QUANTIFICATION OF LEPIDOPTERAN ACTIVITY IN A 2-GENE PRODUCT: A 2-YEAR SUMMARY OF BOLLGARD II®

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Abstract

A 4 field site study was conducted in which Bollgard® II (containing 2 lepidopteran active Bt proteins: Cry1Ac and Cry2Ab) and Bollgard® (DP50B containing only Cry1Ac) cotton tissue samples were collected throughout the growing season and evaluated for total lepidopteran activity using a *Heliothis virescens* quantitative bioassay (Greenplate 1999). In addition, protein-specific ELISA assays were performed upon the same tissue samples to determine relative levels of both insect control proteins. Total lepidopteran activity expressed in Cry1Ac equivalents was much higher in Bollgard® II tissue samples. Overall mean lepidopteran activity levels were 3.5 as great for Bollgard® II as for Bollgard®. This relative increase in mean lepidopteran activity levels was seen at every sampling time (from 2 weeks post-pinhead square to 8 weeks post-pinhead square) and at all field sites. ELISA assays were used to estimate the levels of each toxin. The amount of Cry1Ac protein in Bollgard® II was the same as in Bollgard®. A main-effect ANOVA determined that, in addition to the Bollgard® II-Bollgard® difference, sampling time and tissue type were significant contributors to the variability among lepidopteran activity levels; although the tissue type variability can be explained by the difference between younger tissue (terminal leaf, square) and older tissue (large leaf). There was no significant difference in lepidopteran activity between terminals and squares of Bollgard® II. These data suggest that the greatest effect of the expression of Cry2Ab in Bollgard® II is likely to be greatly increased and prolonged lepidopteran activity in all tissue types, especially reproductive tissue. A summary of the overall mean lepidopteran activity for 1998 and 1999 is shown.

Introduction

In an effort to develop the next generation of Bollgard® cotton, plants containing an additional insect protection gene encoding the lepidopteran active protein Cry2Ab have been produced. The Bollgard® cotton variety DP50B (Delta and Pine Land) expressing Cry1Ac was transformed with the *cry*2Ab gene. The two genes have been shown to segregate independently. The proposed name of the product containing both genes is Bollgard® II; it has not yet received EPA registration. Studies have been conducted to establish the baseline expression of Cry1Ac in the transformation parent DP50B and to evaluate the activity and expression of plants containing both genes. The following study represents a second year of data on the quantitative evaluation of lepipopteran activity of specific Bollgard® II tissues over time and in comparison with its Bollgard® parent variety (DP50B). A summary of the overall activity seen in 1998 and 1999 is shown.

Materials and Methods

Cotton plant tissue samples from 4 field sites were collected and shipped to the Monsanto Chesterfield laboratories where they were processed and evaluated in a tobacco budworm, *Heliothis virescens* quantitative bioassay (Greenplate, 1999). This very sensitive bioassay utilized a purified Cry1Ac standard in a dilution series for comparison with the individual dilutions of lyophilized plant tissue. Developmental arrest caused by the plant samples was compared with that caused by the known concentrations of Cry1Ac to give an estimate of the activity in Cry1Ac equivalents. At each site plant samples were collected at 2 week intervals starting at 2 weeks post-pinhead square and ending at 8 weeks post-pinhead square. Tissues sampled were the main terminal leaf, pre-candle square and the largest leaf associated with that square. Statistical analysis was performed on the lepidopteran bioactivity data using JMP® (version 3.2.6) statistical software (SAS Institute, Cary NC). A main effect ANOVA was used to test the influence of field site, sampling time, tissue type and variety on variability among mean lepidopteran activity levels. In addition, all the tissue samples were evaluated in Cry1Ac- and Cry2Ab-specific quantitative ELISA tests

Results

A main-effect ANOVA determined that variety (Bollgard® vs Bollgard® II), sampling time and tissue type were significant contributors to the variability among lepidopteran activity levels (Table 1.); although the tissue type variability can be explained by the difference between younger tissue (terminal leaf, square) and older tissue (large leaf) (Figure 5.). There was no significant difference in lepidopteran activity between terminals and squares of Bollgard® II and the lower activity of large leaf tissue was still 2-3 times higher than the activity of any tissue type from Bollgard plants. The overall mean lepidopteran activity of Bollgard® II was 3.5 times greater than the overall mean lepidopteran activity of Bollgard® (DP50B)(Figure 1.). Figure 2. illustrates a similar relationship between overall activity of Bollgard and Bollgard® II during 1998. As shown in Figure 4. the activity levels of Bollgard® II declined as the season progressed but these levels were still 2-3.5 times greater than the late season activity levels of Bollgard®. There was no difference in the levels of activity of Bollgard II at each individual field site (Figure 5).

ELISA evaluations confirmed the presence of both proteins in Bollgard II plant tissues. Figure 6. shows the relative expression levels Cry1Ac in Bollgard® and Bollgard® II and Cry2Ab in Bollgard® II. Cry1Ac expression in Bollgard® II by site, time and tissue type are all similar to Bollgard® Cry1Ac expression (Figures 7, 8 and 9). The level of Cry2Ab expression measured in the ELISA is > 10 times the level of Cry1Ac expression seen in Bollgard® II plants. This relationship is consistent and is seen for all sites, sampling times, and tissue types.

Discussion

The addition of the second gene into Bollgard® II cotton provides overall increased lepidopteran activity levels over Bollgard® on the order 3.5 times in 1999 and 4 times in 1998. Data from both years shows that these increased activity levels can be seen at all sites, sampling times and in all tissue types. The variability associated with differences in tissue type during 1999 found by the ANOVA can be explained by the lower activity of large leaves compared to terminal or square activity. While the activity in older tissue is lower it remains higher than activity in any Bollgard® tissue.

The protein-specific ELISA quantification confirms the expression of both proteins in Bollgard® II. The expression of Cry2Ab in Bollgard® II does not appear to compromise expression of the original Bollgard® protein (Cry1Ac). The relatively higher level of expression of Cry2Ab compensates for its lower unit activity against the target pests.

This data suggests that co-expression of the two insect control proteins is likely to result in increased and prolonged lepidopteran activity in all tissue types, especially in reproductive tissues.

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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	Nparm	DF	Sum of Squares	F Ratio	Prob>F
site	3	3	2550.502	1.9912	0.1213
sampling time	3	3	17321.002	13.5228	<.0001
variety	1	1	70200.708	164.4203	<.0001
tissue type	2	2	9109.584	10.668	<.0001

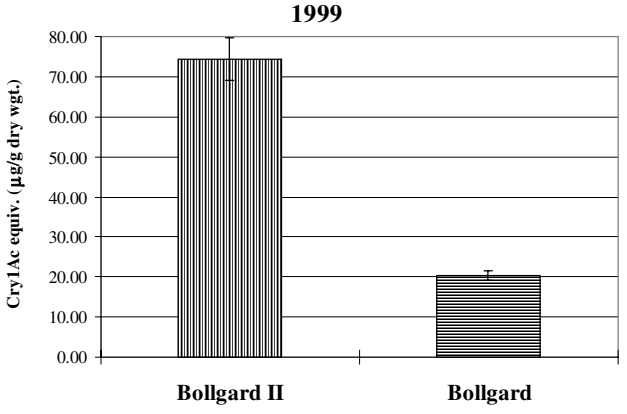

Figure 1. Overall mean lepidopteran activity levels in μg Cry1Ac equivalents/g dry weight for Bollgard and Bollgard II during 1999.

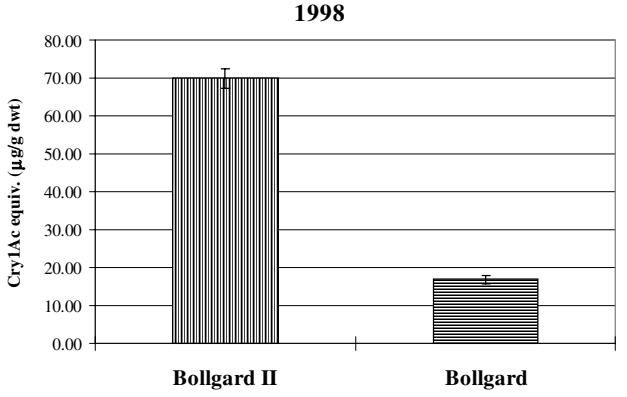

Figure 2. Overall mean lepidopteran activity levels in ug Cry1Ac equivalents/g dry weight for Bollgard and Bollgard II during 1998.

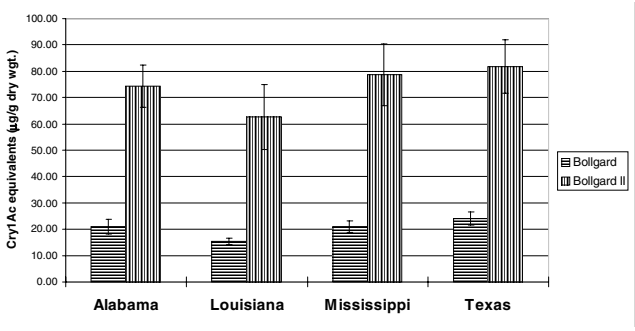

Figure 3. Mean lepidopteran activity levels of Bollgard and Bollgard II in ug Cry1Ac equivalents/g dry weight for each site during 1999.

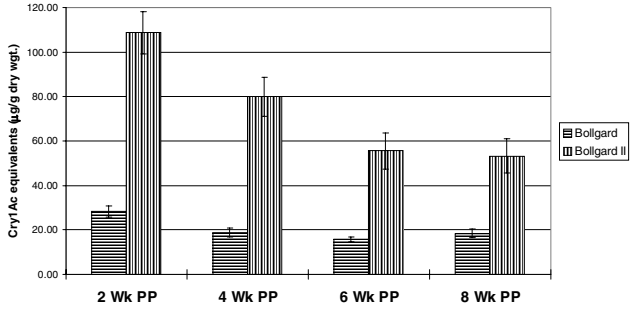

Figure 4. Mean lepidopteran activity levels of Bollgard and Bollgard II in ug Cry1Ac equivalents/g dry weight by sampling time during 1999.

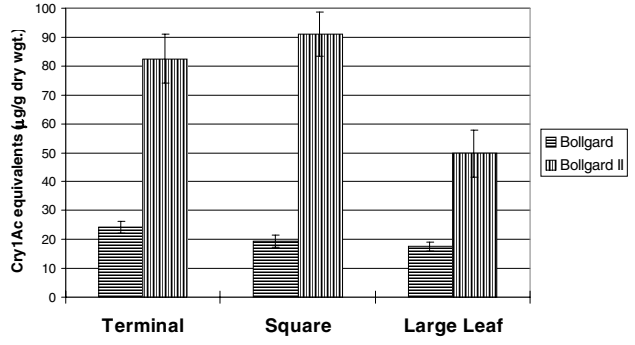

Figure 5. Mean lepidopteran activity levels for Bollgard and Bollgard II in ug Cry1Ac equivalents/g dry weight for each tissue type sampled during 1999.

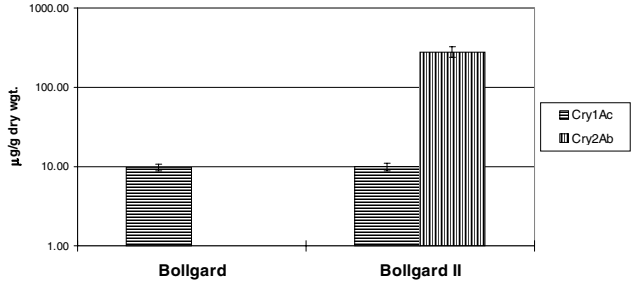

Figure 6. Overall mean concentrations of Cry1Ac in Bollgard and Bollgard II and Cry2Ab in Bollgard II as measured by ELISA.

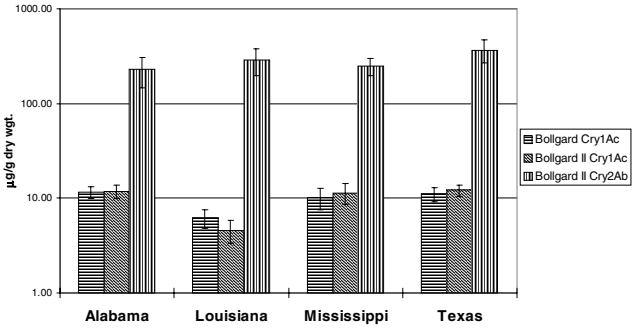

Figure 7. Mean concentrations of Cry1Ac in Bollgard and Bollgard II and Cry2Ab in Bollgard II from individual field sites as measured by ELISA.

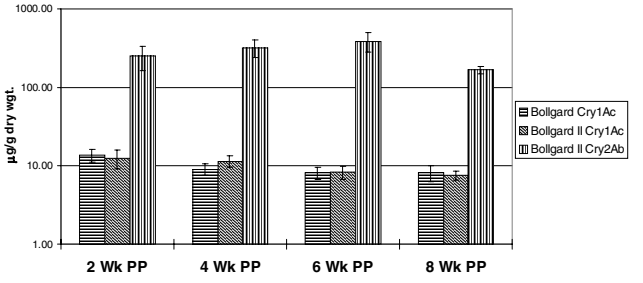

Figure 8. Mean concentrations of Cry1Ac in Bollgard and Bollgard II and Cry2Ab in Bollgard II from specific sampling times as measured by ELISA.

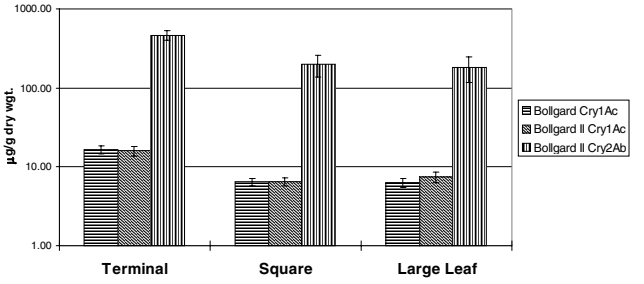

Figure 9. Mean concentrations of Cry1Ac in Bollgard and Bollgard II and Cry2Ab in Bollgard II in specific tissue types as measured by ELISA.