BACILLUS THURINGIENSIS FOR CONTROLLING THREE SPECIES OF MOTHS IN STORED GRAIN

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Abstract

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In laboratory studies, an aqueous suspension of a commercial wettable powder formulation of *Bacillus thuringiensis* var. *kurstaki* (strain HD-1) containing at least 25 billion viable spores/g and 16000 International Units of Potency (*Trichoplusia ni* (Hübner))/mg prevented infestations of Indian meal moths, *Plodia interpunctella* (Hübner), and almond moths, *Cadra cautella* (Walker), in corn and wheat at a dose of ca. 120 mg of formulation/kg of grain. Treatment of a 100-mm-deep surface layer was more effective than treatments 33 or 67 mm deep and as effective as treatment of the entire grain mass. Lack of uniformity in applying *B. thuringiensis* to the grain kernels caused a small decrease in effectiveness. Dust and bait formulations at about the same doses were as effective as the suspension, but the bait was undesirable because it supported the development of beetle larvae. The formulation was less effective in controlling the Angoumois grain moth, *Sitotroga cerealella* (Olivier): doses that gave complete control of the Indian meal moth and the almond moth reduced emergence of adult Angoumois grain moths by only about one-third.

Introduction

Because of the development of resistance to malathion and synergized pyrethrins, the Indian meal moth, *Plodia interpunctella* (Hübner), and the almond moth, *Cadra cautella* (Walker), have become difficult to control in stored grain and grain products (Zettler *et al.* 1973). Alternative methods of control are therefore needed. McGaughey (1975*a*) demonstrated the effectiveness of a granulosis virus for controlling the Indian meal moth, but this virus does not infect other stored-grain pests (Hunter 1970) so its usefulness would be limited. *Bacillus thuringiensis*, a bacterium that is toxic to many species of Lepidoptera, has been studied extensively in recent years (Burges and Hussey 1971; Heimpel 1967). Its compatibility with fumigants that might be used to control infestations of other pest species (McGaughey 1975*b*) and its wider activity spectrum would be desirable for use in controlling stored-product insects because infestations are frequently composed of several insect species.

Steinhaus and Bell (1953) reported that several strains of *B. thuringiensis* were relatively ineffective against the rice weevil, *Sitophilus oryzae* (L.), the granary weevil, *S. granarius* (L.), the confused flour beetle, *Tribolium confusum* Jacquelin duVal, the lesser grain borer, *Rhyzopertha dominica* (F.), and the Angoumois grain moth, *Sitotroga cerealella* (Olivier), but highly effective against the Mediterranean flour moth, *Anagasta kuehniella* (Zeller). Subsequently, Kantack (1959) demonstrated the susceptibility of Indian meal moths to *B. thuringiensis* on wheat and corn, and Burges (1964), van der Laan and Wassink (1964), and Godavaribai *et al.* (1962) demonstrated the susceptibility of several stored-product Lepidoptera including *A. kuehniella*, *Ephestia elutella* (Hübner), *C. cautella*, *P. interpunctella*, *Galleria mellonella* (L.), and *Achroia grisella* (F.) to *B. thuringiensis*. However, these studies dealt only with the susceptibility of the insects. No studies have been made of methods for using commercial formulations containing the highly virulent strain HD-1 (Dulmage 1970; de Barjac and LeMille 1970) of *B. thuringiensis* var. *kurstaki* to control infestations of moths in stored grain. The present report summarizes the results of laboratory studies made to determine the doses required and appropriate methods for using one of these formulations for preventing Indian meal moth and almond moth infestations in wheat and corn. The susceptibility of the Angoumois grain moth was also investigated. Because I previously showed (McGaughey 1975*a*) that application of a granulosis virus to the surface layer of grain was almost as effective as bulk treatment and might be more easily applied, surface-layer treatments were evaluated in detail.

Materials and Methods

Dipel®,[1] a commercial wettable powder formulation containing spores and crystals of *B. thuringiensis* var. *kurstaki* (strain HD-1), was used in the study. According to the label, the product contained at least 25 billion viable spores/g and 16000 International Units of Potency (*Trichoplusia ni* (Hübner))/mg. An experimental bait that contained 5% Dipel in corn-germ cake-meal was also tested.

The experimental techniques used were similar to those previously developed for evaluating a granulosis virus for Indian meal moth control (McGaughey 1975*a*). Aqueous suspensions were prepared by suspending dry *B. thuringiensis* formulation in distilled water with a tissue grinder; a dust was prepared by intimately mixing wheat flour with dry formulation. The suspension was transferred to the diet used in our laboratory for rearing Indian meal moth and almond moth larvae, and incorporated by vigorous mixing in wide mouth Mason jars with a spatula. The aqueous suspension, the dust, and the bait were incorporated in grain in 150-mm-diam. 4-l. jars by rolling the jars of grain plus formulation on a ball mill roller and occasionally turning them end over end by hand. Smaller samples of grain were treated in Mason jars by shaking the jars by hand until incorporation appeared uniform. The suspensions were prepared in concentrations appropriate for application at rates of 20 ml/200 g of diet or 20 ml/kg of wheat, corn, or soybeans. The dust was added to grain at a rate of 5 g/kg. The amount of bait was varied to obtain different doses because the concentration of *B. thuringiensis* in the bait was constant.

After the diet or grain was treated, the samples were held in 1-pt or 2-qt Mason jars, in 150-mm-diam. 4-l. jars, or in 150-mm-diam. × 600-mm metal columns with filter paper covers at 25°C and 60% relative humidity. Then eggs of the appropriate insect were placed on top of each sample. Overcrowding and cannibalism were minimized by using 100 eggs in each grain sample of 1 kg or larger, 50 eggs in each 500-g sample, and 25 eggs in each smaller sample of diet or grain. Indian meal moth and almond moth eggs were obtained from the laboratory colonies by confining adults over a screen and recovering and counting the eggs with a small aspirator under a dissecting microscope. Eggs of the Angoumois grain moth were obtained by confining adults from the laboratory colony in jars containing double strips of black paper between which they attached their eggs. The strips were then separated and cut into segments containing the appropriate numbers of eggs; these segments were placed on top of the treated grain. Percentage mortality for each species was calculated from the difference in numbers of eggs added and of adults that emerged. Values were corrected for mortality in untreated samples. Percentage hatch of the eggs was monitored for each test and was consistently > 95% for each moth species.

Tests of Bulk Treatment with Aqueous Suspension, Dust, and Bait

Aqueous suspensions of *B. thuringiensis* were first evaluated as bulk treatments in which the entire grain mass was treated with the suspension. The mortalities of Indian meal moths on 100-g samples of treated diet and 150-g samples of treated soybeans, corn, and wheat were compared. The test with corn was replicated 4 times; those with the other commodities were replicated twice. Also, the mortalities of Indian meal moths, almond moths, and Angoumois grain moths were compared on 150-g samples of corn treated in the same manner (4 replications). All samples were held in 1-pt Mason jars.

A second test was made to compare the efficacy of the aqueous suspension (20 ml/kg) and the wheat flour dust (5 g/kg) for controlling Indian meal moths. Five

[1] Mention of a proprietary product does not imply endorsement by the U.S. Department of Agriculture.

doses were tested, and the test was replicated 3 times using 1000-g samples of wheat in 4-l. jars.

The third test was made to determine the efficacy of the bait formulation of *B. thuringiensis* in controlling Indian meal moths. Five doses were tested using 500-g samples of wheat in 2-qt Mason jars (4 replications). Two methods of applying the bait were compared: mixing throughout the sample (bulk treatment) and sprinkling on top of the sample. An additional test was made to compare the bait with equivalent doses of aqueous suspension. In this test the suspension and the bait were applied as bulk treatments to 1000-g samples of wheat (3 replications) in 4-l. jars.

Because the corn-germ cake-meal in the bait formulation might contribute to the development of infestations of non-susceptible species of stored-grain beetles, tests were made to determine the suitability of the bait for beetle larval development. Eggs of the red flour beetle, *Tribolium castaneum* (Herbst), the confused flour beetle, the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), the flat grain beetle, *Cryptolestes pusillus* (Schönherr), and the cigarette beetle, *Lasioderma serricorne* (F.), were obtained from the laboratory colonies in finely-sifted flour, removed with sieves, counted out with a small aspirator, and placed in 15-cc samples of bait, autoclaved bait, and cornmeal in 25-mm-diam. × 95-mm shell vials (4 replications). Percentage adult emergence was used as the index of the suitability of the bait for beetle development.

Tests of Surface-Layer Treatment

To determine whether the aqueous suspension of *B. thuringiensis* formulation was effective for controlling Indian meal moths when the treatment was concentrated in the upper layer of grain, bulk treatments (all grain in each test container uniformly treated), surface-layer treatments (uniformly treated grain layered over untreated grain), and mixed treatments (treated grain gently mixed into untreated grain) were compared. Each 150-mm-diam. 4-l. test jar contained the same amount (134 mm deep) of corn (1800 g) or wheat (2000 g). In surface-layer treatments, the entire dose was uniformly applied to half the sample, which was then layered over the untreated half. In mixed treatments, the entire dose was uniformly applied to half the sample, which was then gently mixed with the remaining half. In bulk treatments, the dose was uniformly applied to the entire sample. Therefore, in the mixed and bulk treatments, the dose was half as concentrated as in the treated layer of the surface-layer treatment, but the total amounts of insecticide were the same. Three doses of aqueous suspension of the formulation were used (20, 70, and 120 mg/kg) and the test was replicated 3 times.

Additional tests with the same doses of the aqueous suspension were made to determine the effect of depth of the treated surface layer on the level of Indian meal moth control. Treated wheat was placed in a layer 33, 67, or 100 mm deep over untreated wheat in 4-l. jars (3 replications).

A similar test was made using a single dose (70 mg/kg) of the aqueous suspension on wheat to determine the effect of uniformity of application on the level of Indian meal moth control. The dose was applied to 10, 50 or 100% of the grain kernels in a 67-mm-deep treated surface layer over untreated wheat in 4-l. jars. Three replications were used.

Finally, 100-mm-deep surface-layer treatments of wheat and corn were compared in the 150-mm-diam. 4-l. jars and in the larger 150-mm-diam. × 600-mm metal columns to determine the effect of container configuration. Two doses (20 and 120 mg/kg) of aqueous suspension of *B. thuringiensis* formulation were uniformly applied to all the kernels in the treated layers, and each test was replicated 3 times. Tests were made first in wheat and corn with the Indian meal moth, and then in corn with the almond moth and the Angoumois grain moth.

Table I. Mortality of Indian meal moths on 100-g samples of diet and 150-g samples of soybeans and grain treated with an aqueous suspension of *Bacillus thuringiensis* formulation (4 replications for corn; 2 replications for diet, soybeans, and wheat)

Dose (mg/kg)	% mortality on: Diet	Soybeans	Corn	Wheat
1	4	50	44	0
10	26	100	75	76
20	45	100	80	90
40	54	100	92	89
80	72	100	99	96
100	76	100	96	95
120	87	100	100	100
160	99	100	99	96

Results and Discussion

Efficacy of Bulk Treatments with Aqueous Suspension, Dust, and Bait

The efficacy of aqueous bulk applications of *B. thuringiensis* to diet, soybeans, corn, and wheat in controlling Indian meal moths is summarized in Table I. A dose of 120 mg/kg prevented insect infestation in corn and wheat. More rapid larval development was observed in diet and this may account for the lower mortality levels. Much slower larval development was observed in soybeans and very low doses gave good Indian meal moth control. The efficacy of aqueous bulk treatments of corn in controlling Indian meal moths, almond moths, and Angoumois grain moths is summarized in Table II. The Indian meal moth showed the same susceptibility to doses of *B. thuringiensis* in this test as in the first test. The almond moth was so susceptible that all doses completely prevented development. No dose was completely efficacious against the Angoumois grain moth. Though mortality increased as the dose was increased up to about 100 mg/kg, higher doses produced no further increase in mortality. Thus, Angoumois grain moth larvae are probably susceptible, but they enter a kernel so soon after hatching that their exposure to the *B. thuringiensis* is brief. This brief exposure period, not the dose, is probably the limiting factor for the Angoumois grain moth.

Table II. Mortality of Indian meal moths, almond moths, and Angoumois grain moths on 150-g samples of corn treated with an aqueous suspension of *Bacillus thuringiensis* formulation (4 replications)

Dose (mg/kg)	% mortality of: Indian meal moth	Almond moth	Angoumois grain moth
10	68	100	21
25	98	100	27
50	98	100	48
75	100	100	46
100	98	100	65
125	98	100	53
150	100	100	62
200	100	100	53
250	100	100	63

When bulk treatments with the dust and aqueous suspension of *B. thuringiensis* were compared, the mortalities of Indian meal moths were:

Dose (mg/kg)	% mortality	
	Suspension	Dust
5	33	48
10	52	79
50	96	99
100	97	99
150	100	100

The two methods of treatment appear to be about equally effective. A dose between 100 and 150 mg/kg of either would be expected to prevent Indian meal moth infestation.

The efficacy of surface and bulk treatments of wheat with the bait formulation in preventing Indian meal moth infestation was as follows:

Dose (mg/kg)	% mortality	
	Surface	Bulk
5	53	53
10	52	76
40	84	95
80	88	99
120	86	100

Bulk treatment with a dose of ca. 120 mg/kg (actual Dipel in 5% bait) prevented Indian meal moth infestation, but sprinkling the bait on the grain surface was less effective. However, in the test in which bulk treatment with bait was compared with bulk treatment with the aqueous suspension, the bait appeared less effective: doses of 10, 40, 80, 120, and 160 mg of actual Dipel in 5% bait/kg of wheat produced Indian meal moth mortalities of 0, 19, 63, 72, and 76%, while the same doses of aqueous suspension produced mortalities of 33, 69, 97, 97, and 98%.

Although Indian meal moths could probably be controlled with somewhat higher doses of the bait formulation, the bait would be an undesirable additive in the grain because it readily supported the development of three of five species of beetles that commonly infest stored grain (Table III). Even small populations of the two species that did less well could be detrimental in stored grain. Perhaps low doses of an insecticide could be added to the bait to prevent beetle development.

Table III. Beetle development in 15-cc samples of corn-germ cake-meal bait containing 5% *Bacillus thuringiensis* formulation, autoclaved bait, and cornmeal (4 replications)

Insect (number of eggs/sample)	% adult emergence in:		
	5% bait	Autoclaved bait	Corn meal
Red flour beetle (25)	93	74	91
Confused flour beetle (25)	80	73	82
Sawtoothed grain beetle (10)	75	65	68
Flat grain beetle (25)	20	0	89
Cigarette beetle (25)	45	86	73

Surface-Layer Treatment

As was previously shown in studies with a granulosis virus for Indian meal moth control in grain (McGaughey 1975*a*), application of microbial insecticides to the surface layer of grain may be as effective as treating the entire grain mass for preventing surface-layer infestations of moths. When bulk, surface-layer, and mixed treatments of grain with the aqueous suspension of *B. thuringiensis* formulation were compared (Table IV), the surface-layer treatment was as effective as bulk treatment. The only statistically significant (analysis of variance) difference was between surface-layer and mixed treatments in corn, and this difference was only apparent at the low dose. However, when the depth of the treated surface layer of wheat was varied, the results showed that treatment of the deeper layers was more effective for controlling Indian meal moths, and that a dose greater than 120 mg/kg may be required with a 100-mm-deep surface-layer treatment:

Dose (mg/kg)	% mortality at depths of:		
	33 mm	67 mm	100 mm
20	27	36	66
70	51	74	87
120	67	84	91

When the aqueous suspension of *B. thuringiensis* formulation (70 mg/kg) was applied to 10, 50, or 100% of the kernels within a 67-mm-deep surface layer of wheat, the mortalities of Indian meal moths were 60, 72, and 84%. Thus, lack of uniformity of treatment reduced the level of effectiveness. (This was also indicated in the results with mixed treatments in Table IV.) However, a slight increase in dose might increase efficiency.

The results of these tests in jars encouraged larger-scale studies to determine whether treatment of the surface layer of grain 100 mm deep would be effective in larger grain masses. The results are summarized in Table V. In corn and wheat, treatment of the surface layer was as effective against Indian meal moths in the larger (600-mm-deep) columns as in the jars. A similar result was obtained in tests with the almond moth in corn. Because the almond moth is highly susceptible to the *B. thuringiensis*

Table IV. Efficacy of bulk, mixed, and surface-layer treatments of corn and wheat with an aqueous suspension of *Bacillus thuringiensis* formulation for control of Indian meal moths (3 replications)

Method of treatment	% mortality at doses (mg/kg) of:		
	20	70	120
	Corn		
Layered	77*	94	96
Mixed	56*	93	97
Bulk	69	93	99
	Wheat		
Layered	56	86	91
Mixed	52	88	96
Bulk	66	89	96

* Differ from one another significantly at 5% level of confidence (analysis of variance).

Table V. Effect of container configuration on the effectiveness of 100-mm-deep surface layers of grain treated with an aqueous suspension of *Bacillus thuringiensis* formulation for control of moths (3 replications)

Insect	Grain	Dose (mg/kg)	% mortality in: Jars	Columns
Indian meal moth	Wheat	20	58	74
		120	93	99
Indian meal moth	Corn	20	89	93
		120	100	100
Almond moth	Corn	20	98	100
		120	100	100
Angoumois grain moth	Corn	20	7	14
		120	34	30

formulation used, this method of treatment should completely prevent almond moth infestations in bulk stored grain. However, only about one-third of the Angoumois grain moths, shown in earlier tests to be relatively more difficult to control with *B. thuringiensis*, were killed by the dose (120 mg/kg) that effectively controlled Indian meal moths and almond moths. Complete protection is probably not attainable, but a reduction of one-third may be of value in delaying the build-up of infestations of Angoumois grain moths.

Conclusions

The aqueous suspension of the formulation of *B. thuringiensis* tested may be an effective protectant against Indian meal moths and almond moths in bulk stored grain. The limited effectiveness against Angoumois grain moths is of questionable value, but it should be assessed in actual field studies.

Uniform application of a dose of 120 to 150 mg/kg of the aqueous suspension of *B. thuringiensis* formulation to the 100-mm-deep surface layer of grain could probably be achieved by alternating two or three spray applications with mixing or raking of the grain surface with a hand scoop or garden rake. The slight increases in dose that might be necessary to overcome inadequate depth or uniformity should be more than offset by the large savings realized from confining the treatment to the surface layer of large masses of grain that are already in storage, possibly with no available means of turning for bulk treatment.

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