg masses of either species were deposited on nylon cloth in the laboratory. An egg mass of equal size (ca. 2.54 cm^2 cloth sample) was stapled to the underside of a mature leaf with 4 replications/variety. For fall armyworms, each infested plot received 24 egg masses over a two-week period. For beet armyworms, each plot received 10 egg masses on July 17, 2000.

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Natural infestations of many lepidopterous species [BAW; FAW; tobacco budworm (TBW), *Heliothis virescens* (F); soybean looper (SBL), *Pseudoplusia includens* (Walker); salt marsh caterpillar (SMARSH), *Estigmene acrea* (Drury); bollworm (BW), *Helicoverpa zea* (Boddie)] occurred in all plots. Scouting of whole plants and individual plant parts was conducted only in one replicated plot/block (4 replications/variety). Cotton was mechanically harvested for all rows in all plots and weighed.

Bt Quantification

For each sample date, a single terminal leaf was randomly harvested from 10 plants/plot for all varieties (4 replications/variety). Leaves were transported to the laboratory and within a few hours after being harvested, one sample (ca. 5-8 mg) was taken from each leaf using a standard 6-mm paper ticket punch. The leaf samples were weighed to accurately determine the amount of starting material and combined for each variety into a 1.5 ml microcentrifuge tube containing extraction buffer (EnviroLogic, Inc.; Portland, ME). The tissue was then homogenized using a mini-beadbeater-8™ (Biospec Products, Inc., Bartlesville, Ok.) using 6.4 mm steel ball bearings. To quantify the amount of Bt present for each variety (either Cry1Ac in DP50B or Cry1Ac and Cry2Ab in DP50BII), a commercial quantification plate kit was utilized as described in Adamczyk et al. (2000). For all sample dates, varieties were always identically compared in a side-by-side experiment. The proper standard curve, dilution factors, positive and negative controls, and calculations were conducted as dictated in the kit protocol.

Statistics

All means were log-transformed and analyzed using REML – ANOVA. The means were then separated using the LSMEANS option of PROC MIXED (Littell et al. 1996).

Results and Discussion

Drop cloth samples indicated that many lepidopteran pests were reduced in BollgardII® plots (cv. DP50BII) compared to Bollgard® (cv. DP50B) or conventional cotton (cv. DP50) (Table 1). In addition, very few fall armyworms were found in BollgardII® plots compared to Bollgard® or conventional cotton (Table 2). Because fall armyworms are primarily associated with flower damage and subsequent boll damage in Bollgard® cotton, BollgardII® may have increased efficacy against certain Lepidoptera that primarily feed on fruiting structures. The addition of Cry2Ab to Cry1Ac transgenic cotton could increase lepidopteran activity because of increased expression in reproductive tissues (Greenplate 2000). In the present study, the addition of Cry2Ab to transgenic cotton containing Cry1Ac significantly increased activity against pests such as SBL and to a lesser extent SMARSH, FAW, BAW, and BW. Activity against TBW remained superior (Table 3). However, no yield increase was observed with cv. DP50BII compared to cv. DP50B (Table 4).

The lepidopteran bioactivity and protein expression levels of Cry2Ab appear to be quite different than Cry1Ac. Sims (1997) noted that soybean loopers were highly sensitive (LC_{50} = 0.06 ug/ml) to purified Cry2A incorporated into artificial diet, while other studies have shown that they are not as sensitive to Cry1Ac in Bollgard® (Sumerford and Solomon 2000). Greenplate (2000) showed that Cry2Ab is less potent than Cry1Ac against TBW, but overall expression of Cry2Ab over Cry1Ac is 10 times higher. In our study, we also observed that Cry2Ab is expressed at a higher level than Cry1Ac throughout the growing season (Figure 1). Thus, depending on the species, the increased activity of BollgardII® compared to Bollgard® can be due to increased potency of Cry2Ab, increased overall expression level of Cry2Ab, or possibly a synergistic combination.

Achieving season-long dual-protein activity in BollgardII® will be a critical element in resistance management strategies for TBW and BW. Expression profiles of both proteins in BollgardII® appeared to be independent on one

another (Figure 1). Our data further indicated that the addition of Cry2Ab had no deleterious effect on levels of Cry1Ac in BollgardII®. Because cv. DP50BII results from cloned plants regenerated from transformed tissue of cv. DP50B (Bollgard®), backcrosses with elite varieties will have to be conducted before commercialization. Thus, future research must be conducted to ensure that expression profiles of both proteins are adequate to provide season-long dual-protein activity against a multitude of Lepidoptera.

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Table 1. Mean number of Lepidoptera found in Bollgard® and BollgardII® plots using a 1.24 m^2 drop cloth.

	Mean for 3 drops/plot				
Variety	**BAW**	**FAW**	**TBW**	**SBL**	**SMARSH**
DP 50	42.00 a	3.00 a	7.00 a	32.00 a	8.75 a
DP 50B	35.50 a	1.75 a	0.25 b	37.75 a	2.75 a
DP 50BII	2.50 b	0.75 a	0 b	0.25 b	0.25 a
F-value	36.11	2.33	9.92	106.77	1.90
df	2, 9	2, 9	2, 9	2, 9	2, 9
P > F	<0.001	0.153	0.005	<0.001	0.205

Table 2. Mean number of fall armyworms found in Bollgard® and BollgardII® flowers and whole plants.

	25 flowers/plot		10 Whole
Variety	**White Flowers**	**Pink Flowers**	**Plants**
DP 50	1.50 a	2.25 a	2.75 a
DP 50B	0.25 a	0.75 ab	2.50 ab
DP 50BII	0.25 a	0 b	0.25 b
F-value	1.13	6.14	4.25
df	2, 9	2, 9	2, 9
P > F	0.368	0.021	0.050

Table 3. Cumulative percent reduction, relative to DP50, of larvae found in field plots of Bollgard® and BollgardII® cotton in Stoneville, MS 2000.

Species	**DP50B**	**DP50BII**
BW	57.0	86.0
TBW	99.9	99.9
SBL	0	99.0
FAW	20.0	87.0
BAW	30.0	86.0
SMARSH	29.0	97.0

Table 4. Mean number of damaged plant parts and yield in Bollgard® and BollgardII® plots.

Variety	**Squares[1]**	**25 Pink Flowers[2]**	**Bolls[3]**	**Yield[4] (lbs/A)**
DP 50	7.75 a	7.75 a	22.5 a	872 a
DP 50B	6.25 a	6.50 a	1.0 b	1182 b
DP 50BII	0.75 a	3.50 b	0.25 b	1172 b
F-value	2.17	5.18	54.55	9.10
df	2, 9	2, 9	2, 9	2, 21
P > F	0.170	0.032	<0.001	0.001

[1]Damage significant to cause fruit loss. 10 whole plants/plot.
[2]Lepidopterous feeding on bracts or petals. 25 flowers/plot.
[3]Damage significant to cause fruit loss. 20 whole plants/plot.
[4]Based on 35% gin turnout.

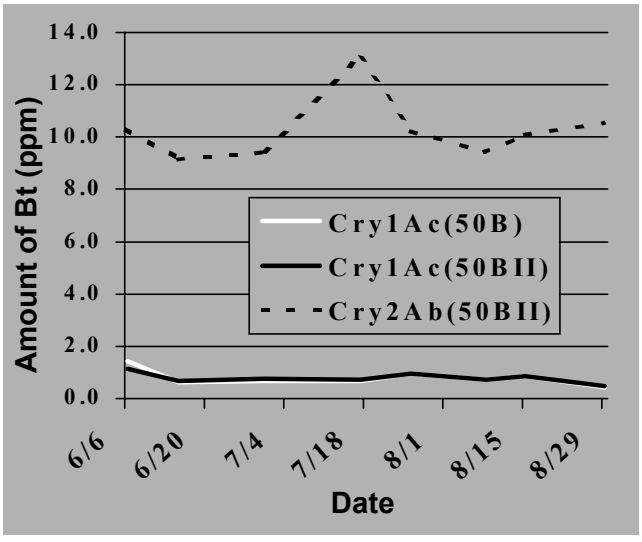

Figure 1. Season-long expression of Bt protein(s) in Bollgard® and BollgardII®.