EFFICACY OF BT COTTON EXPRESSING TWO INSECTICIDAL PROTEINS OF *BACILLUS THURINGIENSIS* BERLINER ON SELECTED CATERPILLAR PESTS

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Abstract

In 1999, field and laboratory assays were performed to compare the relative efficacy of near-isogenic cultivars of cotton (*Gossypium hirsutum* L.), expressing none, one or two insecticidal proteins of *Bacillus thuringiensis* Berliner, on various caterpillar pests (Lepidoptera: Noctuidae). Bollworm, *Helicoverpa zea* (Boddie), and soybean looper, *Pseudoplusia includens* (Walker), populations were high enough to determine that Bt cotton, expressing both CryIAc and CryX endotoxins of *B. thuringiensis*, was more effective in controlling these pests than was cotton expressing only CryIAc. CryX is a pseudonym, and the actual 'Cry' classification of this toxin is unreported. Cotton expressing both toxins is currently being referred to as Bollgard II. Laboratory assays where various plant parts were fed to insects indicated that the dual-toxin Bt cultivar was more toxic to bollworm, fall armyworm (*Spodoptera frugiperda* [Smith]) and beet armyworm (*Spodoptera exigua* [Heubner]) than the single-toxin cultivar. It appears that the newly-developed stacked Bt cultivars will be more effective and have a wider range of activity on the various caterpillar pests commonly encountered in cotton. The implications of this improved activity are addressed.

Introduction

Transgenic Bt cotton, *Gossypium hirsutum* (L.) expressing the CryIAc insecticidal endotoxin of *Bacillus thuringiensis* Berliner, has been commercially available in the United States since 1996. Since this time, Bt cotton has demonstrated remarkable control of some lepidopteran pests, particularly tobacco budworm (*Heliothis virescens* F.) and pink bollworm (*Pectinophora gossypiella* [Saunders]). Control of bollworm, *Helicoverpa zea* (Boddie), has been less dependable, and economically-damaging infestations of this pest can occur on Bt cotton, particularly after plants have begun blooming and when insecticides have disrupted populations of insect predators and parasites. The bollworm is inherently more tolerant than tobacco budworm to the CryIAc endotoxin expressed in currently available Bt cultivars. Additionally, low expression of the toxin in flower parts has been implicated in increased survival of the bollworms on Bt cotton. Other common caterpillar pests (e.g., fall armyworm, *Spodoptera frugiperda* [Smith]; beet armyworm, *Spodoptera exigua* [Heubner]; and soybean looper, *Pseudoplusia includens* [Walker]), are even more tolerant than the bollworm to the current, commercially-available Bt cultivars.

One advantage of Bt cotton is that it greatly reduces plant injury and insecticide use associated with tobacco budworm and bollworm. However, in low-spray environments, pests like fall armyworm can thrive, even in Bt cotton. As already mentioned, soybean looper and beet armyworm can also cause economic damage to Bt cotton. The addition of a second Bt gene, encoding for a toxin with greater activity on these pests, as well as bollworm, would improve the pest-control value of Bt-transgenic cotton. Another potential advantage of having two Bt toxins expressed in cotton is that the risk of insect pests developing resistance to Bt cotton may be reduced, especially if resistance to one toxin does not confer resistance to the other (i.e., cross resistance).

Although not yet commercially available, cotton lines expressing two Bt toxins are under development. This paper reports the results of field and laboratory assays of dual-toxin Bt cotton (expressing CryIAc and CryX) compared with single-toxin Bt cotton and non-Bt cotton. CryX is a pseudonym, and the actual 'Cry' classification of this toxin is unreported. Cotton expressing both toxins is currently being referred to as Bollgard II. Our primary objective was to compare the efficacy of single- and dual-toxin Bt cultivars on various caterpillar pests found in cotton. The potential implications of our results on the management of caterpillar pests are also discussed.

Materials and Methods

In 1999, field and laboratory assays were performed to compare the relative efficacy of near-isogenic cotton cultivars expressing none, one or two insecticidal proteins of *Bacillus thuringiensis* Berliner, on various caterpillar pests. Five varieties of cotton were planted: DPL50 (non Bt), DPL50B and DPL428B (expressing CryIAc), and 15813 and 15985 (expressing both CryIAc and CryX). The cultivars 15813 and 15985 have different insertion events for the gene encoding production of CryX protein. All Bt cultivars, except DPL428B, are near-isogenic lines of DPL50. Four main plots of each cultivar were planted on 20 May in a randomized complete block at the North Farm, Mississippi Agricultural and Forestry Experiment Station, Mississippi State, MS. Cotton was planted at a density of 11.7 seeds/m

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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 cotton cultivar, such that, whenever the DPL50 plots exceeded a treatment threshold for any caterpillar pests, an insecticide application was made to all varieties. After the initial insecticide application, only insect samples in sprayed subplots were used to trigger subsequent applications. All insecticide applications were made with a high-clearance, small-plot tractor calibrated to deliver a spray volume of 68.0 l/ha. Unsprayed subplots were not treated for lepidopteran pests with insecticide.

No at-planting insecticides were used, but Orthene® (acephate, 227 g ai/ha, Valent) was applied to all plots about one week after the plants emerged. Also, several applications of malathion (222 g ai/ha) were made to all plots during the course of the season as part of the existing boll weevil, *Anthonomus grandis* Boheman, eradication efforts. Applications for the control caterpillar pests (primarily bollworm and tobacco budworm) were made to sprayed subplots on 29 July and 4, 13 and 27 August, although the primary target of the 27 August application was for soybean loopers. Tracer® (spinosad, 76 g ai/ha, Dow Agrosciences) and Baythroid® (cyfluthrin, 37 g ai/ha, Bayer) was applied on each of these dates. Based on insect control recommendations in Mississippi (Layton 1999), no other insecticide applications were needed. Plots were furrow irrigated a total of four times from late July though early September.

Field Data

In order to determine the relative efficacy of the different cultivars under field conditions, we monitored naturally occurring populations of pests using visual, sweep-net and drop-cloth samples. Samples were usually taken twice weekly. Prior to the initiation of insecticide applications for bollworm and tobacco budworm (29 July), samples were distributed randomly throughout each main plot. Samples were taken in the four sprayed rows and the four adjacent unsprayed rows subsequent to this first application. The remaining unsprayed rows were used as a source of plant material for laboratory assays (see below).

Visual samples consisted of examining the top five nodes of 15 plant terminals in sprayed and unsprayed subplots for heliothines (i.e., bollworm and tobacco budworm). We also examined 15 one-half grown or larger squares and 15 one-half grown or smaller bolls for damage and the presence of lepidopteran pests (particularly bollworm, tobacco budworm and fall armyworm). On most sampling dates, one drop-cloth sample (1.8 m of row) and 15 sweeps with a 38 cm-diameter sweep net were also made in each subplot. For drop cloth and sweep net samples, we recorded numbers of tarnished plant bug, soybean loopers and beet armyworms.

On one occasion (11 August), we made additional samples of damaged fruiting structures and heliothine caterpillars (bollworm and tobacco budworm) by visually counting their numbers in 0.9 m of row at three locations in each of the unsprayed subplots. This sample corresponded to a time when a population of heliothine larvae had caused considerable damage to the non-Bt plots during the previous 10-15 days. We also collected 20 larvae from unsprayed, non-Bt plots to verify their identity, and mandible extraction indicated all larvae were bollworms.

Intensive sampling of the cotton plots for insects was terminated in mid August, but on 26 August, we made two drop-cloth samples in each of the unsprayed subplots to document cultivar effects on a large population of soybean looper. Five days later, a single drop-cloth sample was taken in the sprayed and unsprayed parts of each plot. By this time, however, soybean looper populations had diminished considerably. Seed cotton yields were estimated by harvesting the center two rows of each cultivar, including sprayed and unsprayed subplots, on 8 October.

Data from the DPL428B cultivar was omitted because, unlike the other varieties, it is not a near-isogenic line of DPL50. Also before analysis, we grouped our cultivars by the toxins they expressed (i.e., non Bt [DPL50], CryIAc [DPL50B], and CryX [15985 and 15813]). Data were analyzed using split-plot analysis of variance procedures and using linear contrasts for mean separation (α = 0.05, Proc GLM Contrasts, SAS 1988).

Feeding Assays with Plant Tissue

Two similar methods were used to assay plant material from the DPL50, DPL50B and 15985 cultivars for activity on bollworm, fall armyworm and beet armyworm. The two assays methods differed only in that, in one method, insects were allowed to feed on plant tissue for 48 h and were then transferred to artificial diet (cite); and in the other method, new plant tissue was provided to larvae every 48 h. Larvae used in testing were from colonies maintained at the USDA-ARS Harned Lab, Mississippi State, MS. Plant materials were collected, equally in each replicate, from the unsprayed portions of our field plots. The plant parts were returned to the laboratory, rinsed with tap water and air dried before use in assays.

Second-instar larvae, previously maintained on artificial diet, were fed various plant parts (Table 1). A $\approx$ 0.5 cm layer of sterile agar was poured into Petri® dish bottoms (9 cm diameter), and autoclaved paper toweling was placed over the agar. This paper was cut to fit the bottom of the dishes and served as a wick for the moisture in the agar. The plant

material was trimmed so that it would fit into the dish and maintain contact with the toweling. The plant material and one larva were placed on top of the paper, and the dish was covered. Each larva represented a replicate. Larvae were maintained at 28 ± 2°C and a 14L: 10D photoperiod throughout the testing.

After 48 hours, mortality and the relative amounts of feeding damage (0-5 scale) to the plant parts were recorded for each individual. Surviving insects were either transferred to rearing cups containing artificial diet, or larvae were provided additional plant material at 48-h intervals until death or pupation (Table 1). Mortality was again determined 24-h later, and subsequently, at 48-h intervals until pupation. The lengths of larvae (mm) were recorded 5 and 7 days following their initial placement on plant tissue. We also recorded dates of pupation, pupal weights and dates of moth emergence. Our intent was to compare the impacts of the two Bt-cotton cultivars, relative to non-Bt cotton, on common caterpillar pests in cotton.

During the course of monitoring moth emergence, it was found that ≈50% of the moths emerging from one of our 'bollworm' assays (i.e., where terminal tissue was provided) were tobacco budworm. Thus, our initial source of bollworm eggs was heavily contaminated with tobacco budworm. Subsequent assays with bollworm did not have this problem.

Our primary interest was cultivar effects on insect survival and development. However, we were also interested in comparing the two assay methods. Statistical comparison about how different plant parts influenced insect survival and development were not done because experiments with different plant parts were not always done at the same time or with the same 'batch' of insects. Partly for the same reason, and also because different plant parts were used for testing, we did not attempt to make statistical comparisons among the species tested. Thus, cultivar and assay methods were the two factors included in analysis. Mortality data was categorical (dead or alive), so we used categorical data analysis (Proc CATMOD, SAS 1988). Other data were analyzed with standard analysis of variance techniques using linear contrast comparisons for mean separation ($\alpha = 0.05$, Proc GLM Contrasts, SAS 1988).

Results

Field Data

The only species of caterpillars, which occurred at densities high enough to evaluate cultivar effects on their populations, were the heliothines (primarily bollworm) and soybean looper. Relative to non-Bt cotton, our data showed that both single- and dual-toxin cultivars of Bt cotton caused a significant reduction in the numbers of heliothine larvae, damaged terminals, and damaged squares and bolls found during visual sampling (Table 2). In terminal samples, we also found lower numbers of heliothines in the dual-toxin cultivars (15985 and 15813) than in the single-toxin cultivar (DPL50B).

For numbers of heliothine larvae found in square and boll samples, there was a significant interaction between cultivars and whether or not insecticides were applied for caterpillar pests (Table 2). This interaction is easily explained in that about four times the larvae were found in unsprayed non-Bt subplots than in insecticide-treated subplots, whereas in the Bt cultivars, the already low numbers of larvae were not greatly affected by insecticide application.

Samples made in unsprayed subplots on 11 August indicated a significant reduction in square and boll damage in Bt cotton relative to non-Bt cotton (Table 3). The dual-toxin cultivars (15985 and 15813) had lower numbers of damaged squares and bolls than the single-toxin cultivar (DPL50B). Based on our collection of larvae and lack of other pests, this damage was clearly caused by bollworms. The number of bollworms found in the dual-toxin cultivars was statistically less than in non-Bt cotton but not less than DPL50B (Table 3).

Soybean looper populations were low in our plots most of the season (data not shown). However, a large population of larvae did occur in late August. Samples on 26 August, in unsprayed plots only, indicated that the dual-toxin Bt cultivars were highly effective in reducing looper populations compared to either the single-toxin or non-Bt cultivars (Table 3). Interestingly, there were more loopers in the single-toxin Bt cultivar (DPL50B) than in the non-Bt cotton on this date. For samples taken on 31 August, in both sprayed and unsprayed subplots, the dual-toxin Bt plots again had fewer loopers (≈ 80%) than the other plots ($F = 14.3$; $df = 2,31$; $P < 0.001$). However, the number of loopers in DPL50 (4.1± 1.0 per drop cloth) and DPL50B (4.0 ± 1.6 per drop cloth) plots were similar. Fewer larvae were in sprayed subplots (1.1 ± 0.6 larvae) than in unsprayed subplots (3.6 ± 0.8) ($F = 20.9$; $df = 1,31$; $P < 0.001$), and at this time, an interaction was detected between cultivars and subplots ($F = 3.8$; $df = 2,31$; $P < 0.05$). This interaction resulted from a decrease in looper numbers in insecticide-treated subplots of DPL50 and DPL50B, relative to unsprayed subplots that did not occur in the 15985 and 15813 cultivars. This interaction would be expected considering looper numbers were already low in unsprayed subplots of 15985 and 15813.

Cotton cultivars had a significant effect on seed cotton yields ($F = 11.3$; $df = 2, 31$; $P < 0.001$). Those expressing one or two Bt toxins yielded better than the non-transgenic cultivar, DPL50, but there was not a difference in yield between the Bt-transgenic cultivars (Fig. 1). Interestingly, seed cotton yield was reduced ≈17% in sprayed subplots relative to those not treated with insecticide (Fig. 1). Also, there was no

interaction between the cultivar and insecticide treatments (F = 0.8; df = 2, 31; P > 0.46). Thus, the apparent decrease in yield observed in plots treated with insecticide occurred across all cultivars.

Feeding Assays with Plant Tissue

Generally, our results indicate that the dual-toxin Bt cotton (15985), compared to the non-Bt and the single-toxin cultivars, reduced the survival of all the caterpillar species (Table 4). When averaged across all species and assays, 62% fewer larvae survived to the pupal stage in assays with 15985 than with DPL50. DPL50B had less of an impact than 15985, reducing survival by 40% relative to DPL50 (15985 vs. DPL50B, X^2 = 19.2, df = 1, P < 0.001). The relatively greater affect of the dual-toxin cultivar on survival was especially apparent for beet armyworms. Only 37% of the beet armyworms fed tissue from the 15985 cultivar survived to pupation, but 83% of larvae survived when fed tissue from DPL50B. Fifteen days after the assay was initiated, the survival of both bollworm and fall armyworm was statistically greater when fed DPL50B than when fed 15985.

The dual-toxin cultivar also had greater sublethal effects than did the single-toxin cultivar (Table 5). For both fall and beet armyworms, there was an obvious reduction in the size of larvae fed 15985 versus DPL50 and DPL50B, and corresponding delays in pupation were also observed. A similar but less pronounced effect was observed for bollworms fed terminal tissue. Except for fall armyworms, there was little evidence that the weight of pupae from individuals fed Bt cultivars was reduced. However, for bollworms fed blooms and beet armyworms continuously fed plant tissue, too few larvae survived to pupation to adequately evaluate cultivar effects on pupal size.

The assay method used often had a significant impact on survival and other variables measured in our experiments, and interactions between cultivar effects and assay method were commonly detected (Tables 4 and 5). Interactions were particularly common for the sublethal effects (see Table 5). Averaged across all assays, effects on survival and sublethal variables were greater when larvae were fed plant materials continuously than when transferred to diet after 48 h of exposure to plant material. For example, fewer larvae survived to the pupal stage when continuously fed plant material (40%) than when transferred to diet (60%) (X^2 = 54.0, df = 1, P < 0.001). Similarly, five days after originally infested on plant tissue, larvae were longer when transferred to diet (16.1 mm) than when kept on tissue (14.9 mm) (F = 11.31; df = 1,554; P < 0.001).

Discussion

Field Data

We saw less damage and had fewer heliothine larvae in field plots of 15985 and 15813 relative to DPL50B. Although we had only moderate populations, these data suggests that the dual-toxin Bt cultivars will give better control of bollworms than those expressing only CryIAc. The control of a large and naturally occurring populations of soybean looper by the dual-toxin cultivars was very good (> 90%). Currently-available, single-toxin Bt cottons sometimes require applications of foliar insecticides to control soybean looper populations, but it seems unlikely that dual-toxin cultivars (expressing CryX) would require treatment for this pest. It is unknown why, on 26 August, there were more loopers in plots of DPL50B than in the non-Bt cotton, DPL50. Lethal effects of DPL50B on soybean looper larvae were apparently very small or non-existent. It is possible that the development of soybean loopers on DPL50B was sufficiently delayed to cause a bottlenecking of larval populations.

In the face of these insect populations, the Bt cultivars had better yields than non-Bt cotton. However, we can not explain why all cultivars had lower yields when they were treated with insecticides. Sampling indicated that pests, other than loopers and heliothines, occurred in very low numbers throughout the season, and the subplots were treated similarly prior to 29 July. It also seems unlikely that insecticide phytotoxicity or the additional tractor traffic in sprayed plots had substantial, negative effects on yield.

Feeding Assays with Plant Tissue

Although statistical comparisons between species and plant parts were not made, some insights can be drawn from examining our assay data. It is apparent that, when fed blooms, fall armyworm larvae were not greatly affected by the CryIAc toxin present in DPL50B but were affected by the 15985 cultivar containing both CryIAc and CryX. When fed leaf tissue, both Bt cultivars caused 100% mortality of fall armyworm. Similarly, DPL50B had very little effect on beet armyworm, but 15985 had dramatic effects on beet armyworm survival and development. This suggests that the CryX toxin expressed in 15985 has greater activity on armyworms than the CryIAc toxin in DPL50B, and the expression of Bt toxins (at least CryIAc) in white blooms is less than that in the canopy leaves. However, our assays with bollworm do not support the conclusion that white blooms express lower doses of Bt toxin than green tissue. We observed more mortality when bollworms were fed white blooms than when fed terminal tissues, even when bollworms fed blooms were transferred to diet after 48 h. This is especially surprising considering that a high percentage of our test insects used in assays with terminal tissue were actually tobacco budworm, rather than bollworm.

In our laboratory assays, the distinction between DPL50B and 15985 on the development and survival of bollworm was somewhat less obvious than it was for the armyworms, especially the beet armyworm. This probably results from higher a sensitivity of bollworm, compared with armyworms, to the CryIAc toxin. Compared with armyworms, bollworm development and survival was already greatly affected by the CryIAc in DPL50B. Thus, the addition of the CryX toxin had a relatively greater impact on armyworms.

The interactions detected between cultivar effects and assay method could indicate that one of the assay methods was not as reliable in separating treatment effects. These interactions apparently resulted from the recovery of larvae after they were transferred from Bt-cotton tissue to diet (particularly 15985). Recovery did not occur when insects were continuously fed plant materials. For example, we observed ≈3% survival of beet armyworms to the pupal stage when continuously fed plant materials from cultivar 15985, but 70% survived when they were transferred to diet. In contrast, beet armyworm survival was similar on DPL50B when larvae were transferred to diet (93% survival) or continuously fed plant material (83% survival).

Despite the interactions, similar conclusions concerning cultivar effects on larval survival and development would generally be made based on the results of either assay method. However, cultivar effects were more dramatic when larvae when kept on plant tissue throughout their lives. Reductions in larval length and pupation delays were perhaps most diagnostic of the increased activity of the dual-toxin cultivar on the various caterpillar species. Besides indicating the direct effects of the Bt toxins, these sublethal variables also reflected the indirect impact of reduced feeding on toxic plant tissue.

Conclusion

It is unknown how much additional protection from insects the insertion of this second Bt toxin-producing gene will give to cotton until it is introduced and used on a large scale. However, our data suggest that dual-toxin Bt (i.e., Bollgard II) cottons will provide substantially better control of lepidopteran pests compared with the existing, single-toxin Bt cultivars. The superiority of these dual-toxin cultivars will probably be most apparent for soybean looper, beet armyworm and fall armyworm because these species are affected less than bollworm by the existing, single-toxin cultivars. However, control of bollworm populations should also be improved.

References

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Table 1. Plant parts fed to the various species of insects during laboratory assays. Number of second-instar larvae assayed, including those transferred to artificial diet (after 48-h) and those continually fed plant material, are shown.

Species tested	Plant parts	Cultivar[3]	No. larvae assayed	
			Transferred to diet	Plant material
Bollworm	Terminals[1]	DPL50	59	0
		DPL50B	55	0
		15985	57	0
	White blooms	DPL50	30	29
		DPL50B	28	30
		15985	30	28
Fall armyworm	White blooms	DPL50	30	30
		DPL50B	30	30
		15985	30	30
	Canopy leaves[2]	DPL50	28	28
		DPL50B	29	29
		15985	29	30
Beet armyworm	Canopy leaves[2]	DPL50	29	28
		DPL50B	30	27
		15985	28	30

[1] Over 50% of moths emerging from this assay were actually tobacco budworm. Terminals consisted of the top 2-3 nodes of plants, including flower buds, collected about one week before flowering began.

[2] Canopy leaves were fully-expanded leaves 4-6 nodes below the uppermost node.

[3] DPL50 (non-Bt transgenic), DPL50B (Bt transgenic for CryIAc), 15985 (Bt transgenic for CryIAc and CryX).

Table 2. Numbers of heliothines (bollworm and tobacco budworm) and heliothine damage. Seasonal means (± SE) are shown by cultivar (non-Bt [DP50], single-toxin Bt [DP50B], and dual-toxin Bt [15985 and 15813]) and for subplots that were sprayed or not sprayed with insecticides to control caterpillar pests.

Treatment factor	Larvae per 15 terminals	No. damaged per 15 terminals
Cultivars		
DPL50	0.46 (0.10) a	1.89 (0.30) a
DPL50B	0.09 (0.04) b	0.17 (0.05) b
15985/15813	0.02 (0.01) c	0.22 (0.05) b
Subplots (Insecticide)		
Unsprayed	0.14 (0.03) a	0.64 (0.10) a
Sprayed	0.16 (0.07) a	0.60 (0.19) a
	Larvae per 30 squares and bolls[1]	**No. damaged per 30 squares and bolls**
Cultivars		
DPL50	0.58 (0.17) a	1.75 (0.41) a
DPL50B	0.04 (0.03) b	0.33 (0.12) b
15985/15813	0.04 (0.02) b	0.27 (0.10) b
Subplots (Insecticide)		
Unsprayed	0.26 (0.09) a	0.80 (0.20) a
Sprayed	0.09 (0.04) b	0.51 (0.15) a

Means not followed by a common letter are significantly different ($P < 0.05$, Proc GLM Contrasts, SAS 1988).

[1] A significant interaction ($F = 6.1$; $df = 2, 191$; $P < 0.01$) between cultivar and insecticide treatment was observed for this variable.

Table 3. Mean numbers (± SE) of damaged squares and bolls, bollworms, and soybean loopers per 2.7 m of row in unsprayed plots of non-Bt (DP50), single-toxin Bt (DP50B), and dual-toxin Bt (15985 and 15813) cotton.

Cultivar	No. damaged squares and bolls[1]	No. bollworms[1]	No. soybean loopers[2]
DPL50	10.0 (3.94) a	2.25 (1.31) a	19.3 (4.4) b
DPL50B	2.25 (0.85) b	0.50 (0.50) ab	36.2 (5.9) a
15985/15813	0.13 (0.13) c	0.00 (0.00) b	1.3 (0.5) c

Means not followed by a common letter are significantly different ($P < 0.05$, Proc GLM Contrasts, 1988).

[1]Data were collected on 11 August by visual inspection of plants.

[2]Data were collected on 26 August using a drop cloth.

Table 4. Survival of bollworm, fall armyworm and beet armyworm fed plant tissue from one of three cotton cultivars. Data are shown for second-instar larvae infested on selected plant parts. The relative amount of feeding on plant tissue (0-5 scale) 48 h after plant parts were infested is also shown.

Species tested[1]	Plant part	Cultivar	Relative feeding	Proportion larvae surviving to pupa
CEW (TBW)[2]	Terminals	DPL50	2.98 a	0.85 a
		DPL50B	1.45 b	0.52 b
		15985	1.42 b	0.47 b
CEW	Blooms	DPL50	1.72 a	0.60 a [m]
		DPL50B	0.47 b	0.07 b
		15985	0.40 b	0.00 b
FAW	Blooms	DPL50	2.25 a	0.73 a
		DPL50B	1.82 b	0.90 b
		15985	1.35 c	0.67 a
FAW	Leaves	DPL50	1.72 a	0.75 a
		DPL50B	0.17 b	0.00 b
		15985	0.08 b	0.00 b
BAW	Leaves	DPL50	1.25 a	0.88 a [m,i]
		DPL50B	1.02 b	0.83 a
		15985	0.82 c	0.37 b
			Proportion larvae surviving	
			3 days	**7 days**	**15 days**
CEW (TBW)[2]	Terminals	DPL50	0.93 a	0.90 a	0.86 a
		DPL50B	0.77 b	0.62 b	0.60 b
		15985	0.67 b	0.58 b	0.58 b
CEW	Blooms	DPL50	0.85 a	0.82 a	0.67 a [m]
		DPL50B	0.26 b	0.19 b	0.16 b
		15985	0.10 c	0.07 b	0.02 c
FAW	Blooms	DPL50	1.00 a	0.90 a [i]	0.86 ab [i]
		DPL50B	1.00 a	0.95 a	0.95 a
		15985	0.90 b	0.86 a	0.83 b
FAW	Leaves	DPL50	0.89 a	0.82 a	0.75 a
		DPL50B	0.00 b	0.00 b	0.00 b
		15985	0.00 b	0.00 b	0.00 b
BAW	Leaves	DPL50	1.00 a	1.00 a	0.93 a
		DPL50B	0.98 a	0.96 a	0.89 a
		15985	0.86 b	0.81 b	0.65 b

Means within a column not followed by a common letter are significantly different ($P < 0.05$, Proc Catmod Contrasts). Superscripted letters indicate significant effects of assay method (m) or an interaction (i) between cultivar and the assay method.

[1] Corn Earworm (CEW), Tobacco Budworm (TBW), Fall Armyworm (FAW), Beet Armyworm (BAW).

[2] Over 50% of moths emerging from this assay were actually tobacco budworm.

Table 5. Larval lengths (5 and 7 days after infestion), delays in pupation (relative to DPL50), and pupal weights of bollworm, fall armyworm and beet armyworm fed plant tissue from one of three cotton cultivars. Data are for second-instar larvae infested on selected plant parts.

Species tested[1]	Plant part	Cultivar	Larval length (mm) 5 Days	Larval length (mm) 7 Days
CEW (TBW)[2]	Terminal	DPL50	16.5 a	20.8 a
		DPL50B	10.0 b	15.0 b
		15985	8.1 c	13.4 b
CEW	Blooms	DPL50	15.6 a [m]	24.1 a [m]
		DPL50B	6.3 b	16.5 b
		15985	7.0 b	17.8 b
FAW	Blooms[2]	DPL50	19.3 a [m,i]	21.0 a [i]
		DPL50B	18.8 a	19.8 a
		15985	14.2 b	17.0 b
BAW	Leaves	DPL50	19.1 a [m,i]	21.1 a [m,i]
		DPL50B	17.7 b	19.7 a
		15985	11.1 c	14.4 b
			Pupation delay (d)	**Pupal wt. (g)**
CEW (TBW)[2]	Terminal	DPL50	0.00 a	0.31 a
		DPL50B	2.06 b	0.33 a
		15985	2.96 c	0.31 a
CEW	Blooms	DPL50	0.00 a	0.30 a
		DPL50B	8.60 a	0.29 a
		15985	-----	-----
FAW	Blooms[3]	DPL50	0.00 a [m,i]	0.20 ab [m,i]
		DPL50B	-0.76 a	0.22 a
		15985	1.50 b	0.19 b
BAW	Leaves	DPL50	0.00 a [m,i]	0.13 a
		DPL50B	0.46 a	0.11 a
		15985	1.29 b	0.14 a

Means within a column not followed by a common letter are significantly different ($P < 0.05$, Proc GLM Contrasts, SAS 1988). Superscripted letters indicate significant effects of assay method (m) or an interaction (i) between cultivar and assay method.

[1] Corn Earworm (CEW), Tobacco Budworm (TBW), Fall Armyworm (FAW), Beet Armyworm (BAW).

[2] Over 50% of moths emerging from this assay were actually tobacco budworm.

[3] Leaf tissue was also tested, but all fall armyworm larvae fed leaves of Bt cultivars were dead 48 h after the assay began. Thus, data is not shown.

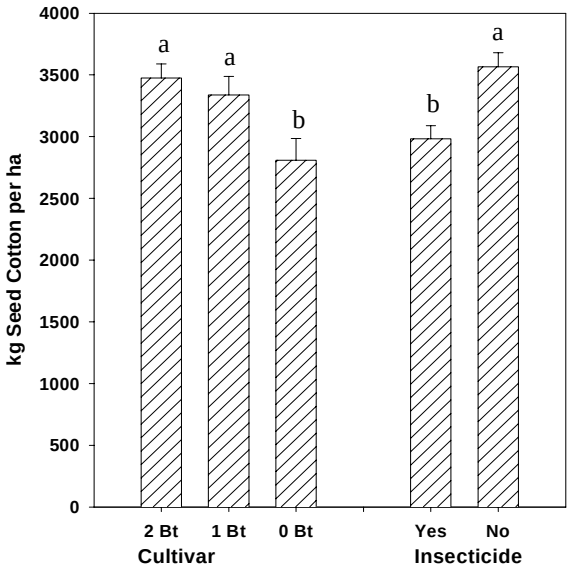

Figure 1. Mean seed cotton yields of near-isogenic lines of DPL50 expressing two, one and no Bt toxins. Yields, averaged across all cultivars, are also shown for subplots that were either treated or not treated with insecticides to control caterpillar pests.