Mortality and Development Effects of Transgenic Cotton on Pink Bollworm Larvae

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ABSTRACT

Pink bollworm (PBW), Pectinophora gossypiella (Saunders), larval mortality after different times of confinement on NuCOTN 33BÒ (Bt) cotton bolls were compared with larval mortality on Delta and Pineland 5415 cotton bolls as controls. We also compared larval mortality on different age cotton fruiting forms and determined the Bt susceptibility of different age PBW larvae.

Infesting Bt bolls with PBW eggs that hatched within 24 h resulted in 92% larval mortality after 48 h and 100% mortality in 4 days or longer. There were no differences between cultivars in numbers of larval entrances holes into bolls. Generally, days to pupation for both males and females were longer on Bt bolls compared with non-Bt cotton. There were no significant mortality differences for larvae feeding on Bt fruiting forms of different ages ranging from one-half grown flower buds to 40-day old immature green bolls.

Introduction

Experimental cotton, *Gossypium hirsutum* L., lines carrying the gene that mediated production of insecticide crystalline protein from *Bacillus thuringiensis* Kurstaki (Berliner) were shown in 1990 in Arizona to be resistant to pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Wilson et al. 1992). Subsequent research (Flint et al. 1995; Watson 1995; Ellsworth et al. 1995a, b, 1996; Flint et al. 1996; Flint and Parks 1999) culminating with the evaluation of the commercial transgenic cultivar NuCOTN 33® (Bt) (Monsanto Co., St. Louis, MO) corroborated the early results. Commercial production of Bt for seed in Arizona was initiated in 1995 and for commercial lint production in 1996. These and subsequent plantings were carefully monitored for PBW infestations (Flint et al. 1996, Flint and Parks 1999). Although PBW resistance to Bt is of concern (Bartlett 1995, Simmons et al. 1998, Patin et al. 1999), the results have shown PBW infestations of < 0.1% for Bt cottons demonstrating a continuing high degree of control efficacy in the field.

Arizona cotton growers have readily accepted Bt technology and over 65% of the cottons grown in 1997, 1998, and 1999 were transgenic types. We conducted field and laboratory studies to determine the relative susceptibilities of PBW larvae to feeding on Delta and Pineland 5415 (DPL 5415) (Delta and Pineland Co., Scott, MS) (non-Bt) and Bt (NuCOTN 33B®, Monsanto Co., St. Louis, MO) flower buds (squares), and 10, 20, 30, or 40-day-old bolls.

Methods and Materials

Greenhouse - Laboratory. Bt or DPL 5415 seed [one per 3.8 cm diameter x 3.8 cm tall Jiffy Pot (Jiffy Products of America, Inc., Batavia, IL)] or 2 to 3 seeds per 10 cm^2 x 12 cm tall plastic flower pot filled with potting soil were planted at about one week intervals to assure a continuous supply of plant material. Emerged seedlings, thinned to one plant, were transplanted to 0.31 m diameter x 0.47 m tall flowerpots when plants were in the four to six leaf

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stage of development. Flowers were tagged as they occurred on the plants. Three weeks following each flower tagging (21 day old bolls), bolls were harvested and placed individually in 5 cm diameter x 7.5 cm tall polyethylene containers. Container lids had 2.54 cm diameter screen covered holes for ventilation. All PBW eggs and larvae used for artificially infesting cotton bolls were from a laboratory colony reared on artificial diet (Bartlett and Wolf 1985) at the Western Cotton Research Laboratory.

We compared PBW larval mortalities and successful entrance into DPL 5415 and Bt fruiting forms after egg infestation by placing 0.6 cm^2 pieces of oviposition cage substrate with PBW eggs under the bracts of bolls that developed from tagged flowers. Each 0.6 cm^2 paper towel had 10 to 25 PBW eggs. For larval infestations, we placed five 1st instar larvae on each boll using camel's hair brushes. Infested bolls were examined with the aid of a microscope and all PBW larval entrance holes in the carpel walls and living and dead larvae were counted.

Field - Laboratory. DPL 5415 and Bt cotton seeds were planted on 13 April 2000 in 17 rows wide by 19 m long plots at the Western Cotton Research Laboratory in Phoenix, AZ. Plots were arranged in a randomized block design with four replications.

Larval mortalities in Bt and DPL 5415 cotton flower buds (squares) were determined by picking 25, 0.95 cm diameter squares from each plot. Each square was placed individually in a 5.5 cm x 1.5 cm deep petri dish on a moistened filter paper on the dish bottom. Pieces (0.6 cm^2) of paper towel oviposition paper with 10 to 20 PBW eggs were placed under the square bracts. Numbers of hatched eggs on oviposition paper and PBW larval entrance holes and living and dead larvae on squares were counted in seven to ten days.

On 29 June 2000, we tagged 250 cotton flowers per plot of each cultivar on plant nodes six to ten. Effect of Bt boll age on toxicity to PBW was determined by harvesting 25 bolls per plot on each of 10, 20, 30 and 40 days following flower tagging. Bolls were placed individually in polyethylene containers (previously described). We placed 0.6 cm^2 pieces of oviposition cage substrate with PBW eggs under the bracts of bolls of each age. Each 0.6 cm^2 paper towel had 10 to 20 PBW eggs. Bolls were held for two weeks after egg infestation. Each boll was examined with the aid of a microscope and PBW larval entrance holes in the carpel walls were counted. Bolls were then dissected and all living and dead larvae were recorded. Controls were DPL 5415 bolls.

Results

When bolls of either cultivar were infested with eggs there were no significant differences between cultivars for entrance holes per hatched egg (Table 1). However, in the case of entrance holes per larvae found in the bolls, significantly fewer occurred in Bt compared with DPL 5415 bolls. There were also no significant differences between cultivars for entrance holes per hatched egg or per larvae found in bolls infested with 1st instar larvae.

For DPL 5415 cotton, bolls infested with PBW eggs, 49% larval mortality occurred within the first 48 h of feeding compared with 92% mortality for larvae feeding 48 h on Bt bolls (Table 2). No larvae survived 4, 6, or 8 h feeding on Bt bolls compared with 47 to 85% larval mortality when feeding on DPL 5415 bolls. Larval mortality decreased as the age of the larvae (instar) at the time of boll infestation increased. Mortality increased with increasing feeding time on Bt but not DPL 5415 bolls. Some larvae placed on bolls in the 1st instar of development survived following feeding periods of 2, 4, or 6 days.

Numbers of days to death for larvae on DPL 5415 bolls were 9.3 and 8.1 and to pupation 17.0 and 17.4, respectively, for eggs and 1st instar larval infestations (Table 3). In contrast, for Bt bolls numbers of days to larval death were 12.0 and 10.3 and for bolls infested with PBW eggs and 1st instar larvae, respectively. For larvae surviving from egg infestations (6) and 1st instar larvae (5) infestations of Bt bolls, days to pupation were 19.4 and 19.5, respectively.

Field – Laboratory. There was no significant difference in the numbers of entrance holes per DPL 5415 or Bt square (Table 4). Mortality of larvae entering Bt squares was 99% compared with 38% for larvae entering DPL 5415 squares.

For different age bolls and cultivars, there were no significant differences for entrance holes (Table 4). Fewer living larvae were found in Bt compared with DPL 5415 bolls and percentages of larval mortality were higher for Bt compared with DPL 5415 for all age bolls.

Discussion

Transgenic cottons with gene expression of the crystalline insecticidal protein of *B. thuringiensis* have become major contributors to efficient PBW management in Arizona, Southern California and West Texas. PBW has been a key pest in Arizona and Southern California cotton production for more than 35 years. Excessive reductions in yield and quality and high costs of control have occurred (Watson and Fullerton 1969, Burrows et al. 1982, Gonzales 1990). Insecticide costs alone have been estimated to exceed 1.2 billion dollars (Roberson 1998). Carrière et al. (2000) reported that Arizona pesticide use analyses by W. Sherman and K. Agnew (USDA Arizona Agricultural Statistics) and P. B. Boher (Arizona Pesticide Information Training Office) indicated 2.2 insecticide applications per cotton acre in 1999 as opposed to 6.3 in 1995. A major factor in the reduction has been the adoption of Bt cotton and reduced PBW infestations.

In summary, Bt cotton had a major adverse effect on PBW larval mortality, growth and development. We found no significant differences in larval mortalities on difference age cotton fruiting forms.

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Tables

Table 1. Means (± SE) Numbers of Pink Bollworm Larval Entrance Holes Per Hatched Egg on Greenhouse Grown DPL 5415 or Bt Bolls in Each Case Infested with Eggs and 1st Instar Larvae.

Infested with:	Cultivar	No./Boll ($\bar{X}$)	Cultivar Entrance Holes $\bar{X}$
Eggs	DPL 5415	13.7 ± 1.0	0.99 ± 0.09 a
	Bt	11.3 ± 0.9	1.04 ± 0.17 a
	DPL 5415	13.7 ± 1.05	6.43 ± 0.69 a
	Bt	11.3 ± 0.9	4.46 ± 0.66 b
Larval instar 1st	DPL 5415	5.1 ± 0.6	1.15 ± 0.12 a
	Bt	5.4 ± 0.2	1.11 ± 0.14 a
	DPL 5415	5.1 ± 0.1	3.32 ± 0.71 a
	Bt	5.4 ± 0.2	3.58 ± 0.83 a

[a] Means of 8 and 4 replications for eggs and 1st instars respectively. Means that occur in cultivar columns with the same letters are not significantly different $P \leq 0.05$.

Table 2. Pink Bollworm Larval Mortality Feeding Periods on DPL 5415 or Bt Greenhouse Grown Cotton Bolls after 2, 4, 6, or 8 Day Larvae.

Bolls Infested With:		Days After Infestation (No.)			
	Cultivars	2	4	6	8
Eggs	DPL5415	48.9 ± 8.1	84.9 ± 5.1	66.7 ± 13.6	47.4 ± 11.4
	Bt	91.5 ± 3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	t, df, *P*[a]	2.1, 26, 0.00	1.7, 24, 0.01	9, 4, 0.13 ns[b]	4.4, 24, 0.00
Larval instar 1st	DPL5415	36.2 ± 5.7	47.6 ± 17.2	53.6 ± 11.0	45.8 ± 20.8
	Bt	95.8 ± 4.2	85.7 ± 15.4	94.7 ± 1.3	100.0 ± 0.0
	t, df, *P*	3.5, 10, 0.01	1.8, 12, 0.10 ns	3.7, 14, 0.00	2.6, 6, 0.04

[a] Student's *t* value, df = degrees of freedom, *P* = probability. Means of 6 to 28 paired comparisons.
[b] ns = not significantly different.

Table 3. Total Numbers of Pink Bollworm Live Larvae from DPL 5415 or Bt Greenhouse Grown Cotton Bolls Infested as Eggs or 1st Instar Larvae.

Bolls Infested With:	Cultivars	N (Total)	Days to Death (No. ± SE)	Days to Pupation (No.)
Eggs				
	DPL5415	854	9.3 ± 1.1	17.0 ± 0. 5
	Bt	754	12.0 ± - -[a]	19.4 ± 1.6 (5 pairs)
	t, df, *P*	- -	- -	1.76, 5, 0.12 ns
Larval instar 1st	DPL5415	218	8.1 ± 1.6	17.4 ± 0.7
	Bt	230	10.3 ± 3.8	19.5 ± 1.5[b] (3 pairs)
	t, df, *P*	- -	0.69, 3, 0.5 ns	No analysis

[a] Two insects.
[b] Three pairs.

Table 4. Mean (± SE) Numbers of Pink Bollworm Entrance Holes and Larval Mortality Percentages Following Egg Infestations of DPL 5415 and Bt Field Grown Cottons.

Cultivar	Boll Age	Entrance Holes	% Larval Mortality
		Flower Buds[a]	
DPL5415	- -	3.06 ± 0.41 a	37.5 ± 3.8 b
Bt	- -	2.80 ± 0.11 a	98.9 ± 1.1 a
F	- -	0.34	97.4
P	- -	0.60 ns	0.00
		Bolls[b]	
DPL 5415	10	8.42 ± 0.27 a	49.73 ± 1.92 f
Bt	10	10.10 ± 1.22 a	88.30 ± 4.74 d
DPL 5415	20	10.75 ± 0.36 a	75.55 ± 8.43 e
Bt	20	11.11 ± 0.72 a	90.57 ± 7.65 a
DPL 5415	30	16.97 ± 1.02 a	90.19 ± 3.98 ad
Bt	30	14.59 ± 0.77 a	95.27 ± 3.42 ab
DPL 5415	40	7.00 ± 1.29 -	95.58 ± 2.68 bc
Bt	40	12.44 ± 2.74 -	100.00 ± 0.00 a
F	- -	3.11	3.22
P	- -	0.07	0.04

[a] Means of 4 replications, 16 squares per replication. Means in a column not followed by the same letter are significantly different.
[b] Means of 4 replications, 22 bolls per replication. Means in the sample column not followed by the same letter are significantly different (40 day old bolls developed mold).