

## Moth Control in Stored Grain: Efficacy of *Bacillus thuringiensis* on Corn and Method of Evaluation Using Small Bins<sup>1,2</sup>

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### ABSTRACT

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Surface-layer application of a commercial formulation of *Bacillus thuringiensis* Berliner was evaluated for controlling Indian meal moths, *Plodia interpunctella* (Hübner), and almond moths, *Ephesia cautella* (Walker), in corn stored in 2-m<sup>3</sup> (ca. 60-bu) capacity bins. The bins were artificially infested, and efficacy was monitored by making weekly moth counts, collecting larvae and pupae in strips and spools of corrugated paper, periodically assaying samples of the grain, and evaluating insect feeding damage at the end of the storage season. The test method proved to be satisfactory, and doses of 100, 125, and 150 mg of *B. thuringiensis* formulation/kg of corn in the 10-cm-deep surface layer reduced moth populations by  $\geq 92\%$ , reduced insect feeding damage by  $>82\%$ , and prevented webbing of the grain surface.

A series of laboratory studies (McGaughey 1975, 1976, Kinsinger and McGaughey 1976, Schesser 1976) demonstrated the potential of formulations of the bacterium, *Bacillus thuringiensis* Berliner, for controlling infestations of Indian meal moths, *Plodia interpunctella* (Hübner), and almond moths, *Ephesia cautella* (Walker), in stored grain. In bulk-stored grain, larvae of these insects essentially confine their activity to the surface of the grain where they feed on the grain kernels and spin silken threads that mat the kernels and form a silken web over the grain surface (Cotton 1961). Repeated applications of insecticides are sometimes made on the surface of the grain mass to prevent infestations (Quinlan and Miller 1958). They are not fully effective due, in part, to high levels of insecticide resistance in the moth populations (Zettler et al. 1973).

The study reported here was made to determine whether treating the surface layer of stored corn with *B. thuringiensis* would prevent infestations of these species. For this study, a method of testing was developed in which bins of 2-m<sup>3</sup> (ca. 60-bu) capacity were used. This bin size permitted adequate replication, artificial infestation, maintenance of untreated check bins, and frequent and thorough monitoring of infestation levels—requirements that cannot be readily met using full-size bins.

### Methods and Materials

Cylindrical corrugated steel bins, 1.435 m diam and 1.26 m high, with steel bottoms and removable steel lids were used (Fig. 1). The lids and sides were commercially available parts of storage bins for animal feeders. The bottoms were specially fabricated. The bins were placed on wood pallets so that they could be moved with a forklift. On Oct. 15, 1976, 20 bins were filled to a level 10 cm below the top (ca. 1450 kg/bin) with 1976 crop-year shelled yellow corn that had been dried by aeration to 12.5% moisture content

without supplemental heat. The bins were arranged in 2 rows with the bins ca. 0.6 m apart. Three treatment levels and an untreated check were assigned to the 4 bins within each of 5 blocks. The onset of cool weather reduced the likelihood that infestations would develop before the following spring. Therefore, treatment was delayed until March 1977.

On Mar. 7, 8, and 9, 1977, the 10-cm-deep surface layer of corn (124 kg plus an additional 4 kg for laboratory evaluation) was removed from each of the bins to be treated, and an aqueous suspension of a WP formulation of *B. thuringiensis* (Dipel<sup>®</sup> Lot No. 51-186-BJ) was applied to that grain. The dosages were 100, 125, and 150 mg of formulation/kg of grain in the treated surface layer. A narrow-mouth bottle was used to sprinkle the suspension, at a rate of 10 ml/kg, onto the corn from each bin as the grain was being mixed in a cement mixer in 3 equal batches. Each batch of grain was mixed for ca. 1 min after the suspension had been added to simulate the mixing that could be expected when the formulation was applied at the inlet of a grain auger on larger quantities of grain. The treated grain was then returned to the appropriate bin and layered evenly over the surface. The extra 4-kg sample of the treated grain from each bin was stored in filter-paper-capped jars at 25°C and 60–70% RH. Corn in the untreated check bins was left undisturbed.

The temperature of the grain in 2 bins was monitored with recording thermometers with sensors placed 10 cm below the surface near the center of the bins. The temperature at the grain surface was monitored with max-min thermometers which were read weekly. The grain temperature was 14°C when treatment was started; during the season it ranged from  $-8^{\circ}$  to  $38^{\circ}$ C at the grain surface and from  $3^{\circ}$ – $32^{\circ}$ C at the 10-cm depth. Because the bins were not ventilated, on May 31, the lids were painted white to prevent excessively high temperatures.

The moisture content of the corn in the surface layer in 2 bins was measured periodically. At the time of treatment the moisture content was 12.02%, on June 1 it was 11.45%, on Sept. 13 it was 12.26%, and on Nov. 23 it was 12.55%.

Infestation pressure was maintained throughout the warm months of 1977 by distributing 1000 eggs each of the Indian

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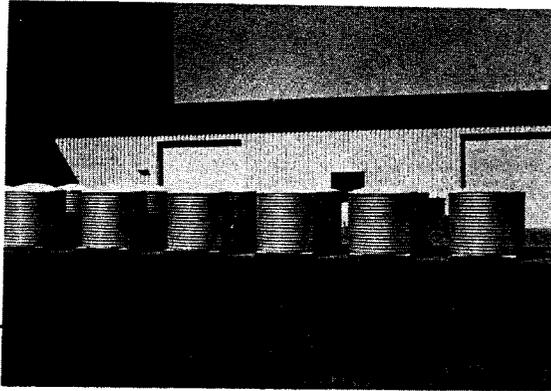


FIG. 1.—Bins used for the tests.

meal moth and almond moth over the surface of the grain in each bin according to the following schedule:

<i>Indian meal moth:</i>	<i>Almond moth:</i>
Mar. 21, all bins	May 9, all bins
Apr. 13–14, all bins	June 6, all bins
May 9, all bins	July 25, treated bins
June 6, treated bins	Sept. 15, all bins
July 25, treated bins	Oct. 11, all bins
Sept. 19, all bins	
Oct. 13, all bins	

Eggs for infesting the bins were obtained from laboratory colonies maintained on a diet of cracked wheat and wheat shorts supplemented with brewers yeast, wheat germ, glycerol, honey, water, and mold inhibitors. The Indian meal moth colony was started May 5, 1976, from larvae and pupae collected from stored wheat in Kingman and Reno Counties, Kans. The almond moth colony was started June 26, 1975, from larvae and pupae collected from rice hulls in Jefferson Co., Tex. Both colonies were known to be intermediate in susceptibility to *B. thuringiensis* relative to several other available colonies (R. A. Kinsinger and W. H. McGaughey, unpublished data).

Infestation levels were monitored weekly by counting moths resting on the grain surface (1.617 m<sup>2</sup>) and bin wall above the grain (0.458 m<sup>2</sup>). Moths resting on the lid were not counted. Total counts were usually possible. When populations exceeded ca. 50/bin, moths were counted on the grain surface in three 929-cm<sup>2</sup> areas defined by a randomly placed wire frame. The avg of the 3 counts was multiplied by 17.41 to obtain the total for the bin. All recorded counts were the avg of independent counts by 2 persons.

A separate measure of infestation levels was made by placing strips and spools of corrugated paper on the grain surface of each bin to provide pupation sites for the larvae. Five 60-cm-long × 19-mm-wide strips and 2 additional strips rolled into spools were used in each bin. These were replaced weekly, returned to the laboratory (25°C and 60–70% RH), and placed in jars. Moths that emerged were identified and enumerated. When moths ceased emerging (ca. 4 wk), the corrugated papers were taken apart so diapausing larvae could be identified and enumerated. The numbers of larvae in the corrugated paper that had died as a result of parasitization by *Bracon hebetor* Say also were noted at that time.

Immediately after treatment and at 90-day intervals thereafter, two 250-g samples of grain were removed from the surface of each bin and returned to the laboratory for bioassay (one sample for each insect species). The samples were held in jars at –23°C for 10 days to destroy existing infestation. Fifty eggs were added to each jar in the initial (March) bioassay, and 40 eggs were used in the subsequent bioassays. The infested jars were closed with filter paper caps and held at 25°C and 60–70% RH until the moths had emerged and had been enumerated. For comparative purposes, parallel bioassays were made on 250-g samples of grain from the small lots withheld from each bin at the time of the treatments.

On Dec. 12, 1977, a 10-cm-diam × 2.54-cm deep sample of grain was aspirated from the surface in each quadrant of each bin. The samples from each bin were pooled and a Gamet Precision Divider was used to reduce the sample size to between 100 and 200 kernels (including broken kernels). These kernels were then examined for insect feeding damage.

### Results and Discussion

The results presented here are for the entire 1977 storage season, from treatment in Mar.–Nov. 29. The 1st infestation was detected May 17. Table 1 summarizes infestation levels that were determined by using weekly moth counts in the bins, weekly collection of larvae and pupae in corrugated paper, and the 2 methods combined. Relationships between infestation levels in treated and untreated bins were similar whether comparisons were between highest weekly avg counts or means over the entire season and whether moth counts, collection of larvae and pupae, or a combination of the 2 methods were used. However, it appeared that combining moth counts with the numbers of larvae and pupae collected the previous week would provide more nearly accurate population estimates than would either method used alone because a larger proportion of the population would be sampled. Analysis of variance and Duncan's multiple range test showed that the season means of these combined counts differed significantly between treatments (Table 1). Each successively higher dose produced significantly greater Indian meal moth control ( $P=0.05$ ). Similar differences occurred for the almond moth, except that the intermediate dose level was neither inferior to the highest dose nor superior to the lowest. The means for almond moth and Indian meal moth in the treated bins were  $\leq 8$  and  $\leq 4\%$ , respectively, of the means in the check bins. The highest weekly averages for the combined counts indicated similar high levels of moth control in the treated bins.

Fig. 2 and 3 show seasonal trends of infestation by the Indian meal moths and almond moths, based on weekly combined counts. (To simplify the illustrations and because trends were similar at each treatment level, only the intermediate dose level and the check were plotted.) In the check bins, maximum levels of infestation by both species occurred in July (avg of 846.6 Indian meal moths observed July 5 and avg of 512.2 almond moths observed July 19). Thereafter, the populations declined, probably because of parasitism by *B. hebetor* and a mite, *Blattisocius tarsalis* (Berlese), both of which appeared in large numbers at ca. the time of maximum infestation.

Counts of the numbers of larvae that died as a result of parasitism by *B. hebetor* in the corrugated paper showed

Table 1.—Summary of weekly (May through Nov., 1977) insect counts in bins of corn treated with *Bacillus thuringiensis*.

Dose (mg/kg)	Weekly avg insect counts (5 bins/dose)					
	adults <sup>a</sup>		larvae and pupae <sup>b</sup>		combined <sup>c</sup>	
	highest	mean	highest	mean	highest	mean <sup>d</sup>
<i>Almond moth</i>						
Check	100.4	8.8	481.8	36.6	515.2	45.4a
100	11.4	1.8	16.8	1.9	20.0	3.7b
125	13.6	1.7	9.4	1.2	16.2	2.9bc
150	11.8	1.5	12.8	1.3	16.0	2.7c
<i>Indian meal moth</i>						
Check	823.8	74.5	242.0	12.7	846.6	87.3a
100	18.6	2.9	0.8	0.2	18.6	3.1b
125	9.6	1.8	0.8	0.2	9.8	1.9c
150	4.8	1.3	0.4	0.1	5.2	1.4d

<sup>a</sup> Moths resting on the grain surface (1.617 m<sup>2</sup>) and bin wall (0.458 m<sup>2</sup>) were counted once each week.  
<sup>b</sup> Five 60-cm × 19-mm strips of corrugated paper plus 2 strips coiled into spools were placed each week on the grain surface in each bin. Moths that subsequently emerged from these were identified and counted. Larvae that diapaused also were counted.  
<sup>c</sup> Moth counts plus larvae and pupae. The highest weekly averages for adults and for larvae and pupae did not necessarily occur the same weeks. Therefore, these values are not the sum of the individual highest weekly averages.  
<sup>d</sup> For each insect species means that are followed by the same letter do not differ significantly at the 0.05 level (Duncan's multiple range test).

that the levels of parasitism were proportional to those of moth infestation. Though their effects were not determined, the mites were most noticeable on adult moths, and commonly, they immobilized the moths. Immediately after moth populations in the check bins had declined, mites were seen in great abundance. They were seen only occasionally and then in very limited numbers, however, in the treated bins. Neither parasite had a discernable effect on the relative insect population levels or the efficacy of the treatments.

Mortality levels of both Indian meal moths and almond moths, as observed in the bioassay tests of 250-g samples from the surface layer of grain in the bins, compared favorably with those in grain that had been stored in the laboratory at 25°C and 60–70% RH. Mortality levels in all treated samples from the initial, June, and Sept. sampling times ranged from 98.5–100%. Environmental conditions in the bins, marked by temperatures as high as 38°C at the grain surface, apparently caused little or no deterioration in the effectiveness of the bacterial deposits on the grain.

In the Sept. bioassay, mortality of both moth species in samples from the check bins increased from levels of 33–55 to 90–100%. The hemocoels of dead larvae examined from these samples were found to be filled with bacterial spores and crystals, presumed to be *B. thuringiensis*. These grain samples had been taken from the bins after the infestations in the checks had declined (Fig. 2, 3) and after the grain surface in the check bins had been extensively webbed and contaminated by many dead insects. Presumably the check bins had become infected with *B. thuringiensis*, and the amount present had increased, possibly through cannibalism among the very dense larval populations during June and July. That infection, in addition to infestation by the parasitic wasps and predaceous mites, was probably a factor in the decline of the moth infestations in the check bins; moth infestations probably cannot be reestablished in these bins. Similar problems in reinfesting check bins following decline of a heavy moth infestation have been observed by other workers (J. K. Quinlan, pers. comm.). It should be noted, however, that when such a situation occurs, exten-

sive webbing, insect contamination, and grain damage already have occurred.

The mean numbers of insect-damaged kernels in the 2.54-cm-deep surface layer of grain in the bins at the end of the storage season (Table 2) show that the treatments had reduced the number of damaged kernels by 82–88%. Analysis of variance and Duncan's multiple range test indicated that the percentages of damaged kernels in the treated bins were significantly lower ( $P=0.05$ ) than those in the checks. Very slight insect feeding damage did occur

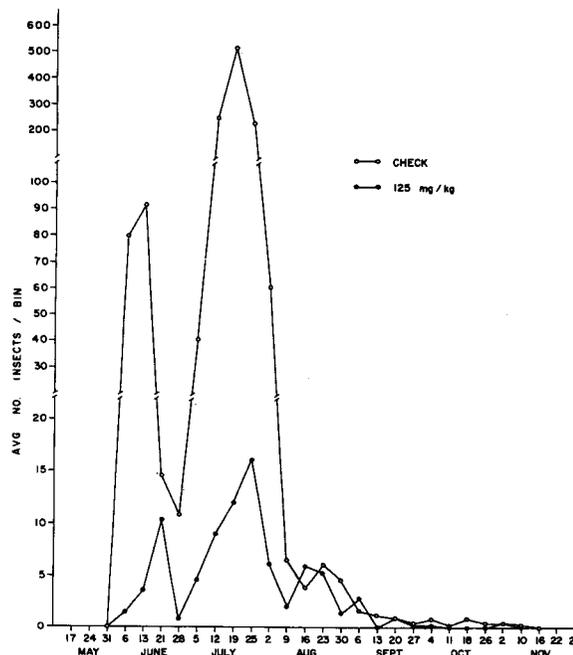


FIG. 2.—Weekly combined Indian meal moth counts (moth counts plus larvae and pupae collected in corrugated paper) in treated (125 mg/kg) and untreated bins of corn (avg 5 bins/treatment).

Table 2.—Percent insect damaged kernels at the end of the 1977 storage season in the 2.54-cm-deep surface layer of corn in bins treated with *Bacillus thuringiensis* (avg 5 bins/treatment).<sup>a</sup>

Dose (mg/kg)	Percent of kernels damaged <sup>b</sup>	Percent reduction in treated bins
Check	27.78a	
100	4.10b	85.2
125	3.44b	87.6
150	4.88b	82.4

<sup>a</sup> Determined by examining a sample of 100–200 kernels from each bin. No damage was present at the beginning of the test.

<sup>b</sup> Means followed by the same letter do not differ significantly at the 0.05 level (Duncan's multiple range test).

despite the use of *B. thuringiensis*. In actual use, the level should be lower than that observed in these tests because natural infestation pressures should usually be lower. However, the doses used here, or the effects of *B. thuringiensis* on the larvae, do permit some feeding before the larvae die. The amount of feeding should be minimal, however, and few or no larvae should complete development and cause a population increase.

*B. thuringiensis* provided, in these tests, an acceptable level of control for both almond moths and Indian meal moths. Differences among treatment levels were noted when mean insect counts were compared, but not when comparisons were based on kernel damage at the end of the 1977 storage season. However, the latter appears to be a much less sensitive method of measuring efficacy. Severe webbing and insect contamination occurred in all check bins. No detectable webbing occurred in any of the treated

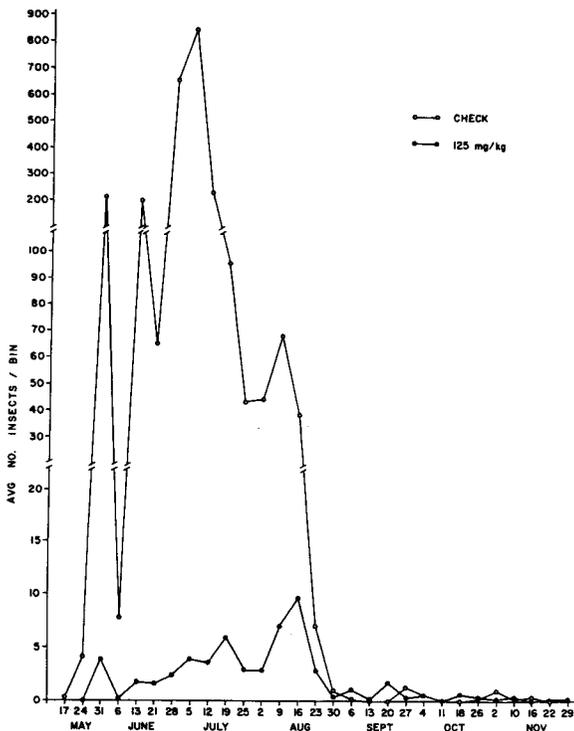


FIG. 3.—Weekly combined almond moth counts (moth counts plus larvae and pupae collected in corrugated paper) in treated (125 mg/kg) and untreated bins of corn (avg 5 bins/treatment).

bins. Insect contamination in the surface layer of treated grain was not noticeable, and under practical conditions probably would be undetectable because the natural infestation pressures would be much lower.

Although the lowest dose (100 mg/kg) gave good insect control in these tests, laboratory studies (McGaughey 1976) suggested the need for using a dose of at least 125 mg/kg. Also, populations of both these insect species have a wide range of susceptibility to *B. thuringiensis* (R. A. Kinsinger and W. H. McGaughey, unpublished data). (The colonies used in these tests were intermediate in susceptibility.) For these reasons, a dose of at least 125 mg/kg should be used. The results of these tests indicate that there would be little benefit in using a dose of 150 mg/kg unless there were problems in achieving efficient and uniform application under actual use conditions.

The test method proved to be a convenient, economical, and successful way to evaluate the efficacy of surface-layer applications of moth control agents. The volume of treated grain was adequate to permit the periodic removal of samples for laboratory bioassay and was not disproportionately large in comparison with the volume of untreated grain. It was possible most of the time to make total counts of emerged moths. Although simultaneous comparisons with full-size bins of similar construction were not made, fluctuations in temperature and grain-moisture content in these bins did not appear to be extreme when compared with those observed in wheat in a 42.3-m<sup>3</sup> (1200-bu) bin at the same site during 1974 (Kinsinger and McGaughey 1976). The bins were not large enough to evaluate the actual procedure of applying the insecticide to the grain as it entered the grain auger for transfer into the bins. (That would be possible if deeper surface layers or the entire bulk were treated.)

The methods used for monitoring infestation levels were acceptable. However, the collection of larvae and pupae in the corrugated paper was density dependent and species specific (Table 1). Thus, the difference would appear to be greater than actually existed between the check and the treatments. The technique was so specific for almond moths that a substantial proportion of the populations (particularly in the checks) was removed, thereby reducing subsequent moth counts as well as limiting population increases that would have occurred had the moths been allowed to emerge and oviposit. Thus, it might be desirable to limit the quantity of paper exposed in the bins. The corrugated paper was useful for collecting diapausing larvae that would not be detected by monitoring only moth emergence, and it did insure detection of peak periods of insect activity because the pupal stadium exceeded 7 days at all times. During warm months, weekly moth counts occasionally missed periods of peak moth emergence because the life span of the moths at the highest temperatures was less than 7 days. Using the 2 sampling methods together appeared to provide greater precision than did either method used alone because a greater proportion of the populations was sampled by using both methods together and because each method appeared to counter weaknesses of the other.

Periodically comparing bioassays of samples of grain from the bins with those of samples held in the laboratory would appear to be an acceptable means of evaluating deterioration of the toxicity of the insecticide deposits on the stored grain. However, because efficacy did not decline

during these tests the assays were of little value in this study.

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