EFFECT OF TRANSGENIC BT-COTTON ON LARVAL MORTALITY AND DEVELOPMENT OF BEET ARMYWORM, *SPODOPTERA EXIGUA* (LEPIDOPTERA: NOCTUIDAE)

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Abstract

Studies were conducted to determine the effects of transgenic Bt-cotton on three larval instars of beet armyworm, *Spodoptera exigua*. First, third and fifth instars were fed field collected Bt-cotton leaves for 1, 2, 3, 4 and 7 d or until pupation, and then transferred to artificial diet. Larval mortality to pupation, larval survival to adults, larval and pupal periods and pupal weights were recorded. Larval mortality at pupation was low for all instars tested, and for the time period on Bt-cotton. Larval period increased with the increase of feeding time on Bt-cotton in 1st and 3rd instars, but not in 5th instar, and significantly differed among three instars when fed continuously on Bt-cotton leaves. Pupal weight was reduced with increase in feeding time on Bt-cotton, and a greater reduction was recorded in larger instars when they were transferred to diet from Bt-cotton. Feeding time on Bt-cotton effected the survival to adults in 1st and 3rd instars but not in 5th instars. These data indicate that Bt-cotton results in a low level of mortality in *S. exigua* larvae, however, it does adversely effect them in other ways such as reduction in pupal weight and extension in larval developmental time.

Introduction

The acreage of transgenic cotton, carrying a *Bacillus thuringiensis* var. *kurstaki* insecticidal protein (CryIA(c) gene, has increased since it was developed in 1996. It has proven effective against some major lepidopterous pests of cotton (Benedict et al.1991, 1993, Ring et al. 1993, Mahaffey et al. 1994).

Cry IA(c) endotoxin, although highly toxic to many lepidopteran pests, is not very efficacious against *Spodoptera* species (Moar et al. 1990, Inagaki et al. 1991). Moar et al. (1990) reported that CryIA(c) protein gave less mortality of *S. exigua* than its sister proteins CryIA(a) and CryIA(b) when incorporated into artificial diet. Inagaki et al. (1991) found that the larvae of *S. litura* had >3600-fold less susceptibility to crystal δ-endotoxin of *B. thuringiensis* var. *kurstaki* HD-1 than a susceptible insect *Bombyx mori*.

Bacillus thuringiensis has been reported to have sub-lethal effects on *S. exigua* when applied in sub-lethal doses (Bai et al. 1993). Dabbs (1995) reported that sub-lethal doses of Condor (*B. thuringiensis*) resulted in low mortality, however, it caused a significant decrease in larval and pupal mass, and a delay in pupation in *S. exigua* larvae. Stapel et al. (1997) observed a lower pupal weight and longer developmental time in *S. exigua* fed on Bt-containing diet. In cases of sub-lethal doses the insect may recover, regenerate columnar cells and complete development (Chiang et al. 1986).

Transgenic Bt-cotton has been reported to cause significant mortality in *Helicoverpa zea* and *Heliothis virescens* (Benedict et al. 1991), and also sub-lethal effects in heliothines (Jenkins et al. 1993, Halcomb et al. 1996) and *S. frugiperda* (Adamczyk et al. 1998). Research has been conducted on the effects of different formulations of *B. thuringiensis* on *S. exigua*, but not much is known of the effect of transgenic cotton on this pest. The objective of these laboratory studies was to evaluate the effects of transgenic Bt-cotton on the mortality, survival and development of *S. exigua*.

Materials and Methods

The experiment was conducted in 1998 in the Department of Entomology, University of Arkansas, Fayetteville AR. The transgenic Bt-cotton (DPL 32B) and regular cotton (DPL 51) were planted in the field on 17 May 1998 and furrow irrigated as needed. Fully expanded, undamaged leaves were removed from the top canopy of plants for the test. The leaves were kept fresh in closed plastic bags, and used within 2 h of excision.

Insects
The *S. exigua* eggs were obtained from ARS, the USDA laboratory Stoneville MS. Eggs were held in an environmental chamber at 25 ±1 ^{0}C and a photoperiod of 14:10 (L:D) h cycle until hatch.

Experimental Procedures
Three larval instars (1st, 3rd and 5th) were employed in the study. Each instar group (125 larvae) was fed transgenic Bt-cotton for 1, 2, 3, 4, or 7 days or until pupation. One group of 25 neonates was reared on artificial diet (Burton 1969) and another group of 25 neonates was fed on non-Bt cotton (DPL 51) until pupation which served as the controls. The experiment was repeated five times. All larvae in the test were held in an environmental chamber set at 30 ± 1 ^{0}C and a photoperiod of 14:10 (L:D) h.

Spodoptera exigua exposed to Bt-cotton as 1st instar were placed individually as neonates in each of 125, 30-ml transparent plastic cups containing one leaf per cup. Larvae exposed to Bt-cotton as 3rd or 5th instar were reared individually in plastic cups containing 10 ml of artificial diet. When the desired instar, 3rd or 5th, was reached, 125 larvae were placed individually in 30-ml cups with one Bt-cotton leaf. The leaf in each cup was replaced with a fresh

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leaf every other day. Larvae in each instar group were inspected after 1, 2, 3, 4 and 7 days, mortality was recorded and the survivors were transferred from Bt-cotton leaves to artificial diet. A larva was considered dead when it was unable to move after being prodded. Fifth instar larvae were transferred to artificial diet at 1 and 2 d only, because they were pupating after 2 d. Pupal weight was recorded after 24 h by using a top loading electric balance (Mettler PL 300). Time to pupation, larval survival to pupation, pupal survival to adult eclosion and number of days to adult emergence was recorded.

Statistical Analysis

All data were analyzed using analysis of variance (ANOVA) (PROC GLM, SAS Institute 1989). Means were separated using the Fisher protected least significant difference (LSD) for all the data (PROC GLM, Option LSD, SAS Institute 1989).

Results

Mortality at pupation in larvae placed as 1st instar on Bt-cotton leaves increased with an increase in feeding period on these leaves (Table 1). In this instar mortality of larvae on Bt-cotton until pupation was higher than mortality at shorter feeding periods. Mortality at pupation was also significantly ($P < 0.05$) higher in 3 to 7 d groups than those on Bt-cotton only for 1 and 2 d or the control. Mortality in larvae exposed to Bt-cotton as 3rd and 5th instar was not effected by the feeding period on Bt-cotton. Mortality in larvae placed on Bt-cotton leaves as 1st instars was also significantly higher than those placed on leaves as 3rd and 5th instars at longer feeding periods or until pupation (Table 1).

The larval developmental period was extended with an increase in larval feeding time on Bt-cotton in 1st and 3rd instar, but not 5th instar (Table 2). In larvae exposed as 1st instar, the developmental period increased significantly when the larvae fed on Bt-cotton leaves for 3 or more days. The developmental period was significantly longer when larvae were transferred to diet after 7 d than those which were transferred after 4 days or less. The 1st instars took a significantly ($P < 0.05$) longer time to pupate when they completed their development on Bt-cotton than those which were transferred to diet. In 3rd instar, the larval period was significantly ($P < 0.05$) longer in all the feeding periods on Bt-cotton than the control on diet. Within feeding periods for 3rd instar there was no difference between 3 d and all the other longer feeding periods, however, 7 d and until pupation were significantly longer than 1 and 2 d. The feeding period did not effect the larval developmental time in 5th instar. Among instars, 1st instar had a significantly ($P < 0.05$) longer developmental period than 3rd instar at all the feeding periods except 7 d. It was longer than 5th instar when they were maintained on Bt-cotton until pupation (Table 2).

A significant ($P < 0.05$) reduction in pupal weight occurred in larvae exposed to Bt-cotton at all ages (Table 3). The 1st instar pupal weight was significantly less when on Bt-cotton for 7 days than for 1 or 2 days, but not for those on it for 3 or 4 days. The pupal weight in 1st instar left on Bt-cotton leaves until pupation was significantly less than for 1st instar on Bt-cotton for all shorter feeding periods. Pupal weight reduction occurred when 3rd instar were fed on Bt-cotton for only 2 d. In the 3rd instar group, larvae left on Bt-cotton 4 to 7 d had a significantly lower pupal weight than those on Bt-cotton 2 or 3 d. The pupal weight in 3rd instar left on Bt-cotton leaves until pupation was not different for those larvae on it for 7 days , though it was significantly ($P < 0.05$) less than when on it for 4 d or less. In 5th instar the reduction in pupal weight was significantly greater in larvae which completed their development on Bt-cotton than those on 1 d. A significantly ($P < 0.05$) higher reduction was recorded at all the feeding periods in 3rd instar than 1st instar, but there was no difference between them when both instars were left on Bt-cotton until pupation. The 5th instar did not differ from 3rd instar at the 1 and 2 d feeding period, but had a higher pupal weight than 1st and 3rd instar when left of Bt-cotton until pupation (Table 3).

The percentage of larval survival to adult (adult emergence) in larvae exposed as 1st instar was only reduced when fed Bt-cotton leaves until pupation. The survival to adults in larvae exposed as 1st instar was significantly ($P < 0.05$) reduced only when larvae completed their development on Bt-cotton leaves. Survival to adults in larvae exposed to Bt-cotton leaves as 3rd instar was significantly lower when allowed to remain on it for 7 d. Survival to adults in the 3rd instar group was significantly ($P < 0.05$) less in larvae left on Bt-cotton until pupation than those transferred to diet. The 5th instar group's survival to adults was not effected by the feeding time on Bt-cotton leaves. All the instars differed significantly ($P < 0.05$) for larval survival to adult when they were left on Bt-cotton until pupation, with a maximum survival (85.6%) in 5th instar and minimum (50.4%) in 1st instar.

Discussion

The mortality at pupation in *S. exigua* larvae feeding on Bt-cotton was low, and it was less in larger instars. Previously, Moar et al. (1990) reported that *B. thuringiensis* protein CryIA (c) was less toxic to *S. exigua* than the other two *B. thuringiensis* proteins CryIA (a) and CryIA (b) when incorporated in diet. Also Inagaki et al. (1990) found that larvae of *S. litura* were less susceptible to crystal δ-endotoxin than the other lepidopterous insects tested in in-diet tests. Chiang et al. (1986) had found that some lepidopteran larvae fed *B. thuringiensis* may recover, and complete their development once they are removed from *B. thuringiensis*. We found that once the larvae were removed from Bt-cotton leaves their chance of survival was greater than those which were left on Bt-cotton leaves until pupation.

The developmental time was extended in the earlier instars when *S. exigua* fed on Bt-cotton for only a few days. Dabbs (1995) reported that *S. exigua* larvae reared on diet containing sub-lethal doses of *B. thuringiensis* had a longer larval stage. Similar results were found by Stapel et al. (1997), who reported a longer developmental time in *S. exigua* when fed Bt-containing diet. Extension in larval developmental time has also been reported in *H. zea* and *H. virescens* on transgenic Bt-cotton by Jenkins et al. (1993) and Halcomb et al. (1996). Our results show that 3rd instar was more effected than 1st instar regarding larval developmental time at shorter feeding periods. This might be due to more consumption of Bt-cotton by 3rd instar than the 1st instar over a short period of time.

We found a reduction in pupal weight in all instars of *S. exigua* fed Bt-cotton, but this reduction was greater in larger instars than 1st instar in larvae fed Bt-cotton only 7 days or less. This may be because larger instars had less time than did 1st instar to recover and gain the required weight after being transferred to Bt-free diet. Dabbs (1995) reported that sub-lethal doses of *B. thuringiensis* reduced the pupal weight in *S. exigua*. Adamczyk et al. (1998) reported a reduction in pupal weight in *S. frugiperda* larvae fed Bt-cotton. Also Halcomb et al. (1996) observed a pupal weight reduction in *H. zea* and *H. virescens* on Bt-cotton. Our results with *S. exigua* were similar to the above findings by other research workers for other noctuid insects on Bt-cotton.

Spodoptera exigua survival to adults was reduced only in larvae fed Bt-cotton until pupation beginning in the 1st or 3rd instar. However, only 1st instar fed Bt-cotton throughout the larval stage had a survival rate as low as 50%. Previously, Jenkins et al. (1993) and Halcomb et al. (1996) found little or no *H. zea* and *H. virescens* survival to adult stage on Bt-cotton. This finding shows that *S. exigua* is more tolerant of Bt-cotton than the heliothines.

In conclusion, the results of the studies show that transgenic Bt-cotton causes low mortality in *S. exigua*. However, larval developmental time was increased and pupal weight was reduced in *S. exigua* surviving Bt-cotton. In light of these sub-lethal effects of Bt-cotton further studies are needed to evaluate these effects on fecundity of adult survivors, and in succeeding generations.

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Table 1. Percent larval mortality at pupation in three instars of *S. exigua* fed Bt-cotton leaves for different time periods.

Days on Bt-cotton leaves [a]	Instar at exposure to Bt-cotton		
	1st	3rd	5th
1 d	11.2 aC	10.4 aA	2.4 bA
2 d	11.2 aC	11.2 aA	3.2 bA
3 d	19.2 aB	10.4 bA	-[b]
4 d	20.0 aB	9.6 bA	-[b]
7 d	20.8 aB	12.0 bA	-[b]
Until pupation	29.6 aA	13.6 bA	5.6 cA

Means in a column not sharing an upper case letter or in a row not sharing a lower case letter are significantly different ($P < 0.05$).
[a] Mortality in control was 10.4 for 1st instar on diet and 12.0 on non-Bt cotton.
[b] Larvae died or pupated before day 3.

Table 2. Days for development of *S. exigua* larvae fed on Bt-cotton for different time periods.

Days on Bt-cotton leaves [a]	Instar at exposure to Bt-cotton		
	1st	3rd	5th
1 d	9.7 bD	10.6 aB	10.2 ab A
2 d	10.1 bCD	11.0 aB	10.3 bA
3 d	10.4 bCD	11.6 aAB	-[b]
4 d	10.8 bC	11.7 aAB	-[b]
7 d	12.5 aB	12.1 aA	-[b]
Until pupation	13.6 aA	12.1 bA	10.3 cA

Means in a column not sharing an upper case letter or in a row not sharing a lower case letter are significantly different ($P < 0.05$).
[a] Developmental time in controls was 9.6 days in 1st instar on diet and 11.2 days on non-Bt-cotton.
[b] Larvae died or pupated before day 3.

Table 3. Pupal weight (mg) for three instars of *S. exigua* when fed on Bt-cotton for different time periods.

Days on Bt-cotton leaves [a]	Instar at exposure to Bt-cotton		
	1st	3rd	5th
1 d	135.4 aA	121.2 bA	115.7 bA
2 d	135.9 aA	109.1 bB	107.2 bAB
3 d	132.5 aAB	102.4 bB	-[b]
4 d	132.2 aAB	91.1 bC	-[b]
7 d	124.3 aB	85.3 bCD	-[b]
Until pupation	86.8 aC	79.1 aD	105.4 bB

Means in a column not sharing an upper case letter or in a row not sharing a lower case letter are significantly different ($P < 0.05$).
[a] Pupal weight in cotrols was 148.1 mg for 1st instar on diet and 116.6 mg on non-Bt cotton.
[b] Larvae died or pupated before day 3.

Table 4. Larval survival to adults (percentage) in three instars of *S. exigua* fed on Bt-cotton leaves for different time periods.

Days on Bt-cotton leaves [a]	Instar at exposure to Bt-cotton		
	1st	3rd	5th
1 d	84.8 aA	83.2 aAB	93.6 aA
2 d	81.6 aA	86.4 aA	87.2 aA
3 d	76.8 aA	81.6 aAB	-[b]
4 d	76.0 bA	87.2 aA	-[b]
7 d	74.4 aA	74.4 aB	-[b]
Until pupation	50.4 cB	63.2 bC	85.6 aA

Means in a column not sharing an upper case letter or in a row not sharing a lower case letter are significantly different ($P < 0.05$).
[a] Larval survival to adults in controls was 85.6 % in 1st instar on diet and 75.2 % on non-Bt cotton.
[b] Larvae died or pupated before day 3.