The susceptibility of the bertha armyworm *Mamestra configurata* Walker (Lepidoptera, Noctuidae) to 61 strains of *Bacillus thuringiensis* Berliner.

A Thesis

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of Graduate Studies

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by

© Marc Roger Trottier

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THE SUSCEPTIBILITY OF THE BERTHA ARMYWORM, MAMESTRA CONFIGURATA WALKER (LEPIDOPTERA, NOCTUIDAE) TO 61 STRAINS OF BACILLUS THURINGIENSIS BERLINER

BY

MARC ROGER TROTTIER

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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To good friends,

and especially for Dad.

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ABSTRACT

Trottier, Marc Roger, M.Sc., The University of Manitoba, May, 1986

The susceptibility of the bertha armyworm, *Mamestra configurata* Walker (Lepidoptera, Noctuidae) to 61 strains of *Bacillus thuringiensis* Berliner.

Sixty-one strains of *B. thuringiensis (B.t.)* belonging to varieties *thuringiensis, alesti, kurstaki, dendrolimus, kenyae, galleriae, canadensis, entomocidus, aizawai,* and *tolworthi* were bioassayed against early third instar larvae of *M. configurata.* Larvae were only weakly susceptible to the reference standard, *B.t.* var. *kurstaki* strain HD-1-S-1980. A median lethal concentraion of 15400 IU/ml was determined. Eleven strains, which mostly belonged to variety *aizawai*, were significantly more toxic for larvae than the reference standard. Strains HD-133, HD-551, and HD-854, were most potent overall with two to four fold greater potency than the reference standard. Survivors of *B.t.* treatments gained significantly less weight than did survivors of control treatments. Survivors of treatments with more toxic strains gained signicantly less weight than did survivors of treatments of the reference standard. The implications of *B.t.* strains with two to four fold greater potency are dicussed in terms of their potential role in the management of populations of *M. configurata.*

1. Introduction

1.1 *M. configurata*: Economic Importance, Life History, and Population Dynamics

Outbreaks of the bertha armyworm, *Mamestra configurata* Walker (Lepidoptera, Noctuidae) have occurred periodically in the parkland area of the Canadian prairies since 1921 (King 1928; Beirne 1971; Turnock and Philip 1977; Turnock 1984). The larvae feed on a variety of cultivated plants and introduced weeds (Beirne 1971), and as of 1971 this noctuid has been recognized as a major pest of rapeseed, *Brassica napus* (L.) and *B. campestris* L., the dominant oilseed crops of Canada. There have been two recent outbreaks of *M. configurata* on these crops, a severe and widespread one from 1970 to 1973, and a smaller and more restricted one from 1979 to 1982 (Turnock and Philip 1977; Anonymous 1969-1983; Lamb *et al.* 1985).

In Canada, *M. configurata* is univoltine and overwinters in the pupal stage (King 1928). The adults emerge from early June until early August, and eggs are oviposited on the underside of the leaves of host plants soon after adult emergence. The eggs hatch within a week and larvae pass through six larval instars. There is synchrony between *M. configurata* and the rapeseed crop in that the last two larval instars are usually present when the plants have finished flowering and the pods are maturing (Bracken 1984). Most of the rapeseed yield loss is attributed to direct feeding on the pods by these two larval instars which ascend the plants and feed indiscriminantly on leaves, bracts and pods (Bracken 1984). In late August and September, fully developed larvae enter the soil, form a cell at depths down to 15 cm from the soil

surface, and pupate after a short prepupal period.

The population dynamics of *M. configurata* on the rapeseed crop is characterized by a short period of very abundant populations followed by an interval with generally low populations and local outbreaks (Turnock 1977). The spatial distribution of outbreaks is attributed to winter mortality due to low soil temperatures, while the timing of outbreaks is affected by undetermined weather factors (Lamb *et al.* 1985). Factors which appear to contribute to the termination of outbreaks are: heavy parasitism, reduced adult emergence and oviposition as a result of inclement weather, reduced early larval survival due to cool wet weather and invertebrate predation, and early crop harvest, pathogens, and bird predation of later instars (Turnock 1977).

The two most important parasitoids of the 15 identified for *M. configurata* are the ichneumonid wasp, *Banchus flavescens* Cress., which killed over 40% of larvae collected during 1973-1975, and the tachinid, *Athrycia cinerea* (Coq.), which killed over 20% of larvae collected from 1972-1975 (Wylie and Bucher 1977; Turnock 1984). The main larval pathogens are fungi of the genus *Entomophthora* and a nuclear polyhedrosis virus which contributed 7.5 and 16.4% of the mortality respectively of field-collected larvae (Wylie and Bucher 1977). Other pathogens include a granulosis virus and a microsporidian which probably belongs to the genus *Nosema* (Wylie and Bucher 1977).

Recommended control measures for *M. configurata* in Manitoba are the application of methomyl (1.25 l/ha Lannate L®), chlorpyriphos (0.75-1.0 l/ha Lorsban 4E®), or methamidophos (0.55-1.1 l/ha Monitor 4.8®) when larval population densities exceed 20-30/m^2 (Holliday *et al.* 1985). This economic threshold is a recent revision after an evaluation of the

cost-benefits of insecticide control of *M. configurata* on rapeseed indicated that the previous threshold was low by a factor of two (Bracken and Bucher 1984). Based on an average yield over five years and the current rapeseed value, a loss of 5.8-8.7% seed yield or 19-29 $/ha is predicted at the present threshold before control measures return a net economic gain (Anonymous 1983b; Bracken and Bucher 1984). Insecticides were applied on the Canadian prairies to about 38000 ha in 1971, 31000 ha in 1972, and 49000 ha in 1980, which comprised 17.7, 23.3, and 2.4% of the seeded area respectively during these three peak years for *M. configurata* populations (Turnock 1984).

The importance of natural enemies including invertebrate predators and parasitoids in the control of *M. configurata*, and the susceptibility of these and other non-target invertebrates and vertebrates to the recommended insecticides has inspired the investigation of more specific agents to suppress damaging infestations of this pest on rapeseed. This search is also promoted by the recommendation that two parasitoids of *Mamestra brassicae* (L.), the closely-related Eurasian species, be imported into Canada for control of *M. configurata* (Turnock 1984). Entomopathogens, by the nature of their relationships with hosts, are very specific control agents.

In recent years pathogens for control of *M. configurata* have been sought. The nuclear polyhedrosis virus of *M. configurata* has potential as a microbial control agent for this pest (Bucher and Turnock 1983), even though the large scale use of viruses for insect control is impeded by the high cost of production and the underdevelopment of application technology (Morris 1980). *M. configurata* larvae are susceptible to the entomogenous nematodes *Steinernema feltiae* Filipjev and *Heterorhabditis*

bacteriophora Poinar and these are potential candidates for control (Morris 1985). The control potential for *M. configurata* by the entomopathogenic bacterium *Bacillus thuringiensis* is unresolved and nothing has been published on the susceptibility of *M. configurata* to this pathogen.

1.2 *Bacillus thuringiensis* Against *M. configurata*

Microbial insecticides based on the bacterium *Bacillus thuringiensis (B.t.)* are recognized as specific and effective agents for the control of larvae of several species of Lepidoptera. Since the early 1970's, commercial formulations of potent preparations of *B.t.* variety *kurstaki* strain HD-1, which are prevalent among products available in Canada, have become competitive alternatives to chemical insecticides against certain Lepidoptera. There has been no published information on the susceptibility of *M. configurata* to *B.t.* Trial applications of commercial formulations of the HD-1 strain of *B.t.* have not resulted in consistent suppression of populations at recommended rates. In 1980 an application of 10 Billion International Units of potency (BIU)/ha of an oil-based emmulsion against *M. configurata* on rapeseed in Manitoba did not control the pest (D.L. Smith, G.E. Bucher, and G.K. Braken, Winnipeg; unpublished data). However, the same formulation applied at 13.2 BIU/ha and a wettable powder formulation applied at 14.4 BIU/ha against *M. configurata* on rapeseed in Alberta performed as well or better than the recommended treatment of methomyl at 227 g AI/ha (H.G. Philip, Vegreville; unpublished data). Inconsistent field results, such as those experienced for *M. configurata* are frequently obtained for species which are only marginally susceptible to

B.t. The results of preliminary laboratory investigations of the susceptibility of *M. configurata* to these commercial formulations also suggest a limited susceptibility to this strain of *B.t.* (O.N. Morris, Winnipeg; pers comm).

A research program examining the potential for suppressing populations of *M. configurata* on rapeseed with *B. thuringiensis* has been initiated at Winnipeg. Given the suspected marginal susceptibility of *M. configurata* to commercial *B.t.* var. *kurstaki* strain HD-1, an integral part of this research program is to investigate methods of improving the potency of *B.t.* preparations for *M. configurata*. The greatest improvement in the potency of *B.t.* has been obtained through strain selection, which is the testing of various isolates to select the most potent ones for a particular host. At the time of their introduction in 1970, preparations of the *B.t.* var. *kurstaki* strain HD-1 represented a 200 fold improvement in potency over commercial preparations based on other *B.t.* strains for some pest lepidopterous larvae (Dulmage 1970). Since that time, other strains have indicated increases in potency of up to six fold greater than the potency of the HD-1 strain for larvae of several species of Lepidoptera, including some noctuids (Dulmage *et al.* 1981).

1.3 The Project

The present study represents the first attempt to find strains of *B.t.* more potent for *M. configurata* than the HD-1 strain. A total of 61 isolates of *B.t.* were chosen as candidates based on reported activity for other noctuid larvae and also on activity for larval Lepidoptera in general. These candidate isolates were also chosen to include

representatives of the varieties and crystal types with greatest activity for lepidopterous larvae. The aims of this study were to screen the 61 isolates and identify any with significantly greater activity for *M. configurata* than the standard *B.t.* var. *kurstaki* strain HD-1, to estimate the potency of the superior strains for *M. configurata*, and to quantify the susceptibility of *M. configurata* to the HD-1 standard strain.

2. Literature Review: Comparative Susceptibility of Larval Noctuidae to *Bacillus thuringiensis*

2.1 *Bacillus thuringiensis* Berliner (Eubacteriales, Bacillaceae)

Bacteria belonging to the genus *Bacillus* have rod-shaped cells and form endospores. No more than one endospore is formed in each sporangial cell, and the endospores differ from the vegetative cells in having greater resistance to heat and other destructive agents (Buchanan and Gibbons 1974). In addition, members of this genus are strict aerobes or facultative anaerobes; the majority stain gram-positive and are motile by flagella. *Bacillus thuringiensis* is distinguished from other bacilli by the production during the sporulation cycle of a crystalline protein body which is released with the spore when the cell lyses at the termination of sporulation. A constituent compound of this crystal is toxic *per os* to a restricted range of insects.

Bacillus thuringiensis had previously been assigned to the *Bacillus cereus* Frankland and Frankland group. The designation of *B. thuringiensis* as a separate species based on stable biochemical characteristics, of which one is the production of parasporal crystals with entomocidal properties, is disputed because acrystalliferous variants of *B. thuringiensis* have been produced in laboratory cultures. Attempts have been made to reorganize the taxonomy to give *B. thuringiensis* varietal status within *B. cereus* (Gordon 1975); however, most authorities recognize two separate species (Buchanan and Gibbons 1974). From an ecological viewpoint, it is also relevant that *B. thuringiensis* is primarily an insect pathogen while *B. cereus* is primarily a soil saprophyte (Burges 1984).

Berliner in 1915 (cited by Dulmage and Aizawa 1982) described a new species of entomopathogenic bacteria which he had isolated from diseased larvae of the Mediterranean Flour moth, *Ephestia (Anagasta) kueniella* Zeller, collected in the German province of Thuringia. The new species was presented as *Bacillus thuringiensis* Berliner which following convention is abbreviated as *B.t.* (Burges 1984). Berliner is credited with the first microbiologically valid descriptions of *B.t.*; however, the first recorded isolations of this bacillus were made at the turn of the century in Japan from diseased larvae of the silkworm, *Bombyx mori* L. (Ishiwata 1901 cited by Dulmage and Aizawa 1982). This bacterium was recognized as the causitive agent of sotto disease which posed a considerable threat to the silkworm industry in Japan. Since that time *B.t.* has been isolated by several researchers from locations all around the world.

Isolations of *B.t.* have been made from a variety of naturally infected insects, including insects on crops in Canada (Smirnoff and Juneau 1973; Morris 1983c), from grain dust samples (DeLucca *et al.* 1982), from sericulture litter (Dulmage 1970), and from soil (Ohba and Aizawa 1978). *B.t.* is common in soils although it may comprise only a minute portion of the soil microbial complex (Dulmage and Aizawa 1982). When introduced into a soil environment, populations of *B.t.* rapidly decline to levels at which the specific environment will sustain; however, reisolations may be made for a long time after the introduction (Dulmage and Aizawa 1982). Several researchers maintain their own collections of *B.t.* isolates, and H.T. Dulmage maintains the largest group of *B.t.* isolates in the International Collection of *B.t.* Isolates held at the USDA Laboratories, Brownsville, Texas (Dulmage

et al. 1981). This international collection is continually growing in numbers as samples of new isolations are contributed. Currently there are about 1000 individual isolates of *B.t.* held in this collection.

Bacillus thuringiensis plays a limited role in the natural regulations of insect populations, although it is commonly found in insects (Dulmage and Aizawa 1982). It is a pathogen of a variety of insect species including members of the Lepidoptera, Diptera, and a few sawflies (Hymenoptera, Tenthredinidae) Coleoptera, and Orthoptera; however, epizootics due to *B.t.* are rare and limited to septicaemia in populations of only a few species (Burgerjon and Martouret 1971). The reason for this is that *B.t.* has a very low capacity to spread in insect populations. Localized outbreaks of infection with *B.t.* have been reported only in stored products environments and insectaries where cadavers are allowed to remain in close proximity to healthy individuals and foodstuffs (Dulmage and Aizawa 1982). The possibility of the spread of *B.t.* infection is similarly enhanced when cannibalism occurs.

The nutritional requirements of *B.t.* have been the subject of few studies. Good growth has been obtained using media supplemented with yeast extract or casein hydrolysate (Luthy *et al.* 1982). In an environment with a good supply of oxygen, carbon, nitrogen, and phosphorus the bacilli grow vegetatively and sporulation is repressed. As the nutrients in the medium become exhausted by the growing population of bacilli, spore formation is initiated. During the sporulation process, the spore protoplast is dehydrated, thus leaving the spore in a dormant state which is extremely resistant to heat, irradiation, and desiccation (Luthy *et al.* 1982). In this resting state the spore is able to survive harsh conditions for a long time.

The protein crystal of *B. thuringiensis* is synthesized within the cytoplasm during the engulfment process of sporulation (Bechtel and Bulla 1976). The crystal is composed of protein subunits and about 5% carbohydrate content which is glucose and mannose. As a rule only one crystal is produced per sporulating cell. At the end of sporulation, the cells lyse releasing spores and crystals. If spores and crystals are extracted from the culture medium and freeze dried, or lyophilized, they can be stored as a powder for several years without loss of viability or toxicity. It is in this state that isolates in the International Collection of *B.t.* are held.

2.2 Varieties of *Bacillus thuringiensis*

Research on *Bacillus thuringiensis* has increased exponentially over the past two decades and by the same rate so has the number of known isolates. Throughout much of this effort a dominant theme has been the variability of individual isolates of *B.t.* Isolates may differ by a number of criteria including the production of the proteinaceous crystal, production of extracellular compounds, susceptibility to different bacteriophages, characteristics of their biochemistry, and their degree of pathogenicity for specific insects (Burgerjon and Martouret 1971; Dulmage *et al.* 1981). It has become increasingly important that reported research on *B.t.* include a complete identification of the isolate or isolates investigated so that valid comparisons with other research can be drawn, and also so that erroneous conclusions can be avoided. Confusion exists surrounding the nomenclature of *B.t.* and Burges (1984) has responded to this confusion by offering an authoritative opinion on the appropriate conventions.

Several characteristics of *B.t.* have been found to give comparable results and allow a relatively clear subdivisional structure to be established within the species. Varieties of *B.t.* are defined mainly on the basis of H-serotype which is the serology of the flagellar proteins or H-antigens of the vegetative cells (de Barjac 1981). There are currently 21 H-serotypes (Table 1) and distinct antigenic subfactors exist for five of these, H-serotypes 3, 4, 5, 8 and 11. This has resulted in their subdivision into two subtypes each (de Barjac 1981). The electrophoretic patterns of esterases produced by the vegetative cells are employed to distinguish two varieties within H-serotype 6 and subtype 4a, 4b (Table 1), and also to distinguish two varieties, *wuhanensis* and *fowleri* which do not possess flagella. By this system there are currently 30 varieties of *B.t.* (Table 1). There is little doubt that these varieties represent the natural first order of subgroups of *Bacillus thuringiensis* because of the close correspondence of classifications based on the flagellar H-antigens with those based on esterase patterns (Norris and Burges 1965) and classical biochemistry (de Barjac 1981). These subgroups have been referred to as "subspecies" (Buchanan and Gibbons 1974); however, this designation is being discouraged in favour of "varieties" (Burges 1984).

The serology of the crystal antigens is the basis for grouping *B.t.* isolates into subgroups within many varieties, and these subgroups are referred to as crystal types or crystovars (Krywienczyk *et al.* 1978). There exists about an 85% correspondence between variety and crystal type, and crystal types are identified by the first three letters of the varietal name with which they are most commonly associated. Table 2 lists the *B.t.* varieties which contain isolates highly pathogenic for

Table 1: Varieties of *Bacillus thuringiensis* Berliner.

Variety	H-serotype	Source
thuringiensis	1	*Ephestia kuehniella*
finitimus	2	*Malcosoma disstria*
alesti	3a	*Bombyx mori*
kurstaki	3a,3b	*Ephestia kuehniella*
sotto	4a,4b	*Bombyx mori*
dendrolimus	4a,4b	*Dendrolimus sibiricus*
kenyae	4a,4c	*Ephestia cautella*
galleriae	5a,5b	*Galleria mellonella*
canadensis	5a,5c	*Diparopsis* sp.
subtoxicus	6	*Plodia interpunctella*
entomocidus	6	*Paralipsa gularis*
aizawai	7	*Ephestia cautella*
morrisoni	8a,8b	*Galleria mellonella*
ostriniae	8a,8c	*Ostrinia nubilalis*
tolworthi	9	*Plodia interpunctella*
darmstadiensis	10	*Galleria mellonella*
toumanoffi	11a,11b	*Galleria mellonella*
kyushuensis	11a,11c	*Bombyx mori*
thompsoni	12	*Galleria mellonella*
pakistani	13	*Cydia pomonella*
israelensis	14	dried stream bed soil
dakota	15	field crop soil
indiana	16	field crop soil
wuhanensis	*	*Anomis flava*
fowleri	*	water basin
tohokuensis	17	*B. mori* litter
kumamotoensis	18	*B. mori* litter
tochigiensis	19	*B. mori* litter
tenebrionis	20	*Tenebrio molitor*
colmeri	21	grain dust sample

* no flagella

(Adapted and revised from Dulmage and Aizawa 1982).

larval Lepidoptera, and lists the crystal types ranked in order according to the number of isolates belonging to each crystal type within the variety. *Bacillus thuringiensis* var. *kurstaki* isolates are essentially divisible into two crystal types *k-1* and *k-73* and these identifications correspond to the culture accession numbers of the isolate with which each crystal type was first identified. Some *B.t.* isolates produce crystals which posses two types of antigens and therefore belong to both crystal types; for example, a *B.t. kurstaki* isolate exists with k-1/k-73 type crystals (Table 2).

A strain of *B.t.* is a representative of the species (Gordon 1975), and is the lowest subdivision in which isolates are grouped. Isolates of one strain of *B.t.* may differ as a result of modifications of culture and reisolation techniques, or times. Strains of *B.t.* are best identified with the culture accession numbers assigned to the representatives in the international collection of *B.t.* isolates, the "HD number". In addition, or if the strains are not held in the international collection, the culture accession numbers for the strains in other collections are given.

2.3 Toxins of *Bacillus thuringiensis*

Bacillus thuringiensis plays a limited role in the natural regulation of populations because it is not readily transmitted between insects (Dulmage and Aizawa 1982). Spores will develop within the haemocoel of a dead insect, while transmission to a healthy individual may only occur by ingestion of foodstuffs contaminated by the cadaver. This is a rare event under natural conditions and limited only to special situations such as stored product environments and insectaries.

Table 2: *Bacillus thuringiensis* varieties with the greatest pathogenicity for larval Lepidoptera.

Variety	H-serotype	Crystal types
thuringiensis	1	*thu, k-1, k-1/thu*
alesti	3a	*ale, k-73*
kurstaki	3a,3b	*k-1, k-73, k-1/k-73*
dendrolimus	4a,4b	*den, sot*
kenyae	4a,4c	*ken,thu, k-1/thu*
galleriae	5a,5b	*gal, k-1, aiz*
entomocidus	6	*ent, thu*
aizawai	7	*aiz*
tolworthi	9	*tol*

Toxins are produced by *B.t.* which, once in the midgut of susceptible individuals, neutralize host defences to favour retention and germination of spores and, with the death of the host, a cycling through of the *B.t.* population. *Bacillus thuringiensis* has attracted considerable interest for use as the basis of microbial insecticides because the pathogen or specific toxins applied to the ingestible foodstuffs can suppress populations of a susceptible species.

Several different insecticidal toxins have been reported for varieties of *B. thuringiensis* (Faust 1975, Faust and Bulla 1982), although the chemical nature, site and mode of action are not resolved for some. The principal entomodical toxin of *B.t.* is the delta-endotoxin which is a subcomponent of the parasporal crystal, while a group of other toxins which are exoenzymes or exotoxins also have important roles in the toxicity of *B.t.*

2.3.1 The exotoxins of *B. thuringiensis*

Among the several different toxins reported for *B.t.* there are five exotoxins or lytic exoenzymes, including phospholipase C (alpha-exotoxin), beta-exotoxin, gamma-exotoxin, a "labile exotoxin", and a "mouse factor" exotoxin (Faust and Bulla 1982). The individual compounds are synthesized and secreted extracellularly only by particular strains belonging to certain varieties of *B.t.* within specific nutritional environments and during the active phase of vegetative growth (Faust 1975; Faust and Bulla 1982). Their role in the pathogenicity of *B.t.* is secondary to the action of the delta-endotoxin, and they contribute to the overall toxemia only when the conditions of the host midgut favour germination of the spore and vegetative multiplication (Heimpel and

Angus 1963). For the most part, these compounds are released by growing populations of *B.t.* in the haemocoel of a dead insect. For this reason these compounds can also be interpreted as agents of an amensalism or allelopathic relationship with other microbes (Atlas and Bartha 1981). In other words, the release of these compounds during the vegetative growth phase within a dead insect may alter the microenvironment or act as antagonists and confer on *B.t.* a competitive edge over saprophytic microbes.

One of the five exotoxins, the beta-exotoxin, has attracted the most attention for its insectidical properties. Its chemical nature and mode of action are known and have been the subject of reviews by Sebesta *et al.* (1981), and Lecadet and de Barjac (1981). The beta-exotoxin is produced solely by strains of *B.t.* belonging to varieties *thuringiensis, galleriae, canadensis, aizawai, morrisoni, tolworthi, darmstadiensis, toumanoffi,* and *kumamotoensis* (Faust and Bulla 1982). It is an adenine nucleotide and ATP analogue with a molecular weight of 700 daltons. This compound is water soluble and heat stable, and has been referred to as the thermostable exotoxin of *B.t.*, or the "fly factor", or thuringiensin.

The beta-exotoxin of *B.t.* is a broad spectrum toxin affecting invertebrates, vertebrates, and even microogranisms. The mode of action is related to its structure; being an adenine nucleotide and ATP analogue, it intrudes into reactions requiring the biochemically energetic molecule ATP. The processes with which thuringensin could interfere are many and it has been found causing inhibition of DNA dependent RNA polymerase, with the polymerase preferring the beta-exotoxin over ATP (Faust and Bulla 1982). Thuringiensin has also

been reported preventing protein synthesis at the translation level, and as a competitive inhibitor of adenyl cyclase.

The beta-exotoxin of *B.t.* is not registered for use in Canada either as a product in which it is the primary active ingredient, or as a synergist in a *B.t.* spore and crystal based product. Along with its broad spectrum of activity including mammalian toxicity, it was feared that that the beta-exotoxin was a mutagen and carcinogen. It has been proven not to be a mutagen based on the results of Ames mutagenicity tests, and a positive correlation exists between carcinogenicity and mutagenicity (Ames *et al.* 1975; Cantwell *et al.* 1983). Preparations of beta-exotoxin have recently been reported very effective against pests resistant to other insecticides, such as the Colorado potato beetle, *Leptinotarsa decimlineata* (Say) (Cantwell *et al.* 1983), and the Mexican bean beetle, *Epilachna varivestis* Mulsant (Cantwell and Cantelo 1982). Insecticides containing beta-exotoxin will possibly be registered in the near future for these kinds of pests in North America. In the Soviet Union such products have been in use for several years (Luthy *et al.* 1982).

2.3.2 The delta-endotoxin of *B. thuringiensis*

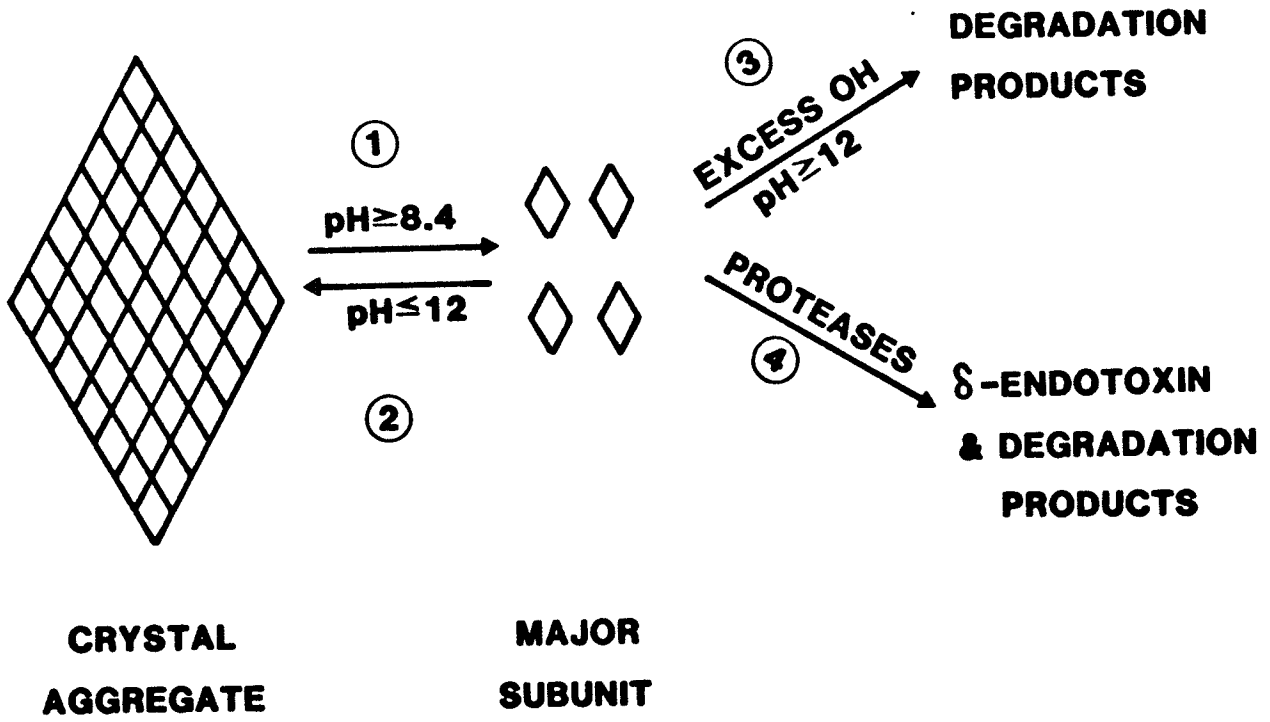
The principal entomodical toxin of *B.t.* is the delta-endotoxin which is a component of the parasporal crystal produced at sporulation. This toxin is a complex entity whose chemical composition and mode of action are not completely defined (Fast 1981; Huber and Luthy 1981; Luthy and Ebersold 1981; Faust and Bulla 1982).

The parasporal crystal is an assembly of a single glycoprotein subunit which is a dimer composed of two identical polypeptide chains,

and has a molecular weight of approximately 230000 daltons (Huber and Luthy 1981). The stability of the crystal structure is maintained by disulfide bonds and noncovalent interactions, and at an alkaline pH the crystals dissolve into the major glycoprotein subunits as illustrated by reaction 1 of Figure 1. The major subunits reaggregate slowly at pH <12 (reaction 2, Figure 1), while in a highly alkaline environment of pH >12 these major subunits are degraded to smaller polypeptide fragments (reaction 3, Figure 1). The crystal and major glycoprotein subunits do not possess any insectidical activity, and therefore represent protoxin. In the presence of specific proteases the major subunits are slowly digested and the active toxin, and, or inactive degradation products are produced (reaction 4, Figure 1). Proteolytic activation of the protoxin to produce delta-endotoxin occurs under natural conditions by the action of digestive juices in the midgut of a susceptible insect larva. The combined influences of adequately high pH, presence of specific proteases, and content of reducing agents such as ascorbic acid in the digestive juices determines the favourability of protoxin activation within the environment of the specific midgut (Naryanan *et al.* 1976).

The delta-endotoxin is a protein or protein complex with toxic activity not related to a defined molecular weight but lying within a rather broad range. By varying the duration of exposure to midgut juice proteases, toxic fragments with molecular weights ranging from 10000 to 80000 daltons have been found (Luthy *et al.* 1982). The specific midgut proteases within an individual larva may affect the speed of activation and the formation and stability of fragments with delta-endotoxin activity; however, it is not yet known what relationship exists between the kind of host proteases used for activation and the subsequent

Figure 1: *B. thuringiensis* parasporal crystal dissolution and delta-endotoxin production. (Adapted from Faust and Bulla 1982).


DEGRADATION
PRODUCTS
3
EXCESS OH
pH≥12
1
pH≥8.4
pH≤12
PROTEASES
4
δ-ENDOTOXIN
& DEGRADATION
PRODUCTS
2
CRYSTAL
AGGREGATE
MAJOR
SUBUNIT

activity of the toxin (Luthy *et al.* 1982). It is known that the delta-endotoxins produced by different varieties or isolates of *B.t.* vary in their toxicity to a particular insect species (Burgerjon and Martouret 1971; Dulmage *et al.* 1981). The reason for this may be that the particular crystal proteins influence the speed of activation, the molecular weight of the intermediate fractions, and the specific activity against a given host, but this relationship is also not yet understood (Luthy *et al.* 1982). This view is supported by the improvements which can be obtained in delta-endotoxin activity for particular hosts by varying the constituents of media from which the spores and crystals are extracted; crystals produced in differing media have different composition (Salama *et al.* 1983a).

The delta-endotoxin of *B.t.* is regarded as a membrane poison, mainly specific for larval Lepidoptera, mosquitoes, black flies and chironomids. In the midgut of a susceptible lepidopterous larva the delta-endotoxin induces rapid swelling of the epithelial cells, accompanied by destruction of the vital cell organelles such as microvilli, endoplasmic reticullum, and mitochondria. This is followed by sloughing off of the midgut epithelium, disruption of ionic control of the haemolymph, and finally loss of midgut integrity with leakage of midgut contents into the haemocoel.

The action of the delta-endotoxin at the molecular level has not been resolved; however, several hypotheses have been advanced (Fast 1981). The delta-endotoxin may act as a respiratory uncoupler of midgut mitochondria, uncoupling oxidative phosphorylation resulting in a depletion of the ATP needed for many metabolic functions including the maintenance of cell membrane integrity. This toxin may also be an

ionophore, a substance which facilitates or causes transport of ions across cell membranes, or alternatively it may act to interfere with the mechanisms by which the gut epithelial cells are protected against autodigestion by contents of the gut lumen. The exact site of action of the delta-endotoxin has yet to be clarified. Specific toxin receptors may be present within the epithelial cell membranes of susceptible individuals, and actions within the cell mediated by second messengers, possibly cyclic nucleotides (Fast 1981).

2.4 Susceptibility of Insects to *Bacillus thuringiensis*

Species of insect are defined as susceptible to *Bacillus thuringiensis* by the pathological response of individuals following the ingestion of spores and crystals. Responses are studied quantitatively and determined under carefully controlled conditions (Burgerjon and Martouret 1971).

2.4.1 Insect types

Species of susceptible insects differ in their responses to *B.t.* and, according to their responses, larval Lepidoptera have been placed into four categories or insect types (Heimpel and Angus 1963). A summary of the symptoms exhibited by the four categories is presented in Table 3. Insects of Type I and Type II are very susceptible to the delta-endotoxin, but differ in the intensity of their reactions, with Type I insects more susceptible than Type II. A comprehensive discussion of symptoms for these two insect types is given by Luthy and Ebersold (1981). A limited number of larval Lepidoptera are of Type I possessing a very high midgut pH such as the silkworm, *Bombyx mori* L.

Table 3: Summary of the different categories of susceptibility of larval Lepidoptera to *B.t.*

Types	Conditions of susceptibility	Response	Representatives
Type I	- very alkaline midgut, pH>10 - proteases favour production of delta-endotoxin	- rapid general paralysis - haemolymph pH increases	*Bombyx mori*
Type II	- alkaline midgut, pH>8.4 - proteases favour production of delta-endotoxin	- gut paralysis - feeding inhibition - starvation, dehydration, and septicemia	most Lepidoptera
Type III	- weakly or non-alkaline midgut, pH<8.4 - spore is essential/germination favoured	- feeding inhibition - morbidity - starvation, dehydration, and septicemia	*Ephestia kueniella* *Lymantria dispar*
Type IV	- alkaline midgut - weak proteolytic action for delta-endotoxin production - weakly susceptible to the delta-endotoxin	- feeding inhibition - morbidity - starvation, dehydration, and septicemia	certain Noctuidae eg. *Spodoptera litura* *Mamestra brassicae*

These larvae exhibit rapid general paralysis accompanied by an increase in blood alkalinity and death. A sublethal dose of delta-endotoxin in the midgut of a Type I insect induces symptoms approaching those of Type II insects. Larvae belonging to the second category exhibit gut paralysis minutes after ingestion of *B.t.* with cessation of feeding within hours. Most Lepidoptera belong to this second category in which general paralysis and an increase in blood alkalinity do not occur. These individuals starve and dehydrate while a slow decrease in midgut alkalinity favours germination of spores and septicemia.

Type III insects include only a few Lepidoptera such as the Mediterranean flour moth, *Ephestia kueniella*; the gypsy moth, *Lymantria (Porthetria) dispar* (L.); and the European corn borer, *Ostrinia nubilalis* (Hubner). These larvae are killed by both *B.t.* spores and delta-endotoxin interacting; the presence of the spore is essential. The crystal protoxin is not readily activated in the midgut of these larvae where the pH of the digestive juices is just above neutrality; an intestinal pH below 8.4 may not allow the crystal to dissolve, but it does favour germination of the spores. Bacillary growth appears to establish conditions favourable to the production of delta-endotoxin (Burgerjon and Martouret 1971). The lavae exhibit cessation of feeding, accompanied by a morbid state during which they succumb to the combined activity of bacillary multiplication and delta-endotoxin, or die from starvation or dehydration.

The fourth category contains larval Lepidoptera which are weakly susceptible to the delta-endotoxin of *B.t.* while their midgut chemistry may not favour protoxin activation nor spore germination (Lebrun and Vlayen 1979). The limited susceptibility of the tobacco caterpillar,

Spodoptera litura (Fabricius) has been attributed to midgut conditions of mild alkalinity (pH 8.2 to 8.5), a deficiency of reducing agents such as ascorbic acid, and poor proteolytic activity (Narayanan *et al.* 1976). Another Type IV insect the cabbage armyworm, *Mamestra brassicae* (L.), may also derive some protection against *B.t.* from the frequent presence of bacteriophages in the midgut (Lebrun and Vlayen 1979). Type IV insects are affected by high doses of *B.t.* which induce feeding inhibition and morbidity, and eventually they succumb to starvation, dehydration, and septicemia.

2.4.2 Host Spectrum of Activity of *B. thuringiensis*

The range of species susceptible to *B.t.* under carefully controlled conditions approaches the fundamental or theoretical niche breadth of *B. thuringiensis* as an entomopathogen and this has been termed the host spectrum of activity of *B. thuringiensis*. The properties of this host spectrum have been summarized by Burgerjon and Martouret (1971) and were revised by Dulmage *et al.* (1981) in a preliminary summary of results from the International Cooperative Program on the Spectra of Activity of *Bacillus thuringiensis* (Dulmage and Beegle 1978).

The first property of the spectrum is that the entomopathogenic activity of a particular isolate of *B.t.* differs for various host species and the range of susceptible hosts for a given isolate establishes the specificity spectrum of that isolate. The second property is that several different isolates of *B.t.* may exhibit different degrees of entomopathogenic activity in the same host species, and the differences in susceptibility displayed by a single host species to different isolates of *B.t.* defines the susceptibility spectrum of the

particular host species. These two properties of the spectrum are interrelated because they indicate that different isolates of *B.t.* vary in their entomopathogenic character and that various host species differ in the nature of their susceptibilities.

This variation is evident from the existence of four categories of susceptibility for larval Lepidoptera as previously outlined (Section 2.4.1). The delta-endotoxins of many different isolates of *B.t.* differ in their degrees of activity (Dulmage *et al.* 1981), and this may be due to differences in rates of protoxin activation or in the size and stability of the intermediate peptides with delta-endotoxin activity. In addition, varying responses to *B.t.* may be related to the differential production of certain exotoxins by particular strains of *B.t.*, and also to factors associated with the host gut including pH, proteolytic enzymes, redox potential, bacteriocidal and bacteriostatic factors, and bacteriophage (Burgerjon and Martouret 1971). These characteristics demonstrate the diversity of processes which govern the host *B. thuringiensis* relationship.

Another property of the spectrum of activity of *B.t.* is that susceptibility to preparations of *B.t.* spores and crystals is limited to Lepidoptera, mosquitoes, blackflies, chironomids, and certain sawflies, Coleoptera, and Orthoptera. Predators and parasites of lepidopterous larvae do not exhibit a direct response to these preparations and no toxic effects in vertebrates have been found. Two incidents of soft tissue infection resulting from accidental inoculation of workers handling *B.t.* preparations serve to reinforce the necessity for appropriate precautions when handling pathogens (Samples and Buettner 1983; Warren *et al.* 1984).

2.4.3 Host Defences to *B. thuringiensis*

The toxins produced by *B. thuringiensis* allow it to circumvent by varying degrees the host defence mechanisms directed against potential pathogens in the midgut of susceptible individuals. Host defences and sources of host resistance to microbial pathogens are the subjects of good reviews by Burges (1971), Harshbarger and Faust (1973), Boman (1981), and Briese (1981). One mechanism of host defence against microbes invading via the midgut is the rate of food passage. Non-storage feeders such as larval Lepidoptera pass food through the alimentary tract in 1-3 hours (Harshbarger and Faust 1973); however, the delta-endotoxin of *B.t.* induces midgut paralysis which results in retention of the pathogen within the midgut.

Microorganisms generally have a low tolerance to extremes of pH at which cell components may be hydrolyzed or enzymes denatured (Atlas and Bartha 1981), and certain insects maintain an elevated or depressed pH in the midgut which discourages microbial growth. Some larval Lepidoptera such as the silkworm, *B. mori*, maintain a midgut pH in excess of 10 (Heimpel and Angus 1963) which is not protection against *B.t.* because delta-endotoxin activation is favoured in very alkaline environments. The delta-endotoxin action produces near neutral pH in the midgut environment which favours *B.t.* growth; at values less than pH 9 and approaching neutrality the germination of *B.t.* spores and vegetative growth is increasingly favoured.

The contents of the midgut include as defence mechanisms antimicrobial substances which originate either with the host gut juices or ingested food (Burgerjon and Martouret 1971). In addition, compounds

may be secreted or ingested such as enzymes to degrade the delta-endotoxin or reduce the stability of toxic intermediates with delta-endotoxin activity.

Resistance of insect populations to *B. thuringiensis* has been reported mostly for populations of species subjected to artificial selection. Low to moderate degrees of resistance have been reported for a few populations in insect rearing programs and stored product environments (Briese 1981). The development of resistance to a *B.t.* commercial formulation by a stored grain pest, the Indian meal moth *Plodia interpunctella* (Hubner), was demonstrated in the laboratory and also detected in small bins of *B.t.*-treated grain (McGaughey 1985). *B.t.* is stable in this environment (McGaughey 1978) and in insect cultures; therefore, populations are in continuous exposure for several generations, while in agricultural and forest environments *B.t.* is rapidly degraded by sunlight (Morris 1983b). The complex nature of the host-*B. thuringiensis* interaction suggests that processes by which a population in a particular environment has developed resistance are probably more characteristic of the individual species rather than foreshadowing a similar development of resistance broadly across the range of susceptible species (Briese 1982). This view is supported by reports of unsuccessful attempts to induce development of resistance to *B.t.* in populations through artificial selection (Briese 1982; Sneh and Schuster 1983).

2.5 Bioassay and Standardization

Standardized bioassay methods have been adopted to quantify the potency of spore and crystal extracts of different *B.t.* isolates for specific hosts. Viable spore counts were initially a convenient approach to estimating the toxicity of these preparations; however, spore counts do not correlate with insecticidal activity (Burges 1967). Simply measuring the toxicity of a given *B.t.* isolate to a particular host is not sufficient because of the considerable variability in the susceptibility of individuals examined from the same population at different times. Individuals may differ in their responses to *B.t.* as a result of variations in temperature, humidity, light and food, during both breeding and testing; also, in size, age, and instar of larvae, in physiological state, particularly in relation to the phase within the instar, in race and degree of inbreeding, as a result of latent infections, and in factors arising from the application of the pathogen (Burges 1967). The variation can be minimized through careful breeding and choice of test individuals, and by controlled experimental technique including a parallel assay each day of a stable reference standard *B.t.* The ratio of the toxicities of the test isolate to the reference standard varies appreciably less from one parallel bioassay to the next than do the individual respective toxicities (Dulmage 1973a). The potency of the standard is expressed in units of activity, conventionally international toxicity units (IU), which are arbitarily defined and agreed upon by the scientific community. The potencies of test isolates for particular hosts are calculated from the ratio of the toxicities of test sample and reference standard in parallel bioassays,

multiplied by the arbitrarily assigned potency of the reference standard in IU/mg.

A measure of toxicity is the median lethal dose (LD_{50}) or concentration (LC_{50}), which is the dose which will produce mortality in half of the population as determined from dosage-mortality response curves. The regression of the probit transformed corrected mortalities against the logarithm of the dose gives a straight line from which the median lethal dosage can be obtained along with a measurement of variation such as the confidence interval (Finney 1971).

Studies on the spectrum of activity of *B.t.* within the Lepidoptera as part of the International Co-operative Program utilize bioassay procedures which are outlined by Dulmage and Beegle (1978) and Dulmage *et al.* (1981) and model bioassays have been published for the cabbage looper, *Trichoplusia ni* (Hubner) (Dulmage *et al.* 1971), and the tobacco budworm, *Heliothis virescens* (Fabricius) (Dulmage *et al.* 1976). These bioassays specify the incorporation of sporulated cultures of *B.t.* into an artificial diet upon which larval Lepidoptera of a specified age are allowed to feed *ad libitim* for a fixed length of time. Modifications are made to accommodate the particular biology of the test insect, such as specific artificial diets. Each artificial diet is essentially a relatively homogeneous and reproducible semi-defined medium.

The original reference standard of *B.t.* for parallel bioassays against larval Lepidoptera is E-61, an isolate of *B.t.* var. *thuringiensis*, which was arbitrarily assigned a potency of 1000 IU/mg in 1966. With the discovery in the early 1970's of *B.t.* var. *kurstaki* isolates possessing considerable potency for a range of larval

Lepidoptera, another reference standard HD-1-S-1971 belonging to this variety was accepted in 1973 and assigned a potency of 18000 IU/mg based on parallel bioassays with E-61 against *T. ni* (Dulmage 1973b). Similarly a more recent reference standard has been accepted HD-1-S-1980 with an assigned potency of 16000 IU/mg (Beegle 1982).

The objective of standardizing bioassay techniques has primarily been to outline a convention for the standardization of commercial insecticides based on *B. thuringiensis*. Standard methods promote predictability in the suppression of larval lepidopteran populations with products marketed at different times or by different companies. These same procedures are also used in the screening of isolates of *B.t.* to select the most potent ones for a particular host.

2.6 Comparative Susceptibility of Selected Noctuidae

The selection of potent strains of *B.t.* for a specific host is a matter of judgement and some luck given the large number of isolates in existence. Few research programs have the resources to be able to screen all of the isolates in a reasonable length of time, and this approach would be a questionable investment because the existence of more potent isolates is not guaranteed. An optimal approach is the screening of a reasonably large group of candidate isolates which are chosen to represent the greatest liklihood of success at that time.

Standardized methods permit comparisons of the susceptibility spectra of different hosts and also the specificity spectra of particular *B.t.* isolates. Such comparisons are the basis for developing hypotheses on the susceptibility of larval Lepidoptera to *B.t.*, possibly correlating a system of grouping or categorizing isolates with their

specificity spectra of insecticidal activity. These hypotheses are useful in the choosing of a group of candidate isolates which may offer the greatest chance of success in identifying potent ones for a particular host species.

One approach to choosing candidate isolates to screen against the noctuid, *M. configurata*, is to examine the susceptibility spectra of closely related phytophagous Noctuidae. It is valuable to establish a frame of reference for each host at the outset, such as the susceptibility to the reference standard strain HD-1-S-1971. Also useful is a comparison of the relative improvements in potency for each host derived through the selection of potent strains with the relative improvements obtained by other methods including the integration of *B.t.* with other control measures for these hosts.

2.6.1 *Trichoplusia ni* (Hubner)

The cabbage looper, *T. ni*, is a serious pest of cruciferous crops in central North America. It is moderately sensitive to the primary U.S. reference standard strain (*B.t.* var. *kurstaki* strain HD-1-S-1971), a feature which, with its ease of rearing in the laboratory has made it the conventional bioassay insect for the standardization of preparations of *B.t.* for pest Lepidoptera in North America (Dulmage *et al.* 1971). In terms of median lethal concentrations, Ignoffo *et al.* (1977) reported a value of 28.7 IU/cm^2 for four day old larvae feeding on the standard HD-1-S-1971 applied to the surface of an artificial diet. This level is comparable to approximately 800 IU/ml by the more conventional method of incorporating the isolates into the artificial diet. Commercial preparations of *B.t. kurstaki* strain HD-1 have been extensively studied

for the suppression of *T. ni* on cabbage, and generally good control has been indicated (Schuster 1979; Workman *et al.* 1980; Creighton *et al.* 1981; Sears *et al.* 1983). Most of the *B.t. kurstaki* used in the United States is against *T. ni* primarily on lettuce and cole crops (Dulmage and Aizawa 1982). *T. ni* is a good representative of a noctuid for which *B.t. kurstaki* has become an integral part of control programs and consequently its susceptibility to *B.t. kurstaki* strain HD-1 is a good reference level to compare the susceptibility of other noctuids (Table 4).

Other *B.t.* varieties are moderately active against *T. ni* including isolates of varieties *thuringiensis* (Tanada 1956; Rogoff *et al.* 1969; Creighton and McFadden 1975), *galleriae* (Rogoff *et al.* 1969; Creighton *et al.* 1971; Jaques 1972), and *aizawai* (Dulmage *et al.* 1981).

Beegle *et al.* (1982) produced spore-crystal preparations of five strains: HD-1 (var. *kurstaki* crystovar *k-1*), HD-73 (var. *kurstaki* crystovar *k-73*), HD-153 and HD-196 (var. *galleriae)*, and HD-264 (var. *thuringiensis*). The potencies of these preparations for neonate larvae were 21300, 13400, 4370, 2020, and 2220 IU/mg respectively, as determined by standardized bioassay with isolates incorporated in an artificial diet. When the preparations were applied against *T. ni* on cabbage in the field, the ranking from most to least efficacious in terms of crop protection was var. *galleriae*, *thuringiensis*, and *kurstaki*. The reversed order of relative potency ranking obtained in field tests may be attributed to the interaction of the delta-endotoxins produced by individual strains of *B.t.* with compounds liberated from the ingested foliage.

Varying results have been obtained with the addition of feeding

Table 4: Susceptibility of selected Noctuidae to *B.t.*

Larval Noctuidae	~LC_{50} (IU/ml) of HD-1-S-1971 for third instar larvae	Relative susceptibility[a]	Most potent isolates were of these varieties[b]
Trichoplusia ni	800	1	Ku, Ga, Ai, Th
Heliothis armigera	1350	1/2	Ku, Ai, En, Th
H. virescens	2000	2/5	Ku, Ga, Th, Ai
H. zea	2200	1/3	Th, Ku, Ga
Spodoptera exigua	2250	1/3	Ai, En, Th, Ku
S. litura	4500	1/5	Ai, To, Ku
S. littoralis	6000	1/7	En, Ai, Ke, Ga
Agrotis ipsilon	14000*	1/17	To, En, Th

a) susceptibility to HD-1-S-1971 relative to *T. ni*

b) order from the left is equivalent to general ranking; varietal abreviations: Ai(*aizawai*), En(*entomocidus*), Ga(*galleriae*), Ke(*kenyae*), Ku(*kurstaki*), Th(*thuringiensis*), To(*tolworthi*)

* neonates

stimulants to *B.t.* preparations for control of *T. ni* on cabbage; these ranged from no improvement (Schuster 1979) to significant improvement (Smith and Hostetter 1982), depending somewhat on the adjuvant used. The combination of a *B.t.* preparation with the entomopathogenic fungus *Nomuraea rileyi* Samson produced an additive effect against *T. ni* (Ignoffo *et al.* 1980).

2.6.2 *Heliothis armigera* Hubner

The old world bollworm or African cotton bollworm, *H. armigera*, is a pest of cotton, tobacco, tomato and other crops in Africa and the Mediterranean region, central Europe through to southeast Asia, the Philippines, Australia, and New Zealand (Anonymous 1983a). It is half as susceptible to the standard *B.t.* var. *kurstaki* HD-1-S-1971 as *T. ni* (Table 4), with an LC_{50} of 1350 IU/ml for third instar larvae (Salama *et al.* 1983d). Commercial preparations of *B.t.k* HD-1 have been moderately effective in the field against *H. armigera* (Roome 1975), and rates of 12 and 16 BIU/ha were effective for third and fifth instar larvae respectively on chick-pea (*Cicer arietinum* L.) (Dabi *et al.* 1979).

Salama *et al.* (1981b) examined 29 strains of 14 different varieties of *B.t.* by the conventional bioassay methods, and found that several strains of var. *aizawai*, *entomocidus*, and *kurstaki* were the most toxic to third instar *H. armigera*. The best of these exhibited a potency of 18000 IU/mg, equivalent to the potency of the standard. With modifications to the bacterial culture media, the potencies of the most effective strains became 65000, 56000, and 43000 IU/mg for HD-1 (var. *kurstaki* crystover *k-1*), HD-73 (var. *kurstaki* crystover *k-73*) and HD-133 (var. *aizawai*), respectively (Salama *et al.* 1983d).

A mixture of the var. *kurstaki* isolate HD-1 and the var. *aizawai* isolate HD-133 was found to have synergistic action against *H. armigera*, with a potency of 96000 IU/mg (Salama *et al.* 1983b). This combination represents a five fold improvement over the potency of the standard, and if problems associated with mixed culture interactions such as competitive exclusion can be avoided then this method of improving the potency of B.t. agents may be effective.

Matter and Zohdy (1981) reported the results of investigations of a commercial preparation of B.t. var. *thuringiensis* incorporated in an artifical diet against *H. armigera*. Bioassays produced LC_{50} values of 990 UI/ml for first instar larvae, 1400 IU/ml for the third instars, and 12000 IU/ml for fifth instarts, indicating that this preparation is no improvement over the standad HD-1 isolate for *H. armigera* (Table 4).

2.6.3 *Heliothis virescens* (Fabricius)

The tobacco budworm or tomato budworm, *H. virescens*, is a major pest of tobacco, cotton and tomato, and an occasional pest of soybean in central North America and parts of central South America (Oliver and Chapin 1981; Anonymous 1983a). The *B.t.* var. *kurstaki* standard HD-1-S-1971 is less active against *H. virescens* than against *T. ni* (Table 4). Commercial formulations of this strain are effective against this noctuid on tobacco, however, for *H. virescens* on cotton, the performance of these products at recommended rates of 4.5 to 18 BIU/ha has been marginal and variable (Bull *et al.* 1979; Ali and Watson 1982a, 1982b; Johnson 1982; Mohamed *et al.* 1983b). Adequate season-long suppression of *Heliothis* spp. on cotton has been obtained, but at required rates which were excessively high, 48 to 71 BIU/ha, and

therefore not economically practical (McGarr *et al.* 1970).

Many strains of *B.t.* have been bioassayed for toxicity to *H. virescens* as a result of its use as a second reference host, *T. ni* the first, for the computation of potency ratios which are used to compare *B.t.* strains (Dulmage *et al.* 1976, 1981). Dulmage *et al.* (1981) reported that an isolate belonging to *B.t.* var. *kurstaki* crystovar *k-73* had a potency against *H. virescens* of 70600 IU/mg, which represents more than a three fold improvement over the standard. Beegle (1982) investigated three var. *kurstaki* isolates, HD-241 and HD-263 (crystovar *k-1*), and HD-244 (crystovar *k-73*), which were superior to the standard against *H. virescens*. The relative improvements over the standard isolate varied with different bacterial culturing techniques, and field testing suggested that a six or seven fold improvement may be required before significant differences are shown against *H. virescens* on cotton.

Research efforts have taken several routes to more effectively employ *B.t.* insecticides as part of control programs for *H. virescens* on cotton. The addition of feeding stimulants and sunlight protectants to spray mixtures of commercial *B.t.* products has considerably improved their performance against *H. virescens* on cotton (Bell and Romine 1980; Johnson 1982; Luttrell *et al.* 1982). Spray mixtures of *B.t.* with certain chemical insecticides including diflubenzuron, chlordimeform, and methoprene at reduced rates have produced synergistic action (Mohamed *et al.* 1983a, 1983b). Synergism against *H. virescens* on cotton has also been found for *B.t.* agents combined with *Heliothis* virus preparations (Bell and Romine 1980; Johnson 1982; Luttrell *et al.* 1982). Significantly improved control was obtained for the application of *B.t.* var. *kurstaki* combined with the release of a predator, *Geocoris punctipes*

Say (Hemiptera, Lygaeidae) (Ali and Watson 1982b).

2.6.4 *Heliothis zea* (Boddie)

The corn earworm, *H. zea*, is one of the more important noctuid pests of the Americas incurring damage to cotton, maize, tomato, alfalfa, clover, soybeans, chrysanthemums, peas, peppers, and sorghum (Oliver and Chapin 1981; Anonymous 1983a). It is weakly susceptible to the *B.t.* var. *kurstaki* standard strain HD-1-S-1971 with an LC_{50} in artificial diet of approximately 2200 IU/ml against third instar larvae (Ignoffo *et al.* 1977). This level of susceptibility is comparable to that of *H. virescens* (Table 4), and both species are found on cotton where their control with *B.t.k.* strain HD-1 formulations at recommended rates is marginal (Bull *et al.* 1979; Johnson 1982).

In laboratory evaluations of a commercial *B.t.* var. *kurstaki* HD-1 preparation on leaf discs of cotton, cabbage, and soybean, Smith and Hostetter (1982) found that mortality among 1 day old *H. zea* larvae was lower when cotton leaves were the substrate. The marginal efficacy of *B.t.* against *Heliothis* spp. on cotton may be related to interference of *B.t.* action by leaf substrates in the host midgut. There are reports of *in vitro* inhibition of *B.t.* growth by some leaf extracts (Pinnock *et al.* 1977).

Strains of varieties *thuringiensis*, *galleriae*, and *kurstaki* have indicated a moderate degree of toxicity for *H. zea* (Hall and Dunn 1958; Creighton *et al.* 1971). Beegle (1982) found that strain HD-263 was about twice as toxic as HD-1 (both var. *kurstaki* crystovar *k-1*).

The potency of commercial *B.t.k.* strain HD-1 products against *H. zea* on cotton was improved by the addition of feeding stimulants

(Johnson 1982; Luttrell *et al.* 1982; Smith and Hostetter 1982). Synergism has been indicated for *B.t.* with *Heliothis* viral agents against *H. zea* (Johnson 1982; Luttrell *et al.* 1982).

2.6.5 *Spodoptera exigua* (Hubner)

The beet armyworm or Lucern caterpillar, *S. exigua*, is a defoliator of cotton, alfalfa, crucifers, and various weeds in Southern Africa and Asia, Australia, the Mediterranean region, and the southern U.S.A. (Oliver and Chapin 1981; Anonymous 1983a). *S. exigua* is weakly susceptible to the *B.t.* var. *kurstaki* standard HD-1-S-1971, and is three times less susceptible to this standard than *T. ni* (Table 4). Salama *et al.* (1983d) reported an LC_{50} of 2250 IU/ml for the reference standard HD-1-S-1971 incorporated in an artificial diet against third instar larvae.

Sublethal effects of *B.t.* ingestion by *S. exigua* larvae include increased larval and pupal duration, and decreased pupal weight, percentage pupation, and longevity and egg production of adults (Salama *et al.* 1981b). The severity of the sublethal effects increases with increasing concentrations of *B.t.* spores and crystals in the larval diet.

Strains belonging to several varieties of *B.t.* have been investigated for their toxicity relative to the reference standard against *S. exigua*. Salama *et al.* (1981a) examined 29 different strains belonging to 14 different varieties and found that the most toxic ones were HD-635 and HD-198 (var. *entomocidus*), and a var. *alesti* strain, for which the potencies were 79995, 46650, and 62077 IU/mg respectively. Dulmage *et al.* (1981) found 18 strains of *B.t.* with high toxicity for

S. exigua, and that 14 of the 18 strains belonged to var. *aizawai*. The most potent var. *aizawai* isolate among the 14 represented a 650% improvement in potency over the var. *kurstaki* standard HD-1, and surpassed the four fold improvement reported for HD-635 by Salama *et al.* (1981a).

The potencies of strains of *B.t.* can be altered and improved with modifications of the bacterial fermentation medium. Salama *et al.* (1983c) found the potencies of the var. *entomocidus* strains HD-635 and HD-198, and the var. *kurstaki* strains HD-1 and HD-73, cultured in a kidney bean based medium, increased to 49000, 40000, 32000, and 18000 IU/mg, respectively, for third instar *S. exigua*. The relative potencies of isolates changed, but the overall ranking of most toxic isolates was not altered. When different agro-industrial by-products such as slaughterhouse residues and legume seeds were used in *B.t.* fermentation media, the toxicity of strains to *S. exigua* varied considerably (Salama *et al.* 1983a).

Strains belonging to other varieties of *B.t.* notably *aizawai* and *thuringiensis* are moderately toxic to *S. exigua* (Table 4) (Krieg and Langenbruch 1981; Dulmage *et al.* 1981; Salama *et al.* 1981a) Combinations of isolates have indicated synergism against *S. exigua*. Mixtures of the var. *entomocidus* strain HD-635 with any one of HD-198, HD-1, or HD-73 were found to result in synergism (Salama *et al.* 1983c).

2.6.6 *Spodoptera litura* (Fabricius)

S. litura is a polyphagous defoliator of concern in Southeast Asia, the Philippines, and Australia, notably in cotton and tobacco fields (Anonymous 1983a). This species is weakly susceptible to the *B.t.* var.

kurstaki standard strain HD-1 with an LC_{50} in artificial diet of approximately 4500 IU/ml against third instar larvae (Dulmage *et al.* 1981). Poor susceptibility to commercial HD-1 preparations has been reported for all larval instars over a range of concentrations in castor (*Ricinus communis* L.) leaf dip tests (Govindarajan *et al.* 1975), and for fifth instar larvae force fed the *B.t.* suspension alone (Tiwari and Mehrotra 1981). The poor susceptibility of *S. litura* may be attributed to a midgut environment of low alkalinity, low ascorbic acid and phenol contents, and poor proteolytic activity (Narayanan *et al.* 1976).

Among 297 strains belonging to several varieties of *B.t.* examined, only 15 caused high mortality (>80%) incorporated in an artificial diet at the comparatively high concentration of 500 ug/ml against third instar *S. litura* (Dulmage *et al.* 1981). The most toxic strains belonged to var. *aizawai* (H-serotype 7), and the superior strain was four times more potent than the var. *kurstaki* standard strain HD-1 (Dulmage *et al.* 1981).

Sublethal effects to *S. litura* from ingestion of *B.t.* var. *thuringiensis* on green gram foliage were positively correlated with *B.t.* concentration (Sareen *et al.* 1983). These sublethal effects included prolongation of the larval period, progressive decrease in pupal weight, and reduced consumption of treated foliage.

2.6.7 *Spodoptera littoralis* (Boisduval)

The prospects for control of the Egyptian cotton leaf worm, *S. littoralis*, using preparations of *B.t.* has been the subject of extensive investigation (Salama 1983; Salama 1984; Salama and Zaki 1985). This noctuid is a major pest of cotton and other crops in the Mediterranean

region and Africa (Anonymous 1983a; Salama 1983; Sneh and Gross 1983). *S. littoralis* is not very sensitive to strains of *B.t.* var. *kurstaki*; Salama *et al.* (1983c) found an LC_{50} of 6000 IU/ml for the reference standard HD-1-S-1971 incorporated in an artificial diet against third instars. In comparison to other noctuids, *S. littoralis* is almost three times less susceptible to this standard than *S. exigua* and at least seven times less susceptible than *T. ni* (Table 4). Alfalfa leaf dip tests with commercial *B.t.* var. *kurstaki* HD-1 preparations resulted in only low mortalities of second instar larvae (Sneh *et al.* 1981). Mortality of fifth instar larvae feeding on field-sprayed alfalfa plants to which a commercial preparation of *B.t.* var. *kurstaki* HD-1 was applied indicated that effective control of *S. littoralis* may be expected at four to six times the recommended rate of 1-3 kg/ha (Navon *et al.* 1983).

Many strains of *B.t.* have been bioassayed for toxicity to *S. littoralis*. Moore and Navon (1973) reported weak activity of strains belonging to varieties *galleriae, darmstadiensis, sotto, morrisoni, tolworthi,* and *kurstaki*. Salama *et al.* (1981a) examined 29 isolates of 14 varieties incorporated in an artificial diet against four day old *S. littoralis* and found the most toxic ones were HD-635 (var. *entomocidus*), and an isolate of var. *alesti*. Strain HD-635 was also the most toxic for *S. exigua*. The potencies of these isolates were 67000 and 23000 IU/mg, respectively, which for HD-635 represents a four fold improvement over the reference standard HD-1. The study was extended to include isolates from a total of 17 varieties and the most toxic strain was still HD-635, for which an LC_{50} of 78.5 ug/ml diet was indicated for four day old larvae (Salama and Foda 1982).

Sneh *et al.* (1981) tested 50 strains of *B.t.* of which some had been isolated from the cadavers of *S. littoralis* collected in the field in Israel. Leaf dipping tests using alfalfa leaves, with second instar larvae as subjects, indicated that the most effective strain belonged to var. *entomocidus*. That two independent screenings of strains of *B.t.* both found the most toxic for *S. littoralis* to be a strain of var. *entomocidus* is indicative of the importance of isolates belonging to var. *entomocidus* in terms of their relative toxicity to this noctuid. Acceptable control of *S. littoralis* in cotton and alfalfa fields in Israel was obtained with a preparation of a strain belonging to var. *entomocidus* (*B.t.* No. 24), and the control improved markedly with the addition of a phago-stimulant and sunlight protectant to spray mixtures (Sneh and Gross 1983).

A rapid screening of more than 900 strains belonging to 21 H-serotypes of *B.t.*, incorporated in artificial diet against first instar *S. littoralis* was made by Kalfon and de Barjac (1985). Isolates for which a high level of toxicity was indicated belonged to varieties *aizawai, kenyae*, and *entomocidus*. One of the best isolates belonging to var. *aizawai* was field tested against *S. littoralis* on alfalfa, and at reasonable rates of 5 or 2.5 l/ha it was effective (Kalfon and de Barjac 1985).

Modified fermentation techniques have been examined as a means of improving the potency of *B.t.* preparations for *S. littoralis* and reducing the costs of production (Goldberg *et al.* 1980; Salama 1984). The potency of the var. *kurstaki* strain HD-1 was considerably improved when cultured in media composed of different agro-industrial by-products, as were the potencies of other isolates examined (Salama *et*

al. 1981a). The use of whey as a fermentation medium for *B.t.* has resulted in isolates with good potency and represents a favourable approach to the recycling of this by-product of the cheese industry (Salama *et al.* 1983c).

Sublethal effects of *B.t.* ingestion by *S. littoralis* include reduction in the rate of development through larval stages and decreased pupal weight (Salama *et al.* 1981b; Sneh and Schuster 1983). Sneh and Schuster (1983) were unable to induce resistance to a *B.t.* var. *entomocidus* isolate (*B.t.* No. 24) in any of ten generations of *S. littoralis*. Each generation tested was the progeny of the 2-30% survivors feeding as second instar larvae on castor leaves dipped in a suspension of this *B.t.* strain.

The dominant factor affecting the very short effective residual life of *B.t.* in the field is the ultraviolet radiation component of sunlight (Salama *et al.* 1983e) and some progress has been made in the control of *S. littoralis* with *B.t.* by improving the persistence of the pathogen in the field. The addition of sunlight protectants to spray mixtures has improved the degree of control obtained with *B.t.* (Sneh and Gross 1983). The isolation of mutants resistant to physical factors such as sunlight has not as yet developed to the point of showing improvements in the control of *S. littoralis* with *B.t.* (Salama *et al.* 1984a).

Certain feeding stimulants such as host plant volatiles added to preparations of *B.t.* increase their potency for *S. littoralis* in laboratory bioassays (Salama *et al.* 1985). Similar increases are also indicated for phago-stimulants added to spray mixtures of *B.t.* for suppression of *S. littoralis* in cotton and alfalfa fields (Sneh and

Gross 1983). The potency of *B.t.* preparations for *S. littoralis* in the laboratory has also been increased with some chemical additives such as alkaline compounds, general proteolytic activators, and mildly toxic inorganic compounds. Most notable were two to four fold increases in the potency of strain HD-635 resulting from the addition to artificial diets of 0.5% sodium borate, tannic acid, or potassium carbonate, or 1.0% magnesium chloride (Salama *et al.* 1984b). These chemical additives and others yet to be investigated may enhance potencies by optimizing the conditions of the host midgut required for the production of crystal protein fragments with delta-endotoxin activity (Salama 1984).

A significant synergistic effect was obtained with combinations of a strain of *B.t.* var. *entomocidus* (*B.t.* No. 24) and chitinolytic bacteria, one of which was identified as *Serratia marcescens* Bizio (Eubacteriales, Enterobacteriaceae), on castor leaf discs fed to second instar *S. littoralis* (Sneh *et al.* 1983). These bacteria release chitinase which in the host midgut may digest the chitin of the peritrophic membrane increasing the penetration of the delta-endotoxin to the susceptible tissues of the midgut epithelium.

Combinations of low dosages (LC_{25}) of pyrethroids and some organophosphorous insecticides with low dosages of preparations of *B.t.* strains HD-635 (var. *entomocidus*) and HD-129 (var. *galleriae*) were synergistic when incorporated into an artificial diet or applied to castor leaves against neonate *S. littoralis* (Salama *et al.* 1984c). The pyrethroids were the most compatible with *B.t.*, having the least deleterious effects on sporulation.

2.6.8 *Agrotis ipsilon* (Hufnagel)

The greasy or black cutworm, *A. ipsilon*, is a pest of potato, tobacco, cotton, and crucifers and has been recorded through Europe, Central and Southeast Asia, Central North America, and in areas of South America, Africa, Australia and New Zealand (Anonymous 1983a). In southern Ontario and the Atlantic provinces this cutworm is almost annually a serious pest of seedling potato, tomato, turnip, radish, sugar-beet, cabbage, cauliflower, lettuce, sunflower, corn and other crops (Beirne 1971). *A. ipsilon* is weakly susceptible to the *B.t.* var. *kurstaki* standard strain HD-1 with an approximate LC_{50} of 14000 IU/ml for HD-1-S-1971 when incorporated in an artificial diet and fed to third instar larvae (Salama and Foda 1984). This degree of susceptibility to the standard strain is about one-seventeenth that of *T. ni* (Table 4), suggesting that control of *A. ipsilon* with preparations of the HD-1 strain would be less than marginal. Conventional spray methods of applying *B.t.* are impractical against this cutworm because of its feeding behaviour at the soil surface; however, the development of granular formulations and baits may allow control of cutworms with potent strains of *B.t.* (Henry 1982; Lewis 1982).

Among 319 strains of several varieties of *B.t.* examined, only 9 strains caused high mortality (>80%) when incorporated in an artificial diet at the comparatively high concentration of 500 ug/ml and fed to third instar *A. ipsilon* (Dulmage *et al.* 1981). Their toxicity for *A. ipsilon* may be due to an exotoxin with some degree of heat stability (Dulmage *et al.* 1981).

Salama and Foda (1984) investigated 25 strains belonging to 16

varieties of *B.t.* and found that the most potent incorporated in an artificial diet against neonate *A. ipsilon* were strains of varieties *tolworthi, entomocidus, finitimus, dendrolimus, sotto,* and *thuringiensis*. The superior strain was a strain of var. *tolworthi* (Institut Pasteur strain) with a potency of approximately 122000 IU/mg giving a six to seven fold improvement over the potency of the reference standard HD-1-S-1971 (Salama and Foda 1984).

2.6.9 A Synthesis

Investigations of the susceptibility of noctuid larvae to *B. thuringiensis* have progressed the farthest for the eight preceding representative species. The susceptibility of these noctuids to the *B.t.k.* reference standard HD-1-S-1971 and commercial preparations of the standard strain HD-1 range from moderate such as *T. ni* to very poor such as *A. ipsilon* (Table 4). Members of other families of the order Lepidoptera are comparatively much more susceptible to this standard strain. The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera, Lymantriidae) is about ten times more sensitive to the reference standard than *T. ni* (Table 4), exhibiting an LC_{50} of approximately 77 IU/ml for HD-1-S-1971 incorporated in an artificial diet against second instar larvae (Wallner *et al.* 1983). The spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera, Tortricidae) is also very susceptible to the standard strain with a median lethal dosage (LD_{50}) of 6.7 ± 0.9 IU for HD-1-S-1980 against fifth instar larvae, as determined by a dosing technique using balsam fir needles (Moore and Morris 1982). This degree of susceptibility is greater than ten fold that of *T. ni*, the most susceptible noctuid examined. *C. fumiferana* and *L. dispar* are both

serious forest defoliators whose populations are successfully managed with commercial preparations of the *B.t.k.* strain HD-1 at acceptable rates of 20-30 BIU/ha (Andreadis *et al.* 1982, 1983; Dimond 1982; Reardon *et al.* 1982, 1984; Morris 1983a, 1984; Morris *et al.* 1984). For both species the standard strain HD-1 is among the most potent; an examination of 50 isolates belonging to 10 varieties of *B.t.* found none significantly more potent against *C. fumiferana* than the reference standard HD-1-S-1980 (Morris and Moore 1983).

The European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera, Pyralidae) is very sensitive to the *B.t.k.* standard HD-1-S-1971 with an LD_{50} of 6.3 IU reported for four day old larvae (Lynch *et al.* 1977). The sunflower moth, *Homeosoma electellum* (Hulst) (Lepidoptera, Phycitidae) is one of the most susceptible to the strain HD-1 with an LC_{50} of 19.8 IU/ml determined for a *B.t.* preparation incorporated in an artificial diet against second and third instar larvae (Lidstone *et al.* 1985). *H. electellum* is about 40 fold more susceptible to this strain than *T. ni* and about 700 fold more susceptible than the least susceptible noctuid examined *A. ipsilon* (Table 4).

A number of individual strains of *B.t.* have been identified as superior in their potencies for particular noctuid species and these are good candidate isolates to screen against other noctuids. Only for *S. exigua* and *S. littoralis* has one single strain been identified as the most potent for more than one species of host (Salama *et al.* 1981a), although several strains are frequently included in the lists of most potent strains for a number of noctuids (Dulmage *et al.* 1981). For noctuid species weakly susceptible to the reference standard HD-1-S-1971, the more potent isolates were largely of varieties

entomocidus, *aizawai*, *tolworthi*, *thuringiensis*, and *kurstaki* (Table 4). Other candidate strains for screening against noctuids may be effectively chosen with a bias for strains belonging to these varieties.

The determination of potent strains is one of the most effective methods of improving the potency of *B.t.* preparations for particular pests. A six to seven fold improvement over the potency of the standard strain HD-1 has been achieved for the noctuid *S. litura* (Dulmage *et al.* 1981), and improvements of this magnitude may be possible for others. The magnitude of such an increase in potency is great because a difference of two or three fold susceptibility can be the difference between reasonable control such as with *T. ni* on cabbage (Sears *et al.* 1983) and marginal or variable control such as experienced for *Heliothis* spp. on cotton (Luttrell *et al.* 1982).

The potencies of *B.t.* preparations for noctuids have been improved or enhanced by other techniques which logically follow the selection of potent strains. Increases of two to four fold potency have been derived from each of: modifying bacterial fermentation techniques, adding sunlight protectants to increase the stability of the pathogen in the field, adding feeding stimulants to increase the feeding activity on the *B.t.* preparation, and adding compounds intended to optimize the conditions within the host midgut to favour the production of the delta-endotoxin (Salama 1984).

In the control of noctuid larvae, the application of *B.t.* preparations is not directly detrimental to natural enemies, including parasitoids and predators of the pest (Ali and Watson 1982b; Salama *et al.* 1982; Salama and Zaki 1983, 1984; Sneh *et al.* 1983). In addition, combinations of *B.t.* preparations and other microbial insecticides such

as fungal and viral agents have been effective against noctuids (Ignoffo *et al.* 1980; Bell and Romine 1980; Johnson 1982; Luttrell *et al.* 1982). *B.t.* preparations are compatible with certain chemical insecticides and combined applications at reduced rates of both have acted in synergism against noctuid larvae (Yearian *et al.* 1980; Mohamed *et al.* 1983a, 1983b; Salama *et al.* 1984c).

The scientific literature recounted holds encouraging progress in improving or enhancing the potency of *B. thuringiensis* preparations for larval Noctuidae. From this is obtained some of the direction for a comprehensive research program examining the potential of *B. thuringiensis* for controlling populations of *M. configurata*. One of the first steps in such a program is logically the determination of potent strains.

3. Methods and Materials

3.1 Rearing *M. configurata*

A non-diapause population of *M. configurata* has been reared continuously in the laboratory at the Winnipeg Research Station of Agriculture Canada since 1973. The artificial diet and rearing technique described by Bucher and Bracken (1976) were utilized with minor modifications in the present study.

Eggs and early instar larvae were maintained in a light/dark regime of 16:8 h, temperature of 25 $\pm$ 1°C, and a relative humidity of 60 $\pm$ 5%. From eggs collected the morning after oviposition, masses of 30-60 eggs were separated 3 days later and placed within a plastic ribbed creamer cup, with a capacity of 23 ml, containing rearing diet. Eggs hatched the next day and generally 4 days later the larvae had moulted into the third instar. The early third instar larvae used in tests were selected from cups containing individuals visually determined to be healthy; individuals were not chosen from cups which were overcrowded, or in which the larvae were of uneven size or poor colour.

Larvae for testing that day were gently removed from the cups using blunt forceps and placed within a plastic bell jar with screened vents. The larvae ascended the walls of the jar assorting themselves on the walls and ceiling, and were later removed through the bottom with a fine brush. The intent of this exercise was to promote the random allocation of siblings, individuals from the same original rearing cup and hence egg mass, to treatments within the same assay. All subsequent transfers of larvae were made with a fine brush.

3.2 The Bioassay

Lyophilized extracts, each containing spores and crystals of one of the 61 candidates strains of *B.t.* or the reference standard strain HD-1-S-1980, were supplied by H.T. Dulmage (USDA, Brownsville, Texas) and N.R. Dubois (USDA-FS, Hamden, Connecticut). The strains had been fermented and recovered by the method described by Dulmage and Beegle (1978), and once received, these powders were stored at 4°C in sealed vials. The 61 candidate strains have been listed in Table 5 by culture accession number in the international collection, with variety and crystovar indicated.

The bioassay procedures proposed by Dulmage and Beegle (1978) were adopted with minor changes to accommodate the particular biology of *M. configurata*. The artificial diet used in bioassays was the rearing diet (Bucher and Bracken 1976), except for the omission of the antibiotic, chlorotetracycline hydrochloride, which may affect the response of larvae (Beegle *et al.* 1981). The bioassay diet in sealed containers was maintained as a liquid for short periods of a few hours, at 55°C in a water bath.

Specified weights of the lyophilized extracts containing *B.t.* spores and crystals of the particular strain were resuspended in a buffered saline solution (Dulmage and Beegle 1978). These samples were then homogenized with a probe sonicator (MSE) rated at 60 watts, for a period of 1 min. (Bucher 1963). One tenth of a ml of 1% Tween 80, a wetting agent, was added for every 10 ml of sample. Samples were incorporated in the bioassay diet at a sample to total volume ratio of 1:25 or 1:10 and mixed in a blender at high speed for 4 min. Treated

Table 5: The 61 candidate strains of *B.t.* chosen to be screened for toxicity against larvae of *M. configurata*.

HD# = the culture accession number in the International Collection of *B.t.* Isolates

process = fermentation code (Dulmage and Beegle 1978)

NI = not identified

blank = not known

HD#	Process	Variety	Crystovar
1	R2069A	*kurstaki*	*k-1*
7	R924A	*dendrolimus*	*den*
8		*galleriae*	*gal*
34	R625A	*dendrolimus*	NI
35	R726A	*dendrolimus*	*den*
37	R911A	*dendrolimus*	*sot*
48		*dendrolimus*	*sot*
52	R925A	*aizawai*	*aiz*
73	R916A	*kurstaki*	*k-73*
84	R66A	*alesti*	*k-73*
87	R651B	*kurstaki*	*k-1*
89	R642B	*kurstaki*	*k-1*
110	R944B	*entomocidus*	*ent*
112	R650B	*aizawai*	*aiz*
120	R906B	*thuringiensis*	*k-1*
121	R1035A	*tolworthi*	*tol*
123	R958A	*kenyae*	*ken*
124		*tolworthi*	*tol*
125	R945A	*tolworthi*	*tol*
129	R945B	*galleriae*	*gal*
133	R2019B	*aizawai*	*aiz*
137	R908A	*aizawai*	*aiz*
166	R677B	*galleriae*	*gal*
176	R683C	*galleriae*	*gal*
181	R588B	*kurstaki*	*k-73*
182	R588C	*kurstaki*	*k-73*
184	R589A	*galleriae*	*gal*
186	R686A	*galleriae*	*gal*
187		*kurstaki*	*k-73*
198	R861C	*entomocidus*	*ent*
203	R939B	*kurstaki*	*k-1*
210	R592B	*galleriae*	*aiz*
228	R697A	*aizawai*	*aiz*
231	R639B	*kurstaki*	*k-1*
232	R639C	*galleriae*	*k-1*
234		*galleriae*	*aiz*
240	R662B	*galleriae*	*gal*
244	R702B	*kurstaki*	*k-1*
255	R920A	*kurstaki*	*k-1*
262		*kurstaki*	*k-1*
263	R1069A	*kurstaki*	*k-1*
269	R706B	*kurstaki*	*k-1*
276		*aizawai*	*ent*
282		*aizawai*	*aiz*
283	R978A	*aizawai*	*aiz*
285	R712B	*tolworthi*	*tol*
287	R664B	NI	*k-1*
320	R1020C	*entomocidus*	*ent*
337		*kurstaki*	*k-1*
341		*kurstaki*	*k-1*
363	R753B	NI	*k-1/thu*
498		NI	NI
551	R1087A	*kenyae*	NI
554	R1089A	*canadensis*	NI
562	R936A	*kurstaki*	NI
582	R972B	NI	NI
588	R2048A	*kenyae*	NI
635	R1021C	*entomocidus*	*ent*
703	R1032A	*thuringiensis*	*k-1/thu*
854	R2053B	*aizawai*	NI
855	R2054B	NI	NI

diet was then poured into plastic creamer cups, approximately 5 ml per cup, within an ultra-violet light hood and allowed to set. Bioassay diets for control treatments received only the sample volume of buffered saline solution and wetting agent.

A single larvae was placed in each cup; the cups were then sealed with cardboard lids and inverted onto rearing boards. The cups on boards were placed in an incubator of 25°C, 60% relative humidity, and a light/dark cycle of 16:8, and in this environment the larvae were allowed to feed *ad libitim* for 7 or 10 days.

3.3 Preliminary Screening

All 61 strains were screened at a relatively high concentration of 500 ug isolate/ml diet (Dulmage and Beegle 1978). For each screening of a single strain, 100 larvae were exposed to each treatment of the candidate strain, the reference standard strain, and the control. Sample volumes of 20 ml were incorporated in liquid bioassay diet to a total volume of 500 ml per treatment for a ratio of 1:25.

Immediately prior to being transferred into the cups, the larvae removed from the bell jar were weighed in five groups of 20 individuals per treatment. Mortality was recorded after 4 and 7 days continuous exposure and after 7 or 10 days the survivors were weighed in the 5 original units. A natural response rate of greater than 5% after 7 days was deemed unacceptable and screenings in which the mortality for the control treatment exceeded this level were repeated.

These methods were modified for strains HD-129, HD-554, and HD-635 because of insufficient quantities of spore-crystal extracts. The amount of powder of strain HD-554 permitted a screening of 80

individuals, in 4 groups of 20, while quantities of strains HD-129 and HD-635 necessitated screenings of single groups of 30 individuals.

Mortalities were corrected for the natural response rate by Abbott's formula (Abbott 1925). The independence of mortalities for the treatments of the candidate strain and reference standard strain was tested by a 2 x 2 contingency table analysis (Steel and Torrie 1980). The weight gain of survivors in mg/individual for each experimental unit of 20 individuals was natural log transformed to reduce the dependence of the variance on the mean survivor weight gain for treatments. An ANOVA with completely randomized design was performed on the original and transformed weight gains to test for treatment effects. Single missing values were estimated by minimizing the error sum of squares in the ANOVA (Steel and Torrie 1980). Planned comparisons by a method of orthogonal contrasts, each with a single degree of freedom, were performed to test the main effect of *B.t.* in the diets on the survivor weight gain, and the specific effects of the candidate isolates versus that of the reference standard treatment on survivor weight gain (Steel and Torrie 1980).

3.4 Concentration - Mortality Response Bioassays

The five most toxic strains based on a primary criterion of toxicity relative to the reference standard after 7 days, were bioassayed with concurrent bioassays of the reference standard HD-1-S-1980, and were repeated as quantities of lyophilized extracts permitted. Seven concentrations were selected for each bioassay at the predicted 25, 35, 40, 50, 60, 65 and 75% mortality levels, with the objective of arriving at precise estimates of median lethal

concentration (Robertson *et al.* 1984). Thirty larvae were exposed to each concentration of the candidate strain and reference standard, and five groups of 30 larvae were exposed to control treatments for each concurrent bioassay. Sample volumes of 15 ml were incorporated in liquid bioassay diet to a total volume of 150 ml for a ratio of 1:10.

Prior to being transferred into the cups, the larvae were weighed in the groups of 30 individuals per treatment. Mortality was recorded daily during the 7 days continuous exposure and the survivors after 7 days were weighed in their original groups.

Estimates of median lethal concentration and 95% confidence intervals were determined from regressions of probit mortality after 7 days against log concentration, and an estimate of relative potency and 95% confidence interval for each bioassay of a candidate strain was calculated from a parallel line regression with the concurrent bioassay of reference standard. Estimates of median lethal time (LT_{50}) for each concentration were determined from regressions of probit mortality against time in days; for each bioassay a regression of the estimates of median lethal time against log concentration was performed to reveal the concentration-time response. The percent reduction in weight gain of survivors at each concentration was the difference between the survivor weight gain in mg/individual and the mean control weight gain in mg/individual, expressed as a percentage of the mean control weight gain. For each bioassay of a candidate isolate or the reference standard, a regression of probit percent reduction in weight gain of survivors against log concentration was performed to reveal the concentration-survivor weight gain response.

3.5 Assessing the Bacteriophage Content of Isolates

Bacteriophages are occasionaly found in *B.t.* preparations and may affect their pathogenicity (Burgerjon and Martouret 1971); for this reason it was decided to test for bacteriophage in the isolates. The 61 isolates and reference standard HD-1-S-1980 were examined for the presence of bacteriophages by a method of surface plating (Billing 1969). The surface plating of serial dilutions of the reference standard indicated that a dilution of 10 mg isolate in 10 ml solution produced satisfactory bacterial lawns for this purpose. This dilution of each isolate was prepared by adding 10 mg to 10 ml buffered saline solution, and the mixture sonicated for 1 minute. Trypticase soy agar plates (BBL) were poured and dried closed for 48 hours at 32°C. Four replications of 2 ml of each isolate in solution were surface plated and plates were incubated at 32°C for 24 hours, after which bacteriophage plaques, or plaque forming units (PFU), were counted.

4. Results

4.1 Preliminary Screening of the 61 Strains

During the screening of larvae at 500 ug/ml, eight bioassays were repeated because the natural response rate after seven days was unacceptably high, the mortality among controls greater than 5%. At this concentration, 11 strains were significantly more toxic after 7 days than the reference standard (Table 6) with confidence of at least 95% determined by contingency table tests. Ten of these eleven strains belonged to varieties *aizawai*, *kenyae*, *kurstaki*, and *entomocidus*, while the variety of one was not known. Four of the eleven strains, HD-133, HD-112, HD-551, and HD-854, were also significantly more toxic after 4 days (Table 6). Corrected mortalities for larvae exposed to treatments of the most toxic strain, HD-133, at this concentration were 25.0 and 72.7% after 4 and 7 days respectively.

The weighted mean corrected mortalities and standard errors for individuals exposed to treatments of the reference standard in all of the 61 screenings were 1.54 ± 1.59 and 9.68 ± 2.48% after 4 and 7 days respectively. Summaries of the mortalities observed for all 61 strain treatments at 500 ug/ml with corresponding treatments of the reference standard are given in the Appendix.

A varietal profile of the toxicities of the 61 strains of *B.t.* screened, relative to the toxicity of the reference standard, is presented in Figure 2. This histogram was formed from the independence test values, the Chi-square values (X^2), for the mortalities from candidate and reference standard treatments at 500 ug/ml diet after 7 days. Five of the nine strains screened which belonged to var. *aizawai*

Table 6: Eleven strains of *B.t.* significantly more toxic than the reference standard at 500 ug/ml diet for third instar larvae of *M. configurata* after 7 days. They are ranked with the most potent at the top, according to the extremeness of the X^2 value after 7 days.

a) Mortality (%) for test isolates corrected for the natural response rate by Abbott's formula (Abbott 1925).

b) X^2 independence of test and reference standard mortalities.

(df=1) significant p(.05) = 3.84

highly significant p(.01) = 6.64

very higly significant p(.001) = 10.8

(Steel and Torrie 1980).

c) Average difference in weight change (mg/individual) for larvae ingesting test isolate compared with larvae ingesting the reference standard.

d) F (1,12) very highly significant p(.001) = 18.6 (Remmington and Schork 1970).

NI = not identified

mv = missing value in ANOVA

* = survivors weighed at 10 days.

HD#	Variety	Crystovar	4 Days		7 Days		Weight gain: Contrast of Candidate vs Standard			
			Mortality[a]	X^2 [b]	Mortality	X^2	$\bar{X}$[c]	SE		F[d]
133	*aizawai*	*aiz*	25.0	22.7	72.7	73.4	-59.3	6.43		892
112	*aizawai*	*aiz*	6.00	6.19	50.5	49.7	-66.7	3.69		209
551	*kenyae*	NI	14.3	12.0	58.8	35.2	-112	mv	*	155
582	NI	NI	6.12	0.072	67.4	27.4	-66.3	17.0	*	28.1
854	*aizawai*	NI	16.0	14.5	37.8	26.7	-70.1	13.1	*	335
262	*kurstaki*	*k-1*	3.03	1.85	34.7	16.5	-32.4	3.03		72.6
337	*kurstaki*	*k-1*	6.00	3.70	37.0	7.09	-20.4	1.8		28.8
198	*entomocidus*	*ent*	3.00	0.521	43.3	5.49	-48.2	11.0		67.4
282	*aizawai*	*aiz*	2.02	0.148	51.0	4.54	-44.4	6.05		97.2
562	*kurstaki*	NI	4.00	1.85	21.2	4.39	-119	11.9	*	33.9
52	*aizawai*	*aiz*	10.0	1.80	48.0	4.05	-56.0	5.28		125

Figure 2: Varietal profile of toxicity of 61 strains of *B.t.* compared with the toxicity of the reference standard at 500 ug/ml for third instar larvae of *M. configurata* after 7 days.

Al = var. *alesti*

Ca = var. *canadensis*

De = var. *dendrolimus*

En = var. *entomocidus*

Ke = var. *kenyae*

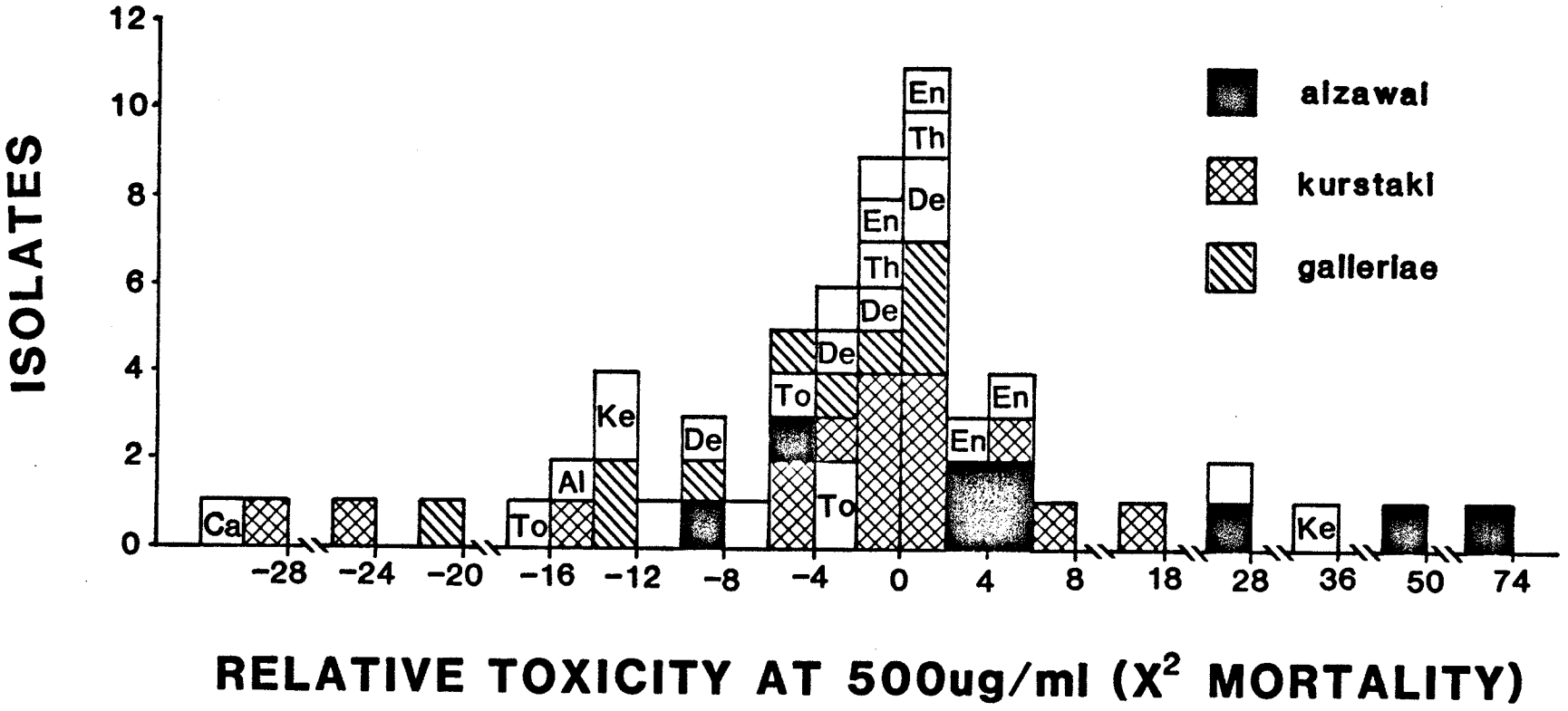
Th = var. *thuringiensis*

To = var. *tolworthi*

blank = variety is not identified

X^2 (df=1) significant p(.05) = 3.84

NB: Each block represents one isolate.


ISOLATES
12
10
8
6
4
2
0
Ca
To
Al
Ke
De
To
De
To
De
En
Th
De
En
Th
De
En
En
En
Ke
-28
-24
-20
-16
-12
-8
-4
0
4
8
18
28
36
50
74
aizawai
kurstaki
galleriae
RELATIVE TOXICITY AT 500ug/ml (X² MORTALITY)

were significantly superior to the reference standard, as were one of the three var. *kenyae* strains, one of the four var. *entomocidus* strains, and three of the 17 var. *kurstaki* strains.

The initial weight of the early third instar larvae chosen for the 61 screenings weighed in groups of 20 larvae ranged from 2.32 to 11.3 mg/individual. The overall mean and standard error of the mean initial weights for the screenings at 500 ug/ml, excluding the screenings of HD-129 and HD-635, was 5.59 $\pm$ 1.35 mg/individual. A natural log transformation reduced the dependence of the variance on the mean survivor weight gain for treatments and other ANOVA assumptions were satisfied by the transformed data. For the 59 unaltered screenings the null hypothesis that the candidate isolate, reference standard, and control treatment means of survivor weight gain were equal was rejected with highly significant F-ratio values calculated. The least significant F-ratio was calculated for the screen of HD-363 and had a probability of being exceeded of only 0.00195%.

Orthogonal contrasts of the main effect, the effect of *B.t.* in the diets on the survivor weight gain, all rejected the null hypothesis of equality of *B.t.* and control treatment means with significant F-ratio values. The least significant ratio was calculated for the screen of strain HD-554 and had a probability of being exceeded of less than 0.05%. The orthogonal contrasts for the effect of the individual candidate isolates versus the effect of the reference standard on survivor weight gain for the 11 superior strains are summarized in Table 6. For all eleven strains very highly significant F-ratio values were calculated rejecting the null hypotheses of equality of candidate isolate and reference standard treatment means. The average effect

calculated for each of the 11 superior strains was a reduction in survivor weight gain compared with reference standard treatments. For the screening of strain HD-133, survivors of the candidate isolate treatment gained on average 59.3 $\pm$ 6.43 mg/individual less than did survivors of the reference standard treatment and this difference was the most highly significant observed (Table 6).

4.2 Bioassays of the Five Most Toxic Strains

The results of concentration mortality response bioassays of the five most toxic strains are summarized in Table 7. For the candidate strains significant linear regressions of probit mortality against log concentration were obtained for all except the first bioassay of strain HD-582. Significant parallel line regressions were obtained for the candidate strains and synchronous bioassays of the reference standard, with the exception of the second bioassay of strain HD-582 and the only bioassay of strain HD-112. The most potent strains overall were HD-133, HD-551, and HD-854. Their estimates of median lethal concentration with 95% confidence intervals and corresponding estimates of potency with 95% confidence intervals are illustrated in Figure 3. The slopes (b) of the probit regression lines for these three strains were all greater than two and the lengths of confidence intervals were all less than twice the magnitude of the median lethal concentrations (Figure 3). Estimated potencies for the three were in the range of 40000 to 50000 IU/mg and the original ranking was maintained with strain HD-133 the most potent.

The eleven bioassays of the reference standard for which significant probit regressions were obtained are summarized in Figure 4. The weighted mean of the estimates of median lethal concentration was

Table 7: Summary of concentration-mortality responses of third instar larvae of *M. configurata* to the five strains of *B.t.* which were most toxic at 500 ug/ml.

nsr = non-significant regression

( ) = approximate of potency based on non-significant regression.

		Single Line Probit Analysis				Parallel Line Probit Analysis With Reference Standard	
HD#	Starting Date	LC_{50} (ug/ml)	95% Confidence Interval for LC_{50}	Slope (b)	SE_b	Relative Potency	95% Confidence Interval for Potency
133	25 Mar	362.8	262.5 - 830.2	3.409	0.970	2.958	1.903 - 3.987
133	26 May	296.4	262.6 - 326.9	4.963	0.746	2.773	2.367 - 3.236
551	3 Jun	349.3	287.6 - 417.9	2.659	0.630	2.713	2.112 - 3.467
551	8 Apr	367.4	326.9 - 404.4	4.452	0.698	2.535	1.894 - 3.364
854	25 Apr	447.6	374.9 - 501.1	4.420	0.754	2.469	2.094 - 3.069
582	30 May	509.0	438.4 - 659.9	3.354	0.681	(1.506)	----- nsr ----
582	15 Apr	---------- nsr ----------				1.664	0.9419 - 2.193
112	5 Apr	757.4	561.1 - 10850	1.380	0.698	(1.123)	----- nsr ----

Figure 3: Median lethal concentrations with 95% confidence intervals and corresponding estimates of potency with 95% confidence intervals for the three strains which were the most potent.

b = slope of the probit regression line.

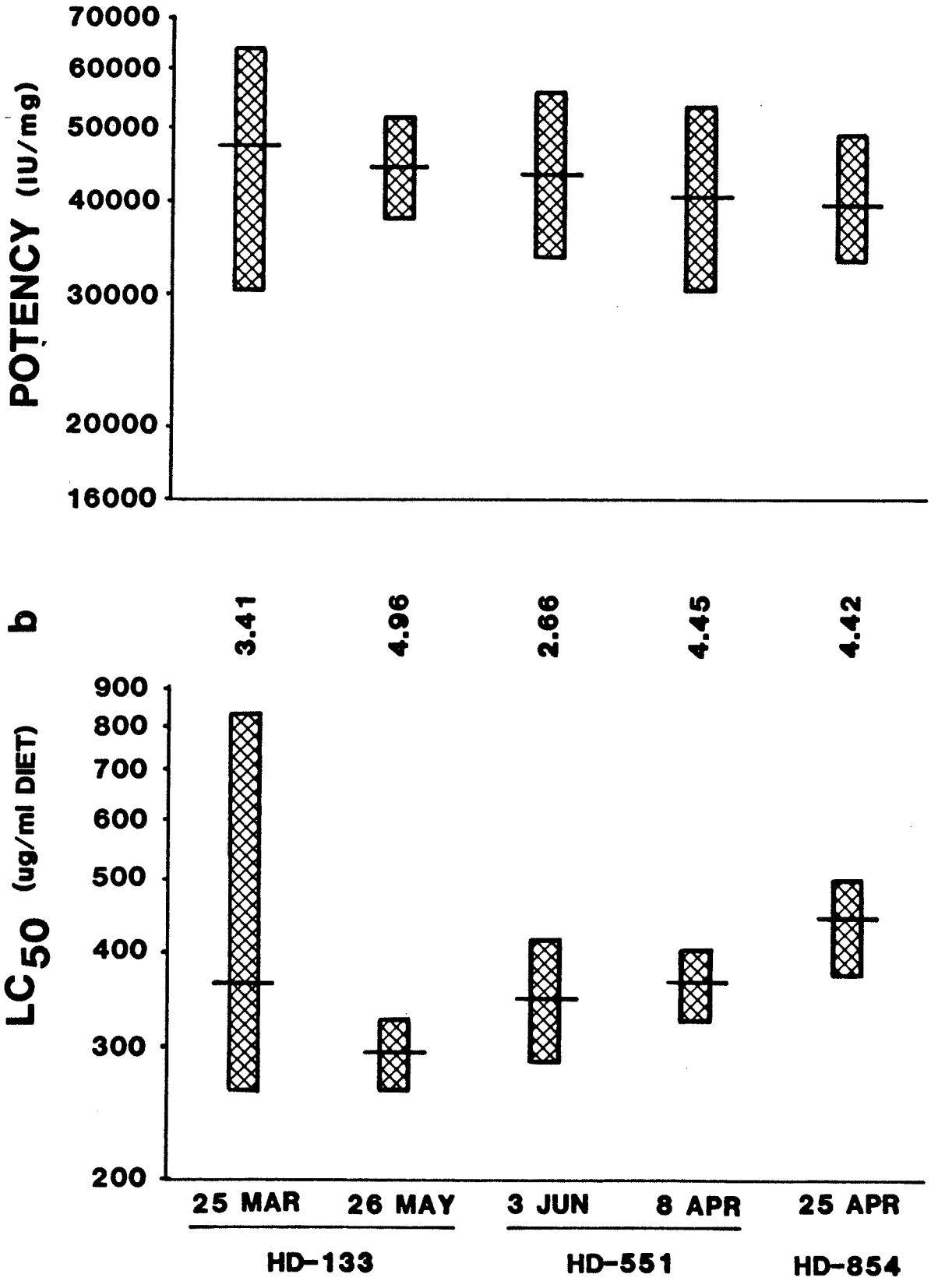
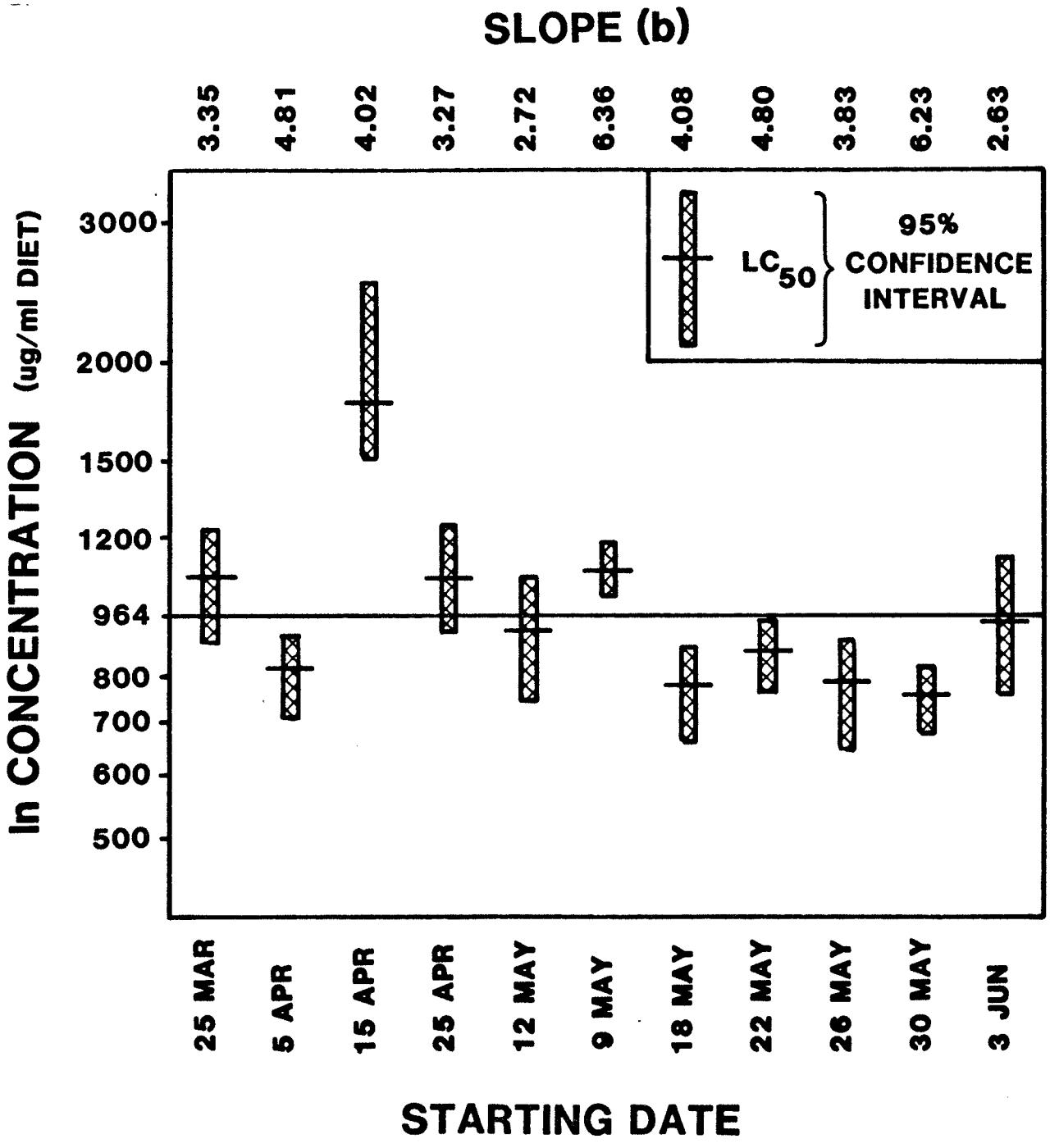

70000
60000
50000
40000
30000
20000
16000
POTENCY (IU/mg)
b
3.41
4.96
2.66
4.45
4.42
900
800
700
600
500
400
300
200
LC50 (ug/ml DIET)
25 MAR
26 MAY
3 JUN
8 APR
25 APR
HD-133
HD-551
HD-854

Figure 4: Concentration mortality responses of third instar larvae of *M. configurata* to the reference standard strain HD-1-S-1980.


SLOPE (b)
3.35
4.81
4.02
3.27
2.72
6.36
4.08
4.80
3.83
6.23
2.63
95% CONFIDENCE INTERVAL
LC50
ln CONCENTRATION (ug/ml DIET)
3000
2000
1500
1200
964
800
700
600
500
25 MAR
5 APR
15 APR
25 APR
12 MAY
9 MAY
18 MAY
22 MAY
26 MAY
30 MAY
3 JUN
STARTING DATE

calculated to be 964 ug/ml diet.

For each of the three most potent strains a significant regression of median lethal time against log concentration was obtained (Figure 5). A significant regression of this type was also obtained for the bioassay of the reference standard corresponding to the bioassay of strain HD-133, presented in Figure 5.

The mean weight gain of individuals exposed to the control treatments over the 7 days of concentration-mortality response bioassays was 241.5 $\pm$ 61.87 mg. Significant regressions of probit percent reduction in weight gain of survivors against log concentration were obtained for a bioassay of each of the three most potent strains, and also the bioassay of reference standard corresponding to that of strain HD-551 (Figure 6).

4.3 The Bacteriophage Content of the Isolates

The isolates which were determined to contain bacteriophage have been listed in Table 8. Two of the three most potent strains, HD-551 and HD-854, and the reference standard contained bacteriophage. Four isolates HD-89, HD-110, HD-263, and HD-320 were found to have high degrees of bacteriophage contamination relative to other bacteriophage contaminated isolates.

Figure 5: Regression of median lethal time against log concentration for bioassays of each of the three most potent strains.

( ) = bioassay starting date.

r = correlation coefficient

b = slope

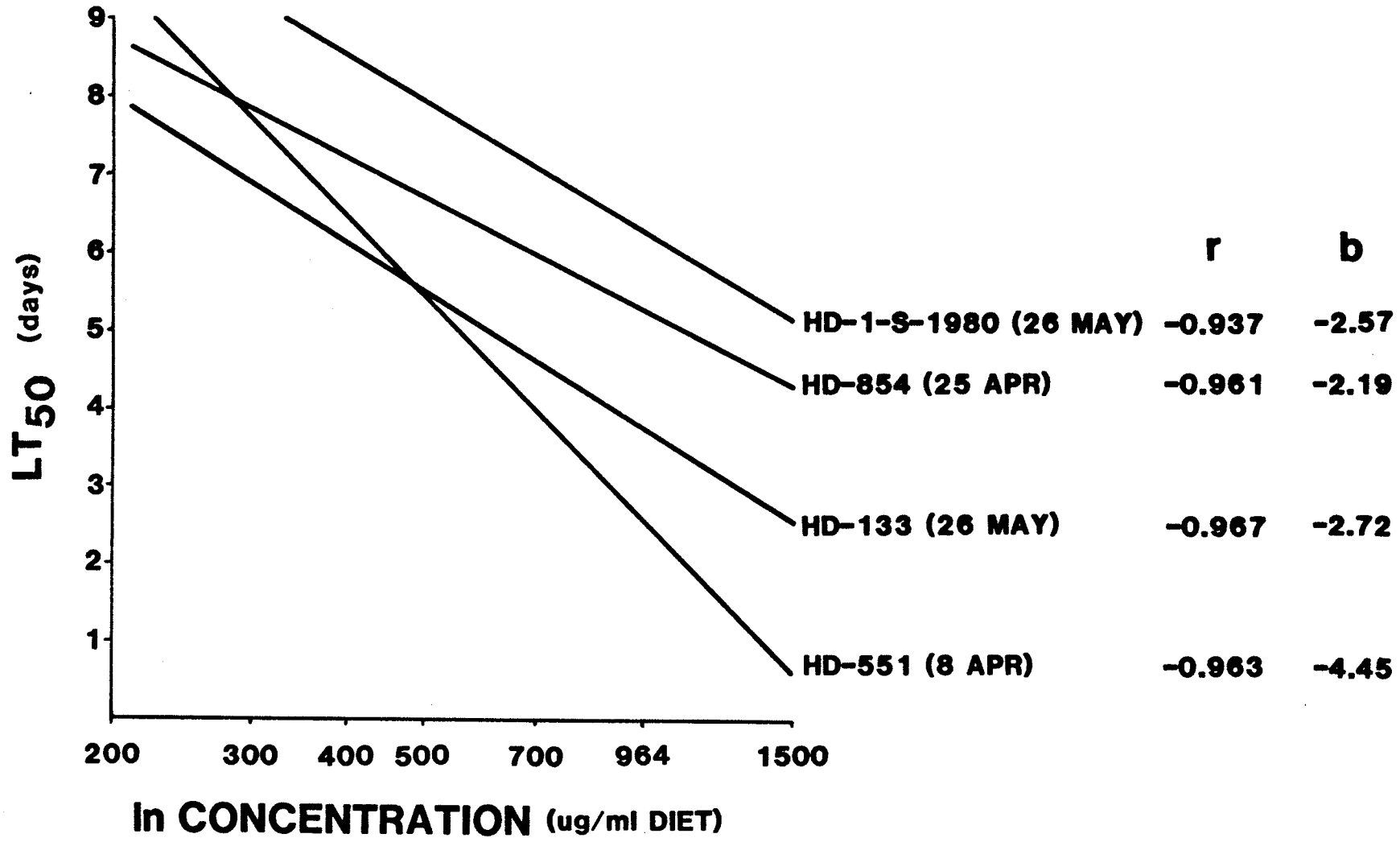

LT50 (days)
9
8
7
6
5
4
3
2
1
200
300
400
500
700
964
1500
ln CONCENTRATION (ug/ml DIET)
r
b
HD-1-S-1980 (26 MAY) -0.937 -2.57
HD-854 (25 APR) -0.961 -2.19
HD-133 (26 MAY) -0.967 -2.72
HD-551 (8 APR) -0.963 -4.45

Figure 6: Regression of probit percent reduction in weight gain of survivors against log concentration for bioassays of each of the three most potent strains.

( ) = bioassay starting date.

r = correlation coefficient

b = slope

r	b	
0.846	0.876	HD-133 (25 MAR)
0.870	0.973	HD-551 (8 APR)
0.858	1.19	HD-854 (25 APR)
0.939	0.754	HD-1-S-1980 (8 APR)

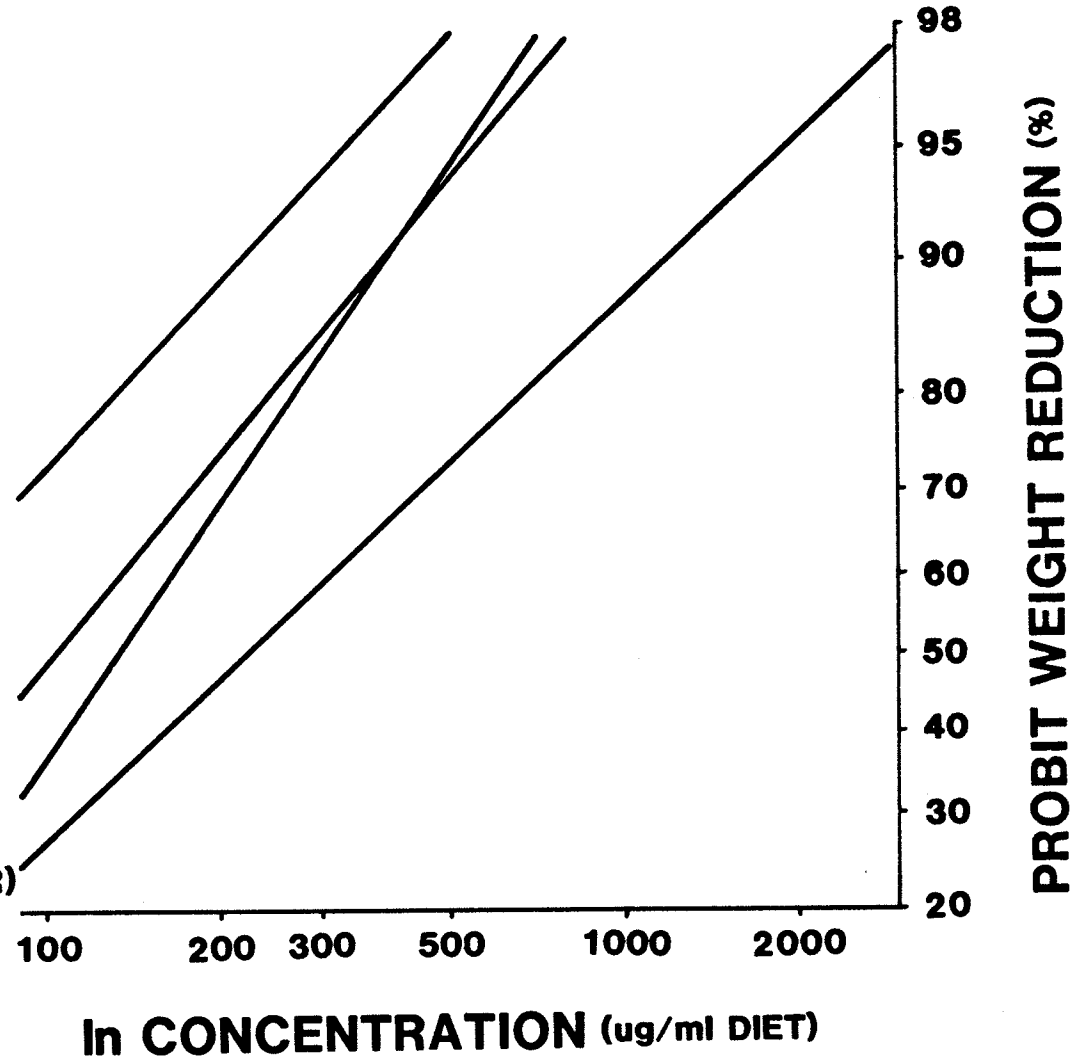


Table 8 : *Bacillus thuringiensis* isolates found to contain bacteriophage.

HD#	Variety	Bacteriophage content in PFU/0.002g isolate Mean ± SE[a]
1	*kurstaki*	1.3 ± 1.5
8	*galleriae*	2.3 ± 1.7
89	*kurstaki*	(100-200)[b]
110	*entomocidus*	(1000-2000)
263	*kurstaki*	(100-200)
276	*aizawai*	0.5 ± 0.68
283	*aizawai*	15 ± 3.4
320	*entomocidus*	(100-200)
551	*kenyae*	67 ± 12
562	*kurstaki*	0.75 ± 0.5
854	*aizawai*	7.3 ± 3.0
1-S-1980	*kurstaki*	15 ± 5.2

a) Mean ± SE from counts of 4 plates

b) Estimated PFU in parentheses

NB : PFU = plaque forming units.

5. Discussion

5.1 The Susceptibility of *M. configurata* to the *B.t.k.* Reference Standard Strain HD-1

It is evident that larvae of *M. configurata* are only very weakly susceptible to the *B.t.* var. *kurstaki* reference standard strain HD-1. This was apparent early in the investigation because in the preliminary screenings the comparatively high concentration of 500 ug/ml of HD-1-S-1980 inflicted on average only 9.68% mortality to early third instar larvae after 7 days feeding. Another isolate of the HD-1 strain was not significantly different from HD-1-S-1980 in its toxicity for larvae of *M. configurata* in these screenings (Table 10 in the Appendix). Concentration-mortality responses confirmed what was suspected; the weighted mean of 11 estimates of median lethal concentration, determined from acceptable bioassays performed over a period of two months, was calculated at 964 ug/ml diet (Figure 4). This concentration of the reference standard HD-1-S-1980 is equivalent to 15400 IU/ml diet which is one of the highest median lethal concentrations reported for larval Noctuidae (Table 4). The susceptibility of *M. configurata* to the strain HD-1 is comparable to that of *A. ipsilon* (Salama and Foda 1984) while both are less than half as susceptible to this strain as *S. littoralis* (Salama *et al.* 1983c) and less than 15 times as susceptible as *T. ni* (Ignoffo *et al.* 1977) (Table 4).

5.2 Potent Strains for *M. configurata*

Eleven strains were significantly more toxic for larvae of *M. configurata* than the reference standard in preliminary screenings (Table 6). Of these eleven, five strains belonged to variety *aizawai* including three of the five most toxic which were bioassayed further, and two of the three strains which were most potent overall (Figure 3). The prominence in terms of toxicity of strains belonging to variety *aizawai* in the susceptibility spectrum of *M. configurata* is illustrated by the varietal profile of relative toxicities for the 61 strains (Figure 2). The distribution of the nine *aizawai* strains is oriented towards the higher toxicity end of the spectrum, relative to the toxicity of the reference standard. The prospects of other potent strains for *M. configurata* existing among strains of variety *aizawai* may be good based on this sample of the susceptibility spectrum.

Strains belonging to variety *entomocidus* were also distributed towards the greater toxicity end of the profile with one strain out of the four falling amongst the 11 more toxic strains (Figure 2). One of the three most potent strains overall was of variety *kenyae* while the broad distribution of strains belonging to this variety across the susceptibility spectrum sample does not provide any information concerning the prospects of other potent strains. The same is true of the wide distribution of strains belonging to variety *kurstaki*, of which three of the 17 strains screened were significantly more toxic than the reference standard.

These findings are consistent with the reported susceptibility spectra of other larval Noctuidae which are weakly susceptible to the

reference standard HD-1; strains of varieties *aizawai* and *entomocidus* are notable as ranked nearer the top of their lists of more potent strains (Table 4). Of 18 strains of *B.t.* with high toxicity for *S. exigua*, Dulmage *et al.* (1981) found 14 were of variety *aizawai* including the most potent strain with 650% more potency than the reference standard. The most toxic strain which Salama *et al.* (1981a) indicated for *S. exigua*, in an examination of 29 strains, was four times more potent than HD-1-S-1971 and belonged to variety *entomocidus*. The most toxic strains of 15 highly toxic ones amongst 297 examined for *S. litura* belonged to variety *aizawai*, *kenyae* and *entomocidus*. The most potent strains for *S. littoralis* determined by Salama *et al.* (1981a) and Sneh *et al.* (1981) were both of variety *entomocidus*. Among potent strains indicated for *A. ipsilon* (Salama and Foda 1984), the second most potent was of variety *entomocidus*.

Three strains, HD-133, HD-551, and HD-854 were determined to be most potent overall for larval *M. configurata* based on a number of measures. All were in the order of two to four times more potent than the reference standard as revealed by concentration mortality responses (Figure 3). The significant parallel regressions indicate that within the realm of statistical probabilities, the three strains are more toxic than the reference standard at all concentrations after 7 days (Finney 1971). In the preliminary screening these three strains were more toxic than the reference standard with very highly significant tests of independence calculated after both 4 days and 7 days (Table 6).

The concentration-time responses illustrated in Figure 5 clarify the relative improvements of the three strains in terms of rate of kill. At least for a bioassay of the best strain HD-133, and its corresponding

reference standard, the significant regressions indicate that the median lethal time at any reasonable concentration of HD-133 was less than that of the reference standard. The rate of kill of strain HD-854 may be intermediate between the rates for HD-133 and the reference standard. The concentration-time response for a bioassay of strain HD-551 suggests that this strain was more rapid in its kill at higher concentrations than strain HD-133; however, this possibility would have to be further examined (Figure 5).

Sublethal effects of reduced survivor weight gain as a result of *B.t.* treatments were indicated in the preliminary screenings. The 11 strains which were significantly more toxic than the reference standard after 7 days produced the effect of an average reduction in survivor weight gain compared with treatments of the reference standard, and all of the differences were very highly significant (Table 6). The reductions relative to the reference standard for strains HD-133, HD-551, and HD-854 were among the most highly significant. Significant linear responses of concentration-weight reduction were obtained which disclose the relative improvements in terms of sublethal effects of these three strains which were most potent overall (Figure 6). The percent reductions in weight gain for survivors of a bioassay of strain HD-551 were higher, over the effective range of concentrations, than survivors of the corresponding bioassay of reference standard. Reductions in weight gain for survivors of HD-133 treatments may be higher yet; however, further investigation of this relationship is warranted. The positive correlation of certain sublethal effects with *B.t.* concentration has also been demonstrated for *S. litura* (Sareen *et al.* 1983).

Strains HD-112 and HD-582 were not among those determined most potent overall, although they were two of the five most toxic strains after preliminary screening (Table 6). The significant probit regression of a parallel bioassay for strain HD-582 estimated a relative potency of 1.664, however the 95% confidence interval for potency included that of the reference standard (Table 7). The only bioassay of strain HD-112 which quantities of isolate would permit produced a significant linear response of concentration-mortality with such a low slope that the predicted value of the median lethal concentration was greatly exceeded, and consequently estimated with little precision. This low slope was atypical of slopes for the bioassays overall (Table 7, Figure 3). The parallel regression was not significant; therefore, the relative potency of this strain is unresolved.

5.3 The Effect of Bacteriophage Content

The effect of bacteriophage content on the toxicity of strains of *B.t.* for larval Lepidoptera has not been extensively investigated. It has been hypothesized without proof that bacteriophage observed in the gut of several larval Lepidoptera might reduce their susceptibility to infection by ingested spores of *B.t.* (Burgerjon and Martouret 1971, Lebrun and Vlayen 1979). For the limited study undertaken here, the presence of bacteriophage in 11 of the 62 isolates examined did not appear to be associated with a loss of toxicity. Two of the three strains which were most potent overall and the reference standard strain were among those containing bacteriophage (Table 7). Of the four strains HD-89, HD-110, HD-263, and HD-320 which were determined to have high degrees of bacteriophage contamination, three were as toxic for

M. configurata as the reference standard. Nineteen of the isolates which were not indicated as contaminated by bacteriophage were significantly less toxic than the reference standard.

Bacteriophage replication studies suggest that a threshold density of host cells exists at approximately 10^4 colony forming units (CFU)/ml below which bacteriophage replication is not favoured (Wiggins and Alexander 1985). Lyophilized extracts of *B.t.* may contain in the order of 10^8 CFU/mg (Sneh and Gross 1983). Concentrations within the range of 0.1 to 3.3 mg isolate/ml diet were fed to larvae; therefore, threshold densities were exceeded in the treated diet. The effect of bacteriophage content on the potency of isolates may be obscured by the dominance of the delta-endotoxin action in the pathogenicity of *B.t.* for *M. configurata*. Further investigations comparing the potencies of the same isolates with and without bacteriophage contamination are needed to adequately determine the effect of bacteriophage content on the potency of isolates of *B.t.*

5.4. Confirmation of the Bioassay System

The precision with which estimates of median lethal concentration were often obtained, the reproducibility of potency ratios, and the generally high slopes, which for insect pathogens are typically less than two (Burges and Thomson 1971) all reflect favourably on the bioassay technique (Figure 3, 4). Careful control of the physiological state of the insects, even distribution of pathogen in the diet, random allocation of larvae to treatments, and the choice of concentrations and larval numbers were key factors affecting the reduction of variation (Burges and Thomson 1971). Aseptic technique improved with time as a bioassay

exceeding the restriction on the acceptability of bioassays, natural response rate not greater than 5%, became a rare event.

5.5 Implications of the Results for IPM of *M. configurata*

Control of *M. configurata* with preparations of the *B.t.k.* standard strain HD-1 can be predicted to be marginal at best, given the median lethal concentration of this strain was determined to be 15400 IU/ml against early third instar larvae (Figure 4). A two to four fold improvement in potency relative to the reference standard was estimated for the three most potent strains. This can be equated with a reduction in median lethal concentration of from 964 ug/ml of the reference standard to approximately 325 ug/ml of strain HD-133 (Figure 3).

A further two to four fold improvement may be achieved through techniques which logically follow the selection of potent strains. Modifications to the fermentation technology, the addition of feeding stimulants and ultra-violet light protectants, and the incorporation of synergists have each indicated relative improvements of this magnitude for other larval Noctuidae (Salama 1984). One such further improvement can reduce the median lethal concentration of HD-133 to just over 100 ug/ml. This would bring a strain of *B.t.* into the range of effectiveness which exists for larval Noctuidae that can be controlled with preparations of *B.t.k.* strain HD-1 at rates which are economically competitive with other insecticides; the median lethal concentrations of HD-1-S-1971 for third instar larvae of *T. ni* and *H. virescens* are approximately 44 and 111 ug/ml respectively (Table 4). The possibility of a *B.t.* preparation assuming a large role in an integrated pest management program for *M. configurata* is consequently not improbable;

although, there remains aspects of the field ecology of *M. configurata*, with respect to its control by *B. thuringiensis* preparations, which also require investigating.

6. Conclusions

(i) Larvae of *M. configurata* are only weakly susceptible to the reference standard, *B.t.* var. *kurstaki* strain HD-1-S-1980. A median lethal concentration of 15400 IU/ml was determined for early third instar larvae.

(ii) Strains which are more toxic than the reference standard at concentrations of 500 ug/ml belong to varieties *aizawai*, *kenyae*, *kurstaki*, and *entomocidus*.

(iii) The prospects are good that additional potent strains for *M. configurata* exist among other strains of the variety *aizawai*.

(iv) Strains HD-133, HD-551, and HD-854 are the most potent among the 61 strains examined, possessing two to four fold more potency for *M. configurata* than the reference standard.

(v) A sublethal effect of exposure to *B.t.* for larvae of *M. configurata* is a reduced rate of growth. Survivors of *B.t.* treatments gain less weight over the duration of the bioassay than do survivors of control treatments.

(vi) Strains which are more toxic than the reference standard cause a greater reduction in the rate of growth. Survivors of treatments of the more toxic strains gain less weight than do survivors of treatments of the reference standard.

(vii) A linear relationship exists for probit percent reduction in weight gain increasing with the natural logarithm of the *B.t.* concentration.

(Viii) Isolates of *B.t.* may occasionally be contaminated by bacteriophage. Contaminated strains retained their potency for

larvae of *M. configurata*; however, the effect of this contamination on the potency of strains requires further investigation.

LITERATURE CITED

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18**, 265-267.

Ali, A.-S.A., and Watson, T.F. 1982a. Survival of tobacco budworm (Lepidoptera: Noctuidae) larvae after short term feeding periods on cotton treated with *Bacillus thuringiensis*. *J. Econ. Entomol.*, **75**, 630-632.

Ali, A.-S.A., and Watson, T.F. 1982b. Efficacy of Dipel and *Geocoris punctipes* (Hemiptera: Lygaeidae) against the tobacco budworm (Lepidoptera: Noctuidae) on cotton. *J. Econ. Entomol.*, **75**, 1002-1004.

Ames, B.N., McCann, J., and Yamaski, E. 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. *Mutat. Res.*, **31**, 347-364.

Andreadis, T.G., Dubois, N.R., Moore, R.E.B., Anderson, J.F. and Lewis, F.B. 1983. Single applications of high concentrations of *Bacillus thuringiensis* for control of gypsy moth (Lepidoptera: Lymantriidae) populations and their impact on parasitism and disease. *J. Econ. Entomol.*, **76**, 1417-1422.

Andreadis, T.G., Dubois, N.R., Weseloh, R.M., Moore, R.E.B., Anderson, J.F., and Lewis, F.B. 1982. Aerial spray tests with *Bacillus thuringiensis* for control of the gypsy moth in Connecticut. *Conn. Arg. Exp. Sta., New Haven, Bull.*, **807**.

Anonymous. 1969-1983. "The Canadian Agricultural Insect Pest Revue". Vols. **47-61**, Agriculture Canada, Ottawa.

Anonymous. 1983a. "Distribution Maps of Pests". Commonwealth Institute of Entomology, Commonwealth Agricultural Bureau, London.

Anyonymous. 1983b. "1983 Manitoba Agriculture Yearbook". Manitoba Agriculture, Agdex 850.

Atlas, R.M., and Bartha, R. 1981. "Microbial Ecology: Fundamentals and Applications". Addison-Wesley Publ. Co., Don Mills, Ontario.

de Barjac, H. 1981. Identification of H-serotypes of *Bacillus thuringiensis*. *In* "Microbial Control of Pests and Plant Diseases, 1970-1980" (H.D. Burges, ed.), pp. 35-43. Academic Press, New York.

Bechtel, D.B., and Bulla, L.A. Jr. 1976. Electron microscope study of sporulation and parasporal crystal formation in *Bacillus thuringiensis*. *J. Bacteriol.*, **127**, 1472-1481.

Beegle, C.C. 1982. Studies on the bioassay of *Bacillus thuringiensis* vs. lepidopterous insects. Paper presented at the Third International Workshop on *B.t.*, Bellagio, Italy, 30 Aug.- 3 Sept.

Beegle, C.C., Dulmage, H.T., and Wolfenbarger, D.A. 1982. Relationship between laboratory bioassay-derived potencies and field efficacies of *Bacillus thuringiensis* isolates with different spectral activities. *J. Invertebr. Pathol.*, **39**, 138-148.

Beegle, C.C., Lewis, L.C., Lynch, R.E., and Martinez, A.J. 1981. Interaction of larval age and antibiotic on the susceptibility of three insect species to *Bacillus thuringiensis*. *J. Invertebr. Pathol.*, **37**, 143-153.

Beirne, B.P. 1971. Pest insects of annual crop plants in Canada, I, Lepidoptera; II, Diptera; III, Coleoptera. *Mem. Entomol. Soc. Can.*, **78**.

Bell, M.R., and Romine, C.L. 1980. Tobacco budworm field evaluation of microbial control in cotton using *Bacillus thuringiensis* and a nuclear polyhedrosis virus with a feeding adjuvant. *J. Econ. Entomol.*, **73**, 427-430.

Billing, E. 1969. Isolation, growth, and preservation of bacteriophages. *In* "Methods in Microbiology" (J.R. Norris and D.W. Ribbons, eds.), Vol. 3B, pp. 315-329. Academic Press, New York.

Boman, H.G. 1981. Insect responses to microbial infections. *In* "Microbial Control Pests and Plant Diseases, 1970-1980" (H.D. Burges, ed.), pp. 769-784. Academic Press, New York.

Bracken, G.K. 1984. Within plant preferences of larvae of *Mamestra configurata* (Lepidoptera: Noctuidae) feeding on oilseed rape. *Can. Entomol.*, **116**, 45-49.

Bracken, G.K., and Bucher, G.E. 1984. Measuring the cost benefit of control measures for bertha armyworm (Lepidoptera: Noctuidae) infestations in rapeseed. *Can. Entomol.*, **116**, 591-595.

Briese, D.T. 1981. Resistance of insect species to microbial pathogens. *In* "Pathogenisis of Invertebrate Microbial Diseases" (E.W. Davidson, ed.), pp. 511-545. Allanheld, Osmun, New Jersey.

Buchanan, R.E., and Gibbons, N.E., eds. 1974. "Bergey's Manual of Determinative Bacteriology, 8th Edition". The Williams and Wilkins Co., Baltimore.

Bucher, G.E. 1963. Ultrasonic cleaning of spores of an obligate bacterial pathogen. *J. Insect Pathol.*, **5**, 427-433.

Bucher, G.E., and Bracken, G.K. 1976. The bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Artificial diet and rearing technique. *Can. Entomol.*, **108**, 1327-38.

Bucher, G.E., and Turnock, W.J. 1983. Dosage responses of the larval instars of the bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), to a native nuclear polyhedrosis. *Can. Entoml.*, 115, 341-349.

Bull, D.L., House, U.S., Ables, J.R., and Morrison, R.K. 1979. Selective methods for managing insect pests of cotton. *J. Econ. Entomol.*, 72, 841-846.

Burgerjon, A., and Martouret, D. 1971. Determination and significance of the host spectrum of *Bacillus thuringiensis*. *In* "Microbial Control of Insects and Mites" (H.D. Burges, ed.), pp. 305-325. Academic Press, New York.

Burges, H.D. 1967. The standardization of products based on *Bacillus thuringiensis*. *In* "Insect Pathology and Microbial Control" (P.A. van der Laan, ed.), pp. 306-338. Proceedings of the International Colloquium on Insect Pathology and Microbial Control, Wageningen, The Netherlands, 1966. North Holland Publ. Co., Amsterdam.

Burges, H.D. 1971. Possibilities of pest resistance to microbial control agents. *In* "Microbial Control of Insects and Mites" (H.D. Burges and N.W. Hussey, eds.), pp. 445-457. Academic Press, New York.

Burges, H.D. 1984. Nomenclature of *Bacillus thuringiensis* with abbreviations. *Mosquito News*, 44, 66-68.

Burges, H.D., and Thomson, E.M. 1971. Standardization and assay of microbial insecticides. *In* "Microbial Control of Insects and Mites" (H.D. Burges and N.W. Hyssey, eds.), pp. 591-622. Academic Press, New York.

Cantwell, G.E., and Cantelo, W.W. 1982. Potential of *Bacillus thuringiensis* as a microbial agent against the Mexican bean beetle. *J. Econ. Entomol.*, 75, 348-350.

Cantwell, G.E., Dougherty, E., and Cantelo, W.W. 1983. Activity of the beta-exotoxin of *Bacillus thuringiensis* against the Colorado potato beetle, (Coleoptera: Chrysomelidae) and the bacterial mutagenic response as determined by the Ames test. *Environ. Entomol.*, 12, 1424-1427.

Creighton, C.S., and McFadden, T.L. 1975. Cabbage caterpillars: Efficacy of chlordimeform and *Bacillus thuringiensis* in spray mixtures and comparative efficacy of several chemical and *B. thuringiensis* formulations. *J. Econ. Entomol.*, 68, 57-60.

Creighton, C.S., McFadden, T.L., and Cuthbert, R.B. 1971. Control of caterpillars on tomatoes with chemicals and pathogens. *J. Econ. Entomol.*, 64, 737-739.

Creighton, C.S., McFadden, T.L., and Robbins, M.L. 1981. Comparative control of caterpillars on cabbage cultivars treated with *Bacillus thuringiensis*. *J. Ga. Entomol. Soc.*, **16**, 361-367.

Dabi, R.K., Sharma, H.C., and Shinde, V.K. 1979. Bioefficacy of *Bacillus thuringiensis* Berliner against *Heliothis armigera* Hubner on gram (*Cicer arietinum* L.). *Entomon.*, **4**, 343-345.

DeLucca, A.J. III, Palmgren, M.S., and Ciegler, A. 1982. *Bacillus thuringiensis* in grain elevator dusts. *Can. J. Microbiol.*, **28**, 452-456.

Dimond, J.B. 1982. Effects of aerial sprays of undiluted *Bacillus thuringiensis* formulations on spruce budworm. *Maine Agr. Exp. Sta.*, Misc. Rep. 274.

Dulmage, H.T. 1970. Insectidical activity of HD-1, a new isolate of *Bacillus thuringiensis* var. *alesti*. *J. Invertebr. Pathol.*, **15**, 232-239.

Dulmage, H.T. 1973a. Assay and standardization of microbial insecticides. *Ann. N.Y. Acad. Sci.*, **217**, 187-199.

Dulmage, H.T. 1973b. *B. thuringiensis* U.S. assay standard. Report on the adoption of a primary U.S. reference standard for assay of formulations containing the delta-endotoxin of *Bacillus thuringiensis*. *Bull. Entomol. Soc. Am.*, **19**, 200-202.

Dulmage, H.T., and Aizawa, K. 1982. Distribution of *Bacillus thuringiensis* in nature. *In* "Microbial and Viral Pesticides" (E. Kurstak, ed.), pp. 209-237. Marcel Dekker, Inc., New York.

Dulmage, H.T., and Beegle, C.C. 1978. Cooperative program for studies on the spectrum of activity of the delta-endotoxins produced by *Bacillus thuringiensis*. USDA-SEA-AR, Brownsville, Texas 78520, mimeograph 9 pp.

Dulmage, H.T., Boening, O.P., Rehnborg, C.S., and Hansen, G.D. 1971. A proposed standardized bioassay for formulations of *Bacillus thuringiensis* based on the international unit. *J. Invertebr. Pathol.*, **18**, 240-245.

Dulmage, H.T., and Cooperators. 1981. Insecticidal activity of isolates of *Bacillus thuringiensis* and their potential for pest control. *In* "Microbial Control of Pests and Plant Diseases, 1970-1980" (H.D. Burges, ed.), pp. 193-222. Academic Press, New York.

Dulmage, H.T., Martinez, A.J., and Penna, T. 1976. Bioassay of *Bacillus thuringiensis* (Berliner) delta-endotoxin using the tobacco budworm. *U.S. Dep. Agr. Tech. Bull.*, 1528.

Fast, P.G. 1981. The crystal toxin of *Bacillus thuringiensis*. *In* "Microbial Control of Pests and Plant Diseases, 1970-1980" (H.D. Burges, ed.), pp. 223-247. Academic Press, New York.

Faust, R.M. 1975. Toxins of *Bacillus thuringiensis*: Mode of action. *In* "Biological Regulations of Vectors" (J.D. Briggs, ed.), pp. 31-48. *U.S. Dep. Health, Education & Welfare Publ.* (NIH) 77-1180.

Faust, R.M., and Bulla, L.A. Jr. 1982. Bacteria and their toxins as insecticides. *In* "Microbial and Viral Pesticides" (E. Kurstak, ed.), pp. 75-208. Marcel Dekker, Inc., New York.

Finney, D.J. 1971. "Probit Analysis". Cambridge University Press, London.

Goldberg, I., Sneh, B., Battat, E., and Klein, D. 1980. Optimization of a medium for a high yield production of spore-crystal preparation of *Bacillus thuringiensis* effective against the Egyptian cotton leaf worm *Spodoptera littoralis* Boisd. *Biotechnololgy Letters*, **2**, 416-426.

Gordon, R.E. 1975. Some taxonomic observations on the genus *Bacillus*. *In* "Biological Regulations of Vectors" (J.D. Briggs, ed.), pp. 67-82. *U.S. Dep. Health, Education & Welfare Publ.* (NIH) 77-1180.

Govindarajan, R., Jayaraj, S., and Narayanan, K. 1975. Observations on the nature of resistance in *Spodoptera litura* (F.) (Noctuidae: Lepidoptera) to infection by *Bacillus thuringiensis* Berliner. *Indian J. Exp. Biol.*, **13**, 548-550.

Hall, I.M., and Dunn, P.H. 1958. Susceptibility of some insect pests to infection by *Bacillus thuringiensis* Berliner in laboratory tests. *J. Econ. Entomol.*, **51**, 296-298.

Harshbarger, J.C., and Faust, R.M. 1973. Environmental factors internal to the host that affect the success of microbial insecticides. *Ann. N.Y. Acad. Sci.*, **217**, 131-140.

Heimpel, A.M., and Angus, T.A. 1963. Diseases caused by spore forming bacteria. *In* "Insect Pathology, An Advanced Treatise" (E.A. Steinhaus, ed.), Vol. 2, pp. 21-73. Academic Press, New York.

Henry, J.E. 1982. Use of baits in microbial control of insects. *In* "Invertebrate Pathology and Microbial Control" pp. 45-48. Proceedings of the 3rd International Colloquium on Invertebrate Pathology, University of Sussex, Brighton, U.K.

Holliday, N.J., Smith, D.L., Kolach, A.J., and McCullough, D.J. 1985. Insect control on field crops: 1985-1986. Manitoba Agriculture Agdex 622.

Huber, H.E., and Luthy, P. 1981. *Bacillus thuringiensis* delta-endotoxin: Composition and activation. *In* "Pathogenisis of Invertebrate Microbial Diseases" (E.W. Davidson, ed.), pp. 209-234. Allanheld, Osmun, New Jersey.

Ignoffo, C.M., Hostetter, D.L., Pinnel, R.E., and Garcia, C. 1977. Relative susceptibility of six soybean caterpillars to a standard preparation of *Bacillus thuringiensis* var. *kurstaki*. *J. Econ. Entomol.*, **70**, 60-63.

Ignoffo, C.M., Zuber, M.S., Garcia, C., Darrah, L.L., and Deutscher, D. 1980. Evaluation of baculovirus heliothis, *Bacillus thuringiensis*, *Nomuraea rileyi*, and carbaryl against *Heliothis zea* on hand-pollinated sweet corn ears. *J. Kans. Entomol. Soc.*, **53**, 485-489.

Jaques, R.P. 1972. Control of the cabbage looper and the imported cabbageworm by viruses and bacteria. *J. Econ. Entomol.*, **65**, 757-760.

Johnson, D.R. 1982. Suppression of *Heliothis* spp. on cotton by using *Bacillus thuringiensis*, *Baculovirus heliothis*, and two feeding adjuvants. *J. Econ. Entomol.*, **75**, 207-210.

Kalfon, A.R., and de Barjac, H. 1985. Screening of the insecticidal activity of *Bacillus thuringiensis* strains against the Egyptian cotton leaf worm *Spodoptera littoralis*. *Entomophaga*, **30**, 177-186.

King, K.M. 1928. *Barathra configurata* Wlk., an armyworm with imporant potentialities on the northern prairies. *J. Econ. Entomol.*, **21**, 279-293.

Krieg, A., and Langenbruch, L.C. 1981. Susceptibility of arthropod species to *Bacillus thuringiensis*. *In* "Microbial Control of Pests and Plant Diseases, 1970-1980" (H.D. Burges, ed.), pp. 837-898. Academic Press, New York.

Krywienczyk, J., Dulmage, H.T., and Fast, P.G. 1978. Occurrence of two serologically distinct groups within *Bacillus thuringiensis* serotype 3ab var. *kurstaki*. *J. Invertebr. Pathol.*, **31**, 372-375.

Lamb, R.J., Turnock, W.J., and Hayhoe, H.N. 1985. Winter survival and outbreaks of bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), on canola. *Can. Entomol.*, **117**, 727-736.

Lebrun, P., et Vlayen, P. 1979. Quelques developpements recents en matiere de lutte microbiologique par l'ulitization de *Bacillus thuringiensis*. *Rev. Quest. Sci.*, **150**, 531-545.

Lecadet, M., and de Barjac, H. 1981. *Bacillus thuringiensis* beta-exotoxin. *In* "Pathogenisis of Invertebrate Microbial Diseases" (E.W. Davidson, ed.), pp. 293-321. Allanheld, Osmun, New Jersey.

Lewis, L.C. 1982. Use of granules and dusts to disseminate insect pathogens. *In* "Invertebrate Pathology and Microbial Control" pp. 66-70. Proceedings of the 3rd International Colloquium on Invertebrate Pathology, University of Sussex, Brighton, U.K.

Lidstone, R.G., Goerzen, D.W., and Khachatourians, G.G. 1985. A standardized bioassay for larvicidal activity of *Bacillus thuringiensis* in sunflower moth *Homeosoma electellum* (Lepidoptera: Phycitidae). *Can. Entomol.*, **117**, 15-22.

Luthy, P., Cordier, J., and Fischer, H. 1982. *Bacillus thuringiensis* as a bacterial insecticide: Basic considerations and application. *In* "Microbial and Viral Pesticides" (E. Kurstak, ed.), pp. 35-74. Marcel Dekker, Inc., New York.

Luthy, P., and Ebersold, H.R. 1981. *Bacillus thuringiensis* delta-endotoxin: Histopathology and molecular mode of action. *In* "Pathogenisis of Invertebrate Microbial Diseases" (E.W. Davidson, ed.), pp. 235-267. Allanheld, Osmun, New Jersey.

Luttrell, R.G., Young, S.Y., Yearian, W.C., and Horton, D.L. 1982. Evaluation of *Bacillus thuringiensis*-spray adjuvant-viral insecticide combinations against *Heliothis* spp. (Lepidoptera: Noctuidae). *Environ. Entomol.*, **11**, 783-787.

Lynch, R.E., Lewis, L.C., Berry, E.C., and Robinson, J.F. 1977. European cornborer control with *Bacillus thuringiensis* standardized as cornborer international units. *J. Invertebr. Pathol.*, **30**, 168-174.

Matter, M.M., and Zohdy, N.Z.M. 1981. Biotic efficiency of *Bacillus thuringiensis* Berl. and a neclear-polyhedrosis virus on larvae of the American bollworm, *Heliothis armigera* Hbn. (Lepid., Noctuidae). *Z. Angew. Entomol.*, **92**, 336-343.

McGarr, R.L., Dulmage, H.T., and Wolfenbarger, D.A. 1970. The delta-endotoxin of *Bacillus thuringiensis*, HD-1, and chemical insecticides for the control of the tobacco budworm and the bollworm. *J. Econ. Entomol.*, **63**, 1357-1358.

McGaughey, W.H. 1978. Moth control in stored grain: Efficacy of *Bacillus thuringiensis* on corn and a method of evaluating using small bins. *J. Econ. Entomol.*, **71**, 835-839.

McGaughey, W.H. 1985. Insect resistance to the biological insecticide *Bacillus thuringiensis*. *Science*, **229**, 193-195.

Mohamed, A.I., Young, S.Y., and Yearian, W.C. 1983a. Effects of microbial agent-chemical pesticide mixtures on *Heliothis virescens* (F.) (Lepidoptera: Noctuidae). *Environ. Entomol.*, **12**, 478-481.

Mohamed, A.I., Young, S.Y., and Yearian, W.C. 1983b. Susceptibility of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) larvae to microbial agent-chemical pesticide mixture on cotton foliage. *Environ. Entomol.*, **12**, 1403-1405.

Moore, A., and Morris, O.N. 1982. An improved technique for dosing larvae of the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae), with measured quantities of *Bacillus thuringiensis* var. *kurstaki*. *Can. Entomol.*, **114**, 89-91.

Moore, J., and Navon, A. 1973. Studies on the susceptibility of the cotton leaf worm *Spodoptera littoralis* (Boisduval) to various strains of *Bacillus thuringiensis*. *Phytoparasitica*, **1**, 23-32.

Morris, O.N. 1980. Entomopathogenic viruses: Strategies for use in forest insect pest management. *Can. Entomol.*, **112**, 573-584.

Morris, O.N. 1983a. Comparative efficacy of Thuricide 16B and Dipel 88 against the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae) in balsam fir stands. *Can. Entomol.*, **115**, 1001-1006.

Morris, O.N. 1983b. Protection of *Bacillus thuringiensis* from inactivation by sunlight. *Can. Entomol.*, **115**, 1215-1227.

Morris, O.N. 1983c. Microorganisms isolated from forest insects of British Columbia. *J. Entomol. Soc. Brit. Columbia*, **80**, 29-36.

Morris, O.N. 1984. Comparative efficacies of single and double applications of commercial *Bacillus thuringiensis* against the spruce budworm *Choristoneura fumiferana* in balsam fir stands. *Can. Entomol.*, **116**, 101-102.

Morris, O.N. 1985. Susceptibility of 31 species of agricultural insect pests to the entomogenous nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora*. *Can. Entomol.*, **117**, 401-407.

Morris, O.N., Dimond, J.B., and Lewis, F.B. 1984. Guidelines for the operational use of *Bacillus thuringiensis* against the spruce budworm. *U.S. Dep. Agr.*, Agr. *Handb.* 612.

Morris, O.N., and Moore, A. 1983. Relative potencies of 50 isolates of *Bacillus thuringiensis* for larvae of the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Can. Entomol.*, **115**, 815-822.

Narayanan, K., Jayaraj, S., and Govindarajan, R. 1976. Further observations on the mode of action of *Bacillus thuringiensis on Papilio demoleus* and *Spodoptera litura*. *J. Invertebr. Pathol.*, **28**, 269-270.

Navon, A., Wysoki, M., and Keren, S. 1983. Potency and effect of *Bacillus thuringiensis* preparations against larvae of *Spodoptera littoralis* and *Boarmia (Ascotis) selenaria*. *Phytoparasitica*, **11**, 3-11.

Norris, J.R., and Burges, H.D. 1965. The identification of *Bacillus thuringiensis*. *Entomophaga*, **10**, 41-47.

Ohba, M., and Aizawa, K. 1978. Serological identification of *Bacillus thuringiensis* and related bacteria isolated in Japan. *J. Invertebr. Pathol.*, **32**, 303-309.

Oliver, A.D., and Chapin, J.B. 1981. Biology and illustrated key for the identification of twenty species of economically important noctuid pests. *La. Agr. Exp. Sta. Bull.* 733.

Pinnock, D.E., Milstead, J.E., Kirby, M.E., and Nelson, B.J. 1977. Stability of entomopathogenic bacteria. *In* "Environmental Stability of Microbial Insecticides". *Misc. Publ. Entomol. Soc. Am.*, **10**, 77-98.

Reardon, R.C., Johnson, D.R., Berndt, M., Platta, M., and Heyd, R. 1984. *Bacillus thuringiensis* applied to spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), in various dosage-volume combinations in Michigan, USA. *Prot. Ecol.*, **6**, 271-277.

Reardon, R.C., Johnson, D.R., Narog, M.G., Banash, S.E., and Hubbard, H.B. Jr. 1982. Efficacy of two formulations of *Bacillus thuringiensis* on populations of spruce budworm (Lepidoptera: Tortricidae) on balsam fir in Wisconsin. *J. Econ. Entomol.*, **75**, 509-514.

Remmington, R.D., and Schork, M.A. 1970. "Statistics With Applications to the Biological and Health Sciences". Prentice Hall, Inc., Englewood Cliffs, New Jersey.

Robertson, J.L., Smith, K.C., Savin, N.E., and Lavigne, R.J. 1984. Effects of dose selection and sample size on the precision of lethal dose estimates in dose-mortality regression. *J. Econ. Entomol.*, **77**, 833-837.

Rogoff, M.H., Ignoffo, C.M., Singer, S., Gard, I., and Prieto, A.P. 1969. Insecticidal activity of thirty-one strains of *Bacillus* against five insect species. *J. Invertebr. Pathol.*, **14**, 122-129.

Roome, R.E. 1975. Field trials with a nuclear polyhedrosis virus and *Bacillus thuringiensis* against larvae of *Heliothis armigera* (Hb.) (Lepidoptera: Noctuidae) on sorghum and cotton in Botswana. *Bull. Entomol. Res.*, **65**, 507-514.

Salama, H.S. 1983. Cotton pest management in Egypt. *Crop Prot.*, **2**, 183-191.

Salama, H.S. 1984. *Bacillus thuringiensis* Berliner and its role as a biological control agent in Egypt. *Z. Angew. Entomol.*, **98**, 206-220.

Salama, H.S., and Foda, M.S. 1982. A strain of *Bacillus thuringiensis* var. *entomocidus* with high potential activity on *Spodoptera littoralis*. *J. Invertebr. Pathol.*, **39**, 110-111.

Salama, H.S., and Foda, M.S. 1984. Studies on the susceptibility of some cotton pests to various strains of *Bacillus thuringiensis*. *Z. Pflanz. Pflanz.*, **91**, 65-70.

Salama, H.S., Foda, M.S., Dulmage, H.T., and El-Sharaby, A. 1983a. Novel fermentaiton media for production of delta-endotoxins from *Bacillus thuringiensis*. *J. Invertebr. Pathol.*, **41**, 8-19.

Salama, H.S., Foda, M.S., and El-Sharaby, A. 1981a. Potency of spore-delta-endotoxin complexes of *Bacillus thuringiensis* against some cotton pests. *Z. Angew. Entomol.*, **91**, 388-398.

Salama, H.S., Foda, M.S., and El-Sharaby, A. 1983b. Biological activity of mixtures of *Bacillus thuringiensis* varieties against some cotton pests. *Z. Angew. Entomol.*, **95**, 69-74.

Salama, H.S., Foda, M.S., El-Sharaby, A., Matter, M., and Khalafallah, M. 1981b. Development of some lepidopterous cotton pests as effected by exposure to sublethal levels of endotoxins of *Bacillus thuringiensis* for different periods. *J. Invertebr. Pathol.*, **38**, 220-229.

Salama, H.S., Foda, M.S., El-Sharaby, A., and Selim, M.H. 1983c. A novel approach for whey recycling in production of bacterial insecticides. *Entomophaga*, **28**, 151-160.

Salama, H.S., Foda, M.S., and Selim, M. 1984a. Isolation of *Bacillus thuringiensis* mutants resistant to physical and chemical factors. *Z. Angew. Entomol.*, **97**, 139-145.

Salama, H.S., Foda, M.S., Selim, M., and El-Sharaby, A. 1983d. Utilization of fodder yeast and agro-industrial by-products in production of spores and biologically active endotoxins of *Bacillus thuringiensis*. *Zentralbl. Microbiol.*, **138**, 553-564.

Salama, H.S., Foda, MS., and Sharaby, A. 1984b. Novel biochemical avenues for enhancing *Bacillus thuringiensis* endotoxin potency against *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Entomophaga*, **29**, 171-178.

Salama, H.S., Foda, M.S., and Sharaby, A. 1985. Role of feeding stimulants in increasing the efficacy of *Bacillus thuringiensis* versus *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Entomol. Gener.*, **10**, 111-119.

Salama, H.S., Foda, M.S., and Zaki, F.N. 1983e. Persistence of *Bacillus thuringiensis* Berliner spores in cotton cultivations. *Z. Angew. Entomol.*, **95**, 321-326.

Salama, H.S., Foda, M.S., Zaki, F.N., and Moawad, S. 1984c. Potency of combinations of *Bacillus thuringiensis* and chemical insecticides on *Spodoptera littoralis* (Lepidoptera: Noctuidae). *J. Econ. Entomol.*, **77**, 885-890.

Salama, H.S., and Zaki, F.N. 1983. Interaction of *Bacillus thuringiensis* Berliner and the parasites and predators of *Sodoptera littoralis* in Egypt. *Z. Angew. Entomol.*, **95**, 425-429.

Salama, H.S., and Zaki, F.N. 1984. Impact of *Bacillus thuringiensis* Berl. on the predator complex of *Spodoptera littoralis* (Boisd.) in cotton fields. *Z. Angew. Entomol.*, **97**, 485-490.

Salama, H.S., and Zaki, F.N. 1985. Application of *Bacillus thuringiensis* Berliner and its potency for the control of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Z. Angew. Entomol.*, **99**. 425-431.

Salama, H.S., Zaki, F.N., and Sharaby, A. 1982. Effect of *Bacillus thuringiensis* Berl. on parasites and predators of the cotton leaf worm *Spodoptera littoralis* (Boisd.). *Z. Angew. Entomol.*, **94**, 498-504.

Samples, J.R., and Buettner, H. 1983. Corneal ulcer caused by a biologic insecticide (*Bacillus thuringiensis*). *Amer. J. Opthamol.*, **95**, 258-260.

Sareen, V., Rathore, Y.S., and Bhattacharya, A.K. 1983. Influence of *Bacillus thuringiensis* var. *thuringiensis* on food utilization of *Spodoptera litura* (Fabricius). *Z. Angew. Entomol.*, **95**, 253-258.

Schuster, D.J. 1979. Adjuvants tank-mixed with *Bacillus thuringiensis* for control of cabbage looper larvae on cabbage. *J. Ga. Entomol. Soc.*, **14**, 182-186.

Sears, M.K., Jaques, R.P., and Laing, J.E. 1983. Utilization of action thresholds for microbial and chemical control of lepidopterous pests (Lepidoptera: Noctuidae, Pieridae) on cabbage. *J. Econ. Entomol.*, **76**, 368-374.

Sebesta, K., Farkas, J., Horska, K., and Vankova, J. 1981. Thuringiensin, the beta-exotoxin of *Bacillus thuringiensis*. *In* "Microbial Control of Pests and Plant Diseases, 1970-1980" (H.D. Burges, ed.), pp. 249-281. Academic Press, New York.

Smirnoff, W.A., et Juneau, A. 1973. Quinze annees de recherches sur les micro-organismes des insects forestiers de la Province de Quebec (1957-1972). *Ann. Soc. Entomol. Quebec*, **18**, 147-181.

Smith, D.B., and Hostetter, D.L. 1982. Laboratory and field evaluations of pathogen adjuvant treatments. *J. Econ. Entomol.*, **75**, 472-476.

Sneh, B., and Gross, S. 1983. Biological control of the Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) in cotton and alfalfa fields using a preparation of *Bacillus thuringiensis* ssp. *entomocidus* supplemented with adjuvants. *Z. Angew. Entomol.*, **95**, 418-424.

Sneh, B., Gross, S., and Gasith, A. 1983. Biological control of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) by *Bacillus thuringiensis* subsp. *entomocidus* and *Bracon hebetor* Say (Hym., Braconidae). *Z. Angew. Entomol.*, **96**, 408-412.

Sneh, B., and Schuster, S. 1983. Effect of exposure to sublethal concentration of *Bacillus thuringiensis* Berliner ssp. *entomocidus* on the susceptibility to the endotoxin of subsequent generations of the Egyptian cotton leaf worm *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae). *Z. Angew. Entomol.*, **96**, 425-428.

Sneh, B., and Schuster, S., and Broza, M. 1981. Insecticidal activity of *Bacillus thuringiensis* strains against the Egyptian cotton leaf worm *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Entomophaga*, **26**, 179-190.

Steel, R.G.D., and Torrie, J.H. 1980. "Principles and Procedures of Statistics, A Biometrical Approach, Second Edition". McGraw-Hill Book Co., Toronto.

Tanada, Y. 1956. Microbial control of some lepidopterous pests of crucifers. *J. Econ. Entomol.*, **49**, 320-329.

Tiwari, L.D., and Mehrotra, K.N. 1981. Effect of *Bacillus thuringiensis* on the pH of gut and haemoloymph as well as on the sodium-potassium concentration of the haemolymph of *Achoea janata* L. and *Spodoptera litura* F. larvae (Lepidoptera: Noctuidae). *Z. Angew. Entomol.*, **92**, 61-66.

Turnock, W.J. 1977. Adaptability and stability of insect pest populations in prairie agricultural ecosystems. *In* "Insect Ecology-Papers Presented in the A.C. Hudson Lectures" (H.M. Kulman and H.C. Chiang, eds.), pp. 89-101. *Minn. Agri. Exp. Sta. Tech. Bull.* 310.

Turnock, W.J. 1984. *Mamestra configurata* Walker, bertha arymworm (Lepidoptera: Noctuidae). *In* "Biological Control Programs Against Insects and Weeds in Canada 1969-1980" pp. 49-55. Commonwealth Agricultural Bureau, London.

Turnock, W.J., and Philip, H.G. 1977. The outbreak of bertha armyworm *Mamestra configurata* (Lepidoptera: Noctuidae), in Alberta, 1971-1975. *Manitoba Entomol.*, **11**, 10-21.

Wallner, W.E., Dubois, N.R., and Grinberg, P.S. 1983. Alteration of parasitism by *Rogas lymantriae* (Hymenoptera: Braconidae) in *Bacillus thuringiensis*-stressed gypsy moth (Lepidoptera: Lymantriidae) hosts. *J. Econ. Entomol.*, **76**, 275-277.

Warren, R.E., Rubenstein, D., Ellar, D.J., Kramer, J.M., and Gilbert, R.J. 1984. *Bacillus thuringiensis* var. *israelensis* protoxin activation and safety. *Lancet*, **8378**, 678-679.

Wiggins, B.A. and Alexander, M. 1985. Minimum bacterial density for bacteriophage replication: Implications for significance of bacteriophages in natural ecosystems. *Appl. Environ. Microbiol.*, **49**, 19-23.

Workman, R.B., Chalfant, R.B., and Schuster, D.J. 1980. Management of cabbage looper and diamondback moth on cabbage by using two damage thresholds and five insecticide treatments. *J. Econ. Entomol.*, **73**, 757-758.

Wylie, H.G., and Bucher, G.E. 1977. The bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Mortality of immature stages on the rape crop, 1972-1975. *Can. Entomol.*, **109**, 823-837.

Yearian, W.C., Luttrell, R.G., Stacy, A.L., and Young, S.Y. 1980. Efficacy of *Bacillus thuringiensis* and *Baculovirus heliothis* - chlordimeform spray mixtures against *Heliothis* spp. on cotton. *J. Ga. Entomol. Soc.*, **15**, 260-271.

APPENDIX

Summaries of mortalities for the preliminary screenings of the 61 strains of *B. thuringiensis*.

Table 9: Summary of the preliminary screening of isolates belonging to varieties *thuringiensis* and *alesti*

		4 Days			7 Days		
HD#	Crystovar	Test Mortality	Standard Mortality	X^2	Test Mortality	Standard Mortality	X^2
var. *thuringiensis*							
703	*k-1/thu*	2.02	2.02	0.0	32.0	27.9	0.368
120	*k-1*	3.0	3.0	0.0	7.0	11.0	0.977
var. *alesti*							
84	*k-73*	0.0	6.06	4.69	-3.16	13.7	14.2

1. Mortalities were corrected for the natural response rate with Abbott's formula (Abbott 1925)
2. The X^2 independence of test and reference standard mortalities:
   (df=1) significant p(.05)= 3.84
   highly significant p(.01)= 6.64
   very highly significant p(.001)= 10.8
   (Steel and Torrie 1980)
3. Isolates are ranked within the variety in terms of toxicity according to the X^2 values after 7 days

NI = not identified.

Table 10: Summary of the preliminary screening of isolates belonging to variety *kurstaki*

HD#	Crystovar	4 Days			7 Days		
		Test Mortality	Standard Mortality	X^2	Test Mortality	Standard Mortality	X^2
262	*k-1*	3.03	0.0	1.85	34.7	8.42	16.5
337	*k-1*	6.0	1.0	3.70	37.0	20.0	7.09
562	NI	4.0	1.0	1.85	21.2	10.1	4.39
263	*k-1*	6.06	2.02	1.68	9.09	6.06	0.579
341	*k-1*	2.0	2.0	0.0	4.08	2.04	0.421
73	*k-73*	-1.03	-1.03	0.0	0.0	0.0	0.0
203	*k-1*	0.0	1.0	1.01	3.0	3.0	0.0
1	*k-1*	0.0	1.0	1.01	2.04	4.08	0.421
187	*k-73*	5.0	2.0	1.33	14.0	18.0	0.595
181	*k-73*	0.0	2.02	1.02	0.0	2.04	0.687
231	*k-1*	-1.01	0.0	1.01	-1.02	1.02	1.02
87	*k-1*	0.0	0.0	0.0	0.0	4.04	2.75
182	*k-73*	0.0	2.0	2.02	0.0	4.0	4.08
269	*k-1*	-1.01	1.01	2.02	2.06	11.3	4.71
244	*k-1*	0.0	0.0	0.0	1.0	16.0	14.46
89	*k-1*	0.0	0.0	1.0	1.0	24.0	24.2
255	*k-1*	1.0	1.0	0.0	3.0	32.0	29.1

* For footnotes see Table 9.

Table 11: Summary of the preliminary screening of isolates belonging to varieties *dendrolimus*, *kenyae*, and *galleriae*

		4 Days			7 Days		
HD#	Crystovar	Test Mortality	Standard Mortality	X^2	Test Mortality	Standard Mortality	X^2
var. *dendrolimus*							
37	*sot*	2.06	0.0	0.521	12.6	9.47	0.344
7	*den*	0.0	1.0	1.01	9.09	7.07	0.244
34	NI	0.0	0.0	0.0	1.02	2.04	0.148
48	*sot*	0.0	0.0	1.0	4.0	11.0	3.53
35	*den*	-2.04	0.0	2.02	-2.04	6.12	8.33
var. *kenyae*							
551	NI	14.3	0.0	13.0	58.8	16.5	35.2
588	NI	0.0	1.0	1.01	0.0	12.0	12.8
123	*ken*	0.0	2.0	2.02	2.0	17.0	13.1
var. *galleriae*							
129	*gal*	26.7	16.7	0.884	43.3	36.7	0.278
232	*k-1*	2.02	1.01	0.205	21.2	17.2	0.50
8	*gal*	2.0	2.0	0.0	15.0	14.0	0.0403
184	*gal*	1.0	3.0	1.02	0.0	2.02	1.02
166	*gal*	0.0	4.0	4.08	0.0	5.05	3.70
176	*gal*	0.0	0.0	1.0	-1.01	4.04	5.13
240	*gal*	0.0	0.0	1.0	0.0	9.0	9.42
234	*aiz*	0.0	3.0	3.05	6.06	24.2	12.1
210	*aiz*	0.0	0.0	1.0	1.0	14.0	12.2
186	*gal*	0.0	2.02	1.02	1.02	23.5	21.1

* For footnotes see Table 9.

Table 12: Summary of the preliminary screening of isolates belonging to varieties *canadensis*, *entomocidus*, and *aizawai*

		4 Days			7 Days		
HD#	Crystovar	Test Mortality	Standard Mortality	X^2	Test Mortality	Standard Mortality	X^2
var. *canadensis*							
554	NI	0.0	2.04	1.28	1.03	39.2	30.7
var. *entomocidus*							
198	*ent*	3.0	5.0	0.521	43.3	26.8	5.49
110	*ent*	1.01	2.02	0.205	14.3	6.12	3.03
320	*ent*	-1.02	0.0	0.338	4.08	4.08	0.0
635	*ent*	0.0	0.0	1.0	-3.45	0.0	1.02
var. *aizawai*							
133	*aiz*	25.0	2.0	22.7	72.7	12.1	73.4
112	*aiz*	6.0	0.0	6.19	50.5	5.05	49.7
854	NI	16.0	1.0	14.5	37.8	6.12	26.7
282	*aiz*	2.02	3.03	0.148	51.0	35.4	4.54
52	*aiz*	10.0	5.0	1.80	48.0	34.0	4.05
228	*aiz*	0.0	0.0	1.0	9.0	3.0	3.19
283	*aiz*	10.0	5.0	1.80	52.0	40.8	2.43
276	*ent*	3.0	1.0	1.02	3.0	11.0	4.92
137	*aiz*	36.4	22.2	4.67	55.6	75.8	8.91

* For footnotes see Table 9.

Table 13: Summary of the preliminary screening of isolates belonging to variety *tolworthi* and isolates for which the variety is not identified

		4 Days			7 Days		
HD#	Crystovar	Test Mortality	Standard Mortality	X^2	Test Mortality	Standard Mortality	X^2
var. *tolworthi*							
121	*tol*	1.04	3.13	0.355	1.04	7.29	2.45
125	*tol*	1.0	0.0	1.01	1.0	5.0	2.75
285	*tol*	0.0	0.0	1.0	5.0	15.0	5.56
124	*tol*	0.0	6.0	6.19	-1.01	14.1	16.2
variety not identified							
582	NI	6.12	5.10	0.072	67.4	29.6	27.4
855	NI	1.01	3.03	0.688	14.1	20.2	1.22
287	*k-1*	0.0	0.0	1.0	0.0	3.0	3.05
363	*k-1/thu*	1.0	1.0	0.0	1.0	10.0	7.72
498	NI	0.0	6.0	6.19	3.0	17.0	10.9

* For footnotes see Table 9.