EVALUATION OF SEEDLING TRANSGENIC COTTON CONTAINING BACILLUS THURINGIENSIS TOXINS TO SALTMARSH CATERPILLAR, ESTIGMENE ACREA (DRURY)

Bo Kesey
David Kerns
Texas AgriLife Research and Extension Center, Texas A&M System
Lubbock, TX

Abstract

The saltmarsh caterpillar, *Estigmene acrea* (Drury), is an occasional pest of cotton, *Gossypium hirsutum* L., in the U.S. Although saltmarsh caterpillar is most often encountered late in the season, the most damaging populations are usually dispersing late instars infesting seedling cotton. Three cotton varieties, a non-Bt, a Bollgard 2 (Cry1Ac + Cry2Ab), and a Widestrike (Cry1Ac + Cry1F), were evaluated at the two true-leaf stage for resistance to feeding by neonate and late fourth-instar saltmarsh caterpillars. The Bollgard 2 and Widestrike varieties were very resistant to neonate saltmarsh caterpillars, killing 100% with no visible damage after 3 days of exposure. Mortality on the non-Bt variety was 0% and damage was evident. When exposed to fourth-instar larvae, the Widestrike and Bollgard 2 varieties killed 80 and 90%, respectively, after 7 days of exposure. Mortality by the non-Bt variety was 10%. Leaf consumption by fourth-instar saltmarsh caterpillars on the Bt varieties was negligible, while a mean of 19.7-cm^2 of the non-Bt variety was consumed per larva. Based on relative leaf area of cotton on the Texas High Plains and the estimate that cotyledon- to two true-leaf-stage cotton can withstand 75% damage without significantly impacting yield, treatment thresholds for late-instar saltmarsh caterpillars may approximate 0.5, 1.0, and 1.5 larva per plant on cotyledon, one true-leaf, and two true-leaf-stage cotton, respectively.

Introduction

The saltmarsh caterpillar, *Estigmene acrea* (Drury), is an occasional pest of cotton, *Gossypium hirsutum* L., in the U.S., particularly in the Southwest (Capinera 2008). In most cases, outbreaks of saltmarsh caterpillars in cotton occur late in the season, and damage caused by defoliation of cotton is usually considered negligible and may be beneficial, causing the cotton canopy to become more open, hastening boll opening, and reducing the likelihood of boll rot. However, occasionally saltmarsh caterpillars infest seedling cotton where substantial defoliation may result in significantly less yield.

Infestation by saltmarsh caterpillar early in the season typically does not manifest from ovipositional colonization, but rather from the dispersal of primarily late-instar larvae from weedy hosts into adjacent cotton fields. Subsequently, damage from these dispersing larvae tends to be most severe along field margins adjacent to infested weedy habitat. In Arkansas in 2001, 242,811 hectares of primarily seedling cotton were infested with saltmarsh caterpillar, 60,700 of which were treated with insecticide (Williams 2002). On the High Plains of Texas in 2007, 127,600 hectares of seedling cotton were infested with saltmarsh caterpillars, and 12,763 hectares were treated (Williams 2008).

Fortunately, dispersing saltmarsh caterpillars have not been difficult to control using insecticide, and transgenic cotton varieties have been reported to convey resistance to saltmarsh caterpillars. Tindal et al. (2008) performed leaf bioassays and found Bollgard 2 and Widestrike varieties very resistant to neonate saltmarsh caterpillar. In field studies late in the season, cotton varieties containing the Widestrike traits were infested with fewer saltmarsh caterpillars than were non-Bt varieties (Parker and Livingston 2005, Haygood et al. 2006). However, information concerning the ability of Bollgard 2 or Widestrike varieties to prevent damage from late-instar saltmarsh caterpillars to seedling cotton has not been reported. Even if the Bollgard 2 and Widestrike events can kill early- and late-instar saltmarsh caterpillars, it is conceivable that late instars may consume enough plant tissue to kill or stunt a seedling cotton plant.

In this study, we report the impact of early- and late-instar saltmarsh caterpillars on non-Bt, Bollgard 2, and Widestrike varieties of cotton. The primary purpose of this study was to determine if seedling Bt cotton can withstand migratory infestations of primarily late-instar saltmarsh caterpillars.

Materials and Methods

Damage by saltmarsh caterpillar to seedling-stage cotton expressing Cry1Ac + Cry2Ab, Cry1Ac + Cry1F, and a non-Bt variety was evaluated in a greenhouse at the Texas A&M System, AgriLife Research and Extension Center, Lubbock, TX, in 2007. Saltmarsh caterpillar larvae were collected from various weedy habitats in Lubbock County, TX, and reared on Stonefly *Heliothis* Diet (Ward's Natural Science, Rochester, NY). F_2 generation larvae were used for this study.

Cotton seeds were planted in 115-mm square x 89-mm tall plastic pots containing standard potting soil. The cotton varieties evaluated were DP174RF (non-Bt), DP141B2RF (Cry1Ac + Cry2Ab, Bollgard 2) (Deltapine, Monsanto Company, St. Louis, MO), and PHY 375 WRF (Cry1Ac + Cry1F, Widestrike) (Phytogen, Dow AgroSciences LLC, Indianapolis, IN). Four cotton seeds were planted per pot, but were thinned after emergence to two plants per pot. Plants were maintained throughout the duration of the study at 25.5 ± 2°C and a photoperiod of 14:10 (L:D) hours in a greenhouse.

The experiment was a randomized complete design with 10 replications; each replication consisted of a single pot. Treatments included each of the aforementioned cotton varieties infested with two saltmarsh caterpillar larvae or left noninfested. One test consisted of neonate larvae, while another test consisted of fourth-instar larvae.

At the two true-leaf stage, just before infestation, plants in each pot were enclosed in a cage constructed from an 89-mm diameter x 133-mm tall Styrofoam cup with the bottom excised. Fitted plastic lids with 38-mm square openings covered with fine cloth mesh were used to enclose the top of each cup. The saltmarsh caterpillar neonates and fourth-instar larvae were allowed to feed for 3 and 7 days, respectively, after which mortality was evaluated. Larvae unable to move upon prodding with a sharpened pencil were considered dead. Missing larvae were considered to have died.

In addition to mortality, at 3 days post infestation with neonate larvae, leaf damage was rated using a 1 to 12 scale where 1 = no damage, 2 to 11 = approximate leaf area (mm^2) consumed or window paned, and 12 = 12 mm^2 leaf area or greater consumed or window paned. At 7 days post infestation with fourth-instar larvae, the cotton plants were removed from the pots and the leaf area was measured using a LI-3100 area meter (Li-Cor Biosciences, Lincoln, NE).

All data were analyzed using GLM (SAS Institute 2004). Means were separated using an *F*-protected LSD ($P \leq 0.05$).

Results and Discussion

Neonate Larvae. Neonate saltmarsh caterpillar larvae were extremely sensitive to the cotton varieties containing either Bollgard 2 or Widestrike transgenic traits, each causing 100% mortality (Table 1). No neonate larvae feeding on the non-Bt variety DP 174RF died. Consequently, the non-Bt variety exhibited a mean damage rating of 9.4 ± 0.97, while those containing the Bt traits suffered no visible damage. These findings are consistent with that reported by Tindal et al. (2008) where survival of neonate saltmarsh caterpillar larvae feeding on a Bollgard 2 cotton variety was 0% at 2 days after infestation. Thus, it is evident that cotton varieties containing the Bollgard 2 and Widestrike Bt traits are safe from foliar feeding saltmarsh caterpillars originating from egg masses deposited directly.

Table 1. Leaf-feeding Damage Ratings and Percentage Mortality of First-instar Saltmarsh Caterpillars Exposed to Non-Bt and Transgenic Bt Cotton Varieties

Variety	Bt trait	n	Percent mortality	Damage rating (1-12)
DP174RF	None	9	0 a	9.4 ± 0.97 a
DP141B2RF	Cry1Ac & Cry2Ab	10	100 b	1.0 b
PHY375WRF	Cry1Ac & Cry1F	10	100 b	1.0 b

Means followed by the same letter in a column are not significantly different (*F*- Protected LSD, $P \leq 0.05$).

Fourth-Instar Larvae. Significantly more fourth-instar saltmarsh caterpillar larvae feeding on varieties containing Bollgard 2 and Widestrike Bt traits died than did those feeding on the non-Bt variety, but mortality did not differ between the two Bt varieties (Table 2). Although the Bt varieties were exposed to large larvae, mortality after 7 days of non-preferential exposure resulted in 80 and 90% mortality on the Widestrike and Bollgard 2 varieties, respectively. Non-Bt plants allowed to grow in the absence of saltmarsh caterpillars had a mean leaf area of 75.6 cm^2, while those exposed to two, fourth-instar larvae had a significantly smaller mean leaf area of 36.3 cm^2, a 48% reduction. Neither the Bollgard 2 nor the Widestrike varieties suffered a significant reduction in leaf area relative to noninfested plants, indicating the surviving saltmarsh caterpillar larvae fed very little. Under field conditions, it is probable that dispersing larvae encountering Bollgard 2 or Widestrike cotton varieties would not feed substantially on those plants but continue to move until death, starvation-induced precocious pupation, production of supernumerary molts, or until a suitable host was encountered (Jones et al. 1980, Safranek and Williams 1984). Leaf area did not differ among the varieties when not infested, but both infested Bt varieties had more leaf area than the non-Bt variety.

Table 2. Leaf Feeding Damage and Percentage Mortality of Fourth-instar Saltmarsh Caterpillars Exposed to Non-Bt and Transgenic Bt Cotton Varieties

				Leaf area (cm^2)	
Variety	Bt trait	n	Percent mortality	Larvae absent	Larvae present
DP174RF	None	10	10.00 ± 10.00 a	75.60 Aa	36.29 Bb
DP141B2RF	Cry1Ac & Cry2Ab	10	90.00 ± 10.00 b	60.85 Aa	64.11 Aa
PHY375WRF	Cry1Ac & Cry1F	10	80.00 ± 13.33 b	79.63 Aa	73.85 Aa

Means followed by the same lower-case letter in a column and the same upper-case letter in a row are not significantly different (*F*- Protected LSD, $P \leq 0.05$).

Damage potential and impact on yield by defoliation of seedling cotton is variable and unclear. Destruction of 50% of one cotyledon on cotyledon-stage cotton resulted in a 4 to 6% increase in yield, while cotton with one cotyledon removed suffered no effect, and when 1.5 or both cotyledons were removed, yield was reduced 11 to 33 and 81 to 100%, respectively (Verhalen et al. 2008). Thus, yield loss was not significant until 75% of the leaf tissue was removed. Similarly, Lane (1959) found that a cotton seedling must suffer more than 75% leaf area reduction before yield was affected. Wanjura and Upchurch (1998) reported in a 2-year study that on cotton averaging 2.8 nodes, yield was reduced both years only when all the cotyledons and true leaves were removed, and during 1 year, yield was significantly less when the true leaves were removed. However, Wilson et al. (2003) reported that cotton defoliated as much as 87% at the node 2 and 4 stages suffered no reduction in boll dry weight, but crop maturity might be affected. Although conflicting information exists, it seems a reduction in leaf area of 75% or more may adversely affect yield.

Nondamaged cotton on the Texas High Plains will typically have an approximate leaf area of 20.0 ± 4.14, 33.8 ± 7.43, and 45.3 ± 8.27 cm^2 at the cotyledon, 1-true-leaf, and 2-true-leaf stages, respectively (Kerns, unpublished data) (Table 3). In our study, a single fourth-instar saltmarsh caterpillar larva consumed a mean of 19.7-cm^2 leaf tissue in a 7-day period, which ended near or at the onset of pupation. Thus, if seedling cotton can tolerate approximately 75% defoliation without significantly impacting yield, it is plausible that on healthy cotton with an adequate plant population, cotyledon-stage cotton fed on by late fourth-instar saltmarsh caterpillars can withstand about 0.5 larva per plant, 1 true-leaf cotton about 1 larva per plant, and 2 true-leaf stage cotton about 1.5 larvae per plant. However, these values need to be validated in the field.

Table 3. Calculated Leaf Area cm^2 (% Reduction) of Three Stages of Seedling Cotton Fed On by Fourth-instar Saltmarsh Caterpillar[a]

	Leaf area cm^2 (% reduction)		
Larvae/plant	Cotyledon	One true leaf	Two true leaf
0.0	20.03 (0.00)	33.76 (0.00)	45.30 (0.00)
0.5	10.20 (49.09)	23.93 (29.11)	35.47 (21.70)
1.0	0.37 (98.17)	14.10 (58.23)	25.64 (43.40)
1.5	0.00 (100)	2.07 (93.87)	15.81 (65.11)
2.0	0.00 (100)	0.00 (100)	5.98 (86.81)

[a]Consumption based on a mean of 19.66-cm^2 leaf area by a single fourth-instar larva over a 7-day period with temperature averaging 25.5 ± 2°C.

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