2005 Beltwide Cotton Conferences, New Orleans, Louisiana - January 4 - 7, 2005

LEPIDOPTEROUS LARVAL MORTALITIES AND CRY1AC TOXIC PROTEIN IN BOLLGARD®, NON-BOLLGARD® AND ROUNDUP READY® COTTONS

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Abstract

Cottons containing genes that mediate production of insect toxic crystalline protein have had a major positive impact on pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), and other lepidopterous insects integrated pest management. Over 70% of Arizona's cotton acreage is now planted with Bt cottons. We evaluated several Bt and two non-Bt cottons for effects on PBW, cabbage looper (CL), *Trichoplusia ni* (Hübner), beet armyworm (BAW), *Spodoptera exigua* (Hübner) and tobacco budworm (TBW), *Heliothis virescens* (Fabricius) mortalities. PBW and TBW were highly susceptible to all Bt cottons tested during 72 h feeding periods. BAW was the most tolerant to effects of the toxic proteins, although feeding periods were of minimal time and high mortality would probably occur with longer exposure periods. High mortality of CL occurred following feeding on the BGII® cotton compared with other Bt cottons tested.

Introduction

NuCOTN 33B®, Bollgard® (BG) transgenic cotton containing the gene encoding the Cry1Ac insect toxic protein was first available in Arizona for pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), control commercially in 1996. Since 1997, the preponderance of Arizona's upland cotton acreage has been in Bt cottons. Cotton yields have increased and farmers accepting Bt technology have realized increased profits per farm and reduction in conventional insecticide for PBW control (Frizvold et al. 2000). A second Bt gene (Bollgard II® (BGII), Monsanto Co., St. Louis, MO) encodes for a different insect toxic crystalline protein. Commercial cottons with BG alone or BGII in each case with the Roundup Ready (RR) herbicide resistance gene have dramatically increased the options for incorporating the new technologies into the cotton grower's arsenal. In 2004, we compared PBW larval mortalities and measured insect Cry1Ac toxins in Delta and Pineland non-BG cotton (Delta and Pineland Company, Scott, MS), BG- or BGII-Roundup Ready® cottons. We also compared cabbage looper (CL), *Trichoplusia ni* (Hübner), beet armyworm (BAW), *Spodoptera exigua* (Hübner) and tobacco budworm (TBW), *Heliothis virescens* (Fabricius) mortalities feeding on leaves of the BG and non-BG cottons in laboratory bioassays.

Methods and Materials

Cotton seeds of Delta and Pineland (DPL) DPL 5415, DPL 5415 RR®, NuCOTN 33B®, DPL 449 BG/RR®, and DPL 424 BGII RR® were planted in four rows wide by 18 m long plots at the Western Cotton Research Laboratory, Phoenix, AZ on 23 April 2004. The experiment was conducted as a randomized complete block experiment with four replications of each cultivar. Standard grower practices were used during the season and cottons, after planting, were irrigated every 14 days from 22 April to 16 September 2004.

PBW larvae were obtained from the Western Cotton Research Laboratory (WCRL) colony raised on artificial diet (Bartlett and Wolf 1985). On 5 August, firm, immature green bolls (three weeks old) were harvested from each cotton plot and placed individually in 5 cm diameter x 7.5 cm tall polyethylene containers. Containers had 2.54 cm diameter, screen-covered holes for ventilation. Five, first-instar PBW larvae were placed on each boll. On day 10 following infestation, each boll was examined with the aid of a microscope and PBW larval entrance holes in carpel walls were counted. Bolls were dissected and all living and dead larvae counted.

On 10 September, leaves were picked at random from the top half of plants in each plot and trimmed to fit in 15.0-cm diameter x 1.5-cm deep plastic Petri dishes lined on the bottom with moist filter paper. BAW, CL and TBW were from the WCRL colonies reared on artificial diet (Henneberry and Kishaba 1966). Ten, second-instar larvae of each species were placed on foliage in each of five Petri dishes. Living and dead larvae were counted after 3 days.

The amounts of Cry1Ac protein in Bt cotton bolls on 5 August and cotton leaves of Bt cotton on 10 September were determined to compare amounts of the toxic protein using commercial enzyme-linked immunosorbent assays (ELISA). Materials, sample preparations, solutions, extractions, dilutions and assays were as described in the

Envirologix, Inc. Cry1Ab/Cry1Ac Plate kit (Envirologix, Portland, ME). Controls were DPL 5415 immature cotton bolls and leaves. Tissue samples were taken from the same bolls and leaves used for larval infestations. Tissue samples from bolls and leaves were weighed and placed in 1.5 cm microcentrifuge tubes. Samples were homogenized in extraction buffer with a fitted pestle. Cry2Ab toxic protein was not determined in DPL 424 BG II RR® cotton.

ANOVAs were conducted for all data when appropriate, and means were separated using the method of LSDs ($P \leq 0.05$) only when a significant F test occurred.

Results and Discussion

No living PBW larvae survived in Bollgard® cotton leaves compared with 53.2 and 31.2% survival on 5 August on DPL 5415 and 5415 RR non-Bollgard® cottons (Table 1). Numbers of entrance holes per boll were not significantly different. Less Cry1Ac toxic protein occurred in DPL 424 BG II RR® cotton compared with NuCOTN33B® or DPL 449 BG® RR® cottons, but due to variation the results were not statistically significant.

Following 72 h feeding periods on the experimental cottons, 97.5% CL larval mortality occurred on DPL 424 BGII RR cottons compared with 0.00 to 5.00% on all other cottons (Table 2). BAW mortalities ranged from 0.00 to 5.90% and the difference between cottons were not statistically significant. Total (100%) mortality of TBW larvae occurred following 72 h feeding on Bt cottons compared to 0.00% mortality on non-Bt cottons. Cry1Ac insect toxic protein found in leaf foliage was highest in DPL 449 BG® RR® (0.338 ppm) followed by NuCOTN33B® and least in DPL 424 BGII® RR® cotton leaves. The reasons are unexplained, but differences in extraction efficiency of the toxic protein may occur with different cultivars.

Some cabbage looper larvae tested positive for Cry1Ac protein following 72 h feeding periods on all Bt cottons tested (Table 3). None of the TBW larvae tested positive for Cry1Ac protein. PBW and BAW tested positive following 72 h feeding on two of the three Bt cottons. Again, the reasons remain unexplained, but may be related to larval size.

References

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Table 1. Mean percentages of pink bollworm (PBW) entrance holes and Cry1Ac protein in cotton boll samples.

Treatment	Percent Mortalities[1]	Entrance holes (No.)	Cry1Ac[2] (ppm)
	5 August 2004		
NuCOTN33B® (Bt)	100.0 a	2.5	0.572
DPL 424 BGII® RR® (Bt)	100.0 a	2.5	0.393
DPL 449 BG® RR® (Bt)	100.0 a	3.1	0.588
DPL 5415	53.2 b	2.7	0.000
DPL 5415 RR®	31.2 c	4.7	0.000
F; df 4,19	64.57	3.22	--
F; df 2,11			1.29
P[3]	0.000**	0.052 (NS)	0.34 (NS)

[1] Mean percentages of pink bollworm larval mortality on Bt and non-Bt cottons and mean number of PBW entrance holes, 4 replications. Means in the same column and sampling date not followed by the same letter are significantly different.
[2] Mean ppms of Cry1Ac insect toxic protein from cotton boll samples. Cry2Ab toxic protein in BGII cotton was not determined.
[3] ** statistically significant, NS = not significant.

Table 2. Mean percentage mortalities of cabbage looper, beet armyworm and tobacco budworm larvae following 72 h feeding periods on Bt and non-Bt cottons and Cry1Ac protein analysis (10 September 04, sampling).

Treatment	Species[1] CL	BAW	TBW	Cry1Ac (ppm)
NuCOTN33B® (Bt)	5.00 b	0.00	100.00 a	0.260 b
DPL 424 BGII® RR® (Bt)	97.50 a	5.90	100.00 a	0.072 c
DPL 449 BG® RR® (Bt)	0.00 b	0.00	100.00 a	0.338 a
DPL 5415	5.00 b	0.00	0.00 b	0.000 –
DPL 5415®	2.50 b	2.50	0.00 b	0.000 –
F[3]; df 4,19	21.40**	2.05 (NS)	>100.00**	28.49**
P	< 0.00	> 0.05	< 0.00	< 0.00

[1] CL = cabbage looper; BAW = beet armyworm; TBW = tobacco budworm
[2] Means of 5 replications in the same column not followed by the same letter are significantly different.
[3] ** statistically significant, NS = not significant.

Table 3. Numbers of cabbage looper (CL), beet army worm (BAW), tobacco budworm (TBW), or pink bollworm (PBW) negative for Cry1Ac insect toxic protein following 72 h feeding periods on Bt cotton foliage or bolls.

	Cultivar		
Species	NuCOTN33B®	DPL 424 BG II® RR®	DPL 449 BG® RR®
CL	0/4[1]	0/1	0/1
BAW	0/4	4/4	0/4
TBW	4/4	4/4	4/4
PBW	3/5	2/2	5/9

[1] No. negative/No. analyzed replications, 5 replications total, in all cases, except 9 for PBW.