

Histopathological Effects of *Bacillus thuringiensis* on Larvae of the Indianmeal Moth¹ and the Almond Moth^{1,2,3}

R. A. KINSINGER⁴ AND WM. H. MCGAUGHEY⁵

ABSTRACT

Ann. Entomol. Soc. Am. 72: 787-790 (1979)

Histopathological studies demonstrated that the midgut epithelium in larvae of both the Indianmeal moth, *Plodia interpunctella* (Hübner), and the almond moth, *Ephestia cautella* (Walker), was damaged within 1/2 to 1 h after they ingested spores and parasporal crystals of *Bacillus thuringiensis* Berliner subsp. *kurstaki*. Midgut epithelial cells progressively degenerated until the midgut was totally disrupted and the larvae died. Larval death preceded extensive septicemia, and thus, apparently was caused by the gut wall's loss of integrity.

The histopathology of *Bacillus thuringiensis* Berliner has been studied in relatively few insect species. Heimpel and Angus (1959) described the effects of the bacterium on the midgut of the silkworm, *Bombyx mori* (L.). They reported gut paralysis and separation of epithelial cells from the basement membrane and from one another within 45 min after *B. thuringiensis* was ingested, with extensive damage to the entire midgut within 60 min. Later studies with other Lepidoptera agreed in general with Heimpel and Angus' findings on the sequence of pathological events (Hoopingartner and Materu 1964, Ramakrishnan and Tiwari 1967, Sutter and Raun 1967, Atwa and Abdel-Rahman 1974, Narayanan and Jayaraj 1974). However, times required for the effects to take place differed among species, possibly because of differences in susceptibility, age of larvae, bacterial formulations used, mode of action of the bacterium, or method of feeding the bacterial preparation.

We attempted to determine the histopathology of *B. thuringiensis* in the Indianmeal moth, *Plodia interpunctella* (Hübner), and the almond moth, *Ephestia cautella* (Walker), species of stored-product moths that can be controlled in stored grain with *B. thuringiensis* (McGaughey 1976, 1978a).

Methods and Materials

Larvae of the Indianmeal moth and the almond moth (populations MKS-L-IMM and FCA-L-AM, Kinsinger and McGaughey 1979) were reared on a ground-wheat larval diet fortified with honey, glycerin, yeast, and wheat germ, until they reached the penultimate instar (3rd and 4th, respectively). They were then fed an aqueous suspension of spores and parasporal crystals of *B. thuringiensis* subsp. *kurstaki* (Dipel[®], 16000 International Units of potency and $\geq 25 \times 10^6$ viable spores/mg), prepared at 100 mg/ml, by placing a droplet on their mouthparts with a micropipette while viewing them through a dissecting microscope. One-half, 1, 2, 4, 8, 16, and 24 h later, the anterior and posterior segments were dissected from larvae to facilitate penetration of liquid, and they were fixed overnight (12 - 24 h) in alcoholic Bouin's fixative. The fixative was washed out

by passing the larvae through changes of Lenoir's fluid until the yellow color disappeared. Larvae then were dehydrated by passing them through a graded ethanol series and were cleared with methyl benzoate, infiltrated with xylene, and embedded in paraffin. Embedded larvae were sectioned longitudinally at a thickness of ca. 8 μ m. Sections were mounted on microscope slides, stained with Harris' hematoxylin and eosin, and prepared for photomicrography.

Results and Discussion

Histopathological investigations showed extensive damage to the midgut in both Indianmeal moth and almond moth. The sequence of events appeared to be similar in both species; however, in Indianmeal moth, gross effects occurred sooner and larvae died sooner than with almond moth.

Midgut epithelial cells from untreated (control) Indianmeal moth larvae were closely associated with one another and with the basement membrane (Fig. 1a). One-half to 1 h posttreatment, the distal ends of the columnar epithelial cells had become distended and bulbous (Fig. 1b, 1c). Two h after larvae ingested *B. thuringiensis*, the midgut epithelium showed localized regions of cell destruction; in other areas the cells remained intact, although exhibiting signs of damage (Fig. 1d). Some vegetative rods could be seen in the gut lumen by 2 h posttreatment. Four h after treatment, sloughed epithelial cells and cell contents nearly filled the gut lumen (Fig. 1e). Cell contents toward the distal ends of cells did not stain as well as previously (compare Fig. 1a and 1e). Heimpel and Angus (1959) reported that this suggested cell membrane breakdown; however, because the cells appeared to be intact we believe that the membranes were still whole and that disruption of the internal cell structures was responsible for the reduced staining. Degeneration of the epithelium continued so that after 8 h it consisted of a thin layer of compact cuboidal cells, in contrast to their previous elongated, columnar shape (compare Fig. 1a and 1f). The gut lumen was filled with debris from disrupted cells, with extensive bacterial growth apparent. Indianmeal moth larvae were dead at 16 h, and desquamation of the epithelial layer was complete, leaving only remnants of the basement membrane and prolific bacterial growth marking the midgut's location (Fig. 1g). Bacterial growth, previously localized in the gut lumen, extended into the hemocoel at 16 h. The gut had totally disintegrated by 24 h and profuse septicemia was observed (Fig. 1h).

In almond moth, as in Indianmeal moth, midgut epi-

¹ Lepidoptera: Pyralidae.

² U.S. Grain Marketing Research Laboratory, Agricultural Research, SEA, USDA, 1315 College Ave., Manhattan, KS, 66502, in cooperation with the Dept. of Entomology, Kans. Agric. Exp. Stn., Kansas State Univ., Manhattan 66506 (Contribution 79-237-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 June 7, 1979.

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

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

⁵ Res. Entomologist, U.S. Grain Marketing Res. Lab., and Adjunct Asst. Entomologist, Agric. Exp. Stn., Kansas State Univ.

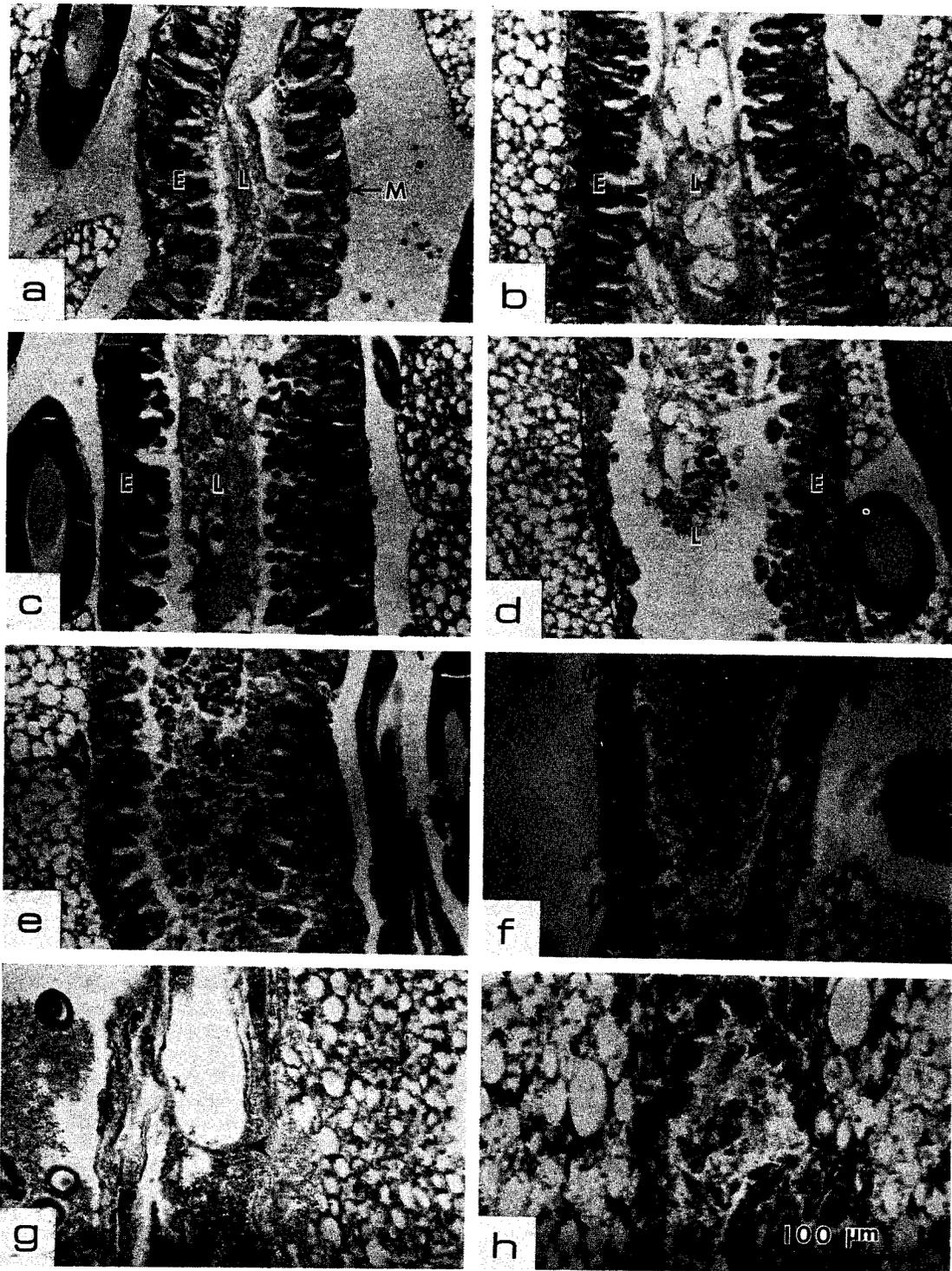


FIG. 1.—Indianmeal moth larvae fed *B. thuringiensis* subsp. *kurstaki*. Longitudinal sections, ca. 8 μ m thick, a, control. b, 1/2 h posttreatment. c, 1 h posttreatment. d, 2 h posttreatment. e, 4 h posttreatment. f, 8 h posttreatment. g, 16 h posttreatment. h, 24 h posttreatment. E = gut epithelium, L = gut lumen, M = basement membrane, ◄ = bacterial rods.

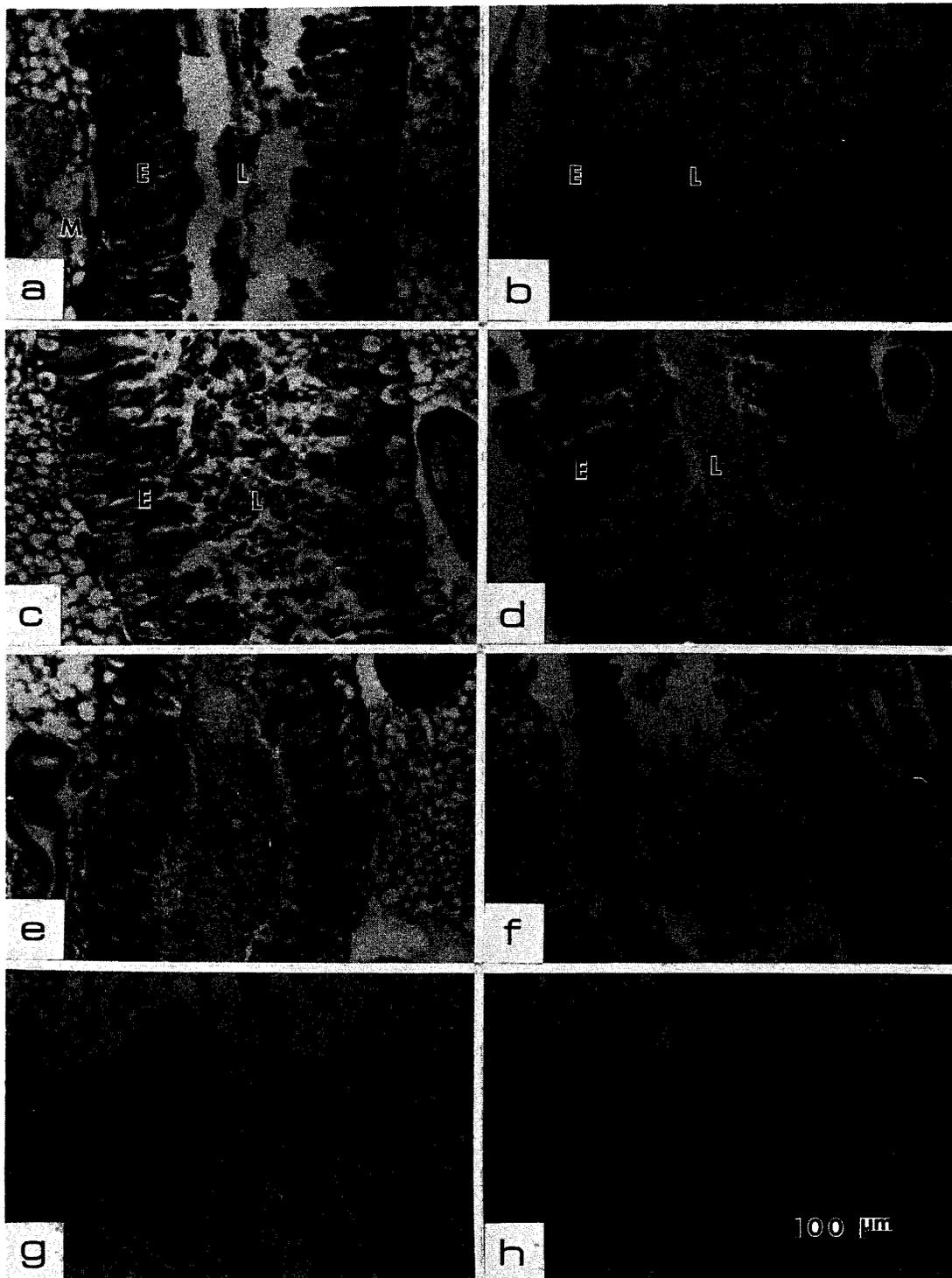


FIG. 2.—Almond moth larvae fed *B. thuringiensis* subsp. *kurstaki*. Longitudinal sections, ca. 8 μ m thick. a, control. b, 1/2 h posttreatment. c, 1 h posttreatment. d, 2 h posttreatment. e, 4 h posttreatment. f, 8 h posttreatment. g, 16 h posttreatment. h, 24 h posttreatment. E = gut epithelium, L = gut lumen, M = basement membrane, \blacktriangle = bacterial rods.

thelium from untreated (control) larvae consisted of columnar epithelial cells closely associated with one another and with the basement membrane (Fig. 2a). At 1/2 h posttreatment, cells were starting to become distended (Fig. 2b) and 1–2 h after *B. thuringiensis* was ingested, the epithelial cells had noticeably distended and some had sloughed into the lumen (Fig. 2c, 2d). Four h posttreatment some bacterial growth in the lumen was noted, the epithelium was more disorganized, and as was observed in Indianmeal moth larvae, the cell contents failed to stain as previously (compare Fig. 2a and 2e). After 8 h the midgut epithelium showed localized areas of destruction and bacterial growth had increased (Fig. 2f). The midgut epithelium was almost completely disrupted after 16 h with only the basement membrane and a few epithelial cells remaining (Fig. 2g). Unlike Indianmeal moth, in which bacterial growth had extended to the hemocoel by 16 h, bacterial growth was still confined to the midgut (compare Fig. 1g and 2g). By 24 h death, total disruption of the midgut, and septicemia had occurred (Fig. 2h).

Our results agree, in general, with those from studies of other insect species. However, some differences were found. Midgut epithelial cells in both species we studied remained closely associated with the basement membrane until total cell disruption; whereas in studies with other species the epithelial cells separated from the basement membrane shortly after the larvae ingested the bacterium as reported for the silkworm (Heimpel and Angus 1959), the European corn borer, *Ostrinia nubilalis* (Hübner) (Sutter and Raun 1967), and the "citrus leaf caterpillar," *Papilio demoleus* L. (Narayan and Jayaraj 1974). Circular muscles of the gut of both moth species we studied were very small and could not be observed in detail. Thus, we could not detect midgut paralysis by light microscopy, as indicated by the relaxation of the circular muscle (Heimpel and Angus 1959). We did not notice general body paralysis in either species we studied. Body muscles maintained normal appearance after treatment and did not become relaxed and fenestrated as reported in silkworms (Heimpel and Angus 1959).

Death appeared to be due primarily to effects of the bacterial formulation (probably the crystal component) on the gut epithelium and the gut's loss of integrity. Earlier bacterial growth in the gut and larval death in Indianmeal moth than in almond moth may have been because the Indianmeal moth population we used was more susceptible than the almond moth population (Kinsinger

and McGaughey 1979); but our results, which were obtained with a mixture of spores and crystals, also may support the finding of McGaughey (1978b) that spores are of little or no consequence against almond moths, while against Indianmeal moths they are ca. 1/3 as toxic as crystals and when added to a crystal preparation cause a slight increase in toxicity.

Heimpel and Angus (1959) proposed a scheme for separating species into 3 types according to their response to *B. thuringiensis*. Although our methods prevented our detecting gut paralysis, the species we tested probably are Type II in their scheme. General body paralysis, typical for Type I insects, was not evident in either species, and both have been reported to respond to pure crystals (McGaughey 1978b), which is atypical of Type III insects.

REFERENCES CITED

- Atwa, W. A., and H. A. Abdel-Rahman. 1974. Histopathological effects of *Bacillus thuringiensis* Berl. on larvae of *Pieris rapae* (L.) (Lep., Pieridae). *Z. Angew. Entomol.* 76: 326–31.
- Heimpel, A. M., and T. A. Angus. 1959. The site of action of crystalliferous bacteria in Lepidoptera larvae. *J. Insect Pathol.* 1: 152–70.
- Hoopingartner, R., and M. E. A. Materu. 1964. The toxicology and histopathology of *Bacillus thuringiensis* Berliner in *Galleria mellonella* (Linnaeus). *Ibid.* 6: 26–30.
- Kinsinger, R. A., and W. H. McGaughey. 1979. Susceptibility of populations of Indianmeal moth and almond moth to *Bacillus thuringiensis*. *J. Econ. Entomol.* 72: 346–9.
- McGaughey, W. H. 1976. *Bacillus thuringiensis* for controlling three species of moths in stored grain. *Can. Entomol.* 108: 105–12.
- 1978a. Moth control in stored grain: Efficacy of *Bacillus thuringiensis* on corn and method of evaluation using small bins. *J. Econ. Entomol.* 71: 835–9.
- 1978b. Response of *Plodia interpunctella* and *Ephesia cautella* larvae to spores and parasporal crystals of *Bacillus thuringiensis*. *Ibid.* 71: 687–8.
- Narayanan, K., and S. Jayaraj. 1974. Mode of action of *Bacillus thuringiensis* Berliner in citrus leaf caterpillar, *Papilio demoleus* L. (Papilionidae: Lepidoptera). *Indian J. Exper. Biol.* 12: 89–91.
- Ramakrishnan, N., and L. D. Tiwari. 1967. Histological changes in *Plusia orichalcea* caused by *Bacillus thuringiensis*. *J. Invertebr. Pathol.* 9: 579–80.
- Sutter, G. R., and E. S. Raun. 1967. Histopathology of European-corn-borer larvae treated with *Bacillus thuringiensis*. *Ibid.* 9: 90–103.

Reprinted from the

ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA