Activity of *Bacillus thuringiensis* Berliner Against Different Ages and Stages of *Helicoverpa zea* (Lepidoptera: Noctuidae) on Cotton[1]

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ABSTRACT This study was done to compare the mortality of *Helicoverpa zea* (Boddie) eggs and larvae of different ages on cotton treated with *Bacillus thuringiensis* Berliner var. *kurstaki*. Mortality of *H. zea* neonates hatching from eggs collected from *B. thuringiensis*-treated cotton plots was significantly ($P \leq 0.01$) higher for eggs 0 than 2 d in age at the highest (1.12kg/ha) rate, but did not differ at lower rates. However, across all rates the median lethal concentration (LC_{50}) was 3.2-fold lower for larvae hatching from eggs that were 2 d in age than those from eggs 0 d in age. Mortality for larvae 1 d in age placed on *B. thuringiensis*-treated cotton terminals was significantly higher than for larvae 3 and 5 d in age at 4.48 and 8.96 kg/ha rates of *B. thuringiensis*. The LC_{50} for larvae 1 d in age (2.1 kg/ha) was 3 and 5.7-fold lower than for larvae 3 and 5 d in age, respectively (based on 95% confidence intervals). These data indicate that in cotton pest management systems, *B. thuringiensis* applications need to be directed at maximum oviposition and eclosion of *H. zea* neonates because control is very low against larger larvae.

KEY WORDS *Gossypium hirsutum, Helicoverpa zea, Bacillus thuringiensis,* egg age, larval age

The cotton bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.), are important pests of cotton in the United States. Both may occur simultaneously on cotton. Pyrethroids have been considered to be most effective against lepidopteran pests, including these heliothine species on cotton. Although pyrethroid resistance has not been reported in *H. zea*, it has become imperative to more closely manage the use of pyrethroids because of the high level of resistance exhibited by *H. virescens* (Leonard et al. 1987, Luttrell et al. 1987, Plapp and Campanhola 1986) and the necessity to direct controls against both species simultaneously.

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Commercial *Bacillus thuringiensis* Berliner var. *kurstaki* preparations have been evaluated for use in control of heliothine species on cotton and are included in current recommendations for control early in the season (Delta Agricultural Digest 1994, Johnson and Jones 1994). Most studies indicate that *B. thuringiensis* reduces populations of heliothine species on cotton (Bell and Romine 1980, McGarr et al. 1970, 1972, Ali and Watson 1982, Luttrell et al. 1982, Yearian et al. 1980, Johnson 1982), but overall control has not been adequate under high population pressure.

Studies also have shown that *H. zea* larvae are more tolerant to *B. thuringiensis* than *H. virescens*. MacIntosh et al. (1990) reported that median lethal concentrations (LC_{50}s) for purified proteins from *B. thuringiensis* on semisynthetic diet were higher for *H. zea* than for *H. virescens* for HD-73 and HD-1 (both isolates of *B. thuringiensis* var. *kurstaki*). Luttrell et al. (1982) reported that *H. virescens* was more susceptible to lower rates of *B. thuringiensis* on cotton leaves than *H. zea*. Ali and Young (1993b) found that LC_{50} for *H. zea* 1 d in age is 2.4-fold higher than for *H. virescens* at 4 d after treatment.

Various *B. thuringiensis* preparations and formulations have been evaluated against *H. virescens* (Dulmage and Martinez 1973, Dulmage et al. 1978, Beegle et al. 1981, Ali and Watson 1982, Stone et al. 1989), but very few data are available on age-specific mortality in *H. zea*. The objective of these studies was to determine the activity of *B. thuringiensis* against different ages and stages of *H. zea* when applied to cotton.

Materials and Methods

Studies were conducted during 1992 in Washington Co., AR, on the Arkansas Agricultural Experiment Station Research Farm, Fayetteville. Cotton 'DPL 50' was planted 10 June and thinned to a density of ≈74,100 to 98,800 plants per ha at the pre-squaring stage. Plant growth was maintained by furrow irrigation as needed. Both eggs and larvae of *H. zea* were obtained from a laboratory colony maintained at the Department of Entomology, University of Arkansas, Fayetteville. No insecticides were used 10 d before or during these studies.

Effect of Egg Age. Plots consisted of two rows, each 3 m in length, with buffers 7 m and five rows between the ends and sides of the plots, respectively. Plots were arranged in a randomized complete block with four replications. Eggs oviposited on cheesecloth, in a 8-h period (2200 to 0600 hours CST), were aged in the laboratory at 29 ± °C so that their age and hatching times could be determined. Plants in all plots were artificially infested with eggs 0 d and 2 d in age (5 eggs per terminal). The 0-d eggs were 0 to 8 h in age and 2-d eggs were 48 to 56 h in age and near hatching. Eggs were placed on plant terminals using 'Plantguard' (Nordlund et al. 1974) as a sticker.

Immediately after egg placement plants were treated with *B. thuringiensis* (Javelin WG 52,863 Spodoptera units/mg], Sandoz Crop Protection, Des Plaines, IL) at 0.14, 0.28, 0.56, or 1.12 kg/ha; lambda-cyhalothrin (Karate 1EC, Zeneca Ag Products, Wilmington, DE), a standard pyrethroid insecticide, at 0.028 kg(AI)/ha; or water (control plots). Applications were made with a CO_2-powered backpack sprayer equipped with two TX-6 nozzles per row. Treatments were applied at a volume of 93 liters/ha and at a nozzle pressure of 2.8 kg/cm^2.

Eggs were monitored daily, and terminals containing eggs were cut and transported to the laboratory just before eggs hatched. Twenty terminals, excluding fully-expanding leaves, that originally contained 100 eggs per treatment per replication were placed individually on moist filter papers in 30-ml plastic containers and incubated at 28°C. Terminals were searched after 48 h to record the number of eggs that did not hatch and the number of larvae. Surviving larvae were transferred to a semisynthetic diet (Burton 1969) in 30-ml plastic containers and returned to the incubators. These larvae were then observed every 4 d, and larvae that died were recorded until all had died or pupated.

Effect of Larval Age. A randomized complete block with four replications was used to determine the activity of *B. thuringiensis* and lambda-cyhalothrin against *H. zea* larvae 1, 3, and 5 d in age (first, second, and third instars, respectively) on cotton. Plants in the field were sprayed with *B. thuringiensis* (Javelin WG) at 0.56, 1.12, 2.24, 4.48, and 8.96 kg/ha; lambda-cyhalothrin at 0.028 kg(AI)/ha; or water (control plants). Treatments were applied in the morning, and terminals were collected after the spray dried (10-20 min). The application method was as previously described.

Terminals were brought to the laboratory in plastic bags, then placed individually on moist filter papers in 30-ml plastic containers after removing the fully-expanded leaves. Twenty-five *H. zea* larvae of 1, 3, or 5 d of age were placed individually on terminals from each treatment. Larvae were allowed to feed for 48 h at 28°C, after which the terminals in individual containers were searched for larvae. Mortality was recorded, and the surviving larvae were transferred to semisynthetic diet in 30-ml plastic containers and returned to the incubators. Larvae were then observed at 4-d intervals, and mortality was recorded until pupation.

Mortality data for all tests were analyzed by analysis of variance (ANOVA), and means were separated by Ryan-Einot-Gabriel-Welsch-*F*-test (REGWF) (SAS Institute 1988). Data were corrected for control mortality using Abbott's formula (Abbott 1925). LC_{50} values were calculated by using the probit procedure (SAS Institute 1988).

Results and Discussion

Effect of Egg Age. Interaction between the age of egg at treatment and insecticidal treatments was not significant ($F = 1.67$; df = 4, 27; $P = 0.186$). Mortality of larvae hatching from eggs that were treated at the age of 0 d was significantly lower than that in the larvae hatching from eggs treated at the age of 2 d only at 1.12 kg/ha; however, percentage mortality was generally higher in larvae hatching from older than from younger eggs (Table 1). Percentage mortality increased with increasing rates of *B. thuringiensis*. Mortalities in both age groups for lambda-cyhalothrin were significantly higher than for any of the *B. thuringiensis* rates. The LC_{50} for neonates hatching from eggs that were treated at 2 d of age was 3.2-fold less than larvae hatching from eggs that were treated at 0 d of age (Table 2). Based on 95% confidence intervals, we concluded that the LC_{50} for neonates hatching from eggs that were treated at 0 or 2 d of age was significantly lower than that for any group treated as larvae.

Table 1. Effect of age of *H. zea* eggs at the time of application on activity of *B. thuringiensis* against hatching larvae on cotton.

Treatment	Rate*	% corrected mortality ± SEM†		
		0 d in age		2 d in age
Javelin WG	0.14	9.12 ± 4.02 c		16.62 ± 3.52 c
Javelin WG	0.28	21.50 ± 10.34 bc		22.32 ± 7.76 bc
Javelin WG	0.56	30.99 ± 12.08 bc		43.08 ± 8.57 b
Javelin WG	1.12	37.15 ± 7.30 b	*	69.45 ± 2.59 b
Lambda-cyhalothrin	0.028**	83.91 ± 9.37 a		95.03 ± 2.27 a

Means within a column not followed by the same letter are significantly different ($P \leq 0.05$; REGWF).• Significant difference ($P < 0.05$; t-test) between means for each age, within a row.

* Amount of formulated material in kg/ha.

** Amount of active ingredient in kg/ha.

† Control mortalities for eggs 0 and 2 d in age were 11.6 ± 4.7 and 5.0 ± 5.0%, respectively.

These results show the same trends in mortality as the findings of Ali and Watson (1982) who reported higher mortality in larvae hatching from *H. virescens* eggs treated when 3 d in age than in those treated when 1 or 2 d in age. Because *B. thuringiensis* must be ingested by the insect to be effective (Miller et al. 1983), differential mortality in larvae hatching from older versus younger eggs may be explained partially by rapid degradation of its activity on cotton (Beegle et al. 1981). *Helicoverpa zea* eggs take more than 2 d to hatch, depending upon temperature (Fye and McAda 1972) and the half-life of *B. thuringiensis* is approximately 2 d (Ali and Young 1993a). Consequently, larvae hatching 2 d later from eggs treated at 0 d are exposed to a lesser amount of active *B. thuringiensis* than larvae hatching from eggs treated at 2 d of age.

Effect of Larval Age. Interaction between larval age and treatment was significant ($F = 3.65$; df = 10, 51; $P = 0.001$). Mortality in each age group increased with increase in *B. thuringiensis* rate (Table 3). Lambda-cyhalothrin mortality in larvae 1 d of age was similar to that at the 4.48 and 8.96 kg/ha rates of *B. thuringiensis*. Percentage mortality from lambda-cyhalothrin (0.028 kg (AI)/ha) was significantly higher than any of the *B. thuringiensis* rates in larvae 3 and 5 d of age. Percentage mortality at 8.96 and 4.58 kg/ha rates was significantly higher for larvae 1 d in age than for those 5 d in age. LC_{50} for larvae 1 d in age was 3.0- and 5.6-fold lower than that for those 3 or 5 d in age, respectively (Table 2). Based on 95% CI, LC_{50} for larvae 1 d in age was significantly lower than for older larvae. These results corroborate the findings of Ali and Young (1993b) who reported an LC_{50} of 1.43 (0.45-12.7) kg/ha in *H. zea* larvae 1 d in age at 7 d after treatment. These results also support the previous findings in bioassays on semisynthetic diets, that larger *H. zea* larvae require a higher concentration of *B. thuringiensis* (S. Y. Young, unpubl. data) than smaller larvae.

Table 2. Concentration-mortality response of various ages and stages of *H. zea* on cotton to *B. thuringiensis*.

Insect age and stage	n	Slope ± SE	LC_{50} (95% CI)	LC_{90} (95% CI)	X^2
0 d, egg**	100	1.005 ± 0.214	2.0 (1.2 - 6.4)	38.2 (10.1 - 940.4)	22.0
2 d, egg**	100	1.702 ± 0.209	0.6 (0.5 - 0.8)	3.6 (2.3 - 7.3)	66.0
1 d, larva†	100	1.939 ± 0.162	2.1 (1.8 - 2.4)	9.4 (7.3 - 13.0)	143.6
3 d, larva†	100	1.537 ± 0.164	6.2 (5.0 - 8.3)	42.5 (26.0 - 88.7)	88.2
5 d, larva†	100	0.837 ± 0.224	11.7 (4.6 - 8673)	397.00 (____)	13.9

* kg/ha formulated material of Javelin.

** Cotton was sprayed immediately after attaching eggs to the terminal.

† Cotton was sprayed in the field, and terminals were brought to the laboratory. Larvae of desired age were allowed to feed on terminals for 48 h and then transferred to artificial diet.

Table 3. Effect of age of *H. zea* larvae on activity of *B. thuringiensis* on cotton.

Treatment	Rate*	% corrected mortality ± SEM†		
		1-d old	3-d old	5-d old
Javelin WG	0.56	15.0 ± 8.0 ef	8.5 ± 3.8 f	16.0 ± 4.7 ef
Javelin WG	1.12	30.3 ± 5.5 def	10.0 ± 6.5 f	11.8 ± 4.7 f
Javelin WG	2.24	46.5 ± 5.4 cd	21.3 ± 5.0 def	30.5 ± 4.7 def
Javelin WG	4.48	81.5 ± 7.9 ab	39.1 ± 9.0 cde	41.5 ± 4.7 cd
Javelin WG	8.96	87.0 ± 4.6 ab	63.8 ± 6.7 bc	42.0 ± 1.9 cd
Lambda-cyhalothrin	0.03**	100.0 ± 0 a	88.8 ± 0 a	97.0 ± 2.2 a

Cotton was sprayed in the field and terminals were brought to the laboratory. Larvae of desired age were allowed to feed on terminals for 48h and then transferred to artificial diet. Means across rows and columns not followed by the same letter are significantly different ($P \leq 0.05$; REGWF).

* Amount of formulated material (kg/ha).

** Amount of active ingredient (AI) (kg/ha).

† Control mortalities for larvae 1, 3, and 5d in age were 9.0 ± 3.4, 4.0 ± 4.0, 3.0 ± 1.9%, respectively.

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