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BOLLGARD® II: DUAL TOXIN EXPRESSION AND INTERACTION

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Abstract

Comparisons of lepidopteran toxicity made among genetic isolines of Monsanto cotton variety 15985, expressing Cry1Ac only, Cry2Ab only, and both toxins, demonstrated a significant increase in activity of the 2-toxin isoline over the Bollgard® isoline (Cry1Ac-only) with Cry2Ab contributing the larger proportion of activity in the 2-toxin line. Diet overlay studies revealed responses to the 15985 isolines indicating an additive interaction of the 2 Bt toxins in the 2-gene (Bollgard® II) isoline. A 10-site field study showed that co-expression of Cry1Ac and Cry2Ab resulted in 3 to 6-fold increases in bioactiviy levels over those found in cotton plants expressing Cry1Ac only (Bollgard®).

Protocols and Results

This study was designed to quantify the bio-efficacy of Cry1Ac/Cry2Ab (Bollgard®II) cotton and compare with that of Cry1Ac (Bollgard®) cotton in a *Heliothis virescens* (F.) bioassay. Genetic near-isolines of a cotton variety (Monsanto 15985), containing Cry1Ac only, Cry2Ab only, and both toxins, were used to examine the relative contributions of each toxin to the total efficacy of Bollgard®II. The nature of the interaction (synergistic/antagonistic or additive) of the individual toxins in the 2-gene cotton was also explored. All tissue samples for these studies were collected in a 10-site field study in 2000. Cotton tissue samples were evaluated in a sensitive *H. virescens* growth inhibition bioassay as previously described (Greenplate 1999), with activity levels being quantified by comparison with concurrently-run concentration-response curves using purified Cry1Ac protein. A quantitative estimate of *H. virescens* activity was made for tissues containing Cry1Ac, Cry2Ab, or both, and expressed in Cry1Ac equivalents, since purified Cry1Ac was used as the standard for comparison. Analyses of variance were performed using JMP© (version 4.1) statistical software (SAS Institute, Cary NC). Mean comparisons utilized Tukey-Kramer HSD at P = 0.05 (Kramer 1956). Quantitative ELISA data for individual toxins were used to link relative levels of each toxin in the 2-gene isoline to the levels found in the respective single-gene isolines and to respective bioactivity levels.

Where mean comparisons were made (Figures 1 & 2), 2-toxin cotton (Bollgard®II) exhibited significantly more lepidopteran activity than did Cry1Ac-only cotton (Bollgard®). Seasonal means were generated by tissue type, with results appearing in Figures 2. Similar results were generated when multi-site means for individual sampling times were compared (Figure 1). Roughly calculated, lepidopteran activity, as measured in the *H. virescens* quantitative assay was 3-6 times higher in the 2-gene cotton (Figures 1 & 2). These data show that the addition of Cry2Ab to Cry1Ac in cotton provided a highly significant and uniform increase in lepidopteran bioactivity.

In a study designed to evaluate the nature of the interaction of Cry2Ab and Cry1Ac in the double-gene cotton variety, single-toxin isolines of 15985 were evaluated separately and compared with the double-toxin isoline. Overall mean values for these assessments appear in Table 1. Using the X^2 statistic, after Salama et al (1984), the calculated expected value for the 2-gene isoline was compared with the observed response and found to be statistically similar, indicating an acceptance of the hypothesis of simple, additive interaction of the 2 toxins against this insect. To accept these conclusions, it was necessary to assume that levels of neither toxin were influenced by the presence of the other, or its gene. This was confirmed by performing a toxin-specific quantitative ELISA (enzyme-linked immunosorbent assay) test on every lyophilized plant sample used in the study. ELISA results (Table 2) clearly show that the level of each toxin in the 2-gene isoline is identical to the level found in its single-gene isoline.

References

Finney, D. J. 1971. Probit analysis. Cambridge University Press. London.

Greenplate, J. T. 1999. Quantification of Bacillus thuringiensis insect control protein Cry1Ac over time in Bollgardâ 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.

Salama, H.S., M.S. Foda, F.N. Zaki, and S. Moawad. 1984. Potency of Combinations of Bacillus thuringiensis and Chemical Insecticides on Spodoptera littoralis (Lepidoptera: Noctuidae). J. Econ. Entomol. 77: 885-890.

Table 1. Evaluation of interaction of Cry1Ac and Cry2Ab against *H. virescens* in a cotton variety expressing both proteins (after Salama et al, 1984.). Expected values for activity were calculated based upon the observed responses to single-protein isolines (Co-treatments in same experiment). Each sample (n) represents 96 larvae challenged with a 20,000-X dilution of lyophilized cotton tissue powder as a diet overlay. a) Proportion of population responding by developmental arrest at or before 2nd larval stadium; b) Proportion of population expected to respond if activity of Cry1 and Cry2 proteins are additive ($PR_{Expected} = PR_{Cry1} + PR_{Cry2}(1-PR_{Cry1})$); c) $X^2 = (PR_{Observed} - PR_{Expected})^2/PR_{Expected}$; compared with X^2 tabular value at P = .05, df = 1. The observed proportions responding for the Cry2Ab and 2-gene isolines were statistically similar and significantly higher than the mean for the Cry1Ac isoline (Tukey-Kramer HSD, P = 0.05).

Isoline	n	PR Observed[a]	SEM	Lower 95%	Upper 95%	PR Expected[b]	X^2 value[c]	Combined effect
Cry1Ac isoline	88	0.121	0.014	0.093	0.150			
Cry2Ab isoline	84	0.581	0.027	0.527	0.635			
2-gene isoline	83	0.580	0.027	0.526	0.635	0.632	0.004 ns	**Additive**

Table 2 Seasonal means (mg/g dry weight) for individual Bt d-endotoxins in isolines of cotton line 15985 as measured in protein-specific ELISA assays.

Isoline	n	Cry1Ac	SEM	Cry2Ab	SEM
Cry1Ac isoline	87	7.2	0.5	0.0	0.0
2-gene isoline	84	7.0	0.6	412.0	25.4
Cry2Ab isoline	86	0.0	0.0	417.7	26.9

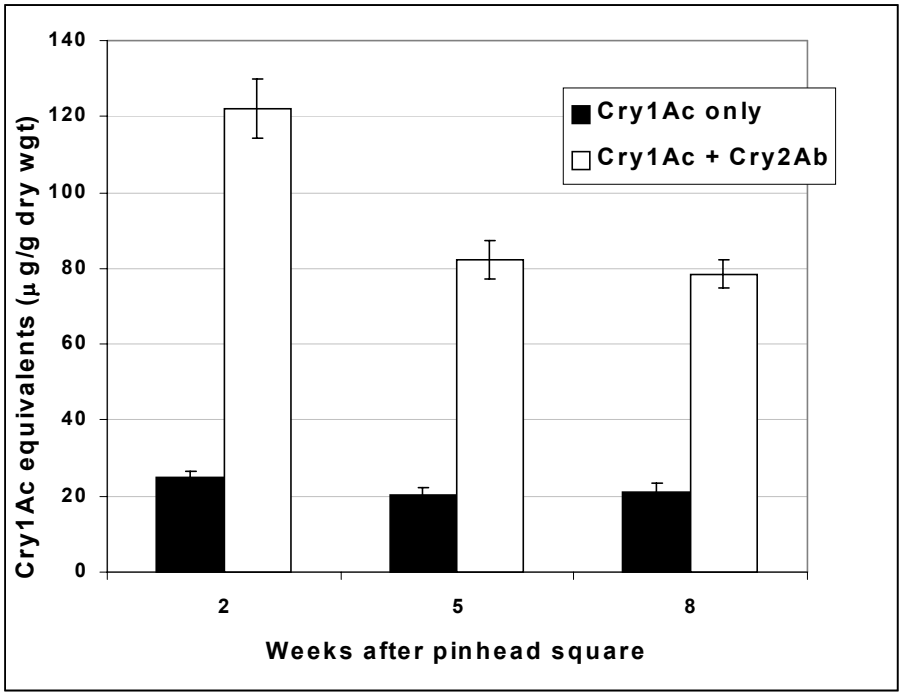

Figure 1. Means (with SEM) for Lepidopteran Activity Levels at individual sampling times. Each bar represents the mean for all field sites, tissue types, and varieties. Within each sampling time, the mean difference was statistically significant (Tukey-Kramer HSD, P = 0.05).

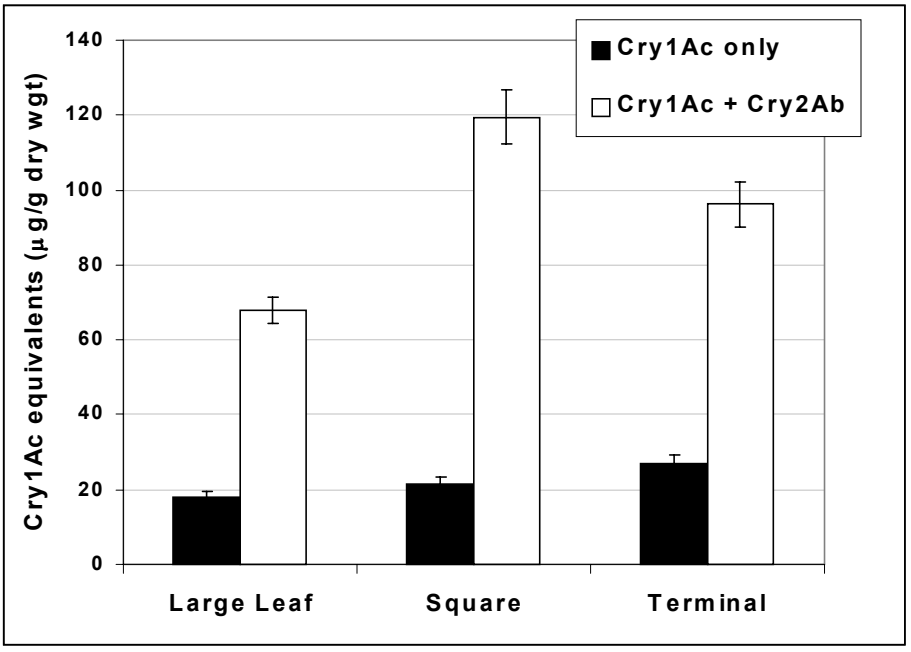

Figure 2. Seasonal mean Lepidopteran Activity Levels (with SEM) for specific tissue types. Each bar represents the mean for all field sites, sampling times, and varieties. Within each tissue type, the mean difference was statistically significant (Tukey-Kramer HSD, P = 0.05).