FIELD EFFICACY OF COTTON EXPRESSING TWO INSECTICIDAL PROTEINS OF *BACILLUS THURINGIENSIS*

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Abstract

Field studies were conducted in 1999 and 2000 to evaluate field efficacy of cotton (*Gossypium hirsutum* L.) expressing two insecticidal proteins of *Bacillus thuringiensis* Berliner (Bt) on various lepidopteran pests. Near-isogenic varieties including non-transgenic, single-toxin, and dual-toxin cotton were compared for heliothine numbers, numbers of occasional lepidopteran pests, damaged terminals, bolls, and squares, and seed cotton yield. Relative to the non-Bt cotton cultivar, both single and dual-toxin cotton caused a significant reduction in the numbers of heliothine larvae, damaged terminals, and damaged squares and bolls found during visual sampling. Generally, fewer heliothines were found in MON15985 than in DPL50B or DPL50 (Table 1, Figure 1). Additionally, fewer heliothines were found in unsprayed subplots with cotton expressing both Cry1Ac and Cry2Ab than non-Bt subplots treated with insecticide. There was no significant difference in damaged terminals, squares, or bolls (predominantly due to bollworm) between single and dual-toxin varieties, but both Bt cultivars exhibited less damage than non-Bt cotton. Cotton expressing one and two insecticidal proteins reduced cumulative bollworm populations 79% and 96% for both years, respectively. Although single-toxin cotton only incited a 14% cumulative reduction of fall armyworms, populations were reduced by 96% in cotton expressing two Bt toxins compared with non-Bt cotton and cotton expressing Cry1Ac alone. Dual-toxin Bt cotton caused a 94% cumulative reduction in soybean loopers, when compared to non-Bt cotton. More soybean loopers were found in the single-toxin Bt cotton than the non-Bt cultivar. Although both single and dual toxin improved seed cotton yield over non-Bt cotton, there was no significant difference between the two varieties.

These data suggest that the insertion of the Cry2Ab gene will provide substantially better control of a wide range of lepidopteran pests when compared to cotton expressing Cry1Ac alone. Since armyworms and loopers are not as affected by the single-toxin cultivar as heliothines, the true benefit of this second toxin-producing gene will likely be more apparent for these occasional pests. However, control of bollworms should also be improved.

Introduction

For many years, lepidopteran pests have been a major source of economic damage in cotton throughout the United States. Much of the total economic damage induced by insects and control costs involved can be attributed to the tobacco budworm (*Heliothis virescens*), bollworm (*Helicoverpa zea*), fall armyworm (*Spodoptera frugiperda*), beet armyworm (*Spodoptera exigua*), and soybean looper (*Pseudoplusia includens*) (Williams 2000).

The use of insecticides has been an effective method of controlling caterpillar pests in cotton. The issues of insecticide resistance (Felland et al. 1990, Graves et al. 1994) and the negative impact on non-target, beneficial arthropod populations, however, has spawned an interest in alternate control methods for these pests. In 1996, transgenic cotton expressing the Cry1Ac δ-endotoxin of *Bacillus thuringiensis* var. *kurstaki* was introduced for commercial production (i. e., Bollgard™; Monsanto Company, St. Louis, MO). This technology provides season-long control of lepidopteran pests, particularly the heliothine complex. Although this new technology has proven to be quite effective, some weaknesses have been noted, such as lack of efficacy against occasional pests (e.g., armyworms, loopers) and high populations of bollworm.

Cotton expressing two insecticidal proteins of *Bacillus thuringiensis* (i.e., Bollgard II™) has recently been developed by Monsanto Company. This transgenic cotton not only expresses the Cry1Ac toxin, but an additional gene expressing Cry2Ab toxin has been added to aid in control of occasional pests and high populations of bollworm. The addition of this second toxin to the original Bt cotton variety can potentially provide additional control of occasional pests as well as bollworms present in high populations.

Materials and Methods

In 1999 and 2000, field studies were conducted to compare the relative efficacy of near-isogenic lines of cotton expressing none, one, or two insecticidal proteins of *Bacillus thuringiensis* Berliner on certain lepidopteran pests. Three varieties of cotton were planted: DPL50 (non-Bt), DPL50B (expresses Cry1Ac), and MON15985 (expresses Cry1Ac and Cry2ab). Four main plots of each cultivar were planted on 20 May, 1999 and 16 May, 2000 in a randomized complete block design at the Plant Science Research Center, Mississippi State, MS. Both years, cotton was planted at 11.7 seeds/m using a cone planter. Each plot was 12 rows wide (97 cm row spacing) and 15-m long and was divided into a four-row sprayed subplot and an eight-row unsprayed subplot. Sprayed subplots were treated with insecticide for control of lepidopteran pests based on average insect counts made in the non-Bt cultivar, such that whenever the DPL50 plots exceeded a treatment threshold for any lepidopteran pests, insecticide was applied to all varieties. After the initial insecticide application, only insect samples in sprayed subplots were used to trigger subsequent applications. Insecticide treatments contained 76 g ai/ha spinosad + 37 g ai/ha cyfluthrin. All insecticide applications were made with a high-clearance, small plot tractor calibrated to deliver a spray volume of 68 l/ha. Unsprayed subplots were not treated for lepidopteran pests with insecticide.

No at-planting insecticides were used either year, but 227 g ai/ha acephate was applied to all plots approximately one week after plant emergence. Additionally, several applications of ULV malathion (222g ai/ha) were made to all plots during the course of the season as part of the existing boll weevil eradication program. Applications for the control of caterpillar pests (primarily bollworm and tobacco budworm) were made to sprayed subplots on 29 July and 4, 13, and 27 August of 1999 (latter application for soybean loopers). In 2000, insecticide applications were made on 12 and 29 July and 5 August. Based on insect control recommendations for Mississippi cotton (Layton 1999, 2000) no other insecticide applications were necessary, either in 1999 or 2000. All plots were furrow irrigated four times in 1999 and five times in 2000.

In order to determine relative efficacy of the different cultivars under field conditions, naturally occurring populations were monitored twice weekly via visual, sweep-net, and drop-cloth samples. Prior to the initiation of insecticide applications for heliothines (29 July in 1999 and 12 July in 2000), samples were taken randomly throughout each main plot. Following the first application, samples were taken in the four sprayed rows and the four adjacent unsprayed rows. The remaining unsprayed rows were used as a source of plant material for use in laboratory assays.

Visual samples consisted of examining the top five nodes of fifteen plant terminals in sprayed and unsprayed subplots for heliothines. Also examined were fifteen half-grown or larger squares and fifteen half-grown or smaller bolls for damage and the presence of lepidopteran pests, particularly bollworms, tobacco budworms, or fall armyworms. On most sampling dates, fifteen sweeps with a 38-cm diameter sweep net were made

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in each subplot in 1999 and 2000. For drop cloth samples, one sample (1.8 m of row) was taken in each subplot in 1999 and two were taken in each subplot in 2000. For both years, numbers of soybean loopers and beet armyworms were recorded. Seed cotton yields were estimated by harvesting the center two rows of each subplot on 8 October in 1999, and 29 September in 2000.

Data were pooled for both years, and were analyzed using split-plot analysis of variance procedures and using linear contrasts for mean separation (α=0.05, Proc GLM Contrasts, SAS 1998).

Results

Relative to the non-Bt cotton cultivar, our data indicated that both single and dual-toxin cultivars caused a significant reduction in the numbers of heliothine larvae, damaged terminals, and damaged squares and bolls found during visual sampling. There was no interaction between cultivar and insecticide in either year. Generally, fewer heliothines were found in dual-toxin cotton than in single-toxin or non-Bt cotton (Table 1, Figure 1). Fewer heliothines were also found in unsprayed subplots of MON15985 compared to DPL50 subplots treated with insecticide (Table 1). For damaged terminals, squares, and bolls (predominantly caused by bollworm), less damage was noted in the single and dual-toxin varieties than in the non-Bt variety (Table 2).

For other lepidopteran pests, only soybean loopers were present in high numbers during August of 1999, and moderate levels of fall armyworm were found in 2000. Cumulative counts represent numbers of total insects in visual, sweep net and drop cloth samples in sprayed and unsprayed subplots for both years. Cumulative counts showed a 79% and 96% reduction of bollworm larvae in Bollgard II plots, compared to those with original Bollgard and non-Bt, respectively (Figure 1). In both years, only one bollworm >1/4 inch was found in MON15985 as opposed to >10 that were found in DPL50B. For fall armyworm, plots containing dual-toxin cotton reduced larvae by 96% over both non-Bt and single-toxin cotton in 1999 and 2000 (Figure 2). Populations of soybean looper were also significantly reduced in plots containing dual-toxin cotton (96%). There were 41% more soybean loopers in the single-toxin variety than the non-Bt cotton (Figure 3).

Seed cotton yield for both 1999 and 2000 showed an increase by at least 15% in single or dual-toxin cotton, compared to the non-Bt variety (Figure 4). However, unsprayed subplots out-yielded those treated with insecticide by 8% (Figure 5). This is due to unusually high yield in unsprayed subplots in 1999. There was no interaction between cultivar and insecticide in either year.

References

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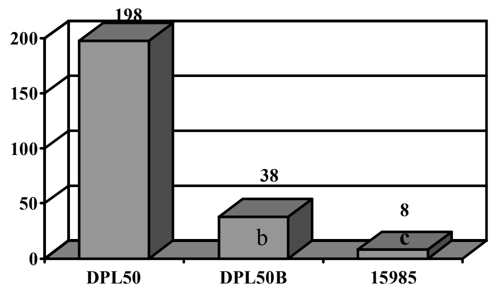
Table 1. Percent heliothine infestation in non-Bt, single-toxin, and dual-toxin transgenic cotton varieties in 1999 and 2000.

		% Heliothine-infested terminals	**% Heliothine-infested squares/bolls**	**% Heliothines in all samples**
DPL50	unsprayed	2.39 a	2.22 a	2.19 a
	sprayed	1.16 b	0.74 b	0.68 b
DPL50B	unsprayed	0.39 bc	0.35 bc	0.35 bc
	sprayed	0.47 bc	0.28 bc	0.24 cd
15985	unsprayed	0.04 c	0.08 c	0.06 d
	sprayed	0.30 bc	0.19 bc	0.15 cd

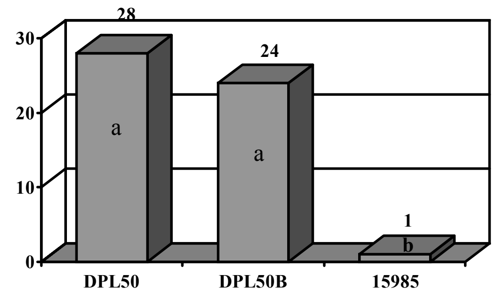
Table 2. Percent damaged terminals, squares, and bolls in non-Bt, single toxin, and dual-toxin transgenic cotton varieties in 1999 and 2000.

	% Damaged terminals	**% Damaged squares**	**% Damaged bolls**
DPL50	9.44 a	0.92 a	0.83 a
DPL50B	1.76 b	0.18 b	0.13 b
15985	1.14 b	0.14 b	0.05 b



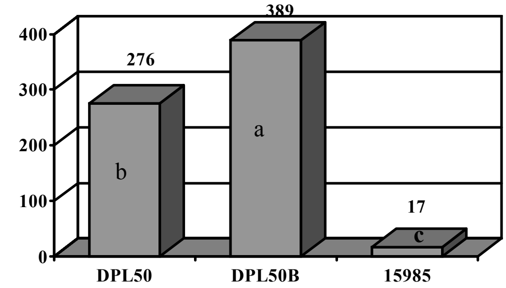
Means not followed by the same letter are significantly different (X^2, P<0.05)

Figure 1. Cumulative counts for bollworm in non-Bt, single toxin, and dual-toxin transgenic cotton varieties in 1999 and 2000.



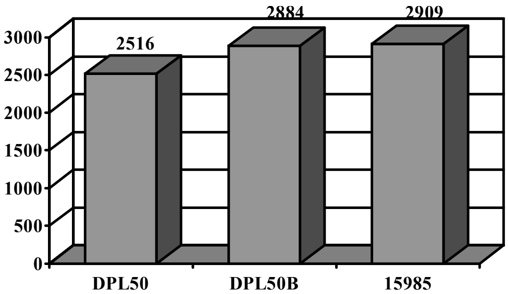
Means not followed by the same letter are significantly different (X^2, P<0.05)

Figure 2. Cumulative counts for fall armyworm in non-Bt, single toxin, and dual-toxin transgenic cotton varieties in 1999 and 2000.



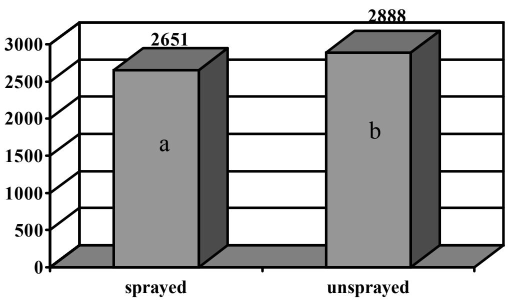
Means not followed by the same letter are significantly different
(X^2, P<0.05)

Figure 3. Cumulative counts for soybean looper in non-Bt, single toxin, and dual-toxin transgenic cotton varieties in 1999 and 2000.



Means not followed by the same letter are significantly different
[P<0.05, Proc GLM (LSmeans, pdiff), SAS Institute 1998}

Figure 4. Seed cotton yield from non-Bt, single toxin, and dual-toxin transgenic cotton varieties in 1999 and 2000.



Means not followed by the same letter are significantly different
[P<0.05, Proc GLM (LSmeans, pdiff), SAS Institute 1998}

Figure 5. Seed cotton yield from sprayed and unsprayed subplots in 1999 and 2000.