2003 BELTWIDE COTTON CONFERENCES, NASHVILLE, TN – JANUARY 6-10

PROFILING SEASONAL EXPRESSION OF CRY2AB IN BOLLS OF DUAL-TOXIN BT COTTON

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Abstract

Laboratory studies were conducted to quantify toxin levels in bolls located at various locations throughout dual-toxin Bt cotton plants. Results of ELISA techniques indicate that there was a significant quadratic relationship between node location and Cry2Ab concentration, with expression levels highest in bolls from the central portion of the plant. For bolls at node 9 and above (averaged over positions 1 and 2), there was a significant decline in Cry2 expression as the season progressed. A more dramatic reduction of Cry2Ab levels was noted above node 16 in bolls from position 1and node 14 in bolls from position 2. Since bolls from these locations are from the same cohort (i.e., the same phenological age), this would indicate that environmental factors may play a significant role in expression of Bt toxins throughout the plant.

Introduction

Since the introduction of transgenic Bt cotton (i.e. Bollgard®, Monsanto Company, St. Louis, MO) in 1996, growers in the southeastern U.S. have adopted this technology at an increasing rate for control of tobacco budworm, *Heliothis virescens* (F.) and moderate infestations of bollworm, *Helicoverpa zea* (Boddie). In 2002, 81% of all cotton acreage in Mississippi was planted to Bollgard varieties (USDA 2002), compared to the 42% that was planted to Bollgard in 1996 (Layton et al. 1997).

As mentioned, the Cry1Ac toxin in Bollgard cotton controls tobacco budworm, but supplemental insecticide applications have been necessary for occasional pests (e.g. fall armyworm, *Spodoptera frugiperda* [J. E. Smith]) and high populations of bollworm (Layton 2003). As of late, cotton expressing an additional protein (in addition to the Cry1Ac protein) has been developed. This second protein (Cry2Ab) should provide control of occasional pests, as well as enhance control of bollworms. Cotton containing this second-generation Bt gene is expected to enter the market in selected varieties in 2003. It is generally believed that once this dual-toxin Bt cotton (i.e. Bollgard II®, Monsanto Company) is introduced, it will likely replace the current single-toxin Bt cotton varieties within a few years.

Several studies have been conducted with Bollgard cotton investigating differences in Bt toxin expression levels among plant parts (Akin et al. 2002), seasonal patterns in terminal leaves (Adamczyk et al. 2001a), and expression among various transgenic Bt cotton varieties (Adamczyk et al. 2001b). Differences in toxin levels among these studies have been noted. Few studies to date, however, have been conducted to evaluate seasonal expression profiles in bolls.

In upland cotton, young bolls (i.e. <350 DD60s) are particularly susceptible to attack by heliothines, as well as fall armyworms. Although heliothine larvae often begin feeding in the terminal of non-Bt cotton plants and flowers of single-toxin Bt cotton (Gore et al. 2001), it is important to investigate toxin expression in bolls, since heliothines and fall armyworms eventually move to young bolls to feed in both non-Bt and Bt cotton. According to Jenkins and McCarty (1995), ca. 75% of total yield from the cotton plant is derived from first position bolls, and an additional 18-20% comes from second position bolls. Therefore, of all bolls produced by the plant, it is imperative to investigate toxin expression levels in those with economic importance (i.e., those that contribute significantly to yield).

Determining the temporal or spatial pattern of Cry protein expression in Bollgard and Bollgard II cotton plants will help us better understand strengths or weaknesses related to location on the plant (i.e., time of the growing season). Scouting protocols can then be modified to better correlate with times of the season when toxin expression is at its lowest. Implications with regard to temporal resistance in lepidopteran larvae can also be considered.

Materials and Methods

Young bolls were collected from two varieties of cotton planted on May 14, 2002 in Starkville, Mississippi (Plant Science Research Center)--NuCOTN 33B ('Bollgard', Cry1Ac alone) and NuCOTN 33BII ('Bollgard II', Cry1Ac and Cry2Ab). The experimental design was a randomized complete block with three replications. Plot size was 8 rows (96-cm spacing) x 15 m,

with 3 m alleys. The soil type is a well-drained Leeper silty clay loam, and plots were irrigated 6 times during the growing season. Three times weekly, during the predominant portion of anthesis (8 July-19 August), white blooms in each plot were marked with color-coded snap-on tags (Hummert International, Earth City, MO 63045). Resulting bolls were collected 7 d later and stored in –80 C until processing could take place. Collection of bolls took place from 15 July through 26 August. First position bolls from nodes 6-19 and second position bolls from nodes 7-17 were subjected to ELISA for toxin expression levels.

The growth of tagged bolls was recorded in heat units as described in Bagwell and Tugwell (1992) as follows: (maximum + minimum daily temperature/2) – 60, where 60 is considered the minimum temperature at which development occurs. The age of cotton bolls at collection was calculated by accumulating heat units from the pink-bloom stage (1 d after tagging) to time of collection (range=136-157 DD60's; mean=146.2; s. d.=6.1).

Processing of bolls consisted of dissecting each boll and discarding the internal contents (i.e. seed and lint). Five bolls were chosen at random from each plant location (node x position) for each rep/variety. After processing, boll walls were then placed back into –80C until lyophilization could be performed. Boll walls were freeze-dried using a Genesis 25LL lyophilizer (Virtis Company, Inc., Gardiner, NY 12525). Once lyophilized, boll walls from each sample were homogenized and crushed with a mortar and pestle into a fine powder, and stored in scintillation vials at -20 C until immunoassays could be performed. From each plant location, two weighed subsamples of 20 mg were used for analysis.

The technique used to quantify Bt toxins in this experiment utilizes enzyme-linked immunosorbent assay (ELISA) (Envirologix, Inc., Portland, ME 04103), as described by Adamczyk et al. (2001). This is a commercially available test that uses a color development step where color is proportional to amount of antigen (i.e. Bt protein) in the sample extract. Optical densities were read using a Thermomax® microplate reader (Molecular Devices, Inc., Sunnyvale, CA, 94089), then converted to concentrations using SOFTmax Pro®2.1.1 computer software (Molecular Devices, Inc.). All data were analyzed using Proc Mixed and Proc Reg (SAS Institute, 1998).

Results

Based on Proc mixed analysis, there was no significant difference in Cry1Ac expression due to node or position in single or dual-toxin Bt cottons. There was, however, a node*position interaction in both Bollgard (F=2.52; df=10; p=0.0258) and Bollgard II (F=2.29; df=10; p=0.0301). In Bollgard cotton, differences in expression of Cry1Ac were near significant in second position bolls (F=2.17; df=10; p=0.0511). In Bollgard II cotton, there were significant differences in the level of Cry1Ac expression for first position bolls (F=2.14; df=13; p=0.0320).

Using regression analysis, for Cry2Ab expression in first-position bolls of Bollgard II cotton, a significant quadratic trend was found (r^2=0.4396; F=4.31; p=0.0414) (Figure 1). There was no significant quadratic trend for second position bolls of Bollgard II cotton (r^2=0.3071; F=1.77; p=0.2305) (Figure 2), but there was a significant fit (quadratic) to the average toxin expression pattern of both positions 1 and 2.

When only data from nodes 9-19 were included in the analysis, there was a slight, but significant linear decline in Cry2Ab expression in first position bolls as the season progressed (r^2=0.4584; F=7.62; p=0.0221) (Figure 3). No significant linear fit was noted to second position bolls (Figure 4), but again, there was a significant linear fit when both positions were averaged (r^2=0.5968; F=9.58; p=0.0032). These results indicate that Cry2Ab expression in Bollgard II cotton gradually declines as the season progresses.

Future Research

Bioassays will be conducted using *H. zea* to investigate correlation between the observed trend in toxin expression and deleterious effects on larvae. Bollworms (L2) will be infested onto meridic diet incorporated with tissue used in aforementioned ELISA's, and larval survival and/or stunting will be observed. Further statistical analysis of Cry1Ac in Bollgard and Bollgard II cotton boll tissue will also be performed.

Acknowledgements

The author would like to thank Debbie Boykin (USDA-ARS) for assistance with data analysis, as well as Kathy Knighten for her assistance in the laboratory. A great deal of gratitude also goes out to Bobbie Boyd (MSU College of Veterinary Medicine) for use of spectrophotometer and software. The author would also like to thank Doug Dollar (USDA-ARS) for use of lyophilization equipment.

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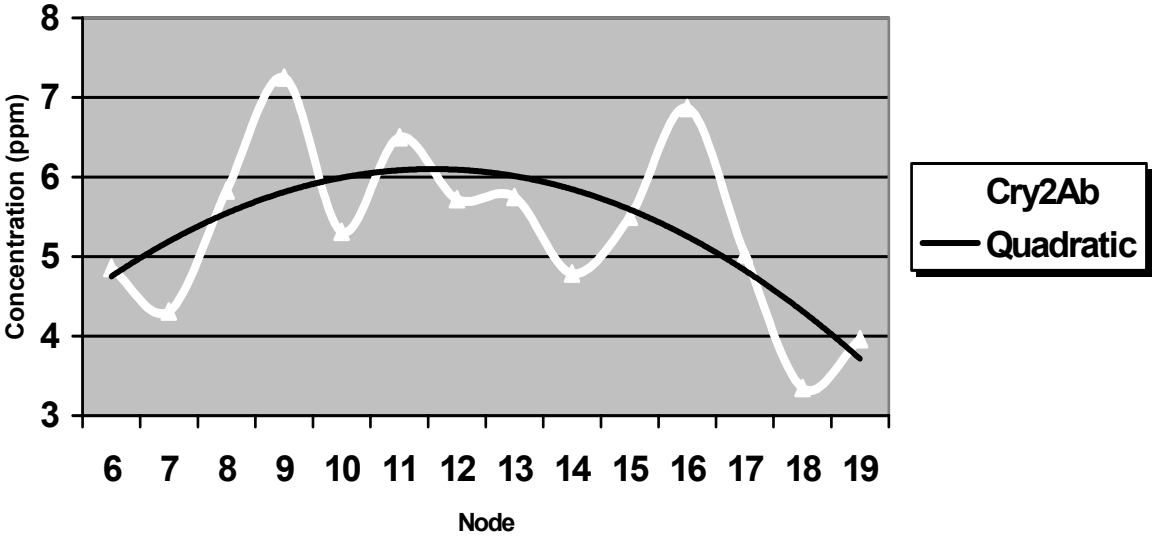

Figure 1. Cry2Ab expression levels in position 1 bolls (nodes 6-19) of dual-toxin Bt cotton (r^2=0.4396; F=4.31; p=.0414).

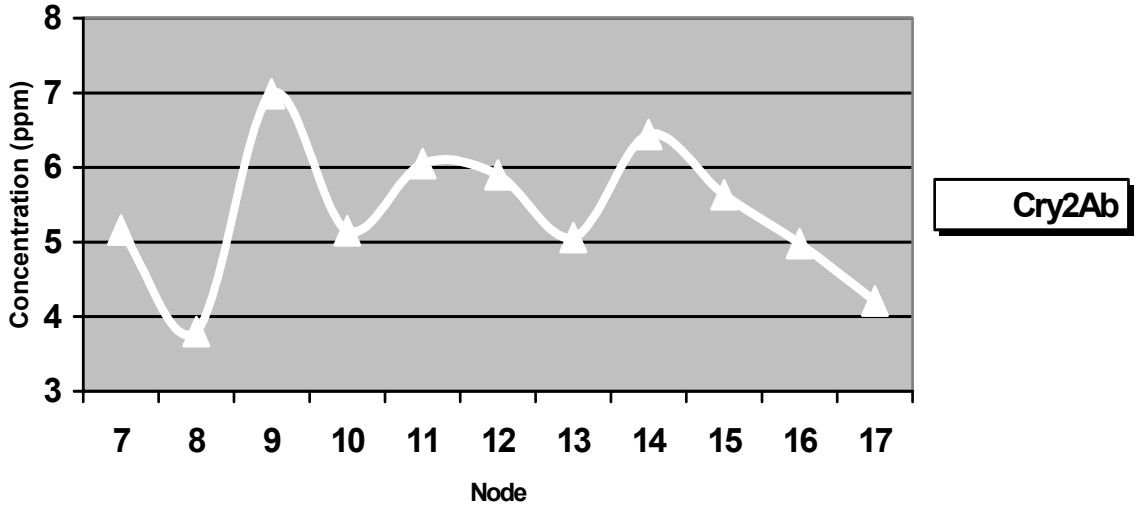

Figure 2. Cry2Ab expression levels in position 2 bolls (nodes 7-17) of dual-toxin Bt cotton (r^2=0.3071; F=1.77; p=.2305).

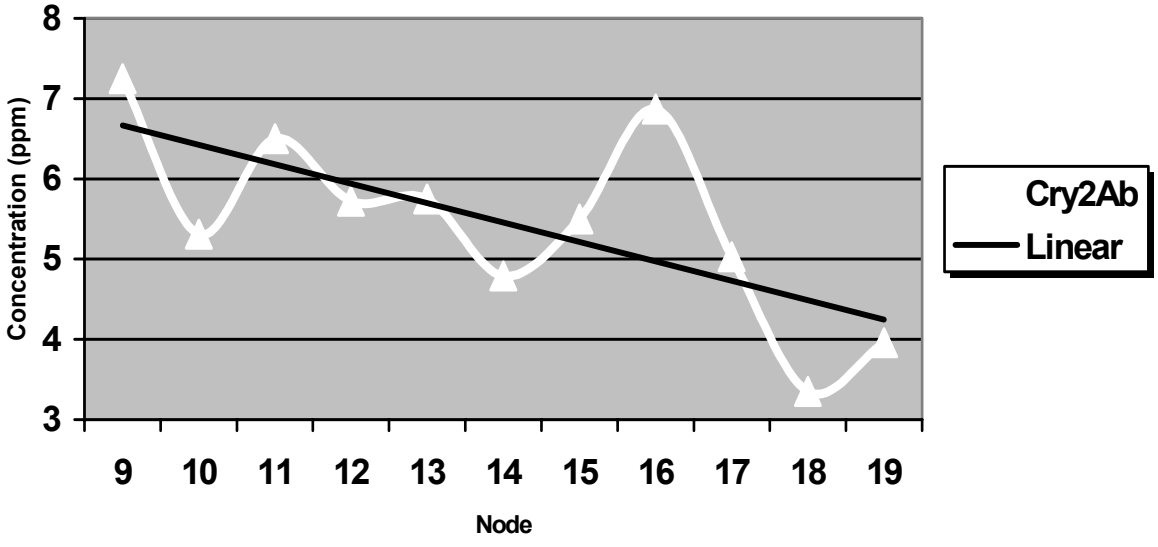

Figure 3. Cry2Ab expression levels in position 1 bolls (nodes 9-19) of dual-toxin Bt cotton (r^2=0.4584; F=7.62; p=.0221; [ ] = 8.66 - .2301 x node).

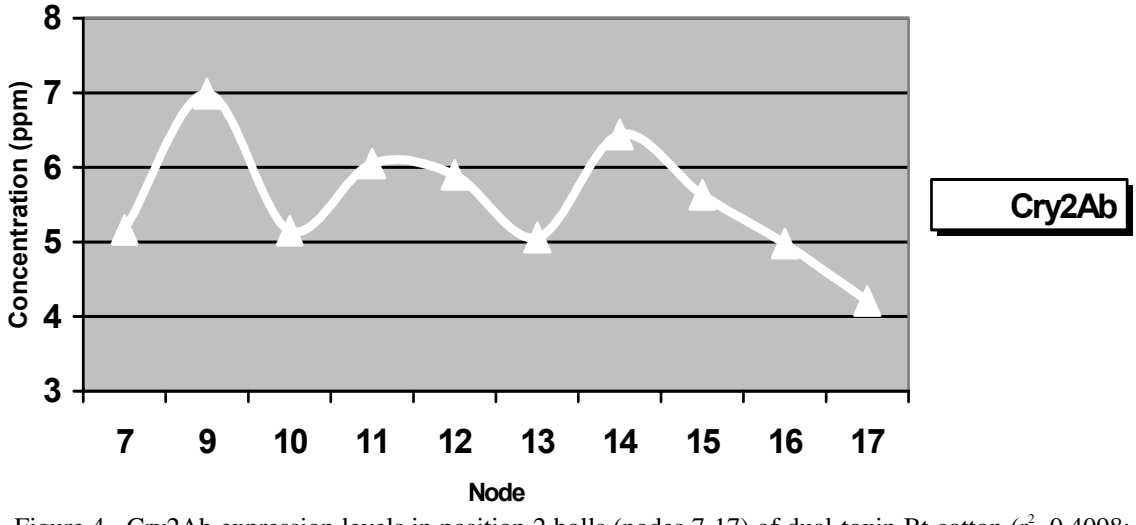

Figure 4. Cry2Ab expression levels in position 2 bolls (nodes 7-17) of dual-toxin Bt cotton (r^2=0.4098; F=4.86; p=.0633; [ ] = 7.97 - .1810 x node).