

Susceptibility of Populations of Indianmeal Moth¹ and Almond Moth¹ to *Bacillus thuringiensis*^{2,3}

R. A. KINSINGER⁴ AND WM. H. MCGAUGHEY⁵

ABSTRACT

J. Econ. Entomol. 72: 346-349 (1979)

Concentration-mortality studies with several populations of the Indianmeal moth, *Plodia interpunctella* (Hübner), and the almond moth, *Ephestia cautella* (Walker), were conducted with *Bacillus thuringiensis* subsp. *kurstaki* Berliner incorporated into a ground wheat diet. Indianmeal moth and almond moth populations differed as much as 6- and 10-fold, respectively, in responses to *B. thuringiensis*. The differences in both cases appeared to be related, in part, to population vigor. Midgut pH did not relate to population susceptibility in either species.

The Indianmeal moth, *Plodia interpunctella* (Hübner), and the almond moth, *Ephestia cautella* (Walker), the major lepidopteran pests of stored grain in the USA, are resistant to malathion and synergized pyrethrins, the chemical grain protectants now used (Zettler et al. 1973). The potential for using *Bacillus thuringiensis* Berliner as an alternative to chemical insecticides to control these moth species in stored grain has been demonstrated (McGaughey 1975, 1976, Kinsinger and McGaughey 1976). It has not been reported whether differences in susceptibility to *B. thuringiensis* exist among populations of these insect species, however, Hunter and Hoffmann (1973) observed a 7-fold difference in the susceptibilities of 2 populations of the Indianmeal moth to a granulosis virus.

We studied susceptibilities of several isolated populations of Indianmeal moth and almond moth to a commercial formulation of *B. thuringiensis* and determined concentration-mortality curves for the different populations. To try to account for susceptibility differences found, we measured population vigor and larval midgut pH.

Materials and Methods

Origin of Insect Populations

The Indianmeal moth and almond moth populations were obtained as laboratory colonies from Kansas State Univ. and several USDA laboratories; and wild populations were collected from commodity-storage sites. Table 1 lists origins and code designations used in referring to these populations. Some of the populations may have originated from the same parent stock, but they had been maintained separately for several years. Two of the Indianmeal moth populations, SMO-L-IMM and BNB-L-IMM, had been maintained separately for ca. 7 yr on diet containing 20-80 ppm malathion and were presumed resistant to malathion. We maintained all populations on an insecticide-free ground-wheat larval diet

fortified with wheat germ, yeast, honey, and glycerol for ca. 3 mo prior to and during these studies.

Concentration-Mortality Tests

To determine the relative susceptibilities of the moth populations, we suspended Dipel[®], a commercial WP formulation of the spore- δ -endotoxin complex of *B. thuringiensis* subsp. *kurstaki* Berliner, in water at a rate calculated to produce the highest concentration desired. Serial 1:2 dilutions were then used to produce 7 concentrations of the formulation. The suspensions were thoroughly incorporated into a ground wheat larval diet at a rate of 10 ml/100 g. A water-treated control was used.

Each population was tested during 3, not always consecutive, generations. To test the 1st generation, we treated 100 g of diet, and divided each sample into 2 replications of ca. 50 g each in glass jars with filter paper covers. For the other 2 generations, samples were divided into 3 replications of ca. 33 g each. Fifty eggs were added to each jar. (Egg hatchability was always $\geq 95\%$.) The jars were held at $25 \pm 1^\circ\text{C}$ and 60% RH until the adults emerged.

Mortality percentages were calculated from the difference between the number of eggs added and adults that emerged, corrected for mortality in untreated samples. The corrected mortality percentages were used to calculate concentration-mortality curves for each replicate by probit analysis. Median lethal concentrations (LC_{50} values) and slopes of the concentration-mortality curves were then compared by analysis of variance and Duncan's multiple range test. The LC_{50} value was chosen for comparisons because concentration-mortality curves vary less there than at any other point.

Vigor of Populations

To quantify factors related to moth-population vigor, we measured developmental time and size, 2 factors believed to indicate vigor or lack of vigor. The small size of the insects and the way they were maintained made it difficult to collect data on developmental time of each stage of the life cycle, so we used the total developmental times (egg to adult emergence) as measures. The number of days between the 1st eggs collected to maintain the colonies to the 1st appreciable adult emergence in the next generation was recorded for 15 generations of each population.

Size measurements were made on pupae because in-

¹ Lepidoptera: Pyralidae.

² U.S. Grain Marketing Res. Lab., Agric. Res. SEA, USDA, Manhattan, Kans., in cooperation with the Dept. of Entomology, Kans. Agric. Exp. Stn., Kansas State Univ., Manhattan (Contribution 79-58-J). Part of a dissertation submitted by the 1st author in partial fulfillment of requirements for the Doctor of Philosophy degree in Entomology. Received for publication Oct. 11, 1978.

³ Mention of a proprietary product does not constitute an endorsement by the USDA.

⁴ Graduate Res. Asst., Dept. of Entomology, Kansas State Univ., Manhattan 66506.

⁵ Res. Entomologist, U.S. Grain Marketing Res. Lab., 1515 College Ave., Manhattan, KS 66502, and Adjunct Asst. Entomologist, Agric. Exp. Stn., Kansas State Univ., Manhattan 66506.

Table 1.—Origin of Indianmeal moth and almond moth populations and code designations assigned to each.

Code designation ^a	Obtained from ^b	Origin	Date colonized
Indianmeal moth			
HKS-W-IMM	—	Haven and Kingman, KS	May, 1976
MKS(1)-W-IMM	—	Manhattan, KS	May, 1975
MKS(2)-W-IMM	—	Manhattan, KS	July, 1975
PMO-W-IMM	—	Pattonsburg, MO	June, 1975
BNB-L-IMM	1	Beatrice, NB	1968
BTX-L-IMM	2	Fresno, CA	before 1974 ^c
DCA-L-IMM	3	Durham, CA	Jan, 1973
FCA-L-IMM	3	Savannah, GA	before 1962 ^c
KSU-L-IMM	4	Unknown	before 1970 ^c
MCA-L-IMM	3	Modesto, CA	Nov, 1967
MKS-L-IMM	1	Fresno, CA	before 1972 ^c
SGA-L-IMM	5	Unknown	before 1970 ^c
SMO-L-IMM	1	Slater, MO	1968
Almond moth			
BTX-W-AM	2	Beaumont, TX	June, 1975
LFL-W-AM	6	Leesburg, FL	July, 1975
BTX-L-AM	2	Savannah, GA	before 1974 ^c
FCA-L-AM	3	Riverside, CA	before 1967 ^c
GFL-L-AM	6	Leesburg, FL	1973
MKS-L-AM	1	Manhattan, KS	before 1974 ^c
SGA-L-AM	5	Unknown	before 1970 ^c

^a Where obtained - wild (W) or laboratory (L) colony - Indianmeal moth (IMM) or almond moth (AM).

^b 1 = U.S. Grain Marketing Research Laboratory, Manhattan, KS; 2 = Texas A&M Research and Extension Center, Beaumont, TX; 3 = Stored-Product Insects Research Laboratory, Fresno, CA; 4 = Department of Entomology, Kansas State Univ., Manhattan, KS; 5 = Stored-Product Insects Research and Development Laboratory, Savannah, GA; and 6 = Insect Attractants and Biological Research Laboratory, Gainesville, FL.

^c Exact date unknown.

dividual pupae fluctuate little in size (Silhacek and Miller 1972) and are easily observed. In preliminary studies, we found that head capsule width and width at the base of the wings were not adequate measures of the variability in pupal size. Weight and length both were reliable measures of relative size but were so closely related that we used weight alone as the measure of variability among populations. The mean pupal weight of 30 ♂ and 30 ♀ from a single generation of each population was used.

Midgut pH

Midgut pH has been reported to be a major factor related to susceptibility of different species of Lepidoptera to *B. thuringiensis* (Heimpel and Angus 1959, 1960, Burgerjon and Martouret 1971). To determine whether pH also contributes to differences in susceptibility of different populations of the same species, we measured the midgut pH of mature last-stadium larvae, the only instar large enough to measure. The larvae were cooled a short time to anesthetize them, then pinned down at both ends, grasped on both sides near the 1st abdominal segment with fine forceps, and a tear was made in the dorsum. A loop of the midgut extruding through the tear was pulled to one side where all hemolymph and other tissues were removed with a small piece of tissue paper. A small piece of pH indicator paper (pHydrion®—narrow range) was touched to the loop of gut, then a puncture was made to allow a droplet of gut fluid to wet the paper. The pH was estimated by comparing color changes in the indicator paper with a standard color chart.

Results and Discussion

Concentration-Mortality Tests

Populations of both the Indianmeal moth and the al-

mond moth differed in responses to *B. thuringiensis*. Comparisons of the slopes of the concentration-mortality curves show the relative heterogeneity of the responses to *B. thuringiensis* within the populations, and the relative LC₅₀ values show the differences in susceptibility between populations.

SMO-L-IMM obviously differed from the other Indianmeal moth populations in response to *B. thuringiensis* (Table 2), and the variation between assays was so large that it could not be compared with the other populations in an analysis of variance. Its mean LC₅₀ was 270.11 mg/kg, almost 7 times that of any other population. Thus, this population appeared to be less susceptible than other Indianmeal moth populations to *B. thurin-*

Table 2.—Median lethal concentrations (LC₅₀ values) and slopes of concentration-mortality curves for populations of the Indianmeal moth on a ground-wheat diet treated with *Bacillus thuringiensis*.^a

Population	LC ₅₀ ±SD (mg/kg)	Slope±SD
SMO-L-IMM ^b	270.11±361.82	0.74±0.27
BNB-L-IMM ^c	40.00± 7.88a	1.46± .29a
MCA-L-IMM	30.61± 8.18ab	1.72± .33a
MKS(2)-W-IMM ^c	29.24± 14.68abc	1.97± .40a
MKS-L-IMM	28.08± 5.72abc	1.74± .34a
BTX-L-IMM	27.72± 22.66abc	2.12± .36a
SGA-L-IMM	19.98± 12.96abc	1.84± .20a
HKS-W-IMM	17.91± 3.43abc	1.60± .21a
MKS(1)-W-IMM	14.23± 7.31bc	1.71± .29a
KSU-L-IMM	13.66± 2.02bc	1.70± .24a
DCA-L-IMM	12.12± 2.53bc	2.05± .28a
PMO-W-IMM ^c	10.32± 5.47bc	1.52± .34a
FCA-L-IMM	6.43± 3.15c	2.18± .55a

^a Means of 8 replications over 3 generations; means not followed by the same letter differ significantly ($P < 0.05$) by Duncan's multiple range test.

^b Not included in the analysis of variance or Duncan's multiple range test.

^c Means of only 6 replications over 2 generations.

giensis. But, responses of individuals within this resistant population varied widely; the mean slope of its concentration-mortality curves was 0.74, smaller than that of any other population. So, at low concentrations (i.e., concentrations causing <20% mortality) SMO-L-IMM might appear to be more susceptible than other populations, but at high concentrations, more than 7 times less susceptible.

Mean slopes of the concentration-mortality curves calculated for the remaining populations of the Indian-meal moth did not differ significantly ($P \geq 0.05$). Because the relative variation in responses to *B. thuringiensis* of individuals within each population was similar, concentration-mortality curves could be assumed to be parallel, so, conclusions drawn about the LC_{50} values should hold true for all points along the curves.

The mean LC_{50} value of the most susceptible population, FCA-L-IMM, was only $\frac{1}{6}$ that of the least susceptible population, BNB-L-IMM. The mean LC_{50} for FCA-L-IMM, 6.43 mg/kg, was significantly smaller ($P < 0.05$) than those of 2 of the other 11 populations. The mean of 40.00 mg/kg for BNB-L-IMM was significantly greater ($P < 0.05$) than those of 5 of the other 11 populations. Scheffé's test (Snedecor and Cochran 1967) showed that the mean LC_{50} value for the wild populations (17.66 mg/kg) did not differ ($P \geq 0.05$) from that of populations maintained in the laboratory for a number of years (21.75 mg/kg).

One presumably malathion-resistant population, SMO-L-IMM, was obviously less susceptible to *B. thuringiensis*, and the other resistant population, BNB-L-IMM, was slightly less susceptible (though not significantly so) than other populations. Both apparently differ from the others in a way that affects susceptibility to *B. thuringiensis*. The lower slopes and higher LC_{50} values exhibited by the 2 resistant populations follow the pattern that Hoskins and Gordon (1956) attributed to populations in the process of developing true resistance. Some of the other 11 populations showed differences like those Hoskins and Gordon (1956) attributed to vigor tolerance, differences in LC_{50} values without differences in slope. We did not collect enough data, however, to determine whether malathion resistance and resistance to *B. thuringiensis* are related.

The slope of the concentration-mortality curve for almond moth population MKS-L-AM (2.39) was larger ($P < 0.05$) than that of 4 of the other 6 populations (Table 3) indicating that the responses of individuals in the population to *B. thuringiensis* were more homogeneous than in other populations. MKS-L-AM also was more susceptible than other populations at all doses except at doses which produced a theoretical mortality of less than 20%. Thus, we assumed that our comparisons were valid even though the concentration-mortality curves for that population and some of the others were not parallel.

Median lethal concentrations of *B. thuringiensis* differed as much as 10-fold among the almond moth populations. FCA-L-AM, with a mean LC_{50} of 55.88 mg/kg, was significantly less susceptible ($P < 0.05$) to *B. thuringiensis* than any other population. MKS-L-AM, with a mean LC_{50} of 5.60 mg/kg was more susceptible ($P < 0.05$) than populations BTX-L-AM and GFL-L-

Table 3.—Median lethal concentrations (LC_{50} values) and slopes of concentration-mortality curves for populations of the almond moth on a ground-wheat diet treated with *Bacillus thuringiensis*.^a

Population	$LC_{50} \pm SD$ (mg/kg)	Slope $\pm SD$
FCA-L-AM ^b	55.88 \pm 19.30a	1.68 \pm 1.24ab
BTX-L-AM	28.54 \pm 16.34b	1.86 \pm .50ab
GFL-L-AM	27.51 \pm 8.72b	1.07 \pm .31b
SGA-L-AM	21.47 \pm 9.69bc	1.05 \pm .43b
BTX-W-AM	15.02 \pm 4.63bc	1.28 \pm .32b
LFL-W-AM ^c	14.37 \pm 6.69bc	1.07 \pm .14b
MKS-L-AM	5.60 \pm 3.46c	2.39 \pm .41a

^a Means of 8 replications over 3 generations; means not followed by the same letter differ significantly ($P < 0.05$) by Duncan's multiple range test.

^b Means of only 7 replications over 3 generations.

^c Means of only 5 replications over 2 generations.

AM with mean LC_{50} values of 28.54 and 27.51 mg/kg, respectively. A comparison of the mean LC_{50} of the wild populations (14.77 mg/kg) with that of the populations maintained in the laboratory for a number of years (27.08 mg/kg) showed that there was no difference in the responses of the 2 groups ($P > 0.05$).

Vigor of Populations

Duncan's multiple range test separated the populations into groups based on mean developmental times or mean pupal weights (Tables 4 and 5). However, our main interest was to determine whether those 2 measurements and susceptibility to *B. thuringiensis* were related, so we calculated correlation coefficients, which indicated mutual relationships (Table 6). Although none of the coefficients differed significantly ($P \geq 0.05$) from 0, some trends could be seen. In both moth species developmental time appeared to be more closely related to LC_{50} than did pupal weight. However, both developmental time and pupal weight of Indianmeal moths correlated negatively with LC_{50} (i.e., as developmental time and pupal weight increased, LC_{50} decreased) while

Table 4.—Developmental time, pupal weight, and midgut pH of Indianmeal moth populations reared on a ground wheat diet.^a

Population	Developmental time (days) ^b	Pupal weight (mg) ^c	Midgut pH ^d
FCA-L-IMM	32.2a	16.32a	9.24f
MKS(2)-W-IMM	28.0b	12.72ef	9.61abc
PMO-W-IMM	27.9b	11.75fg	9.57bc
SMO-L-IMM	27.4bc	14.09cd	9.50cd
BNB-L-IMM	27.4bc	15.81ab	9.36ef
DCA-L-IMM	27.0bcd	14.36cd	9.62ab
MKS(1)-W-IMM	26.9bcd	14.77bc	9.67a
KSU-L-IMM	26.5bcde	13.62de	9.32f
SGA-L-IMM	26.2cde	15.74ab	9.34f
BTX-L-IMM	25.9cde	10.48h	9.37ef
MCA-L-IMM	25.3def	—	9.40def
MKS-L-IMM	24.9ef	10.86gh	9.42de
HKS-W-IMM	24.0f	—	—

^a Means not followed by the same letter differ significantly ($P < 0.05$) by Duncan's multiple range test.

^b Means of 15 generations; days from beginning of egg collections to maintain colonies to first appreciable adult emergence in subsequent generation.

^c Means of 30 ♂ and 30 ♀ removed from colonies.

^d Means of 20 mature last-instar larvae removed from colonies.

Table 5.—Developmental time, pupal weight, and midgut pH of almond moth populations reared on a ground wheat diet.^a

Population	Developmental time (days) ^b	Pupal weight (mg) ^c	Midgut pH ^d
BTX-L-AM	34.2a	13.00b	8.44b
FCA-L-AM	32.5b	12.93b	9.27a
MKS-L-AM	31.1bc	10.98c	9.13a
SGA-L-AM	30.5c	14.83a	8.63b
BTX-W-AM	26.8d	12.64b	—
LFL-W-AM	26.6d	—	—
GFL-L-AM	26.4d	12.16b	8.97a

^a Means not followed by the same letter differ significantly ($P < 0.05$) by Duncan's multiple range test.

^b Means of 15 generations; days from beginning of egg collection to maintain colonies to first appreciable adult emergence in subsequent generation.

^c Means of 30 ♂ and 30 ♀ removed from colonies.

^d Means of 20 mature last-instar larvae removed from colonies.

both measurements correlated positively with LC_{50} values of almond moths (i.e., as developmental time and pupal weight increased, LC_{50} increased). These trends, though inconclusive, provide evidence, in addition to differences in the response of larvae of these moths to spores and crystals (McGaughey 1978a) and differences in the effects of *B. thuringiensis* on the rate of larval development (McGaughey 1978b), that *B. thuringiensis* may have different modes of action in these 2 species.

Midgut pH

Tables 4 and 5 summarize data on midgut pH of larvae of Indianmeal moth and almond moth. Midgut pH did not correlate significantly ($P \geq 0.05$) with LC_{50} or slope in either moth species, and coefficients were smaller than those for population vigor (Table 6). Thus, it appeared that susceptibility of populations could not be related to midgut pH as we measured it.

Differences in susceptibility between populations of these 2 moth species are large enough that wide ranges in application rates may be required for effective control. Differences that we found were comparable to those reported by Hunter and Hoffmann (1973) for responses of Indianmeal moth populations to a granulosis virus. Some concern about the development of resistance to *B. thuringiensis* also may be warranted. Although the susceptibility of populations of both species appeared to be related, in part, to population vigor and not to larval midgut pH, other factors, such as biochemical or immunological factors, also may contribute to the overall susceptibility and may interact with the factors which we measured. More knowledge of the obviously complex mode of action of *B. thuringiensis* in these species is needed before a more precise explanation can be made.

Table 6.—Correlation coefficients for relationships of developmental time, pupal weight, and midgut pH with LC_{50} and slope of the concentration-mortality curves for populations of the Indianmeal moth and the almond moth.

	Developmental time	Pupal weight	Midgut pH
Indianmeal moth			
LC_{50}	-0.41	-0.21	-0.12
Slope	0.43	-0.01	0.03
Almond moth			
LC_{50}	0.41	0.31	0.26
Slope	0.66	-0.64	0.26

REFERENCES CITED

- Burgerjon, A., and D. Martouret. 1971. Determination and significance of the host spectrum of *Bacillus thuringiensis*. P. 305-25. In H. D. Burges and N. W. Hussey [eds.]. Microbial Control of Insects and Mites. Academic Press. London. 861 pp.
- Heimpel, A. M., and T. A. Angus. 1959. The site of action of crystalliferous bacteria in Lepidoptera larvae. J. Insect Pathol. 1: 152-70.
1960. Bacterial insecticides. Bacteriol. Rev. 24: 266-88.
- Hoskins, W. M., and H. T. Gordon. 1956. Arthropod resistance to chemicals. Annu. Rev. Entomol. 1: 89-122.
- Hunter, D. K., and D. F. Hoffmann. 1973. Susceptibility of two strains of Indian meal moth to a granulosis virus. J. Invertebr. Pathol. 21: 114-5.
- Kinsinger, R. A., and W. H. McGaughey. 1976. Stability of *Bacillus thuringiensis* and a granulosis virus of *Plodia interpunctella* on stored wheat. J. Econ. Entomol. 69: 149-54.
- McGaughey, W. H. 1975. Compatibility of *Bacillus thuringiensis* and granulosis virus treatments of stored grain with four grain fumigants. J. Invertebr. Pathol. 26: 247-50.
1976. *Bacillus thuringiensis* for controlling three species of moths in stored grain. Can. Entomol. 108: 105-12.
- 1978a. Response of *Plodia interpunctella* and *Ephesia cautella* larvae to spores and parasporal crystals of *Bacillus thuringiensis*. J. Econ. Entomol. 71: 687-8.
- 1978b. Effects of larval age on the susceptibility of almond moths and Indianmeal moths to *Bacillus thuringiensis*. Ibid. 71: 923-5.
- Silhacek, D. L., and G. L. Miller. 1972. Growth and development of the Indian meal moth, *Plodia interpunctella* (Lepidoptera:Phycitidae), under laboratory mass-rearing conditions. Ann. Entomol. Soc. Am. 65: 1084-7.
- Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. 6th ed. Iowa State University Press. Ames, Iowa. 593 pp.
- Zettler, J. L., L. L. McDonald, L. M. Redlinger, and R. D. Jones. 1973. *Plodia interpunctella* and *Cadra cautella* resistance in strains to malathion and synergized pyrethrins. J. Econ. Entomol. 66: 1049-50.