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RESPONSE OF BOLLWORM OFFERED SELECTED PLANT STRUCTURES FROM WIDESTRIKE COTTON

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Abstract

WideStrike (Dow AgroSciences, Indianapolis, IN) cotton produces *Bacillus thuringiensis kurstaki* (Bt) insecticidal proteins, c*ry1Ac* and c*ry1F*, that provide protection against lepidopteran pests. Laboratory studies evaluated the susceptibility of bollworm larvae fed various plant structures of conventional cotton plants and transgenic plants expressing c*ry1Ac* and c*ry1F*. One-day old larvae (L1 stage) were provided white flowers, squares, and terminal leaves at two, four, and six weeks after flower initiation. Additionally, second instars (L2 stage) were supplied with leaf tissue (discs) from five and eight nodes below the plant terminal and quarter-sized bolls. Mortality was assessed at 48, 72, and 96 hours after plant structures were infested. Mortality was higher in L1 larvae than in L2 larvae. WideStrike produced greater mortality than the non-Bt line.

Introduction

Since the introduction of insect-resistance transgenic cotton in 1996, improvements in the technology offer a broader spectrum of lepidopteran pest control and new genes for expression of different proteins to assist in resistance management. WideStrike (Dow AgroSciences, Indianapolis, IN), has two separate insecticidal crystal proteins from the soil bacterium *Bacillus thuringiensis kurstaki* (Bt), *cry1Ac* and *cry1f.* WideStrike has good to excellent activity against tobacco budworm (*Heliothis virescens*), bollworm (*Helicoverpa zea*), pink bollworm (*Pectinophora gossypiella*), cabbage looper (*Trichoplusia ni*), soybean looper (*Pseudoplusia includens*), saltmarsh caterpillar (*Estigmene acrea*), European corn borer (*Ostrinia nubilalis*), beet armyworm (*Spodoptera exigua*), fall armyworm (*Spodoptera frugiperda*), and southern armyworm (*Spodoptera eridania*) and moderate activity against black cutworm (*Agrotis ipsilon*) (Haile et al. 2004).

Previous studies investigating Bollgard and Bollgard II technologies (Monsanto, St. Louis, MO) have shown there is considerable variation in the plant's expression of Bt proteins. Bioactivity of cry proteins are influenced by location of plant tissue on the plant (Greenplate 1999, Greenplate et al. 2000, Akins et al 2003), type of plant structure (reproductive vs foliage) (Greenplate 1999, Gore 2001), age of leaves (Penn et al 2000), and presence or absence of flower tissue attached at the tip of bolls (Abel and Adamczyk 2004). Additionally, studies have shown that bollworm larvae are more likely to be found lower in the plant canopy on white flowers and bolls of Bollgard plants than in terminals and squares of non-Bollgard plants (Gore et al. 2002). Larval survival is higher on white flower components than squares and bracts (Gore et al. 2001). Although there have been differences in quantity of Bt proteins in Bollgard and that differences have been associated with variation in larval mortality, bollworm survival does not always correlate with protein expression levels (Gore et al. 2001). Conversely, Akins et al. (2004) observed *cry1Ac* levels were inversely related to mean larval weight when comparing results from ELISA to those from bollworm bioassays on fresh tissue. Protein expression and insect behavior on WideStrike has not been defined as thoroughly as that on Bollgard. Observations suggest that bollworm larvae on WideStrike plants behave differently from those on Bollgard plants. Therefore, efforts were made to characterize bollworm mortality when feeding on various plant structures from WideStrike plants expressing *cry1Ac + cry1f* proteins.

Materials and Methods

This experiment was conducted at the Macon Ridge Research Station near Winnsboro, LA (Franklin Parish) in 2004. Plots consisted of three rows (centered on 40 inches) and were 30 ft in length. Treatments were arranged in a randomized complete block with four replications. Two lines were examined for toxicity against bollworm: Phytogen 440W (*cry 1Ac + cry 1f*), and Phytogen 355 (non-Bt parent line). To ensure that each plant used in the feeding assays expressed the appropriate genes, ELISA techniques were used to assay every plant on row two for Bt

proteins. A leaf disk from a fully expanded cotton leaf located on the lower 2/3rd of the plant was removed using a hole punch. Any plant that did not show the correct expression was removed from the row.

Two, four, and six weeks after flower initiation, plant structures (i.e., terminal leaves, white flowers, squares, leaf discs from five and eight nodes below the terminal leaf, and quarter-sized bolls) were removed from plants on row two. Leaf discs were collected by using a metal cork borer (7/8 inch diameter) to cut discs of tissue. While in the field, all plant tissues, except white flowers and bolls, were placed individually in 1 oz plastic diet cups with a thin layer of agar as a source of moisture and transported to the laboratory in an ice chest. White flowers and bolls were removed from plants, placed in plastic bags, and transported to the laboratory on ice. Flowers were dissected because the presence of petals led to a rapid growth of fungal pathogens. Only staminal columns and ovules of white flowers were presented to larvae in 1 oz diet cups. Bolls were placed in plastic specimen vials. A range of 10-15 structures was sampled for each plant part on each harvest date. Each terminal leaf, white flower, and square was infested with three 1-day old bollworm larvae (L1); leaf discs and bolls were infested with one second instar (L2). Mortality of L1 larvae was assessed at 48 and 72 hours. Mortality of L2 larvae was assessed at 48, 72, and 96 hours after larval infestation.

Data were averaged over plant structure to determine if there were differences in mortality of L1 and L2 larvae offered plant tissue from WideStrike plants and the non-Bt parent. Additionally, mortality of larvae at the same age was examined to detect differences between different plant structures. The two comparisons were analyzed using PROC MIXED as a two-way ANOVA and means were separated using Tukey's studentized range test (SAS Institute 1998).

Results and Discussion

Regardless of cotton line, mortality was greater in L1 stage larvae than L2 stage larvae (Table 1). At 48h differences in bollworm mortality on WideStrike plants was numerically greater than that on conventional cotton for both L1 and L2 larvae. At 72h, mortality of L1 bollworms consuming WideStrike plant tissue was significantly greater than that on non-Bt cotton. Similarly, mortality of L2 fed WideStrike tissue was greater than that on the non-Bt line at 72h.

Table 1. Mortality of 1-day old (L1) and second instar (L2) bollworm presented plant tissue from plants with WideStrike (PHY440W) and the non-Bt parent line (PHY355) averaged over plant structures offered.

Treatment	% Mortality ± SE 48h[a]	% Mortality ± SE 72h
PHY440W		
L1	78.4 ± 1.5	91.6 ± 1.5a[b]
L2	30.1 ± 3.1	67.9 ± 3.6b
PHY335		
L1	46.0 ± 4.3	72.8 ± 5.4b
L2	9.2 ± 1.3	23.5 ± 2.1c

[a] Mortality of larvae averaged across plant structure.
[b] Means in the same column followed by different letters are significantly different (Tukey's studentized range test $\alpha = 0.05$).
Treatment effects:
48h – Plant lines: $F = 88.1$; $df = 1,9$; $P < .0001$; Insect stage: $F = 224.9$; $df = 1, 9$; $P < .0001$; Plant line*Insect stage: $F = 4.12$; $df = 1,9$; $P = 0.0731$
72h – Plant lines: $F = 81.8$; $df = 1,9$; $P < .0001$; Insect stage: $F = 109.0$; $df = 1, 9$; $P < .0001$; Plant line*Insect stage: $F = 13.5$; $df = 1,9$; $P = 0.0052$

Mortality of L1 larvae fed flowers or squares from WideStrike plants, was significantly higher than those fed flowers or squares from conventional plants at 48h (Table 2). At 72h, mortality of larvae fed non-Bt tissues was similar to that in the WideStrike line with the exception of those fed flowers due to high mortality of larvae fed non-

Bt tissue. Mortality of larvae that consumed flowers from transgenic plants was greater than those fed non-Bt flowers.

Table 2. Mortality of 1-day old (L1) bollworm larvae presented selected plant structures from WideStrike (PHY440W) and the non-Bt parent line (PHY355).

Treatment	% Mortality ± SE 48h	% Mortality ± SE 72h
PHY440W		
Flowers	79.2 ± 3.1a	91.6 ± 3.0a
Squares	77.4 ± 1.5ab	91.7 ± 3.5a
Terminal leaves	78.6 ± 3.4ab	91.4 ± 2.3a
PHY355		
Flowers	32.1 ± 4.7d	49.7 ± 4.5b
Squares	45.8 ± 4.7cd	87.6 ± 2.4a
Terminal leaves	60.2 ± 5.3bc	81.1 ± 4.5a

Means in the same column followed by different letters are significantly different (Tukey's studentized range test $\alpha = 0.05$).
Treatment effects:
48h – Plant lines: $F = 97.92$; $df = 1, 15$; $P < .0001$; Plant structure: $F = 5.88$; $df = 2, 15$; $P = 0.0130$; Plant line* Plant structure: $F = 6.42$; $df = 2, 15$; $P = 0.0077$
72h – Plant lines: $F = 44.25$; $df = 1, 15$; $P < .0001$; Plant structure: $F = 17.17$; $df = 2, 15$; $P = 0.0001$; Plant line* Plant structure: $F = 17.17$; $df = 2, 15$; $P = 0.0001$

At 48, 72, and 96h, mortality of L2 larvae fed tissue from WideStrike plants was greater than that for larvae offered conventional cotton tissue. (Table 3). Plant structure did not significantly affect larval mortality averaged across treatments (Table 3) or on WideStrike cotton (Table 4).

Table 3. Mortality of second instar (L2) bollworm presented selected plant structures from WideStrike (PHY440W) and the non-Bt parent line (PHY355).

	% Mort. ± SE 48h	% Mort. ± SE 72h	% Mort. ± SE 96h
Plant Line (across structures)			
PHY440W	30.1 ± 3.1a	67.9 ± 3.6a	83.1 ± 2.8a
PHY355	9.2 ± 2.3b	23.5 ± 2.6b	36.1 ± 3.0b
Plant Structure (across lines)			
5-Node leaf	18.4 ± 4.1	39.5 ± 7.6	54.8 ± 8.2
8-Node leaf	20.0 ± 4.8	46.9 ± 9.7	58.4 ± 11.1
Bolls	20.5 ± 5.8	50.7 ± 9.6	65.6 ± 9.1

Means in the same column followed by different letters are significantly different (Tukey's studentized range test $\alpha = 0.05$).
Treatment effects:
48h – Plant lines: $F = 13.7$; $df = 3, 33$; $P < .0001$; Plant structure: $F = 2.43$; $df = 2, 33$; $P = 0.1032$; Plant line* Plant structure: $F = 1.1$; $df = 6, 33$; $P = 0.3600$
72h – Plant lines: $F = 52.7$; $df = 3, 33$; $P < .0001$; Plant structure: $F = 9.1$; $df = 2, 33$; $P = 0.0007$; Plant line* Plant structure: $F = 1.8$; $df = 6, 33$; $P = 0.1303$
96h – Plant lines: $F = 48.3$; $df = 3, 33$; $P < .0001$; Plant structure: $F = 4.7$; $df = 2, 33$; $P = 0.0157$; Plant line* Plant structure: $F = 2.0$; $df = 6, 33$; $P = 0.1014$

Table 4. Mortality of second instar (L2) bollworm presented quarter-sized bolls and leaf discs from leaves five nodes and eight nodes below the terminal of WideStrike plants.

	% Mort. ± SE 48h	% Mort. ± SE 72h	% Mort. ± SE 96h
Plant Structure			
Leaves (5 Nodes)	26.9 ± 5.1	58.2 ± 4.7	74.7 ± 5.8
Leaves (8 Nodes)	30.6 ± 5.4	70.6 ± 7.2	87.0 ± 3.8
Bolls	32.8 ± 6.8	74.9 ± 4.2	87.7 ± 1.1

Means in the same column followed by different letters are significantly different (Tukey's studentized range test $\alpha = 0.05$).
Treatment effects:
48h – Plant structure: $F = 0.27$; $df = 2, 6$; $P = 0.7708$
72h – Plant structure: $F = 3.26$; $df = 2, 6$; $P = 0.1101$
96h – Plant structure: $F = 4.14$; $df = 2, 6$; $P = 0.0740$

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