

Effects of Larval Age on the Susceptibility of Almond Moths¹ and Indianmeal Moths¹ to *Bacillus thuringiensis*^{2,3}

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ABSTRACT

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Larvae of the almond moth, *Ephestia cautella* (Walker), were highly susceptible to *Bacillus thuringiensis* Berliner in their diet if exposure began during the 1st stadium; they were less susceptible if exposure began during the later stadia. Larvae of the Indianmeal moth, *Plodia interpunctella* (Hübner), also were highly susceptible if exposure began during the 1st or 2nd stadium, but only the last (4th) stadium showed low susceptibility. *B. thuringiensis* caused a large increase in the development time of *E. cautella* larvae but caused little increase in that of *P. interpunctella*. The bacterium had no discernible effect on the sex ratio or reproductive capacity of moths that developed from surviving larvae.

A formulation of *Bacillus thuringiensis* Berliner prevented infestation of Indianmeal moths, *Plodia interpunctella* (Hübner), and almond moths, *Ephestia cautella* (Walker), when it was applied to uninfested grain that was then artificially infested with insect eggs (McGaughey 1976). The effectiveness of the bacterium has not been reported when larvae are initially exposed as 2nd, 3rd, 4th, or 5th instars, as might occur if an infestation is present when applications are made, although mature larvae are known to be less susceptible than neonate larvae (Nwanze et al. 1975). Also, the effects on reproduction by moths that emerge from survivors of the treatments have not been reported. The laboratory study reported here was made to determine these effects.

Methods and Materials

Larvae of laboratory colonies of both insect species were reared at 25°C and 60–70% RH on a diet that consisted of cracked wheat, wheat shorts, wheat germ, brewers' yeast, glycerin, honey, water, and mold inhibitors. At such conditions, the Indianmeal moth was determined by head capsule measurements to have 4 larval instars and the almond moth to have 5. By making daily measurements of the head capsule width of the larvae, it was determined on which days large numbers of the desired instars could be expected to be ready for use in tests (Table 1). On the appropriate days, larvae with darkened head capsules, but of the earliest possible age, were individually selected for testing.

The effects of *B. thuringiensis* on almond moth larvae were determined by treating larval diet with *B. thuringiensis* (Dipel®) suspended in water (10 ml/100 g) in concentrations that gave doses of 0, 6.25, 25, and 100 µg of formulation/g of diet. The effects on Indianmeal moth larvae were determined by treating diet with 0, 12.5, 50, and 200 µg of formulation/g of diet. The suspensions were mixed with the diet in round-bottom mixing bowls with an electrically driven stirrer. Five 9-g samples of each lot of treated diet were placed in 25×95-mm shell vials. Then, 10 almond moth or Indianmeal moth larvae of each instar were placed in each vial and the vials were capped with plastic foam stoppers. (Eggs from the laboratory colonies were used instead of 1st instars to avoid the need to handle the small larvae,

since experience had shown that the egg hatchability for both species was always >95%.) All vials were held at 25°C and 60–70% RH until the adults emerged. Numbers of male and female moths that emerged from each vial were recorded daily.

To determine the effects of *B. thuringiensis* on reproduction by almond moths that emerged from treated diet, two 120-g samples of larval diet were treated with *B. thuringiensis* formulation at a rate of 12 ml/120 g. The formulation was suspended in water at a concentration that would give a dose of 4 µg of formulation/g of diet. Two additional samples were treated with water only (12 ml/120 g). Effects on the Indianmeal moth were determined by treating diet with water only or with a dose of 40 µg of formulation/g of diet. The jars of water-treated diet were infested with 125 eggs each, and jars of *B. thuringiensis*-treated diet were infested with 250 eggs each (ca. 50% mortality was expected in the treated samples). All jars were held at 25°C and 60–70% RH. As moths emerged, i.e., over several days, copulating pairs of newly emerged moths were removed from the jars with an aspirator and placed in jars containing ca. 50 g of untreated diet. The other moths were discarded at the end of each day to assure that only newly emerged moths were used. Pairs of moths from either or both of the jars receiving a treatment were used when available. Jars infested with these pairs of F₁ moths were held at 25°C and 60–70% RH until F₂ adults emerged and could be counted. Other pairs obtained in the same way from a 2nd set of treated and untreated jars of diet were placed in plastic vials with 20-mesh screen bottoms over glass petri dishes and held as before for oviposition. Eggs laid by the female of each pair were counted every 2 days, removed to clean vials, and examined for hatching after 6–7 days. Total moth emergence (F₁) in the parent jars also was recorded.

Results and Discussion

Almond moth larvae exposed initially in the 1st stadium were more susceptible to *B. thuringiensis* than larvae exposed later (Fig. 1); at the 25 µg/g dose, 98% died before adult emergence. Larvae exposed initially in the 2nd, 3rd, 4th, and 5th stadia were much less susceptible, and doses of 6.25 or 25 µg/g did not differ significantly in effect. Also, a dose of 100 µg/g did not produce significantly different mortality in 3rd, 4th, or 5th instars. However, 2nd instars showed a response intermediate between that of the 1st instar and the last 3 instars.

¹ Lepidoptera: Pyralidae.

² Reference to a company or product does not imply approval or recommendation of the product by the USDA.

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Table 1.—Instar, head capsule width, and days during which most Indianmeal moth and almond moth larvae were within these size ranges (reared on a ground-wheat diet at 25°C).^a

Instar	Indianmeal moth		Almond moth	
	Days ^b	Head capsule width (mm) ^c	Days ^b	Head capsule width (mm) ^c
1	3-6	0.14-0.18	3-6	0.17-0.18
2	8-9	0.31-0.33	9-10	0.25-0.28
3	11-12	0.52-0.55	12-13	0.37-0.42
4	16-17	0.78-0.87	15-16	0.54-0.60
5			20-23	0.83-0.87

^a Measurements were made on 10 larvae each day.
^b Days after ≤24-h-old eggs were placed on diet.
^c No intermediate sizes were found.

The earlier instars of the Indianmeal moth were ca. equally susceptible (Fig. 1). At the lowest dose, there was no significant difference among instars, but at 50 and 200 μg/g, the 1st and 2nd instars showed high levels of susceptibility, and the 3rd showed an intermediate level; only the 4th instars showed a low level of susceptibility. Thus, an application of *B. thuringiensis* to grain infested with larvae of mixed ages might be more effective against an infestation of Indianmeal moths than against an infestation of almond moths. Adult almond moths probably would emerge despite the treatment, and control would be dependent upon mortality among the early larval instars of the next generation.

The time required for almond moth larvae to complete development and for adults to emerge was greater when exposure to *B. thuringiensis* was begun in the earlier stadia and also was greater when the dose was higher (Fig. 2). When exposure began in the last 2 stadia, there was little

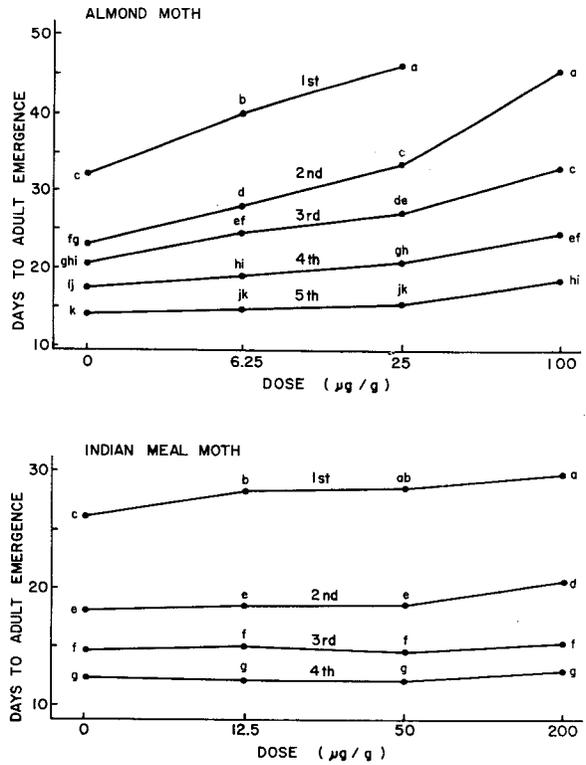


FIG. 2.—Avg no. of days to adult emergence of almond moth and Indianmeal moth larvae exposed initially in the 1st, 2nd, 3rd, 4th, or 5th stadia to *Bacillus thuringiensis*-treated diet. For each species, points denoted by the same letter do not differ significantly at the 0.05 confidence level (Duncan's multiple range test).

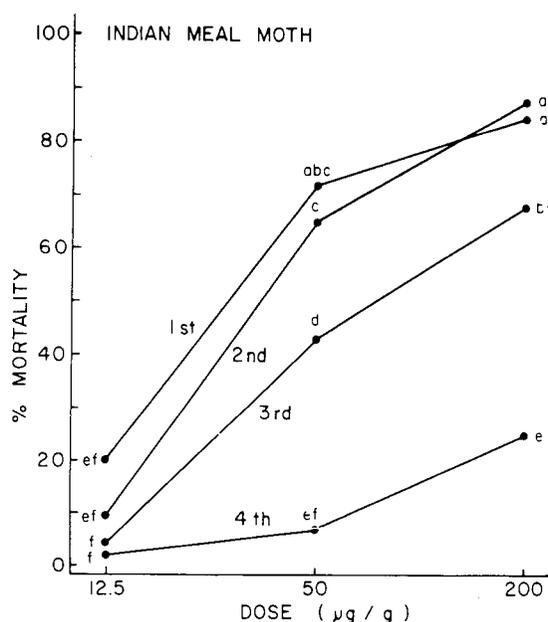
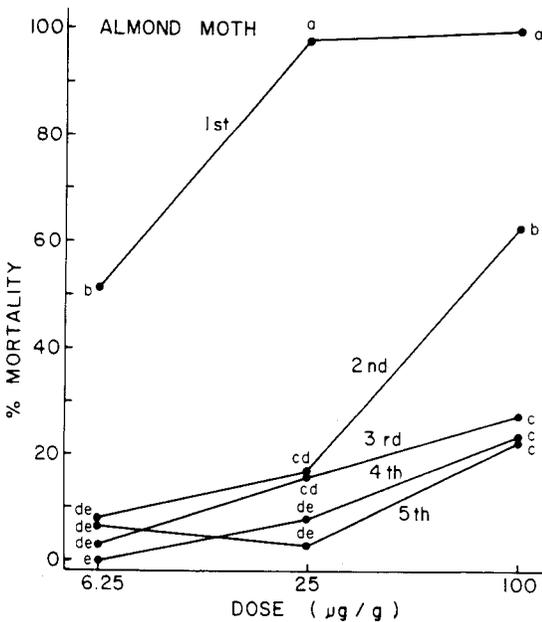


FIG. 1.—Percent mortality prior to adult emergence when almond moth and Indianmeal moth larvae were exposed initially in the 1st, 2nd, 3rd, 4th, or 5th stadia to *Bacillus thuringiensis*-treated diet. For each species, points denoted by the same letter do not differ significantly at the 0.05 level (Duncan's multiple range test).

increase in developmental time except at the highest dose. With Indianmeal moths, the time to complete development increased slightly with each successively higher dose if exposure was begun in the 1st stadium (Fig. 2). When exposure was begun in the 2nd stadium, the highest dose produced only a slight increase in developmental time, but the lower doses caused no increase. Also, when exposure began in the 3rd and 4th stadia, dose had no significant effect.

A similar effect of *B. thuringiensis* on developmental time was reported for the Mediterranean flour moth, *Anagasta kuehniella* (Zeller), by Jacobs (1951) and Afify and Matter (1968, 1969), and for the Indianmeal moth and almond moth by van der Laan and Wassink (1964) and Nwanze et al. (1975). The increased development time for the almond moth was sufficient to be of possible economic value, particularly since all but the 1st instar of this species showed relatively low levels of susceptibility (Fig. 1). The increased time for the Indianmeal moths was so slight that it is probably inconsequential.

The formulation had no appreciable effects on the sex ratio of moths of either species that survived. Likewise,

there were no significant differences between the numbers of F_2 adult progeny produced on untreated diet, the total numbers of eggs, or the numbers of viable eggs produced by pairs of moths of either species when the F_1 generation was reared on untreated or treated diet (Table 2).

Delayed development (particularly for the almond moth) and larval mortality are thus the principal effects of *B. thuringiensis* on these species. A dose high enough to control late-instar larvae may be impractical. Selection of a dose that will control 1st and 2nd instars would be satisfactory if no infestation or only a light infestation was present at the time of treatment. When heavy infestations are present, fumigation prior to application of the *B. thuringiensis* may be desirable. Otherwise, adult emergence and oviposition may persist for several days or weeks after treatment.

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Mr. Edwin B. Dicke of the U.S. Grain Marketing Research Laboratory assisted in conducting these tests and Mr. Robert A. Kinsinger of Kansas State University assisted with the statistical analyses.

Table 2.—Eggs laid and F_2 adult progeny produced on untreated diet by pairs of F_1 adults that were reared from eggs on untreated or *Bacillus thuringiensis*-treated diet.^a

Diet	Egg production test				F_2 adult progeny test		
	% F_1 mortality	No. pairs tested	Avg no. eggs produced	Avg no. hatched	% F_1 mortality	No. pairs tested	Avg no. F_2 adults
<i>Almond moth</i>							
Untreated	25	16	177	151	42	25	123
4 mg/kg	55	16	160	151	71	25	113
<i>Indianmeal moth</i>							
Untreated	15	12	226	218	3	25	265
40 mg/kg	58	12	200	190	67	25	257

^a Separate tests and sources of F_1 adults were used in evaluating egg production and progeny production.

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