

Stability of *Bacillus thuringiensis* and a Granulosis Virus of *Plodia interpunctella*¹ on Stored Wheat^{2,3}

R. A. KINSINGER⁴ and W. H. MCGAUGHEY⁵

ABSTRACT

The activity of 2 microbial insecticides on small samples of wheat did not decrease appreciably during a year of storage in a farm bin of wheat in which temperatures ranged from -19° to 48°C. *Bacillus thuringiensis* var. *kurstaki* spore viability decreased ca. 25%, primarily during the summer months. In the laboratory, insecticidal activity of the granulosis virus of *Plodia interpunctella* (Hübner) and *B. thuringiensis* decreased to ca. 10 and

85% of the original levels, respectively, when treated wheat was stored at a constant temperature of 42.0°C for 42 weeks. The viability of *B. thuringiensis* spores on wheat decreased rapidly immediately after application and at a lower rate throughout the laboratory-storage period. The decreases were directly proportional to storage temperature.

The Indian meal moth, *Plodia interpunctella* (Hübner), a cosmopolitan insect pest of stored grain and grain products, is resistant to the chemical grain protectants now in use (Zettler et al. 1973), but some insect pathogens have shown potential as alternative methods of control. The larvae of this pest are susceptible to *Bacillus thuringiensis* Berliner (Kantack 1959, Burges 1964) and a granulosis virus (GV) (Hunter 1970, Hunter et al. 1973) described by Arnott and Smith (1968); laboratory studies have shown that both microorganisms will protect stored grain from infestation (McGaughey 1975a, 1976). However, no field studies of the efficacy or stability of either microorganism on stored grain have been reported. Because long-term protection of grain from moth infestations is essential, we evaluated the stability of an experimental formulation of the GV and a commercial formulation of *B. thuringiensis* on wheat stored in a farm bin. Concurrently we designed laboratory studies to determine more clearly the effects of storage temperature on the persistence of the microorganisms during extended storage.

METHODS AND MATERIALS.—The microbial insecticides were Dipel®, a commercial WP formulation of the spore-δ-endotoxin complex of *B. thuringiensis* var. *kurstaki*, strain HD-1 (Dulmage 1970, de Barjac and LeMille 1970), and GV formulated in our laboratory by coprecipitation with lactose using acetone, a procedure similar to that described by Dulmage et al. (1970) for formulating a nuclear polyhedrosis virus. The *B. thuringiensis* formulation was labeled as containing 16,000 IU of potency/mg and at least 25 billion viable spores/g, but viable spore counts made using a spread-plate technique on 1/2-strength nutrient agar revealed ca. 49 billion spores/g. A count of the virus capsules in the dry GV formula-

tion, made using a Petroff-Hausser counting chamber and a bright-field microscope, showed ca. 32 billion capsules/g. Aqueous suspensions of the insecticide formulations were applied to wheat at 20 ml/kg in 3.9-liter jars and incorporated by tumbling on a ball-mill roller until they were evenly distributed and free moisture was absorbed.

Bin Study.—To determine the stability of the microbial insecticides under farm grain storage conditions, wheat was treated in November 1973 with the formulated GV at doses of 0, 0.059, 0.469, and 1.875 mg/kg, and the *B. thuringiensis* formulation at doses of 0, 25, 50, and 150 mg/kg. Two 1500-g quantities of wheat were treated at each dose for each replication. Twenty-four 100-g samples of treated wheat, each in a small cotton bag, were divided into 4 groups and placed in a 1200-bu farm-type steel grain storage bin (4.6 m diam × 2.4 m) containing 760 bu of wheat. Bags in each group were placed in the bin at depths of 0, 7.5, 15.0, 22.5, 30.0, and 37.5 cm in the wheat and allowed to age for periods of 90, 180, 270 or 360 days. Columns of bags were placed in the grain mass using a 0.6-m section of 10-cm-diam plastic drain pipe, which was pushed into the grain and evacuated using a vacuum grain sampler. After evacuation, bags with strings attached to facilitate removal were positioned at the proper depth and the pipe was removed leaving the bags buried in the grain. The columns of bags of each dose were positioned in the bin to permit removing 1/4 of the bags from each quadrant at each sampling time without disturbing the remaining bags. The samples were replicated in each quadrant of the bin. Five 100-g samples of the treated wheat were placed in 1-pt Mason jars and held as checks in the laboratory at 25°C and 60% RH for measuring insecticidal activity immediately after treatment and after 90, 180, 270, and 360 days. These samples were also replicated 4 times.

In May 1974, wheat was again treated with GV and *B. thuringiensis* and placed in bags in the bin and in Mason jars in the laboratory to replace the samples that had been removed at the 90- and 180-day sampling intervals. Thus, it was possible to compare the effects of the high summer temperatures on fresh and aged treatments.

¹ Lepidoptera: Pyralidae.

² U. S. Grain Marketing Research Center, Agric. Res. Serv., USDA, 1515 College Ave., Manhattan, KS 66502, in cooperation with the Dept. of Entomology, KS Agric. Exp. Stn., Kansas State Univ., Manhattan (Contribution No. 1160). Part of a thesis submitted by the 1st author in partial fulfillment of requirements for the Master of Science degree in Entomology. Received for publication Oct. 28, 1975.

³ Mention of a proprietary product does not constitute an endorsement by the USDA.

⁴ Graduate Res. Asst., Dept. of Entomology, Kansas State Univ., Manhattan 66506.

⁵ Res. Entomologist, U. S. Grain Marketing Res. Ctr., and Adjunct Asst. Entomologist, Agric. Exp. Stn., Kansas State Univ., Manhattan 66502.

Insecticidal activity on the samples of wheat immediately after treatment and after aging in the laboratory or in bags in the bin was measured by placing them in 1-pt Mason jars and adding 25 Indian meal moth eggs. The eggs, obtained from laboratory colony adults, were counted using a small aspirator and a dissecting microscope. Egg hatchability was monitored each time and was always greater than or equal to 95%. 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 samples.

B. thuringiensis spore viability immediately after treatment and at each sampling interval was estimated on the 50 mg/kg treatment using a spread-plate technique on 1/2-strength nutrient agar. Small samples of wheat, removed from the samples used to test for insecticidal activity, were refrigerated at $2\pm 1^\circ\text{C}$ until they could be plated. At the time of plating, 21 whole kernels were weighed and shaken for 1 min at 50 ml of distilled water from which serial dilutions (1, 1:10, 1:100) were made. Three plates were then smeared with 0.1 ml of each dilution spread evenly over each plate with a bent glass rod. Colonies were counted after 30–40 h incubation at 25°C and the numbers of spores/mg of wheat calculated. As an additional measure of the decrease in spore viability on wheat stored in the bin, wheat was sterilized with gamma irradiation (1250 krad) (at the Stored-Product Insects Research and Development Laboratory, Agric. Res. Serv., USDA, Savannah, GA), and in February 1974, was treated with an aqueous suspension of the *B. thuringiensis* formulation at 50 mg/kg. Small samples (ca. 15 g) were placed in plastic vials with snap-on caps fitted with millipore filters (0.20- μ pore size) to prevent contamination but allow air exchange. The vials were placed in small cotton bags and arranged in columns in the farm-storage bin at the same depths and in the same manner as were the bagged wheat samples. Replicated samples held in the laboratory at 25°C and 60% RH served as controls. At intervals of 90, 180, and 270 days samples were removed and viable spores counted.

Temperatures in the bin were monitored throughout the study using 7-day temperature recorders with remote sensing elements placed 0.6 m above the grain surface, at the grain surface, and 7.5, 15.0, and 30.0 cm below the surface. Temperature variations around the perimeter of the bin were monitored using thermocouple cables placed horizontally in the grain 0.7 m from the bin wall at depths of 15 and 30 cm; temperature sensors were spaced to allow daily readings at 12 locations at each depth. Readings were taken between 1000 and 1400 h. The accuracy of all temperature measurements was $\pm 1^\circ\text{C}$. Maximum and minimum daily outdoor temperatures for Manhattan, KS, were obtained from U.S. Department of Commerce reports (Anon. 1965–1974).

Moisture content of the wheat in the bin was determined at each sampling time (0, 180, 270, and 360 days) using twenty-four 300-g samples of un-

treated wheat in small cotton bags. The placement of the bags of wheat was replicated in each quadrant of the bin at depths of 0.0, 7.5, 15.0, 22.5, 30.0, and 37.5 cm. The moisture content of those samples, and of 4 corresponding samples held in the laboratory in Mason jars with filter-paper covers at 25°C and 60% RH, was measured using a Steinlite® Electronic Moisture Tester Model RCT. Samples were returned to their original positions in the bin immediately after their moisture contents were determined.

Laboratory Study.—To define further the temperature limits for treatment persistence, wheat was treated with the formulated GV at doses of 0, 0.007, 0.059, and 0.469 mg/kg, and *B. thuringiensis* formulation at doses of 0, 10, 50, and 150 mg/kg, and stored in 2-qt Mason jars in continuous darkness at 16.5° , 25.0° , 33.5° , and 42.0°C and at 60% RH. Three replications were used. Immediately after treatment and at 21-day intervals, a 100-g sample was removed from each jar and used to measure insecticidal activity and, on the 50-mg/kg dose of *B. thuringiensis*, spore viability.

RESULTS AND DISCUSSION.—Temperatures during the test year at Manhattan did not deviate greatly from the 10-yr averages (Fig. 1A). Therefore, the results of this study should be representative of a normal storage year. Temperature variation, as determined from the thermocouple readings, was negligible around the bin perimeter so the temperature levels recorded at single points by the 7-day recorders were considered to be representative of the temperature levels around the bin at each depth. The air temp above the grain in the bin (Fig. 1B) remained warmer than the outside air during the winter and became much warmer during the summer due to the heating effect of the sun on the metal roof and the heat given off by the grain mass. The insulating capacity of the grain prevented the temperature at the surface of the grain mass (Fig. 1C) from reaching the extreme daily or seasonal levels recorded in the bin head space or outside air. That insulating effect increased at greater depths in the grain (Fig. 1D–F) with the least daily and seasonal fluctuation occurring at 30.0 cm below the surface.

The moisture content of samples of wheat stored in the farm grain bin decreased from an initial level of ca. 13.5% and equilibrated near 12.0% in ca. 270 days. Because the moisture of the grain mass was lower than the levels which Ignoffo (1964) found to adversely affect *B. thuringiensis* activity, little effect on the microorganisms was expected.

B. thuringiensis Insecticidal Activity.—The toxicity of *B. thuringiensis* to the Indian meal moth was not appreciably reduced by storing wheat in the grain bin (Table 1). There was no apparent difference in insecticidal activity of samples held in the laboratory as checks or at different depths in the bin. Even though the temperature fluctuated at the surface of the grain mass more than at other depths, and reached as high as 38°C on 39 days during the summer, no deterioration in toxicity was noted. At the

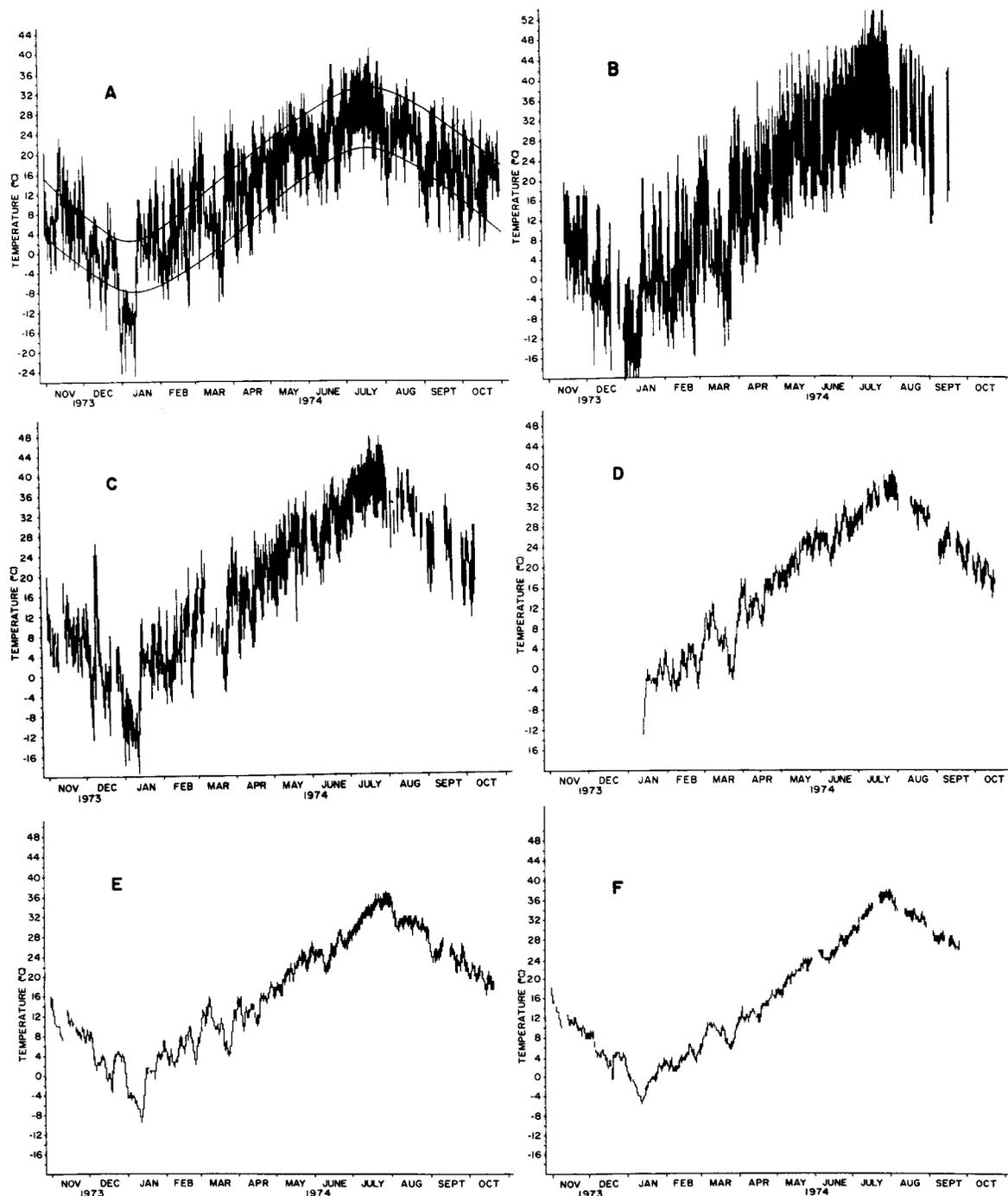


FIG. 1.—Daily temperature ranges during the test year at Manhattan, KS, with the 10-yr average maximum and minimum temperatures (A); in the bin head space (B); at the grain surface (C); and at depths of 7.5 cm (D); 15.0 cm (E); and 30.0 cm (F).

50-, and 150-mg/kg doses (data not tabulated) mortality was greater than or equal to 97% throughout the test; no decrease in toxicity was noted. No difference was noted between samples prepared in November and those prepared in May. (Because no dif-

ferences were observed, data for the samples treated in May are not tabulated.)

B. thuringiensis was also relatively stable on wheat stored at constant temperatures in the laboratory (Table 2). Insecticidal activity did not decrease ap-

Table 1.—Persistence of insecticidal activity of *Bacillus thuringiensis* and a granulosis virus against Indian meal moths on 100-g samples of wheat stored in a farm grain bin and in the laboratory at 25°C and 60% RH (check).^a

Dose (mg/kg)	Depth (cm)	% mortality on samples prepared in November 1973 after storage for (days)				
		0 (Nov)	90 (Feb)	180 (May)	270 (Aug)	360 (Nov)
<i>B. thuringiensis</i>						
25	Check	97	84	84	92	88
	0.0		94	92	97	98
	7.5		98	95	94	89
	15.0		94	96	95	95
	22.5		93	88	93	96
	30.0		96	95	94	94
	37.5		96	96	97	99
Granulosis virus						
0.059	Check	97	100	96	94	91
	0.0		100	97	72	97
	7.5		100	97	86	91
	15.0		94	95	89	80
	22.5		100	89	96	96
	30.0		100	88	95	93
	37.5		100	97	87	100

^a Samples of treated wheat were removed from storage, 25 Indian meal moth eggs were added to each, and they were held at 25°C and 60% RH until adults emerged; values are means of 4 replications and are corrected for mortality in untreated samples. Mortality on samples treated with *B. thuringiensis* at 50 and 150 mg/kg remained above 97 and 100%, respectively, throughout the test. Mortality on samples treated with granulosis virus at 0.469 and 1.875 mg/kg remained at 100%. Mortality on samples prepared in May 1974 did not differ noticeably from those prepared in November 1973.

preciously at storage temperatures of 16.5° (data not tabulated), 25.0° or 33.5°C, but did decrease on wheat after storage for ca. 15 wk at 42.0°C. At that

temperature, insect mortality in samples treated with doses of 10 and 50 mg/kg was reduced to 82 and 97% of the original level, respectively, after ca. 15 wk of storage and gradually decreased to ca. 45 and 85%, respectively, during the remainder of the study (42 wk). (In both cases, the high mortality at 24, 27, and 30 wk was attributed to a temporary decrease in vigor of the insect colony which was also noted in the GV data, Table 2). At the 150-mg/kg dose (data not tabulated), mortality persisted at 100% throughout the test. In the field study, the temperature in the surface layer of grain in the bin reached 42°C on only 15 days. Thus, high temperatures in the grain bin apparently did not persist long enough to cause the insecticidal activity of the *B. thuringiensis* treatments to deteriorate.

B. thuringiensis Spore Viability.—Spore viability on treated wheat stored in the grain bin decreased ca. 25% during storage. The decrease was not influenced significantly ($P>0.05$) by depth in the grain mass and, except for the samples prepared in November and placed at the grain surface in the bin, was not significantly greater than that on the check samples stored in the laboratory at 25°C. Therefore, data for samples stored at different depths in the bin were pooled (Table 3). Most of the decrease in spore viability occurred during the summer months (May to August). Regardless of the time samples were prepared, spore viability on those samples removed in August was significantly less ($P<0.01$) than on those removed in May, but no further decrease occurred between August and November. Spore viability on sterilized samples of wheat did not differ noticeably from that on unsterilized samples and little contamination by other

Table 2.—Persistence of insecticidal activity of *Bacillus thuringiensis* and a granulosis virus against Indian meal moths on wheat stored at constant temperature in the laboratory.^a

Dose (mg/kg)	Storage temp (°C)	% mortality after storage for weeks)														
		0	3	6	9	12	15	18	21	24	27	30	33	36	39	42
<i>B. thuringiensis</i>																
10	25.0	55	70	79	70	57	62	59	69	58	44	64	70	77	50	57
	33.5		79	63	72	46	60	57	57	57	47	80	69	56	58	45
	42.0		78	66	63	45	49	24	42	55	70	53	49	3	25	38
50	25.0	95	95	100	100	98	97	98	100	94	98	100	94	98	93	92
	33.5		100	100	98	96	98	89	100	100	96	100	97	92	89	100
	42.0		98	94	94	95	92	88	91	83	94	100	88	95	79	74
Granulosis virus																
0.059	25.0	94	88	92	87	82	81	88	91	67	90	90	88	98	98	86
	33.5		80	92	70	31	63	48	65	49	96	80	65	89	43	69
	42.0		44	26	35	0	30	0	3	30	27	62	16	7	5	29
0.469	25.0	100	100	100	100	100	100	98	100	100	100	100	100	100	100	100
	33.5		100	100	100	100	100	100	100	100	100	96	100	92	97	100
	42.0		97	98	88	92	94	79	48	86	51	48	15	4	3	11

^a Samples of treated wheat were removed from storage, 25 Indian meal moth eggs were added to each, and they were held at 25°C and 60% RH until adults emerged; values are means of 3 replications and are corrected for mortality in untreated samples. Mortality on samples treated with *B. thuringiensis* at 150 mg/kg remained near 100% throughout the test. Mortality on samples treated with granulosis virus at 0.007 mg/kg was extremely variable and conclusions could not be drawn. No decrease in activity of either insecticide was noted at 16.5°C.

Table 3.—Viability of *Bacillus thuringiensis* spores on 100-g samples of wheat stored in a farm grain bin and in the laboratory at 25°C and 60% RH.^a

Sample position	Viable spores/mg of wheat on samples removed in (1974) ^b		
	May	Aug	Nov
	Samples prepared in November 1973		
Laboratory	887	692	686
Bin	854	556	577
	Samples prepared in February 1974		
Laboratory	821	510	542
Bin	798	495	523
	Samples prepared in May 1974		
Laboratory	873	630	786
Bin		505	693

^a Samples of treated wheat were removed from storage, washed with distilled water, and the number of viable spores was estimated using a spread-plate technique on 1/2-strength nutrient agar; laboratory values are means of 4 replications; bin values are pooled means of 4 replicate values at 6 depths.
^b Spore counts in August were significantly lower ($P < 0.01$, Duncan's multiple range test) than in May on each group of samples. The decrease was not significantly greater ($P > 0.05$) on samples stored in the grain bin than on those stored in the laboratory.

species of bacteria appeared on plates from wheat samples not sterilized prior to treatment.

Under constant-temperature storage, spore viability decreased sharply during the 1st 3 wk, then decreased gradually during the remainder of the test (Fig. 2). The amounts of the initial decrease and subsequent gradual decrease were directly related to storage temperature ($42.0^\circ > 33.5^\circ > 25.0^\circ > 16.5^\circ\text{C}$), and reduction in spore viability was not accompanied by a corresponding decrease in insecticidal activity when samples were stored at 16.5°, 25.0°, and 33.5°C. At 42.0°C, insecticidal activity decreased, although proportionately less than did spore viability, and only after the number of viable spores decreased by ca. 80%, in this case to below 200 spores/mg. Thus the small decrease in spore viability noted in the bin study was probably too small to affect insecticidal activity. Inactivating spores by methyl bromide fumigation (McGaughey 1975b) or by UV or gamma irradiation (Burges et al. 1975) did not cause a corresponding decrease in insecticidal activity, indicating that the effects of high temperature on the *B. thuringiensis* spore- δ -endotoxin complex differed

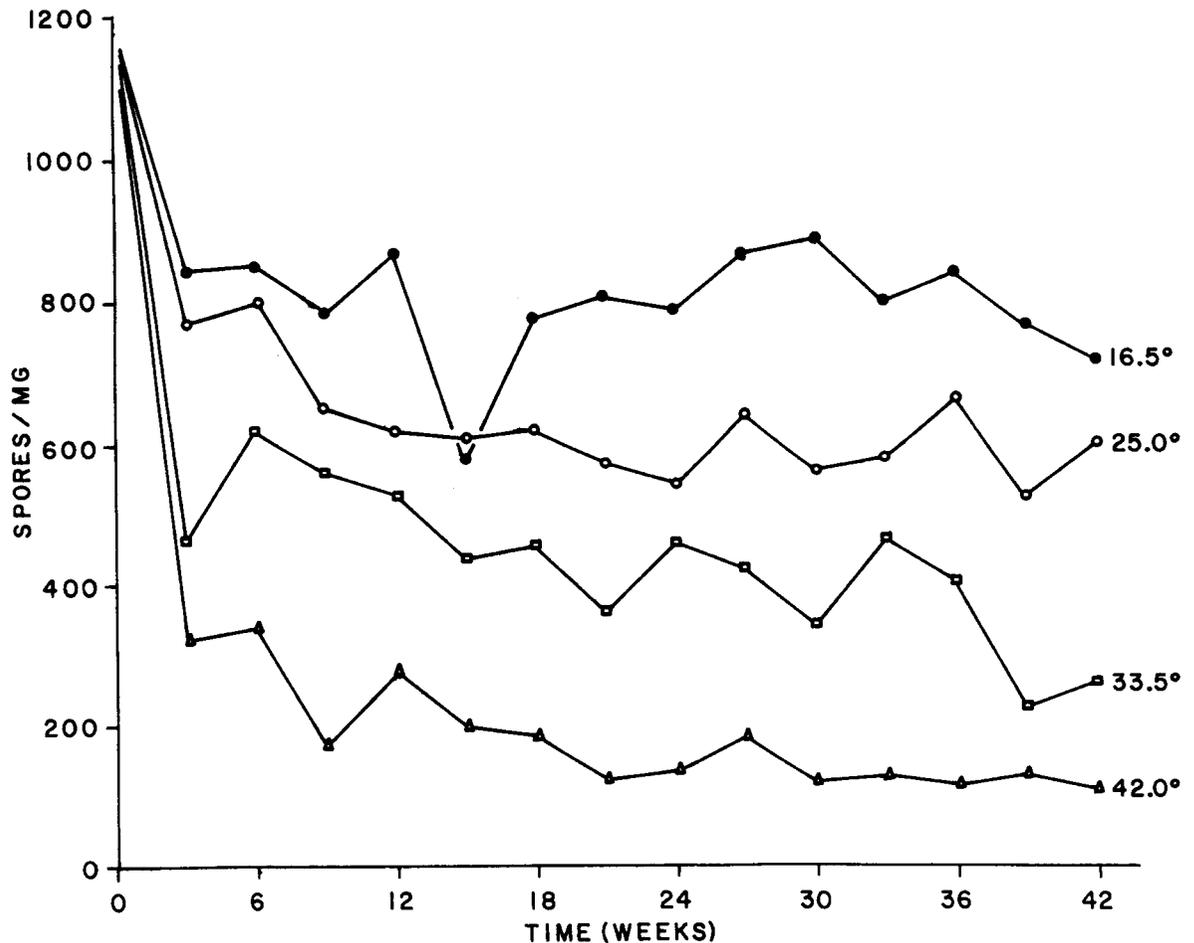


FIG. 2.—Viability of *Bacillus thuringiensis* spores on wheat stored at constant temperatures.

from those of methyl bromide and UV and gamma irradiation.

GV Insecticidal Activity.—The GV appeared to be slightly more susceptible to the storage-bin environment than *B. thuringiensis* (Table 1). At a dose of 0.059 mg/kg activity of the virus deposit decreased slightly during the year the treated samples were stored in the grain bin and in the laboratory. Loss in activity was greatest between 90 and 360 days of storage on samples placed in the bin in November. No activity was lost during 180 days on samples placed in storage in May (data not tabulated), suggesting that decreased activity was probably related more to time than to temperature or that old samples were more susceptible than fresh ones to the high summer temperatures. However, the virus doses used produced mortality at or near 100% on most of the samples. Thus, decreases in activity may have occurred which could not have been detected in this test. At the 0.469- and 1.875-mg/kg doses (data not tabulated) mortality was 100% throughout the test. As with *B. thuringiensis*, depth in the grain mass apparently did not affect the extent of the activity loss.

Under constant-temperature storage, the GV was more susceptible to high temperatures than was *B. thuringiensis*, but differences were more difficult to assess, particularly at the lowest dose, because mortality in the samples was much more variable. At the 0.059-mg/kg dose (Table 2), storage at 16.5° (data not tabulated) or 25.0°C did not decrease the insecticidal activity of the virus. However, at 42.0°C, and to a lesser extent at 33.5°C, insecticidal activity did decrease. At the higher temperature, activity was reduced to ca. 1/2 the initial level within 3–9 wk and activity further decreased with storage time. At 33.5°C activity had decreased to ca. 70% of the original level by the end of 42 wk of storage. (The increased mortality that occurred at 24, 27, and 30 wk was attributed to a temporary decrease in vigor of the insect colony.) At 0.469 mg/kg activity decreased to ca. 10% of the original level over 42 wk of storage at 42.0°C. However, there was little or no reduction in activity at the 3 lower temperatures. Because even brief storage at 42.0°C caused a large decrease in virus activity, possibly the 15 days on which the temperature of the surface layer of grain in the farm bin reached 42°C caused the slight deterioration observed on samples stored in the grain bin.

The insecticidal activity of both the GV and *B. thuringiensis* was only slightly reduced by storing treated wheat in the farm grain bin; it was reduced under constant-temperature storage only at high temperatures which can be expected to occur infrequently in farm grain-storage bins in Kansas. Both microbial insecticides were sufficiently stable, in the

formulations tested, to be used for long-term control of Indian meal moths in stored grain. With proper timing of applications, either pathogen could be expected to protect the grain from Indian meal moth infestation for 1 yr, and enough residual activity might be present to extend protection longer.

REFERENCES CITED

- Anonymous.** 1965–1974. Climatological data: Kansas, vols. 79–88. U.S. Dept. Commerce, Natl. Oceanic Atmos. Admin., (Environ. Sci. Serv. Admin.), Environ. Data Serv.
- 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.
- Barjac, H. de, and F. LeMille.** 1970. Presence of flagellar antigenic subfactors in serotype 3 of *Bacillus thuringiensis*. *J. Invertebr. Pathol.* 15: 139–40.
- Burges, H. D.** 1964. Insect pathogens and microbial control of insects in stored products. I. Test with *Bacillus thuringiensis* Berliner against moths. *Entomophaga Mem. hors Ser. No. 2*, pp. 323–7.
- Burges, H. D., S. Hillyer, and D. O. Chanter.** 1975. Effect of ultraviolet and gamma rays on the activity of δ -endotoxin protein crystals of *Bacillus thuringiensis*. *J. Invertebr. Pathol.* 25: 5–9.
- Dulmage, H. T.** 1970. Insecticidal activity of HD-1, a new isolate of *Bacillus thuringiensis* var. *alesti*. *Ibid.* 15: 232–9.
- Dulmage, H. T., A. J. Martinez, and J. A. Correa.** 1970. Recovery of the nuclear polyhedrosis virus of the cabbage looper, *Trichoplusia ni*, by coprecipitation with lactose. *Ibid.* 16: 80–83.
- Hunter, D. K.** 1970. Pathogenicity of a granulosis virus of the Indian-meal moth. *Ibid.* 16: 339–41.
- 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.
- Ignoffo, C. M.** 1964. Effects of temperature and water on viability and virulence of *Bacillus thuringiensis* var. *thuringiensis* Berliner spores. *Entomophaga Mem. hors Ser. No. 2*, pp. 293–8.
- Kantack, B. H.** 1959. Laboratory studies with *Bacillus thuringiensis* Berliner and its possible use for control of *Plodia interpunctella*. *J. Econ. Entomol.* 52: 1226–7.
- McGaughey, W. H.** 1975a. A granulosis virus for Indian meal moth control in stored wheat and corn. *Ibid.*, 68: 346–8.
- 1975b. Compatibility of *Bacillus thuringiensis* and granulosis virus treatments of stored grain with four grain fumigants. *J. Invertebr. Pathol.* 26: 247–50.
1976. *Bacillus thuringiensis* for controlling three species of moths in stored grain. *Can. Entomol.* 108: 105–12.
- Zettler, J. L., L. L. McDonald, L. M. Redlinger, and R. D. Jones.** 1973. *Plodia interpunctella* and *Cadra cautella* resistance in strains to malathion and synergized pyrethrins. *J. Econ. Entomol.* 66: 1049–50.