EFFECT OF *BACILLUS THURINGIENSIS* ON MORTALITY, SURVIVORSHIP AND MOVEMENT OF COTTON BOLLWORM (*HELICOVERPA ZEA*) (LEPIDOPTERA: NOCTUIDAE) ON COTTON

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Abstract

The relationship between *Bacillus thuringiensis* dose and bollworm, *Helicoverpa zea* larval mortality, survivorship and movement on and away from cotton leaves was investigated. *Bacillus thuringiensis* (Costar) was applied to the upper surface of cotton leaves using a spray table in five concentrations i. e., 0.0, 0.14, 0.29, 0.58, and 1.15 kg/ha. Three-day-old *H. zea* larvae were released on treated leaves, kept at 30 ^{0}C, and transferred to artificial diet after 12 h, 24 h, and 48 h. Higher numbers of larvae were found on lower than upper leaf surface at all three transfer times, but the numbers in all treatments were similar to the control. Larval movement from leaf to cup surface was significantly ($P < 0.05$) higher in *B. thuringiensis*-treated leaves than the control. Mortality of larvae when transferred from leaves to diet and 7 days after treatment was significantly higher in all treatments than control and highest at the highest rates (0.58 and 1.15 kg/ha). Data on survival of larvae at different locations suggests that at least for the first 24 h, the increase in the percentage of larvae on the inner cup surface in *B. thuringiensis* treatments was not due to larvae that had consumed a lethal dose, but appeared to be an attempt by larvae to avoid the *B. thuringiensis* on the upper leaf surface. Highest larval weight was recorded in the control for all transfer times. The length of the larval period increased with dose in the 12-h transfer. Pupal weight reduction was found with the highest doses.

Introduction

Synthetic insecticides have been effective against *H. zea* and *H. virescens* on cotton. After development of resistance to pyrethroids by *H. virescens* (Leonard et al. 1987, Luttrell et al. 1987), interest increased in use of alternative strategies for management of heliothines on cotton. Interest in the use of *Bacillus thuringiensis* for their management has increased due to improved strains and recent advances in genetic engineering, including insertion and expression of *B. thuringiensis* toxin genes in some major crop plants such as tobacco, cotton and tomato (Gasser and Fraley 1989).

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1339-1343 (1998)
National Cotton Council, Memphis TN

Although *B. thuringiensis* sprays may provide some control (Johnson 1982), they are not always effective, especially against heliothine populations that are predominantly *H. zea* (Young et al. 1997). *B. thuringiensis* inhibits larval feeding, but at sublethal doses larvae may recover from initial exposure and continue feeding and pupate normally, unless further treatment is applied (Ali and Watson 1982; Fast and Regniere 1984). Ali and Watson (1982) proved that *H. virescens* survived various feeding periods on cotton treated with *B. thuringiensis*. The ability of larvae to recover decreased with an increase in dosage rate or exposure time. *B. thuringiensis* is also found to reduce the weight of surviving *H. virescens* larvae (Luttrell et al. 1982; Bell and Romine 1980). Ali and Watson (1982) reported an extension in *H. virescens* larval and pupal periods when fed *B. thuringiensis*-treated cotton leaves. They also mentioned a decrease in pupal weight.

Susceptible and toxin-adapted strains of *H. virescens* avoided moderate and high concentrations of the *Bt* toxin when it was added to semisynthetic diet (Gould and Anderson 1991). Application of *B. thuringiensis* to cotton terminals resulted in movement of some first instars of heliothine from meristems to other sites on the terminals, and this movement increased with increasing *B. thuringiensis* rates (Jyoti et al. 1996). In their results, Jyoti et al. (1996) have also reported that *B. thuringiensis* resulted in greater movement of larvae from leaves and terminals of cotton plants. However, they did not determine the fate of larvae that moved from the leaves.

Reported herein are results of experiments designed to determine *B. thuringiensis* effects on *H. zea* larval location and mortality, length of larval and pupal periods, and larval and pupal weights on excised leaves of cotton plants.

Materials and Methods

The experiment was conducted at the Agricultural Experiment Station, Fayetteville AR during 1997. *H. zea* larvae used in the experiment were obtained from a laboratory colony maintained at the Department of Entomology, University of Arkansas, Fayetteville AR.

Artificial diet was prepared (Burton 1969) and put in 270-ml wax coated paper cups. *H. zea* neonates were released in groups of 25 to 30 per cup three days before initiating the experiment.

Cotton (Deltapine 50) was planted in 4-liter plastic pots in a 7:7:5 mixture of sand, soil, and peat moss and held in the greenhouse at a photoperiod of 14:10 (L:D) h. Plantings were made at different dates to allow testing over an extended period. The experiment was initiated when plants were 45 to 50 days old. Fully expanded leaves along with petiole (5 to 8 cm) from the middle to top canopy of the plants were removed, put in plastic bags and brought for use in the spray table test. Seventy-five leaves per treatment

were pinned on a Styrofoam sheet (50×50 cm) covered with paper and treated with *B. thuringiensis* on a motorized spray table (Luttrell et al. 1987).

The spray apparatus was a Co_2-pressurized boom-type sprayer (R & D Sprayers, Inc., Riverside, CA) with a one-row boom equipped with two TX-6 hollow-cone nozzles 0.46 m apart and 30.5 cm above the surface of leaves. The apparatus was calibrated to deliver the equivalent of 96 liters of water/ha.

Treatments were aqueous suspensions of Costar (Novartis Inc., San Diego, CA) at rates equivalent to 0.0 (control), 0.14, 0.29, 0.58 and 1.15 kg/ha. *B. thuringiensis*-treated leaves were allowed to dry, brought to the laboratory and placed individually in 270-ml wax-coated paper cups containing a moistened filter paper disk. A single 3-d-old larva was placed on the upper surface on each of 75 leaves per treatment with a pair of fine forceps. The cups were covered with a plastic Snap-On lid and held in an incubator at 30 ± 1^0C and photoperiod of 14:10 (L:D) h.

Each treatment consisted of five replications of 75 leaves. Leaves were divided into 3 groups and twenty-five leaves from each treatment were examined after 12, 24 and 48 h. Mortality and larval location on lower or upper leaf surface, petiole, or cup surface (off-leaf) were recorded at each interval and surviving larvae were transferred individually to 30-ml plastic cups containing artificial diet. The cups were kept in trays, and held in an incubator at 30 ± 1^0C and 14:10 (L:D) h. Larvae were examined daily to record mortality. Larval weight was taken at the 10th day of their life cycle and pupal weight was recorded 3 days after larvae pupated in each treatment. An electric balance (Mettler 300 SL) was used for taking weights. Pupal and adult emergence dates were recorded by observing insects daily in the cups.

Data Analysis

For each sampling time on each replicate of each treatment, cumulative relative frequency for mortality, based on 25 larvae, and relative frequencies for larval locations, based on live larvae at that sampling time were determined. For each sampling time, data were analyzed according to a randomized complete block design using the general linear model (GLM) procedure of the SAS system (SAS institute 1989). Pupation mortality for each treatment and each set of transfer time was based on 25 larvae. For larval locations, the number of live larvae at the sampling time was used as a weighting factor in a weighted analysis. Survivorship for each location was based on the number of live larvae found at that location at each transfer time. For larval period and pupal weight survivorship was used as a weighting factor. When the *F* test for treatments was significant ($P < 0.05$), treatments were compared by multiple *t*-tests.

Results

B. thuringiensis rate altered the percentage of live *H. zea* larvae on leaf surfaces and increased movement onto the holding cup inner surface. At all 3 transfer times the number of live larvae on the lower leaf surface was significantly lower in all treatments than control, except for the 0.29 kg/ha rate after 12 h, and this difference increased with higher doses (0.58 and 1.15 kg/ha). The percentage of larvae located on the cup-surface (off-leaf) was significantly greater in all *B. thuringiensis* treatments than in the control at 12, 24 and 48 h after treatment. The 0.58 and 1.15 kg/ha rate further increased the percentage of larvae on the cup surface over lower *B. thuringiensis* rates after 24 h (Figure 1). The percentage of larvae on the petiole was less than 5 % in all treatments. Overall larval mortality after 12 h did not differ with treatment, but after 48 h at all *B. thuringiensis* rates was greater than that in the control, and increased significantly with each increase in *B. thuringiensis* rate ranging from 12.8 % in control to 79.2% at 1.15 kg/ha (Figure 2). Larval mortality at pupation was also significantly higher in all *B. thuringiensis* rates than the control, at all the transfer times. Mortality, however, differed among *B. thuringiensis* rates, with a higher mortality in 0.58 and 1.15 than in 0.14 and 0.29 kg/ha at 12 and 24 h (Figure 3).

Larvae transferred from the upper surface had a higher survival rate in the control and 0.14 kg/ha than the highest 2 *B. thuringiensis* treatments at all 3 transfer times. Survival of larvae transferred from the lower leaf surface was higher in the control than in *B. thuringiensis* treatments at 12, and 24 h transfer, except at 12 h in the 0.29 kg/ha treatment. At the 48 h transfer, *B. thuringiensis* treatments did not effect the survival of larvae transferred from the lower leaf surface. *B. thuringiensis* treatment reduced the survivorship of larvae transferred from the cup-surface at 48 h. (Figure 4).

The larval period was significantly longer in all *B. thuringiensis* treatments than control, in the 12, 24 and 48 h transfer (Table 1). At 24 and 48 h transfer larval period did not differ with *B. thuringiensis* rate. Larval weight was higher in control than at 0.14 and 1.15 kg/ha at 12 h, and higher than in all the treatments at the 48 h transfer (Table 1). The length of pupal period did not show any difference at the 12 and 24 h transfer at all *B. thuringiensis* rates and control, but was significantly longer in 0.58 kg/ha than control at 48 h transfer. Pupal weight was significantly higher in the control than in one of the *B. thuringiensis* treatments at all transfer times (Table 2).

Discussion

Results on effect of *B. thuringiensis* treatment on larval movement cooberated those of Jyoti et al. (1996). In both the studies the percentage of *H. zea* larvae was higher on the lower than upper leaf surface and this was not altered with

B. thuringiensis treatment. *B. thuringiensis* did, however, increase the percentage of live larvae on the cup surface as well as larval mortality.

Larval survival at each location (upper and lower leaf surface, and cup surface) was not examined by Jyoti et al. (1996). Results of this study showed that survival at highest *B. thuringiensis* rates was lower than in the control except on the lower leaf surface at 48 h. However, the results showed that survival of these larvae removed from leaves (or cup surface) and transferred to diet was not necessarily influenced by their location. At some highest rates with larvae transferred from leaves at 48 h, however, survival of larvae recovered from the lower leaf surface was higher than that of larvae recovered from the upper leaf surface or cup surface. Data suggests that at least for the first 24 h, the increase in the percentage of larvae on the inner cup surface in *B. thuringiensis* treatments was not due to larve that had consumed a lethal dose, but appeared to be an attempt by larvae to avoid the *B. thuringiensis* on the upper leaf surface.

The extended larval period of *B. thuringiensis* -treated *H. virescens* reported by Ali and Watson (1982) was observed in our study, however, the extended pupal period they reported was not noted. We also obtained a decrease in both larval and pupal weight in *B. thuringiensis*-treated larvae.

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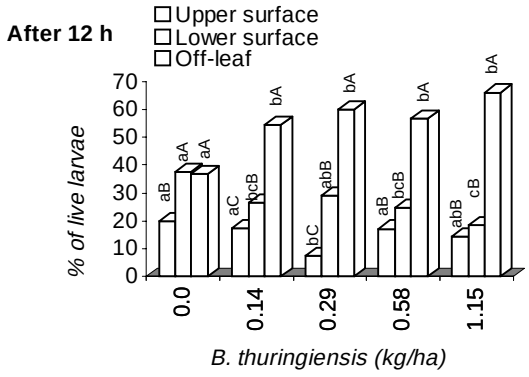
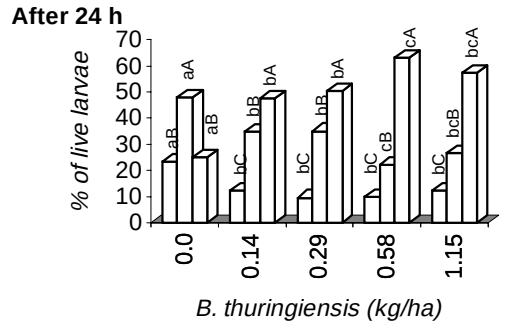




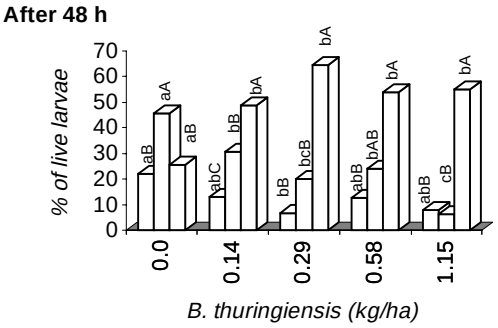


Figure 1. Distribution of larvae on *B. thuringiensis*-treated leaves at different time after exposure. Same colored bars with same lower case letters indicate the treatments are not significantly different within location ($P < 0.05$); bars with same upper case letters indicate the locations are not significantly different within treatment ($P < 0.05$). The percentage of live larvae on petioles was less than 5 % in all treatments and not included in the analysis.

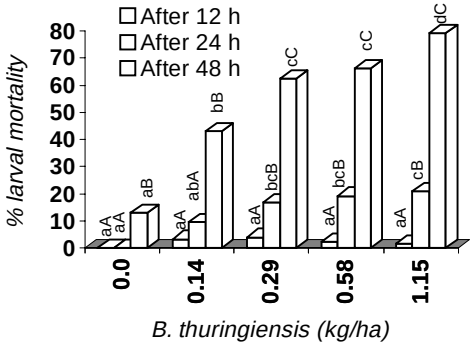


Figure 2. Mortality of *H. zea* larvae at the time of removal from leaves exposed to *B. thuringiensis* treated leaves for different time periods. Same colored bars with lower case letters indicate the treatments are not significantly different within time ($P < 0.05$); bars with same upper case letters indicate that exposure time is not significantly different within treatment ($P < 0.05$).

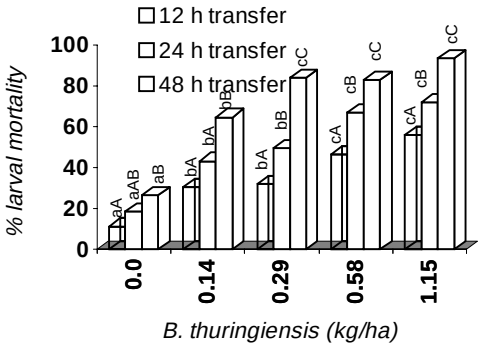
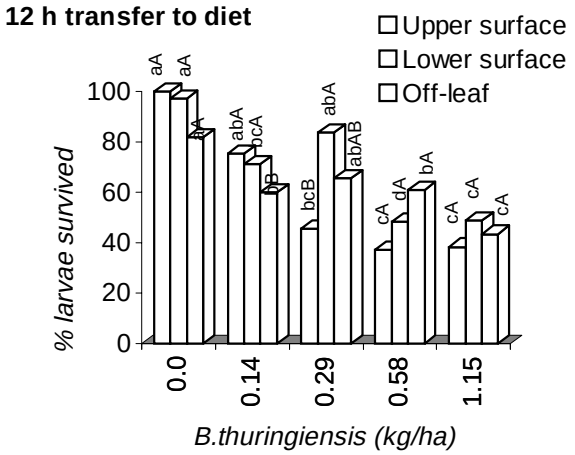
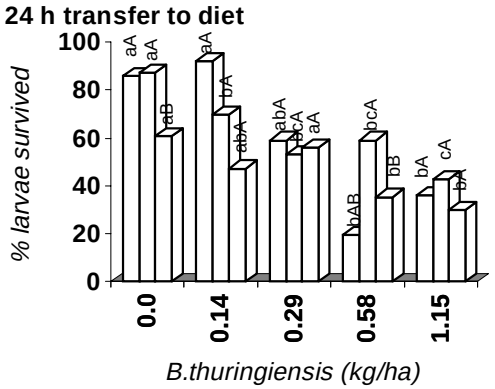


Figure 3. Mortality of *H. zea* larvae at pupation transferred at different time periods from *B. thuringiensis*-treated leaves. Same colored bars with same lower case letters indicate the treatments are not significantly different within time ($P < 0.05$); bars with same upper case letters indicate that exposure time is not significantly different within treatment ($P < 0.05$).





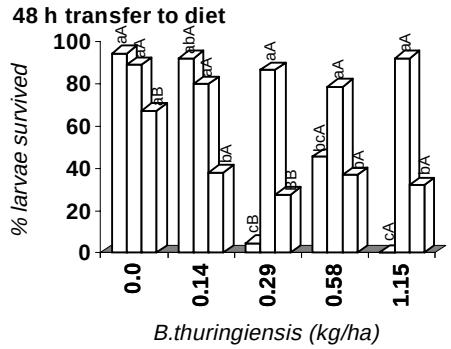


Figure 4. Survivorship of larvae transferred to semisynthetic diet from different locations of *B. thuringiensis*-treated leaves at different time intervals. Same colored bars with same lower case letters indicate that treatments are not significantly different within location ($P < 0.05$); bars with same upper case letters indicate the locations are not significantly different within treatment ($P < 0.05$).

Table 1. larval period and weight *H. zea* at 10 days of age after fed on *B. thuringiensis*-treated cotton for different time periods

Bt rate	Larval period (days)			Larval weight (mg)		
(kg/ ha)	12 h♣	24 h	48 h	12 h	24 h	48 h
0.0	12.9a	13.9a	14.7a	590.1a	536.9a	492.0a
0.14	14.0b	15.8b	16.4b	512.6b	443.1a	389.0b
0.29	14.7bc	16.0b	16.7b	546.3ab	469.1a	278.4b
0.58	14.2b	15.3b	16.1b	567.5ab	541.3a	328.4b
1.15	15.2c	15.8b	16.8b	483.4b	420.0a	338.7b

♣Feeding period on *B. thuringiensis*-treated cotton prior to transfered to semisynthetic diet.
Means within a column followed by the same letter are not significantly different
($P < 0.05$)

Table 2. Pupal period and weight of *H. zea* after larvae fed on *B.thuringiensis*-treated cotton leaves for different time periods

Bt rate	Pupal period (days)			Pupal weight (mg)		
(kg/ ha)	12 h♣	24 h	48 h	12 h	24 h	48 h
0.0	9.9a	9.8a	9.8a	466.2a	456.7ab	457.9a
0.14	9.9a	9.6a	9.4ab	454.6ab	459.7a	439.7ab
0.29	9.8a	9.8a	9.8ab	460.4a	433.5b	424.1ab
0.58	9.7a	9.7a	9.1b	475.3a	458.7ab	418.9b
1.15	9.9a	9.6a	9.2ab	435.3b	440.5ab	427.6ab

♣Feeding period on *B. thuringiensis*-treated cotton before transfered to semisynthetic diet.
Means within a column followed by the same letter are not significantly different
($P < 0.05$)