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EFFECTS OF LEAF AGE ON EFFICACY OF BOLLGARD II AGAINST BEET ARMYWORM, *SPODOPTERA EXIGUA*

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Abstract

Three cotton varieties were grown in the field and bioassayed in the laboratory against the beet armyworm (BAW), *Spodoptera exigua*. A non-B.t. standard, DPL 5415, was compared to NuCotton 33B, which produces the Cry1Ac protein, and NuCotton 33BII, which produces both the Cry1Ac and Cry2Ab proteins. Four leaf ages (3rd, 6th, 9th and 12th leaf from the terminal) were used in the bioassay to evaluate effects of leaf age. Bioassays were initiated with one day old larvae and run for 10 days or 5 day old larvae and run for 8 days. The bioassays generally showed increased mortality and decreased weight of surviving larvae with increased leaf age for all three varieties. NuCotton 33BII showed excellent activity against BAW. The greatest survival in NuCotton 33BII occurred on the younger leaves, but surviving larvae had grown little or none and were generally about 1/10 the weight of larvae on DPL 5415. It is extremely unlikely that any of the larvae on NuCotton 33BII would have been able to develop to the adult stage.

Introduction

Bollgard cotton varieties, which produce Cry1Ac protein with activity against lepidopterous insects, have found wide acceptance throughout much of the cotton belt with acreage planted to these varieties growing from about 1.8 million acres in 1996 to over 4.4 million acres in 2000. These varieties have shown excellent activity against tobacco budworm and pink bollworm, but have not provided as high an efficacy against other lepidopterous pests such as the beet armyworm and loopers. A new transgenic variety, that is scheduled for commercial production in the near future, has an additional gene insertion that codes for production of a second protein, Cry2Ab. This variety produces both proteins and has maintained its efficacy against previously controlled species while showing increased efficacy against a much wider range of lepidopterous pests including bollworm, armyworms, loopers and leaf perforators (Adamezyk et al. 2001, Norman and Sparks 2001, Ridge et al. 2000).

Studies on protein expression in B.t. cottons have typically indicated variability with plant structure and plant age. Within leaf tissue, protein expression has been shown to decline in terminal leaves throughout the season (Adamezyk et al. 2000, Greenplate et al. 2001), as well as within individual leaves as they age (Penn et al. 2001). Thus, foliage feeding insects would encounter lower protein levels as they moved down the plant. Bollworm larvae have shown behavioral responses to B.t. cotton with increased movement down the plant (Gore, et al. 2001). If defoliating larvae respond similarly, they may have greater chance for survival as they move down the plant. The purpose of our studies were to investigate potential survival of beet armyworm on young and old leaves of B.t. cottons.

Materials and Methods

Field plots were planted on 20 March, 2001, at the Texas A&M Research and Extension Center's Hiler Farm, Weslaco, Texas. Individual plots were 4 rows (on 40 inch centers) by 50 feet. Each variety was replicated four times in a randomized complete block design. Three varieties were planted: DPL 5415, DPL NuCotton 33B and DPL NuCotton 33BII. DPL 5415 does not contain the genetics for production of B.t. toxins. NuCotton 33B is a commercially available variety that produces the Cry1Ac protein. NuCotton 33BII was developed by insertion of the genetics for production of the Cry2Ab protein into NuCotton 33B, thus, this variety produces both proteins.

Two laboratory bioassays were conducted to evaluate the activity of the field grown B.t. cottons against beet armyworm. Bioassays were conducted on two dates, with leaves of four different ages on each date, to evaluate leaf age effects. The first bioassay was initiated on 14 June with one day old larvae. The second bioassay was initiated on 3 July with five day old larvae in order to reduce mortality and allow for comparisons of leaf age effects. The beet armyworm larvae used in these tests were obtained from a laboratory colony maintained at the USDA-ARS laboratory in Weslaco, Texas. Larvae were held on artificial diet until used.

For collection of leaf material, five plants were randomly selected in each field plot. On each plant, the 3rd, 6th, 9th and 12th leaf from the terminal were collected and placed in bags. The leaves of each age were combined for all five plants and all four

replications (i.e. all 3rd leaves from a variety were combined; all 6th leaves from a variety were combined; etc.). In the laboratory, 7/8 inch leaf disks were cut from the leaves (3 or 4 from each leaf) and placed in plastic bags. Again, leaf disks were combined by variety and leaf age.

Individual leaf disks were placed in 3/4 ounce plastic cups which contained a 7/8 inch diameter filter paper moistened with 2 drops of reverse osmosis water. After the leaf was placed in the cup, a single beet armyworm larva was placed on the leaf and a paper lid was placed on the cup. Each variety/leaf age treatment was replicated four times, with 15 individual larvae per replication. Larvae were held at room temperature.

Larvae were checked for mortality every two days. At each mortality check, surviving larvae were supplied with a freshly collected leaf disk corresponding to the variety and leaf age of the original treatment, and the filter paper was moistened if needed. At 10 days (for the bioassay initiated with 1 day old larvae) or 8 days (for the bioassay initiated with 5 day old larvae) after the initial exposure, surviving larvae for each replication of each variety/leaf age treatment were placed in a petri dish and weighed to determine average larval weights (total weight divided by number of surviving larvae).

Larval mortality and surviving larval weights were analyzed with PROC GLM of PC-SAS. Where significant differences were detected ($P<0.05$), means were separated with Duncan's Multiple Range Test ($P<0.05$).

Results and Discussion

Mortality was generally low in the first check (2 days after exposure) in each test and generally remained so for the younger leaves of DPL 5415, the non-B.t. variety. Thus, it can be assumed that the majority of the mortality in these studies resulted from treatment effects rather than handling or disease (no visual evidence of disease was noted as well). Analyses indicated that cotton variety and leaf age had significant effects on percent mortality and larval weights on the last check date in both experiments. There was also a significant interaction of these two factors. In general, NuCotton 33BII provided the highest mortality and lowest surviving larval weights across all leaf ages (Table 1). Older leaves tended to also provide higher mortalities and lower surviving larval weights across all varieties (Table 2).

Additional analyses were conducted to evaluate effects of varieties within leaf age categories (Tables 3 and 5) and leaf age effects within varieties (Table 4 and 6). The trends are similar for both bioasssays (although they are 'cleaner' for the bioassay initiated with 5 day old larvae), and are similar to the results of the combined analysis. Within each leaf age category, NuCotton 33BII generally provided higher mortality and lower surviving larval weights and within varieties the older leaves provided higher mortality and lower larval weights.

The interaction of variety and leaf age shows as a more rapid increase in mortality with increased leaf age in the NuCotton 33BII (as compared with smaller increases seen in the other two varieties) and a decrease in leaf age effect on larval weights within NuCotton 33BII (as compared to the same effect in the other two varieties). The rapid increase in mortality with older leaves within NuCotton 33BII suggests that the mortality factors associated with older leaves are likely synergistic with B.t. proteins. This is best seen in the bioassay initiated with 5 day old larvae. Within the other two varieties, mortality remained near zero even with the oldest leaves. Within NuCotton 33BII, mortality increased dramatically with older leaves, with an increase of 45% between the 6th and 9th leaf. If the B.t. proteins were acting independently of the leaf age factors, mortality should have remained relatively constant or reduced as protein levels are reported to decline with leaf age. Contrary to the mortality data, weight of surviving larvae were not affected as much by leaf age within NuCotton 33BII as compared to the other varieties. This appears to be an effect of severe weight reduction with the youngest leaves, resulting in very little room for additional impact from older leaves.

These data support earlier work that shows high efficacy of Bollgard II against the BAW. However, contrary to decreased B.t. protein expression in older foliage, these results suggest that terminal growth is the most likely location for survival of B.t. tolerant defoliating species.

References

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Table 1. Mortality and surviving larval weights of beet armyworm larvae feed B.t. and non-B.t. cotton foliage, data averaged across four leaf ages.

	1 day old larvae at 10 DAE		5 day old larvae at 8 DAE	
Variety	**% mortality**	**g/larvae**	**% mortality**	**g/larvae**
NuCotton 33BII	88.3 a	0.0017 c	46.3 a	0.0059 c
NuCotton 33B	20.4 b	0.0178 b	0.4 b	0.0327 b
DPL 5415	12.9 b	0.0285 a	0.4 b	0.0377 a

Means within columns followed by the same letter are not significantly different (DMRT, P=0.05).
DAE = days after (initial) exposure.

Table 2. Mortality and surviving larval weights of beet armyworm larvae feed cotton leaves of different ages, data averaged across three cotton varieties including B.t. and non-B.t. varieties.

Leaf number	1 day old larvae at 10 DAE		5 day old larvae at 8 DAE	
(from terminal)	**% mortality**	**g/larvae**	**% mortality**	**g/larvae**
3	28.3 b	0.0200 b	3.3 c	0.0364 a
6	33.9 b	0.0259 a	8.9 b	0.0285 b
9	45.6 a	0.0231 ab	23.9 a	0.0223 c
12	54.4 a	0.0086 c	26.7 a	0.0145 d

Means within columns followed by the same letter are not significantly different (DMRT, P=0.05).
DAE = days after (initial) exposure.

Table 3. Percent mortality and surviving larval weights of beet armyworm larvae feed B.t. and non-B.t. cotton. Bioassay initiated with one day old larvae.

	Percent Mortality at indicated days after exposure (DAE)					
Variety	**2 DAE**	**4 DAE**	**6 DAE**	**8 DAE**	**10 DAE**	**g/larvae at 10 DAE**
3rd leaf from the terminal						
DPL 5415	0.0 a	1.7 a	1.7 a	1.7 a	1.7 b	0.0470 a
NuCotton 33B	1.7 a	11.7 a	18.3 a	18.3 a	18.3 b	0.0116 b
NuCotton 33B II	21.7 a	38.3 a	50.0 a	51.7 a	65.0 a	0.0014 c
6th leaf from the terminal						
DPL 5415	3.3 a	11.7 b	11.7 b	11.7 b	13.3 b	0.0206 b
NuCotton 33B	0.0 a	0.0 b	0.0 b	0.0 b	0.0 b	0.0429 a
NuCotton 33B II	20.0 a	51.7 a	68.3 a	75.0 a	88.3 a	0.0024 c
9th leaf from the terminal						
DPL 5415	0.0 b	0.0 c	1.7 c	1.7 c	1.7 c	0.0377 a
NuCotton 33B	18.3 b	25.0 b	25.0 b	31.7 b	35.0 b	0.0084 b
NuCotton 33B II	61.7 a	98.3 a	100.0 a	100.0 a	100.0 a	----
12th leaf from the terminal						
DPL 5415	3.3 b	30.0 b	33.3 b	35.0 b	35.0 b	0.0088 a
NuCotton 33B	10.0 b	23.3 b	23.3 b	26.7 b	28.3 b	0.0085 a
NuCotton 33B II	63.3 a	100.0 a	100.0 a	100.0 a	100.0 a	----

Means within columns and leaf age followed by the same letter are not significantly different (DMRT, P=0.05).
DAE = days after (initial) exposure.

Table 4. Percent mortality and surviving larval weights of beet armyworm larvae feed leaf material of four ages. Bioassay initiated with one day old larvae.

Leaf number	**Percent mortality at indicated days after exposure (DAE)**					
(from terminal)	**2 DAE**	**4 DAE**	**6 DAE**	**8 DAE**	**10 DAE**	**g/larvae at 10 DAE**
DPL 5415						
3	0.0 a	1.7 b	1.7 b	1.7 b	1.7 b	0.0470 a
6	3.3 a	11.7 b	11.7 b	11.7 b	13.3 b	0.0206 c
9	0.0 a	0.0 b	1.7 b	1.7 b	1.7 b	0.0377 b
12	3.3 a	30.0 a	33.3 a	35.0 a	35.0 a	0.0088 d
NuCotton 33B						
3	1.7 a	11.7 a	18.3 a	18.3 a	18.3 a	0.0116 b
6	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0429 a
9	18.3 a	25.0 a	25.0 a	31.7 a	35.0 a	0.0084 b
12	10.0 a	23.3 a	23.3 a	26.7 a	28.3 a	0.0085 b
NuCotton 33B II						
3	21.7 b	38.3 b	50.0 b	51.7 c	65.0 b	0.0014 a
6	20.0 b	51.7 b	68.3 b	75.0 b	88.3 a	0.0024 a
9	61.7 a	98.3 a	100.0 a	100.0 a	100.0 a	----
12	63.3 a	100.0 a	100.0 a	100.0 a	100.0 a	----

Means within columns and variety followed by the same letter are not significantly different (DMRT, P=0.05).
DAE = days after (initial) exposure.

Table 5. Percent mortality and surviving larval weights of beet armyworm larvae feed B.t. and non-B.t. cotton. Bioassay initiated with five day old larvae.

	Percent Mortality at indicated days after exposure (DAE)				
Variety	**2 DAE**	**4 DAE**	**6 DAE**	**8 DAE**	**g/larvae at 8 DAE**
3rd leaf from the terminal					
DPL 5415	0.0 a	0.0 a	0.0 a	0.0 a	0.0482 a
NuCotton 33B	0.0 a	0.0 a	0.0 a	0.0 a	0.0509 a
NuCotton 33B II	0.0 a	1.7 a	5.0 a	10.0 a	0.0100 b
6th leaf from the terminal					
DPL 5415	0.0 a	0.0 a	0.0 b	0.0 b	0.0418 a
NuCotton 33B	0.0 a	0.0 a	0.0 b	0.0 b	0.0379 a
NuCotton 33B II	0.0 a	6.7 a	16.7 a	26.7 a	0.0059 b
9th leaf from the terminal					
DPL 5415	0.0 a	0.0 b	0.0 b	0.0 b	0.0374 a
NuCotton 33B	0.0 a	0.0 b	0.0 b	0.0 b	0.0253 b
NuCotton 33B II	0.0 a	20.0 a	65.0 a	71.7 a	0.0042 c
12th leaf from the terminal					
DPL 5415	0.0 a	1.7 b	1.7 b	1.7 b	0.0234 a
NuCotton 33B	0.0 a	0.0 b	0.0 b	1.7 b	0.0166 b
NuCotton 33B II	0.0 a	31.7 a	65.0 a	76.7 a	0.0036 c

Means within columns and leaf age followed by the same letter are not significantly different (DMRT, P=0.05).
DAE = days after (initial) exposure.

Table 6. Percent mortality and surviving larval weights of beet armyworm larvae feed leaf material of four ages. Bioassay initiated with five day old larvae.

Leaf number	**Percent mortality at indicated days after exposure (DAE)**				
(from terminal)	**2 DAE**	**4 DAE**	**6 DAE**	**8 DAE**	**g/larvae at 8 DAE**
DPL 5415					
3	0.0 a	0.0 a	0.0 a	0.0 a	0.0482 a
6	0.0 a	0.0 a	0.0 a	0.0 a	0.0418 ab
9	0.0 a	0.0 a	0.0 a	0.0 a	0.0374 b
12	0.0 a	1.7 a	1.7 a	1.7 a	0.0234 c
NuCotton 33B					
3	0.0 a	0.0 a	0.0 a	0.0 a	0.0509 a
6	0.0 a	0.0 a	0.0 a	0.0 a	0.0379 b
9	0.0 a	0.0 a	0.0 a	0.0 a	0.0253 c
12	0.0 a	0.0 a	0.0 a	1.7 a	0.0167 d
NuCotton 33B II					
3	0.0 a	1.7 b	5.0 b	10.0 b	0.0100 a
6	0.0 a	6.7 b	16.7 b	26.7 b	0.0059 b
9	0.0 a	20.0 a	65.0 a	71.7 a	0.0042 bc
12	0.0 a	31.7 a	65.0 a	76.7 a	0.0036 c

Means within columns and variety followed by the same letter are not significantly different (DMRT, P=0.05).
DAE = days after (initial) exposure.