

# Correlating Differences in Larval Survival and Development of Bollworm (Lepidoptera: Noctuidae) and Fall Armyworm (Lepidoptera: Noctuidae) to Differential Expression of Cry1A(c) $\delta$ -Endotoxin in Various Plant Parts Among Commercial Cultivars of Transgenic *Bacillus thuringiensis* Cotton

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**ABSTRACT** Differences in larval survival and development of bollworm, *Helicoverpa zea* (Boddie), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith), respectively, were found to exist among commercially available Cry1A(c) transgenic *Bacillus thuringiensis* Berliner (Bt) varieties. Using a quantification assay (ELISA) to measure the levels of  $\delta$ -endotoxin in two of these varieties ('DP 451B/RR' and 'NuCOTN 33B'), differences in the amount of  $\delta$ -endotoxin present in various plant parts was correlated with larval survival of bollworms and larval development of fall armyworms throughout the growing season. Larvae that were fed on DP 451B/RR completed development faster and exhibited better survivorship than those larvae fed NuCOTN 33B, whereas lower levels of  $\delta$ -endotoxin were generally detected in plant parts from DP 451B/RR compared with NuCOTN 33B. These differences may impact population dynamics of these pests which may be a critical factor in managing resistance to Bt. Furthermore, the utility of using this system for providing information to the grower concerning varietal choices may be more common in the future.

**KEY WORDS** *Bacillus thuringiensis* quantification, transgenic varieties, enzyme-linked immunosorbent assay

SINCE THE FIRST transgenic Cry1A(c) *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 >25 transgenic varieties. These varieties can contain the Cry1A(c) Bt gene (Bollgard, Monsanto, St. Louis, MO), herbicide-resistance genes (Roundup Ready, Monsanto, 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 Cry1A(c)  $\delta$ -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.

Resistance management guidelines have been developed to prolong the usefulness of transgenic insect control products. Primarily based on modeling data, these recommendations suggest that planting non-Bt

cotton will serve as a refuge for Bt susceptible Lepidoptera and thus delay resistance (Caprio 1994). These models rely on a high-dose strategy for insects such as the tobacco budworm, *Heliothis virescens* (F.), but delaying resistance developing in insects that are intrinsically tolerant to Bt cotton, such as the bollworm, *Helicoverpa zea* (Boddie), are much debated. Recently, laboratory genetic-model studies have shown that temporal separation of mating can potentially occur among tobacco budworm and pink bollworm, *Pectinophora gossypiella* (Sanders), sub-populations from non-Bt and Bt cotton (Peck et al. 1999). The assortative mating results from a delay in larval development for resistant larvae feeding on Bt cotton relative to larvae feeding on non-Bt cotton (Liu et al. 1999). Therefore, if current Bt varieties express different levels of Cry1A(c)  $\delta$ -endotoxin, then further reproductive isolation of populations of intrinsically tolerant Lepidoptera may occur, complicating recommendations.

Although genetic transformation events often modify the agronomics of the transgenic variety compared with the corresponding parental variety (e.g., plant maturity and mean height) (D&PL Seed Research and Agronomic Services, Scott, MS), few studies have been published that examine differential expression of toxin among different plant parts and varieties (Fitt 1998, Holt 1998, Sachs et al. 1998). Greenplate (1999)

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**Table 1.** Commercially available cotton varieties planted in Elizabeth, MS, 1999

Non-Bt cottons		Bt cottons	
Conventional	Herbicide-Resistance gene <sup>a</sup>	Cry1A(c) <sup>b</sup>	Cry1A(c) <sup>b</sup> + Herbicide-Resistance gene <sup>a</sup>
DP388 <sup>c</sup>	DP425RR <sup>c</sup>	DP20B <sup>c</sup>	DP409B/RR <sup>c</sup>
DP5111 <sup>c</sup>	DP429RR <sup>c</sup>	DP32B <sup>c</sup>	DP422B/RR <sup>c</sup>
DP5415 <sup>c</sup>	DP436RR <sup>c</sup>	DP35B <sup>c</sup>	DP451B/RR <sup>c</sup>
	DP5415RR <sup>c</sup>	DP50B <sup>c</sup>	DP458B/RR <sup>c</sup>
	PM1220RR <sup>d</sup>	DP90B <sup>c</sup>	PM1220B/RR <sup>d</sup>
		DP428B <sup>f</sup>	
		DP448B <sup>f</sup>	
		NuCOTN 33B <sup>f</sup>	
		PM1215BG <sup>d</sup>	
		PM1244BG <sup>d</sup>	
		PM1330BG <sup>d</sup>	
		PM1560BG <sup>d</sup>	

<sup>a</sup> Roundup Ready (Monsanto, St. Louis, MO).

<sup>b</sup> Bollgard (Monsanto, St. Louis, MO).

<sup>c</sup> Delta & Pineland variety (Delta & Pineland, Scott, MS).

<sup>d</sup> Paymaster variety (Delta & Pineland, Scott, MS).

developed a quantification bioassay using tobacco budworms that showed that Cry1A(c)  $\delta$ -endotoxin levels decrease among squares and bolls throughout the growing season. Although numerous studies have shown that intrinsically tolerant Lepidoptera widely differ in their susceptibility to the  $\delta$ -endotoxin (MacIntosh et al. 1990, Jenkins et al. 1992, Wilson et al. 1992, Stone and Sims 1993, Adamczyk et al. 1998a, Luttrell et al. 1999), none have tried to correlate larval survival and development to the amount of  $\delta$ -endotoxin present in commercial varieties. In a preliminary experiment, two Bt varieties widely differed in their effect on bollworm survival ('NuCOTN 33B' and 'DP 451B/RR', Delta & Pine Land, Scott, MS). Therefore, these two varieties were closely examined to determine if differences exist in bollworm survival and fall armyworm development among various Bt plant parts and varieties, and if such differences could be correlated to  $\delta$ -endotoxin concentrations.

### Materials and Methods

**Field Plots.** Eight non-Bt varieties and 17 Bt varieties were planted in experimental plots on 24 May 1999 near Elizabeth, MS (Table 1). Plots consisted of four rows (1.0 m centers)  $\times$  30.5 m. Treatments were arranged in a randomized complete block design with four replications. All plots were nonirrigated. Insecticide applications consistent with local recommendations were made on all varieties for nonlepidopterous insects throughout the season. Only non-Bt varieties received a single application of foliar insecticide (organophosphate) to control a natural infestation of tobacco budworms and bollworms.

**Larval Survival of Bollworms Among Plant Parts from Different Bt Varieties.** *Insects.* To ensure that healthy, near wild-type larvae would be used in all tests, collections were made from whorl-stage field corn near Stoneville, MS, in early May of 1999. Ap-

proximately 300 larvae were collected and reared to pupation on artificial diet. Rearing of adults and egg harvesting were conducted as described in Adamczyk et al. (1998a). The F<sub>2</sub> or F<sub>3</sub> generation of neonates was used in all tests.

**Leaves, Squares, White Flowers.** To compare larval survival of bollworms among 17 Bt and eight non-Bt varieties, 15 leaves (fourth true leaf stage), 10 squares (8–9 node stage; prebloom cotton), and five white flowers (first-position; first week of flowering) were harvested from randomly selected plants from each plot (4 replications) (Table 1). Only a single individual plant part was harvested per plant. Leaves and squares were placed into individual 29.6-ml plastic cups (JetPlastica, Hattisburg, PA), and white flowers were placed into individual 236.8 ml paper wax-lined cups (Chimet, E. Providence, RI). Because of petal desiccation complicating larval assessments in previous studies (Adamczyk et al. 1999), the petals were removed before larval infestation. One neonate was introduced into a cup containing a leaf (60 neonates/variety) or a square (40 neonates/variety), while three neonates were introduced into a cup containing a white flower (60 neonates/variety). The cups were capped with corresponding lids. After 2 d, larvae from leaves were carefully transferred with a camel's-hair brush into individual 9.0-cm petri dishes that each contained a moistened filter paper and a fresh leaf, and the plates were covered with corresponding lids. All cups and dishes were placed inside an autoclave bag to further prevent desiccation, and were held in an environmental chamber (Percival Scientific, Boone, IA) at 27  $\pm$  1°C, 80% RH, and a photoperiod of 14:10 (L:D) h. All plant parts were changed every 2 d. Larval survival among all plant parts was assessed at 4 d after exposure (DAE). Furthermore, larval survival among leaves was assessed at 8, 10, and 14 DAE. Larvae were considered alive if movement was observed after being lightly stroked with a camel hairbrush. Because larvae that were inside the square could not be assessed without causing significant mortality from mutilation, these samples were discarded. The number of alive larvae fed leaves and white flowers was log transformed, whereas percent survival of larvae fed squares was arcsine square-root transformed (Steel and Torrie 1980). Mean survivorship was analyzed using REML-analysis of variance (ANOVA), and means were separated with the LSMEANS option of PROC MIXED (Littell et al. 1996).

**Larval Development of Fall Armyworms Between Two Bt Varieties.** *Insects.* Fall armyworms, *Spodoptera frugiperda* (J. E. Smith), were used in the larval development study because this species is a damaging pest of cotton that is intrinsically tolerant to the Cry1A(c)  $\delta$ -endotoxin and can be easily reared to pupation on Bt varietal tissue (Adamczyk et al. 1998a). Larvae were obtained from the USDA-ARS-CHPRRU at Mississippi State, MS. Females from this colony are annually out-crossed with wild, pheromone trapped males to ensure genetic diversity and traits present in field individuals. Larval and adult rearing as well as egg

**Table 2.** Mean  $\pm$  SE percent larval survival of bollworms fed leaves among cotton varieties and between two commercially available Bt varieties

Assessment (DAE) <sup>a</sup>	Non-Bt cottons <sup>b</sup>	Bt cottons <sup>c</sup>	NuCOTN 33B vs DP451B/RR (P-value)	
4	92.29 NS $\pm$ 1.12	34.32** $\pm$ 2.59	18.33 $\pm$ 10.32	55.00 $\pm$ 13.44
8	87.29 NS $\pm$ 1.68	21.08* $\pm$ 1.97	8.33 $\pm$ 6.31	40.00 $\pm$ 13.61
10	85.83 NS $\pm$ 1.76	15.59** $\pm$ 1.59	3.33 $\pm$ 3.33	30.00 $\pm$ 11.71
14	83.13 NS $\pm$ 1.67	7.84* $\pm$ 1.13	0	16.67 $\pm$ 10.36

Percent survival converted to number alive and log transformed prior to analysis ( $\alpha = 0.05$ ; PROC MIXED [Littell et al. 1996]). \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; NS, not significant.

<sup>a</sup> Days after exposure.

<sup>b</sup> Average from eight non-Bt varieties (Table 1).

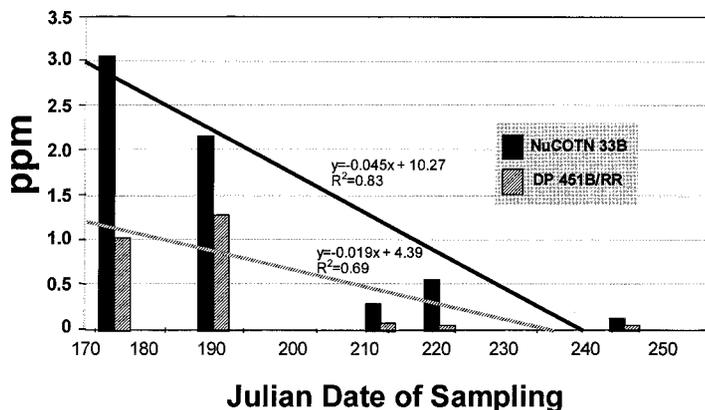
<sup>c</sup> Average from 17 Bt varieties (Table 1).

harvesting were conducted as described by Adamczyk et al. (1998a).

**Leaves.** Two Bt varieties (DP 451B/RR and NuCOTN 33B) were compared with each other and a non-Bt variety served as a control (DP 5415). From each plot, a single leaf from 30 randomly selected plants was harvested as described above (4 replications). One neonate was placed in each dish along with a moistened filter paper, and the dishes were covered with corresponding lids (120 neonates/variety). Leaves were changed every 2 d until pupation. Larval weights were recorded at 16 DAE. Pupal weights, pupal duration (separated by sex), and time to pupation also were recorded. All data were log transformed (Steel and Torrie 1980). Mean survivorship was analyzed using REML-ANOVA, and means were separated with the LSMEANS option of PROC MIXED (Littell et al. 1996).

**$\delta$ -Endotoxin Quantification.** Two Bt varieties (NuCOTN 33B and DP 451B/RR) that differed in larval survival and development of bollworms and fall armyworms, respectively, were analyzed to quantify the amount of  $\delta$ -endotoxin present in various plant parts. In addition, a non-Bt variety (DP 5415) was used in all protocol steps as a control.

**Leaves.** The amounts of  $\delta$ -endotoxin present in leaves from the two Bt varieties (NuCOTN 33B and DP 451B/RR) were quantified throughout the growing season (24 June–2 September 1999). To ensure that all leaves used in these assays were of a known, uniform age, the fourth true leaf from 100 plants in each plot was tagged on 24 June 1999 (Slant 'N Lock; A. M. Leonard, Piqua, OH). For the first three sample dates (24 June 1999, 7 July 1999, and 6 August 1999), a single tagged leaf was randomly harvested from five plants/plot (four replications). Because some of the tagged leaves had shed throughout the growing season, only enough samples from five plants/plot (two replications) could be harvested for the last sample date (2 September). Leaves were transported to the laboratory and within a few hours after being harvested, one sample ( $\approx 5$  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 into a 1.5-ml microcentrifuge tube and homogenized by hand in Cry1A(c) extraction buffer (EnviroLogic, Portland, ME) using a fitted pestle. This entire extraction process for each variety was replicated twice.



**Fig. 1.** Amount of  $\delta$ -endotoxin (ppm) measured in leaves from two Bt varieties throughout the 1999 Mississippi Delta growing season.

**Table 3.** Mean  $\pm$  SE percent larval survival of bollworms fed squares or white flowers from different cotton varieties

Squares		White Flowers <sup>a</sup>	
Non-Bt cottons <sup>b</sup>	Bt cottons <sup>c</sup>	Non-Bt cottons	Bt cottons
65.60 NS $\pm$ 2.38	16.67 NS $\pm$ 1.81	76.67 NS $\pm$ 1.56	62.06 NS $\pm$ 1.81

Percent survival log transformed prior to analysis ( $\alpha = 0.05$ ; PROC MIXED [Littell et al. 1996]). NS, not significant.

<sup>a</sup> Petals removed.

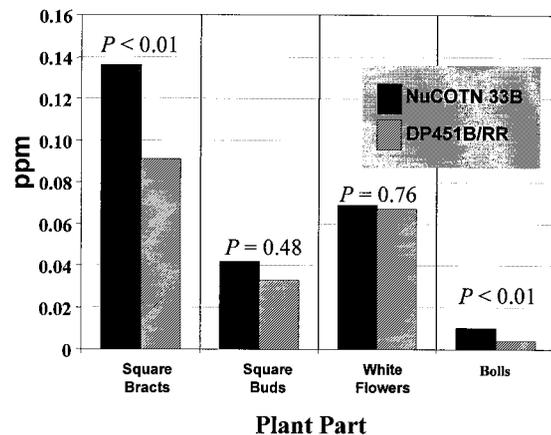
<sup>b</sup> Average from eight non-Bt varieties (Table 1).

<sup>c</sup> Average from 17 Bt varieties (Table 1).

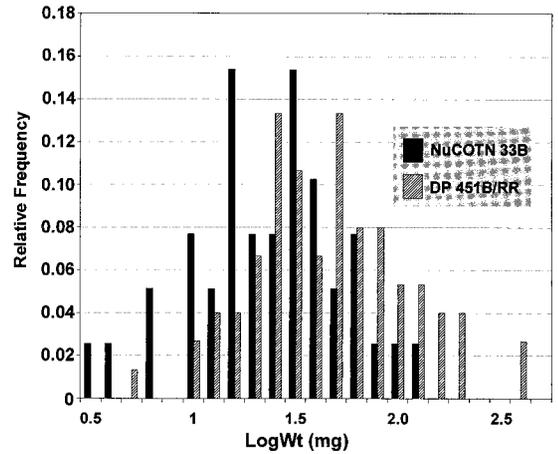
To identify possible geographical isolation affects, an additional location (B. R. Leonard, Macon Ridge Location of the Northeast Research Station, LSU Agricultural Center, Winnsboro, LA) was added. Leaves were harvested on 29 July 1999 and frozen within 1 d at  $-80^{\circ}\text{C}$ . The identical harvesting and sample extraction techniques were followed as described above.

To quantify the amount of  $\delta$ -endotoxin present for each variety, a commercial quantification plate kit was used (EnviroLogic). This "sandwich" enzyme-linked immunosorbent assay (ELISA) uses a color development step where intensity of color production is proportional to CryIA(c) concentration in the sample extract. Therefore, quantification of  $\delta$ -endotoxin is determined spectrophotometrically (Benchmark, Bio-Rad, Hercules, CA). For all sample dates, both 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. Means were analyzed using REML-ANOVA, and the means were separated using the LSMEANS option of PROC MIXED (Littell et al. 1996).

*Square Parts, White Flowers, Bolls.* The amount of  $\delta$ -endotoxin present in plant parts from the two Bt varieties (NuCOTN 33B and DP 451B/RR) were quantified simultaneously when bioassays were being conducted, with the exception of bolls (no bioassay



**Fig. 2.** Amount of  $\delta$ -endotoxin (ppm) measured in various plant parts from two Bt varieties.



**Fig. 3.** Distribution of larval weights (mg) for fall armyworms reared on two Bt varieties.

conducted). To ensure that bolls were the same age, first-position white flowers were tagged as described in Adamczyk et al. (1998b), and the subsequent bolls harvested after 3 d. For bracts of squares, a paper ticket punch was used to obtain samples as mentioned above for leaves. However, a scalpel was used to obtain a cross-section of tissue from square buds, white flowers (petals removed), and boll wall material (i.e., no seeds or lint). The same protocol was used for sample preparation, except that the homogenization step was modified. To maximize uniformity during extraction, a high-speed homogenizer was used which used stainless steel beads (6 mm) to shear the tissue (Mini-Bead Beater; Biospec Prod, Bartlesville, OK). The amount of  $\delta$ -endotoxin was quantified as mentioned above for the leaves.

## Results

**Correlating Differences in Larval Survival of Bollworms to  $\delta$ -Endotoxin Levels in Two Bt Varieties.** *Leaves.* Although there were no significant differences ( $P > 0.05$ ) observed in larval survival among the eight non-Bt varieties at 4 ( $F = 0.46$ ;  $df = 7, 21$ ;  $P = 0.86$ ), 8 ( $F = 1.16$ ;  $df = 7, 21$ ;  $P = 0.36$ ), 10 ( $F = 1.40$ ;  $df = 7, 21$ ;  $P = 0.26$ ), and 14 DAE ( $F = 1.33$ ;  $df = 7, 21$ ;  $P = 0.29$ ), significant differences ( $P < 0.05$ ) were observed in larval survival among the 17 Bt varieties at 4 ( $F = 2.41$ ;  $df = 16, 48$ ;  $P = 0.01$ ), 8 ( $F = 1.99$ ;  $df = 16, 48$ ;  $P = 0.03$ ), 10 ( $F = 3.14$ ;  $df = 16, 48$ ;  $P < 0.01$ ), and 14 DAE ( $F = 2.16$ ;  $df = 16, 48$ ;  $P = 0.02$ ). In particular, significant differences ( $P < 0.05$ ) in larval survival were observed between the two Bt varieties, NuCOTN 33B and DP 451B/RR, at 4 (LSMEANS:  $t = 3.38$ ;  $df = 48$ ,  $P < 0.01$ ), 8 (LSMEANS:  $t = -3.40$ ;  $df = 48$ ,  $P < 0.01$ ), 10 (LSMEANS:  $t = -3.84$ ;  $df = 48$ ,  $P < 0.01$ ), and 14 DAE (LSMEANS:  $t = -2.76$ ;  $df = 48$ ,  $P < 0.01$ ) (Table 2).

Higher levels of  $\delta$ -endotoxin were detected in leaves from NuCOTN 33B than in leaves from DP

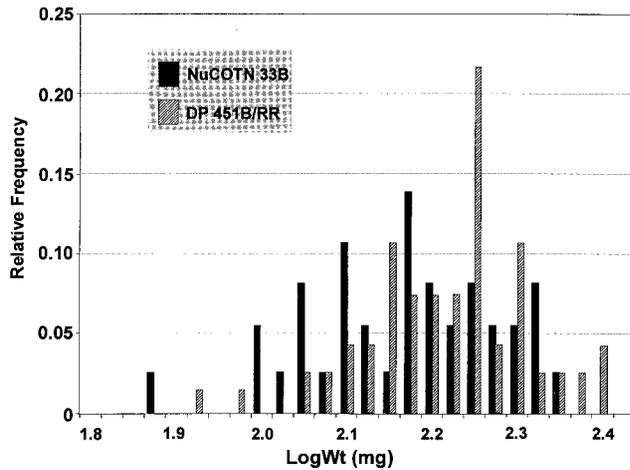


Fig. 4. Distribution of pupal weights (mg) for fall armyworms reared on two Bt varieties.

451B/RR. Significant differences ( $P < 0.05$ ) in the amount of  $\delta$ -endotoxin present in leaves from these varieties were observed for all sample dates from experimental plots in Elizabeth, MS, {[24 June 1999 (175 d):  $F = 143.11$ ;  $df = 1, 18$ ;  $P < 0.01$ ] [7 July 1999 (188 d):  $F = 18.60$ ;  $df = 1, 6$ ;  $P < 0.01$ ] [6 August 1999 (218 d):  $F = 206.69$ ;  $df = 1, 3$ ;  $P < 0.01$ ] [2 September 1999 (245 d):  $F = 803.01$ ;  $df = 1, 2$ ;  $P < 0.01$ ]} including the samples sent from Winnsboro, LA, [29 July 1999 (210 d):  $F = 18.78$ ;  $df = 1, 2$ ;  $P = 0.04$ ]. Trend lines further indicate that  $\delta$ -endotoxin levels appear to decrease throughout the season (Fig. 1).

*Square Parts, White Flowers, and Bolls.* At 4 DAE, there were no significant differences among the eight non-Bt varieties ( $P > 0.05$ ) in the survival of larvae when fed squares or flowers [(squares:  $F = 0.84$ ;  $df = 7, 21$ ;  $P = 0.57$ ) (white flowers:  $F = 0.52$ ;  $df = 7, 21$ ;  $P = 0.81$ )]. Likewise, at 4 DAE there were no significant differences among the 17 Bt varieties ( $P > 0.05$ ) in survival of larvae when fed squares or flowers

[(squares:  $F = 1.27$ ;  $df = 16, 48$ ;  $P = 0.26$ ) (white flowers:  $F = 1.28$ ;  $df = 16, 48$ ;  $P = 0.25$ )]. It is noteworthy that larvae chose to feed only on the bud portion of the square, rather than on the bracts. Therefore,  $\delta$ -endotoxin levels were determined separately for square buds and bracts (Table 3; Fig 2).

Although not always statistically different, a trend toward higher levels of  $\delta$ -endotoxin was detected in all plant parts examined from NuCOTN 33B versus DP 451B/RR. Although there were no significant differences between the two Bt varieties ( $P > 0.05$ ) in  $\delta$ -endotoxin levels in square buds ( $F = 0.56$ ;  $df = 1, 6$ ;  $P = 0.48$ ) or white flowers ( $F = 0.11$ ;  $df = 1, 6$ ;  $P = 0.76$ ), there were significant differences in  $\delta$ -endotoxin levels in square bracts ( $F = 59.84$ ;  $df = 1, 3$ ;  $P < 0.01$ ) and 3-d-old bolls ( $F = 68.73$ ;  $df = 1, 3$ ;  $P < 0.01$ ) (Fig 2).

**Correlating Differences in Larval Development of Fall Armyworms to  $\delta$ -Endotoxin Levels in Two Bt Varieties.** Control data validate that the experimental design and quality of insects was adequate. As in Adamczyk et al. (1998a), larval and pupal weights were significantly higher for larvae reared on a non-Bt variety compared with a Bt variety ( $P < 0.01$ ). In addition, time to pupation for larvae reared on DP5415 was

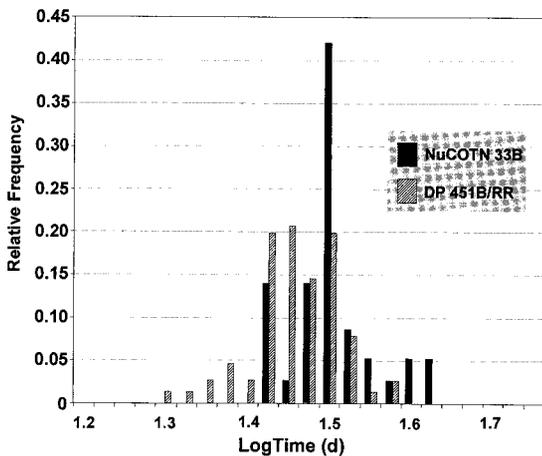


Fig. 5. Distribution of pupation times (d) for fall armyworms reared on two Bt varieties.

Table 4. Mean  $\pm$  SE time (d) in the pupal stage for fall armyworm larvae fed leaves from conventional and Bt varieties

Variety	Males	Females
DP 5415 <sup>a</sup>	8.43a $\pm$ 0.21	7.88a $\pm$ 0.08
DP 451 B/RR <sup>b</sup>	8.25a $\pm$ 0.07	7.29b $\pm$ 0.10
NuCOTN 33B <sup>c</sup>	8.05a $\pm$ 0.09	7.20b $\pm$ 0.11
F-value	2.42	9.76
P-value	0.097	<0.001

Pupal duration time (d) log transformed prior to analysis. Means in column followed by the same letter are not significantly different ( $\alpha = 0.05$ ; PROC MIXED [Littell et al. 1996]).

<sup>a</sup> Non-Bt variety (Table 1).

<sup>b</sup> Bt-variety containing a herbicide-resistance gene (Table 1).

<sup>c</sup> Bt-variety derived from DP 5415 (Table 1).

significantly less than for those larvae reared on NuCOTN 33B or DP 451B/RR ( $P < 0.01$ ).

There were significant differences ( $P < 0.05$ ) in larval and pupal weights, and time to pupation between the two Bt varieties. Mean larval weights at 16 DAE were significantly higher ( $P < 0.05$ ) for those larvae fed DP 451B/RR (66.7 mg) than for those fed NuCOTN 33B (32.0 mg) ( $t = 4.31$ ,  $df = 140$ ,  $P < 0.01$ ) (Fig. 3). Likewise, mean pupal weights were significantly higher ( $P < 0.05$ ) for those larvae fed DP 451B/RR (169.2 mg) than for larvae fed NuCOTN 33B (151.4 mg) ( $t = 2.41$ ,  $df = 124$ ,  $P = 0.02$ ) (Fig. 4). In addition, mean time to pupation was significantly less ( $P < 0.05$ ) for those larvae fed DP451B/RR (29.0 d) than for larvae fed NuCOTN 33B (32.1 d) ( $t = -4.06$ ,  $df = 130$ ,  $P < 0.01$ ) (Fig. 5).

It appears that the  $\delta$ -endotoxin in transgenic Bt cotton may have an effect on the pupal stage. Although not always significant, fall armyworms remained in the pupal stage longer when larvae fed on a non-Bt rather than on either Bt cotton variety. Although there were no significant differences in pupal stage duration ( $P > 0.05$ ) for males among the non-Bt and Bt varieties, there were significant differences for females among the varieties (Table 4).

### Discussion

The assumption that all Bt varieties express similar levels of CryIA(c)  $\delta$ -endotoxin, and thus have an identical effect on intrinsically tolerant Lepidopteran survival and development, appears to be inaccurate. Our study using a commercially available Bt quantification ELISA corroborates the results of Holt (1998), Fitt (1998), Sachs et al. (1998) and Greenplate (1999) that showed that the level of CryIA(c)  $\delta$ -endotoxin decreases in various plant parts throughout the growing season. Information on the precise factors that affect  $\delta$ -endotoxin expression among plants appears to be limited at this time (Sachs et al. 1998). However, studies conducted with Australian Bt varieties have shown that the 35S promoter driving expression of CryIA(c)  $\delta$ -endotoxin decreases in field grown plants as they age throughout the growing season (Finnegan et al. 1998). In addition, our bioassays and  $\delta$ -endotoxin levels among various plant parts support many consultants and grower observations that more bollworms are found feeding and damaging Bt cotton fruit (i.e., square buds, flowers and bolls) compared with green tissue (i.e., leaves, square and boll bracts) (J.J.A., unpublished data).

It is noteworthy that high mortality was observed in larval bioassays involving leaves collected late in the season (data not shown), but very low-levels of  $\delta$ -endotoxin were detected. This discrepancy was attributed to low nutritional value or increased levels of secondary compounds [e.g., condensed tannins (Olsen et al. 1998)] in these late-season leaves compared with early-season leaves, because control mortality using non-Bt leaves was also extremely high (>95%). Thus, solely relying on larval bioassays to assess the effectiveness of  $\delta$ -endotoxin may not always be accu-

rate unless proper controls and high-quality insects are used.

By not providing a high-dose strategy to control the intrinsically tolerant Lepidoptera (i.e., armyworms, loopers, and bollworms), managing resistance to these insects may be further complicated by differential expression of  $\delta$ -endotoxin among plant parts and varieties (Sachs et al. 1998). Although complex interactions are involved, models developed by Peck et al. (1999) showed that delays in larval development time for tobacco budworms feeding on Bt cotton compared with a non-Bt refuge may affect the rate of resistance development. In another study involving the pink bollworm, researchers showed that Bt-resistant larvae feeding on Bt cotton take 5–6 d longer to develop into moths compared with Bt-susceptible larvae feeding on non-Bt cotton (Liu et al. 1999). Our data show that larval development of the fall armyworm was increased by 3 d when fed NuCOTN 33B compared with DP 451B/RR, which was ample time for these populations to differentiate in the laboratory. The implications of differential expression among commercially available Bt cotton varieties need to be further characterized to determine the impact on population dynamics that may be a critical factor in managing resistance.

Because of the relative ease in conducting this quantification assay, the utility of using this system to provide information to the grower concerning varietal choices may be more common in the future. With >25 commercially available transgenic Bt cotton varieties to choose from, more information to determine which variety offers the best insect control is clearly needed.

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### References Cited

- Adamczyk, J. J., Jr., J. W. Holloway, G. E. Church, B. R. Leonard, and J. B. Graves. 1998a. Larval survival and development of the fall armyworm (Lepidoptera: Noctuidae) on normal and transgenic cotton expressing the *Bacillus thuringiensis* CryIA(c)  $\delta$ -endotoxin. *J. Econ. Entomol.* 91: 539–545.
- Adamczyk, J. J., Jr., V. J. Mascarenhas, G. E. Church, B. R. Leonard, and J. B. Graves. 1998b. Susceptibility of conventional and transgenic cotton bolls expressing the *Bacillus thuringiensis* CryIA(c)  $\delta$ -endotoxin to fall armyworm (Lepidoptera: Noctuidae) and beet armyworm (Lepidoptera: Noctuidae) injury. *J. Agric. Entomol.* 15: 163–171.
- Adamczyk, J. J., Jr., B. R. Leonard, and J. B. Graves. 1999. Toxicity of selected insecticides to fall armyworms (Lepidoptera: Noctuidae) in laboratory bioassay studies. *Fla. Entomol.* 82: 230–236.

- Caprio, M. A. 1994. *Bacillus thuringiensis* gene development and resistance management in single- and multi-tactic environments. *Biocontrol Sci. Technol.* 4: 487–497.
- Finnegan, E. J., D. J. Llewellyn, and G. P. Fitt. 1998. What's happening to the expression of the insect protection in field-grown Ingard cotton? pp. 291–297. *In Proceedings, The Ninth Australian Cotton Conference*. The Cotton Research and Development Corporation, Conrad Jupiters, Broadbeach, Australia.
- Fitt, G. P. 1998. Efficacy of Ingard cotton-patterns and consequences, pp. 233–245. *In Proceedings, The Ninth Australian Cotton Conference*. The Cotton Research and Development Corporation, Conrad Jupiters, Broadbeach, Australia.
- 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.
- Holt, H. E. 1998. Season-long monitoring of transgenic cotton plants- development of an assay for the quantification of *Bacillus thuringiensis* insecticidal crystal protein, pp. 331–335. *In Proceedings, The Ninth Australian Cotton Conference*. The Cotton Research and Development Corporation, Conrad Jupiters, Broadbeach, Australia.
- Jenkins, J. N., W. L. Parrott, and J. C. McCarty, Jr. 1992. Effects of *Bacillus thuringiensis* genes in cotton on resistance to lepidopterous insects, pp. 606–607. *In Proceedings, Beltwide Cotton Conferences*. National Cotton Council, Memphis, TN.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Cary, NC.
- Liu, Y.-B., B. E. Tabashnik, T. J. Dennehy, A. L. Patin, and A. C. Bartlett. 1999. Development time and resistance to Bt crops. *Nature (Lond.)* 400: 519.
- Luttrell, R. G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. *J. Econ. Entomol.* 92: 21–32.
- MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. F. Fischhoff, and R. L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. *J. Invertebr. Pathol.* 56: 258–266.
- Olsen, K. M., J. C. Daly, and G. J. Tanner. 1998. The effect of cotton condensed tannin on the efficacy of the CryIac  $\delta$ -endotoxin of *Bacillus thuringiensis*, pp. 337–342. *In Proceedings, The Ninth Australian Cotton Conference*. The Cotton Research and Development Corporation, Conrad Jupiters, Broadbeach, Australia.
- Peck, S. L., F. Gould, and S. P. Ellner. 1999. Spread of resistance in spatially extended regions of transgenic cotton: implications for management of *Heliothis virescens* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 92: 1–16.
- Sachs, E. S., J. H. Benedict, D. M. Stelly, J. F. Taylor, D. W. Altman, S. A. Berberich, and S. K. Davis. 1998. Expression and segregation of genes encoding CryIA insecticidal proteins in cotton. *Crop Sci.* 38: 1–11.
- Steel, R.G.D., and J. H. Torrie. 1980. Enumeration data II: contingency tables, pp. 495–520. *In Principles and procedures of statistics: a biometrical approach*. McGraw-Hill, New York.
- Stone, T. B., and S. R. Sims. 1993. Geographic susceptibility of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis*. *J. Econ. Entomol.* 86: 989–994.
- Wilson, F. D., H. M. Flint, W. R. Deaton, D. A. Fischhoff, F. J. Perlak, T. A. Armstrong, R. L. Fuchs, S. A. Berberich, N. J. Parks, and B. R. Stapp. 1992. Resistance of cotton lines containing a *Bacillus thuringiensis* toxin to pink bollworm (Lepidoptera: Gelechiidae) and other insects. *J. Econ. Entomol.* 85: 1516–1521.

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