STABILITY OF *BACILLUS THURINGIENSIS* AND A GRANULOSIS VIRUS OF *PLODIA INTERPUNCTELLA* (HUBNER) ON STORED WHEAT

by

ROBERT ALLAN KINSINGER

B. S. Northeast Missouri State University, 1973

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1975

Approved by:

William H. McGaughey
Major Professor

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THIS BOOK CONTAINS SEVERAL DOCUMENTS THAT ARE OF POOR QUALITY DUE TO BEING A PHOTOCOPY OF A PHOTO.

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INTRODUCTION

The Indian meal moth, Plodia interpunctella (Hübner), is a cosmopolitan insect pest of stored grain and other stored products. Its level of resistance (Zettler et al. 1973) to the approved chemical grain protectants which are now in use is becoming a matter of increasing concern to those who deal with stored commodities. The use of insect pathogens for controlling insects has been reviewed by Steinhaus (1963a, b), Burges and Hussey (1971a), and others. Some microorganisms have shown promise as alternatives to chemicals for insect control and for use in integrated insect control programs. However, microbial insecticides have been evaluated only to a limited extent against stored-product pests (Steinhaus and Bell 1953, Kantack 1959, Burges 1964, van der Laan and Wassink 1964, Hunter 1973). These authors showed that stored-product moths, including the Indian meal moth, were susceptible to microbial insecticides such as the bacterium, Bacillus thuringiensis, and certain insect viruses, but little has been done to develop methods for using these materials on stored grain or to evaluate the stability of the pathogens under grain storage conditions.

This study was made to evaluate the stability of a commercial formulation of B. thuringiensis and an experimental formulation of a granulosis virus (Arnott and Smith 1968) when used to control Indian meal moths on wheat under farm storage conditions. Supporting studies were made in the laboratory using controlled environmental conditions to more clearly determine effects of constant temperature on the persistence of the pathogens during extended periods of storage.

LITERATURE REVIEW

The first significant example of the use of a microorganism for the destruction of an insect pest was the use of the fungus, Metarrhizium anisopliae (Metchnikoff), in soil against larvae of the wheat cockchafer, Anisoplia austriaca Herbst, by Metchnikoff in 1879 (Steinhaus 1949). Since that time insect pathogens have been studied extensively, and bacteria (Heimpel and Angus 1960, Falcon 1971), viruses (Smith 1967, Stairs 1971, David 1975), fungi (Roberts and Yendol 1971), protozoans (McLaughlin 1971), and other microorganisms are now being considered as potential control agents for many different insect pests. Burges and Hussey (1971c) predicted that spore-forming bacteria and occluded viruses would be the main sources for future microbial insecticides. These groups of microorganisms are more protected from environmental changes and adverse conditions than are many other pathogens; the bacteria by their hardy spores and the viruses by their protein capsules. In most cases, they are safe for man, animals, and beneficial insects. Burges and Hussey suggested that fungi are too susceptible to the environment to be effective except in situations where there are cool temperatures and adequate free moisture or high humidity such as in aquatic situations or moist soils. Some fungi are also unsafe for man and beneficial insects (Heimpel 1971). Burges and Hussey also considered the use of protozoans to be unlikely, even though they are relatively safe, because they are less virulent and slower acting. Thus protozoans would be more suitable for introduction as agents for long-term suppression of populations rather than for short term control. In

order to do this repeated introductions might be necessary or a means of protecting the free-living forms from adverse environmental conditions would need to be developed. Chemical pesticides might also be necessary initially to reduce pest insect populations to levels below the economic threshold.

The prediction made by Burges and Hussey is further supported by the advanced stage of development of certain bacterial and viral insect pathogens. The milky-disease bacteria, Bacillus popillae Dutky and B. lentomorbus Dutky have been used commercially for some time against the Japanese beetle, Popillia japonica Newman. Formulations of the crystalliferous spore-forming bacterium B. thuringiensis Berliner, have been registered for use in the U. S. against several lepidopterous pests on fruit, vegetable, and field crops, forest and shade trees, and ornamentals (Falcon 1971), and have compared favorably to insecticides for insect control in many cases (Rabb et al. 1957, Guthrie et al. 1959, McEwen et al. 1960, Vail et al. 1972). The nuclear-polyhedrosis virus of Heliothis spp. has been tested extensively (Ignoffo 1966a, b, c, Heimpel and Buchanan 1967, Allen and Ignoffo 1969, Meinecke et al. 1970, Ignoffo et al. 1972, 1973) and has been registered for experimental use on cotton (Ignoffo 1973). According to Stairs (1971) other nuclear-polyhedrosis viruses, including those of the cabbage looper, Trichoplusia ni (Hübner), and the Douglas fir tussock moth, Hemerocampa pseudotsugata McDunnough, are being mass produced in the United States but they have not been registered for extensive use.

Microorganisms are being considered as an alternative to chemical methods of insect control for several reasons. In some cases pest insects have become resistant to chemicals and increased dosage rates or

numbers of applications are required. Microorganisms have not yet been used extensively in the field and such resistance has not been reported in field use, although resistant insect strains have been produced in the laboratory (Burges 1971). Use of chemicals may result in detrimental effects on beneficial insects and hazards to man and the environment from persistent toxic residues. Most chemicals are toxic for man, animals, and beneficial insects and some are toxic to plants, whereas, most insect pathogens are safe for man, animals, and beneficial insects and none are known to be phytotoxic or phytopathogenic (Burges and Hussey 1971c). Factors that must be considered before substituting microbial insecticides for chemical insecticides include the narrow host spectrum, variable efficacy, and variable stability of microorganisms.

The narrow host range of many microorganisms would be a valuable attribute in cases of application to field crops or forests where there usually is only one major insect pest to be controlled by each insecticide application. The selection of highly specific control agents would assure safety of naturally occurring parasites and predators. The parasites and predators might then be able to hold the pest population below the economic threshold. In a closed environment, such as in a grain storage bin where there usually is a complex of insect pests to control and parasites and predators are of little or no consequence, the narrow host spectrum of microorganisms may be a disadvantage. Even though they would appear to be too specific for general use in stored-product insect control, microbial insecticides might be considered for use against moth infestations in stored grain. Stored-product moths are frequently controlled with specific chemical treatments which are separate from control measures used against other stored-product pests.

Some of these moths have become resistant to the chemical control measures now available (Zettler et al. 1973), and alternate methods of control are needed. Microbial insecticides appear to be a viable alternative in this case since most of the known insect pathogens are effective against lepidopterous insects.

Insect viruses tend to be quite host specific. Most exhibit generic specificity, infecting insects which are closely related to the "original" host species, and many show a tendency for species specificity (Ignoffo 1968). Ignoffo reported that the granulosis viruses were the most specific of all insect viruses, with only 6 of 52 attempts at cross-transmission succeeding. The least specific insect viruses, according to Ignoffo, are the noninclusion viruses. This was supported by Smith et al. (1961) and Smith (1967) when they demonstrated that the Tipula iridescent virus could be transmitted to at least 7 species of Diptera, 12 species of Lepidoptera, and 3 species of Coleoptera.

B. thuringiensis is one of the least specific of the microbial insecticides, affecting at least 137 species in 4 orders of insects (Heimpel 1967a). B. thuringiensis, unlike many microorganisms affecting insects, does not usually produce disease. Its effect on insects is toxic in nature and Heimpel attributed the organism's lack of specificity to the different toxic fractions which it produces. These include the α-exotoxin, which is known to affect only sawfly larvae; the β-exotoxin, which is rather nonspecific, affecting species from all insect orders; the γ-exotoxin, of which little is known except that it will clear egg yolk agar around developing bacterial colonies and is probably not actually toxic; and the δ-endotoxin, which is responsible

for most of the B. thuringiensis-related mortality in lepidopterous insects. The β-exotoxin is eliminated from most commercial formulations because of its nonspecificity. Since the toxin is water soluble this can be done in the formulation process and, since most commercial formulations are intended for controlling only lepidopterous pests, varieties of B. thuringiensis can be selected which produce primarily the δ-endotoxin and little or no β-exotoxin.

Eleven different serotypes (de Barjac and LeMille 1970) of B. thuringiensis have been described which exhibit differences in their effectiveness against a particular insect species. Different species of insects also show varying degrees of susceptibility to each serotype (Rogoff et al. 1969, Dulmage 1970, 1971, Dulmage and de Barjac 1973, Galowalia et al. 1973). Dulmage (1971) even found variations between fermentations of the same bacterial isolate. Burgerjon and Martouret (1971) pointed out that the amino acid composition of the crystalline -endotoxin varies so little between serotypes and it does not seem possible that such slight variations could be responsible for the pronounced toxicity differences observed. They suggested that the toxicity differences might be explained by differences in the manner in which hydrolysis of the crystalline protein occurs in the insect gut. Galowalia et al. (1973) also proposed that differences in the target tissues involved in different insect species might be another source of variability in toxicity. Much is yet to be learned about the composition of the -endotoxin and its role in the specificity of B. thuringiensis. Its variation may be responsible for much of the difficulty in predicting the effectiveness of a B. thuringiensis formulation against a pest insect population.

The effectiveness of insect viruses applied to control an insect pest can be affected by various host-pathogen-environment interactions. In some insects the lethal time for virus infections becomes shorter as the temperature is increased, within reasonable limits (Canerday and Arant 1968, Okada 1969, Biever and Hostetter 1971, Hunter and Hartsell 1971), but in others, as the temperature approaches the upper limits for insect development the virus infection becomes inapparent even though the insect still harbors the virus and the virus itself is not inactivated (Thompson 1959, Ignoffo 1966b, Tanada and Chang 1968, Bullock 1972, Hunter and Hartsell 1971). When the temperature is again lowered the infection becomes active once again and may cause death. Susceptibility has also been shown to be related to the age of the larvae when they were initially infected. As larval age increases, less mortality occurs as a result of virus infection (Tanada 1956, Ignoffo 1966a). Stress is another factor that may increase susceptibility as was demonstrated by Jacques (1961, 1962) when he subjected larvae to constant shaking, vibrating, or overcrowding. Poor nutrition is another stress factor which may enhance the effect of a virus infection.

A major factor causing variability in the efficacy of any insecticide is nonuniform distribution of the material. When using microbial insecticides in the field this may be an acute problem. Since most insect pathogens must be ingested to be effective, proper location and thorough coverage of the insecticidal application is usually necessary to achieve a high level of insect control. However, McGaughey (1975a) suggested that thorough coverage is not as critical in the use of microbial insecticides on stored products. He demonstrated in the laboratory that, when a granulosis virus was applied to wheat or corn,

100% of the kernels did not need to be treated to effectively control the Indian meal moth.

Some of the above factors such as variation in toxicity or pathogenicity to different insect species; interaction of host, insecticide, and environment; and nonuniform distribution of materials may be common to chemical and microbial means of insect control. Others, such as the production of toxins and the fact that pathogens are living organisms subject to biological variation, are unique to microorganisms (Burges and Hussey 1971b). This biological variation and resulting variation in efficacy makes standardization of microbial insecticides difficult.

Steinhaus (1957) saw the need for registration and standardization of microbial insecticides to protect the public as well as to protect the manufacturer from unfair competition, and since that time several proposals have been made for methods of standardization, but the subject is still under debate. No methods other than bioassay or counts of spores or virus particles are yet available for standardizing microbial insecticides. Burges (1967) reported that participants in a symposium at the International Colloquium on Insect Pathology and Microbial Control in Wageningen, The Netherlands, recommended that E-61, a formulation of B. thuringiensis from the Institut Pasteur, Paris, France, be adopted as an international standard to which all other B. thuringiensis formulations could be compared. It was assigned an arbitrary value of 1000 International Units of potency/mg. Workers in the U. S. (Dulmage 1973) adopted a formulation of δ-endotoxin produced by HD-1, an isolate of B. thuringiensis var. kurstaki (de Barjac and LeMille 1970), as the primary U. S. reference standard for use in standardization of

formulations of δ-endotoxin produced by B. thuringiensis. It was assigned a value of 18,000 International Units of Potency/mg after comparison with E-61. Dulmage et al. (1971) proposed that the cabbage looper be used as the test insect to standardize B. thuringiensis formulations containing the δ-endotoxin by a bioassay method based on the International Unit. They gave the formula for calculating the potency of test formulations as:

$$\text{IU/mg test formulation} = \frac{LD_{50} \text{ standard}}{LD_{50} \text{ test formulation}} \times \text{IU/mg standard.}$$

The potency of the standard is usually determined by a previous comparison to the international standard, E-61, which was assigned a potency of 1000 International Units/mg. Later, van der Geest and Wassink (1972) proposed that Pieris brassicae (L.) which is quarantined from the United States, be used in countries where the cabbage looper is prohibited. Commercial preparations produced in the U. S. are now standardized by the number of viable spores as well as the number of International Units of potency based on a bioassay using the cabbage looper as the test insect. This method is still rather unsatisfactory since, as was discussed before, different insect species demonstrate variable susceptibility to any one serotype of B. thuringiensis and the resulting susceptibility of each pest insect species to a commercial preparation cannot be predicted without thorough testing.

The standardization of viral formulations may be more complex because each virus must be standardized as to its pathogenicity to each species of insect. Hink and Vail (1973) developed a plaque-assay method for assaying the nuclear polyhedrosis virus of the alfalfa looper,

Autographa californica (Speyer), using a tissue culture from the cabbage looper, but this method has not become widespread because tissue cultures have been developed for few insect species for which viruses are known. Ignoffo (1966d) and Heimpel (1967b) suggested that (1) a reference standard for each insect virus be established, (2) all virus preparations be compared to the standard and their activity expressed in number of activity units/unit weight or volume, and (3) activity of viral preparations be measured as mortality (LD_{50}) of a particular insect species for each virus following strictly defined procedures. So far no uniform method has been adopted.

Environmental stability is a major consideration in predicting the efficacy of any insecticide in the field. Solar radiation, temperature, rainfall, humidity, and other weathering factors may affect the stability of microbial insecticide deposits. Solar radiation appears to be the most critical factor in degradation of microbial insecticides on plants. Spores of B. thuringiensis are rapidly inactivated by exposure to sunlight or to an ultra-violet lamp (Cantwell and Franklin 1966), but similar exposure of the δ-endotoxin did not appear to alter its effect on insect species to which it is toxic (Cantwell 1967, Burges et al. 1975). In field tests other workers found that the bacterial spores are inactivated and insecticidal activity is greatly reduced within one day after application (Frye et al. 1973, Ignoffo et al. 1974). Burges et al. (1975) attributed this loss in insecticidal activity in some cases to the fact that mortality in some insects is influenced by spores as well as by crystals so that a reduction in spore viability could be accompanied by a corresponding reduction in insecticidal activity.

Tests done with various insect viruses (Cantwell 1967, Ignoffo and Batzer 1971, Ignoffo et al. 1972, 1973, 1974, Broome et al. 1974,

Yearian and Young 1974, Hostetter et al. 1973) have shown that most viruses are inactivated by exposure to ultra-violet lamps or sunlight. Ignoffo et al. (1972, 1973) demonstrated that the half-life of the Heliothis nuclear-polyhedrosis virus in the field was <1 day but that this critical period could be doubled if a carbon protectant was added to the formulation before application.

Temperature, in some cases, may be important in microbial stability, but little work has been reported on the effect of physical factors such as temperature, moisture, and the interrelationship of these factors on B. thuringiensis. Ignoffo (1964) exposed dry and aqueous suspensions of B. thuringiensis to temperatures of 10°, 30°, and 50°C and found that the viability of the dry preparation was reduced only 15% at 50°C for 200 days, while aqueous suspensions lost viability at all temperatures with an increase in temperature resulting in a corresponding decrease in viability.

Most thermal inactivation tests on insect viruses have been done at very high temperatures (60°-90°C) and inactivation has taken place after short periods of time (5 min-24 hr) (Tanada 1959, David and Gardiner 1967, Gudauskas and Canerday 1968, MacFarlane and Keeley 1969). When tests were done with the Heliothis nuclear polyhedrosis virus at lower storage temperatures of 5°, 37°, and 50°C (Shapiro and Ignoffo 1969), half the original activity remained after 120, 60, and 30 days, respectively, showing it to be relatively stable under temperature conditions which might be encountered in the field.

The literature reveals little in-depth work using microbial insecticides for the control of stored-product insects. Most reports have been of preliminary observations on insect susceptibility and

cross-infectivity. No work has been reported of use in actual storage situations. Steinhaus and Bell (1953) found, in preliminary studies, that the granary weevil, Sitophilus granarius (L.), the rice weevil, S. oryzae (L.), and the Angoumois grain moth, Sitotroga cerealella (Olivier), were slightly susceptible to strains of B. thuringiensis which had been isolated from various stored-product moths. Kantack (1959) and Afify (1968) found the Indian meal moth to be very susceptible to formulations of B. thuringiensis which probably belonged to serotype I. Burges (1964) and van der Laan and Wassink (1964) found commercial formulations, Bakthane® and Thuricide® toxic to the Mediterranean flour moth, Anagasta kühniella (Zeller), the tobacco moth, Ephestia elutella Hübner, the almond moth, Cadra cautella (Walker), and the Indian meal moth.

In 1968, Arnott and Smith described a granulosis virus of the Indian meal moth, and since that time Hunter and associates have investigated its pathogenicity and cross-infectivity (Hunter 1970, Hunter and Hartsell 1971, Hunter et al. 1972, 1973a). They showed that the virus was effective in controlling the Indian meal moth in the laboratory but that it failed to infect larvae of the tobacco moth, the almond moth, or the raisin moth, Cadra figulilella (Gregson). Hunter and associates have also worked with a granulosis and a nuclear polyhedrosis virus of the almond moth, showing that both were highly pathogenic to the almond moth in laboratory tests and that the Indian meal moth was slightly susceptible to both viruses (Hunter and Dexel 1970, Hunter and Hoffmann 1970, 1972, Hunter et al. 1973b, c).

These preliminary tests have shown the potential for using microbial insecticides to control some stored-product insects, mostly

moths. B. thuringiensis and a granulosis virus appear to be likely candidates for control of the Indian meal moth in stored products. Hunter et al. (1973a) conducted laboratory studies on the feasibility of using the Indian meal moth granulosis virus to control the moth on stored inshell nuts and found it to be very effective. McGaughey (1975a) made laboratory studies of various aspects of using the virus on stored wheat and corn. He proposed that the microbial insecticide would be effective against moths when applied only to the surface layer of grain in bulk storage and that the treatment need not cover 100% of the kernels in the treated layer in order to effect control of moth infestations.

No studies have been reported on the stability of any microorganism under actual grain storage conditions. Instability due to solar radiation, which is the major degradative factor in field applications, should not be a problem in a grain bin. The major degradative factor in a bin would appear to be temperature, which may undergo more extreme fluctuations inside grain storages than outside, especially in the surface layer of grain to which McGaughey (1975a) proposed that the treatment be restricted. To a lesser extent, humidity and grain moisture content, which are directly related, may have some effect on microbial stability.

MATERIALS AND METHODS

Insect Rearing and Handling

A culture of the Indian meal moth, obtained from the Stored-Product Insect Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Fresno, California, in June, 1972, was maintained at the U. S. Grain Marketing Research Center, Agricultural Research Service, U. S. Department of Agriculture, Manhattan, Kansas, for use in the study. Larvae were reared in 1.9-liter jars containing 450-500 g of diet that consisted of 500 g coarse-ground wheat, 500 g shorts, 50 g wheat germ, 40 g brewers' yeast, 2 g sorbic acid, and 2 g methyl p-hydroxybenzoate. The sorbic acid and methyl p-hydroxybenzoate were used to prevent fungal growth. These dry ingredients were mixed thoroughly by hand before incorporating 300 ml of warm liquid that consisted of 120 ml honey, 120 ml glycerin, and 60 ml distilled water. The prepared diet could be refrigerated for up to two weeks before use without significant moisture loss. One batch of diet was sufficient for 3 1.9-liter jars.

The larvae were allowed to pupate in rolls of corrugated paper placed on top of the diet. Rolls of paper with pupae were transferred to empty 1.9-liter jars with 20-mesh screen covers which were inverted over plastic petri "X" plates. Adults, upon emergence, deposited their eggs through the screen into the plate. The eggs were separated from loose scales, surface-sterilized using 4% formaldehyde solution as suggested by Spitler (1970), and rinsed in distilled water. To maintain the cultures 80-100 mg of sterilized eggs were placed in each 1.9-liter

jar of diet.

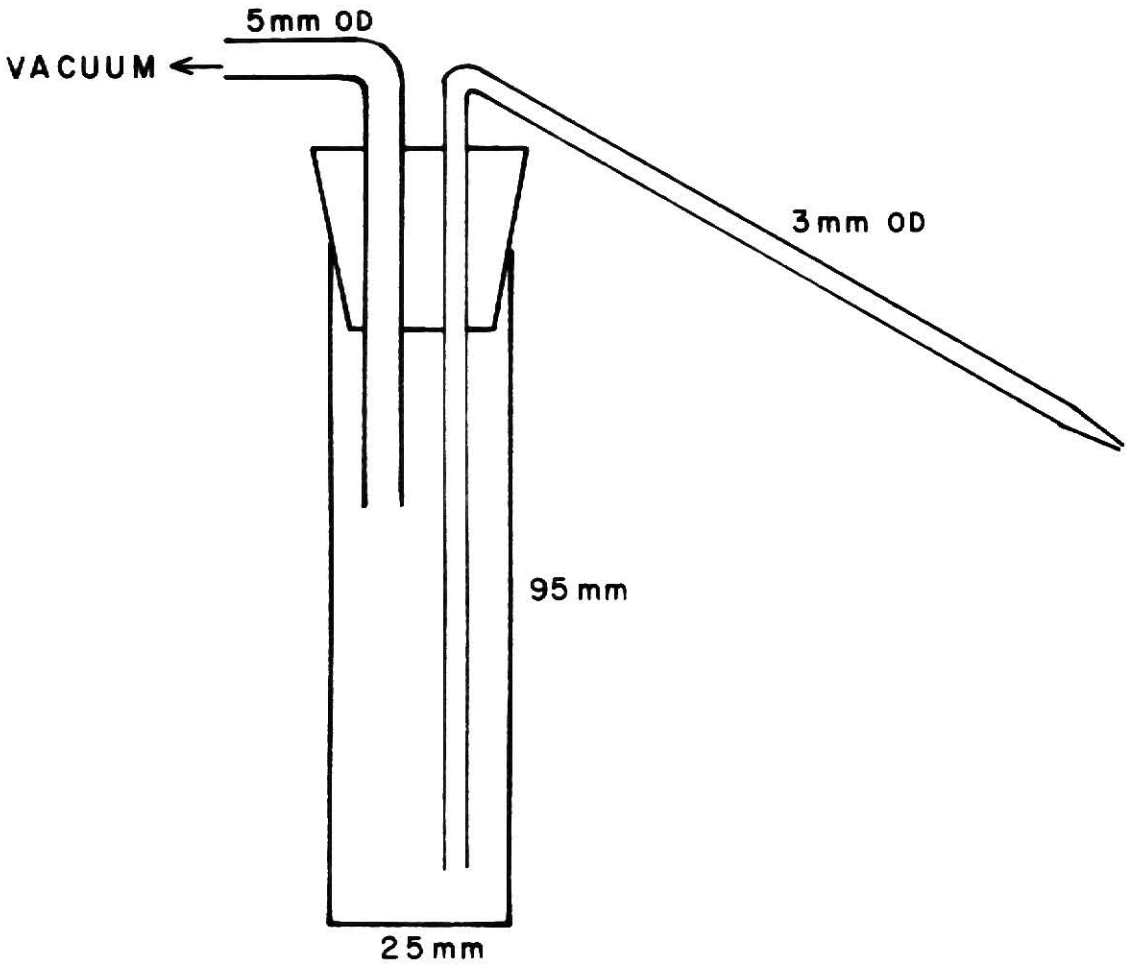
Eggs to be used in tests were not surface sterilized. After separation from scales they were counted under a dissection microscope (10X magnification) as they were collected using a small aspirator (Fig. 1). This aspirator consisted of an 8-dram shell vial (45 x 95 mm) and a 2-hole #4 rubber stopper through which were run lengths of 3-mm and 5-mm OD glass tubing. A vacuum line regulated by a needle valve was attached to the 5-mm tubing and eggs were aspirated through the finely-drawn 3-mm tubing into the vial. A 1-dram shell vial (15 x 25 mm) could be used inside the larger vial to hold the eggs. Eggs were then placed directly into the test samples. This procedure proved to be less time consuming than other methods of counting eggs or neonate larvae and caused no damage to the eggs in handling.

Pathogen Formulation and Application

The microbial insecticides used in this study were Dipel® and a granulosis virus of the Indian meal moth (Arnott and Smith 1968). Dipel is a commercial formulation of B. thuringiensis var. kurstaki, strain HD-1 (Dulmage 1970, de Barjac and LeMille 1970), produced by Abbott Laboratories, North Chicago, Illinois. According to the manufacturer's label it contains 16,000 International Units of potency/mg and at least 25 billion viable spores/g. The number of viable spores present in the preparation was determined during this study using a spread-plate technique on half-strength nutrient agar, and was found to be approximately 49 billion spores/g.

The granulosis virus was produced by macerating diseased Indian meal moth larvae in distilled water using a tissue grinder, and

Figure 1. Aspirator used to count Indian meal moth eggs.


5mm OD
VACUUM
3mm OD
95 mm
25 mm

inoculating diet that contained second instar larvae. The time of inoculation was determined which would produce diseased larvae which grew to maturity. Fully-diseased late instar larvae, which could be recognized by their milky-white appearance and sluggish movements, were collected and frozen until needed. A formulation for testing was produced by blending these diseased larvae in distilled water and coprecipitating the virus capsules with lactose using acetone similar to the method described by Dulmage et al. (1970). This yielded a dry formulation consisting of half lactose and half virus capsules. A count of the virus capsules in this dry formulation, made using a Petroff-Hausser counting chamber and a bright-field microscope, showed approximately 32 billion capsules/g of formulation.

These microbial formulations were suspended in distilled water in concentrations appropriate for application to wheat at the rate of 20 ml of suspension/kg. One liter of suspension was prepared in a volumetric flask at the concentration to be used for the highest dose and a portion was then diluted for the lower doses. The suspensions were pipetted into 3.8-liter jars containing wheat, and the jars were tumbled on a ball-mill roller until the suspension was evenly distributed and all free moisture was absorbed.

Spread-plate Tests

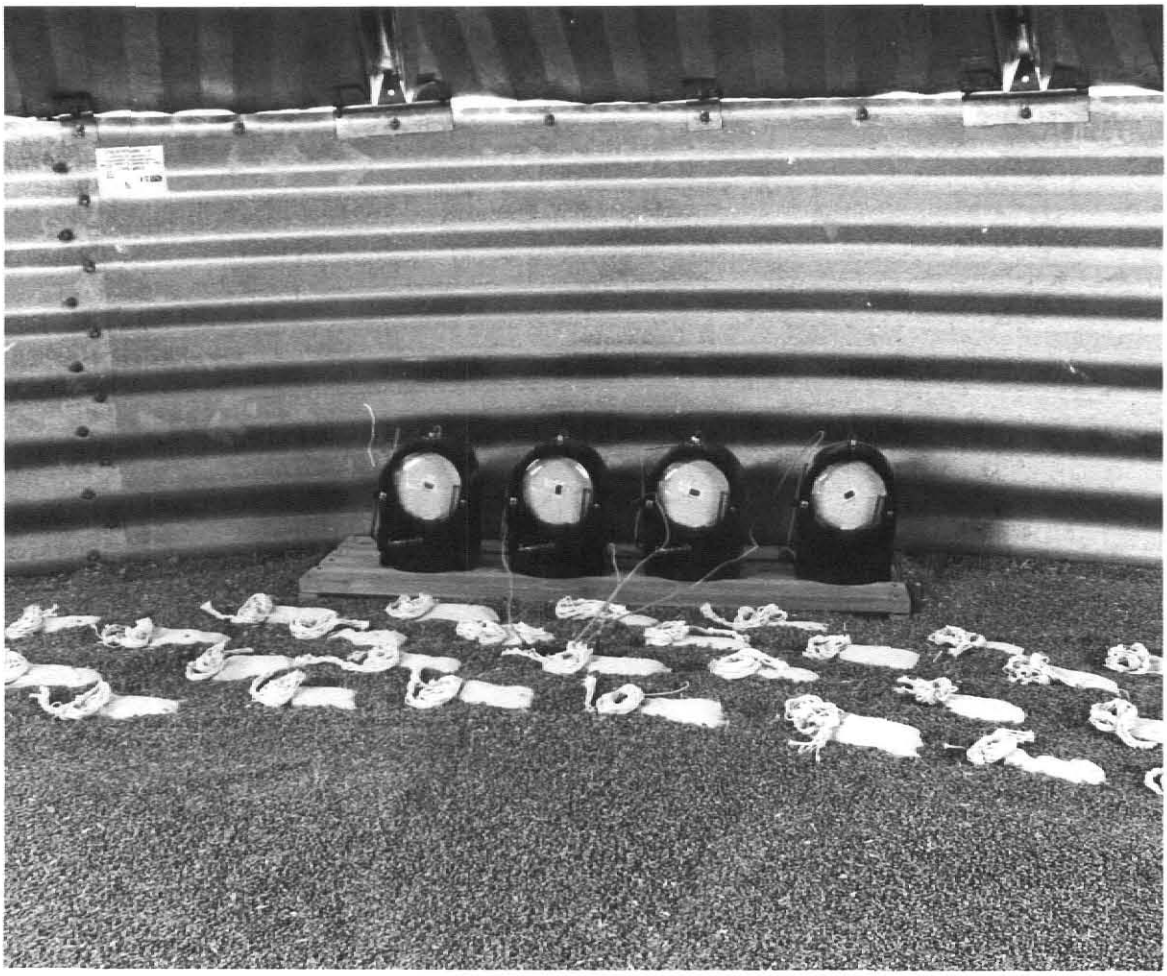
A spread-plate technique using half-strength nutrient agar (11.5 g Difco® nutrient agar plus 7.5 g agar/liter of distilled water) was used to obtain viable spore counts on the wheat samples treated with *B. thuringiensis*. Small quantities of wheat were removed from the samples used to test for insecticidal activity and were refrigerated

at 2°C ± 1°C until they could be plated. At the time of plating, 21 whole kernels (approximately 0.5 g) were weighed and shaken for 1 min in 50 ml of distilled water from which serial dilutions (1, 1:10, 1:100) were immediately made. Three plates of each dilution were then made using 0.1 ml of each dilution spread evenly over the plate with a bent glass rod. Colonies were counted after 30-40 hr incubation at 25°C. Counts of 3 replicate plates were averaged and the numbers of spores/mg of wheat calculated.

Field Study

To determine the stability of microbial insecticides under actual storage conditions, wheat samples treated with the pathogen formulations were placed in a 1200-bu farm-type circular steel bin 4.6 m in diam. It contained 760 bu of wheat harvested in 1973. The bin was located at the U. S. Grain Marketing Research Center, Manhattan, Kansas. Temperatures in the bin were monitored throughout the study. Continuous records were made 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 (Fig. 2). Daily readings were taken from a maximum-minimum mercury thermometer at the grain surface and another suspended 0.6 m above the grain surface to aid in standardizing the recorder readings and to fill in data missed because of recorder malfunctions. 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 15.0 and 30.0 cm. Temperature sensors were spaced every 0.9 m along the cables allowing 12 readings around the perimeter of the bin at each depth. Daily

Figure 2. Seven-day temperature recorders used to obtain continuous records at the grain surface, and 7.5, 15.0, 30.0 cm below the surface of the grain mass in a 1200-bu farm bin containing 760 bu of wheat.



temperature readings were taken between 1000 and 1400 hours using a Hot Spot® portable potentiometer, model PP-62 (Fig. 3). Maximum and minimum daily outdoor temperatures for Manhattan, Kansas, recorded by the Kansas State University Physics Department, were obtained from U. S. Department of Commerce reports (Anonymous 1965-1974). The accuracy of all temperature measurements was $\pm$ 1°C.

Moisture content of the wheat in the bin was monitored throughout the test using 24 300-g samples of wheat in small cotton bags. These bags of wheat were arranged in a column 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 these samples and 4 corresponding samples held in the laboratory in Mason jars with filter-paper covers at 25°C and 60% RH was measured at each sampling interval (0, 180, 270, and 360 days) with a Steinlite® Electronic Moisture Tester Model RCT. The samples were returned to their original position in the bin immediately after determining the moisture content.

Wheat samples with an average moisture content of 11.9% were treated with the microbial insecticides and placed in small cotton bags in the bin of wheat in October and November, 1973. An aqueous suspension of the B. thuringiensis formulation was applied to wheat at doses of 0, 25, 50, and 150 mg/kg and the formulated granulosis virus was applied at doses of 0, 0.059, 0.469, and 1.875 mg/kg. Samples were treated with distilled water for comparison with the B. thuringiensis treatments and with an aqueous solution of lactose for comparison with the granulosis virus treatments. Two 1500-g quantities of wheat were treated with each dose and then divided into 30 100-g samples. Five of the samples were placed in 0.5-liter Mason jars with filter-paper covers and 24 were

Figure 3. Hot Spot® portable potentiometer used to take daily temperature readings from thermocouples placed 15.0 and 30.0 cm below the grain surface in a 1200-bu farm bin containing 760 bu of wheat.


IMPORTANT
BATTERY
hot spot
KEYSTONE SYSTEMS INC.
OPEN

placed in 7.6 x 12.7-cm cotton bags.

The bags were placed in the farm storage bin at depths of 0.0, 7.5, 15.0, 22.5, 30.0, and 37.5 cm in the wheat (Fig. 4). Bags of each dose were placed in 4 columns with the bags in each column positioned at the proper depth so that it was possible to remove entire groups of columns (Fig. 5), representing ¼ of the samples, at each of 4 time intervals (90, 180, 270, and 360 days) without disturbing the remaining samples. The test was replicated 4 times, once in each quadrant of the bin. Columns of bags were placed in the grain mass using a 0.6-m section of 10-cm-diameter plastic drain pipe which was pushed into the grain and evacuated with a vacuum grain sampler (Fig. 6). 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.

Four of the 5 0.5-liter Mason jars containing samples of each treatment were held in the laboratory at 25°C and 60% RH to serve as laboratory controls at each of the 4 sampling times. The fifth sample was tested for insecticidal activity immediately after treatment by introducing 25 Indian meal moth eggs. This jar was also held at 25°C and 60% RH until the adults emerged. Samples held in the laboratory were also replicated 4 times.

After 90, 180, 270, and 360 days, samples were removed from the bin and taken to the laboratory to measure the amount of insecticidal activity remaining. A portion of each sample treated with B. thuringiensis at the 50 mg/kg rate was also set aside to be used for estimating the decrease in spore viability using the spread-plate technique described before. The contents of each bag was placed in a

Figure 4. Grain bin with an enlarged inset illustrating placement of bags and temperature sensors at different depths beneath the grain surface.


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Figure 5. Diagram of grain surface showing placement of columns of treated samples with days indicated at which each group of bags was removed.

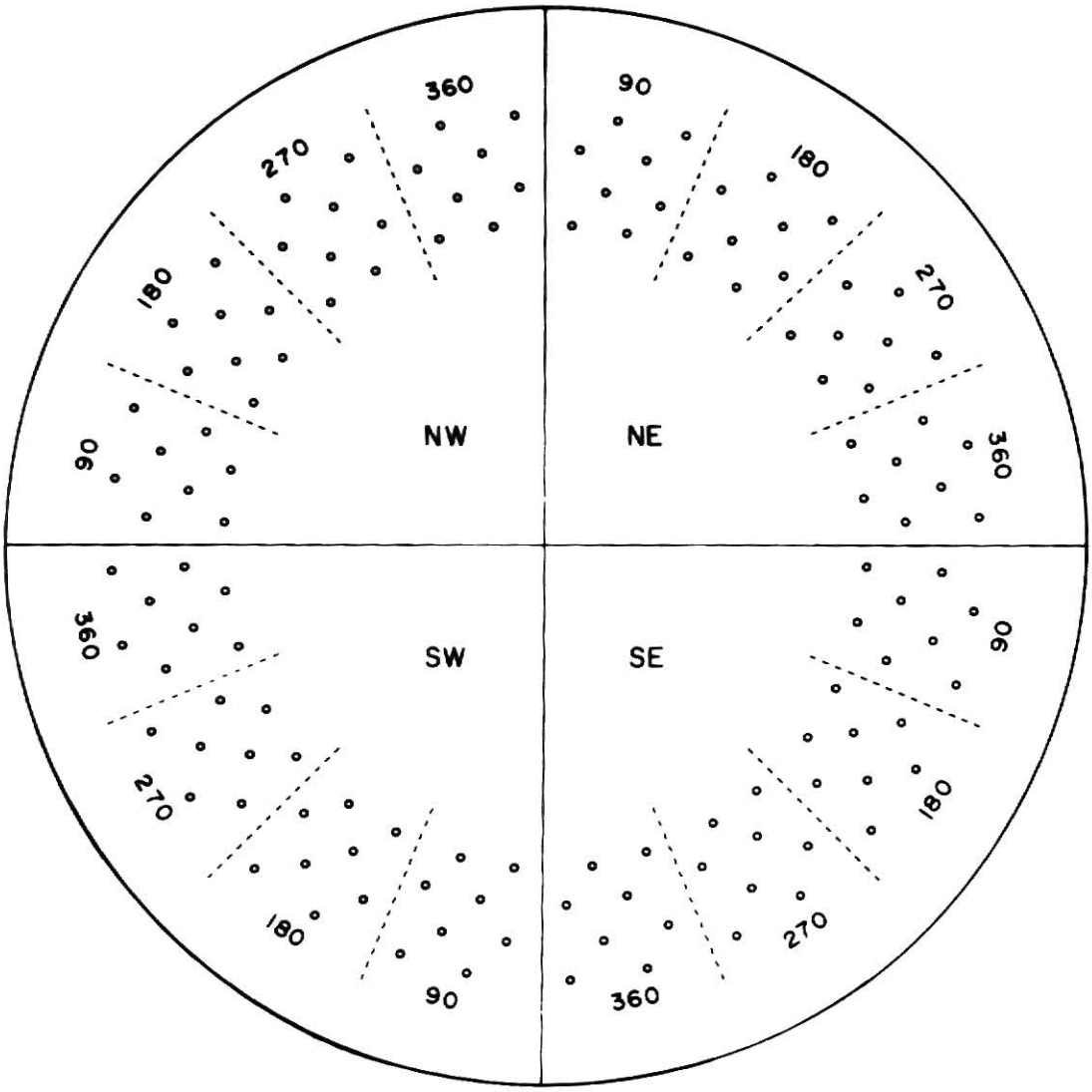
360
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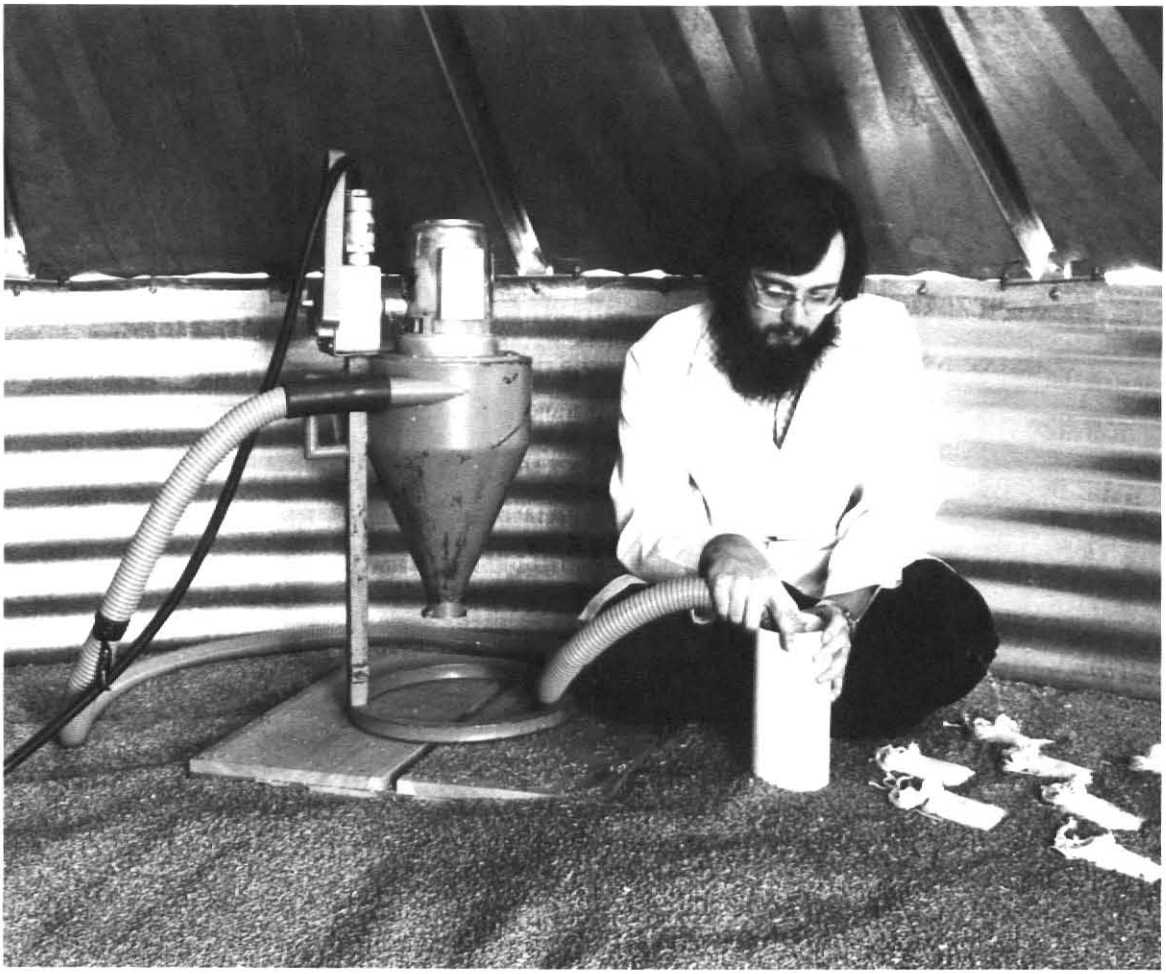
Figure 6. Vacuum grain sampler being used to evacuate a 0.6-m section of 10-cm-diam plastic drain pipe before placement of 100-g samples of treated grain in the grain mass.



0.5-liter Mason jar with a filter-paper cover and 25 Indian meal moth eggs were added. These jars were held at 25°C and 60% RH until the adults emerged, at which time percent mortality was calculated from the difference in the number of eggs added and adults that emerged. Values were corrected for mortality in untreated samples (Abbott 1925).

Additional samples of wheat with an average moisture content of 12.3% were treated and placed in the farm storage bin in May, 1974, to replace samples that had been treated in the fall and removed after 90 and 180 days. The doses and placement in the bin were the same as those used in the fall. One 1500-g quantity of wheat was treated with each dose and then divided into 15 100-g samples. Three of the samples were placed in 0.5-liter Mason jars with filter-paper covers and 12 were placed in 7.6 x 12.7-cm cotton bags. The bags were placed in the bin with bags of each dose positioned at the proper depths in 2 columns to facilitate removal after 90 and 180 days. Two of the 3 samples placed in Mason jars were again held at 25°C and 60% RH to serve as laboratory controls while the remaining sample was immediately tested for insecticidal activity. This test was also replicated in each quadrant of the bin. After 90 and 180 days, coinciding with the 270- and 360-day sampling times for the samples treated in the fall, samples were removed and insecticidal activity was tested in the same manner as the fall samples.

To measure the decrease in B. thuringiensis spore viability on treated samples stored in the bin, wheat with an average moisture content of 11.8% was sterilized at the Stored-Product Insects Research and Development Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Savannah, Georgia, in October, 1973, using

gamma irradiation at the rate of 1.25 million rads. In February, 1974, a 300-g quantity of this wheat was treated with an aqueous suspension of the B. thuringiensis formulation at a dose of 50 mg/kg and divided into 24 samples of 10-15 g each. These samples were placed in 3-dram (23 x 45 mm) plastic vials with snap-on caps. Five holes 5 mm in diam were punched in the caps and they were fitted with 20-mm millipore filters with 0.20-μ pore size. These vials were placed in 7.6 x 12.7-cm cotton bags and placed in the farm storage bin in 4 columns at depths of 0.0, 7.5, 15.0, 22.5, 30.0, and 37.5 cm in the same manner as the bagged wheat samples. The test was replicated in each quadrant of the bin. Replicated samples were held in the laboratory at 25°C and 60% RH to serve as laboratory controls. Samples were removed at intervals of 90, 180, and 270 days, corresponding to the 180-, 270-, and 360-day sampling times for the bagged wheat samples placed in the bin in the fall, and taken to the laboratory where they were used to make viable spore counts using the spread-plate technique. These sterilized treated samples were used in addition to the unsterilized bagged samples to estimate the decrease in spore viability.

Laboratory Study

To more exactly define the temperature limits of viability of the pathogen treatments on wheat, samples were treated and placed in a controlled environment in continuous darkness at 16.5°, 25°, 33.5°, and 42°C and 60% RH. Wheat with an average moisture content of 12.3% was treated with an aqueous suspension of the B. thuringiensis formulation at doses of 0, 10, 50, and 150 mg/kg and the formulated Indian meal moth granulosis virus at rates of 0, 0.007, 0.059, and 0.469 mg/kg. A

single water-treated check was used for both pathogens. The treated samples were held in the controlled environments in 1.9-liter jars. Each test chamber contained 3 shelves and the test was replicated 3 times with 1 replication on each shelf. The jars on each shelf were rotated each week to counteract any deviation in conditions from one part of the shelf to another.

Immediately after treatment and at 21-day intervals, a 100-g sample was removed from each of the 1.9-liter jars and placed in a 0.5-liter Mason jar with a filter-paper cover. Twenty-five Indian meal moth eggs were added and the samples were held at 25°C and 60% RH until the adults emerged. Percent mortality was again used as a measurement of insecticidal activity. Small quantities of wheat were also removed at each interval from the jars containing the 50-mg/kg *B. thuringiensis* treatment and used to make viable spore counts.

RESULTS AND DISCUSSION

Field Study

The daily temperature extremes and seasonal fluctuation which occurred in Manhattan, Kansas, during the study and the 10-year average maximum and minimum temperatures as recorded by the Kansas State University Physics Department and reported by the U. S. Department of Commerce (Anonymous 1965-1974) are shown in Fig. 7. Except for a relatively cold period from December 30 to January 12, a warm period from February 26 to March 9, and a cool period from March 10-25, the temperature followed the 10-year average rather closely. The temperature during the summer, which was expected to be more detrimental to pathogen stability, deviated little from the 10-year average. Thus, the results obtained in this study should be representative of a normal year of storage.

The daily and seasonal temperature extremes in the farm grain storage bin are illustrated in Figs. 8-12. The air temperature inside the bin (Fig. 8) remained warmer than the outside air during the winter, probably due to the heating effect of the sun on the metal roof and from heat given off by the grain mass. It also became much warmer in the summer for the same reasons. The seasonal extremes reached outside the bin were ca. -25°C in the winter and ca. 41°C during the summer, while those in the bin head space were only ca. -20°C in the winter and nearly 55°C during the summer. The mean daily temperature range in the head space (18.1°, SD=6.6°C) was also greater than that outside (11.9°, SD=4.5°C), demonstrating the daily heating effect of the

Figure 7. Daily temperature ranges for the period of November 1, 1973, to October 31, 1974, and the 10-year (1965-1974) average maximum and minimum temperatures for Manhattan, Kansas, recorded by the Kansas State University Physics Department and reported by the U. S. Department of Commerce (Anonymous 1965-1974).

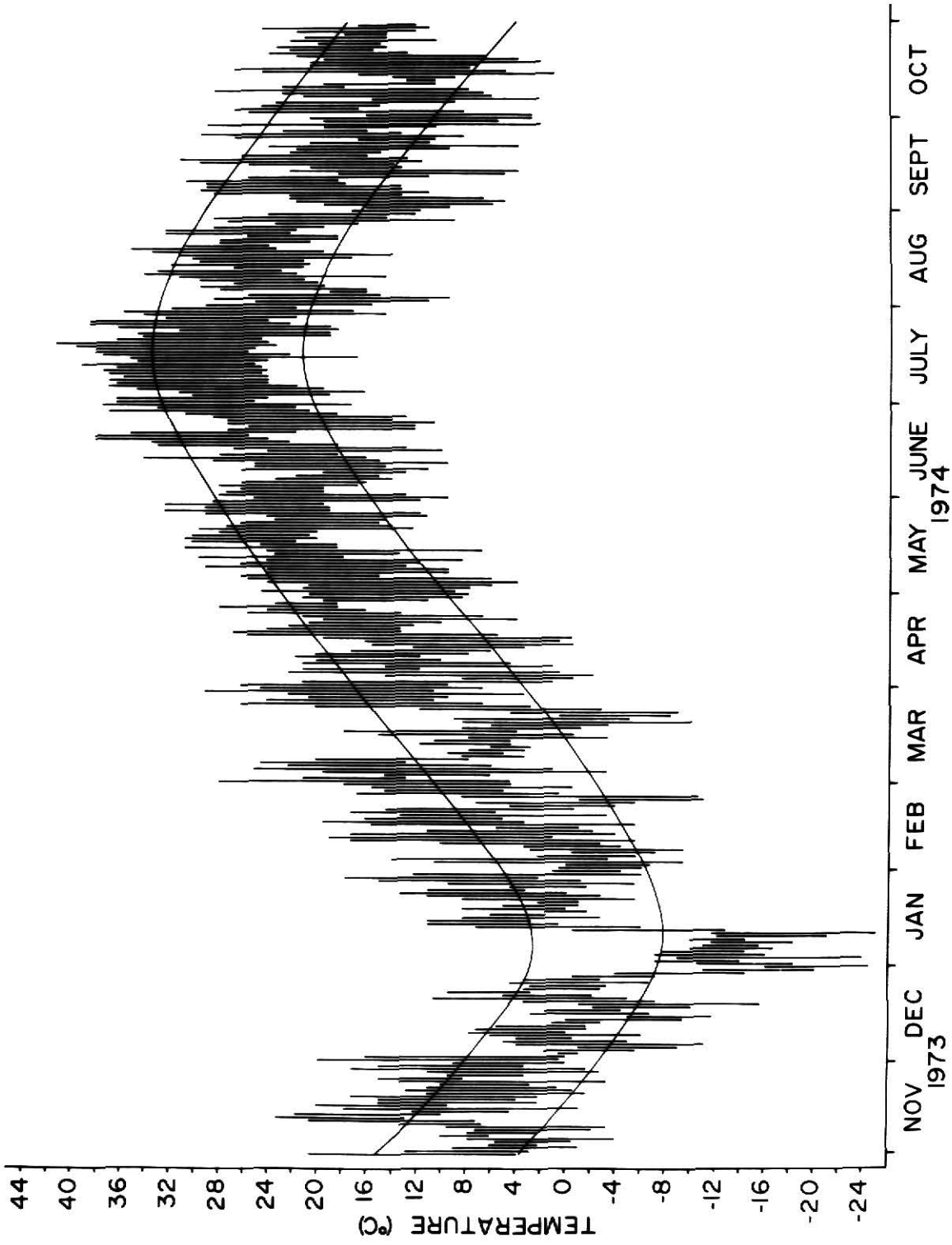
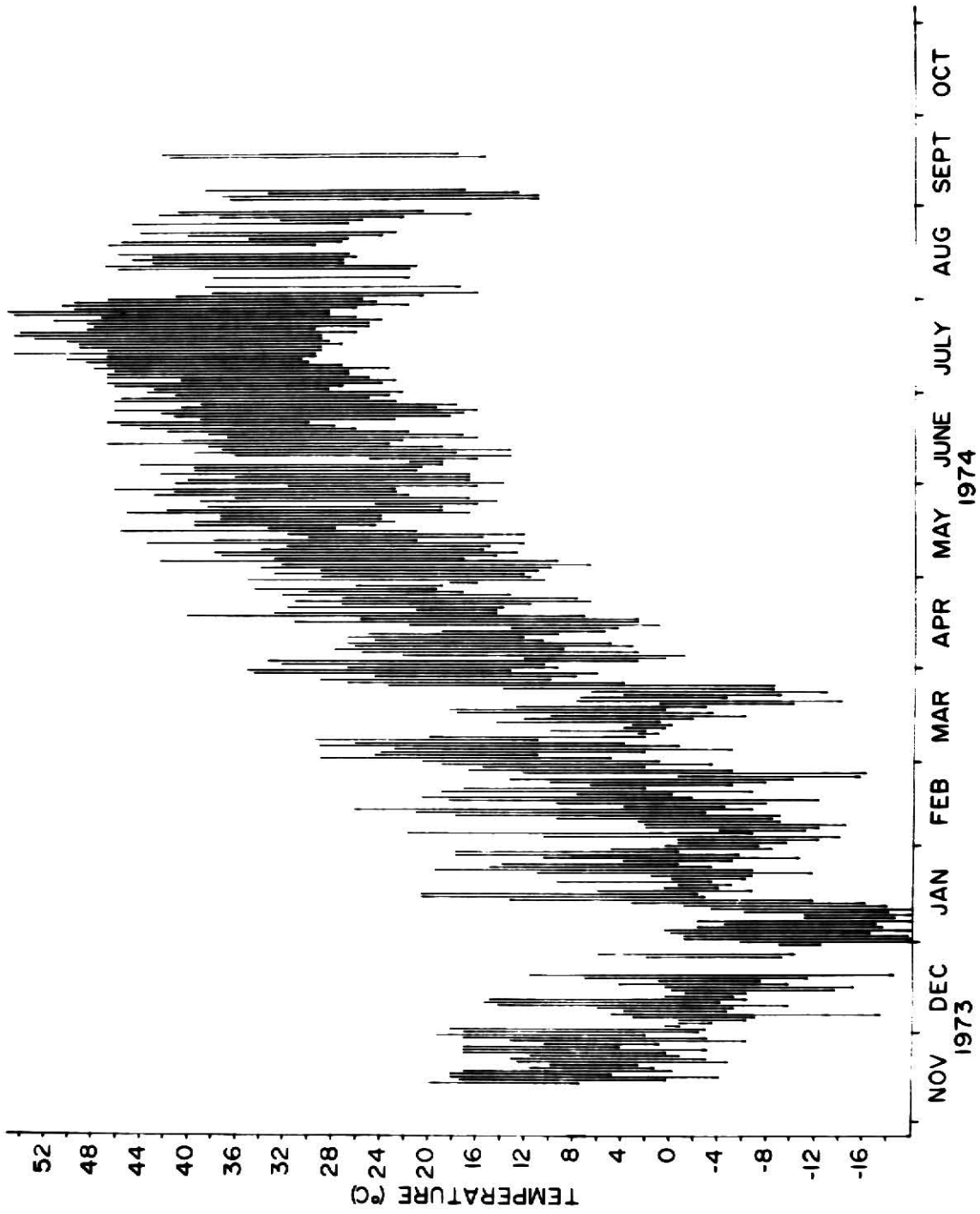

TEMPERATURE (°C)
44
40
36
32
28
24
20
16
12
8
4
0
-4
-8
-12
-16
-20
-24
NOV
1973
DEC
JAN
FEB
MAR
APR
MAY
JUNE
1974
JULY
AUG
SEPT
OCT

Figure 8. Daily temperature ranges for the period of November 1, 1973, to October 31, 1974, recorded by a 7-day temperature recorder with the sensor placed 0.6 m above the grain surface in a 1200-bu farm storage bin containing 760 bu of wheat. Values for the following days were estimated using a maximum-minimum mercury thermometer: Nov 14-Dec 20, 26, 27, 30-Jan 11, Mar 22, July 9, 11, 12, 14, 16-25, 27-31, Aug 5, 8, 11-16, 26-30, Sept 3-6, 17, 18.


TEMPERATURE (°C)
52
48
44
40
36
32
28
24
20
16
12
8
4
0
-4
-8
-12
-16
NOV
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JAN
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MAR
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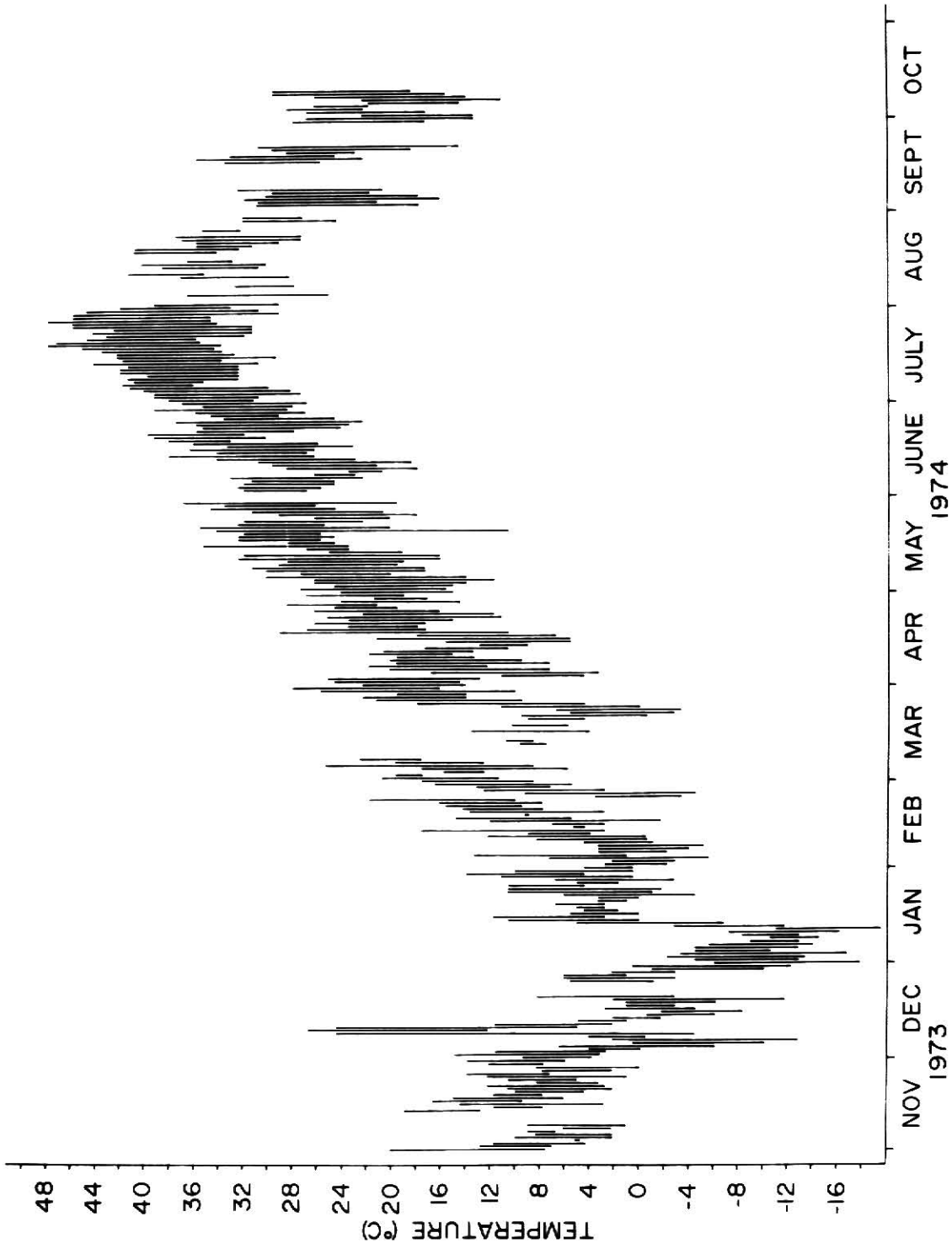
MAY
JUNE
1974
JULY
AUG
SEPT
OCT

Figure 9. Daily temperature ranges for the period of November 1, 1973, to October 31, 1974, recorded by a 7-day temperature recorder with the sensor placed at the grain surface in a 1200-bu farm storage bin containing 760 bu of wheat. Values for the following days were estimated using a maximum-minimum mercury thermometer: Nov 1-4, Dec 8, Feb 13-20, Mar 13, 14, 17, 19, Apr 29, May 14, 22, June 17, 18, July 5-7, 15-21, 28, Aug 5, 8, 11, 16, 26, 29, 30, Sept 3.


TEMPERATURE (°C)
48
44
40
36
32
28
24
20
16
12
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4
0
-4
-8
-12
-16
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DEC
1973
JAN
FEB
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APR
MAY
JUNE
1974
JULY
AUG
SEPT
OCT

Figure 10. Daily temperature ranges for the period of November 1, 1973, to October 31, 1974, recorded by a 7-day temperature recorder with the sensor placed 7.5 cm below the grain surface in a 1200-bu farm storage bin containing 760 bu of wheat.

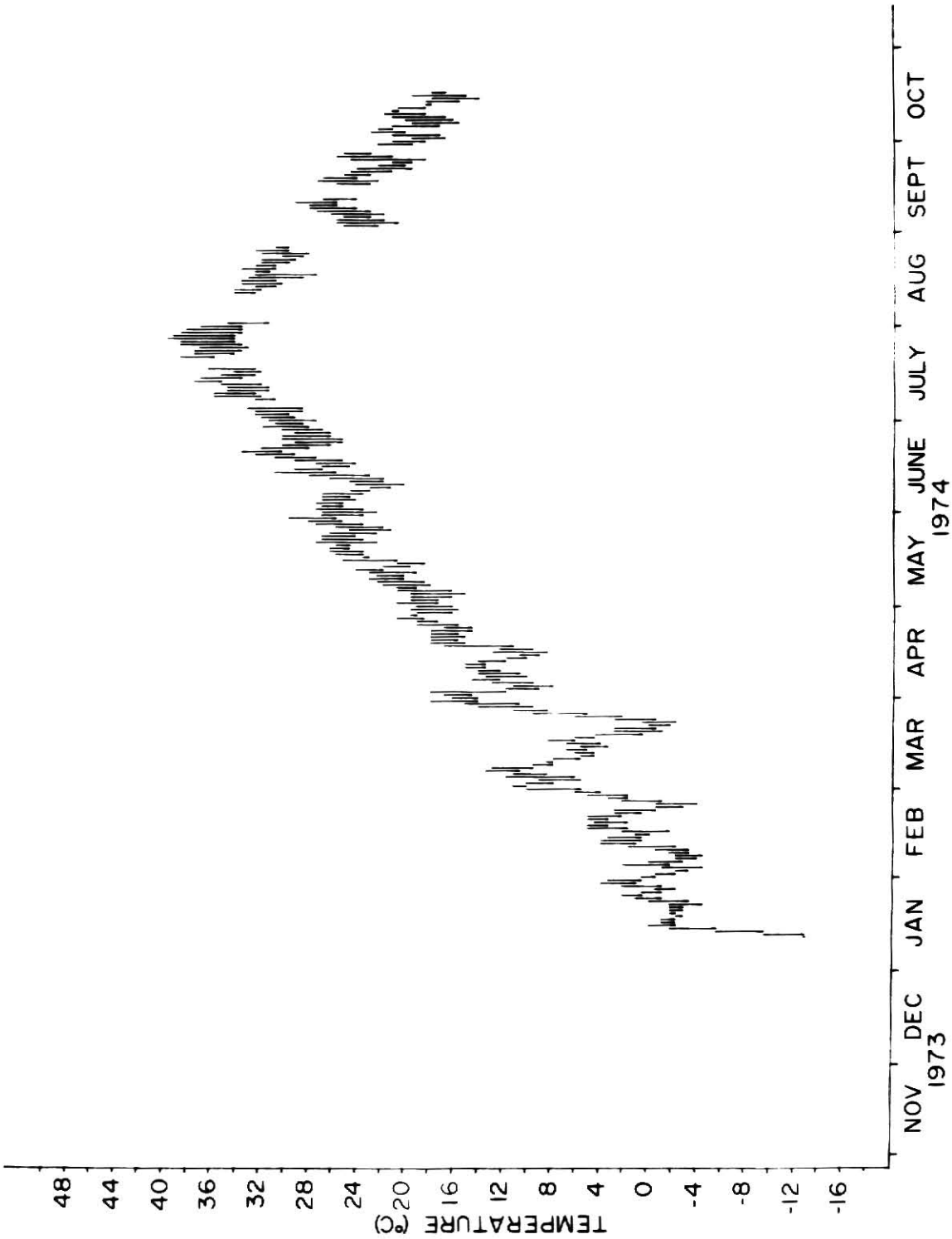
TEMPERATURE (°C)
48
44
40
36
32
28
24
20
16
12
8
4
0
-4
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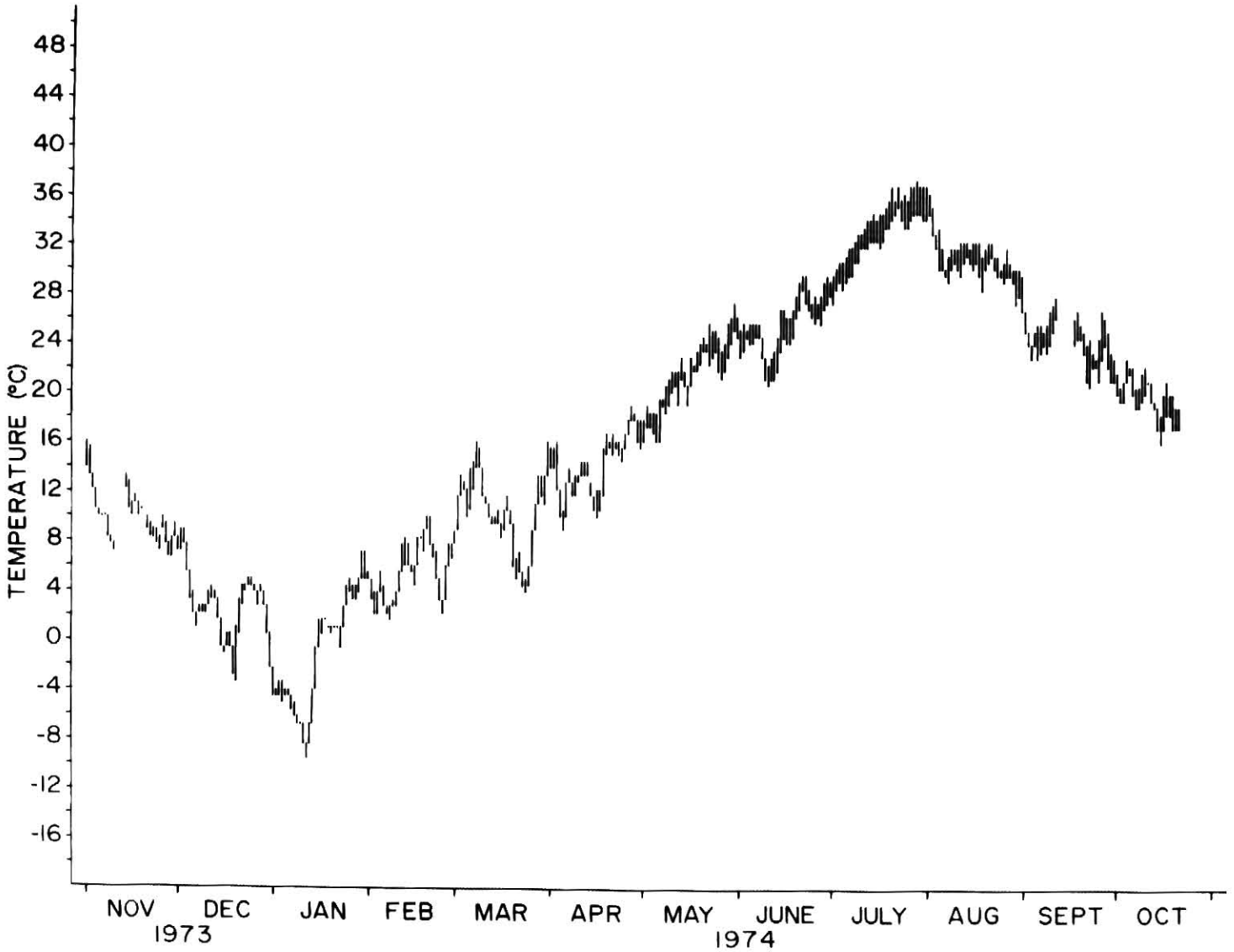
Figure 11. Daily temperature ranges for the period of November 1, 1973, to October 31, 1974, recorded by a 7-day temperature recorder with the sensor placed 15.0 cm below the grain surface in a 1200-bu farm storage bin containing 760 bu of wheat.


TEMPERATURE (°C)
48
44
40
36
32
28
24
20
16
12
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4
0
-4
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-12
-16
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUNE
JULY
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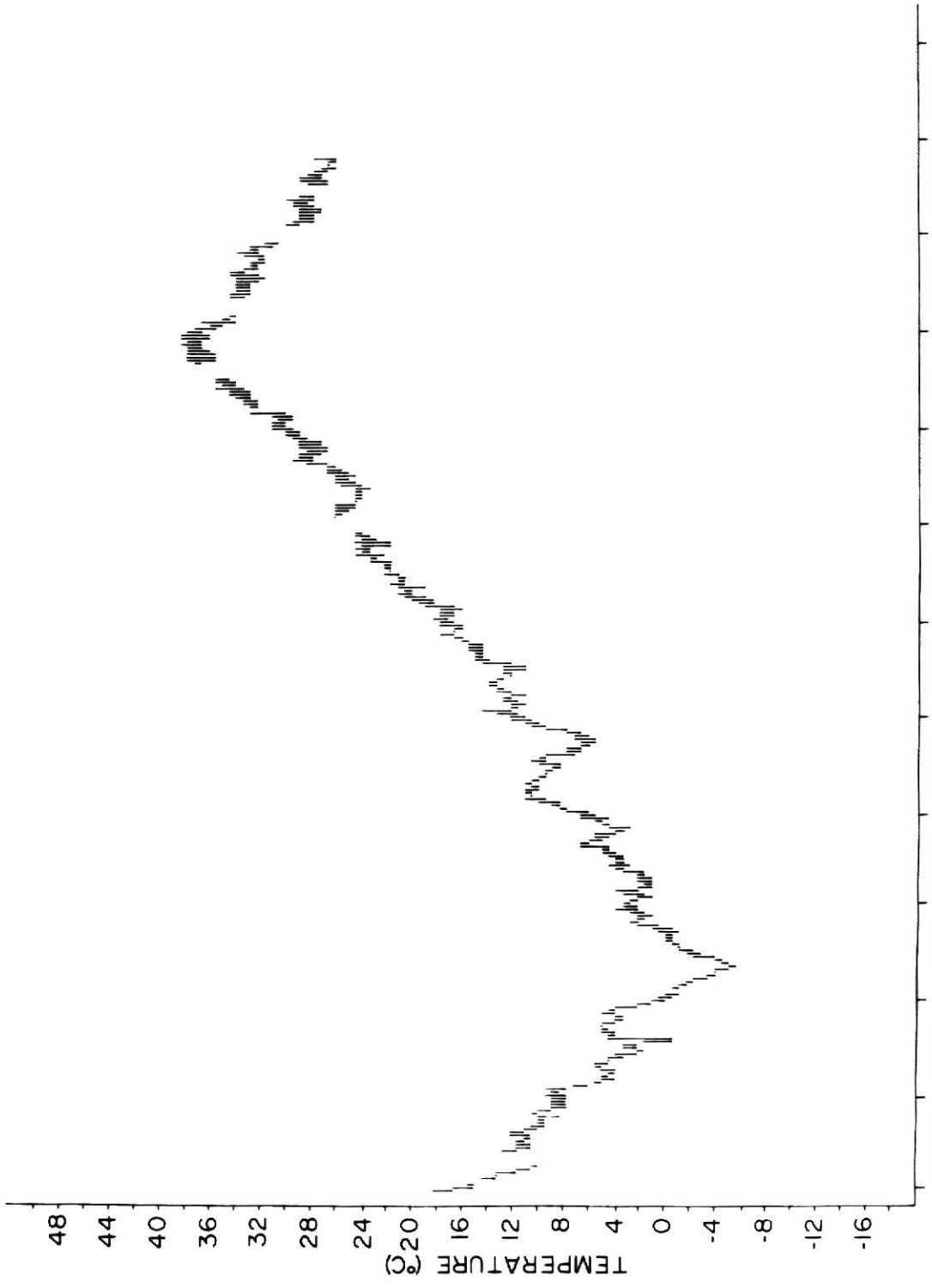
SEPT
OCT
1973
1974

Figure 12. Daily temperature ranges for the period of November 1, 1973, to October 31, 1974, recorded by a 7-day temperature recorder with the sensor placed 30.0 cm below the grain surface in a 1200-bu farm storage bin containing 760 bu of wheat.


TEMPERATURE (°C)
48
44
40
36
32
28
24
20
16
12
8
4
0
-4
-8
-12
-16
NOV
DEC
1973
JAN
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sun and a fairly rapid loss of heat at night. The insulating capacity of the grain prevented the temperature at the surface of the grain mass (Fig. 9) from reaching the daily or seasonal extremes recorded in the bin head space or the outside air. This insulating effect of the grain increased at greater depths in the grain, with the least daily and seasonal fluctuation occurring at 30.0 cm below the surface (Figs. 10, 11, and 12). The mean daily temperature ranges at each depth were: surface, 8.9°, SD=3.8°C; 7.5 cm, 2.9°, SD=1.2°C; 15.0 cm, 1.6°, SD=0.8°C; and 30.0 cm, 1.1°, SD=0.6°C. The lowest winter temperature and the highest summer temperature reached at each depth were: surface, -19° and 48°C; 7.5 cm, -13° and 40°C; 15.0 cm, -10° and 38°C; and 30.0 cm, -6° and 38°C. These temperature extremes were reached infrequently. However, temperatures above 30°C, which might be expected to have a detrimental effect on pathogen stability, were achieved on ca. 100 days at the grain surface, ca. 65 days at a depth of 7.5 cm, ca. 60 days at 15.0 cm, and ca. 55 days at 30.0 cm.

Weekly mean temperatures for each quadrant of the grain bin were calculated from the daily temperature levels indicated by the thermocouples placed around the perimeter of the bin at depths of 15.0 and 30.0 cm (Table 1). The highest weekly mean temperature occurred in the 2 southern quadrants most of the time while the lowest temperature occurred in the 2 northern quadrants (Table 2), but the differences between quadrants were negligible. The average range of the weekly mean temperature between quadrants was only 0.8°C (SD=0.3°C) at the 15.0-cm depth and 1.0°C (SD=0.4°C) at the 30.0-cm depth. Because the variation around the bin perimeter was so slight, the temperature levels recorded at single points by the 7-day recorders were considered to be

Table 1. Weekly mean temperature measured between 1000 and 1400 hr each day at 15.0 and 30.0 cm below the grain surface in each quadrant of a 1200-bu farm storage bin containing 760 bu of wheat (3 thermocouples/quadrant at each depth).

Week beginning	Weekly mean temperature (°C) at depth of...							
	15.0 cm				30.0 cm			
	SE	SW	NW	NE	SE	SW	NW	NE[f]
Nov 4	11.7	11.7	10.8	10.8	15.3	15.6	14.4	14.4
11	13.1	13.1	12.8	12.8	14.4	14.7	13.9	13.9
18	12.5	12.8	12.2	11.9	13.9	14.4	13.3	13.1
25	10.8	11.4	10.6	10.3	11.9	12.5	11.7	11.4
Dec 2	9.7	10.0	9.7	9.7	11.9	12.2	11.7	11.1
9	7.2	7.2	6.9	6.4	8.9	10.0	8.6	7.8
16[a]	4.2	4.2	3.9	3.9	6.7	8.1	6.9	6.1
23[b]	5.0	5.8	5.0	5.3	6.1	6.7	5.0	5.3
30	0.6	0.6	0.3	0.0	3.3	4.2	3.6	3.1
Jan 6	-3.3	-3.1	-3.1	-3.1	-0.6	0.6	-0.3	-1.4
13	-0.8	-0.8	-1.7	-1.4	-1.4	-0.6	-1.7	-1.9
20	2.8	2.5	1.9	2.5	2.2	2.2	1.4	1.4
27	4.7	4.7	3.9	4.4	4.2	4.4	3.3	3.1
Feb 3	3.6	3.6	3.1	3.3	4.2	4.7	3.6	3.3
10	5.0	4.7	3.9	4.4	4.4	4.7	3.6	3.3
17	7.2	6.9	6.1	6.7	6.1	6.1	5.6	5.3
24	6.1	6.4	5.3	5.6	6.1	6.4	5.6	5.3
Mar 3	11.7	11.4	10.3	10.6	9.4	9.4	8.3	8.1
10[a]	11.4	11.1	10.6	11.1	11.4	11.4	10.8	10.6
17	9.2	8.9	8.9	8.9	9.7	10.0	9.4	9.4
24	6.9	8.1	7.5	7.5	7.8	8.1	7.5	7.2
31[a]	13.3	12.8	12.5	12.8	11.9	11.7	11.4	11.1
Apr 7	13.3	13.3	12.8	13.1	12.5	12.5	12.2	11.9
14[a]	13.3	13.6	12.8	13.1	12.8	13.1	12.8	12.8
21	16.4	16.4	15.8	15.8	15.3	15.0	13.9	14.2
28[a]	18.1	18.1	17.5	17.5	17.2	16.9	16.1	16.4
May 5[c]	18.6	18.6	17.8	18.1	17.8	17.5	16.9	17.2
12	21.1	20.6	20.0	20.3	19.7	19.2	18.9	18.9
19	23.6	23.3	22.8	22.8	21.9	21.7	21.4	21.4
26[a]	23.9	23.1	22.8	23.1	23.1	22.8	22.2	22.8
June 2[c]	24.4	23.6	23.9	23.9	23.9	23.3	22.8	23.6
9	23.6	23.1	22.8	22.8	23.3	23.3	22.5	22.8
16	26.1	25.0	25.0	25.3	24.7	24.2	23.9	24.4
23[a]	26.7	25.8	26.1	26.7	26.4	25.6	25.3	26.1
30	28.1	26.9	27.2	27.8	26.9	26.4	26.1	26.9
July 7	30.0	28.9	29.2	29.7	28.6	27.8	27.8	28.6
14[a]	31.7	30.6	30.8	31.4	30.6	29.7	29.7	30.6
21[a]	32.8	31.7	31.9	32.5	31.9	30.8	30.8	31.7
28	31.7	31.7	31.9	32.5	32.2	31.4	31.4	31.9

Table 1. (concluded).

Week beginning	Weekly mean temperature (°C) at depth of...							
	15.0 cm				30.0 cm			
	SE	SW	NW	NE	SE	SW	NW	NE[f]
Aug 4[d]	28.9	28.9	28.9	28.9	30.3	29.7	29.7	30.0
11	28.9	28.6	28.9	28.9	28.9	28.6	28.6	28.9
18[c]	28.6	28.6	28.6	28.6	28.9	28.9	28.6	28.6
25[a]	27.8	27.8	27.5	27.8	28.3	28.3	27.8	27.8
Sept 1[c]	23.3	23.9	23.6	23.6	25.3	25.3	25.0	25.0
8[e]	23.9	24.2	23.6	23.9	24.7	24.7	24.2	24.2
15[b]	23.6	23.9	23.1	23.3	23.9	23.9	23.1	23.3
22	--	--	--	--	--	--	--	--
29[b]	22.8	23.3	22.8	22.5	24.4	24.4	23.9	23.9
Oct 6[b]	23.9	24.2	23.6	23.9	24.7	24.7	24.2	24.2
13[d]	23.3	23.6	23.1	23.1	24.2	24.2	23.3	23.6

a/ Temperature measurements were made on only 6 days.

b/ Temperature measurements were made on only 3 days.

c/ Temperature measurements were made on only 5 days.

d/ Temperature measurements were made on only 4 days.

e/ Temperature measurements were made on only 1 day.

f/ Only 2 thermocouples.

Table 2. Percentage of time that the highest and lowest weekly mean temperatures at depths of 15.0 and 30.0 cm occurred in each quadrant of a 1200-bu farm storage bin containing 760 bu of wheat.

	% Of time the temperature was...			
	Highest		Lowest	
Quadrant	15.0 cm	30.0 cm	15.0 cm	30.0 cm
SE	51	45	9	1
SW	43	51	17	6
NW	2	0	53	43
NE	4	4	21	50

representative of the temperature levels around the bin at each depth.

The moisture content of samples of wheat stored in the farm grain bin decreased throughout the study until they equilibrated at a point near 12.0% (Table 3). The moisture content was slightly higher at the surface of the grain mass than at greater depths in the winter and was lower in the summer. Ignoffo (1964) demonstrated that the insecticidal activity of B. thuringiensis was reduced by very high moisture but was relatively unaffected by low moisture. Because the moisture of the grain mass was relatively low, little effect on the pathogens was expected.

The toxicity of B. thuringiensis to the Indian meal moth was not appreciably reduced by storage of treated wheat in the grain bin (Table 4). Ca. 98% of the original toxicity remained after a year of storage and there was no apparent difference in insecticidal activity of samples held at different depths even though the temperature fluctuations at the surface of the grain mass were more extreme than at other depths. Also, mortality levels in the samples placed in the bin in November and stored for 90 and 180 days (winter months) decreased about the same as the samples placed in the bin in May and stored for the same lengths of time (summer months), and there was no further decrease from 180 to 360 days of storage. Thus, even though the temperature at the grain surface reached levels as high as 38°C on 39 days during the summer months, the rate of deterioration of the B. thuringiensis treatment did not increase. Most of the decrease which occurred appeared to take place during the first 180 days of storage, regardless of temperature. Furthermore, some of the samples stored in the laboratory under constant conditions of 25°C and 60% RH during the

Table 3. Periodic moisture contents of 300-g samples of wheat in Mason jars stored in the laboratory (check) and in cotton bags stored at different depths in a farm grain bin containing wheat.[a/]

Depth (cm)	Samples prepared in November					Samples prepared in May		
	0 (Nov)	90 (Feb)	180 (May)	270 (Aug)	360 (Nov)	0 (May)	90 (Aug)	180 (Nov)
check	--	11.6	11.5	12.1	11.6	13.7	12.6	11.8
0.0	13.6	13.4	12.6	11.0	12.4	13.7	11.2	12.2
7.5	13.6	13.1	12.8	12.0	12.0	13.7	11.8	11.9
15.0	13.6	12.8	12.7	12.2	12.2	13.7	12.4	12.0
22.5	13.4	12.7	12.7	12.5	12.3	13.7	12.6	12.1
30.0	13.5	12.6	12.6	12.5	12.1	13.7	12.7	12.2
37.5	13.6	12.6	12.5	12.6	12.1	13.7	12.6	12.2

a/ Moisture content measured using a Steinlite® Electronic Moisture Tester Model RCT.

Table 4. Persistence of insecticidal activity of Bacillus thuringiensis against Indian meal moths on 100-g samples of wheat stored in a farm grain bin.[a/]

Dose (mg/kg)	Depth (cm)	% Mortality in 2 sets of samples after storage for (days)							
		Samples prepared in November					Samples prepared in May		
		0 (Nov)	90 (Feb)	180 (May)	270 (Aug)	360 (Nov)	0 (May)	90 (Aug)	180 (Nov)
25	check	97	84	84	92	88	90	89	87
	0.0		94	92	97	98		95	98
	7.5		98	95	94	89		94	91
	15.0		94	96	95	95		90	98
	22.5		93	88	93	96		93	92
	30.0		96	95	94	94		92	97
	37.5		96	96	97	99		94	90
50	check	97	95	91	94	100	98	99	89
	0.0		100	100	100	100		100	100
	7.5		100	100	98	98		89	96
	15.0		97	100	100	98		96	96
	22.5		100	98	97	100		98	94
	30.0		100	100	97	98		100	98
	37.5		96	98	96	99		98	98
150	check	100	100	100	100	100	100	99	100
	0.0		100	100	100	100		100	100
	7.5		100	100	100	100		100	100
	15.0		100	100	100	100		100	100
	22.5		100	100	100	100		100	100
	30.0		100	100	100	100		100	100
	37.5		100	100	100	100		100	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 (Abbott 1925).

storage period lost slightly more insecticidal activity than the samples stored in the bin. The loss in activity of these samples also occurred within the first 180 days.

Estimates of the number of viable B. thuringiensis spores on treated samples of wheat after storage in the grain bin for various times are summarized in Table 5. The counts were highly variable, but it appears that a slight reduction in spore viability, amounting to approximately 25% to 30% of the original level, occurred during the storage period. The reduction was not influenced by depth in the grain mass. No data were collected immediately after treatment of samples placed in the grain bin in November, but when the estimated number of viable spores remaining after 180 days of storage are compared to estimates made immediately after treatment of samples prepared in May, it appears that little degradation occurred between November and May. There was a decrease in spore viability from May to November (180 to 360 days) on samples placed in the grain bin in November which compared to the decrease in viability between May and November in the samples prepared in February and in May. Thus, most of the deterioration in spore viability appears to have occurred during the summer months rather than during the winter. There were no noticeable differences between levels of deterioration in spore viability on wheat samples which had been sterilized prior to treatment and those which were unsterilized, and little contamination by other species of bacteria appeared on plates from wheat samples which had not been sterilized prior to treatment.

The granulosis virus appeared to be slightly more susceptible to the storage bin environment than B. thuringiensis (Table 6). The virus deposit at a dose of 0.059 mg/kg lost 5-10% of its original

Table 5. Persistence of Bacillus thuringiensis spores on 100-g samples of wheat stored in a farm grain bin.[a/]

Depth (cm)	Viable spores/mg of wheat in 3 sets of samples after storage for (days)								
	Samples prepared in November			Sterilized samples prepared in February			Samples prepared in May		
	180 (May)	270 (Aug)	360 (Nov)	90 (May)	180 (Aug)	270 (Nov)	0 (May)	90 (Aug)	180 (Nov)
check	887	692	832	821	510	542	873	630	786
0.0	740	454	482	833	413	456		518	620
7.5	879[b/]	592	578	725	546	550		492	699
15.0	822[b/]	534	553	871	481	536		501	686
22.5	913[b/]	598	548	804	498	591		506	593
30.0	908	552	780	786	557	505		513	905
37.5	873	609	600	770	477	500		500	654

a/ Samples of treated wheat were removed from storage, washed with distilled water, and the number of viable spores were estimated using a spread-plate technique on half-strength nutrient agar; values are means of 4 replications.

b/ Mean of only 3 replication.

Table 6. Persistence of insecticidal activity of the granulosis virus against Indian meal moths on 100-g samples stored in a farm grain bin.[a/]

Dose (mg/kg)	Depth (cm)	% Mortality in 2 sets of samples after storage for (days)							
		Samples prepared in November					Samples prepared in May		
		0 (Nov)	90 (Feb)	180 (May)	270 (Aug)	360 (Nov)	0 (May)	90 (Aug)	180 (Nov)
0.059	check	97	100	96	94	91	86	88	100
	0.0		100	97	72	97		72	85
	7.5		100	97	86	91		91	83
	15.0		94	95	89	80		89	76
	22.5		100	89	96	96		92	88
	30.0		100	88	95	93		82	91
	37.5		100	97	87	100		85	88
0.469	check	100	100	100	100	98	100	100	100
	0.0		100	100	100	100		100	100
	7.5		100	100	100	100		100	100
	15.0		100	100	100	100		100	100
	22.5		100	100	100	100		100	100
	30.0		100	100	100	100		98	100
	37.5		100	100	100	100		100	100
1.875	check	100	100	100	100	100	100	100	100
	0.0		100	100	100	100		100	100
	7.5		100	100	100	100		100	100
	15.0		100	100	100	100		100	100
	22.5		100	100	100	100		100	100
	30.0		100	100	100	100		100	100
	37.5		100	100	100	100		100	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 (Abbott 1925).

activity (vs. ca. 2% for B. thuringiensis) during the year of storage of the treated samples in the grain bin and in the laboratory. Most of the loss in activity took place between 180 and 360 days of storage on samples placed in the bin in November. Little loss in activity occurred during 180 days of storage of samples placed in storage in May. This suggests that the decrease in activity was probably related more to time than to temperature or that the older samples were more susceptible to temperature extremes. However, the virus doses used produced mortality levels at or near 100% on most of the samples. Thus, small decreases in activity may not have been detected. As with B. thuringiensis, depth in the grain mass did not appear to affect the amount of the loss in activity.

The data from the field study alone do not permit drawing conclusions as to the precise effects of storage temperature on the persistence of the microbial deposits, but it is apparent that both B. thuringiensis and the granulosis virus will persist on wheat stored in a grain bin under the conditions of this test for up to 1 year without appreciable losses in activity.

Laboratory Study

Under long-term storage at constant temperatures, B. thuringiensis was very stable (Table 7). Insecticidal activity was not appreciably affected by storage of treated wheat at temperatures of 16.5°, 25.0°, or 33.5°C. After storage for 12 weeks at 42.0°C there was a reduction in insecticidal activity. Insect mortality in samples treated with a dose of 50 mg/kg was reduced to 97% of the original activity after 15 weeks of storage and a further gradual reduction to ca. 85% of the original

Table 7. Persistence of insecticidal activity of *Bacillus thuringiensis* against Indian meal moths on wheat stored at constant temperatures.[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
10	16.5		58	36	72	63	60	56	67	73	70	64	71	81	54
	25.0	55	70	79	70	57	62	59	69	58	44	64	70	77	50
	33.5		79	63	72	46	60	57	57	57	47	80	69	56	58
	42.0		78	66	63	45	49	24	42	55	70	53	49	3	25
50	16.5		91	95	95	92	98	98	100	94	97	100	90	100	95
	25.0	95	95	100	100	98	97	98	100	94	98	100	94	98	93
	33.5		100	100	98	96	98	89	100	100	96	100	97	92	89
	42.0		98	94	94	95	92	88	91	83	94	100	88	95	79
150	16.5		100	100	100	98	100	100	100	98	100	100	100	100	100
	25.0	100	100	100	100	100	100	100	98	100	100	100	100	100	100
	33.5		100	100	100	100	100	95	100	97	100	100	100	100	100
	42.0		98	98	98	100	96	100	97	97	100	100	100	95	97

[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 (Abbott 1925).

activity occurred during the remainder of the study. In samples treated with 10 mg/kg the mortality was reduced to ca. 82% of the original activity after 12 weeks of storage with a further reduction to ca. 45% of the original activity during the remainder of the study. Mortality levels in both cases, beginning at 24-27 weeks, returned almost to the original level until after 30 weeks when they again decreased. This is attributed to variation in the test insects. In the field study discussed earlier the temperature in the surface layer of grain in the bin reached 42°C on only 15 days. Thus, the periods of high temperature in the grain storage bin did not persist long enough to cause the same levels of deterioration in insecticidal activity of the B. thuringiensis treatments that occurred in constant high temperature storage.

Spore viability decreased sharply during the first 3 weeks of constant-temperature storage, and further slight reductions occurred during the remainder of the test. This deterioration pattern is similar to that of chemical residues on stored grain (e.g., McGaughey 1971). Table 8 and Fig. 13 illustrate that the amounts of the initial decrease and subsequent gradual decrease were directly related to storage temperature (42.0°>33.5°>25.0°>16.5°C). At 16.5°C the spore viability was reduced to ca. 75% of the original level at 3 weeks with a further decrease to ca. 65% after 42 weeks, while at 42.0°C only ca. 25% of the original spores remained viable after 3 weeks and ca. 10% remained after 42 weeks of storage. The 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 the reduction in spore viability. Reduction of insecticidal activity was noted only

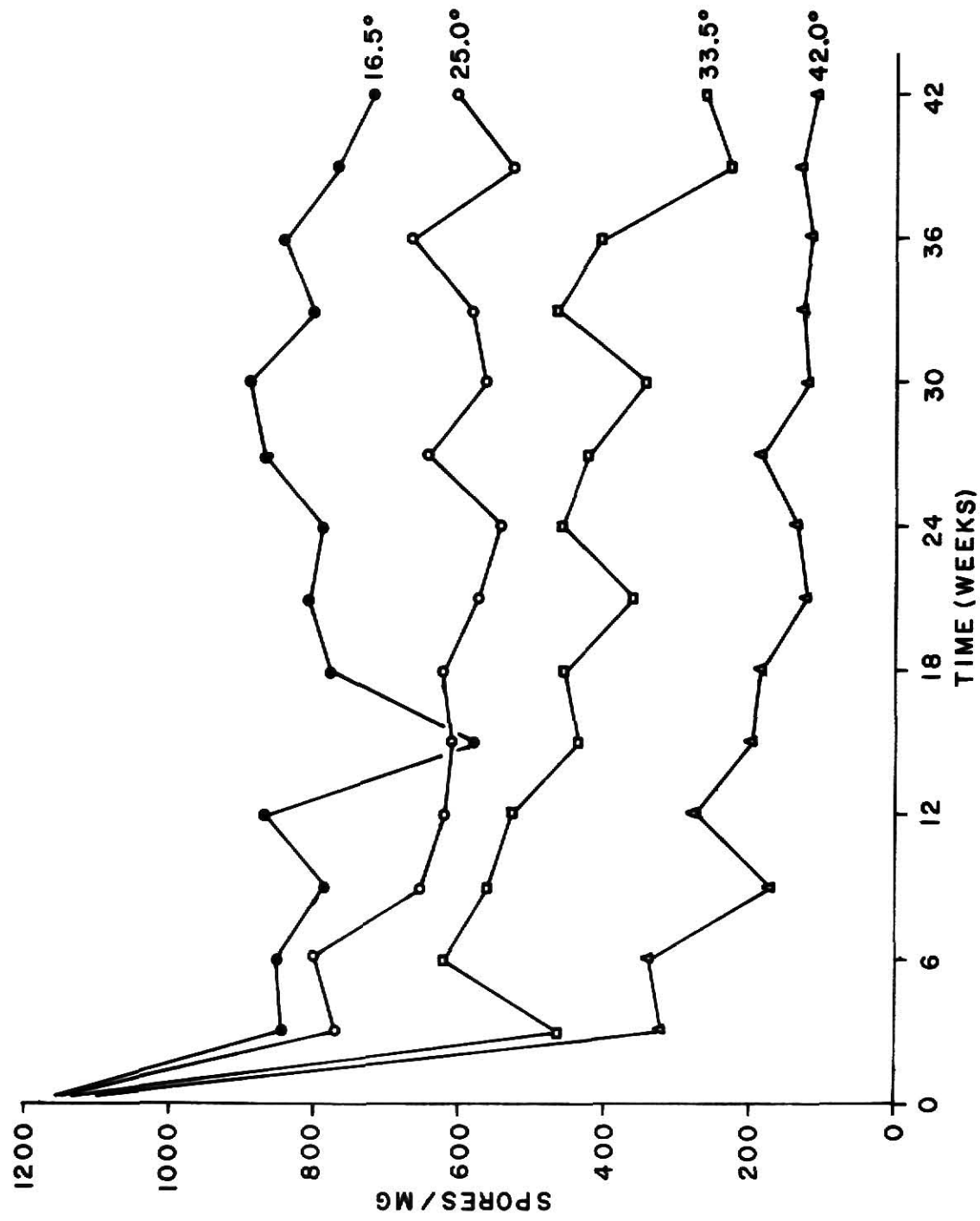
Table 8. Persistence of Bacillus thuringiensis spores on wheat stored at constant temperatures.[a/]

Storage temp.(°C)	% Original number of spores remaining after storage for (weeks)														
	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42
16.5	(1173)[b/]	74	75	69	77	51	69	71	70	77	79	71	74	68	64
25.0		70	73	59	57	55	57	52	49	59	51	53	61	48	55
33.5		40	53	48	45	37	39	31	39	36	29	40	35	20	23
42.0		25	27	14	22	16	14	10	10	15	10	10	9	10	9

a/ Samples of treated wheat were removed from storage, washed with distilled water, and the number of viable spores were estimated using a spread-plate technique on half-strength nutrient agar; values are means of 3 replications.

b/ Viable spores/mg of wheat immediately after treatment.

Figure 13. Persistence of Bacillus thuringiensis spores on wheat stored at constant temperatures.


16.5°
25.0°
33.5°
42.0°
SPORES / MG
TIME (WEEKS)
0
200
400
600
800
1000
1200
6
12
18
24
30
36
42

after the number of viable spores decreased by ca. 80%, in this case to a level below 200 spores/mg. However, when spores are inactivated by methyl bromide (McGaughey 1975b) or by ultra-violet or gamma irradiation (Burges et al. 1975) decreases in insecticidal activity have not been noted. Perhaps the effects of temperature upon B. thuringiensis differ from those of methyl bromide and ultra-violet and gamma irradiation.

The granulosis virus treatment was more susceptible to high temperature storage than was B. thuringiensis, but differences were more difficult to assess, particularly at the lowest dose, because the mortality levels in the samples were much more variable (Table 9). At the 0.059 mg/kg dose, storage at 16.5° and 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. 40-50% of the initial level within 3-9 weeks and a further decrease in activity occurred as storage time increased. At 33.5°C activity decreased to ca. 70% of the original level by the end of 39 weeks of storage. The increased mortality levels that occurred from 24-30 weeks are attributed to variations in the insects. Because even brief storage at 42.0°C caused a large decrease in activity of the virus, it is possible that the 15 days on which the temperature of the surface layer of grain in the farm bin reached 42°C may have caused the slight deterioration observed on samples stored in the grain bin.

Table 9. Persistence of insecticidal activity of the granulosis virus against Indian meal moths on wheat stored at constant temperatures.[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
0.007	16.5		15	0	29	13	41	5	19	51	72	48	38	28	18
	25.0	40	12	31	39	11	45	31	79	9	23	50	0	41	43
	33.5		38	5	2	6	51	14	34	10	61	28	11	12	1
	42.0		45	20	24	11	15	3	6	4	21	24	9	9	16
0.059	16.5		94	87	77	55	80	88	100	98	68	100	92	96	89
	25.0	94	88	92	87	82	81	88	91	67	90	90	88	98	98
	33.5		80	92	70	31	63	48	65	49	96	80	65	89	43
	42.0		44	26	35	0	30	0	3	30	27	62	16	7	5
0.469	16.5		100	100	100	100	100	98	98	100	100	100	100	100	100
	25.0	100	100	100	100	100	100	98	100	100	100	100	100	100	100
	33.5		100	100	100	100	100	100	100	100	100	96	100	92	97
	42.0		97	98	88	92	94	79	48	86	51	48	15	4	3

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 (Abbott 1925).

CONCLUSIONS

The insecticidal activity of B. thuringiensis on wheat is highly resistant to the temperature extremes that are likely to occur in a Kansas farm grain storage bin, but spore viability is less stable. Insecticidal activity did not appear to be related to spore viability, and was reduced only after large reductions in the number of spores had occurred. The periods of high temperature that occurred in the grain storage bin were probably too brief to reduce spore viability to the extent that occurred under constant high temperature storage in the laboratory.

The pathogenicity of deposits of the formulated granulosis virus on wheat was greatly reduced by constant high-temperature storage in the laboratory, but the temperature extremes reached in the grain storage bin were too brief to cause more than slight reductions in activity.

No apparent differences were observed in the stability of either microbial formulation between the surface of the grain mass and the 37.5-cm depth during the storage period.

It appears that both pathogens were sufficiently stable, in the formulations tested, to be used for long-term Indian meal moth control in stored grain. With proper timing of the applications, either pathogen could be expected to afford protection from Indian meal moth infestation for 1 year, and enough residual activity might be present to extend protection for a longer period.

ACKNOWLEDGEMENTS

Financial support and facilities for this study were provided by the U. S. Grain Marketing Research Center, North Central Region, Agricultural Research Service, U. S. Department of Agriculture, through cooperative agreement #12-14-3001-232 with the Department of Entomology, Kansas Agricultural Experiment Station.

I express my gratitude to Dr. William H. McGaughey for his many constructive suggestions and criticisms during the course of this study and preparation of the manuscript.

Dr. Robert B. Mills and Dr. John O. Harris are gratefully acknowledged for reviewing the manuscript and making helpful suggestions.

Acknowledgement is made to Edwin Dicke for assistance in making some insect counts; to Warren Blodgett for help in obtaining grain used in the field study; and to Donald Bechtel for taking the photographs used in the manuscript.

Scientists at the Stored-Product Insects Research and Development Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Savannah, Georgia, are acknowledged for irradiating wheat samples for use in part of this study.

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STABILITY OF BACILLUS THURINGIENSIS AND A GRANULOSIS VIRUS OF PLODIA INTERPUNCTELLA (HUBNER) ON STORED WHEAT

by

ROBERT ALLAN KINSINGER

B. S. Northeast Missouri State University, 1973

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1975

The stability of microbial insecticides on stored grain is a determining factor in their use for controlling stored-product insects. In this study the persistence of Bacillus thuringiensis and a granulosis virus of the Indian meal moth, Plodia interpunctella, was evaluated on stored wheat.

The microorganisms were applied to 100-g samples of wheat which were stored at the grain surface and at depths of 7.5, 15.0, 22.5, 30.0, and 37.5 cm in a 1200-bu farm grain storage bin containing 760 bu of wheat for periods of up to 1 year. Supporting studies were conducted in the laboratory under constant-temperature storage at 16.5°, 25.0°, 33.5°, and 42.0°C. Indian meal moth mortality and B. thuringiensis spore viability were measured at appropriate intervals to estimate the rate of deterioration of the treatments in relation to storage time and temperature.

The microbial formulations proved to be stable on the wheat samples stored in the farm grain bin. The viability of B. thuringiensis spores was reduced by ca. 25-30% during a year of storage but the insecticidal activity decreased only by ca. 2%. The granulosis virus appeared somewhat less stable, but the reduction in insecticidal activity of the virus was only ca. 5-10% over the period of a year.

Under constant-temperature storage the rate of decrease of B. thuringiensis spore viability was directly related to storage temperature, but a corresponding decrease in insecticidal activity was noted only at 42.0°C after a large reduction in spore viability had occurred. Insecticidal activity of the granulosis virus was reduced by <5% at 16.5°C but increasingly greater deterioration occurred at higher temperatures. At 42.0°C nearly total inactivation had occurred by the

end of 39 weeks of storage.

Even though temperatures as high as 42°C were reached on 15 days and 30°C on 50-100 days in the grain bin, these temperature levels apparently did not persist long enough to have an appreciable effect on either microbial formulation. Thus, both B. thuringiensis and the granulosis virus could be expected to persist and afford protection from Indian meal moth infestation for at least 1 year when applied to stored grain.