Development of *Spodoptera exigua* and *Helicoverpa zea* (Lepidoptera: Noctuidae) on Transgenic Cotton Containing CryIAc Insecticidal Protein[1]

Muhammad Ashfaq,[2] S. Y. Young[2] and R. W. McNew[3]

University of Arkansas, Fayetteville AR 72701 USA

J. Entomol. Sci. 35(4): 360-372 (October 2000)

Abstract The effects of transgenic *Bacillus thuringiensis* (Bt)-cotton on three instars of *Spodoptera exigua* (Hübner) and *Helicoverpa zea* (Boddie) were studied. 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 at pupation, length of larval and pupal periods, pupal weights and survival time from hatch to adult were recorded for regular and Bt-cotton. Larval mortality at pupation in *S. exigua* fed Bt-cotton was low for all instars exposed and feeding periods on Bt-cotton. The mortality in *H. zea* was high for first and third instars and significantly increased with the increase in feeding period on Bt-cotton ($P < 0.05$). The length of larval period increased in both species when first and third instars but not fifth instars were fed Bt-cotton. In *H. zea,* the larval period increased when larvae were on Bt-cotton leaves for only 2 d. Pupal weight was reduced with an increase in feeding time on Bt-cotton in all three instars in both species. An increase in length of feeding time on Bt-cotton reduced survival to adults in both species on Bt-cotton in first and third instars but not in fifth instars. Survival to adults was much lower in *H. zea* than *S. exigua* for all instars exposed and feeding periods on Bt-cotton. These results demonstrate the large differences in effectiveness of Bt-cotton against lepidopteran cotton pests, as well as the adverse effects on larvae developing on Bt-cotton.

Key Words *Spodoptera exigua, Helicoverpa zea,* transgenic cotton, *Bacillus thuringiensis*

Bollworm, *Helicoverpa zea* (Boddie), is a frequent and major pest of cotton. The beet armyworm, *Spodoptera exigua* (Hübner), also damages cotton when outbreaks occur (Smith 1989). Management of *H. zea* is generally effective with conventional chemical insecticides (Plapp 1979). However, variable levels of beet armyworm tolerance to chemical insecticides have been reported in numerous studies, and control has been erratic (Cobb and Bass 1976, Meinke and Ware 1978, Brewer et al. 1990). The development of plant transformation technology has allowed the introduction and expression of CryIA genes encoding *Bacillus thuringiensis* Berliner var. *kurstaki* CryIA insecticidal proteins into the cotton genome (Perlak et al. 1990). The development of transgenic Bt-cottons offers an alternative to heavy reliance on conventional insecticides against some lepidopterans.

Transgenic Bt-cotton (Bt-cotton) has been effective against some major lepidopteran pests of cotton (Benedict et al. 1992, 1993, Mascarenhas et al. 1994, Mahaffey

[1]Received 15 November 1999; accepted for publication 28 February 2000.

[2]Department of Entomology.

[3]Agricultural Statistics Laboratory.

et al. 1994, Halcomb et al. 1994, 1996, Meyers et al. 1997) and is widely used in management of these pests on cotton (Layton et al. 1997). Benedict et al. (1996) found that *H. zea* and *Heliothis virescens* (F.) larvae caused much less injury to Bt-cotton lines than the non-transgenic cotton. Important lepidopteran pests of cotton, such as *H. zea, H. virescens, Pectinophora gossypialla* (Sanders), and *Spodoptera frugiperda* (J. E. Smith) differ widely, however, in their susceptibility to the δ-endotoxin found in foliar-applied *B. thuringiensis* products (MacIntosh et al. 1990, Nyouki et al. 1996) as well as in Bt-cotton (Wilson et al. 1992, Halcomb et al. 1996, Adamczyk et al. 1998). CryIA (c) endotoxin, although highly toxic to *H. virescens* and *H. zea,* is not very efficacious against *Spodoptera* species (Moar et al. 1990, Inagaki et al. 1991). However, Davis et al. (1995) and Harris et al. (1996) reported suppression of *S. exigua* activity by Bt-cotton under field conditions. Burris et al. (1994) also determined no effect of Bt-cotton on mortality and feeding of *S. exigua* in the field.

The efficacy of *B. thuringiensis* is greater when applied against early than late instars of target species. For example, Ali and Young (1996) reported that the LC_{50} of *B. thuringiensis* for *H. zea* larvae 1 d in age was 5.7-fold lower than for those 5 d in age. Bt-cotton has also been reported to cause sublethal effects in heliothines (Jenkins et al. 1993, Halcomb et al. 1996, Mascarenhas and Luttrell 1997) and *S. frugiperda* (Adamczyk et al. 1998). The weight of *H. zea* larvae fed Bt-cotton was less than that in larvae fed non-Bt cotton (Halcomb et al. 1996, Mascarenhas and Luttrell 1997). The sublethal doses of *B. thuringiensis* var. *kurstaki* were also reported to cause sublethal effects such as increased larval developmental period in *S. exigua* (Bai et al. 1993). Stapel et al. (1997) observed both a lower pupal weight and longer developmental time in *S. exigua* fed *B. thuringiensis*-containing diet. Sub-lethal doses of *B. thuringiensis* in rice moth, *Corcyra cephalonica* (Stainton), larvae allow the insect to recover, regenerate columnar cells and complete development (Chiang et al. 1986).

The studies reported herein were focused on the effects of Bt-cotton on different instars of *H. zea* and *S. exigua* fed leaves for different periods of time and then transferred to semisynthetic diet.

Materials and Methods

Transgenic Bt-cotton (DPL 32B) and nontransgenic (non-Bt) cotton (DPL 32) were planted in the field on 11 May 1998 at the Agricultural Experiment Station, University of Arkansas, Fayetteville, and furrow irrigated as needed. After plants reached 8 wks of age, fully-expanded, undamaged leaves were removed from the top canopy 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 the ARS, USDA Crop Sciences Research Laboratory, Stoneville, MS. Eggs were held in an environmental chamber at 25 ± 1 °C and a photoperiod of 14:10 (L:D) h cycle until hatching. The *H. zea* larvae were obtained from a laboratory colony maintained in the Department of Entomology, University of Arkansas, Fayetteville, AR.

Experimental procedures. Studies were conducted in two separate but similar experiments for *S. exigua* and *H. zea.* Three larval instars (first, third and fifth) were used in each study. Each instar group (125 larvae) was fed transgenic Bt-cotton leaves for 1, 2, 3, 4, or 7 d or until pupation. One group of 25 neonates was reared on artificial diet (Burton 1969) and another group on nontransgenic cotton leaves until pupation, which served as the controls. The experiments were repeated five times. All larvae in the test were held in an environmental chamber set at 30 ± 1 °C and a photoperiod of 14:10 (L:D) h.

Spodoptera exigua and *H. zea* exposed to Bt-cotton as first instars were placed individually as neonates in each of 125, 30-ml transparent plastic cups containing one leaf per cup. Each group of 25 larvae in 30-ml cups was placed in a plastic container containing moist paper towels to assure adequate humidity. Larvae exposed to Bt-cotton as third or fifth instars were reared individually in plastic cups containing 10 ml of artificial diet. When the desired instar, third or fifth, 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 leaf every other day and treated as for the first instar group. Larvae in each instar group were inspected after 1, 2, 3, 4 and 7 d, 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 instars were not transferred to artificial diet after 1 and 2 d because some were pupating by the third day. Pupal weight was recorded 1 d after pupation. Time and larval mortality to pupation, pupal survival to adult eclosion and number of days from neonates to adult emergence were recorded.

Data analysis. All data were analyzed separately for *S. exigua* and *H. zea* using analysis of variance (ANOVA) with mortality at pupation and larval survival to adults as sources of variation (PROC ANOVA, SAS Institute 1989). Length of larval period, pupal weight and length of pupal period were analyzed in a weighted analysis using the number of larvae reaching pupation as a weighting factor (PROC GLM, SAS Institute 1989). When the F test for treatments was significant ($P < 0.05$), treatments were compared by multiple t-tests (PROC GLM, Option LSD, SAS Institute 1989) ($P < 0.05$).

Results

Spodoptera exigua. The stadium at exposure to Bt-cotton and length of exposure interaction was significant for the percentage of mortality at pupation ($F = 2.45$; df7, 64; $P = 0.0274$). Mortality at pupation in larvae placed on Bt-cotton leaves was low, ranging from 29.6% in the first-instar group to 5.6% in the fifth-instar group. The percentage of mortality did, however, increase significantly with an increase in the time that first instars were on these leaves, and was highest when they were on the leaves until pupation ($P < 0.05$) (Table 1). Mortality in larvae exposed to Bt-cotton from third or fifth instars until pupation did not differ significantly from that in the controls. The percentage of mortality in larvae placed on Bt-cotton leaves as first instars was significantly higher than in those placed on leaves as third and fifth instars, except it did not differ from the third-instar group exposed to Bt-cotton through 2 d (Table 1).

The stadium at exposure to Bt-cotton and length of exposure interaction was significant for length of developmental period ($F = 19.53$; df 7, 64; $P = 0.0001$). The larval developmental period was extended with an increase of up to 3.9 and 1.5 d in larval feeding time on Bt-cotton in first and third instars, respectively, but not the fifth instar (Table 2). In larvae exposed as first instars, the developmental period increased significantly when larvae fed on Bt-cotton leaves for 4 and 7 d, and again when allowed to pupate there ($P < 0.05$). When exposed as third instars, the length of developmental period increased over 1 and 2 d of exposure only after larvae were

Table 1. Percent larval mortality at pupation (±SE) in three instars of *Spodoptera exigua* fed Bt-cotton leaves for different time periods

Instar at exposure to Bt-cotton	Days on Bt-cotton leaves*,**					
	1 d	2 d	3 d	4 d	7 d	Until pupation
First	11.2 ± 1.6 aC	11.2 ± 2.8 aC	19.2 ± 2.8 aB	20.0 ± 2.0 aB	20.8 ± 2.8 aB	29.6 ± 6.4 aA
Third	10.4 ± 2.4 aA	11.2 ± 2.0 aA	10.4 ± 2.8 bA	9.6 ± 1.6 bA	12.0 ± 1.6 bA	13.6 ± 2.8 bA
Fifth	2.4 ± 1.6 bA	3.2 ± 1.6 bA	–†	–†	–†	5.6 ± 2.0 cA

Means in a column not sharing a lower case letter or in a row not sharing an upper case letter are significantly different ($P < 0.05$).
* Days the larvae were exposed to Bt-cotton leaves in cups.
** Mortality in controls was 10.4 ± 2.0% for larvae on diet and 12.0 ± 3.6% on non-Bt cotton.
† Larvae died or pupated before day 3.

Table 2. Days (±SE) for development of *Spodoptera exigua* larvae fed Bt-cotton for different time periods

Instar at exposure to Bt-cotton	Days on Bt-cotton leaves*,**					
	1 d	2 d	3 d	4 d	7 d	Until pupation
First	9.7 ± 0.2 bD	10.1 ± 0.2 bCD	10.4 ± 0.2 bCD	10.8 ± 0.2 bC	12.5 ± 0.2 aB	13.6 ± 0.2 aA
Third	10.6 ± 0.2 aB	11.0 ± 0.2 aB	11.6 ± 0.1a AB	11.7 ± 0.2 aAB	12.1 ± 0.1 aA	12.1 ± 0.1 bA
Fifth	10.2 ± 0.5 abA	10.3 ± 0.5 bA	–†	–†	–†	10.3 ± 0.5 cA

Means in a column not sharing a lower case letter or in a row not sharing an upper case letter are significantly different ($P < 0.05$).
* Days the larvae were exposed to Bt-cotton leaves in cups.
** Larval developmental time in controls was 9.6 ± 0.2 days on diet and 11.2 ± 0.2 days on non-Bt cotton.
† Larvae died or pupated before day 3.

exposed for 7 d or longer. When exposed until pupation, the length of larval period decreased with the larval instar when placed on Bt-cotton (Table 2).

The instar at exposure to Bt-cotton and length of exposure interaction was significant for pupal weight ($F = 12.05$; df = 7, 64; $P = 0.0001$). A significant reduction ($P < 0.05$) in pupal weight occurred in larvae exposed to Bt-cotton at all ages (Table 3). Pupal weight of larvae exposed as first or third instars was significantly reduced from that of larvae on cotton for 1d when on Bt-cotton for 7 or 2 d, respectively, and lowest when on Bt-cotton until pupation. In fifth instars, the reduction in pupal weight was significantly greater in larvae that completed their development on Bt-cotton than those on it for 1 d. The pupal weight in third and fifth instars on Bt-cotton leaves up to 7 d was significantly less than in the first-instar group. However, when on Bt-cotton until pupation, the weight reduction was greater in first and third instars than fifth instars. Feeding Bt-cotton to *S. exigua* did not affect the length of its pupal period ($F = 0.72$; df = 16, 68; $P = 0.763$). The mean (±SEM) pupal period in the control groups was 5.50 ± 0.14 d when larvae completed development on artificial diet and 5.64 ± 0.13 d when on non-Bt cotton.

The instar at exposure to Bt-cotton and length of exposure interaction was significant for survival to adults ($F = 3.28$; df = 7, 64; $P = 0.0049$). The percentage of survival from neonate to adult (adult emergence) in larvae exposed as first instars was reduced significantly only when fed Bt-cotton leaves until pupation ($P < 0.05$). However, if not fed Bt-cotton leaves until the third stadium, survival of larvae fed for only 7 d was lower than that in larvae fed for 1 d (Table 4). The fifth instar group's survival to adults was not altered by the feeding time on Bt-cotton leaves. Survival differed among instars only when on Bt-cotton until pupation, with greatest survival (85.6%) in the fifth-instar and lowest (50.4%) in the first-instar group. Larval survival to adult in the controls was 85.6 and 75.2% on artificial diet and non-Bt cotton, respectively.

Helicoverpa zea. The instar at exposure to Bt-cotton and length of exposure interaction was significant for *H. zea* mortality at pupation ($F = 7.59$; df = 7, 64; $P = 0.0001$). Mortality at pupation in larvae placed as first or third instars on Bt-cotton leaves increased with an increase in feeding period on the leaves ($P < 0.05$) (Table 5). The percentage of mortality at pupation in larvae placed as first or third instars on Bt-cotton leaves was 86.4% and 68.8%, respectively. In the first-instar group, mortality in larvae on Bt-cotton for 2 d or more was significantly higher than in larvae on it for 1 d. Larval mortality was low (12.8%) when fifth instars were exposed to Bt-cotton leaves until pupation and did not differ with length of exposure. All three instar groups differed significantly in the mortality level when remaining on Bt-cotton until pupation, with highest mortality in the first-instar and lowest in the fifth-instar group.

The instar at exposure to Bt-cotton and length of exposure interaction was significant for length of developmental period ($F = 33.33$; df = 7, 63; $P = 0.0001$). The length of developmental period in larvae exposed to Bt-cotton as first or third instars increased significantly between 1 and 2 d of exposure ($P < 0.05$) (Table 6). When first and third instars were exposed to Bt-cotton until pupation, the increase in developmental time over 1 d of exposure was 11.3 and 8.0 d, respectively. The time for larval development when on Bt-cotton until pupation was significantly longer in the first- than third-instar group, but it did not differ at shorter feeding periods. The length of pupal period was not altered in any instar group by feeding on Bt-cotton ($F = 1.64$; df = 16, 63; $P = 0.083$) with a mean (±SEM) pupal period of 9.96 ± 0.09 d when development was completed on artificial diet and 9.93 d ± 0.05 when on non-Bt cotton.

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

Instar at exposure to Bt-cotton	Days on Bt-cotton leaves*,**					
	1 d	2 d	3 d	4 d	7 d	Until pupation
First	135.4 ± 4.1 aA	135.9 ± 3.4 aA	132.5 ± 3.7 aAB	132.2 ± 3.8 aAB	124.3 ± 3.8 aB	86.8 ± 1.6 bC
Third	121.2 ± 1.5 bA	109.1 ± 4.1 bB	102.4 ± 4.1 bB	91.1 ± 3.3 bC	85.3 ± 1.2 bCD	79.1 ± 1.8 bD
Fifth	115.7 ± 5.7 bA	107.2 ± 3.5 bAB	–†	–†	–†	105.4 ± 5.6 aB

Means in a column not sharing a lower case letter or in a row not sharing an upper case letter are significantly different ($P < 0.05$).
* Days the larvae were exposed to Bt-cotton leaves in cups.
** Pupal weights in controls was 148.1 ± 2.6 mg for larvae on diet and 116.6 ± 2.7 mg on non-Bt cotton.
† Larvae died or pupated before day 3.

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

Instar at exposure to Bt-cotton	Days on Bt-cotton leaves*,**					
	1 d	2 d	3 d	4 d	7 d	Until pupation
First	84.8 ± 1.6 aA	81.6 ± 3.2 aA	76.8 ± 4.0 aA	76.0 ± 1.6 bA	74.4 ± 3.6 aA	50.4 ± 5.2 cB
Third	83.2 ± 3.2 aAB	86.4 ± 2.4 aA	81.6 ± 4.8 aAB	87.2 ± 1.6 aA	74.4 ± 5.6 aB	63.2 ± 7.2 bC
Fifth	93.6 ± 2.8 aA	87.2 ± 4.0 aA	–†	–†	–†	85.6 ± 3.6 aA

Means in a column not sharing a lower case letter or in a row not sharing an upper case letter are significantly different ($P < 0.05$).
* Days the larvae were exposed to Bt-cotton leaves in cups.
** Larval survival to adults in controls was 85.6 ± 2.0% in larvae on diet and 75.2 ± 2.8% on non-Bt cotton.
† Larvae died or pupated before day 3.

Table 5. Percent larval mortality at pupation (±SE) in three instars of *Helicoverpa zea* fed Bt-cotton leaves for different time periods

Instar at exposure to Bt-cotton	Days on Bt-cotton leaves*,**					
	1 d	2 d	3 d	4 d	7 d	Until pupation
First	27.2 ± 8.3 aC	50.4 ± 6.6 aB	53.6 ± 7.2 aB	60.8 ± 7.5 aB	76.8 ± 3.4 aA	86.4 ± 3.9 aA
Third	12.0 ± 2.5 bC	12.0 ± 2.2 bC	23.2 ± 4.4 bC	40.0 ± 1.3 bB	56.0 ± 3.3 bA	68.8 ± 5.8 bA
Fifth	7.2 ± 3.2 cA	8.0 ± 1.8 bA	–†	–†	–†	12.8 ± 4.6 cA

Means in a column not sharing a lower case letter or in a row not sharing an upper case letter are significantly different ($P < 0.05$).
* Days the larvae were exposed to Bt-cotton leaves in cups.
** Mortality in controls was 7.2 ± 2.3% for larvae on diet and 20.8 ± 5.1% on non-Bt cotton.
† Larvae died or pupated before day 3.

Table 6. Days (±SE) for development of *Helicoverpa zea* larvae fed Bt-cotton for different time periods

Instar at exposure to Bt-cotton	Days on Bt-cotton leaves*,**					
	1 d	2 d	3 d	4 d	7 d	Until pupation
First	13.6 ± 0.6 aE	14.1 ± 0.2 aD	14.8 ± 0.3 aCD	15.9 ± 0.2 aC	17.7 ± 0.3 aB	24.9 ± 0.5 aA
Third	13.7 ± 0.4 aE	14.7 ± 0.5 aD	15.5 ± 0.3 aCD	15.9 ± 0.2 aC	18.9 ± 0.5 aB	21.7 ± 0.8 bA
Fifth	12.4 ± 0.6 bA	12.9 ± 0.7 bA	–†	–†	–†	12.8 ± 0.7 cA

Means in a column not sharing a lower case letter or in a row not sharing an upper case letter are significantly different ($P < 0.05$).
* Days the larvae were exposed to Bt-cotton leaves in cups.
** Larval period in controls was 12.3 ± 0.2 days for larvae on diet and 15.0 ± 0.4 days on non-Bt cotton.
† Larvae died or pupated before day 3.

The pupal weight of first- and third-instar exposure groups of *H. zea* on Bt-cotton leaves for 4 to 7 d, respectively, was significantly reduced from that in larvae fed Bt-cotton for 1 d ($P < 0.05$) (Table 7). The pupal weight of first or third instars on Bt-cotton leaves until pupation was approximately 150 (>30%) mg less than those on Bt-cotton for 7 d or less. The pupal weight reduction was less in the fifth-instar group on Bt-cotton. There were no differences in pupal weight between first and third instars at any of the feeding periods on Bt-cotton. Pupal weights of earlier instar exposure groups were much reduced from those in the fifth-instar group when on Bt-cotton until pupation. Pupal weight in the controls was 435.7 and 380.6 mg for larvae on artificial diet and non-Bt cotton, respectively.

The instar at exposure to Bt-cotton and length of exposure interaction was significant for *H. zea* survival to adults ($F = 4.42$; df = 7, 64; $P = 0.0005$). Survival from neonates to adults in *H. zea* larvae exposed to Bt-cotton was reduced with an increase in feeding time on Bt-cotton in the first- and third- but not fifth-instar exposure groups ($P < 0.05$) (Table 8). Survival of the first- and third- instar groups that pupated on Bt-cotton was only 10.4%, and 27.2%, respectively, and was significantly lower than for larvae that fed for 4 d or less. Survival of the first-instar group on Bt-cotton was generally lower than that for the third- instar group after 2 d of exposure. Larval survival to adults in the controls was 85.6 and 71.2% for larvae on artificial diet and non-Bt cotton, respectively.

Discussion

The mortality at pupation was low in *S. exigua* larvae of all sizes fed Bt-cotton leaves. This is in agreement with Stapel et al. (1997) who reported that *S. exigua* grown on two Bt-cotton varieties had approximately 75% survival to pupation. Results of our tests also showed that *S. exigua* exposed to Bt-cotton as third or fifth instars had mortality similar to that in the control groups. Previously, Moar et al. (1990) reported that *B. thuringiensis* protein CryIA (c), the toxin in Bt-cotton, was less toxic to *S. exigua* than the other two *B. thuringiensis* proteins CryIA (a) and CryIA (b) when incorporated in diet. In addition, Adamczyk et al. (1998) reported survival of *S. frugiperda* larvae after lengthy exposure on Bt-cotton. Inagaki et al. (1991) had found that larvae of *S. litura* were less susceptible to crystal δ-endotoxin of *B. thuringiensis* than the other lepidopterous insects tested in in-diet tests.

Mortality of first-instar *H. zea* on Bt-cotton leaves in our study was high, but less in larvae exposed as third instars and low when exposed as fifth instars. Halcomb et al. (1994) had reported a high mortality in early instars and a lower mortality in later instars of *H. zea* when exposed to Bt-cotton flower buds. Even in the first-instar group, mortality in *H. zea* was dependent on the time on Bt-cotton, and low when larvae fed on Bt-cotton leaves for only a few days. These results agree with Mascarenhas and Luttrell (1997) who found that *H. zea* mortality increased with the days that larvae were preconditioned on Bt-cotton plants. Mascarenhas et al. (1994) also found that *H. virescens* larvae, which were exposed to Bt-cotton for only 24 h, had higher initial survival than larvae exposed for 48 and 96 h. They also reported that 4-d-old larvae survived at significantly higher rates than 1- and 2-d-old larvae. Mortality for *S. exigua* and *H. zea* was less than previously reported for *H. virescens* on Bt-cotton (Wilson et al. 1992, Halcomb et al. 1994, Mascarenhas et al. 1994). Results of field studies by Mahaffey et al. (1994) also indicated that *Ostrinia nubilalis* (Hübner) was controlled by Bt-cotton. Others have found that some lepidopteran larvae fed *B. thuringiensis* in diet

Table 7. Pupal weight (mg ± SE) for three instars of *Helicoverpa zea* fed BT-cotton for different time periods

Instar at exposure to BT-cotton	Days on Bt-cotton leaves*,**					
	1 d	2 d	3 d	4 d	7 d	Until pupation
First	407.5 ± 12.7 aA	394.7 ± 17.3 aAB	378.9 ± 27.0 aAB	374.0 ± 19.3 aB	368.0 ± 13.4 aB	238.9 ± 13.0 bC
Third	396.8 ± 14.0 aA	384.4 ± 23.3 aAB	377.8 ± 20.3 aAB	374.2 ± 20.8 aAB	356.8 ± 15.7 aB	247.9 ± 16.2 bC
Fifth	410.4 ± 3.2 aA	374.6 ± 17.0 aB	–†	–†	–†	359.7 ± 13.9 aB

Means in a column not sharing a lower case letter or in a row not sharing an upper case letter are significantly different ($P < 0.05$).
* Days the larvae were exposed to Bt-cotton leaves in cups.
** Pupal weight in controls was 435.7 ± 2.0 mg for larvae on diet and 380.6 ± 9.5 mg on non-Bt cotton.
† Larvae died or pupated before day 3.

Table 8. Larval survival to adults (percentage ± SE) in three instars of *Helicoverpa zea* fed Bt-cotton leaves for different time periods

Instar at exposure to Bt-cotton	Days on Bt-cotton leaves*,**					
	1 d	2 d	3 d	4 d	7 d	Until pupation
First	64.8 ± 8.3 aA	39.2 ± 3.8 bB	32.8 ± 6.8 aBC	28.0 ± 4.9 bBC	20.0 ± 2.2 aCD	10.4 ± 3.7 cD
Third	71.2 ± 3.6 aA	69.6 ± 5.8 aA	64.0 ± 2.0 aA	47.2 ± 2.3 aB	32.8 ± 3.4 aBC	27.2 ± 5.9 bC
Fifth	79.2 ± 6.4 aA	76.0 ± 4.2 aA	–†	–†	–†	70.4 ± 8.1 aA

Means in a column not sharing a lower case letter or in a row not sharing an upper case letter are significantly different ($P < 0.05$).
* Days the larvae were exposed to Bt-cotton leaves in cups.
** Larval survival to adults in controls was 85.6 ± 3.2% for larvae on diet and 71.2 ± 2.3% on non-Bt cotton.
† Larvae died or pupated before day 3.

may recover and complete their development after removal from *B. thuringiensis* (Dulmage et al. 1977, Chiang et al. 1986).

The larval developmental time was extended when early instars of *S. exigua* and *H. zea* were fed Bt-cotton for 4 to 7 d. However, this extension in *S. exigua* was small relative to that in *H. zea,* and less than 4 d when larvae were on Bt-cotton leaves until pupation. Stapel et al. (1997) reported developmental time in *S. exigua* fed Bt-containing diet was delayed by up to 5 d. Bt-cotton had a much greater effect on developmental time of *H. zea* than *S. exigua.* We found that the increase in larval developmental time in first-instar *H. zea* that survived until pupation was almost 2-fold greater on Bt-cotton than on the non-Bt-cotton, whereas, this increase in *S. exigua* was only 1.2-fold. The delay in pupation was up to 11 and 8 d in first- and third-instar *H. zea,* respectively. Even 1 d of feeding on Bt-cotton leaves by these younger instars slowed development in *H. zea.* Extension in larval developmental time of *H. zea* on transgenic Bt-cotton was also reported by Jenkins et al. (1993) and Halcomb et al. (1996).

A reduction in pupal weight occurred in all instars of *S. exigua* and *H. zea* fed Bt-cotton until pupation. This reduction in pupal weight was large in both species exposed as first or third instars. However, when larvae were on Bt-cotton for 1 wk or less, the reduction, if any, was typically small. Halcomb et al. (1996) observed a pupal weight reduction in fourth-instar *H. virescens* but not in fifth-instar *H. virescens* and *H. zea* surviving on Bt-cotton. Jenkins et al. (1993) found that larval weight of *H. virescens* that survived on Bt-cotton was significantly lowered. Adamczyk et al. (1998) reported a reduction in pupal weight in *S. frugiperda* fed Bt-cotton. Stapel et al. (1997), however, did not notice a pupal weight reduction in *S. exigua* fed *B. thuringiensis*-treated artificial diet.

These findings showed post-larval mortality in both *H. zea* and *S. exigua.* However, overall mortality was much lower in *S. exigua* than in *H. zea.* Only a small percentage of first-instar *H. zea* survived to adult. Previously, Jenkins et al. (1993) and Halcomb et al. (1996) found little or no *H. zea* and *H. virescens* survival to the adult stage on Bt-cotton. In contrast, we found that only first-instar *S. exigua* on Bt-cotton leaves until pupation had a survival rate as low as 50%. Davis et al. (1995) and Harris et al. (1996) reported some suppression of *S. exigua* activity by Bt-cotton. However, Burris et al. (1994) did not observe an effect of Bt-cotton on mortality and feeding of *S. exigua* in the field. Trumble (1990) reported that formulations of *B. thuringiensis* applied at recommended field rates on tomatoes were not efficacious against *S. exigua.* These studies also suggest that in the field if a refugia option of mixed seed (Bt/Non Bt) were offered, *H. zea* mortality would be changed somewhat with movement of larvae between Bt- and non-Bt-cotton plants. For example, unless larvae were nearing pupation at movement, any that moved from non-Bt-cotton would likely be killed or suffer adverse sublethal effects on Bt-cotton plants.

In conclusion, larval mortality and effects of Bt-cotton leaves on survivors differed for the two pest species examined. *Helicoverpa zea* on Bt-cotton leaves showed high levels of mortality and major effects on survivors in the post-larval stages. In contrast, *S. exigua* on Bt-cotton leaves showed low mortality, and effects on survivors were comparatively low. The differences in susceptibility of these two pests to Bt-cotton illustrate the complexities in generalizing the usefulness of Bt-cotton to control lepidopteran pests on Bt-cotton. The usefulness of Bt-cotton must be evaluated for each pest species. The adverse effects of Bt-cotton on larval survivors also illustrate the need to evaluate effectiveness of Bt-cotton in pest management systems using these factors as well as acute larval mortality. In light of these sublethal effects of Bt-cotton, further studies are needed to evaluate these effects of Bt-cotton on fecundity of adult survivors and in succeeding generations.

Acknowledgments

This manuscript is published with the approval of the Director, Arkansas Agricultural Experiment Station.

References Cited

Ali, A. and S. Y. Young. 1996. Activity of *Bacillus thuringiensis* Berliner against different ages and stages of *Helicoverpa zea* (Lepidoptera: Noctuidae) on cotton. J. Entomol. Sci. 31: 1-8.

Adamczyk, J. J., J. W. Holloway, G. E. Church, B. R. Leonard and J. B. Graves. 1998. Larval survival and development of fall armyworm (Lepidoptera: Noctuidae) on normal and transgenic cotton expressing the *Bacillus thuringiensis* CryIA (c) δ-endotoxin. J. Econ. Entomol. 91: 539-545.

Bai, D., D. Degheele, S. Jansens and B. Lambert. 1993. Activity of insecticidal crystal proteins and strains of *Bacillus thuringiensis* against *Spodoptera exempta*. J. Invertebr. Pathol. 62: 211-215.

Benedict, J. H., D. W. Altman, P. F. Umbeck and D. R. Ring. 1992. Behavior, growth, survival, and plant injury for *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) on transgenic Bt cottons. J. Econ. Entomol. 85: 589-593.

Benedict, J. H., E. S. Sachs, D. W. Altman, D. R. Ring, T. B. Stone and S. R. Sims. 1993. Impact of δ-endotoxin-producing transgenic cotton on insect-plant interactions with *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). Environ. Entomol. 22: 1-9.

Benedict, J. H., E. S. Sachs, D. W. Altman, W. R. Deaton, R. J. Kohel, D. R. Ring and S. A. Berberich. 1996. Field performance of cottons expressing transgenic CryIA insecticidal proteins for resistance to *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Econ. Entomol. 89: 230-238.

Brewer, M. J., J. T. Trumble, B. Alvarado-Rodriguez and W. E. Chaney. 1990. Beet armyworm (Lepidoptera: Noctuidae) adult and larval susceptibility to three insecticides in managed habitats and relationship to laboratory selection for resistance. J. Econ. Entomol. 83: 2136-2146.

Burris, E., J. B. Graves, B. R. Leonard and C. A. White. 1994. Beet armyworm (Lepidoptera: Noctuidae) in northeast Louisiana: observations on an uncommon insect pest. Florida Entomol. 77: 455-459.

Burton, R. L. 1969. Mass rearing the corn earworm in the laboratory. USDA, ARS. 3-8 pp.

Chiang, A. S., D. F. Yen and W. K. Peng. 1986. Defense reaction of midgut epithelial cells in the rice moth larva (*Corcyra cephalonica*) infected with *Bacillus thuringiensis.* J. Invertebr. Pathol. 47: 333-339.

Cobb, P. P. and M. H. Bass. 1976. Beet armyworm: Dosage mortality studies on California and Florida strains. J. Econ. Entomol. 68: 813-814.

Davis, M. K., M. B. Layton, J. D. Varner and G. Little. 1995. Field evaluation of Bt-transgenic cotton in the Mississippi Delta, Pp. 771-775. *In* D. A. Richter and J. Armour (eds), Proc. Beltwide Cotton Conferences, 4-7 January 1995, San Antonio, TX. National Cotton Council of America, Memphis, TN.

Dulmage, H. T., H. M. Graham and E. Martinez. 1977. Interactions between the tobacco budworm, *Heliothis virescens,* and the δ-endotoxin produced by the HD-1 isolate of *Bacillus thuringiensis* var. *kurstaki:* relationship between length of exposure to the toxin and survival. J. Invertebr. Pathol. 32: 40-50.

Halcomb, J. L., J. H. Benedict, D. R. Ring and B. Cook. 1994. Instar specific survival and feeding preference of bollworm and tobacco budworm on mixtures of transgenic Bt and nontransgenic cotton, Pp. 1068-1072. *In* D. J. Herber and D. A. Richter (eds.), 1994 Proc. Beltwide Cotton Conferences, January 5-8, 1994, San Diego CA, National Cotton Council of America, Memphis, TN.

Halcomb, J. L., J. H. Benedict, B. Cook and D. R. Ring. 1996. Survival and growth of bollworm and tobacco budworm on nontransgenic and transgenic cotton expressing a CryIA insecticidal protein (Lepidoptera: Noctuidae). Environ. Entomol. 25: 250-255.

Harris, F. A., R. E. Furr, Jr. and D. S. Calhoun. 1996. Cotton insect management in transgenic Bt-cotton in the Mississippi delta, 1992-1995. Pp. 854-859. *In* D. J. Herber and D. A. Richter (eds.), 1996 Proc. Beltwide Cotton Conferences, January 9-12, 1996, Nashville, TN, National Cotton Council of America, Memphis, TN.

Inagaki, S., M. Miyasono, T. Ishiguro, R. Takeda and Y. Hayashi. 1991. Proteolytic processing of δ-endotoxin of *Bacillus thuringiensis* var. *kurstaki* HD-1 in insensitive insect, *Spodoptera litura:* unusual proteolysis in the presence of sodium dodecyl sulfate. J. Invertebr. Pathol. 60: 64-68.

Jenkins, J. N., W. L. Parrott, J. C. McCarty, Jr., F. E. Callahan, S. A. Berberich and W. R. Deaton. 1993. Growth and survival of *Heliothis virescens* (Lepidoptera: Noctuidae) on transgenic cotton containing a truncated form of the delta endotoxin gene from *Bacillus thuringiensis.* J. Econ. Entomol. 86: 181-185.

Layton, M. B., M. R. Williams and S. Stewart. 1997. Bt-cotton in Mississippi: the first year, Pp. 861-863. *In* P. Dugger and D. Richter (eds.) 1997, Proc. Beltwide Cotton Production, January 6-10, 1997, New Orleans, LA, National Cotton Council of America, Memphis, TN.

MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischhoff and R. L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebr. Pathol. 56: 258-266.

Mahaffey, J. S., J. S. Bacheler, J. R. Bradley, Jr. and J. W. Van Duyn. 1994. Performance of Monsanto's transgenic B. t. cotton against high populations of lepidopterous pests in North Carolina, Pp. 1061-1063. *In* D. J. Herber and D. A. Richter (eds.) Proc. Beltwide Cotton Conferences, January 5-8, 1994, San Diego CA, National Cotton Council of America, Memphis, TN.

Mascarenhas, V. J., R. G. Luttrell and J. C. Schneider. 1994. Activity of transgenic cotton expressing delta-endotoxin against tobacco budworm, Pp. 1064-1067. *In* D. J. Herber and D. A. Richter (eds.), Proc. Beltwide Cotton Conferences, January 5-8, 1994, San Diego CA, National Cotton Council of America, Memphis, TN.

Mascarenhas, V. J. and R. G. Luttrell. 1997. Combined effect of sublethal exposure to cotton expressing the endotoxin protein of *Bacillus thuringiensis* and natural enemies on survival of bollworm (Lepidoptera: Noctuidae) larvae. Environ. Entomol. 26: 939-945.

Meinke, L. J. and G. W. Ware. 1978. Tolerance of three beet armyworm strains in Arizona to methomyl. J. Econ. Entomol. 71: 645-646.

Meyers, H. B., D. R. Johnson, T. L. Singer and L. M. Page. 1997. Survival of *Helicoverpa zea* (Boddie) on Bollgard cotton, Pp. 1269-1271. *In* P. Dugger and D. Richter (eds.), Proc. Beltwide Cotton Conferences, January 6-10, 1997, New Orleans LA, National Cotton Council of America, Memphis, TN.

Moar, W. J., L. Masson, R. Brousseau and J. T. Trumble. 1990. Toxicity to *Spodoptera exigua* and *Trichoplusia ni* of individual P1 protoxins and sporulated cultures of *Bacillus thuringiensis* subsp. *Kurstaki* HD-1 and NRD-12. App. Environ. Microbiol. 56: 2480-2483.

Nyouki, F. F. R., J. R. Fuxa and A. R. Richter. 1996. Spore-toxin interactions and sublethal effects of *Bacillus thuringiensis* in *Spodoptera frugiperda* and *Pseudoplusia includens* (Lepidoptera: Noctuidae). J. Entomol. Sci. 31: 52-62.

Perlak, F. J., R. W. Deaton, T. A. Armstrong, R. L. Fuchs, S. R. Sims, J. T. Greenplate and D. A. Fischhoff. 1990. Insect resistant cotton plants. Bio/Technology 8: 939-943.

Plapp, F. W. 1979. Synergism of pyrethroid insecticides by formamidines against *Heliothis* pests of cotton. J. Econ. Entomol. 72: 667-670.

SAS Institute. 1989. SAS/STAT user's guide. Version 6, 4th ed., Vol. 2. SAS Institute Inc., Cary, NC.

Smith, R. H. 1989. Beet armyworms on cotton. Alabama Coop. Ext. Serv. Circular ANR-538. 4pp.

Stapel, J. O., D. J. Waters, J. R. Ruberson and W. J. Lewis. 1997. Development and behavior of *Spodoptera exigua* (Lepidopera: Noctuidae) larvae in choice tests with food substrates containing toxins of *Bacillus thuringiensis.* Biol. Control 11: 29-37.

Trumble, J. T. 1990. Vegetable insect control with minimal use of insecticides. Hortscience 25: 159-164.

Wilson, F. D., H. M. Flint, W. R. Deaton, D. A. Fischhoff, F. J. Perlak, T. A. Armstrong, R. L. Fuchs, S. A. Berberich, N. J. Parks and B. R. Stapp. 1992. Resistance of cotton lines containing a *Bacillus thuringiensis* toxin to pink bollworm (Lepidoptera: Gelachiidae) and other insects. J. Econ. Entomol. 85: 1516-1521.