

Response of *Plodia interpunctella*¹ and *Ephestia cautella*¹ Larvae to Spores and Parasporal Crystals of *Bacillus thuringiensis*^{2,3}

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ABSTRACT

Parasporal crystals of *Bacillus thuringiensis* Berliner were 30X as toxic to larvae of *Ephestia cautella* (Walker) as were spores. The toxicity of spore-crystal mixtures was predictable from the number of crystals in the mixture. Parasporal crystals were only 3X as toxic as spores to *Plodia interpunctella* (Hübner) larvae, and a 50:50 mixture of spores and crystals was more toxic than crystals alone.

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Recent and ongoing studies (McGaughey 1976, Kinsinger and McGaughey 1976) have demonstrated the potential of formulations of spores and parasporal crystals of *Bacillus thuringiensis* Berliner for controlling larvae of the Indian meal moth, *Plodia interpunctella* (Hübner), and the almond moth, *Ephestia cautella* (Walker), in stored grain. However, the mode of action of these formulations and the relative importance of spores and crystals in causing death of the larvae have not been reported for these species. This study was made to determine the relative toxicity of spores, crystals, and various mixtures of spores and crystals to these species. This information is potentially valuable because modified formulations might be desirable for application to food products and because certain other pest control measures such as fumigation appear to be selectively detrimental to the spores (McGaughey 1975).

Methods and Materials

Crystals and spores of a culture of *B. thuringiensis* var. *kurstaki* (HD-1) isolated from a commercial formulation were produced in modified GYS medium (Nickerson and Bulla 1974) in a 250-liter fermenter. The spores and crystals were separated by renografin-water density gradient centrifugation (Sharpe et al. 1975), and were washed 4 times in water to remove the renografin. Spores and crystals in samples of each preparation were counted by using a Petroff-Hausser counting chamber, and sufficient lactose was added to each preparation so that upon freeze-drying each would contain 25×10^6 particles/mg of dry material. Plate counts, using half-strength nutrient agar, showed that ca. 0.29% spores were viable in the crystal preparation. Microscopic examination of suspensions of the 2 preparations confirmed this level of spore contamination in the crystal preparation and indicated the spore preparation was equally pure.

The preparations were bioassayed with larvae of *P. interpunctella* and *E. cautella* in a diet of cracked wheat and wheat bran supplemented with wheat germ, brewers yeast, glycerol, honey, water, and fungistatic agents. Stock suspensions of the spores and crystals were prepared in distilled water. Three mixtures of the stock suspensions, each with the same total particle concentration, but with spore-crystal ratios of 10:90, 50:50, and 90:10, also were prepared. The concentrations were such that when 3 ml of suspension were added to 30 g of diet a spore, crystal, or spore-crystal dosage of 12.5×10^6 particles/g would be achieved.

Serial 1:2 dilutions of each preparation were made and added to other samples of diet to give a total of 10 doses ranging from 12.5×10^6 particles/g to 2.44×10^4 particles/g each preparation.

The suspensions were incorporated with the diet in round-bottom mixing bowls with an electrically-driven polyethylene stirrer. Each sample of treated diet was divided in half, and the 2 portions were placed in separate mason jars with filter paper caps (1 jar for each insect species). Twenty-five eggs from laboratory insect colonies were added to each jar. The infested jars were held at 25°C and 60-70% RH until adults emerged. Mortality levels were determined by comparing adult emergence with the number of eggs added. Mortality in the treated samples was corrected for that in untreated diet (4% for *P. interpunctella* and 29% for *E. cautella*). The concentrations of each preparation and mixture in diet that were required to kill 50 and 70% (LC₅₀ and LC₇₀) of the larvae were calculated by probit analysis.

Results and Discussion

The LC₅₀ values for *E. cautella* (Table 1) show that crystals are ca. 30X more toxic than spores to this species. The lowest LC₅₀ value (4.8×10^5 particles/g) was obtained with crystals alone. The LC₅₀ values obtained with the mixtures could be predicted almost exactly from the numbers of crystals contained in the mixtures. The low level of toxicity present in the spores (LC₅₀ = 145×10^5 particles/g) may have resulted from crystal contamination or from a small amount of similar toxin in the spore wall (Somerville et al. 1968, Somerville and Pockett 1975, Schesser and Bulla 1978). (A crystal contamination level of 3.3% would be required to produce an LC₅₀ of 145×10^5 particles/g if no toxic activity were associated with the spores.) Although somewhat more variable, because of the lower precision of the estimates, the relative LC₇₀ values for the different preparations show the same response.

The LC₅₀ values for *P. interpunctella* (Table 1) show that both spores and crystals are toxic to this species, and that crystals are only ca. 3X as toxic as spores. Further, the maximum toxicity was obtained with a 50:50 mixture of crystals and spores (LC₅₀ = 31.9×10^5 particles/g). However, reducing the percentage of spores in the mixture had a relatively small effect in comparison with the effect of reducing the percentage of crystals. The LC₇₀ values show similar relationships between the mixtures.

The results show that crystals are toxic to *E. cautella*, but that spores are not appreciably so; they also indicate that crystals are more toxic than spores to *P. interpunctella*, though a 50:50 mixture is the most toxic combination. Re-

¹ Lepidoptera: Pyralidae.

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

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Table 1.—Response of larvae of *E. cauteila* and *P. interpunctella* to spores, crystals, and spore-crystal mixtures of *B. thuringiensis* in larval diet.

Spore-crystal ratio	Slope	No. of particles X 10 ⁵ /g			
		LC ₅₀ & 95% C.I.		LC ₇₀ & 95% C.I.	
<i>Ephestia cauteila</i>					
0:100	1.64	4.8	3.3–7.4	10.0	6.6–18.4
10:90	1.25	5.7	3.5–10.4	14.9	8.5–41.6
50:50	2.91	10.5	7.1–16.3	15.9	11.0–31.2
90:10	2.80	46.0	31.0–73.0	70.8	48.5–143.4
100:0	1.14	145.0	45.9–	417.3	84.8–
<i>Plodia interpunctella</i>					
0:100	3.00	50.5	34.5–79.2	75.6	52.5–150.0
10:90	2.76	38.1	25.4–59.0	59.0	40.5–112.8
50:50	1.95	31.9	22.1–48.9	59.2	39.9–109.4
90:10	1.94	102.5	48.1–	191.1	82.4–
100:0	2.88	147.2		223.9	

duction of viable spores, either by modification of the formulation or by other pest control treatments such as fumigation (McGaughey 1975), should have no effect on the toxicity to *E. cauteila* of spore-crystal deposits. Likewise, toxicity to *P. interpunctella* should not be affected much unless virtually all of the spores are removed or killed.

These results contrast with those obtained in studies with 2 other species of stored-product Lepidoptera, *Galleria mellonella* (L.) and *Anagasta kuehniella* (Zeller), which appear to be more susceptible to spores when crystals are present (Burges et al. 1976, Heimpel and Angus 1959). They also differ from results recently reported from this laboratory for *Manduca sexta* (L.), in which spores and crystals were ca. equally toxic (Schesser et al. 1977, Schesser and Bulla 1978). The response of *P. interpunctella*, however, appears similar to that reported by Fast (1977) for *Choristoneura fumiferana* (Clemens), in which crystals and spore-crystal mixtures were most toxic (and ca. equally so) and spores alone had little toxicity.

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