

# Methods of Applying *Bacillus thuringiensis* to Stored Corn for Moth Control<sup>1,2</sup>

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### ABSTRACT

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Two practical methods of applying *Bacillus thuringiensis* Berliner to the surface layer of stored corn, treating the grain after the bins were filled or during transfer of the grain with an auger, were compared with a laboratory method, treating grain in a cement mixer. The bacterial deposits resulting from use of each method were ca. equally toxic to insect larvae. Spore counts on individual kernels varied only slightly more with the practical methods than with the laboratory method, and all 3 methods provided sufficient mixing to prevent pockets of over- or under-treated kernels. The auger method was more prone to error in dose rate than were the other methods.

*Bacillus thuringiensis* Berliner controls Indianmeal moths, *Plodia interpunctella* (Hübner), and almond moths, *Ephestia cautella* (Walker), in stored grain (McGaughey 1978). The surface layer of grain in bins must be treated to at least the 10-cm depth, and laboratory studies have shown that the bacterium is more effective when applied evenly to all kernels in this treated layer (McGaughey 1976). This study was made to compare the relative uniformity of *B. thuringiensis* deposits that result from 2 practical methods of application and the laboratory application procedure used in earlier studies (McGaughey 1978). *B. thuringiensis* was mixed with the surface layer of corn in bins, added to corn as it was transferred with an auger, or mixed with corn in a cement mixer.

### Materials and Methods

Two lots of shelled yellow corn were treated with aqueous suspensions of 11.98 g of *B. thuringiensis* (Dipel®, lot 51-186-BJ)/liter of water (0.1 lb/gal) at a rate of 8.06 liters/m<sup>3</sup> (0.6 pt/bu) or 0.815 liters/m<sup>2</sup> (2 gal/100 ft<sup>2</sup>) mixed 10 cm (4 in.) deep by each of the 3 application and mixing methods. With the cement mixer, 454 ml (0.96 pt) of suspension were poured onto 0.056-m<sup>3</sup> (1.6-bu) lots of grain as they were mixed for ca. 2 min. For the auger treatment, a garden sprinkler can with some of its holes plugged to adjust the liquid flow rate to the grain capacity of a 15.24-cm (6-in.) diam auger was used to sprinkle 14.2 liters (3.75 gal) of suspension onto the corn as it was dumped from a truck into the auger's receiving hopper. (This quantity of suspension is required to treat 1.762 m<sup>3</sup> (50 bu) of grain, the approximate amount in the 10-cm surface layer of a 4.572-m (15-ft) diam bin.) The flow of grain was stopped after all the suspension had been applied and the treated grain, which was transferred to another truck instead of a bin, was weighed to calculate the actual dosage. The suspension was constantly agitated during application by rotating and shaking the sprinkler can, and the flow of grain from the truck into the auger hopper was stopped while the can was being refilled. For treating grain already in bins, two 1.435-m (56.5-in.) diam bins of corn were

used. A garden sprinkler can was used to sprinkle the suspension (1.32 liters/bin) onto the surface and a scoop was used to mix the surface 10-cm layer. In an effort to achieve thorough coverage of the corn, the dosage was applied in 3 portions. Most of the dosage was applied in 2 portions and mixed with the grain after each application. The 3rd portion, representing ca. 1/10 of the total, was applied to the surface but not mixed in.

Eight 4-liter samples of grain were drawn from the corn before treatment, and within ca. 2 h after treatment, 4 additional 4-liter samples were drawn from each treated lot. All samples were held in jars at room temperature for 48 h to permit moisture equilibration, then they were stored at -23°C to prevent deterioration of the bacterial deposits. After 2 wk, an 800-g subsample of grain was removed from each sample and bioassayed for insecticidal activity. Each subsample was divided into 2 Mason jars with filter paper caps, and 50 almond moth eggs from a laboratory colony were added to each jar. The infested jars of grain were held at 25°C and 60-70% RH until adults emerged. Mortality levels were based upon the differences between the number of eggs added and the number of adults that emerged. (In samples of eggs from this batch, >95% hatched.)

Several weeks after treatment, *B. thuringiensis* spore counts were made on individual kernels from the portion of each sample remaining in storage at -23°C. Ten kernels of average size and uniform shape were selected from each sample and were individually washed in 5 ml water by agitation for 90 sec in vial on a vortex-action mixer. The wash water was diluted 1:100, and 0.1 ml was spread evenly over the surface of a half-strength nutrient agar plate with a bent glass rod. Colony counts were made after the plates were incubated 48 h at 27°C. Very few colonies of bacteria other than *B. thuringiensis* were observed on the plates. The identity of *B. thuringiensis* was confirmed by microscopically examining smears from colonies that had been allowed to grow 2 or 3 additional days for the presence of spores and crystals.

### Results and Discussion

Bioassay data indicated that each of the 3 methods applied lethal deposits of *B. thuringiensis* on the corn. Almond moth mortalities were 97-100% on the subsamples of treated grain and were within the expected range (based upon prior experience with this species) of 40-67% on the untreated grain.

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<sup>2</sup> Mention of a proprietary product does not constitute an endorsement by the USDA.

Table 1.—Estimates of variance components and their confidence intervals for *B. thuringiensis* spores per kernel resulting from 3 different methods of application.

Source of variation	df	Estimate of variance	% each source	95% confidence interval <sup>a</sup>
Auger method				
Samples	7	$2.395 \times 10^8$	15.85	$(0.236, 13.913) \times 10^8$
Kernels/samples	72	$12.719 \times 10^8$	84.15	$(9.402, 18.170) \times 10^8$
Total		$15.114 \times 10^8$		
Bin surface method				
Samples	7	$1.695 \times 10^8$	17.28	$(0.227, 9.564) \times 10^8$
Kernels/samples	72	$8.110 \times 10^8$	82.72	$(5.995, 11.586) \times 10^8$
Total		$9.805 \times 10^8$		
Cement mixer method				
Samples	7	$0.135 \times 10^8$	2.97	$(0.000, 1.941) \times 10^8$
Kernels/samples	72	$4.401 \times 10^8$	97.03	$(3.253, 6.287) \times 10^8$
Total		$4.536 \times 10^8$		

<sup>a</sup> Confidence intervals were calculated using the methods of Snedecor and Cochran (1967).

Mean numbers of viable spores per kernel of corn provide a more precise indication of differences in bacterial deposits produced by the 3 treatment methods than do the bioassay data. The mean number with the auger treatment, 72356, was significantly greater ( $P < 0.05$ ) than the means from the cement mixer, 38444, or bin surface treatment, 37881. In the trials with the auger method, we applied quantities of *B. thuringiensis* suspension suitable for treating 1.762 m<sup>3</sup> (50 bu) of grain, but the entire amount was applied to 1.692 m<sup>3</sup> (48 bu) in the 1st lot and to 1.44 m<sup>3</sup> (43.8 bu) in the 2nd lot because of difficulty in synchronizing flow rates of the grain and suspension. After adjusting for this higher dose rate with the auger method, the 95% confidence intervals for the 3 methods overlap, although the difference between the means is still rather large. Under farm or warehouse conditions more precise rates of application seem unlikely because trial applications are seldom made and time is too short for adjusting grain or liquid flow rate once treatment has started. Also, wide variation seems to be inherent in flow rates of liquid from the sprinkler cans and of grain from the truck and through the auger. Thus, a major problem in treating the grain as it is augered into bins is that of achieving a precise dose rate. Treating the surface layer of grain already in the bin, however, can result in a dosage rate very close to that obtained with the laboratory method if appropriate attention is given to the depth to which the grain is mixed.

Estimates of the variance components and their confidence intervals (Table 1) for spore counts per kernel are useful for comparing the uniformity of grain coverage which the 3 application methods provide. Although 15–17% of the variability in the 2 practical methods and only ca. 3% of the variability in the laboratory (mixer) method were due to differences between samples, the overlapping confidence intervals for sample-to-sample variation within all 3 methods suggest that the grain was mixed to about the same degree with the different methods. Further, the small variability due to sample variation indicates that each method mixed sufficiently to prevent "pockets" of over- or under-treated grain.

The larger source of variability with all 3 treatment methods was between kernels within samples. This may have resulted because the suspension was poured or sprinkled onto the grain using a large volume to achieve coverage of individual kernels rather than dispersing the suspension with a sprayer. The overlapping confidence intervals indicate that the kernel-to-kernel variability was about the same for the 2 practical methods of application. The cement mixer, however, provided slightly lower kernel-to-kernel variability.

As should be expected, the 2 practical methods of applying *B. thuringiensis* varied slightly more than the laboratory (cement mixer) method. However, the auger and bin treatment methods did not appear to differ significantly from each other in terms of sample-to-sample variability or kernel-to-kernel variability. The auger method appeared more prone to error in dose rate, which could affect uniformity of application. Therefore, if used conscientiously, either of the tested field methods will provide satisfactory results, so other factors such as convenience, labor requirements, and kind of facilities and equipment available can be considered in selecting the method to use.

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#### REFERENCES CITED

- McGaughey, W. H. 1976. *Bacillus thuringiensis* for controlling three species of moths in stored grain. Can. Entomol. 108: 105–12.
1978. Moth control in stored grain: efficacy of *Bacillus thuringiensis* on corn and method of evaluation using small bins. J. Econ. Entomol. 71: 835–9.
- Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. 6th ed. Iowa State University Press. Ames, Iowa. 593 pp.