SEASONAL Cry1Ac LEVELS IN DP50B: THE "BOLLGARD® BASIS" FOR BOLLGARD II

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Abstract

A 12 field site study was performed in which DP50B (Bollgard®)cotton tissue samples were collected throughout the growing season and evaluated for Cry1Ac concentrations utilizing a sensitive quantitative bioassay. Annual seasonal means for terminal foliage, pre-candle squares, and young bolls were 22, 14, and 17 μg/g dry weight, respectively. A main-effect ANOVA determined that field site, sampling time, and plant tissue type were all significant sources of variability among Cry1Ac levels in DP50B. Mean Cry1Ac concentrations within specific tissues, although variable from sampling time to sampling time, showed no specific trend over time to either increase or decrease; this suggests that tissues of similar physiological age may express Cry1Ac at levels around a tissue-specific mean throughout the fruiting cycle (the approximate sampling period of this study). Further, this tissue-specific mean may be influenced by environmental conditions at specific sites.

Introduction

In the development of the second generation of Bollgard® products, a second insect control gene encoding another Bt protein, qualitatively different from Cry1Ac (called CryX by Monsanto), was used to transform tissue from the current Bollgard® variety DP50B (Delta & Pineland). Cloned plants regenerated from the transformed tissue expressed both the Cry1Ac protein and the CryX protein. These genes also segregated independently. The proposed name for the 2-gene product is Bollgard II; it has not yet received EPA registration. In an effort to fully understand the insect control significance of the addition of the second gene, a study was conducted to first evaluate the expression of Cry1Ac in the transformation "parent" (DP50B). This would set the "baseline" from which the addition of the second protein could be measured.

Materials and Methods

Cotton tissue samples from 12 field sites were collected and shipped to Monsanto laboratories where they were processed and evaluated in a sensitive quantitative bioassay which utilized purified Cry1Ac as a standard and took advantage of the extreme sensitivity of *Heliothis virescens* to the Cry1Ac protein (Greenplate, 1999). Tissue sample effects on *H. virescens* larval development were compared with effects of known concentrations of Cry1Ac and Cry1Ac levels in cotton tissues were thereby estimated. Within each site, several plants within 3 replicate plots were sampled at 2 week intervals beginning at 2 weeks post-pinhead square stage and ending at 8 weeks post-pinhead square. The specific tissues sampled were main terminal foliage, pre-candle squares (1st position square 2-3 nodes below main terminal), and young bolls (1st position boll 2-3 nodes below top white bloom). The JMP® (version 3.1) statistical software (SAS Institute, Cary NC) was used to perform the statistical evaluations. A main effect ANOVA was used to test the influence of field site, sampling time, tissue type, and replicate plot (within site) on variability among mean Cry1Ac levels. Subsequent mean comparisons were made using Tukey-Kramer HSD (Kramer, 1956).

Results

The main effect ANOVA determined field site, sampling time, and tissue type all were significant sources of variability among Cry1Ac means (Table 1). There was no significant plot effect. Table 2 shows overall means for each field site. Means range from 7.38 μ/g dry weight in East Texas to 31.49 μ/g dry weight in Louisiana. Overall means for Cry1Ac at different sampling times (Table 3) show that Cry1Ac means rise slightly from 2 to 4 weeks post pinhead square (17.34 to 22.08 μ/g dry weight) then drop to around 15.5 μ/g dry weight for 6 and 8 weeks post pinhead square. Overall seasonal means for Cry1Ac in terminals, squares, and bolls were 22.27, 14.09, and 17.04 μ/g dry weight, respectively (Table 4). When individual tissue types were evaluated separately over time, they reflected the trends of overall means to rise at 4 weeks and level off at 6 to 8 weeks.

Discussion

In an effort to determine a Bollgard® baseline from which to evaluate Bollgard II in the future, we successfully measured Cry1Ac levels in DP50B over time, across sites, and among different tissue types. As seen before with Bollgard® (Greenplate, 1999), environmental site, sampling time, and tissue type were all found to contribute significantly to the variability among Cry1Ac levels in DP50B. Terminal tissues were found to have higher levels of Cry1Ac than did reproductive tissues; this has also been reported previously for Monsanto's original experimental Bollgard® variety Coker 312/531 (Greenplate, 1999). Mean Cry1Ac concentrations within specific tissues, although variable from sampling time to sampling time, showed no specific trend over time to either increase or decrease (Figure 1). Greenplate (1999) in his study of Coker 312/531 found that Cry1Ac levels in a specific fruiting position dropped steadily as the plant aged, suggesting that as a specific fruit matures,

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Cry1Ac levels may be reduced. The present report suggests that similar tissues of similar physiological age may express Cry1Ac at levels around a tissue/age-specific mean throughout the fruiting cycle (the approximate sampling period of this study). Further, this tissue/age-specific mean may be influenced by environmental conditions at specific sites.

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References

Greenplate, J. T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard® cotton fruit and terminals. J. Econ. Entomol. 92: 1377-1383.

Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics 12: 309-310.

Table 1. ANOVA main effects table.

Source	DF	Sum of Squares	F Ratio	Prob > F
Site	11	5665.8730	2.2850	0.0107
Time	3	2812.9548	4.1596	0.0066
Tissue Type	2	4490.2227	9.9598	<0.0001
Plot [Site]	23	1891.3466	0.3648	0.9973

Table 2. Overall Cry1Ac means (in µ/g dry weight) at each site. Means with different letters are statistically different at P = 0.05 as measured by Tukey-Kramer HSD.

Site	Cry1Ac	SEM			
E.TX	7.38	1.28	a		
N.AL	11.24	1.14	a	b	
C.AL	11.88	1.48	a	b	
W.TX	13.38	1.63	a	b	
NC	15.16	2.12	a	b	
SC	15.76	1.73	a	b	
AZ	17.03	1.33	a	b	
GA	20.11	1.34		b	c
MS	22.80	3.67		b	c
OK	23.51	3.11		b	c
S.AL	25.00	5.04		b	c
LA	31.49	5.56			c

Table 3. Overall Cry1Ac means (in µ/g dry weight) at each sampling time (Weeks post pinhead square). Means with different letters are statistically different at P = 0.05 as measured by Tukey-Kramer HSD.

Weeks	Cry1Ac	SEM	
2	17.34	1.56	ab
4	22.08	2.19	b
6	15.36	1.16	a
8	15.64	1.39	a

Table 4. Overall Cry1Ac means (in µ/g dry weight) for each tissue type. Means with different letters are statistically different at P = 0.05 as measured by Tukey-Kramer HSD.

Tissue	Cry1Ac	SEM	
Terminal	22.27	1.87	a
Square	14.09	1.05	b
Boll	17.04	1.25	b

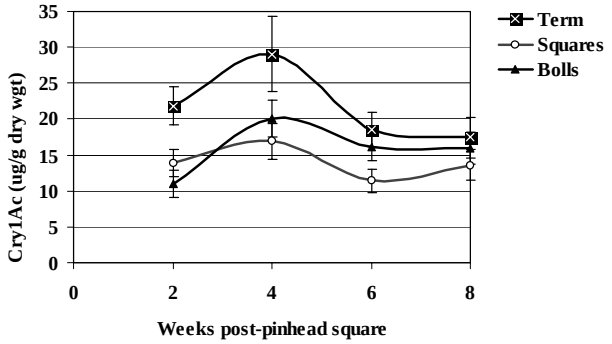

Figure 1. Time course of mean Cry1Ac levels for individual tissue types of Bollgard variety DP50B.