

# **Cry1Ac Toxic Protein in Overwintered Volunteer and Annual Seeded NUCOTN 33B7 (Bt) and Deltapine (DPL) 5415 Cottons: Effects on Pink Bollworm (PBW) and Tobacco Budworm (TBW) Larval Mortalities.**

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## **Abstract**

*Season-long protection from pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), damage has been outstanding each year, since 1996, in NuCOTN 33B7 (Bt) commercial cotton plantings in Arizona. Cotton is a perennial plant and whether or not the insect toxic protein was expressed in overwintered Bt cotton was unknown. This could be a consideration in Arizona Bt resistance monitoring in the field since occasional overwintered volunteer cotton plants may occur. In 2002 we studied Cry1Ac levels in overwintered volunteer Bt cotton plants and determined their effects on PBW and tobacco budworm (TBW), *Heliothis virescens* F., larval mortalities. No TBW larvae survived three-day feeding periods on Bt leaves compared with < 3% TBW larval mortality feeding on DPL 5415 leaves. PBW larval mortality after three-day feeding on flower buds or seven-day feeding on Bt cotton bolls exceeded 98% compared with 40 to 41 % mortality feeding in DPL 5415 fruiting forms. Cry1Ac toxic protein in leaves, flower buds or cotton bolls of overwintered Bt cotton was not significantly different compared with 2002 seeded cotton.*

## **Introduction**

Major improvements in pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), population management have occurred as a result of the adoption of Bollgard<sup>7</sup> (Bt) cottons in Arizona (Flint and Parks 1999, Simmons et al. 1998, Patin et al. 1999, Sims et al 2001). Reduced production costs because of fewer insecticide applications and increased cotton yields have occurred (Frisvold et al. 2002). Bt cotton was first grown commercially in Arizona in 1996. Cry1Ac toxic protein levels in different cotton plant tissue appears to be affected by field site, sampling time, and variety (Greenplate et al. 2000). Significant reductions in Cry1Ac protein level occur with increasing days after planting (Greenplate et al. 1998, Greenplate 1999), but PBW efficacy has not decreased suggesting high susceptibility of PBW larvae to the lowest expressed levels of Cry1Ac toxic protein. (Henneberry and Jech 2000, Henneberry et al. 2001).

## Materials and Methods

**Field plot design.** DPL 5415 and NuCOTN 33B7 (Bt) cotton seeds were planted on 29 April 2002 in replicated (four) field plots that were seven rows wide by 60 feet long at the Western Cotton Research Laboratory at Phoenix, AZ. Overwintered volunteer plants of Bt and DPL 5415 cotton were found randomly occurring in the experimental field. Both Bt and DPL 5415 overwintered volunteer cultivars had been defoliated on 1 November 2001 and stalks cut on 15 January 2002. We tagged the overwintered volunteer plants and incorporated them into the current years experiment to compare Cry1Ac toxic protein levels in leaves, flower buds, and bolls from overwintered volunteer plants with toxic protein levels in cotton leaves, flower buds, and bolls from plants arising from seed planted in 2002. The experiment was conducted as a completely random design with four observations of volunteer and annual seeded cottons of each cultivar. Standard grower practices were used during the 2002 season and cottons were irrigated every 14 days during the experimental period from 3 May to 2 September, 2002.

**Insect bioassays.** PBW and TBW larvae used in the studies were from Western Cotton Research Laboratory colonies reared on artificial diets.

For TBW, we picked leaves from four plants each of volunteer and seeded Bt and volunteer and seeded DPL 5415 cotton plots on 28 June, 12 August and 16 September 2002. Two leaves from each plant were trimmed to fit in four inch diameter x 0.33 inch deep plastic petri dishes lined on the bottom with moist filter paper. Five, first instar TBW larvae were placed on leaves in each petri dish. Living and dead larvae were counted after three days.

For PBW larvae, one-third to one-half grown flower buds were picked on 23 July and 20 September 2002 from Bt and DPL 5415 plants. Each flower bud, on each sampling date, was placed individually in a two inch diameter x one third inch deep petri dish on a moistened filter paper placed on the dish bottom. First instar PBW larvae were placed on each flower bud. On day three following infestation PBW larval entrance holes and living and dead larvae in dissected flower buds were counted with the aid of a microscope.

On 29 August and 24 September 2002 firm, immature green bolls (21 to 28 days-old) were picked from plants in each plot, of volunteer and 2002 seeded Bt and DPL 5415 cotton plants. Bolls were placed individually in 2 inch diameter x three inch tall polyethylene containers. Five, first instar PBW larvae were placed on each boll. Bolls were examined on day seven following infestation with the aid of a microscope. PBW larval entrance holes in the carpel walls were counted. Bolls were then dissected and all living and dead larvae were recorded.

**Cry1Ac toxic protein determinations.** The amounts of toxic protein in flower buds, bolls or leaves of Bt cotton were determined using the enzyme-linked immunosorbent assays (ELISA). Materials, sample preparations, solutions, extractions and dilutions were as described in the Envirologix, Inc. Cry1Ab/Cry1Ac plate kit (Envirologix, Inc., Portland ME). DPL 5415 plant tissue samples in all cases were controls. For flower buds and bolls, 0.25 inch wide slices across the center and through the entire fruiting form were cut with razor blade. Each 0.25 inch flower bud or boll piece was weighed before placing in a microcentrifuge tube and homogenized by hand in extraction buffer with a fitted pestle. For leaves a 0.4 inch diameter leaf disk punch was taken from the leaves weighed and placed in a microcentrifuge tube and homogenized as described.

**Statistical analysis.** ANOVA were conducted for TBW and PBW data in each case, combined for all sampling dates for leaves, flower buds or bolls. Contingent upon significant F tests, means were separated using the method of least significant differences ( $P \# 0.05$ ). All percentages were transformed to arcsines before analysis. Cry1Ac toxic protein data for overwintered cotton and 2002 seeded cotton were compared using Student "t" tests.

## Results

For TBW, no live larvae were found following three-day feeding periods on leaves from Bt plants (Table 1). In contrast, < 3% TBW larval mortality per leaf, occurred on DPL 5415 leaves. No Cry1Ac toxic protein was found in leaves of DPL 5415 plants. Cry1Ac toxic protein amounts found in leaves from overwintered Bt plants was not significantly different compared with amounts in leaves from 2002 seeded plants.

For PBW larvae in flower buds (Table 2), and cotton bolls (Table 3), more dead and fewer live PBW larvae were found in Bt cottons compared with DPL 5415 cottons. The differences in Cry1Ac toxic protein content in flower buds and bolls of overwintered volunteer Bt cotton compared with 2002 seeded cotton were not statistically significant.

## Discussion

Bt cottons continue to maintain high levels of efficacy in PBW control programs in Arizona (Sims et al. 2001). This has occurred even through strains with high levels of Bt resistance have been selected in the laboratory. Understanding development of resistance under laboratory selection conditions may provide keys to explaining the absence of resistance selection under field conditions. Using readily available ELISA technology to compare Cry1Ac toxic protein in overwintered Bt cotton leaves and fruiting forms did not reveal any significant differences compared with current year cotton from seed. Low toxic protein extraction efficiency and interference of other chemicals not showing insecticidal activity may have influenced our quantifications of the toxic protein (Greenplate 1999). However, the insect bioassays provide evidence that sufficient Cry1Ac levels were expressed by overwintered Bt plants that no difference in mortalities of PBW or TBW larvae were detected compared with 2002 Bt cotton from seed.

## References

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Table 1. Means numbers of living and dead tobacco budworm larvae and percentage mortalities after three-day feeding periods on NuCOTN 33B<sup>7</sup> and DPL 5415 cotton leaves from 2001 overwintered and 2002 seeded cottons.

Treatment <sup>a</sup>	Larvae			Cry1Ac Toxic Protein (PPM) <sup>b</sup>
	Live	Dead	% Mortality	
<b>Cultivar Effect</b>				
Bt	0.00 b	3.98 a	100.00 a	0.379 -
DPL	4.13 a	0.10 b	2.40 b	0.000 -
F = <sup>c</sup>	1974.20 -	1423.30 -	2386.10 -	- -
P =	<0.05	<0.05	<0.05	- -
<b>2002 Seeded vs Volunteer Effect</b>				
Seeded	2.10 a	2.02 a	51.98 a	0.416 a
Volunteer	2.02 a	2.06 a	50.42 a	0.342 a
F =	0.46 -	0.01 -	1.43 -	- -
P =	> 0.05 -	> 0.05 -	> 0.05 -	- -

<sup>a</sup> Means of 4 replications, 3 observations per replication in a column in the same group not followed by the same letter are significantly different.

<sup>b</sup> t, 22 df = 0.502 NS.

<sup>c</sup> df = 1,33.

Table 2. Means numbers of living and dead pink bollworm larvae and percentage mortalities after three-day feeding periods in NuCOTN 33B<sup>®</sup> and DPL 5415 cotton flower buds from 2001 overwintered and 2002 seeded cottons.

Treatment <sup>a</sup>	Larvae			Cry1Ac Toxic Protein (PPM) <sup>b</sup>
	Live	Dead	% Mortality	
<b>Cultivar Effect</b>				
Bt	0.03 b	2.06 a	98.75 a	0.189 -
DPL	1.03 a	0.78 b	41.04 b	0.000 -
F =	34.79 -	19.73 -	31.92 -	- -
P =	< 0.05 -	< 0.05 -	< 0.05 -	- -
<b>Seeded vs Volunteer</b>				
Seeded	0.59 a	1.47 a	68.65 a	0.180 a
Volunteer	0.47 a	1.38 a	71.15 a	0.198 a
F =	0.60 -	0.11 -	0.08 -	- -
P =	> 0.05 -	> 0.05 -	> 0.05 -	- -

<sup>a</sup> Means of 4 replications, 2 observations per replication in a row in the same group not followed by the same letter are significantly different.

<sup>b</sup> t, 14 df = 0.296 NS.

Table 3. Means numbers of living and dead pink bollworm larvae and percentage mortalities after seven-day feeding periods in NuCOTN 33B<sup>®</sup> and DPL 5415 cotton bolls from 2001 overwintered and 2002 seeded cottons.

Treatment <sup>a</sup>	Larvae			Cry1Ac Toxic Protein (PPM) <sup>b</sup>
	Live	Dead	% Mortality	
Cultivar Effect				
Bt	0.01 b	1.57 a	99.81 a	0.320 -
DPL	1.00 a	0.65 b	40.20 b	0.000 -
F =	61.72 -	19.17 -	63.71 -	- -
P =	< 0.05	< 0.05	< 0.05	- -
2002 Seeded vs Volunteer Effect				
Seeded	0.49 a	1.23 a	71.18 a	0.332 a
Volunteer	0.52 a	0.99 a	68.83 a	0.307 a
F =	0.05 -	1.29 -	0.08 -	- -
P =	> 0.05 -	> 0.05 -	> 0.05 -	- -

<sup>a</sup> Means of 4 replications, 2 observations per replication in a column not followed by the same letter are significantly different.

<sup>b</sup> t, 14 df = 0.309 NS.