Evaluation of Seedling Transgenic Cotton Containing Bacillus Thuringiensis Toxins to Saltmarsh Caterpillar

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Introduction

The saltmarsh caterpillar, *Estigmene acrea* (Drury), is an occasional pest of cotton, *Gossypium hirsutum* L., in the U.S., particularly in the Southwest. 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 (Figure 1). 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).

Figure 1. Late instar saltmarsh caterpillar feeding on seedling cotton

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. 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.

Objectives

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.

Materials and Methods (continued)

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 (Figure 2). 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.

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 (Figure 3A). 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

Results and Discussion (continued)

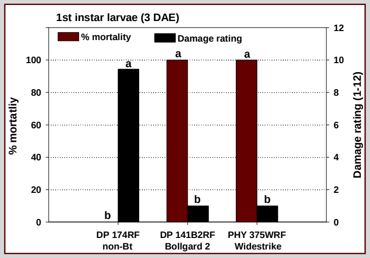

Figure 2. Leaf-feeding damage ratings and percentage mortality of 1[st] instar saltmarsh caterpillars exposed to non-Bt and transgenic Bt cotton varieties. Same colored bars capped with the same letter are not significantly different based on a *F* protected Mixed Procedure (LSD, $P < 0.05$)

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 (Figure 3B). 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. 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 (Figure 3B).

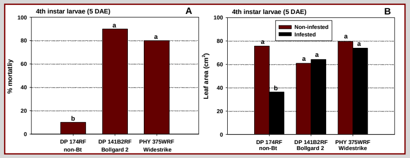

Figure 3. A) Percentage mortality of 4[th] instar saltmarsh and B) Leaf feeding damage of 4[th] instar saltmarsh caterpillars exposed to non-Bt and transgenic Bt cotton varieties. Same colored bars capped with the same letter are not significantly different based on a *F* protected Mixed Procedure (LSD, $P < 0.05$)

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.

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 cm2 at the cotyledon, 1-true-leaf, and 2-true-leaf stages, respectively (Kerns, unpublished data) (Table 1).

Results and Discussion (continued)

Table 1. Calculated leaf area cm^2 (% reduction) of three stages of seedling cotton fed on by 4[th] instar saltmarsh caterpilar[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.

In our study, a single fourth-instar saltmarsh caterpillar larva consumed a mean of 19.7-cm2 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.

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