Evaluation of *Bacillus thuringiensis* for Controlling Indianmeal Moths (Lepidoptera: Pyralidae) in Farm Grain Bins and Elevator Silos[1]

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ABSTRACT Tests were conducted over a five-state area to evaluate the effectiveness of *Bacillus thuringiensis* for controlling Indianmeal moths, *Plodia interpunctella* (Hübner), in stored grain. Infestations in wheat were reduced 50–60%; in corn, infestations were reduced >80%. Dust and wettable powder (WP) formulations were equally effective. Treatment of grain in the auger as it was elevated into the bin gave uniform distribution of both formulations. Application by raking the formulations into the surface layer of filled bins gave acceptable distribution of the dust, but poor distribution of the WP. Control was not as good with rake-in application of the WP to wheat. Spray volume had no effect on distribution or efficacy of WP treatments, but a reduced volume of 326 ml/m² was easier to apply than the current label rate of 815 ml/m².

Bacillus thuringiensis Berliner (*B.t.*) has been tested extensively under laboratory and pilot-scale conditions as a protectant for stored grain and other commodities, and has been found to effectively control Indianmeal moths, *Plodia interpunctella* (Hübner), and almond moths, *Cadra cautella* (Walker) (McGaughey 1976, 1978, 1980, 1982). Formulations of the bacterium are compatible with most other grain treatments and are stable in the grain bin environment (McGaughey 1975, Kinsinger and McGaughey 1976). However, the efficacy of *B.t.* has not been evaluated under farm conditions and, in one study, we detected problems in achieving satisfactory coverage of the grain under practical conditions (McGaughey and Dicke 1980).

This study was done to evaluate application procedures and the performance of *B.t.* under some of the storage conditions existing in Illinois, Iowa, Kansas, Nebraska, and Oklahoma. Dust and wettable powder (WP) formulations, auger and rake-in applications, and reduced spray volumes for the WP formulation were compared.

Materials and Methods

Eight trials were conducted over a 3-year period. Bins of wheat were treated in Kansas and Oklahoma in June and July of 1980 and 1981, and in Illinois in June and July of 1981 and 1982. Wheat in elevator silos was treated in Oklahoma in May 1981. Bins of corn were treated in Nebraska in October of 1980 and 1981, and in Iowa in May 1981. None of the bin sites were large enough to accommodate all treatments, so the various treatments were applied sequentially from bin to bin. Most bin sites had two or more bins that permitted one or more untreated controls at each site. Some grain was sold as the seasons progressed, so data are based on fewer bins later in the seasons than early. A total of 142 bins and silos of wheat and 43 bins of corn was treated, and 158 untreated bins and silos of wheat and corn were used as controls.

The two formulations used were Dipel WP containing 16,000 International Units (IU) per mg and Top-Side Dipel dust containing 4,000 IU per mg in a wheat flour carrier. Dipel WP is registered for application to the top 10-cm layer of grain at a rate of ca. 9.77 g/m² of grain surface and Top-Side Dipel dust at a rate of ca. 39 g/m². These rates were used in this study. Dipel WP was applied in water at the label rate of 815 ml/m² or at one of two lesser volumes, 326 or 108 ml/m².

Rake-in applications were made by spraying the liquid from a pressurized hand sprayer or sprinkling the dust from a shaker can over the leveled surface of the grain and raking the grain with a garden rake to mix the insecticide to a 10-cm depth. To achieve greater uniformity of application, the dosage was applied in three portions (4/10, 4/10, 2/10), and the grain surface was raked after applying each of the first two portions, but left undisturbed after applying the third.

Auger applications were made when the last load of grain was elevated into the bins. The number of bushels per 10 cm of depth in the bins was calculated; when that quantity of grain (estimated) remained on the truck or wagon, the formulation was sprayed (WP) or sifted (dust) into the grain stream in the hopper at the bottom of the

[1] This paper reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

auger. Accurate control of dose was virtually impossible because of the necessity for dealing with estimated quantities of grain and the need to calibrate spray or dust application rates with auger flow rates; however, accuracy improved with experience. Extra suspension or dust was always prepared to avoid exhausting the supply before all the grain was elevated into the bin and leaving an untreated layer of grain at the surface. In bins that were not to be filled above the eaves, the auger was stopped and the grain surface was leveled before elevation of the treated grain into the bin. After treatment, the mound of treated grain was spread evenly over the surface to provide a protective cap. Over-filled bins were left mounded and the protective cap of treated grain was left as it fell from the auger.

Bins were sampled after treatment at five positions, center and north, east, south, and west at ⅓ the distance from the wall to the center. Two sets of samples were obtained. One set was collected with a scoop (10 by 10 cm) and Indianmeal moth larvae were used to bioassay the toxicity of the insecticide deposit. Bioassays were done in 0.946-liter mason jars containing ca. 250 g of grain. Fifty eggs from a laboratory colony of Indianmeal moths were added to each sample and the samples were held at 25°C and 60–70% RH until adults emerged and were counted. The other set of samples was collected with a square tube (10 by 10 cm) inserted 10 cm into the grain and partioned at 2.5-cm intervals to provide four samples from the treated layer of grain. Bacterial plate counts were made on these samples to provide an indication of the amounts of *B.t.* deposited and the vertical uniformity of application within the treated layer of grain. Counts were made by washing 5 g of grain in 50 ml sterile water, preparing serial 1:10 dilutions of the wash water, and spreading 0.1 ml of the dilutions on half-strength nutrient agar plates. Colonies were counted after incubation of the plates at 27°C for ca. 24 h. Mean spore counts were used to compare the levels of *B.t.* deposition for the treatments. For each treatment and method of application, slopes of simple linear regression lines (Draper and Smith 1981) for spore count versus grain depth were calculated to compare the uniformity of deposits within the treated layer of grain. Slopes closer to zero indicated more uniform deposition. The Fisher least significant difference multiple comparison procedure of the Statistical Analysis System (SAS Institute 1982) was used to compare the spore counts and slopes for the treatments at the $P = 0.05$ level.

Bins were inspected at ca. 1-month intervals to evaluate performance of the treatments. Adult counts, larval counts in a 2-liter sample of grain from the surface, and estimates of the amount of webbing on the grain surface were recorded. However, these insect counts were highly variable because infestations were not synchronous and the inspections were too infrequent, and in most cases the amount of webbing was too light to be estimated. Data more suited to comparing infestation levels were obtained by counting larvae, pupae, and empty cocoons in strips of corrugated paper (2 by 50 cm) that were placed on the grain surface in each bin at a rate of one strip per 2.32 m² of surface area at each visit. These strips were taken apart at the bin site and counts were made, or they were returned to the laboratory for processing. The strips provided a continuous record of larval activity in the bins. Counts were expressed as insects per strip per 30 days, and average monthly counts for months 7–12 were used in comparing the treatments. Means for formulations or application methods were obtained by combining means of the respective components in weighted averages. Within a location, the data were analyzed using a one-way analysis of variance with unequal numbers of observations per treatment combination. A two-way analysis of variance was used when locations were combined. The Fisher least significant difference multiple comparison procedure (SAS Institute 1982) was used to compare formulation and application means.

The effect of water volume on the effectiveness of *B.t.* was tested in the laboratory by spraying *B.t.* suspensions onto 1,000-g samples of wheat and 933-g samples of corn with a nasal sprayer as the grain dropped from a funnel onto a metal chute (5 by 15 cm). Water volumes of 10.43, 6.95, 4.17, and 1.39 ml per sample, and *B.t.* doses of 125, 75, 25, 10, 1, and 0 mg per sample were tested. The 10.43-, 4.17-, and 1.39-ml volumes are equivalent to the 815, 326, and 108 ml/m² volumes tested in bins of grain. Samples were held in 3.9-liter jars at 25°C and 60–70% RH and 100 Indianmeal moth eggs were added to each. The numbers of adults that emerged were used to compare the effectiveness of the different water volumes using analysis of variance. To determine whether effectiveness was influenced by uniformity of application, series of samples of wheat and corn were prepared in which the *B.t.* dosages were the same, but the deposits were concentrated on either 20, 40, 60, or 80% of the kernels, or evenly applied to all of them. To accomplish this, each sample was divided; one portion was treated with a concentrated suspension of *B.t.*, and the two portions were recombined and blended. Series of samples were prepared at each of five *B.t.* dosages: 125, 95, 65, 35, and 5 mg/kg. Control series were treated with water. The samples were held in 3.9-liter jars at 25°C and 60–70% RH and 100 Indianmeal moth eggs from laboratory colonies were added to each. The numbers of adults that emerged were used to compare the different levels of application uniformity using analysis of variance.

Results and Discussion

Uniformity of Application. Comparison of the slopes of regressions for spore count versus grain

Vol. 78, no. 5

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Table 1. Effect of application method on uniformity of *B.t.* deposits as indicated by slopes of spore count versus grain depth in the top four 2.5-cm layers of grain[a]

Method	Corn	Wheat	Both grains
WP formulation			
Auger	−14.0a (11)	+3.3a (32)	−1.4a
Rake	−103.4b (9)	−165.8b (42)	−154.8b
Dust formulation			
Auger	−16.4a (8)	+12.7a (31)	+6.1a
Rake	+0.3a (3)	−57.0b (27)	−51.3b

[a] For each grain and formulation, means followed by the same letter are not significantly different at the $P = 0.05$ level (Fisher least significant difference multiple comparison procedure [SAS Institute 1982]). Numbers of bins are indicated in parentheses.

Table 3. Average spore count ($\times 10^4$/g) in top 10 cm of grain using different treatment methods[a]

Method	Corn	Wheat	Both grains
WP formulation			
Auger	46.5a (11)	138.6a (32)	113.5a
Rake	176.0b (9)	246.3b (42)	233.9b
Dust formulation			
Auger	49.3a (8)	85.3a (31)	77.2a
Rake	62.3a (3)	85.0a (27)	82.7a

[a] For each grain and formulation, means followed by the same letter are not significantly different at the $P = 0.05$ level (Fisher least significant difference multiple comparison procedure [SAS Institute 1982]). Numbers of bins are indicated in parentheses.

depth showed that both auger and rake-in application produced reasonably uniform mixing of the dust formulation with the surface layer of grain (Table 1). The slopes were near zero, indicating uniform vertical distribution of spores. In corn, deposits produced by the two methods did not differ significantly. In wheat, there was a significant but modest difference in favor of auger application. In corn, the larger kernels may have permitted the dust to sift down among the kernels more readily as the grain was raked. In experiments with the WP formulation, however, a large difference in uniformity of mixing was observed between the two methods. Auger application of the WP produced a degree of uniformity in both grains that was comparable to that achieved for the dust. Rake-in application of the WP produced poor distribution in both grains, but the distribution was worse in wheat. Part of the lack of uniformity with rake-in application was due to the final 2/10 of the dosage that was applied to the surface without mixing. However, raking apparently does not produce the degree of mixing that occurs in a grain auger, particularly when a WP suspension is used. Perhaps the suspension is quickly absorbed by a small portion of the kernels, whereas the dust remains free to sift down among the kernels during raking.

Water volume had no significant effect on uniformity of the WP deposits on either grain (Table 2). The two reduced water volumes produced deposits that were as uniform as the label rate whether application was by auger or rake-in. However, the intermediate water volume was much more convenient to use under field conditions. For a typical bin, this volume fits in a pressurized garden-type sprayer, and these sprayers can be adjusted without modification to deliver this volume at a rate corresponding to the delivery rate of a typical grain auger. With the lowest water volume, there were frequent problems with nozzle clogging caused by the need to spray the thicker suspension at a slower rate. The highest water volume was impractical for field use. Auger applications had to be interrupted to refill sprayers and this amount of water caused increased friction and occasional clogging in the grain augers. Also, lifting such a large volume of water to the top of bins for rake-in applications was difficult.

Accurate application of a precise dose to individual bins was difficult to achieve with the auger application method because both formulations were

Table 2. Effect of water volume on uniformity of *B.t.* deposits as indicated by slopes of spore count versus grain depth in the top four 2.5-cm layers of grain[a]

Water vol (ml/m²)	Corn	Wheat	Both grains
Auger application			
108	−21.2a (4)	+26.3a (10)	+10.5a
326	−9.4a (5)	−7.8a (20)	−8.1a
815	−7.3a (2)	−1.9a (2)	−4.6a
Rake-in application			
108	−127.1b (2)	−187.0b (14)	−179.5b
326	−119.4b (4)	−161.3b (20)	−154.3b
815	−66.2b (3)	−139.9b (8)	−119.8b

[a] Within each column means followed by the same letter are not significantly different at the $P = 0.05$ level (Fisher least significant difference multiple comparison procedure [SAS Institute 1982]). Numbers of bins are indicated in parentheses.

Table 4. Average spore count ($\times 10^4$/g) in top 10 cm of grain using the WP formulation suspended in different water volumes[a]

Water vol (ml/m²)	Corn	Wheat	Both grains
Auger application			
108	60.9a (4)	254.5b (10)	190.0b
326	38.9a (5)	88.0a (20)	78.2a
816	29.2a (2)	65.3a (2)	47.3a
Rake-in application			
108	195.0b (2)	284.3b (14)	273.1b
326	221.6b (4)	235.9ab (20)	233.5b
816	102.4a (3)	205.7a (8)	177.6a

[a] For each grain and application method, means followed by the same letter are not significantly different at the $P = 0.05$ level (Fisher least significant difference multiple comparison procedure [SAS Institute 1982]). Numbers of bins are indicated in parentheses.

Table 5. Percent control of Indianmeal moth infestations in eight trials

Location	Year	Commodity	% Control[a]
Kansas, Oklahoma, Nebraska	1980	Wheat	0
Kansas, Oklahoma, Nebraska	1981	Wheat	0
Oklahoma (elevator silos)	1981	Wheat	0
Illinois	1981	Wheat	55
Illinois	1982	Wheat	62
Kansas, Oklahoma	1982	Wheat	51
Nebraska, Iowa	1981	Corn	81
Nebraska	1982	Corn	87

[a] In comparison with untreated bins in the same area.

applied by hand. Application required only a few minutes and the use of mechanical applicators that required extensive calibration did not seem practical. Instead, the dust was sifted from a can and the WP suspension was applied from a hand-held pressurized sprayer. Formulation delivery rates were periodically readjusted during application to try to achieve the correct dosage. For individual bins, the actual dosage ranged from ca. 85 to 110% of the intended amount, and the accuracy of application improved with experience. Of course, under practical conditions, serious errors of application could be corrected by applying the remainder of the dosage as a rake-in application.

Average viable spore deposits that resulted from each application method and formulation are summarized in Table 3. Spore counts with the dust formulation were low because the lot used in this study had a low spore count (ca. 35% of the WP count). If the values in Table 3 are adjusted to compensate for this difference, it is apparent that rake-in application produced equivalent deposits of the two formulations. Also, auger and rake-in application produced equivalent deposits of the dust formulation. However, auger application of the WP formulation produced much lower deposits than rake-in application, probably as the result of over-incorporation of the dosage to depths greater than 10 cm. The wet, treated grain tended to sink into the dry grain mass rather than to rest in a discrete layer on top. Grain spreaders in the bins did not alleviate this problem because they do not evenly distribute grain when the bin is full and they did not effectively spread wet grain. The fact that the dust formulation produced comparable deposits whether applied by auger or rake-in tends to support this premise regarding wet grain.

Lower water volumes tended to produce higher spore deposits with the WP formulation (Table 4). However, since this occurred with both auger and rake-in application, it is not clear whether higher water volume caused over-incorporation or reduced spore viability. At the highest water volume, some spore germination and subsequent death during application might have occurred because the grain remained damp for several hours after treatment.

Bin Infestation. Overall levels of Indianmeal moth control in the eight trials, expressed as percent population reduction in comparison with untreated bins in the same area, are summarized in Table 5. Moth infestations were erratic and variable in density during the course of the study. In bins of wheat in Kansas, Oklahoma, and Nebraska during 1980 and 1981, infestations were particularly erratic and no beneficial effects of the treatments were detected. However, no treated or control bins developed troublesome infestations. Consistent infestations developed in the elevator silos during 1981, but heavy moth infestations throughout the elevator caused extensive migration of moths and larvae from adjacent bins into the treated bins. As a result, the data failed to demonstrate beneficial levels of Indianmeal moth control even though large numbers of black, *B.t.*-killed larvae were observed in all of the treated silos.

Table 6. Percent control of Indianmeal moth infestations by different formulations and application methods[a]

Location	Mean infestation untreated bins[b] (no. bins)	% Reduction[c]: All treated (no. bins)	Formulation: WP	Formulation: D	Method: Auger	Method: Rake	WP: Auger	WP: Rake	Dust: Auger	Dust: Rake
Corn										
Nebraska, Iowa—1981	4.61 (32)	81 (17)	87	73	87	76	90	84	84	64
Nebraska—1982	5.37 (10)	87 (9)	86	88	83		86		73	
All corn	4.87 (42)	84 (26)	86	81	84	77	87	85	79	66
Wheat										
Illinois—1981	13.03 (13)	55 (23)	53	61	62	43	59	41	68	48
Illinois—1982	8.68 (21)	62 (27)	67	54	76a	20b	86a	2b	58	42
Kansas, Oklahoma—1982	5.00 (25)	51 (16)	57	36	30	56	100	52		68
All wheat	7.76 (59)	48 (66)	49	45	56	37	61a	34b	47	44
All grain	6.73 (101)	52 (92)	51	52	58a	37b	63a	34b	48	43

[a] For each location and commodity, means for formulations or application methods that are followed by the same or no letter are not significantly different at the $P = 0.05$ level (Fisher least significant difference multiple comparison procedure [SAS Institute 1982]).
[b] Mean number of larvae and pupae collected per corrugated paper strip per month.
[c] Percent population reduction as compared to untreated bins.

Table 7. Percent mortality of Indianmeal moths on grain with the *B.t.* dosage applied in the laboratory to different percentages of the kernels[a]

% Of kernels treated	Dose (mg/kg)				
	125	95	65	35	5
Corn					
100	97a	92a	91a	81a	41a
80	89b	88ab	79b	75ab	27b
60	78b	72c	63c	68bc	29ab
40	82b	76c	62c	59c	29ab
20	86b	80bc	73bc	72abc	35ab
Wheat					
100	97a	98a	94a	90a	39a
80	97a	94ab	91a	77b	38a
60	86b	87bc	87ab	82ab	39a
40	84b	87bc	79b	75b	22b
20	83b	79c	80b	71b	21b

[a] Means of five replications. For each grain, means within a column followed by the same letter are not significantly different at the $P = 0.05$ level (Duncan's [1955] multiple range test).

Table 8. Percent mortality of Indianmeal moths on grain treated in the laboratory with a WP formulation using different water volumes

Water vol (ml/sample)[a]	Dose (mg/kg)				
	125	75	25	10	1
Corn[b]					
10.43	98	95	80	72	42
6.95	98	97	86	69	46
4.17	99	98	88	73	47
1.39	99	99	93	83	58
Wheat[c]					
10.43	99	98	90	74	38
6.95	100	99	89	81	38
4.17	100	98	94	85	35
1.39	99	98	94	77	37

[a] 1,000-g samples of wheat and 933-g samples of corn. The 10.43, 4.17, and 1.39 rates are equivalent to grain bin rates of 815, 326, and 108 ml/m^2. There were no significant differences between water volumes for either grain ($P = 0.05$).
[b] Means of four replications.
[c] Means of three replications.

Infestations were more consistent in wheat in Illinois during 1981 and 1982, and in Kansas and Oklahoma during 1982, but overall levels of control were 50–60%. This is somewhat lower than the control obtained in a pilot study in 2-m^3 bins of wheat (McGaughey 1980). Control was >80% in corn in Nebraska and Iowa during 1981 and 1982; this level is comparable to the control obtained in an earlier pilot study in corn (McGaughey 1978). The variation in levels of control is attributed to the wide range of *B.t.* susceptibility among the moth populations and to development of *B.t.* resistance in populations in the treated bins. Studies with moth colonies collected from the bins showed that susceptibility of the populations to *B.t.* varied 10-fold, and that colonies from treated bins were more resistant than those from untreated bins even though they had been collected within 1–5 months after treating the grain (McGaughey 1985). Laboratory selection confirmed that the Indianmeal moth populations could develop high levels of resistance to *B.t.* within two or three generations.

Average infestation levels for the various combinations of application method and formulation for the five trials in which moth infestations were suppressed are summarized in Table 6. Comparisons were made only within these trials because there was no purpose in comparing treatments where no treatment benefit could be documented. There were few significant differences in levels of Indianmeal moth control between the two formulations and the different methods of application. The development of resistance and the wide range in susceptibility of the populations probably obscured differences that otherwise would have been apparent.

The WP and dust formulations gave equivalent levels of control in all trials in both corn and wheat. Auger and rake-in applications were equivalent for both formulations in corn and for the dust formulation in wheat. However, for the WP formulation on wheat, auger application gave better control than rake-in application. Rake-in application of the WP to wheat produced the poorest incorporation of any of the treatment combinations (Table 1) and appears to have been the only treatment to significantly affect moth control. Water volume had no effect on performance of the WP formulation. Bioassay of samples of grain from the treated bins did not provide any indication of the variation in *B.t.* deposits in the bins. Mortality of laboratory-reared larvae was >90% in all of these samples.

Laboratory Evaluation of Application Methods. The results of laboratory tests to more precisely determine whether nonuniform application of *B.t.* to grain would affect levels of Indianmeal moth mortality are summarized in Table 7. Efficacy was reduced by only a small amount when the dosage was applied to a smaller percentage of kernels. In this experiment, however, the treated kernels were evenly dispersed through the grain samples. Similarly, when the WP formulation was applied to wheat and corn under laboratory conditions using different water volumes, mortality was not affected (Table 8).

Conclusions. In this study, *B.t.* reduced but did not eliminate Indianmeal moth infestations. Infestation levels in wheat were reduced 50–60% and in corn >80%. The two formulations were equally effective. These levels of control are not as good as users might hope for, but they should be beneficial, particularly in the absence of more effective treatments. However, users should be alert to the potential for development of resistance to *B.t.* and closely monitor developing infestations.

Application requirements do not appear to be critical. Efficiency of auger and rake-in application differed significantly, but this difference had little effect on levels of moth control. With the dust formulation, differences in uniformity of application with the two methods were small, spore counts in the treated grain were nearly the same, and no differences in levels of moth control were apparent. With the WP formulation, rake-in application did not mix the *B.t.* uniformly into the surface layer of grain, but the level of moth control was less than with auger application only in wheat. Auger application produced uniform deposits of the WP, but spore counts were lower than with rake-in application, indicating that the dosage was mixed more than 10 cm deep. Labor requirements for the two methods were about equal. Auger application requires leveling the grain in the bin twice; rake-in application requires leveling the grain once and hand-raking to mix the formulation with the grain.

Water volume had no effect on uniformity of the *B.t.* deposits. At the higher volumes there was a small effect on viable spore deposits, but control was not affected. Laboratory tests confirmed that nonuniform application that might result from use of low water volumes should not affect the efficacy of the treatments if the grain is thoroughly mixed. The 326 ml/m² rate was more easily applied than the currently recommended rate.

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