Survival Rates of Tobacco Budworm (Lepidoptera: Noctuidae) Larvae Exposed to Transgenic Cottons Expressing Insecticidal Protein of *Bacillus thuringiensis* Berliner[1]

C. D. Parker, Jr., V. J. Mascarenhas, R. G. Luttrell and K. Knighten

Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762 USA

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Abstract The insecticidal activity of transgenic cottons expressing endotoxin protein of *Bacillus thuringiensis* Berliner (*Bt* cotton) was quantified by measuring survival of tobacco budworm, *Heliothis virescens* (F.), larvae caged on different plant structures for varying lengths of exposure. Percentages of larvae surviving were measured on *Bt* cottons expressing Cry1Ab and Cry1Ac protein. Plant structure (terminal, leaf, square or boll) did not affect larval survival, and survival did not differ significantly between Cry1Ab and Cry1Ac cottons. Larvae exposed to *Bt* cotton for only 24 h had higher initial survival than larvae exposed for 48, 72 and 96 h. Larvae first exposed to *Bt* cotton at 4 d of age had higher survival than those first exposed as neonate or 2-d-old larvae. Survivorship of neonate and 4-d-old larvae exposed to Cry1Ac cotton was significantly reduced with only 48 h of exposure to the insecticidal plants. Seven-day-old larvae exhibited no significant reduction in survivorship with exposure to Cry1Ac cotton for 48 h.

Key Words *Bacillus thuringiensis,* tobacco budworm, *Heliothis virescens,* Cry1Ac, Cry1Ab

Transgenic cotton, *Gossypium hirsutum* L., expressing endotoxin protein of *Bacillus thuringiensis* Berliner (*Bt* cotton) is a highly efficacious alternative to traditional insecticides. Small plot and laboratory efficacy studies (Jenkins and Parrott 1990, Jenkins et al. 1993, Benedict et al. 1992, 1996, Mascarenhas et al. 1994) have shown that the insecticidal activity of *Bt* cotton is sufficient to provide economic control of tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie). Tobacco budworm larvae surviving on *Bt* cotton are rarely found. However, some bollworms have survived on *Bt* cotton long enough to cause damage in field studies. Mahaffey et al. (1995) found that 32% of the bolls were damaged in *Bt* cotton following natural infestation by bollworm, while 75% of the bolls were damaged in non-*Bt* cotton.

Jenkins et al. (1991, 1993), Benedict et al. (1992, 1993), Wilson and Flint (1991) and others have evaluated *Bt* cotton measuring larval mortality, behavior and damage when tobacco budworm and/or bollworm larvae were exposed to excised leaf tissue or greenhouse plants. In general, survival of tobacco budworm exposed to *Bt* cotton as neonates ranged from 0 to 8% (Jenkins et al. 1993) at 6 d post-infestation to less than 2% at 10 d post-infestation (Benedict et al. 1992). Older, larger larvae tend to have higher initial survival on *Bt* cotton tissues (Jenkins et al. 1993, De Spain et al.

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1993). Jenkins et al. (1993) reported survival rates of 26 to 55% for 3-d-old and 50 to 90%, for 6-d-old tobacco budworm larvae placed on *Bt* cotton for 6 d in laboratory assays. However, larvae that survived on *Bt* cotton tended to have lower pupation and eclosion rates. De Spain et al. (1993) showed that all bollworm larval instars, except for fifth (final) instar, failed to pupate when confined to *Bt* cotton flower buds for the remainder of the larval period.

Although *Bt* cotton is highly efficacious against tobacco budworm and bollworm in laboratory and field environments, its impact on population level phenomena and associated interactions with natural control and non-target organisms are not fully understood. The effect of Bt cotton on population dynamics of target and non-target arthropods is an extremely important issue. Potential development of pest populations resistant to *Bt* cotton and the potential benefits associated with argiculutal use of *Bt* cotton, such as reduced insecticide inputs, are being explored. Large scale adoption of *Bt* cotton may reduce the dependence on insecticides and create an environment for natural enemies to have a greater role controlling key pests of cotton (Luttrell and Herzog 1994); however, the extent of this reduction is difficult to estimate on the basis of current information. Strategies for deploying transgenic crops and managing resistance to insecticidal proteins expressed in transgenic crops often rely on results of simulation models to design management plans. These models should be based on empirical data describing the biology and ecology of the organisms involved and the impact of various management options on population growth. The research reported here was conducted to develop quantitative estimates of the survival of tobacco budworm exposed to *Bt* cotton. Resulting data are of value to those interested in modeling insecticide resistance development and use of *Bt* cotton as an integrated tool in an IPM program.

Materials and Methods

To measure the survival of tobacco budworm larvae exposed to *Bt* cotton in the absence of beneficial insects, larvae were exposed to *Bt* and non-*Bt* (CK) cotton in laboratory and field cage experiments. Field studies were conducted during 1992 within adjacent 2 to 3 ha blocks of *Bt* and CK cotton grown on a commercial farm in the delta production region of Leflore Co., MS. MON-81, a transgenic cotton expressing Cry1Ab protein, and 'Coker 312', the non-*Bt* cotton parent line, were used in the 1992 studies. Subsequent laboratory studies conducted during 1993 utilized Event 531, a transgenic cotton expressing Cry1Ac protein that is genetically similar to current commercial *Bt* cottons, and 'Coker 312', the non-*Bt* parent cotton line. All 1993 studies were conducted in plots at the Mississippi Agricultural and Forestry Experiment Station Plant Science Research Farm, Mississippi State, MS. Tobacco budworm larvae used in all studies were obtained from the USDA Crop Science Research Laboratory at Mississippi State, MS. Wild genotypes are annually added to this colony to maintain similarity to wild populations (Jenkins et al. 1995).

Initial field studies in 1992 were conducted to estimate: (a) survival of tobacco budworm neonates caged for 48 h on terminals, squares, leaves and bolls of *Bt* and CK cotton; (b) survival of tobacco budworm neonates caged on cotton terminals of *Bt* and CK cotton for 24, 48, 72 and 96 h; and (c) survival of neonate, 2- and 4-d-old larvae caged for 48 h on terminals of *Bt* and CK cotton. Plants on which tobacco budworm larvae were caged were chosen at random from *Bt* and CK cotton plots. Twenty to 25 larvae of the appropriate test age were bagged individually on the

desired plant structures for the desired length of time using cotton-cloth pouches (8 × 12 cm) with drawstring closures. After a larva was confined on the cotton plant for the designated length of time, larval survival was measured by visual inspection of the material within the bag. Surviving larvae at the end of the treatment period were transferred to a wheat germ-casein diet (King et al. 1985) in 38.3-ml plastic cups and transported to the Clay Lyle Entomology Complex at Mississippi State University, Mississippi State, MS. Larvae were maintained at room temperature (≈25 to 32°C) and at an approximate 14:10 (L:D) photoperiod regime through the remaining larval development period. Larvae were periodically monitored, and mortality data were accumulated at the end of the larval and pupal stages. Each separate test was replicated 3 or 4 times. The percent of larvae surviving was calculated with a correction for the number missing on CK cotton (e.g., number surviving on *Bt* cotton/ [Total infested – number missing on CK cotton]). Percent survival and damage ratings were analyzed by ANOVA (PROC GLM, SAS Institute 1988), and means were separated using Student-Newman-Kuel's multiple comparison test (SAS Institute 1988). Percentage data were transformed by arcsine-square root for analyses. Reported means are not transformed.

In 1993, field studies were repeated at the Leflore Co. location to compare the survival of tobacco budworm larvae caged on cottons expressing Cry1Ab or Cry1Ac proteins. Procedures were the same as those described for 1992 except that a plot of MON-757 cotton was compared to MON-81 and 'Coker 312' cottons. MON-757 was an experimental line of *Bt*-cotton that expressed Cry1Ac protein.

Based on results of 1992 studies, additional experiments were designed in 1993 to measure the effects of short-term exposure to *Bt* cotton on survival of various larval ages of tobacco budworm. The impetus for these studies were concerns that larvae may move between plants in mixed planting of *Bt* and non-*Bt* cotton (Mallet and Porter 1992). Shortened exposure periods may result in higher survivorship. All 1993 studies were conducted using water pic techniques described by Luttrell et al. (1987), and plants were grown in field plots at the Plant Science Research Farm at Mississippi State University. Plant terminals from Event-531 (*Bt*) and 'Coker 312' (CK) were collected prior to first flower from plots not sprayed with an insecticide. Terminals were placed individually in water pics. The water pics were sealed at the top by inserting a plastic holding tube through the center of lids of 240-ml plastic cups. The experimental design was a randomized complete block. Four replicates of 20 larvae for each of 3 different age groups (neonate, 4- and 7-d-old) were placed individually on plant terminals of each cotton type (*Bt* and CK). Neonates were not fed prior to placement on plant terminals, but 4-d and 7-d-old larvae were reared on a wheat germ-casein diet (King et al. 1985) until 24 h prior to placement on terminals. At 24 h before placement on terminals, larvae were transferred from diet to cotton leaves (Coker 312) collected from field plots in an attempt to condition larvae to cotton. A plastic cup with ventilation holes covered with mesh cloth was placed over each terminal. Larvae were caged on the plant terminals for different treatment exposure periods (1.5, 3, 6, 12, 24 and 48 h). After each exposure period, the number of larvae alive, dead and missing were recorded. Surviving larvae were removed from the plant tissue and placed on wheat-germ casein diet. Subsequent observations were made at 4-, 7- and 14-d post initial exposure to determine survival and pupation rates. All data were analyzed by ANOVA (PROC GLM, SAS Institute 1988) with means separated by Fisher's Protected least-significance test. Percent survival of larvae on *Bt*

cotton was corrected by the percent survival on CK cotton and analyzed by linear regression (Norusis 1990).

Results and Discussion

Survival rates of neonate tobacco budworm caged for 48 h on various plant structures of *Bt* and CK cotton illustrate the insecticidal activity of *Bt* cotton (Table 1). Larval survival on CK cotton structures was significantly higher than larval survival on *Bt* cotton structures at the end of the 48 h treatment period and the end of the larval stage, except for larvae caged on squares and bolls. More neonates survived through pupation on CK terminal than on any *Bt* structure, but no differences were measured for CK leaves, squares, or bolls at the end of the pupal stage as compared to *Bt* cotton structures. Tobacco budworm survival on *Bt* cotton did not vary significantly ($P \leq 0.05$) among tissues tested. On CK cotton, initial survival at 48 h was higher on terminals and leaves than on squares or bolls. Higher survival on CK cotton leaves and terminals as compared to CK cotton squares and bolls probably reflects differences in the nutrient content and texture of the plants structures (Table 1).

Larval survival on CK cotton did not vary with length of exposure, while more larvae caged on *Bt* cotton for 24 h survived ($P \leq 0.05$) at the end of the treatment period as compared to larvae caged for 48, 72 and 96 h (Table 2). This difference in mortality may have been due to the fact that larvae exposed to *Bt* cotton for 24 h may have consumed less tissue than those exposed to *Bt* cotton for longer time periods (Table 2), or because each treatment group was observed at different lengths of time after initial exposure. A lethal dose of endotoxin protein may have been ingested without actually killing the larvae within the 24 h treatment period. For example, if

Table 1. Survival [mean ± SEM] of neonate tobacco budworm caged on plant structures of nontransgenic (CK) and transgenic (*Bt*) cotton (MON-81) expressing the delta-endotoxin of *Bacillus thuringiensis* for 48 h. Larvae were transferred to wheat germ-casein diet after the treatment period

Cotton type	Plant structure	End of treatment period, %	End of larval stage, %	End of pupal stage, %
Bt	Terminal	38.1 ± 6.35b	14.9 ± 3.95b	12.1 ± 4.45b
Bt	Leaf	37.9 ± 1.6b	10.9 ± 4.1b	8.2 ± 3.3b
Bt	Square	48.1 ± 5.45b	10.0 ± 7.3b	8.0 ± 5.45b
Bt	Boll	34.4 ± 9.85b	20.6 ± 9.9b	13.3 ± 9.45b
CK	Terminal	75.4 ± 9.5a	51.9 ± 6.7a	41.9 ± 7.3a
CK	Leaf	81.0 ± 4.3a	49.1 ± 9.6a	35.3 ± 7.2ab
CK	Square	57.0 ± 4.1b	38.0 ± 5.3ab	26.6 ± 7.55ab
CK	Boll	36.6 ± 1.35b	26.5 ± 4.35ab	16.8 ± 3.7ab

Means within a column not followed by a common letter differ significantly at $P < 0.05$ (n = 4) according to Student-Newman-Kuel's test.

Table 2. Survival [mean ± SEM] of neonate tobacco budworm caged on terminals of nontransgenic (CK) and transgenic (*Bt*) cotton (MON-81) expressing the delta-endotoxin of *Bacillus thuringiensis* for various treatment intervals. Larvae were transferred to wheat germ-casein diet after the treatment period

Cotton type	Treatment period h	End of treatment period, %	End of larval stage, %	End of pupal stage, %
Bt	24	43.4 ± 3.15b	17.1 ± 2.7b	11.1 ± 4.5b
Bt	48	15.5 ± 7.1c	7.9 ± 3.4b	5.8 ± 2.8b
Bt	72	19.1 ± 1.7c	15.2 ± 4.4b	8.2 ± 1.3b
Bt	96	11.0 ± 4.6c	6.7 ± 3.6b	6.7 ± 3.6b
CK	24	75.3 ± 6.0a	65.1 ± 1.6a	49.9 ± 1.3a
CK	48	67.8 ± 1.3a	57.5 ± 5.5a	48.9 ± 5.8a
CK	72	65.2 ± 7.0a	55.4 ± 7.5a	42.1 ± 10.5a
CK	96	64.5 ± 1.2a	53.1 ± 3.5a	46.7 ± 3.1a

Means within a column not followed by a common letter differ significantly at $P < 0.05$ (n = 4) according to Student-Newman-Kuel's test.

larvae exposed to *Bt* cotton for 24 h had been placed on artificial diet and their mortality measured at 48, 72 and 96 h, mortality levels may have been more similar to those for larvae caged to *Bt* cotton for the same exposure periods, illustrating the delayed effects of *Bt* cotton consumption. This hypothesis is supported by the fact that mortality for the various durations of exposure to *Bt* cotton tended to equalize by the end of the larval and pupal stages.

Larval age at exposure significantly affected survival of tobacco budworm larvae caged on *Bt* cotton terminals. As expected, 4-d-old tobacco budworm larvae caged on *Bt* cotton had higher survival than neonate and 2-d-old larvae (Table 3) after the 48 h treatment period and at the end of the larval stage. With both *Bt* and CK cotton, there was a general trend for increased survival with increased age of larvae. More larvae of all ages caged on CK cotton survived ($P \leq 0.05$) than those caged on *Bt* cotton, with the exception of 4-d-old larvae caged on *Bt* cotton. These results corroborate the findings of De Spain et al. (1993) showing that smaller larvae are more susceptible to *Bt* cotton. Luttrell et al. (1982) showed that older larvae might be able to tolerate dosages of foliar *Bt* endotoxins applied to cotton leaves that kill younger, smaller larvae. These findings have important implications to the seed-mixture strategy of managing resistance to *Bt* cotton (Fischhoff 1992). Larvae developing on non-*Bt* cotton plants within the mixture may conceivably move to *Bt* plants as older larvae and cause economic damage. Jenkins et al. (1992) reported 60 to 96% survival of tobacco budworm larvae reared on artificial diet for 6 d and then transferred to *Bt* cotton tissues for an additional 6 d. This study indicates that 4-d-old larvae caged on *Bt* cotton terminals for 48 h are capable of surviving long enough to cause damage on non-*Bt* cotton.

Survival of tobacco budworm larvae caged for 48 h on cotton terminals did not

Table 3. Survival [mean ± SEM] of tobacco budworm larvae of different ages caged on terminals of nontransgenic (CK) and transgenic (*Bt*) cotton (MON-81) expressing the delta-endotoxin of *Bacillus thuringiensis* for 48 h. Larvae were transferred to wheat germ-casein diet after the treatment period

Cotton type	Initial larval age, d	End of treatment period, %	End of larval stage, %	End of pupal stage, %
Bt	Neonate	38.1 ± 6.4b	14.9 ± 4.0c	12.1 ± 4.5c
Bt	2	46.8 ± 2.9b	27.8 ± 1.5c	22.7 ± 3.1bc
Bt	4	72.4 ± 5.0a	46.3 ± 6.6b	29.4 ± 7.2abc
CK	Neonate	75.3 ± 9.5a	51.9 ± 6.7b	41.9 ± 7.3abc
CK	2	86.6 ± 4.3a	65.4 ± 5.5ab	50.8 ± 7.9ab
CK	4	92.4 ± 1.4a	77.4 ± 7.2a	59.9 ± 12.0a

Means within a column not followed by a common letter differ significantly at $P < 0.05$ (n = 4) according to Student-Newman-Kuel's test.

differ significantly between the two *Bt* cotton lines tested (Table 4), but survival on the Cry1Ab cotton (MON-81) was numerically higher than that on the Cry1Ac cotton (MON-757). This tendency may be due to slight differences in protein expression. MON-81 expresses the Cry1Ab protein at 0.05% of the total soluble protein in the plant, while MON-757 expresses the Cry1Ac protein at 0.1% of total soluble protein (MacIntosh et al. 1990). Overall, the comparison indicates that the Cry1Ac cotton caused mortality of tobacco budworm at levels equal to or greater than the Cry1Ab cotton studied.

Significant ($P \leq 0.05$) linear regression equations ($y = a + bx$; where y = % corrected survival, a = intercept, b = slope, and x = h of initial exposure) were found to describe survival of tobacco budworm exposed as neonate and 4-d-old larvae to Event-531 for different intervals of time (Table 5, Fig. 1). Slopes of the regression

Table 4. Survival [mean ± SEM] of neonate tobacco budworm caged for 48 h on terminals of nontransgenic cotton ('Coker 312') and or one of 2 different transgenic cotton lines (MON-81 and MON-757) expressing delta-endotoxin of *Bacillus thuringiensis*. Larvae were transferred to wheat germ-casein diet following the 48-h exposure period

Cotton genotype	End of treatment period, %	End of larval stage, %
MON-81	46.3 ± 9.4ab	25.6 ± 11.0a
MON-757	31.6 ± 7.5b	16.4 ± 5.7a
Coker 312	63.1 ± 3.1a	29.8 ± 3.2a

Means within a column not followed by a common letter differ significantly at $P < 0.05$ (n = 4) according to Student-Newman-Kuel's test.

Table 5. Regression equation statistics describing percent corrected mortality of neonate, 4, and 7-d-old *H. virescens* larvae as a function of length of exposure to Event 531 (*Bt* cotton) terminals in a laboratory caged environment. Percent mortality on Event 531 was corrected for percent mortality on Coker 312 (non-*Bt* cotton)

Observation period	Larval age (d)	Intercept (±S.E.)	Slope (±S.E.)	*F*-value	$P > F$	r^2
At Recovery	neonate	92.54 (±6.62)	−0.90 (±0.29)	9.52	0.005	0.30
	4	97.89 (±2.92)	−0.75 (±0.13)	33.94	0.001	0.61
	7	96.22 (±3.67)	−0.21 (±0.16)	1.74	0.200	0.07
4-d Post	neonate	86.81 (±6.81)	−1.30 (±0.30)	17.75	0.001	0.45
	4	94.90 (±3.27)	−0.83 (±0.14)	33.10	0.001	0.60
	7	90.75 (±4.61)	−0.12 (±0.20)	0.35	0.56	0.02
7-d Post	neonate	82.43 (±7.87)	−1.42 (±0.35)	16.57	0.001	0.43
	4	92.71 (±4.13)	−0.86 (±0.18)	22.32	0.001	0.50
	7	87.50 (±4.71)	−0.19 (±0.21)	0.84	0.370	0.04
14-d Post	neonate	80.35 (±8.41)	−1.54 (±0.37)	17.11	0.001	0.44
	4	90.69 (±5.77)	−0.97 (±0.26)	14.38	0.001	0.40
	7	83.24 (±5.48)	−0.23 (±0.24)	0.93	0.350	0.04

lines were steepest for neonates and illustrated a consistent increase in slope from initial observations at recovery of larvae (b = −0.902) to observations 14 d post initial exposure (b = −1.537). Thus, observed survival rates decreased with the amount of exposure time. The effect of exposure intervals on survival of 4-d-old larvae was less pronounced although significant regression equations were found (Table 5, Fig. 1). Slopes of the regression lines for 4-d-old larvae ranged from −0.753 to −0.970. Those for 7-d-old larvae were not different ($P \leq 0.05$) from 0 for any exposure period.

The relationships between survival and exposure intervals indicate that neonates are very susceptible to *Bt* cotton, and the effects of initial exposure to *Bt* endotoxin tend to increase as post-exposure interval increases. Four-day-old larvae were less susceptible, and 7-d-old larvae had little or no susceptibility to *Bt* cotton when confined on *Bt* cotton terminals for 48 h. This indicates that larvae that feed and develop on a non-*Bt* cotton plant for 4 to 7 d may survive on *Bt* cotton, if they move from the non-*Bt* plant to a *Bt* plant. Neonates remaining on the *Bt* plant would experience high mortality, and few or no larvae would be expected to pupate.

Extension of the regression predictions beyond the 48 h of exposure observed suggest that all neonates would die after 65 to 110 h (2.7 to 4.6 d) of exposure to *Bt* cotton. We recognize that extrapolation of these regression lines beyond observation points is less reliable. However, given that the normal larval period requires ~17 d (Neunzig 1969), *Bt* cotton seems to be highly efficacious for tobacco budworm neonates remaining on *Bt* cotton. Extension of the regression lines for 4-d-old larvae

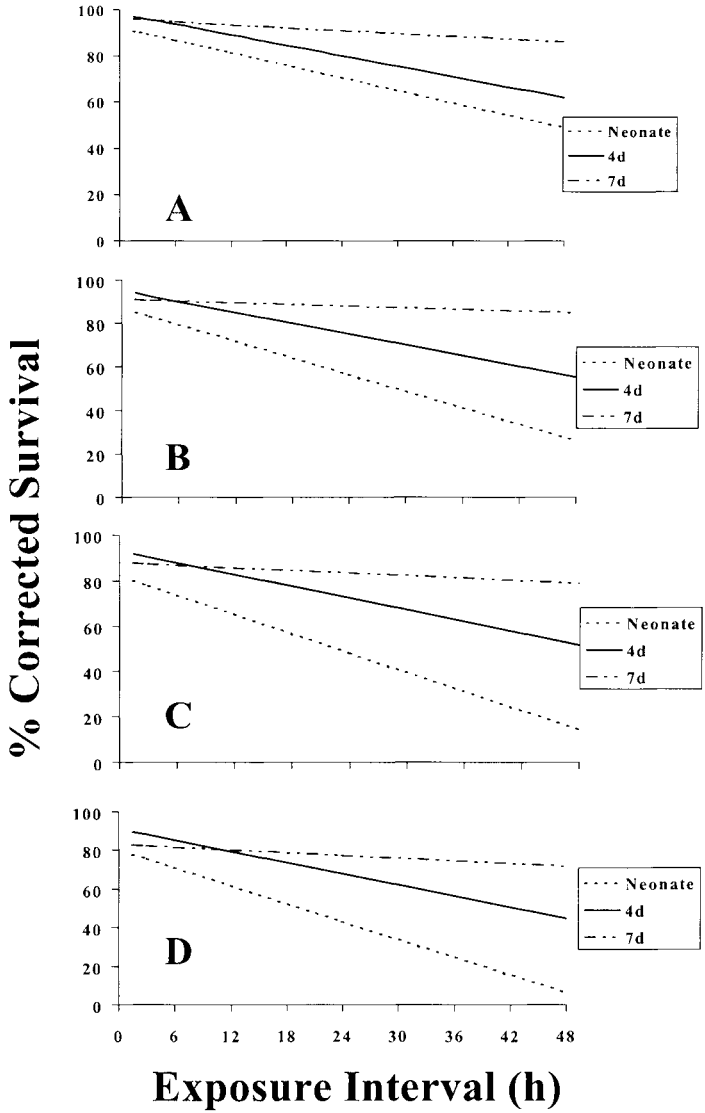

Fig. 1. Percent corrected survival of neonate, 4-, and 7-d-old tobacco budworm larvae exposed to cotton (Event 531) expressing Cry1Ac protein of *Bacillus thuringiensis* for (A) at exposure interval, (B) 4 d post initial exposure, (C) 7 d post initial exposure, and (D) 14 d post initial exposure. Percent survival on *Bt* cotton was corrected for percent survival on non-*Bt* cotton.

indicates that 4-d-old larvae not previously exposed to *Bt* cotton would need to be exposed for 103 to 132 h (4.3 to 5.5 d) to *Bt* cotton before 100% mortality could be achieved. Lack of a significant regression equation for 7-d-old larvae indicates that *Bt* cotton would not reduce survival of larvae moving to *Bt* cotton after they were 7 d of age.

Observations of neonates held on diet for an additional 14 d indicated no differences in survival between larvae exposed to *Bt* and CK cotton for initial periods of plant exposure less than 48 h. However, significantly fewer neonates survived 14 d when they were exposed for 48 h to Event-531 cotton (*Bt*) as compared with 'Coker 312' (CK) (Fig. 2). Percent survival of 4- and 7-d-old larvae on diet 14 d post-exposure showed no significant differences in survival of the larvae on the two genotypes. Dulmage et al. (1978) showed that *H. virescens* neonates exposed to diet containing serial dilutions of *B. thuringiensis* could recover from the toxin effects following exposure times of 24 to 72 h.

Fewer neonates and 4-d-old larvae had reached the pupal stage 14-d post-exposure to Event 531 (*Bt*) as compared to Coker 312 (CK) cotton (Fig. 2). Although the differences in neonates appears to be reflective of survival differences, the data for 4-d-old larvae suggest that sublethal exposure to Cry1Ac cotton may result in slower larval development. This corroborates the research of Dulmage et al. (1978), Luttrell et al. (1982) and Ali and Young (1993) that reported delayed development of *H. virescens* larvae with sublethal exposure to *B. thuringiensis.* This delayed development may have implications to resistance management. If insects surviving expo-

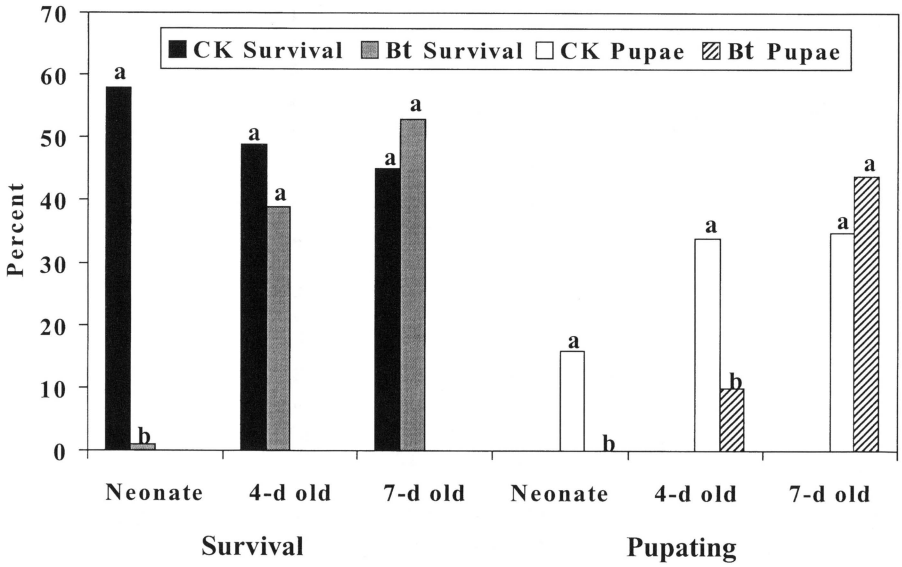

Fig. 2. Mean percent of initial larvae surviving and pupating 14 d post-exposure to Event 531 (*Bt*) and Coker 312 (CK) cotton terminals for 48 h. Paired means with similar lower case letters do not differ ($P \geq 0.05$) according to Fisher's Protected Least-significant difference test.

sure to transgenic cotton expressing Cry1Ac proteins do not develop at the same rate as those produced in a conventional refuge, the goal of diluting resistance via mating of resistant genotypes with susceptible genotypes may be affected (Tabashnik 1994, Klepetka and Gould 1996). No differences were observed in percent of 7-d-old larvae pupating 14 d post-exposure among the different plant genotypes and exposure periods. This suggests again that larger, older larvae moving from conventional to transgenic plants may not be greatly affected by exposure intervals of at least 48 h to *Bt* cotton.

Results of this research indicate that the insecticidal activity of *Bt* cotton against tobacco budworm is high. To relate these results to the collective literature available on survival of heliothines exposed to *Bt* cotton expected larval survival rates were estimated from our data and that of several published reports. Survival of neonates as a function of length of exposure to *Bt* cotton was estimated by linear regression (Freed et al. 1986) of collective data reported by Benedict et al. (1993), De Spain et al. (1993), Jenkins et al. (1993) and those reported in this article. Data included in the analysis were from laboratory, greenhouse, and field studies where levels of natural control varied.

Significant linear-regression equations describing mortality of neonate larvae as a function of exposure length to *Bt* cotton plants were developed (Fig. 3). The first analysis was of uncorrected survival on *Bt* cotton (*Bt* UNC) (slope (SE) = –1.90 (0.26), intercept = 21.97, r^2 = 0.44, and $P > F = 0.001$). The second analysis was of uncor-

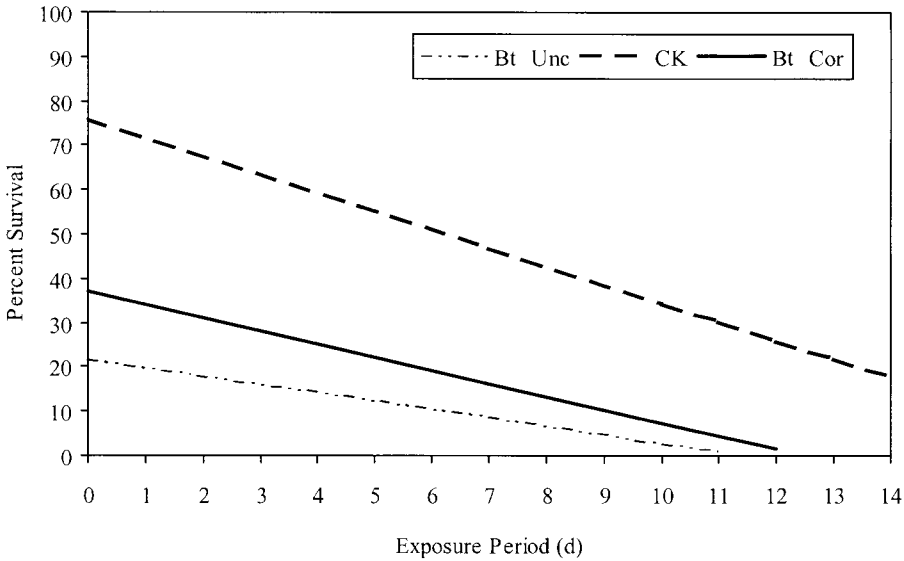

Fig. 3. Survival of neonate tobacco budworm as a function of length of exposure to *Bt* and non-*Bt* cotton. Regression lines for uncorrected *Bt* (*Bt* Unc), uncorrected non-*Bt* (CK), and corrected *Bt* (*Bt* cor) survival were based on collective results reported by Benedict et al. (1993), De Spain et al. (1993), Jenkins et al. (1993), and results from the studies reported in this manuscript.

rected survival on non-*Bt* (CK) (slope (SE) = −4.14 (0.56), intercept = 75.67, $r^2 = 0.52$, and $P > F = 0.001$). The final analysis was of corrected survival on *Bt* cotton (*Bt* COR) (slope (SE) = −2.97 (0.54), intercept = 37.12, $r^2 = 0.30$, and $P > F = 0.001$), where survival levels (inverse of mortality rates adjusted by Abbott's [1925] formula) observed in the non-*Bt* cotton were used to calculate corrected survival on *Bt* cotton. Factors that likely influenced larval survival on CK cotton were plant and/or environmental conditions and beneficial arthropods. These same factors, coupled with the insecticidal activity of *Bt* endotoxin, probably influenced survival of larvae on *Bt* cotton. Corrected survival on *Bt* cotton probably reflects insecticidal activity of *Bt* cotton.

The high insecticidal activity of *Bt* cotton against neonate tobacco budworm is illustrated by differences in regression lines that represent survival on *Bt* and non-*Bt* cotton (Fig. 3). Activity of natural mortality factors, illustrated by the difference between uncorrected and corrected survival on *Bt* cotton, was also an important factor lowering larval survival, particularly during the initial 5 to 6 d of exposure. Literature suggesting that smaller larvae are more susceptible to natural mortality factors (Sterling et al. 1989) corroborates this. At 7 d of exposure, survival of larvae on *Bt* cotton when effects of natural mortality were included (uncorrected survival on *Bt*) or removed (corrected survival on *Bt*) was ≈9 and 16%, respectively. The equations calculated on the collective data of Benedict et al. (1993), De Spain et al. (1993), Jenkins et al. (1993) and data reported in this manuscript indicate that neonate tobacco budworm cannot successfully reach the pupal stage (≈17 d) when larvae are confined to *Bt* cotton throughout the larval stage, regardless of the effects of natural control (Fig. 3). The regression models estimated that ≈5% of the larvae developing on CK cotton would pupate. Quantitative information described here should be useful in developing refined systems of pest and crop management that optimize the use of this important technology.

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