Cabbage Looper, Tobacco Budworm, and Beet Armyworm Larval Mortalities, Development and Foliage Consumption on Bt and Non-Bt Cottons

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Abstract

Tobacco budworm (TBW), Heliothis virescens (F.), larvae were highly susceptible to feeding on Bt cotton leaves or flower buds with 100% and 96% mortality occurring within 4 days, respectively, compared to an average mortality of 95% for cabbage looper (CL), Trichoplusia ni (Hübner), and 57% for beet armyworm (BAW), Spodoptera exigua (Hübner), after 14 days feeding on Bt leaves. Larval weights, of CL and BAW after 7, 10, or 14 days of feeding on Bt leaves were lower compared with those feeding on non-Bt cotton leaves. BAW, CL, and TBW larvae consumed significantly less Bt leaf area per feeding day compared with DPL 5415.

Introduction

In 1990, experimental cotton lines carrying the gene that mediated production of insecticide crystalline protein from *Bacillus thuringiensis* Kurstaki (Berliner) were shown to be nearly immune to pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) and also resulted in significant reductions in other lepidopterous insect populations in Arizona (Wilson et al. 1992). Results were later corroborated with the commercial transgenic cultivar NuCOTN 33B® (Bt) (Monsanto Co., St. Louis, MO) (Flint et al. 1995, 1996; Watson 1995). Arizona cotton growers have readily accepted Bt technology and estimated acreages grown from 1997 to 2000 ranged from 50 to 62%. We conducted greenhouse, field and laboratory studies on leaf feeding and leaf age effects of DPL 5415 and Bt cottons on CL, TBW and BAW mortality, pupation, growth, development, and leaf-area consumption.

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Materials and Methods

Field-Laboratory. DPL 5415 and Bt cotton seeds were planted in 17 rows wide by 60 feet long plots at the Western Cotton Research Laboratory in Phoenix, AZ. Plots were arranged in a randomized block design with four replications. On 22 June, leaves were picked at random from the top (14-16 nodes), middle (11-13 nodes), and bottom (5 to 6 nodes) of three plants in each DPL 5415 and Bt plot planted on 13 April 2000. Leaves were trimmed to fit in 15.0-cm diameter x 1.5-cm deep plastic petri dishes lined on the bottom with moist filter paper. Prior to placing leaves in the petri dishes, the leaf area of each leaf was measured using a leaf meter (LiCor LI-3100 LiCor Inc., Lincoln, NE). For each insect species, five, first-instar larvae were placed on foliage in each of seven petri dishes. Leaves were replaced in dishes at 3-to 4-day intervals and leaf areas remeasured to estimate leaf areas consumed. Living and dead larvae were counted daily until pupation. The entire experiment was repeated on 17 July (top nodes, 22-25), (middle nodes, 14-16), (bottom nodes, 8-10) and 14 August (top nodes, 20-23), (middle nodes, 13-18), (bottom nodes, 10-13). In a second experiment, we compared the effect of leaf-age by picking a leaf from node 7, 14, and 21 on 10 July from three plants in each plot of DPL 5415 and Bt cotton. Leaves were trimmed to fit into petri dishes, leaf area measured, and leaves infested with first-instar larvae of each species described. Insects of each species were from the Western Cotton Research Laboratory colonies reared on artificial diet (Henneberry and Kishaba 1966). Larval mortality and pupation were recorded daily and foliage replaced from the same plant nodes from randomly selected plants. The entire experiment was repeated on 7 August.

Effects of Bt on BAW and CL larval growth. Leaves were picked at random from Bt and DPL 5415 cotton plants, trimmed and placed in petri dishes as described. Five, first-instar BAW or CL larvae were placed on a leaf in each of five to ten petri dishes. Larvae surviving in each case for seven, ten or 14 days were weighed to the nearest one-hundredth of a milligram (Mettler-Toledo, AT/MT/AMT balance, Hightstown, NJ). The experiment was repeated on five occasions.

Data were analyzed using ANOVA and means separated using the methodology of least significant differences following a significant F test or using Student's "*t*" test for paired treatment comparisons (MSTAT-C 1989). For both tests, differences with probabilities of ≤ 0.05 or ≤ 0.01 were considered significantly different.

Results

Field-Laboratory. After 14 days, CL and BAW larval mortalities feeding on Bt foliage were 96 and 57%, respectively, compared with 17 and 10% feeding on DPL 5415 foliage (Table 1). No larvae pupated on Bt cotton compared to 35, 68, and 0% pupation for BAW, CL, and TBW, respectively, on DPL 5415 cotton.

BAW, CL, and TBW consumed more leaf tissue per feeding day compared with Bt leaf tissue (Table 2). For the experiment on leaf-age as determined by identified leaf node, higher mortality of BAW and TBW occurred on leaves from node 7 compared with nodes 14 and 21 (Table 3). Also, BAW and TBW consumed significantly less leaf tissue of leaves from node 7 compared with leaves from nodes 14 or 21 and for BAW on leaves from node 7 versus leaves from node 21 (Table 4).

Effects of Bt on BAW and CL larval growth. Weights of CL and BAW larvae feeding on Bt leaves were significantly less after 7, 10, and 14 days of feeding on Bt leaves compared with feeding on DPL 5415 leaves (Fig. 1). No TBW larvae survived after three days feeding on Bt leaves and no weight comparisons were made. BAW larvae on Bt leaves for 7, 10, or 14 days weighed 1.51, 5.76 and 34.73 mg compared to 3.19, 25.50, and 170.38 mg on DPL 5415. In the case of CL larvae, after 7, 10, or 14 days on Bt leaves, weights were 1.22, 4.42, and 23.07 mg compared to 47.79, 258.99, and 420.98 on DPL 5415 leaves, respectively. Average weights of larvae for cultivars for all feeding periods (7, 10 and 14 days) were for BAW 66.31 mg feeding on DPL 5415 compared with 15.50 for Bt cotton. For CL weights were 242.58 mg for feeding on DPL 5415 leaves and 14.09 mg on Bt cotton.

Discussion

Laboratory leaf bioassays with leaves from field-grown Bt cotton showed a high level of susceptibility. The results corroborate test results by Benedict et al. (1996), and Johnson et al. (2000). High mortality of BAW (57%) and CL (96%) occurred during 14-day feeding periods. Leaf tissue consumption and larval growth of both species were significantly reduced after 7, 10, and 14 days of feeding on Bt vs. DPL 5415 leaves, similar to the results of Nava-Camberas and Ibarra-Frias (2000). Overall, BAW and CL larvae appear less susceptible to the Cry1Ac toxic protein compared to PBW (Henneberry and Forlow Jech, 2000) and TBW. Concern for the occurrence of less susceptibility of these lepidopterous pests to Bt cotton may be unnecessary since the second generation of Bollgard® transgenic cottons with incorporation of a second gene producing a second Bt toxic protein (Greenplate et al. 2000) is more effective against minor cotton pests such as the soybean looper, *Pseudoplusia includens* (Walker), and BAW (Ridge et al. 2000, Stewart and Knight 2000).

In summary, Bt cotton had a major adverse effect on TBW, BAW and CL mortality, growth and development, and foliage consumption.

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Table 1. Mean pupation and mortality percentages of beet armyworm and cabbage looper after 14 days, and tobacco budworm larvae after 3 to 4 days on DPL 5415 and Bt from field grown cotton leaves.

	Species[a]					
	Beet Armyworm		Cabbage Looper		Tobacco budworm[b]	
Cultivar	Mortality	Pupation	Mortality	Pupation	Mortality	Pupation
DPL 5415	10 a	35 a	17 b	68 a	41 b	0 a
Bt	57 a	0 b	96 a	0 b	100 a	0 a
F, df = 1,30	19.9	32.3	93.6	227.5	36.1	--
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	--

[a] Means of 7 replications, 5 larvae per replication. Means in row within a group not followed by the same letter are significantly different. Data for beet armyworm and cabbage looper are for 14 days, but all tobacco budworm larvae died within 3 to 4 days of infestation on Bt leaves.

[b] No tobacco budworm larvae survived longer than three days on Bt cotton.

Table 2. Mean square centimeters of DPL 5415 or Bt cotton leaf tissue from field grown cotton that was consumed per beet armyworm and cabbage looper larvae per day for 14 days or tobacco budworm larvae for 3 to 4 days.

	Species[a]		
Cultivar	Beet armyworm	Cabbage looper	Tobacco budworm[b]
DPL 5415	1.2 a	2.5 a	0.8 a
Bt	0.4 b	0.1 b	0.1 b
F[h]	26.8	176.4	30.8
P =	< 0.05	< 0.05	< 0.05

[a] Means of 12 replications in a column not followed by the same letter are significantly different using the method of least significant differences.

[b] Leaf tissue consumed per day per larvae for 3 to 4 days, no tobacco budworm larvae survived after 4 days.

Table 3. Mean pupation and mortality percentages of beet armyworm, cabbage looper, or tobacco budworm larvae on DPL 5415 and Bt field grown cotton leaves from nodes 7, 14 and 21.

Cultivar and Plant Node Source	Species					
	Beet Armyworm[b]		Cabbage Looper[b]		Tobacco Budworm[b]	
	Mortality	Pupation	Mortality	Pupation	Mortality	Pupation
Cultivar Effect						
DPL 5415	13.1 b	49.1 a	15.0 b	73.3 a	41.4 b	0.0
Bt	68.7 a	0.0 a	92.5 a	0.0 b	100.0 a	0.0
F[c]	29.2	59.1	87.4	326.3	75.4	--
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	--
Node Effect						
21	23.3 b	35.3 a	51.3 a	37.8 a	55.9 b	0.0
14	37.0 b	38.3 a	50.9 a	41.3 a	58.6 b	0.0
7	62.3 a	0.0 b	59.1 a	30.9 a	97.6 a	0.0
F[d]	5.4	14.8	0.2	1.1	16.5	--
P	< 0.05	< 0.05	ns	ns	< 0.05	--

[a] Means 5, 8, and 7 replications for beet armyworm, cabbage looper and tobacco budworm, respectively. Means in a column in a group not followed by the same letter are significantly different. Method of least significant differences $P \leq 0.05$.

[b] Mortality and pupation after 14 days for beet armyworm and cabbage looper and three to four days for tobacco budworm since no larvae survived longer than four days.

[c] df = 1, 20 for BA; 1, 35 for CL and 1, 30 for tobacco budworm.

[d] df = 2, 20 for BA; 2, 35 for CL and 2, 30 for tobacco budworm.

Table 4. Mean square centimeter of DPL 5415 or Bt cotton on leaf tissue from field grown cotton that was consumed per beet armyworm or CL larvae per day for 14 days or tobacco budworm per day for three or four days.

Cultivar/ Plant Node	Species[a]		
	Beet Armyworm	Cabbage Looper	Tobacco Budworm[b]
Cultivar Effect			
DPL 5415	1.6 a	4.2 a	1.0 a
Bt	0.5 b	0.3 b	0.1 b
F[c]	85.9	255.7	32.5
P	< 0.05	< 0.05	< 0.05
Node Effect			
21	1.3 a	1.9 a	0.9 a
14	1.2 a	2.5 a	0.7 a
7	0.6 b	2.2 a	0.2 b
F[d]	12.5	1.6	8.2
P	< 0.05	ns	< 0.05

[a] Means 5, 8, and 7 replications for BAW, CL and tobacco budworm, respectively. Means in a column in a group but not followed by the same letter are significantly different. Method of least significant differences $P \leq 0.05$.

[b] Tobacco budworm larvae did not survive beyond four days.

[c] 1,20; 1,35; and 1,30 for BAW, CL and tobacco budworm, respectively.

[d] 2,20; 2,35; and 2,30 for BAW, CL and tobacco budworm, respectively.

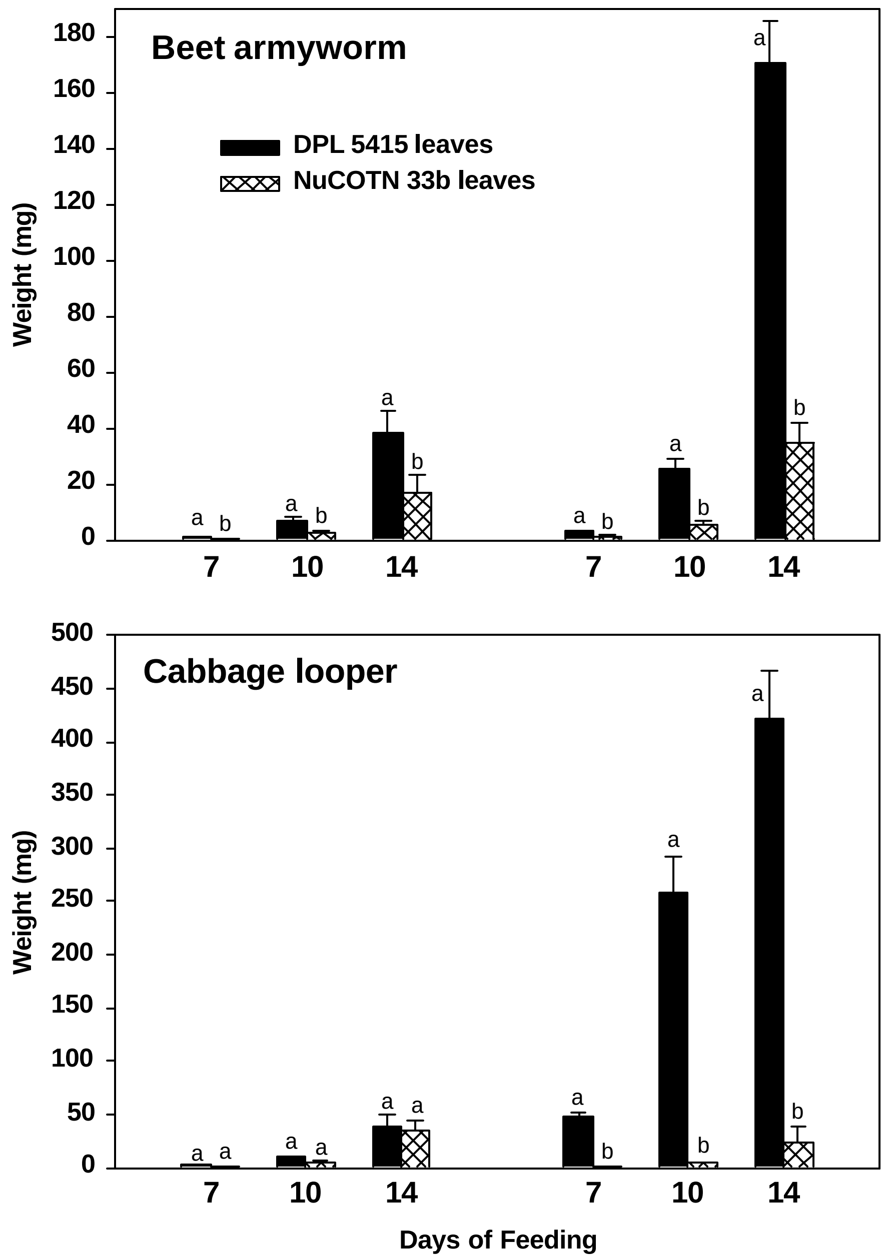

FIG. 1. Mean (± SE) weights (mg) of beet armyworm and cabbage looper larvae after feeding for 7, 10, or 14 days on DPL 5415 or Bt cotton leaves. Means for each day not followed by the same letter are significantly different.