PROFILING SEASON-LONG EXPRESSION OF Cry1Ac IN 13 COMMERCIAL VARIETIES OF BOLLGARD® COTTON: INFLUENCE OF PARENTAL BACKGROUND AND ENVIRONMENTAL FACTORS ON ENDOTOXIN EXPRESSION LEVELS

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Abstract

Transgenic cotton varieties widely differ in the amount of expressed Bt throughout the growing season. These data suggest that the conventional parental background, for which the *cry1Ac* gene is inserted, is a factor contributing to expression differences. Although other environmental factors (e.g. irrigation and plant stress) could influence expression, soil composition did not contribute to Bt expression differences among varieties. These expression data will hopefully contribute to a variety-development program that incorporates high-yielding varieties with the best transgenic insect control.

Introduction

Since the first transgenic Cry1Ac *Bacillus thuringiensis* Berliner (Bt) cotton variety was commercialized in 1996, there have been numerous advancements for insect control with transgenic technology. Where once a single variety contained a single insecticidal gene, growers can now choose from over 25 transgenic varieties. These varieties can contain the c*ry1Ac* Bt gene (Bollgard®, Monsanto Ag. Co., St. Louis, MO), herbicide-resistance genes (Roundup Ready®, Monsanto Ag. Co., St. Louis, MO or BXN®, Aventis, Lyon, France), and Bt varieties stacked or pyramided with a herbicide-resistance gene. Because the number of transgenic cotton varieties being developed annually is increasing, evaluation of varieties that best suit specific geographical regions and growing conditions is becoming increasingly difficult. The advent of commercialized Cry1Ac δ-endotoxin quantification systems will allow more routine evaluations of different Bt varieties. As with any foliar insecticide and herbicide, the efficacy of each variety must be determined to ensure the best recommendation to growers.

Studies have shown that differences in larval survival of bollworms, *Helicoverpa zea* (Boddie) and larval development of fall armyworms, *Spodoptera frugiperda* (J. E. Smith) can be correlated to the differential expression of Cry1Ac in various plant parts among commercial varieties of transgenic Bt cotton (Adamczyk et al. 2000). However, factors that influence the level of expressed Bt among varieties are still not fully understood (Fitt 1998).

The purpose of this research was to profile season-long Bt expression to determine what factors are responsible for differential Bt expression among US commercial varieties.

Materials and Methods

Field Plots

Thirteen transgenic varieties containing Cry1Ac were planted in experimental plots on 17 May 2000 near Elizabeth, MS (Table 1). Plots consisted of 4 rows (1.0 m centers) x 30.5 m treatments arranged in a randomized block design. Two fields that differed by soil composition (silty-loam versus clay) were utilized. Varieties were replicated three times in each field. All plots were non-irrigated.

Bt Quantification

For each sample date, a single terminal leaf or cotyledon was randomly harvested from 10 plants/plot (3 replications/field) for all varieties. 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 Cry1Ac extraction buffer (EnviroLogic, Inc.; Portland, ME). The tissue was then homogenized using a mini-beadbeater-8™ (Biospec Products) using 6.4 mm steel ball bearings. To quantify the amount of Bt present for each variety, 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.

Season-Long Expression Differences: Influence of Parental Background

Bt levels in the cotyledon stage (26 May) were determined for all 13 varieties planted in the silty-loam field. In addition, the amount of Bt present in terminal leaves (13 sample dates: 31 May – 25 August 2000) from the same Bt varieties were quantified. Thus, the season-long differences in Bt levels present in terminal leaves among the 13 varieties could be correlated to Bt levels observed in the cotyledon stage (PROC CORR, SAS Institute 1985). Furthermore, by grouping varieties, the influence of conventional parental background trait (from Delta & Pine Land Co. and Stoneville Pedigree Seed Co.) on differential expression was examined. Differences in Bt levels (ppm) among varieties were analyzed using PROC GLM and varietal expression slopes were analyzed using PROC REG (SAS Institute 1985).

Influence of Soil Composition on Expression among Varieties

The amounts of δ-endotoxin present in terminal leaves from 8 Bt varieties were quantified throughout the growing season (2 June – 11 August 2000) for two fields that differed by soil composition as described above. Thus, the influence of soil composition on varietal expression differences could be examined. Means were analyzed using REML – ANOVA, and the means were separated using the LSMEANS option of PROC MIXED (Littell et al. 1996).

Results and Discussion

It appears that transgenic cotton varieties widely differ in the amount of expressed Bt throughout the growing season. Two varieties (NuCOTN 33B and DP458B/RR) sharing the same parental background (DP5415), expressed Bt at significantly higher levels ($P < 0.05$) compared to other Bt varieties (Figure 1, Table 2). In addition, Bt levels decreased throughout the growing season (see Date Effects, Table 2) while the slopes among varietal expression lines were similar (Figure 2, Table 3).

The amount of Bt was significantly correlated ($P < 0.05$) from the cotyledon stage to samples collected throughout the season for terminal leaves indicating that varietal differences may be under genetic control (Figure 3, Table 4). Furthermore, it appears that parental background plays an important role in expression differences among varieties (Figure 4).

These data suggest that soil composition was not a significant factor in differential expression of Bt among varieties. Overall, there were no significant differences ($P > 0.05$) in the amount of Bt expressed in varieties planted in silty-loam versus clay soil (Figures 5 and 6, Table 5).

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1420-1423 (2001)
National Cotton Council, Memphis TN

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Acknowledgment

The dedicated efforts of Ms. Katrina Bew and numerous student aids are much appreciated. The author would like to thank Drs. D. V. Sumerford and W. R. Meredith for their review of these data.

Disclaimer

Mention of a commercial or propriety product does not constitute an endorsement by the U.S. Department of Agriculture for its use.

Table 1. Commercially available transgenic cotton varieties examined in 2000.

Cry1Ac [a]	Parental Background	Cry1Ac [a] + Herbicide-Resistance Trait [b]	Parental Background
DP 20B [c]	DP 20 [c]	DP 409B/RR [c]	DP 5409 [c]
DP 50B [c]	DP 50 [c]	DP 422B/RR [c]	DP 20 [c]
NuCOTN 33B [c]	DP 5415 [c]	DP 451B/RR [c]	DP 51 [c]
DP 428B [c]	DP 51 [c]	DP 458B/RR [c]	DP 5415 [c]
ST 4691B [d]	ST 474 [d]	SG 125B/RR [e]	SG 125 [e]
		PM 1218B/RR [f]	PM 1220 [f]
		ST 4892B/RR [d]	ST 474 [d]
		PM 2280B/RR [f]	PM 2200 [f]

[a] Bollgard®; Monsanto Co., St. Louis, MO).
[b] Roundup Ready®; Monsanto Co., St. Louis, MO).
[c] Delta & Pineland® variety (Delta & Pineland Co., Scott, MS).
[d] Stoneville Pedigree Seed variety (Memphis, TN).
[e] Sure-Grow® variety (Delta & Pineland Co., Scott, MS).
[f] Paymaster® variety (Delta & Pineland Co., Scott, MS).

Table 2. Interaction of variety planted and date of sampling on expression of Bt in transgenic varieties for Figure 1.

Effect	Num df	Den df	SS	F-Value	P > F
Rep	2	24.9	1.255	3.96	0.032
Variety	12	24	21.792	64.50	<.001
Date	12	24	33.706	18.39	<.001
Date*Variety	144	288	8.290	2.56	<.001
NuCOTN 33B & DP 458B/RR vs. Other 11 Varieties	1	24	20.104*	714.07	<.001

* 92.3% Varietal SS Due to Differences between NuCOTN 33B and DP 458B/RR vs. Other 11 Varieties (PROC GLM, SAS Institute 1985).

Table 3. Slopes for varietal expression lines in Figure 1.

Variety	Slope*	Low CL	High CL	t-value	P > t
DP 20B	-0.0089	-0.0125	-0.0053	-5.396	<0.001
DP 50B	-0.0082	-0.0120	-0.0043	-4.634	<0.001
DP 33B	-0.0119	-0.0175	-0.0063	-4.687	<0.001
DP 428B	-0.0110	-0.0137	-0.0084	-9.220	<0.001
DP 409B/RR	-0.0065	-0.0093	-0.0038	-5.185	<0.001
DP 422 B/RR	-0.0074	-0.0094	-0.0054	-8.112	0.005
DP 451B/RR	-0.0075	-0.0124	-0.0026	-3.400	0.003
DP 458B/RR	-0.0102	-0.0161	-0.0043	-3.788	<0.001
SG 125B/RR	-0.0100	-0.0133	-0.0067	-6.710	<0.001
PM 1218B/RR	-0.0110	-0.0138	-0.0082	-8.583	<0.001
ST 4691B	-0.0098	-0.0131	-0.0066	-6.721	<0.001
ST 4892B/RR	-0.0084	-0.0118	-0.0051	-5.539	<0.001
PM 2280B/RR	-0.0098	-0.0141	-0.0055	-5.009	<0.001

*All slopes are considered not significantly different from one another since the 95% confidence limits (CL) overlapped.

Table 4. Correlating Bt levels in cotyledons to Bt levels in terminal leaves among 13 varieties for 13 sample dates.

Julian Date	r-Coefficient	p-Value
152	0.6301	0.0210
161	0.5717	0.0412
166	0.5387	0.0575
172	0.7868	0.0014
180	0.7394	0.0039
187	0.8592	0.0002
194	0.7674	0.0022
200	0.7653	0.0023
206	0.3655	0.2194
213	0.7387	0.0039
222	0.8086	0.0008
228	0.7454	0.0034
238	0.7973	0.0011
Mean for 13 Sample Dates	**0.7878**	**0.0016**

Table 5. Interaction of variety planted and date of sampling, while accounting for soil composition, on expression of Bt in transgenic varieties for Figures 5 & 6.

Effect	Num df	Den df	F-Value	P > F
Rep (Soil)	4	24.5	0.93	0.464
Variety	7	14	77.19	<0.001
Date	6	24	3.86	0.008
Soil	1	23.2	1.69	0.207
Date*Soil	6	24	2.23	0.075
Date*Variety	42	168	2.39	<0.001
Variety*Soil	7	14	0.79	0.609
Date*Variety*Soil	42	168	0.66	0.944

PROC MIXED, Littell et al. 1996.

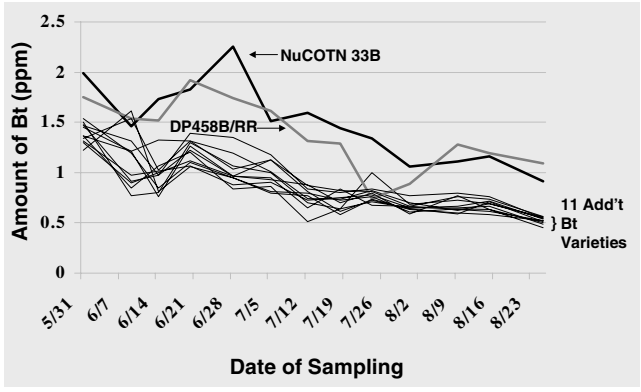

Figure 1. Expression of Bt in terminal leaves throughout the growing season for 13 transgenic varieties (see Table 1).

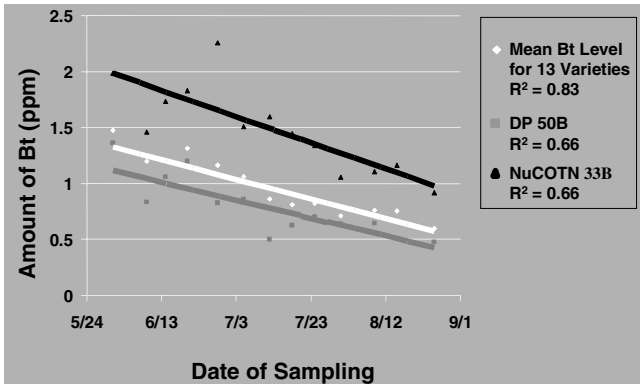

Figure 2. Mean level of expressed Bt for 13 transgenic varieties: comparing a high expressing versus a low expressing variety.

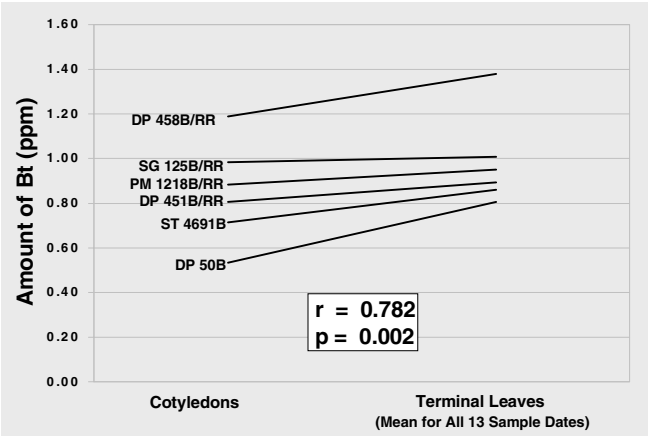

Figure 3. Correlating expression levels of Bt in cotyledons to expression levels of Bt in terminal leaves among 13 varieties. All varieties were included in analysis, 6 chosen for illustration.

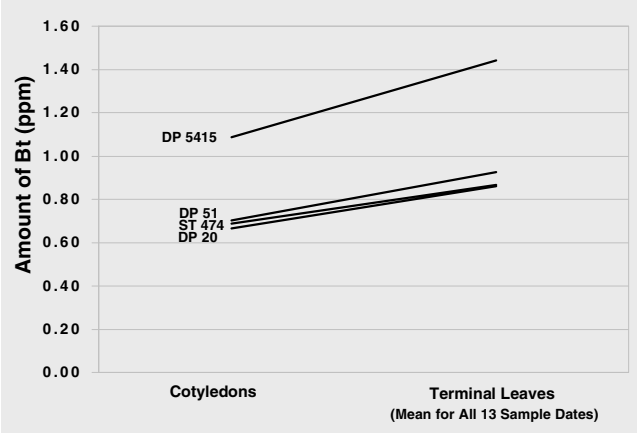

Figure 4. Influence of parental background on expression of Bt in 13 commercial varieties. The expression levels in cotyledons and from the mean of 13 terminal leaf samples was compared.

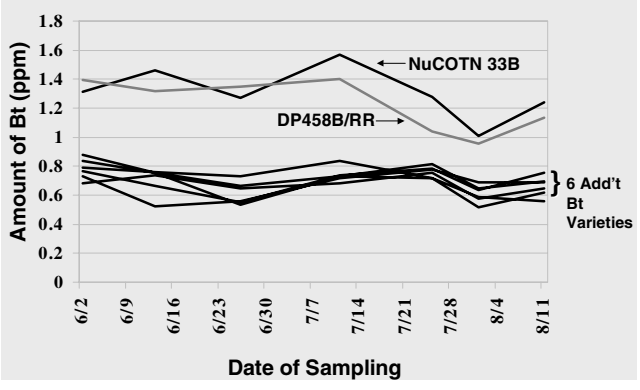

Figure 5. Expression of Bt in terminal leaves for 8 transgenic varieties planted in silty-loam soil. All varieties examined were "DP" (see Table 1).

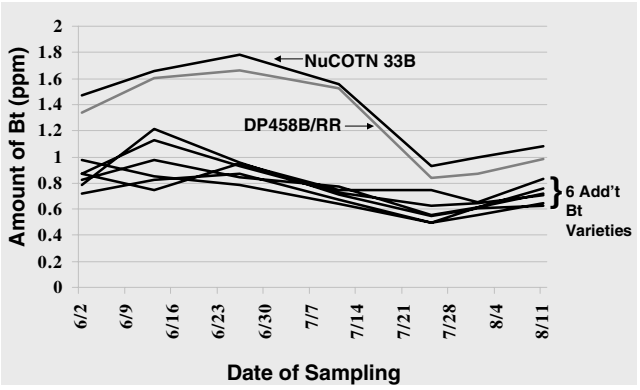

Figure 6. Expression of Bt in terminal leaves for 8 transgenic varieties planted in clay soil. All varieties examined were "DP" (see Table 1).