

Compatibility of *Bacillus thuringiensis* and Granulosis Virus Treatments of Stored Grain with Four Grain Fumigants¹

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Wheat was fumigated with phosphine, methyl bromide, CCl₄-carbon bisulfide (80:20 by volume), or ethylene dichloride-CCl₄ (75:25 by volume) after it had been treated with either of two formulations of *Bacillus thuringiensis* or with a granulosis virus of the Indian meal moth, *Plodia interpunctella*. Only methyl bromide had an adverse effect: the granulosis virus was inactivated. Spores of *B. thuringiensis* washed from treated wheat after fumigation with methyl bromide did not produce colonies on nutrient agar plates, but the activity of the *B. thuringiensis* against Indian meal moths was not affected.

INTRODUCTION

Control of the Indian meal moth, *Plodia interpunctella*, a serious pest of stored grain and cereal products, is difficult because the insect is resistant to such protective chemicals as malathion and synergized pyrethrins (Zettler et al., 1973). Published reports suggest that under laboratory conditions *Bacillus thuringiensis* and a granulosis virus may offer effective control of the Indian meal moth (Kantack, 1959; Hunter, 1970; Hunter and Hoffmann, 1973; Hunter et al., 1973). Because these microorganisms have a narrow host range, it would be necessary to use other control measures to suppress or control populations of other pest species that are not affected by the pathogens. Compatibility of these microorganisms with the other treatments would therefore be essential. Although there are conflicting reports in the literature, *B. thuringiensis* and certain insect viruses appear to be compatible with malathion and synergized pyrethrins (Ignoffo and Montoya, 1966; Dougherty et al., 1971; Sutter et al., 1971; Morris, 1972). Information is not

available regarding their compatibility with grain fumigants. Therefore, tests were made to determine the compatibility of these pathogens with four grain fumigants that might be used to control populations of other stored-product pest species.

METHODS AND MATERIALS

The granulosis virus (Arnott and Smith, 1968) used in the tests was produced by inoculating bran diet containing 2nd-instar Indian meal moth larvae with a virus suspension prepared by grinding infected larvae from a diseased culture in distilled water. By carefully selecting the time of inoculation it was possible to obtain large numbers of diseased larvae which grew to maturity. A dry formulation was then prepared from these diseased larvae by coprecipitation with lactose as described by Dulmage et al. (1970). This formulation contained approximately 3.2×10^7 capsules/mg, as estimated using a Petroff-Hausser bacteria counter and a bright-field microscope.

Two wettable powder formulations of *Bacillus thuringiensis* var. *kurstaki* were used in the studies. These were Dipel®, a commercial formulation containing spores and crystals, and IMC-90007 (Sandoz-Wander,

¹Mention of a proprietary product does not constitute a recommendation or an endorsement by the U.S. Department of Agriculture.

Inc.,² Homestead, Florida), an experimental formulation containing few spores. The Dipel contained approximately 25×10^9 spores/g and 16,000 International Units (IU)/mg³ (7.26 billion IU/lb). The IMC-90007 contained $<10^4$ spores/g and approximately 20 billion IU/lb³.

Aqueous suspensions of the three materials were applied to 1000-g samples of wheat (20 ml suspension/1000 g wheat) in 4-liter jars and incorporated by rolling the jars on a ball mill roller until all free moisture was absorbed by the grain. Then the treated samples of grain were placed in small cotton bags, allowed to dry for about 48 hr, and fumigated.

The fumigants used were phosphine (as it evolved from Phostoxin[®] pellets), methyl bromide, ethylene dichloride-CCl₄ (75:25 by volume), and CCl₄-carbon bisulfide (80:20 by volume). Treated and untreated grain samples were placed in 6.06-ft³ (0.1716 m³) stainless steel fumigation chambers and fumigated for 96 or 144 hr at 25°C and 60% relative humidity. Since four chambers were available, the fumigants were tested one at a time, each at two doses with two replications of each dose. After fumigation for the desired time period the chambers were aerated and fresh samples were placed in them and another fumigant was tested. Samples were held at 25°C and 60% relative humidity while awaiting fumigation. The test was done twice using different pathogen and fumigant doses and fumigant exposure and aeration times in each test. The liquid fumigants were placed in beakers in the chambers and allowed to evaporate. The Phostoxin pellets were placed on pads of wet filter paper in the chambers to speed evolution of gas. The methyl bromide was chilled, introduced as a liquid (using a pipette to measure the appropriate amounts) into flasks connected to the chambers by tubing,

and allowed to evaporate from the flasks into the chambers.

After fumigation, the bags of grain were aired on a laboratory counter at 25°C and 60% relative humidity. In the first test, each 1000-g sample was divided among five jars, and 25 Indian meal moth eggs were placed in each subsample. In the second test, each 1000-g sample was transferred to a single jar, and 100 eggs were added. All jars were held at 25°C and 60% relative humidity until adults emerged, and percentage of mortality was calculated from the difference in numbers of eggs introduced and numbers of adults that emerged. Additionally in the second test, counts were made of viable *B. thuringiensis* spores on fumigated and unfumigated samples of untreated wheat and Dipel-treated wheat. Prior to adding eggs, small subsamples were removed from each sample for plating. Subsamples from the two replications of each treatment were combined to form a 3-g sample of each dose. This combined sample was washed by shaking vigorously in 100 ml of distilled water. Then dilutions of the wash water were immediately plated on half-strength nutrient agar and incubated at 25°C and 60% relative humidity. Colonies were counted after 48 hr.

RESULTS AND DISCUSSION

The results of the first test are shown in Table 1 as average mortality percentages for two replications of five subsamples each; the values are corrected for mortality in samples not treated with pathogens. Phosphine, CCl₄-carbon bisulfide, and ethylene dichloride-CCl₄ did not affect the toxicity of the microorganisms. Methyl bromide did not affect the toxicity of *B. thuringiensis*, but did inactivate the granulosis virus.

Because only two replications were used in the first test and because the mortality levels were so high that small differences in the effects of the fumigants could not be detected, a second test was made with lower doses of the pathogens. Additionally, slightly shorter fumigation times and a higher dose of one of the fumigants (ethylene dichloride-

²Formerly a product of International Minerals and Chemical Corp., Libertyville, Illinois.

³Spore counts and International Units of Potency (IU) against *Trichoplusia ni* were supplied by the manufacturers.

TABLE 1
Corrected Percentage of Mortality of Indian Meal Moths in Wheat Treated With a Pathogen and Then Fumigated Before Infestation With Eggs.^a

Fumigant	Fumigant dose ^b	Granulosis virus (0.375 mg/kg)	<i>Bacillus thuringiensis</i>	
			Dipel (40 mg/kg)	IMC-90007 (80 mg/kg)
Phosphine ^c	0.2 g	100	96	97
	.4 g	100	97	98
CCl ₄ -carbon bisulfide	36.7 ml	100	95	96
	73.4 ml	100	96	96
Ethylene dichloride-CCl ₄	36.7 ml	100	98	98
	73.4 ml	99	95	97
Methyl bromide	5.5 g	13	94	99
	11.0 g	0	95	100
No fumigant		100	93	97

^aAbbott's correction (Abbott, 1925) was applied to values for each fumigant to correct for mortality in fumigated samples that were not treated with pathogen. Values are averages of 2 replications, each with 5 subsamples infested with 25 eggs each.

^bDoses were applied to samples in 6.06-ft³ (0.1716 m³) stainless steel fumigation chambers for 144 hr. Samples were aerated for 96 hr before infestation.

^cPhosphine was evolved from Phostoxin[®] pellets at the rate of 0.2 g hydrogen phosphide/0.6-g pellet.

CCl₄) were used in the second test to make the fumigation conditions more comparable to commercial practice. The results (average mortality corrected for mortality in samples not treated with pathogens) are summarized in Table 2. In this test mortalities were lower

because of the lower doses of pathogen, but the effects of the fumigants were the same as in the first test. The lesser effect on the granulosis virus of the higher dose of methyl bromide appears to be a result of variation in the insects.

TABLE 2
Corrected Percentage of Mortality of Indian Meal Moths in Wheat Treated With a Pathogen and Then Fumigated Before Infestation With Eggs.^a

Fumigant	Fumigant dose ^b	Granulosis virus (0.075 mg/kg)	<i>Bacillus thuringiensis</i>	
			Dipel (10 mg/kg)	IMC-90007 (20 mg/kg)
Phosphine ^c	0.2 g	68	65	64
	0.4 g	80	70	65
	None	76	64	61
CCl ₄ -carbon bisulfide	36.7 ml	81	77	80
	73.4 ml	72	77	76
	None	95	77	64
Ethylene dichloride-CCl ₄	55 ml	92	72	78
	110 ml	91	77	68
	None	84	81	72
Methyl bromide	5.5 g	2	78	75
	11.0 g	21	78	68
	None	84	77	68

^aAbbott's correction (Abbott, 1925) was applied by using an average check mortality derived from 8 fumigated and 8 unfumigated samples that had not been treated with pathogen (av = 38.25% mortality). Fumigation did not appear to influence mortality in the checks. Values are averages of two replications.

^bDoses were applied to samples in 6.06-ft³ (0.1716 m³) stainless steel fumigation chambers for 96 hr. Samples were aerated for 72 hr before infestation.

^cPhosphine was evolved from Phostoxin[®] pellets at the rate of 0.2 g hydrogen phosphide/0.6-g pellet.

TABLE 3
Viability of *Bacillus thuringiensis* Spores on Wheat
Treated with Dipel (10 mg/kg) and Then
Fumigated

Fumigant	Fumigant dose ^a	Spores/mg of wheat
Phosphine ^b	None	96.0
	0.2 g	104.8
	0.4 g	112.6
CCl ₄ -carbon disulfide	None	111.1
	36.7 ml	98.4
	73.4 ml	103.2
Ethylene dichloride-CCl ₄	None	121.8
	55 ml	126.4
	110 ml	139.1
Methyl bromide	None	91.3
	5.5 g	1.0
	11.0 g	0.1

^aDoses were applied to samples in 6.06-ft³ (0.1716 m³) stainless steel fumigation chambers for 96 hr. Samples were aerated for 72 hr before plating.

^bPhosphine was evolved from Phostoxin[®] pellets at the rate of 0.2 g hydrogen phosphide/0.6-g pellet.

The effect of the fumigants on the viability of spores on Dipel-treated samples is summarized in Table 3. Methyl bromide killed or otherwise prevented germination of the *B. thuringiensis* spores. The other fumigants had no appreciable effect.

I conclude that phosphine, CCl₄-carbon disulfide, and ethylene dichloride-CCl₄ fumigations are compatible with the pathogens tested. Methyl bromide fumigation would not be compatible with the granulosis virus, and its use as a grain fumigant could be expected to inactivate the virus. It would appear that methyl bromide would be a suitable fumigant for disinfecting insect rearing facilities, equipment, and diet in the manner recently described for ethylene oxide by Tompkins and Cantwell (1975).

Dipel was more toxic than IMC-90007 to Indian meal moth larvae (Tables 1, 2), suggesting that the presence of spores or related materials increases the toxicity of *B. thuringiensis* formulations to this insect. Because the toxicity of Dipel was independent of spore viability, methyl bromide fumigation to kill spores during formulation may be more desirable than physical removal of

spores or other means of producing spore-free formulations, and its effects on *B. thuringiensis* should be studied in more detail.

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