

A Granulosis Virus for Indian Meal Moth¹ Control in Stored Wheat and Corn²

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ABSTRACT

Aqueous and dust applications of a lactose formulation of a granulosis virus (ca. 3.2×10^7 capsules/mg) were equally effective in protecting wheat against *Plodia interpunctella* (Hübner), and a dose of 1.875 mg of formulated virus/kg of grain gave good control. Treatment of the surface layer of wheat or corn to a depth of 100 mm

was more effective than treatments applied to depths of 33 or 67 mm and was almost as effective as treating the entire grain mass. Grain in the treated layer need not be uniformly treated since the level of insect mortality was unchanged whether the treatment was applied to every kernel or to only part of the kernels.

The Indian meal moth, *Plodia interpunctella* (Hübner), is a serious pest of stored grain and grain products and is difficult to control because of its resistance to malathion and synergized pyrethrins (Zettler et al. 1973). It was, therefore, of interest when Hunter (1970) and Hunter and Hoffmann (1973) found that a granulosis virus was highly pathogenic to larvae of the Indian meal moth. It affects cells of the epidermis, fat, tracheal matrix, and perhaps other tissues (Arnott and Smith 1968, Hunter et al. 1972). Hunter et al. (1973) demonstrated in preliminary tests that the virus would protect inshell nuts from this pest. Reported here are results of studies to establish the efficacy of the virus and to devise methods of using it to control infestations of Indian meal moths in stored wheat and corn.

METHODS AND MATERIALS.—The granulosis virus was formulated from diseased larvae by coprecipitation with lactose by using acetone, a procedure similar to that described by Dulmage et al. (1970). The formulation contained ca. 3.2×10^7 virus capsules/mg, as estimated by use of a Petroff-Hausser bacteria counter and a brightfield microscope. Diseased larvae were obtained by inoculating their diet with a viral suspension prepared by grinding diseased larvae in distilled water. By careful choice of the time of inoculation (when larvae were ca. 2nd instar), it was possible to obtain mature diseased larvae. The diseased larvae were then frozen until needed.

Aqueous suspensions of the formulated virus and the fresh diseased larval material were prepared in a tissue grinder with distilled water. The dust was prepared by intimately mixing wheat flour with dry virus formulation. The suspensions of formulated virus and the fresh material were transferred to the diet by pipet and incorporated by vigorous mixing in wide-mouth 1-qt mason jars with a spatula. The aqueous suspension and the dust were incorporated in grain in 150-mm-diam 4-liter jars, either by rolling the jars on a ball mill roller and occasionally turning them end over end by hand until all visible traces of moisture disappeared, or by shaking the jars by hand until incorporation was complete. The suspensions

were prepared in concentrations appropriate for use at rates of 20 ml/200 g of diet or 20 ml/kg of grain. The dust was added to grain at a rate of 5 g/kg.

After the diet or grain was treated, the samples were held in 1-pt mason jars, in 150-mm-diam 4-liter jars, in 150-mm-diam metal pipes (600 or 1200 mm deep), or in 300×300-mm-diam cans with filter paper or muslin covers at 25°C and 60% RH. Indian meal moth eggs from the laboratory colony were counted out by using a small aspirator and a dissecting microscope and placed on top of the treated diet or grain. Percentage mortality was calculated from the difference in the numbers of eggs added and of adults that emerged. Values were corrected for mortality in untreated diet or grain. The number of eggs used differed in some tests, but 100 eggs usually were placed in each grain sample of 1 kg or larger and 50 eggs in each smaller sample of grain or diet. Thus, overcrowding and cannibalism were minimized.

Tests with Fresh and Formulated Virus.—The effectiveness of aqueous suspensions of the formulated virus and of the fresh diseased larval material were compared. In this test, 200-g samples of diet each containing 1 of 6 doses of one of the materials were prepared. Then each sample was divided into 2 subsamples (replications) that were infested as described with 50 Indian meal moth eggs. The data were analyzed statistically by the Minimum Logit χ^2 method. Analysis was based on 2 replications and 6 doses of each preparation. Also, Indian meal moths were exposed to 8 doses of an aqueous suspension of the formulated virus on 150-g samples of wheat (2 replications).

A 2nd test compared the toxicity of the aqueous suspension (20 ml/kg) and wheat flour dust (5 g/kg). Four doses were tested, and the test was replicated 3 times using 1000-g samples of wheat.

Method of Application of Virus.—Because the Indian meal moth tends to infest only the surface layer of grain in bulk storage, treatment of the entire grain mass may be unnecessary. If only the surface layer was treated after the grain was in storage, simpler equipment would be needed, and less virus would be required; also, only that portion of grain subject to infestation would be treated. Therefore, bulk treatments (all grain in each test container was uniformly

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Table 1.—Efficacy of bulk, mixed, and surface-layer treatments of corn and wheat with granulosis virus formulation for Indian meal moth control.

Method of treatment	% mortality at doses (mg/kg) of			Means
	0.1875	0.9375	1.875	
	<i>Corn</i>			
Layered	75	94	94	88
Mixed	74	96	98	89
Bulk	72	98	100	90
	<i>Wheat</i>			
Layered	78	95	98	90
Mixed	84	99	100	94
Bulk	79	100	100	93

treated with the formulated virus), surface-layer treatments (uniformly treated grain was layered over untreated grain), and mixed treatments (treated grain gently mixed into untreated grain) were compared. Three doses of aqueous suspension of the formulation were used.

All test containers 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 1/2 the sample and layered over the untreated 1/2. In mixed treatments, the entire dose was uniformly applied to 1/2 the sample and then gently mixed with the remaining 1/2. In bulk treatments, the dose was uniformly applied to the entire sample. Therefore, in the mixed and bulk treatments the dose was 1/2 as concentrated as in the treated layer of the surface-layer treatment. Each treatment was replicated 3 times.

Also, tests were made (with the same doses) in which the effect of treating only the upper layer of grain to different depths was compared. The virus was applied uniformly to all the kernels (100%), and this treated grain was placed in a layer 33, 67, or 100 mm deep over untreated grain in the jars. Three doses were tested. All tests with wheat were replicated 3 times; those with corn were replicated twice.

This test of surface-layer treatments also included a comparison of the effect of uniformity of incorporation of the virus, i.e., application of the entire dose to 10, 50, or 100% of the kernels in the treated layer. However, these data were confirmed with a separate test in which the same 3 doses were applied to 10, 50, or 100% of the kernels within a 67-mm-deep treated surface layer over an 83-mm (wheat) or 67-mm (corn) untreated layer. The tests with wheat were replicated 3 times; those with corn were replicated 2 times.

Larger-scale tests of surface treatments were made to compare the effectiveness of 100-mm-deep surface-layer treatments of wheat (2 doses) in the jars, in the 1200-mm metal columns, and in the cans. Similar tests with corn were made in which results in jars and in the 600-mm metal columns were compared. The tests were replicated 3 times.

RESULTS AND DISCUSSION.—*Efficacy of Fresh and Formulated Virus.*—The comparison of the formulated

virus and the fresh diseased larval preparation showed that ca. 15 times as much formulated virus (0.615 mg/kg, 95% limits 0.526–0.729) as fresh preparation (0.039 mg/kg, 95% limits 0.027–0.058) were required to kill 50% of the Indian meal moths, while only ca. 2.5 times as much (5.237 mg/kg, 95% limits 3.554–9.023 vs. 2.081 mg/kg, 95% limits 0.989–5.924) were required to kill 95%. Parallelism SS equalled $19.41 > 6.63 = \chi^2$, 0.01, so the dose-mortality curves were not parallel. The slopes for the formulation and fresh material were 3.164 and 1.707. The lower toxicity of the formulation may have resulted from the 50% lactose in the formulation, a possible loss of virus during the formulation process, or clumping, which would prevent an even distribution of the capsules in the diet. However, the level of potency of the formulation was acceptable, so any other reasons for the differences in toxicity and lack of parallelism in the dose-mortality relationships were not explored.

The mortalities of Indian meal moths exposed to the 150-g samples of wheat treated with an aqueous suspension of the formulated virus at doses of 0.01875, 0.075, 0.09375, 0.1256, 0.1875, 0.375, 1.875, and 187.5 mg/kg were 23, 54, 56, 67, 81, 87, 100, and 100%, respectively. Thus, treatment with 0.375–1.875 mg of formulated virus/kg of wheat effectively controlled an Indian meal moth infestation in these small samples.

Mortalities caused by dust or aqueous applications of formulated virus on 1000-g samples of wheat were:

Dose (mg/kg)	% mortality	
	Suspension	Dust
0.09375	95.7	97.3
.1875	99.7	99.3
.9375	100	99.7
1.875	100	100

The 2 methods of application were equally effective, and the doses required for insect control (100% mortality) were about the same as in the previous test (lower doses did appear somewhat more effective in this test). In view of this finding, aqueous suspensions were used in all subsequent tests.

Method of Application of Virus.—Table 1 summarizes results of the test with bulk, surface-layer, and mixed treatment of wheat and corn with the aqueous formulation of granulosis virus. It made little difference whether the treatment was concentrated in the upper layer or whether it was applied to all (bulk) or part (mixed) of the grain. Analysis of variance showed no significant effect of method. Thus, application need not be particularly uniform, as has often been required in field use of microbial insecticides. However, slightly higher doses appeared necessary when only the surface layer was treated.

Table 2 summarizes results obtained when the surface layer of grain was treated to different depths over untreated grain. In both wheat and corn, mortality of the Indian meal moths was higher with increasing dose and increasing depth of the treated layer. In both grains, good control was obtained by treatment at a rate of 1.875 mg/kg with the treated layer 100

Table 2.—Effect of depth of treated surface layer and of percentage of kernels treated with granulosis virus on mortality of Indian meal moths in wheat and corn.

Depth of treated layer	Percentage of kernels treated	% mortality at doses (mg/kg) of		
		0.1875	0.9375	1.875
<i>Wheat</i>				
33 mm	10%	38	74	83
	50%	49	81	89
	100%	40	74	87
67 mm	10%	77	91	95
	50%	66	95	97
	100%	68	93	95
100 mm	10%	90	98	99
	50%	87	97	100
	100%	87	99	100
<i>Corn</i>				
33 mm	100%	54	72	82
67 mm	100%	75	94	97
100 mm	100%	94	99	100

mm deep. Greater depths were not evaluated because they would be less desirable in an actual storage condition.

Table 2 also reports the effect of uniformity of application to kernels of grain in the treated layer on control of the Indian meal moth. Uniformity of application had no discernible effect at any depth, and mortality was not reduced when only 10% of the kernels were treated. These results were confirmed in separate tests with wheat and corn. Mortalities in wheat when the dose was applied to 10, 50, or 100% of the kernels within a 67-mm-deep treated surface layer were: 0.1875 mg/kg—84, 90, and 82%; 0.9375 mg/kg—95, 99, and 98%; and 1.875 mg/kg—98, 99, and 98%. In corn, a dose of 0.9375 mg/kg produced mortalities of 96, 98, and 97% when the dose was applied to 10, 50, or 100% of the kernels in the treated layer. However, in each test, the treated kernels were evenly distributed through the treated layer.

The results of the several jar tests encouraged larger-scale tests, and Table 3 summarizes the results. Statistical analysis (analysis of variance) revealed a significant difference in control in wheat in the containers. Treatments were significantly more effective (Scheffe's test) in jars than in cans, but there was no statistical difference between the jars and the columns or between the cans and the columns. With corn, there was no difference between containers at either dose. Although the statistical analysis suggested a slightly decreased effectiveness of the virus when applied to grain in larger containers, most of this difference occurred at the lower dose. Differences became less pronounced as the level of insect control neared 100%. The test method therefore appears valid, and I conclude that treatment of the surface layer of grain in bulk storage with a formulation of virus containing

Table 3.—Effect of container configuration on the effectiveness of treating 100-mm-deep surface layers of wheat and corn with granulosis virus.

Container ^a	% mortality at indicated doses (mg/kg) in			
	Wheat		Corn	
	0.09375	1.875	0.09375	1.875
Jar	54	99	83	100
Can	31	94		
Column	40	96	87	100

^a Jars 150×150-mm grain mass, cans 300×300-mm grain mass, columns 150-mm-diam × 1200-mm (wheat) or 150-mm-diam × 600-mm (corn) grain mass.

ca. 3.2×10^7 capsules/mg at a rate of 1.875 mg/kg should effectively control Indian meal moth infestations. The treatment of kernels need not be particularly uniform.

Conceivably, 2 or 3 applications of the formulated virus could be sprayed onto the grain surface, and the surface would be raked or mixed with a garden rake or hand scoop. Such mixing, if done thoroughly, should provide adequate coverage and depth. A slight increase in dose might be useful if adequate depth of incorporation is not feasible. The cost of such an increase should be more than offset by the large savings involved in treating only 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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REFERENCES CITED

- Arnott, H. J., and K. M. Smith. 1968. An ultrastructural study of the development of a granulosis virus in the cells of the moth *Plodia interpunctella* (Hbn.). *J. Ultrastruct. Res.* 21: 251-68.
- Dulmage, H. T., A. J. Martinez, and J. A. Correa. 1970. Recovery of nuclear polyhedrosis virus of the cabbage looper, *Trichoplusia ni*, by coprecipitation with lactose. *J. Invertebr. Pathol.* 16: 80-83.
- Hunter, D. K. 1970. Pathogenicity of a granulosis virus of the Indian-meal moth. *Ibid.*, 16: 339-41.
- Hunter, D. K., and D. F. Hoffmann. 1973. Susceptibility of two strains of Indian meal moth to a granulosis virus. *Ibid.*, 21: 114-15.
- Hunter, D. K., T. D. Dixel, and D. F. Hoffmann. 1972. On the granulosis of the Indian meal moth, *Plodia interpunctella*. *Ibid.*, 20: 361-3.
- Hunter, D. K., S. J. Collier, and D. F. Hoffmann. 1973. Effectiveness of a granulosis virus of the Indian meal moth as a protectant for stored inshell nuts: preliminary observations. *Ibid.*, 22: 481.
- Zettler, J. L., L. L. McDonald, L. M. Redlinger, and R. D. Jones. 1973. *Plodia interpunctella* and *Caduta cautella* resistance in strains to malathion and synergized pyrethrins. *J. Econ. Entomol.* 66: 1049-50.