BEHAVIORAL RESPONSE OF LEPIDOPTERAN PESTS ON COTTON EXPRESSING INSECTICIDAL PROTEINS OF *BACILLUS THURINGIENSIS*

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Abstract

Laboratory studies were conducted to evaluate the behavioral response of bollworm and fall armyworm on non-Bt cotton cultivars versus those expressing one (Bollgard™) or two (Bollgard II™) insecticidal proteins of *Bacillus thuringiensis*. Plastic trays consisting of cells with interconnecting corridors were used to assay larval movement and preference for meridic diet containing freeze-dried leaf tissue. After six hours, there was no significant difference in the variety bollworm or fall armyworm larvae preferred. For all varieties, 75-84% specimens were still in cells containing diet with incorporated leaf tissue. However, only 28% of bollworm larvae had moved from their original cell containing non-Bt tissue, compared to 49% that had vacated cells containing either Bt cotton tissue. About 60% of fall armyworm larvae were found in cells containing non-Bt and dual-toxin tissue, and fewer were found on single-toxin tissue (43%). After 24 hours, 88% of bollworms were found in cells containing non-Bt plant tissue, compared to 68% and 53% of bollworms found in cells containing single-toxin and dual-toxin plant tissue, respectively. Fall armyworms were found in cells containing tissue from non-Bt and single-toxin cotton 61% and 71% of the time, respectively. Only 36% of fall armyworms were found in cells containing both Cry1Ac and Cry2ab toxins. Larvae of both bollworm and fall armyworm were more inclined to move when placed in cells containing dual-toxin Bt plant tissue, as 78% of the larvae moved at least one cell after 24 hours. Approximately 50% of larvae moved when placed in cells containing non-Bt and single-toxin plant tissue.

Introduction

Much of the total economic damage to cotton (*Gossypium hirsutum*) can be attributed to the bollworm (*Helicoverpa zea*) and fall armyworm (*Spodoptera frugiperda*) (Williams 2000). These pests can be especially detrimental if populations are high, feeding on cotton squares and bolls. Transgenic cotton expressing the Cry1Ac δ-endotoxin of *Bacillus thuringiensis* Berliner (Bt) is available for protection against certain lepidopteran pests. In the Mid-South, this technology is used to provide season-long suppression of heliothines in cotton. Although excellent control is exhibited towards tobacco budworm (*Heliothis virescens*), less success has been noted against bollworm (Stone and Sims 1993). This is particularly true after the initiation of blooming and insecticides have disrupted beneficial arthropod populations that normally keep bollworm populations in check. Also, intrinsically tolerant lepidopteran species, such as the fall armyworm, can cause economic damage in Bt cotton (Layton 1996).

Because fall armyworms and high populations of bollworm can be economically detrimental to Bt cotton, supplemental insecticide applications are sometimes necessary (Layton 1996). Insecticides with contact activity should have greater effectiveness in Bt cotton if larvae are more inclined to move while searching for suitable feeding sites. However, this movement may also increase the survival of individuals with some resistance to Bt toxins if it enables them to find lower-expressing plant tissue.

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As of late, cotton expressing two insecticidal proteins of *Bacillus thuringiensis* has been developed (i.e., Bollgard II™). This transgenic cotton not only expresses the Cry1Ac toxin, but an additional gene expressing Cry2Ab toxin has been added to aid in control of occasional pests (e.g., fall armyworm) and high populations of bollworm. This research addresses the effects of cotton expressing one or two Bt toxins on behavior of bollworm and fall armyworm larvae.

Materials and Methods

Studies were conducted to evaluate how ingestion of various Bt toxins would influence the behavior of bollworm and fall armyworm larvae. In the laboratory, trays comprised of 30 cells (6 x 5) with connecting corridors were used as the test arena (BioServ; Frenchtown, NJ). These trays were designed to hold 30-ml larval rearing cups. Cells were filled with meridic diet alone or meridic diet containing lyophilized leaf tissue of non-Bt, single-toxin, or dual-toxin cotton incorporated at a dose of 20 μg tissue/ml. This dose does not cause significant short-term mortality, but for Bt cottons, can greatly affect larval development (Stewart et al., in press). Four cells in each corner of the tray (16 cells total per tray) contained diet with incorporated tissue from one cultivar, and the remaining cells contained diet alone (Figure 1). One larva was placed in each corner of the tray, which was then covered with Mylar® and tack-ironed to prevent escapes. Trays were placed in an incubator (dark) at 29°C. For each of the three varieties and each species, 40 neonates (ten trays, four larvae per tray) were tested. Larval movement from the original cell and its choice of food (i.e., treated vs. untreated diet) was recorded after 6 and 24 hours. Experiments were then repeated using second instar larvae of each species.

Migration among cells should indicate: 1) if larvae can detect Bt toxins, 2) if they avoid sites with Bt toxins, and 3) if larvae preferentially seek lower-toxin food sources. Data were analyzed using categorical data analysis (Proc Genmod, LSmeans, SAS Institute 1998) to determine if the presence of one or two Bt toxins or larval age affected their behavior.

Results

6 Hours

There was no significant difference of preference between variety for either bollworm or fall armyworm (Table 1). Approximately 83% of larvae for both species were on diet containing non-Bt or Bollgard plant tissue, and 75% larvae for both species were found on diet containing dual-toxin tissue (p=0.061). Fewer bollworm and fall armyworm neonates were found on treated diet (71%) than second instar larvae (89%), likely due to the higher sensitivity to the toxin exhibited by neonate larvae. Neonate larvae are also more inclined to move than second instar larvae. Fall armyworms were more likely to be on treated diet (76%) than were bollworms (84%). Fall armyworms may be inherently more mobile than bollworms; thus they are more likely to move several cells after six hours.

Of bollworm larvae, 49% had moved from their original site of placement in trays containing either Bt cotton, compared to only 28% in non-Bt cotton trays (Table 2). Fall armyworm showed unexpected results of movement. Only 43% of larvae had moved at least one cell in Bollgard trays, compared to 59% and 62% of larvae for trays containing non-Bt and dual-toxin cotton tissue, respectively. Neonate larvae for both species were approximately twice as mobile (64%) as second instar larvae (33%) in all trays. Neonates may be more mobile than second instar larvae, or increased sensitivity of neonates to Bt toxins may have caused them to be more active.

24 Hours

The numbers of bollworms found in Bollgard or Bollgard II cells were not significantly different (Table 3). For non-Bt cotton, 88% of bollworms were found on treated diet compared to 68% and 53% for Bollgard and Bollgard II cotton, respectively. For fall armyworm, however, the addition

of the second toxin in Bollgard II had a significant effect on the number of larvae that were found on treated diet. On non-Bt and Bollgard trays, 61% and 71%, respectively, were residing in cells with incorporated plant tissue. For Bollgard II, only 36% of larvae were found in cells containing treated diet. For both species, more second instar larvae (72%) than neonates (53%) were found on treated diet.

The percentage of larvae that had moved by 24 hours was significantly higher in trays containing dual toxin plant tissue compared to non-Bt and single-toxin varieties (Table 4). For bollworm and fall armyworm, 78% of larvae had moved in Bollgard II trays. Approximately 50% of larvae moved at least one cell in trays containing non-Bt or Bollgard tissue. It was also noted that more specimens had escaped the trays containing dual toxin cotton tissue compared with other varieties. For fall armyworm, it is evident that the Cry1Ac toxin alone had little effect on larval behavior, but the addition of the Cry2ab toxin caused increased movement.

Summary

These data indicate that, for bollworm and fall armyworm, ingestion of Bt toxins will result in increased movement as larvae seek low-toxin tissue on which to feed. Increased movement on Bt cottons may increase the efficacy of supplemental insecticide applications. However, it may also increase the chances that larvae which are partially resistant to Bt toxins will survive exposure to Bt plants.

References

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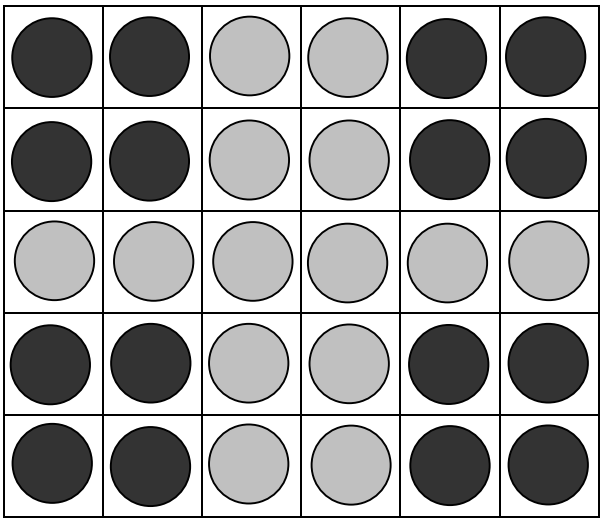

Figure 1. Test arena consisting of meridic diet (gray) and meridic diet incorporated with plant tissue (black). Cells interconnected horizontally and vertically (not diagonally).

Table 1. Percent larvae on plant tissue-incorporated diet after 6 hours.*

	Bollworm & Fall armyworm
Variety	
DPL50	84±3 a
DPL50B	82±3 a
MON15985	75±3 a (p=0.061)
Instar	
Neonates	71±2 a
2nd Instar	89±3 b
Species	
Bollworm	84±2 a
Fall armyworm	76+2 b

Numbers not followed by a common letter are significantly different (Proc GENMOD, Pdiff, SAS Institute 1998)
*There was no interaction between main effects

Table 2. Percent larvae that moved after 6 hours.*

Variety	**Bollworm**	**Fall armyworm**
DPL50	28±5 a	59±5 a
DPL50B	49±5 b	43±5 b
MON15985	49±5 b	62±5 a
Instar	**Bollworm & Fall armyworm**	
Neonates	64±2 a	
2nd Instar	33+3 b	

Numbers not followed by a common letter are significantly different (Proc GENMOD, Pdiff, SAS Institute 1998)
*Variety by species interaction: X^2=16.2, df=2, P<0.0003 (Proc Genmod, SAS)

Table 3. Percent larvae on plant tissue-incorporated diet after 24 hours.*

Variety	Bollworm	Fall armyworm
DPL50	88±5 a	61±5 a
DPL50B	68±6 b	71±5 a
MON15985	53±6 b	36±5 b
Instar	**Bollworm & Fall armyworm**	
Neonates	53±3 a	
2nd Instar	72+3 b	

Numbers not followed by a common letter are significantly different (Proc GENMOD, Pdiff, SAS Institute 1998)

*Variety by species interaction: X^2=7.76, df=2, P<0.021 (Proc Genmod, SAS)

Table 4. Percent larvae that moved after 24 hours.*

Variety	Bollworm & Fall armyworm	
DPL50	47±4 a	
DPL50B	50±4 a	
MON15985	78±4 b	
Instar	**Bollworm**	**Fall armyworm**
Neonates	46±5 a	60±5 a
2nd Instar	37+5 a	77+5 b

Numbers not followed by a common letter are significantly different (Proc GENMOD, Pdiff, SAS Institute 1998)

*Instar by species interaction: X^2=7.63, df=1, P<0.006 (Proc Genmod, SAS)