

## Insecticidal Activity of Spore-Free Mutants of *Bacillus thuringiensis* against the Indian Meal Moth<sup>1</sup> and Almond Moth<sup>1,2</sup>

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Three oligosporogenic mutants of *Bacillus thuringiensis* were assayed for toxicity against larvae of the Indian meal moth, *Plodia interpunctella*, and the almond moth, *Ephestia cautella*. The results were compared with insecticidal activity obtained from the parent strain (HD-1) and two standard *B. thuringiensis* formulations (HD-1-S-1971 and HD-1-S-1980) against the same insect species. The toxicity of the sporeless mutant preparations was significantly diminished against the Indian meal moth (10- to 26-fold increase in LC<sub>50</sub>) but exceeded the toxicity of the standards against the almond moth. The toxicities of the *B. thuringiensis* preparations toward the Indian meal moth were consistent with the number of spores in the test samples, but spores did not contribute to toxicity to *E. cautella* larvae. A rationale for basing dosage on soluble protein was demonstrated for use in situations where spores are not a contributing factor in toxicity.

KEY WORDS: *Bacillus thuringiensis*; Indian meal moth; *Plodia interpunctella*; almond moth; *Ephestia cautella*.

### INTRODUCTION

The Indian meal moth, *Plodia interpunctella*, and the almond moth, *Ephestia cautella*, are major lepidopteran pests of stored grain in the United States. Both insects can be controlled with the entomocidal toxin from *Bacillus thuringiensis* (McGaughey, 1976, 1978a). However, larvae of the almond moth are sensitive only to the parasporal crystals of *B. thuringiensis* var. *kurstaki*, whereas spores from the bacterium contribute significantly to toxicity in Indian meal moth larvae (McGaughey, 1978b).

Mutant strains of *B. thuringiensis* have been isolated which are defective for spore production, resulting in a substantial reduction in sporulation frequency (<0.1%) or complete loss of spore formation (asporogeny) (Somerville, 1971; Nishiitsutsuji-Uwo et al., 1975; Johnson et al., 1980). Some of these mutant strains retain their

entomocidal activity (Nishiitsutsuji-Uwo and Endo, 1980; Johnson and Freedman, 1981) and impart a significantly reduced spore count to the resulting entomocidal formulation. These strains are valuable for use in obtaining crystal protein essentially uncontaminated with spore coat protein and also have potential application for insect control where spores may be undesirable. For instance, it would be advantageous to use a spore-free product for the protection of milled flours and processed cereals intended for human consumption. Although spores of *B. thuringiensis* appear not to survive the baking process in large numbers (McGaughey et al., 1980) and do not constitute an environmental or health hazard, the elimination of spores from the entomocidal formulation should find greater acceptance among the public when such formulations are used to protect processed cereal foods during storage. Consequently, this study was designed to determine whether oligosporogenic mutant strains of *B. thuringiensis* could be used to

<sup>1</sup> Lepidoptera: Pyralidae.

<sup>2</sup> Mention of a proprietary product does not constitute an endorsement by the U.S. Department of Agriculture.

control two major lepidopteran pests of stored grain as effectively as the wild-type strain of *B. thuringiensis* var. *kurstaki*.

#### MATERIALS AND METHODS

Spores and crystals originating from six different preparations of *B. thuringiensis* var. *kurstaki* were used in this study. Three oligosporogenic mutants (NRRL B-4454, B-4457, and B-4458) were derived from the parent (HD-1) by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Johnson et al., 1980). The two standards (HD-1-S-1971 and HD-1-S-1980) were obtained from H. Dulmage, U.S. Department of Agriculture, Brownsville, Texas. All strains were grown on modified nutrient sporulation medium containing phosphate (NSMP) as previously described (Johnson et al., 1980). Spores and crystals were suspended in 1 M NaCl and 0.01% Triton X-100, vortexed, and washed repeatedly with sterile water by centrifugation. Preparations were dried by lyophilization and stored at 4°C. Protein concentration was determined by the method of Lowry et al. (1951) using crystalline bovine serum albumin as a standard. Viable spore counts were performed upon accurately weighed dry quantities of spore-crystal preparations, which were re-suspended at 1 mg/ml in water and heated at 80°C for 20 min. Appropriate dilutions were plated on NSMP, incubated at 28°C for 48 hr, and counted.

The preparations were bioassayed in a larval diet of cracked wheat and wheat bran supplemented with wheat germ, brewer's yeast, glycerol, honey, water, and fungistatic agents. Dry formulations were suspended in water, using a tissue grinder, in concentrations appropriate for addition to the larval diet at a rate of 6 ml/60 g to achieve the highest dosage, 500 mg/kg. Serial 1:2 dilutions were made for eight lower doses. The suspensions were mixed 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 two portions were placed in

separate mason jars with filter paper caps (one jar for each insect species). Fifty eggs from laboratory 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. The concentrations of each preparation in diet that were required to kill 50% (LC<sub>50</sub>) of the larvae were calculated by probit analysis.

#### RESULTS AND DISCUSSION

Because the soluble protein contained in the crystal is responsible for insect toxicity and shares some common properties with spore coat protein (Somerville and Pockett, 1975), we measured the total alkali-soluble protein per gram of crystal preparation (dry wt) and the viable spore population in each of the six samples (Table 1). We found that the three oligosporogenic mutant preparations contained significantly higher protein content, due to fewer spores and inert ingredients per gram dry weight. The two reference standards were lowest in protein content due to a preponderance of inert ingredients and other growth medium constituents. The spore counts were effectively reduced (99.6–99.9%) in the three mutant preparations (as compared with the HD-1 parent). Surprisingly, the spore count for

TABLE 1  
RELATIVE COMPOSITION OF TOTAL PROTEIN AND  
VIABLE SPORES IN *Bacillus thuringiensis*  
PREPARATIONS USED FOR BIOASSAY AGAINST LARVAE  
OF *Ephestia cautella* AND *Plodia interpunctella*

Source	Protein <sup>a</sup>	Spores <sup>b</sup> (× 10 <sup>5</sup> )
HD-1	466	3050.0
B-4454	757	13.3
B-4457	1000	2.2
B-4458	586	2.6
HD-1-1971(s)	241	130.0
HD-1-1980(s)	381	21.8

<sup>a</sup> μg/mg dry wt.

<sup>b</sup> Viable count, number remaining after heating at 80°C for 20 min.

the HD-1-S-1980 standard was also reduced (36.4%, as compared to HD-1-S-1971). Both standards contained fewer viable spores than the laboratory preparation of HD-1.

Mortality data based upon dry weight for *E. cautella* larvae showed that the oligosporogenic strains were as toxic as the parent strain and actually surpassed the two standard reference preparations for toxicity to the almond moth (Table 2). Conversely, LC<sub>50</sub> data for Indian meal moth larvae showed a severely diminished toxicity associated with the oligosporogenic preparations. Likewise, both standards were less potent for the Indian meal moth (LC<sub>50</sub> values were two- to five-fold higher) than for *E. cautella*, while the laboratory preparation of HD-1 maintained a toxicity that was consistently high (LC<sub>50</sub> = 5.21–6.70 µg dry wt/g diet) for both insect species. The expected decrease in toxicity of the mutant preparations toward the Indian meal moth was apparently due to the reduced spore count in these samples. It has been established that spores contribute significantly to toxicity against Indian meal moth larvae (McGaughey 1978b). Indeed,

the HD-1-S-1980 standard was less toxic for the Indian meal moth than the 1971 standard, due to the reduced spore count (no difference was observed between these standards against the almond moth). However, the extent of diminished toxicity (LC<sub>50</sub> increase of 33–65×) due to the reduced spore content in the mutant preparations was greater than previously observed with the Indian meal moth using various spore–crystal ratios (McGaughey 1978b).

Because dosage levels based upon dry weight were inappropriate with samples of differing composition, we considered the measurement of soluble protein as an alternative parameter. We found that dosage based upon protein content was a reliable index of toxicity, at least for insect species susceptible only to *B. thuringiensis* entomocidal protein and not to any secondary contribution from spores (see Table 2). The mortality results based upon dry weight in Table 2 were adjusted for relative protein levels in each bacterial sample, thereby relating all potency values to an equivalent protein content. In the case of mutant strain B-4457, there was no need for adjustment

TABLE 2  
MORTALITY OF *Ephestia cautella* AND *Plodia interpunctella* LARVAE TO *Bacillus thuringiensis* PREPARATIONS IN THE LARVAL DIET BASED UPON DRY WEIGHT AND PROTEIN CONTENT

Source	µg dry wt/g diet			µg protein/g diet	
	Slope	LC <sub>50</sub>	95% C.I.	LC <sub>50</sub>	95% C.I.
<i>Ephestia cautella</i>					
HD-1	2.30	5.21	0.84–12.81	2.43	0.39–5.97
B-4454	1.45	3.85	1.51–7.70	2.91	1.14–5.83
B-4457	1.81	3.85	3.00–4.83	3.85	3.00–4.83
B-4458	1.91	6.15	3.78–9.25	3.60	2.21–5.42
HD-1-S-1971	2.71	17.63	12.15–25.35	4.25	2.93–6.11
HD-1-S-1980	1.54	15.93	5.11–33.75	6.07	1.94–12.87
<i>Plodia interpunctella</i>					
HD-1	2.13	6.70	5.4–8.15	3.12	2.52–3.80
B-4454	0.89	436.78	183.37–	330.64	138.81–
B-4457	0.89	213.12	142.15–	213.12	142.15–
B-4458	1.14	239.61	130.74–	140.41	76.61–
HD-1-S-1971	1.83	39.79	32.58–48.54	9.59	7.85–11.70
HD-1-S-1980	1.43	82.79	64.92–105.76	31.54	24.73–40.29

since all of the sample was accountable as protein (1 mg dry wt equaled 1 mg protein, measured spectrophotometrically). The corrected LC<sub>50</sub> values and confidence intervals based upon protein content related all values on a meaningful basis and revealed a surprising degree of homology between samples. The potency of both standards toward the almond moth were slightly poorer than that of the laboratory strains, perhaps reflecting a contribution from nonspecific (noncrystal) protein originating from extraneous sources. Adjustment of mortality data for Indian meal moth larvae on the basis of protein content failed to significantly change the original findings based on dry weight, however, and demonstrated the significant contribution by spores to mortality for this insect.

These results corroborate previous studies by McGaughey (1978b) and McGaughey and Dicke (1984) concerning relative toxicity of *B. thuringiensis* preparations against the Indian meal moth and the almond moth. The reduced toxicity of the oligosporogenic mutant preparations toward Indian meal moth larvae supports the observations of McGaughey (1978b) and Schesser (1976), who described lower potency for an experimental *B. thuringiensis* formulation which contained few, if any, spores.

Consequently, the oligosporogenic mutants appear to have little utility against insect species like the Indian meal moth in which spores play a significant role in toxicity, yet should provide exceptional protection against insects which are susceptible only to parasporal crystal protein (such as the almond moth). Cereal grain processing facilities, warehouses, and milling plants involved in food production for human consumption would all be likely sites for application of a spore-free insecticidal preparation for the prevention or control of *E. cautella* infestations.

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