BT IN NEW MEXICO: BEET ARMYWORM SUSCEPTIBILITY AND EXPRESSION OF RESISTANCE IN SELECTED CULTIVARS

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Abstract

Bioassays were used to evaluate the effects of plant nitrate levels, diet, instar, and leaf developmental stage on resistance levels in Bt transgenic cotton as shown by mortality of beet armyworm, *Spodoptera exigua*, when fed leaves from three varieties of Bt cotton plants and their recurrent parents. It was found that high nitrate levels may affect plant expression of Bt resistance and that *S. exigua* larvae fed on artificial diet as neonates experienced significantly less mortality than those fed exclusively on Bt or recurrent parent leaves. Only first instar *S. exigua* were consistently susceptible to Bt cotton, though mortality was noted in second instars. Leaf developmental stage had no effect on mortality, but variety may influence resistance levels in Bt cotton.

Introduction

Cotton plants, *Gossypium hirsutum* L., genetically modified to express endotoxin proteins from *Bacillus thuringiensis* Berliner var. *kurstaki* for control of lepidopteran pests, were planted on extensive acreage for the first time in 1996. Available varieties generally performed well, though there were notable exceptions. Multiple insecticide applications were made to Bt cotton for the control of bollworm, *Helicoverpa zea*, and other lepidopteran pests in seven southeastern states and the Brazos River bottom area in Texas (Hardee & Herzog 1997). This year, the southwestern portion of the cotton belt also experienced *H. zea* damage. Field observations have suggested that reduction in plant resistance may be linked to highly stressed plants or high rates of nitrogen (M. Bates, *pers. comm*.). Increased plant nitrate levels have been indirectly associated with increased insect survivorship (Scriber 1984).

In addition to evaluating the affects of insect diet, instar, and leaf developmental stage, our main objective was to determine if nitrogen affects resistance of Bt cotton to a lepidopteran pest. Since Halcomb et al. (1996) have shown that Bt cotton is highly toxic to 1^{st}-4^{th} instar *H. zea* and tobacco budworm, *Heliothis virescens*, we chose to utilize the beet armyworm, *Spodoptera exigua*, a lepidopteran that is naturally less susceptible to Bt *kurstaki* and therefore more likely to be sensitive to changes. The sensitivity of this insect may be used to detect differences that could also affect resistance levels in other more susceptible lepidopterans such as the pink bollworm, *Pectinophora gossypiella*, *H. zea*, and *Heliothis virescens*.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1246-1248 (1998)
National Cotton Council, Memphis TN

Materials & Methods

Field Plots

Field plots were set up in a complete randomized block design with three Bt transgenic varieties (DP33B, DP35B, DP90B) and their respective recurrent parents (DP5415, DP5690, DP90).

Treatments:
- low nitrogen (no N added)
- low nitrogen and pix (a growth regulator)
- high nitrogen (62.8 kg N/ha, June 26,1997)
  (28 kg N/ha, July 21, 1997)
- high nitrogen and pix

Each treatment was replicated six times with petioles collected weekly to determine plant nitrate levels using a Cardy Meter (Spectrum Technologies, Inc. Plainfield, IL) and the ion selective electrode for nitrate nitrogen method of analysis.

Bioassay

One leaf and moistened sheet of filter paper /237 ml cup
Five larvae/cup/treatment (Table 1) replicated a minimum of six times
Checked for mortality every 48 hours until terminated
Data analyzed using GLM (SAS Institute, Inc. 1996)

Results

Test 1

There was no significant difference in mortality due to variety (P<0.46), Bt endotoxin (P<0.84), or nitrogen (P<0.84) with second instar *S. exigua* reared on artificial diet after 144 hrs. (Fig. 1).

Test 2

Again, there was no difference in mortality rates between nitrogen levels (P<0.60) with second instars reared on artificial diet after 240 hrs. (Fig. 2). There was significantly higher, albeit somewhat low, mortality in the Bt varieties compared to the recurrent parents (P<0.03). Bt varieties produced 14% mean mortality compared to 6% in the recurrent parents. Bt variety DP33B produced 27% mortality, significantly higher then DP35B with 6%, and DP90B with 12% (P<0.03). Differences were also noted between DP33B and DP90B at low and high leaf nitrate levels.

Test 3

In the third test, we examined the effects of instar, leaf developmental stage, and leaf nitrate concentration on expression of plant resistance or *S. exigua* susceptibility. There was significantly higher mortality of first instar *S. exigua* when compared to second instars, 28% and 0% respectively, after 120 hrs. (P<0.0008) (Fig. 3). Leaf developmental stage and nitrate concentration had no influence on mortality (*P*<0.69 and *P*<0.63).

Test 4
In the final test, effect of neonate diet and leaf nitrate concentration on expression of plant resistance or *S. exigua* susceptibility was examined. Neonate feeding on artificial diet significantly increased survival of first instars on DP90B and DP90 (*P*<0.004) (Fig. 4). There was a significant interaction between the effect of leaf nitrate concentration and neonate diet on mortality (*P*<0.04). *S. exigua* fed exclusively on DP90B leaves experienced 6% mortality at high and 38% mortality at low nitrate concentrations. There was no significant difference in mortality between insects fed on artificial diet and DP90 at high and low nitrate concentrations as neonates (P<0.21). Neonates fed diet followed by DP90B leaves with low and high nitrate levels plus pix had 4% and 0% mortality respectively.

Discussion & Conclusions

Nitrogen Effects
High plant nitrate levels may affect plant expression of Bt resistance. However, it is likely that nitrate levels will only affect expression of resistance under very particular conditions. High leaf nitrate concentrations were associated with lower mortality in *S. exigua* fed DP35B as second instars and DP90B and as neonates and second instars (Fig. 2 & 4). The opposite was observed for *S. exigua* fed DP33B as second instars (Fig. 2). Nitrogen rates in future studies will exceed the 91 kg/ha introduced this year to produce greater differences in leaf nitrate concentrations.

Diet & Leaf Stage Effects
S. exigua larvae which fed on artificial diet as neonates experienced less mortality then those which fed exclusively on Bt or recurrent parent leaves, even when later fed on Bt leaves as first instars (Fig. 4). In future studies all neonates will feed on leaves. Fully developed leaves produced levels of mortality equivalent to those produced by less fully developed leaves (Fig. 3), thereby removing a concern about leaf uniformity.

Instar Effects
Only first instar *S. exigua* are consistently susceptible to Bt cotton (Fig. 3). While we did see significantly higher mortality in Bt varieties compared to the recurrent parents in Fig. 2, the levels of mortality (6-27%) were too low to be meaningful. Differences in expression by varieties are likely to affect the level of mortality by instar. For example, second instar *S. exigua* were susceptible to DP33B but not DP90B (Fig. 2). DP90B only produced significant mortality in first instars (Fig. 3).

Varietal Effects
There are likely varietal differences in expression of resistance. Although second instar *S. exigua* is not highly susceptible to Bt endotoxins in Bt cotton, we were able to detect significant differences in mortality in Test 2 (Fig. 2). DP33B, which had the highest level of mortality (27%) also had comparatively high mortality (41%) when fed to first instars reared on artificial diet (Fig 3.). DP90B which had the lowest level of resistance (6%) in Test 2 (Fig. 2), did not have significant levels of resistance with first instars reared on artificial diet (Fig. 4).

The potential of environmental factors and plant genotype to affect the expression of resistance in Bt cotton to all target pests needs to be further evaluated. The possibility for such factors to affect field performance must be considered when making scouting, varietal selection, and management recommendations.

Citations

Halcomb, J. L., J. H. Benedict, B. Cook and D. R. Ring. 1996. Survival and growth of bollworm and tobacco budworm on nontransgenic and transgenic cotton expressing a CryIA insecticidal protein (Lepidoptera: Noctuidae). *Environmental Entomology*. 25: 250-255.

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Scriber, J. M. 1984. Nitrogen nutrition of plants and insect invasion. In R. D. Hauck [ed.], *Nitrogen in Crop Production*. American Society of Agronomy, Madison, WI. 441-460.

SAS Institute, Inc. 1996

Acknowledgments

This work was completed in part with funds from Monsanto and Cotton Incorporated. A special thanks to A. M. Flores, C. French, M. Wusaty, and C. E. Barnes for their assistance.

Table 1. Treatments for the four tests discussed within this presentation.

			Cotton Variety		Nitrogen		Neonate
Test	n	Instar	Transgenic	Recurrent Parent	Levels	LDS	Diet
1	100	2	DP33B, DP90B	DP5415, DP90	low, high	fully	artl
2	50	2	DP33B, DP90B DP35B	DP5415, DP90 DP5690	low, high	fully	artl
3	30	1, 2	DP33B	DP5415	low, high	not, fully	artl
4	30	1	DP90B	DP90	low, high	fully	artl, DP90 DP90B

LDS = Leaf Developmental Stage
artl = artificial

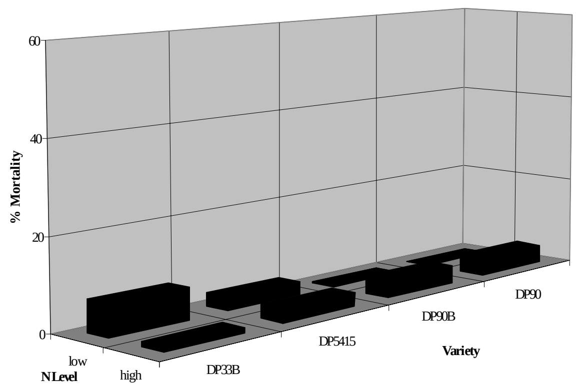

Figure 1. Effect of nitrogen levels on mortality of second instar *S. exigua* feeding on either Bt or recurrent parent cotton leaves.

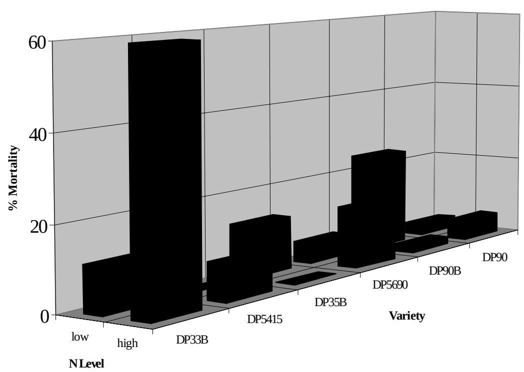

Figure 2. Effect of nitrogen level on mortality of second instar *S. exigua* fed on either Bt or recurrent parent cotton leaves.

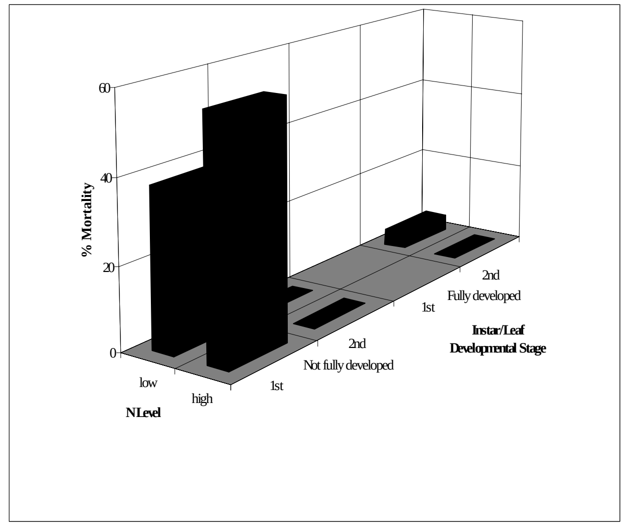

Figure 3. Effects of *S. exigua* instar DP33B leaf developmental stage, and nitrogen on mortality.

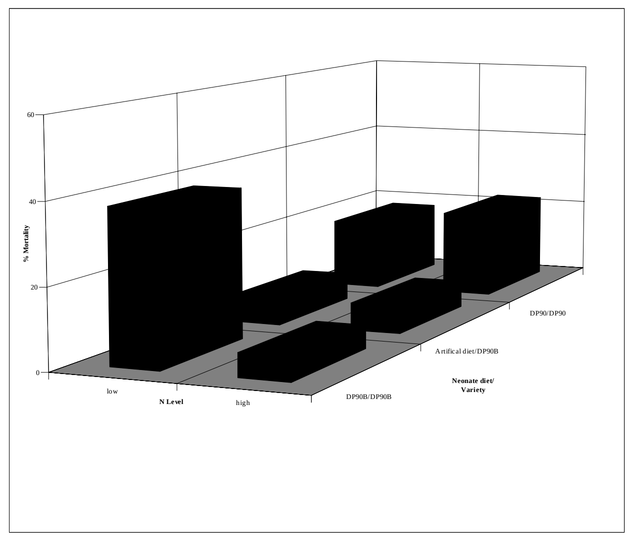

Figure 4. Effect of neonate diet and nitrogen on mortality of first instar *S. exigua* fed on DP90B or the recurrent parent.