BACILLUS THURINGIENSIS: A CRITICAL REVIEW

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Abstract—Bacillus thuringiensis (BT), a bacterium that has wide applications in pest control on crops, ornamentals, and forests, is the first microorganism to be approved for use on stored grain. BT seems ideally suited for use on stored grain and seed. It is compatible with most protectants, fumigants, and seed fungicides, and the deposits on grain remain active indefinitely except under extremely high temperature conditions. BT is available in dust, wettable powder, and liquid formulations that can be mixed with grain in augers or other handling equipment or applied directly to the surface of grain in storage. In the USA BT is exempt from requirements for a tolerance.

Laboratory and pilot studies have shown that BT applied to the surface layer of stored grain will provide up to 95% control of infestations of Plodia interpunctella and Cadra cautella. Sitotroga cerealella is somewhat less susceptible and coleopteran species are not affected. Control levels for P. interpunctella in farm bins and elevators in the USA have varied widely, but usually have been lower than in smaller scale tests. In a few instances BT has not provided any control. We have recently found that P. interpunctella can develop high levels of resistance to BT within a few generations, which may account for the wide variation in control in grain bins. However, the extent to which resistance may become a problem under actual grain storage conditions is not fully known.

For over thirty years pathogenic microorganisms have been studied as potential alternatives to chemical insecticides for controlling pest insects. This interest has been stimulated by the real and perceived problems associated with the post-WW II insecticides, including environmental hazards, applicator safety, destruction of beneficial predators and parasites, and insecticide resistance. Insect pathogens have been advanced as alternatives that were believed to be free of most if not all of these problems because of their relative specificity for a few or sometimes only a single pest species, and because of their natural occurrence in the environment without any observed harmful effects. In addition, many scientists and environmentalists have come to believe that insect pathogens would be free of the resistance problem that has been the ultimate fate of most man-made chemicals. Resistance to microorganisms has not been a problem so far, but recent reports suggest that it will probably become one (Briese, 1981; McGaughey, 1985a).

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Pests of grain and other stored commodities have not been overlooked in this search for pathogens that could be used to mitigate insect damage. However, developmental research on controlling storage pests with microorganisms has lagged because of the narrow host spectrum of these agents. The most promising pathogens affect only the Lepidoptera, which in many instances (grain, for example) are minor pests of stored commodities. Because of this limited host range, for many years pathogens were presumed to be of little value in stored commodities where several insect species frequently occur simultaneously. In fact, over twenty years ago in a discussion of the potential of *Bacillus thuringiensis* for controlling storage pests, Burges (1964) acknowledged this disadvantage of pathogens when he stated that the "specificity of this bacterium almost entirely to Lepidoptera is a great disadvantage in food stores, where Coleoptera are common and where there are no predatory insects worth preserving." Nevertheless, increasing problems with chemical usage, the slow progression of research on other alternatives, and advances in the use of insect pathogens for controlling plant and human pests encouraged continued research on the use of these microorganisms for controlling storage pests.

Most of the attention has focused on the bacteria, viruses, and protozoa. The viruses and bacteria seem to hold the most immediate promise for use against storage pests and have received the most attention because of their relative ease of manipulation and their often acute effects on pest populations. The protozoa are common among both coleopteran and lepidopteran pests of stored commodities, as they are among many other pest species. The protozoa have been shown to have chronic, debilitating effects on pest populations, reducing population vigor and increasing susceptibility to other pathogens, chemical insecticides, and environmental stress. Thus far, however, their potential for manipulation and use to control storage pests has not been extensively explored despite their abundance and several instances of successful use against certain nonstorage pests. The insect-pathogenic fungi and nematodes are rare among storage pests, probably because of the low moisture content of the commodity storage environment.

*Bacillus thuringiensis* (BT) is the most widely used and intensively studied of the microbial insecticides. Dr. Ernst Berliner made the primary description of the bacterium in a culture from diseased Mediterranean flour moths, *Anagasta kuehniella* (Zeller), in 1911, although the bacterium was probably discovered several years earlier in Japan in diseased larvae of the silkworm, *Bombyx mori* (Linnaeus). BT is a spore-forming bacterium that produces several insecticidal materials, two of which have been studied extensively: the δ-endotoxin and the θ-exotoxin. The crystalline δ-endotoxin is the one that is widely used in agriculture.

Most of the literature relevant to the use of BT for controlling pests of stored grain has been reviewed recently by Subramanyam and Cutkomp (1985). The early studies, particularly the comprehensive ones by Jacobs (1950) and Steinhaus and Bell (1953), clearly established the
susceptibility of *Anagasta kuehniella* and the general absence of susceptibility among coleopteran species. Subsequently, studies by Kantack (1959), van der Laan and Wassink (1964), Burges (1964), Godavaribai et al. (1962), and others explored the BT susceptibility of several other lepidopteran storage pests, including *Plodia interpunctella* (Hubner), *Cadra cautella* (Walker), *Achroia grisella* (Fabricius), and *Galleria mellonella* (Linnaeus). In addition, studies by Flanders and Hall (1965) and Burges and Hurst (1977) explored the role of BT epizootics as a regulatory mechanism in self-perpetuating populations of these storage moths. However, the studies done prior to 1972 involved laboratory preparations or commercial formulations of BT that had relatively low potency and often poorly defined composition, and which are no longer available. Since 1972, most commercial formulations have been produced from the much more toxic isolate HD-1 of *Bacillus thuringiensis* subsp. *kurstaki* (Dulmage, 1970; de Barjac and LeMille, 1970). The studies on controlling storage pests with these more potent and better standardized formulations are summarized in Table 1.

The recent studies on BT, most of which were conducted at the USDA, Agricultural Research Service, U.S. Grain Marketing Research Laboratory in Manhattan, Kansas, constitute a body of knowledge as comprehensive as that available for any other stored grain insecticide. As a result of these studies, in 1979 the U.S. Environmental Protection Agency granted approval for the use of Dipel® WP (Abbott Laboratories), a formulation of *Bacillus thuringiensis* subsp. *kurstaki*, for controlling moth infestations in stored grains and soybeans. Approval has subsequently been extended to several other BT formulations and commodity uses. This was the first and remains the only approved use of a microbial formulation for protecting stored commodities from insect infestation.

These USDA studies were concerned with the control of Indianmeal moth, *Plodia interpunctella*, and almond moth, *Cadra cautella*, infestations in bulk stored commodities, primarily grain. In bulk grain these species infest primarily the surface layer of the grain, and in the USA have traditionally been combated by surface applications of malathion or synergized pyrethrins, or the use of DDVP resin strips. Both species have become highly resistant to malathion and pyrethrins. Laboratory studies demonstrated that a top-dressing treatment of the 10-cm deep surface layer of grain with BT at a dosage of ca. 125 mg/kg was an effective preventive measure (McGaughey, 1976). Such treatments were effective against both the almond moth and Indianmeal moth on a wide range of commodities, but showed only limited potential against the Angoumois grain moth, *Sitotroga cerealella* (Olivier), another insecticide resistant pest, because the larvae of that species feed inside the grain kernels (McGaughey, 1976; Nwanze et al., 1975; McGaughey and Kinsinger, 1978). Applications were most effective against all species when made prior to infestation of the grain because the early larval instars were much more susceptible than the later instars (McGaughey, 1978c).
The formulations currently approved for use have little or no effect on the major coleopteran pests of stored commodities (McGaughey et al., 1975; McGaughey, 1976). Earlier reports which suggest the presence of coleopteran activity in BT were undoubtedly based on impure formulations containing other toxins. Because of the absence of toxicity toward coleopteran species, the continued use of other control measures including chemical protectants and fumigants would be necessary to control those pests in the grain. Compatibility of BT with the other insecticides would therefore be essential. Although there are some conflicting reports in the literature, BT appears to be compatible with malathion and synergized pyrethrins (Dougherty et al., 1971; Sutter et al., 1971; Morris, 1972). Data on compatibility of BT with other grain protectants is lacking. BT does appear to be compatible with major grain fumigants, including phosphine (McGaughey, 1975). Methyl bromide causes some decrease in BT spore viability. This is of little consequence when BT is used to control almond moths because this species is affected exclusively by the parasporal crystals (McGaughey, 1978a). However, against Indianmeal moths the BT spores enhance toxicity and methyl bromide could be detrimental although we did not observe any significant effect. BT is also compatible with the seed fungicide captan, which is commonly used for treating crop seed prior to planting (McGaughey, 1983). Thus BT is a suitable protectant for use on crop seeds which are often treated and stored in bags where they are highly susceptible to damage by moth larvae feeding on the germ.

BT is remarkably stable on stored grain where it is protected from the UV radiation which causes rapid loss in activity under outdoor conditions. Studies have shown some loss in spore viability immediately after application, but little or no further decline in either spore viability or efficacy on grain for periods of up to two years (Kinsinger and McGaughey, 1976; McGaughey, 1978b, 1980). Laboratory studies indicate that in some climates storage temperatures could be high enough to reduce longevity, but the storage temperatures prevalent in the central U.S. have not caused any problems.

Dust, wettable powder, and liquid formulations of BT are available for use on stored grain. The liquid formulations are much easier to mix with water for application, but their use so far is limited to seed that is intended for planting. The dust and wettable powder formulations can be used for either crop seed or stored food grain. In our studies they have proved equally effective for protecting stored wheat and corn (McGaughey, 1976, 1985b). However, in some instances the dust formulation was easier to apply, and on inshell peanuts it gave better protection than the wettable powder because it penetrated into cracked pods more readily than the aqueous spray (McGaughey, 1982). Either formulation can be applied to grain or other seeds by spraying, sprinkling, or dusting onto the surface of the grain bulk and mixing with a rake to the recommended depth of 10 cm, or the formulations can be mixed with the grain in augers or other handling equipment as the last layer of the grain is elevated into the storage bins (McGaughey and Dicke, 1980; McGaughey, 1985b). Both methods are labor intensive and have proved to be a detriment to use of BT on grain. A less labor
intensive but not yet approved method for applying the dust formulation involves using high air velocity grain drying fans to draw airborne dust downward from the overspace into the grain (McGaughey, 1986). This technique has been effective in very limited trials in corn, but further testing is needed. This method will probably be more effective in commodities with large kernels or pods.

In pilot-scale tests using bins of ca. 2 m³ capacity surface-layer applications of BT have provided 85-95% control of Indianmeal moths and almond moths in both wheat and corn (McGaughey, 1978b, 1980). These levels of control persisted for two storage seasons. Data from field-scale usage are limited. Studies in Germany using Thuricide® on rye showed good long-term control of Indianmeal moths (Schmidt and Wohlgemuth, 1979). However, in my studies levels of control of Indianmeal moths in farm grain bins and elevator silos treated with Dipel were lower than in the pilot tests and they were quite variable (McGaughey, 1985b). The variable levels of control may have resulted from differences in the susceptibility of the native moth populations to BT. Our studies have shown that the sensitivity of different populations of Indianmeal moths or almond moths to the same BT preparation can differ by as much as 7 to 10 fold (Kinsinger and McGaughey, 1979a).

Moreover, our recent studies have shown that Indianmeal moths have the capacity to develop high levels of resistance to BT within only a few generations (McGaughey, 1985a). This is an unprecedented and unexpected observation. Many scientists believed that insect resistance to the spore and δ-endotoxin complex of BT was unlikely to occur. Our studies showed slight but statistically significant levels of resistance in Indianmeal moth populations collected from grain bins only a few months after treatment of the grain. Laboratory studies with five different colonies of these moths from four states confirmed that resistance levels of nearly 30 fold could develop in as little as two generations of rearing on BT-treated diet, and levels >100 fold could be attained within 15 generations.

The physiological and genetic mechanisms and the practical significance of this BT resistance are unknown. However, both are being intensively studied. Work by Dulmage (1981) and collaborators as well as our own studies (Kinsinger et al., 1980; McGaughey and Dicke, unpublished data) show that BT isolates differ extensively in their host spectrum and potency. This suggests that there is almost unlimited potential for identifying BT isolates and producing formulations which will overcome this as well as future cases of BT resistance.

This resistance phenomenon also provides a new approach for investigating the mechanisms of BT toxicity in insect larvae. Knowledge of the mechanism of this resistance could lead eventually to an understanding of the mechanisms controlling the host specificity of the organism. Once this is achieved there are almost limitless possibilities for using both conventional genetic techniques and genetic engineering technology to produce BT toxins with activity
against a broader range of pest species, including the coleopteran pests of stored grain. The potential for using BT for controlling coleopteran pests has already been demonstrated with the recent reports on *B. thuringiensis* subsp. *tenebrionis* by Krieg *et al.* (1983) in West Germany, and on a similar if not identical Coleoptera-active isolate by Herrnstadt *et al.* (1986) in the U.S.

So far BT has been used only on grain, but there is also great potential for using existing BT preparations for controlling pests in the wide range of raw and processed commodities in which lepidopteran pests predominate or appear exclusively. BT spores are readily killed in cooking cereal products and have no effect on baking properties of flour (McGaughey *et al.*, 1980). For current uses in the USA BT is exempt from requirements for a tolerance. For situations where the bacterial spores are objectionable, sporeless mutants of BT are known (Nishiitsutsuji-Uwo *et al.*, 1975); the potential usefulness of these mutants against both storage and agronomic pests has already been documented along with the relative roles of spores and crystals in controlling a large number of lepidopteran pests, including the major lepidopteran pests of stored commodities (McGaughey, 1978a; Burges *et al.*, 1976; Nishiitsutsuji-Uwo and Endo, 1980; Johnson *et al.*, 1980; Johnson and Freedman, 1981; Johnson and McGaughey, 1984).

The full potential for using microbial insecticides for protecting stored commodities is far from being fully realized. Our discipline has made some significant strides in the discovery and description of viruses, bacteria, and protozoa that infect our pest insects. However, we have seen only one product (BT) registered for use. That attempt has not been without problems, but it has demonstrated that microbial insecticides are a viable alternative to the broad spectrum chemicals that we have relied upon for over 50 years. Furthermore, the work has demonstrated that microorganisms are compatible with other technologies in our pest management systems and that they are cost-competitive with our traditional measures. It has also shown that our storage facilities provide an ideal environment for using microorganisms, an environment where the organisms will remain viable and provide long-term insect control.

The next step is to begin to exploit the great genetic diversity that exists among BT and other pathogenic microorganisms to produce more potent formulations targeted for use in specific situations where susceptible species predominate, where the susceptible species have acquired resistance to existing insecticides, or where the facilities, marketing procedures, management expertise, or socio-economic conditions do not lend themselves to using sophisticated technologies. (The microbial insecticides do not require special facilities, equipment, or safety precautions.) The biotechnology is now available for altering the host specificity of bacteria and viruses to control previously nonsusceptible pest species. The prevailing opinion is that future biological insecticides will not be based on new discoveries. They will most likely result from improvements upon pathogens such as BT that have already demonstrated commercial potential, and about which much is already known (Kirschbaum, 1985). Future success in using BT
and other microorganisms for controlling storage pests does not depend upon changing our marketing system or future scientific breakthroughs, although these factors may be important. Rather, our success with these agents depends upon whether we choose to use the organisms, knowledge, and technology now available.

Table I. Summary of recent studies on commercially-available formulations of Bacillus thuringiensis for controlling storage pests.a

<table>
<thead>
<tr>
<th>Nature of study</th>
<th>References</th>
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<tr>
<td>Toxicology and histopathology</td>
<td>McGaughey, 1978a, 1978c; Kinsinger and McGaughey, 1979b, Johnson and McGaughey, 1984</td>
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<td>Susceptibility of different moth populations</td>
<td>Kinsinger and McGaughey, 1979a, Kinsinger et al., 1980; Mardan and Haretn, 1984</td>
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<td>Compatibility with other insecticides and fungicides</td>
<td>McGaughey, 1975, 1983</td>
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<td>Fate of spores in processed grain</td>
<td>McGaughey et al., 1975, 1980</td>
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<td>Formulation and application to grain</td>
<td>McGaughey, 1976, McGaughey and Dicke, 1980; McGaughey, 1985b, 1986</td>
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<td>Pilot scale tests</td>
<td>McGaughey, 1978b, 1980, Kramer et al., 1985</td>
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<tr>
<td>Field tests</td>
<td>Schmidt and Wohlgemuth, 1979; McGaughey, 1985b</td>
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<td>Insect resistance</td>
<td>Briese, 1981, McGaughey, 1985a, 1985b</td>
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a Primarily formulations produced from isolate HD-1 of Bacillus thuringiensis subsp. kurstaki (Dulmage, 1970, de Barjac and Lemille, 1970).
References


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Morris, O. N. (1972) Susceptibility of some forest insects to mixtures of commercial Bacillus thuringiensis and chemical insecticides, and sensitivity of the pathogen to the insecticides. Can. Ent. 104, 1419-1425.


