MINISTRY OF FORESTS

# Environmental Report and Current Status of *Bacillus thuringiensis var. kurstaki* Use For Control of Forest and Agricultural Insect Pests

by

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Victoria, B.C.

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The size of the document prevented most from reviewing it in its entirety. Our special thanks are extended to those who did. All comments and suggestions were carefully considered, but the ommissions, if any, are the authors' responsibility.

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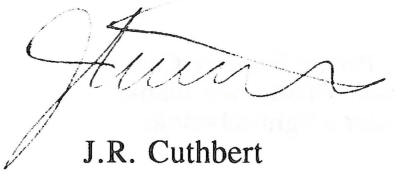
BRITISH COLUMBIA FOREST HEALTH COMMITTEE

During the past three decades the naturally occurring bacterium, ***Bacillus thuringiensis*** var. ***kurstaki*** (***B.t.k.***), has been developed commercially and used with increasing frequency by the British Columbia Forest Service and others in this province to minimize losses caused by insect defoliators. Most of the data to support its operational use has been provided by research specialists with Forestry Canada. The increasing use of ***B.t.k.*** in British Columbia's Silviculture, Program, as well as in the eradication programs conducted against the gypsy moth by Agriculture Canada has raised awareness and caused some concern, especially by some special interest groups.

The British Columbia Forest Health Committee is pleased to endorse this review as a summary of available knowledge on the potential effects of ***B.t.k.*** The committee, composed of representatives from governments, industry, universities and technical colleges, is dedicated to modern and rational forest health practices. It is hoped that the data and information presented in this report will help to promote constructive discussion on the use of ***B.t.k.*** in our forests.

This report constitutes one of the first attempts to bring a large volume of available information together under one cover especially for users and other interested parties in British Columbia. It is the result of the joint effort by the B.C. Forest Service and the Pacific Forestry Centre, Forestry Canada. The contribution of the authors, other contributors and the reviewers are acknowledged with appreciation and sincere thanks.



J.R. Cuthbert
Chairman

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# FOREWORD

In the last three decades, there has been growing public concern about the environmental effects of broad-spectrum synthetic chemical insecticides. In the case of forestry operations, these concerns, as well as the results of intensive research, led to increased use of more specific and environmentally more acceptable alternative agents. The biological insectide, *Bacillus thuringiensis* var. *kurstaki* (*Btk*), for example, has become the most widely used material for direct control of forest insect pests in Canada. *Btk* is widely used against spruce budworms and the gypsy moth both in Canada and the United States. It is also the preferred material for the use on agricultural crops and in home gardens.

There has been a dramatic increase in the use of *Btk* in recent years. In 1980, for example, about 2% of the total area treated for spruce budworm in Canada was sprayed with *Btk*; this proportion increased to about 63% by 1990. The elevation of *Btk* to this position is based on an extensive, world-wide accumulation of data pertaining to research, development, operational experience, and the detailed investigations on potential side effects.

This review of the environmental impact of *Btk* summarizes all the published information which could be found through electronic literature search (Commonwealth Agriculture Bureaux Abstracts, Agricola, and Biosis), up to and including December 1991. Additional information included in this review is based on all authoratative, published references available to us in various libraries. Although the formal literature search was halted at the end of 1991, in a few instances information published in 1992 was also added because it filled in gaps in the information and data (like the effect of *Btk* on aquatic insects), or because the report provided new or additional information (e.g., the effect of *Btk* on immunocompromised people and other health concerns).

Information on the effects of *Btk* on aquatic organisms was and still is relatively sparse. We were almost tempted to substitute information on another strain of *Bt* (i.e. Bti=*Bacillus thuringiensis* var. *israliensis*) because more information was available on it. However, since this strain is used specifically against mosquito and blackfly larvae, inclusion of these data would have confused the issue and the intent of this review. Therefore, we accepted the reviewers comments and eliminated most of the references to *Bti*. Much of the information on the effect of *Bti* on the environment is on file at Pacific Forestry Centre and is available on request.

The contents of this report were primarily assembled for the members of the Forest Health Committee, especially forest health, pesticide, and public health officers, and forest managers, in order to assist them in managing and explaining the use of *Btk* for forest insect suppression. The data assembled here is presented without critical comments or interpretation for the most part. We felt this would be in the best interest of the reader. Instead, we tried to synthesize and organize the available information under various subject headings most fequently raised as concerns. The information is presented by paraphrasing the authors and, whenever possible, providing direct quotations and page numbers to assist interested people in locating the original information.

We may have, inadvertently, missed some pertinent information which may not be cited or which may not have been available through searching and accessing the electronic databases.

Suggestions and concerns regarding future revisions or the general utility of this review would be appreciated. Particularly, we would appreciate notification of omissions (including, if possible, a copy of the missed reference or specific bibliographic information) as we hope to update this report in the near future and make it a more critical review. Please send this information to:

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# I INTRODUCTION

The insecticidal bacterium *Bacillus thuringiensis* (*Bt*) was first introduced into the North American insect control market in 1958, and was given full exemption for use on food and forage crops in 1960 in the United States. *Bt* was registered in Canada in 1961, and in Germany in 1964 (Ignoffo, 1973, p. 143; Krieg, 1978, p. 177). The variety used initially in insect control programs was *Bacillus thuringiensis* variety *thuringiensis* (*Btt*), known at the time as *Bt* Berliner (Ellis, 1991, p. 2).

Since its discovery, *Bacillus thuringiensis* has been divided into several subspecies (or varieties), including the variety *kurstaki* (*Btk*), an isolate commonly used in commercial pest control formulations. Given the large number of varieties which have been isolated, any references to *Bt* are somewhat ambiguous unless the variety is specified.

*Bacillus thuringiensis* variety *kurstaki* is a naturally occurring gram-positive, spore-forming bacterium that produces a proteinaceous parasporal crystal. This crystal contains toxins that are active against the larval stage of certain lepidopterous insects when ingested, but the spore may also contribute to the insecticidal activity of formulations (Forsberg *et al.*, 1976, p. 18). *Btk* has been proven to be exceedingly useful as a microbial control agent in insect abatement programs since its registration in 1967. Over the past 25 years, the use of *Btk* has been increasing rapidly as a result of the progressive shift from the large-scale use of wide spectrum chemical insecticides to the more environment-friendly control measures and integrated pest management strategies.

*Btk* is active against more than 200 target lepidopteran species, and is currently registered for use against many defoliating Lepidoptera in Canada, including agricultural, forestry and home-garden pests. *Btk* is used in forestry in Canada against pests such as the following: the eastern and western spruce budworms, *Choristoneura fumiferana* (Clemens) and *C. occidentalis* Freeman, respectively, (Tortricidae); the gypsy moth, *Lymantria dispar* (Linnaeus) (Lymantriidae); the blackheaded budworm, *Acleris variana* (Fernald) (Tortricidae); jack pine budworm, *Choristoneura pinus pinus* Freeman (Tortricidae); eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Guenée) (Geometridae); and species of tent caterpillar, *Malacosoma* spp. (Lasiocampidae) (Dulmage and Aizawa, 1982, p. 214-215; Forsberg *et al.*, 1976, p. 18; Morris, 1982, p. 241; Morris *et al.*, 1986, p. 7). Its specificity towards lepidopterans allows it to be used in conjunction with other biocontrol measures such as predatory and parasitic insects with minimal or no direct deleterious effects to these beneficials or other non-target organisms in the treated areas (Yousten, 1973, p. 313; Buckner *et al.*, 1974, p. F55; Wilkinson *et al.*, 1975, p. 117; Franz *et al.*, 1980, p. 234; Morris, 1983b, p. 1006; Lim *et al.*, 1986, p. 200; Niwa *et al.*, 1987, p. 752). Furthermore, during the 1970s, in an effort to reduce the quantities of chemicals used in pest control, *Btk* was effectively combined with various chemical insecticides in the laboratory and in field trials to increase its efficacy and decrease the volume of chemical insecticides released into the environment (Ellis, 1991, p. 36; Morris, 1977a; Morris, 1977b; Morris and Armstrong, 1975).

*Btk* is considered to be non-pathogenic towards humans and other non-target organisms. The previous 55 years of production, testing and operational use without any health or environmental problems suggests that *Btk* is safe when applied at the recommended dosages. However, there are still some concerns expressed by some groups over the possible, yet unsubstantiated, environmental effects of extensive *Btk* application, particularly when it is applied adjacent to important waterways or near highly populated areas. Recently, the restrictions concerning applying *Btk* near or over water were removed from the label.

The intent of this review is to bring together published information on the safety and toxicology of *Btk* and its environmental impact on non-target organisms, including mammals, fish, birds, and aquatic and terrestrial insects and other invertebrates. Where little or no information on the effects of *Btk* was available, information on some of the aforementioned topics involving other varieties of *Bt* is provided.

In some earlier works, the variety of *Bt* used in spraying or testing is unknown. This is sometimes the result of an oversight on the part of the authors,

although more often the information cited pertains to the various varieties and effects of *Bt* obtained prior to the 1970s, when the taxonomy of *Bt* was not well known, and the varieties were seldom identified in the literature. Parenthesis are used, where applicable, to denote the absence of information about the variety of *Bt* used in the experiment or application.

Much of the information included in this literature review was obtained through the use of CD-ROM (both at Pacific Forestry Centre and Ocean Sciences Centre) and on-line searches into Commonwealth Agriculture Bureau abstracts (CAB), Agricola and Biosis abstracts. CAB abstracts dating from 1972 until December 1991 were accessed; Agicola abstracts from 1970 until December 1991 were accessed; Biosis abstracts from 1969 until December 1991 were accessed. Other relevant papers were obtained through personal communication with other scientists knowledgeable in the area of *Btk* and biological pest control, and through reference lists within various journal articles already obtained. Two earlier reviews prepared by Surgeoner and Farkas (1990) and Ellis (1991) were also consulted for additional references that were not retrieved from the CD-ROM searches.

# II
# BACKGROUND

## A) DISCOVERY

The bacterium *Bacillus thuringiensis* was discovered by Ishiwata in 1901 in Japan from diseased silkworm larvae, *Bombyx mori* (Linnaeus) (Lepidoptera: Bombycidae). He named the pathogen *Bacillus sotto*. In 1911, Berliner in Germany identified the organism in infested Mediterranean flour moth, *Ephestia* (=*Anagasta*) *kuehniella* (Zeller) (Lepidoptera: Pyralidae), and named it *Bacillus thuringiensis* (*Bt*) (DeLucca *et al.*, 1981, p. 865). Although some interest was expressed in Europe over the potential of this bacterium to control some economically important insects, poor results from early trials resulted in a loss of interest in developing *Bt* as an insect control product. In 1927, however, Mattes re-discovered *Bt* Berliner in the Mediterranean flour moth, which spawned renewed interest in the bacterium as a potential insecticide. This variety was initially employed for commercial use in France (Morris, 1982, p. 211).

The first commercial formulation of *Bt*, developed in 1938 in France, was known as "Sporeine". Following World War II, several United States companies began producing *Bt*, which was marketed under such trade names as Thuricide, Agritrol, Biotrol BTB, Bakthane 1-69 and Parasporin. All of these products contained the variety *thuringiensis*, which was at that time called *Bt* Berliner. Between the 1940s and the 1960s, producers and users of *Bt* encountered various problems associated with the insecticide, such as inadequate standardization of application and production techniques, and high production costs. The results were often poor, and this deterred research and interest in using *Bt* in the field of biological control (Luthy *et al.*, 1982, p. 56-57).

In the 1960s and the early 1970s new and more potent varieties of *Bt* were discovered, initiating a steady increase in its use both in agriculture and forestry. *Bt* var. *kurstaki* was isolated by Kurstak in France in 1962 from diseased *Anagasta kuehniella* (Dulmage and Aizawa, 1982, p. 212). In 1967, a serotype of *Btk* that is 15 times more potent than previous serotypes was isolated by Dulmage from the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). It was named *Btk* HD-1, and this is the serotype used today in forestry (Dulmage and Aizawa, 1982, p. 212). In 1977, Goldberg and Margalit isolated *Bt* var. *israelensis* from soils in Israel, which gave excellent control of mosquito and blackfly larvae (Davidson, 1982, p. 291). *Bt* var. *tenebrionis*, a pathovar of *Bt* var. *morrisoni* which has been found to be pathogenic to coleopterous larvae, was isolated by Kreig *et al.* in 1982 (Kreig *et al.*, 1983, p. 500). A history of the discovery of the various *Bt* varieties is given in Table 1.

## B) TAXONOMY

Initially it was thought that *Bt* was a variant of *Bacillus cereus*, a ubiquitous spore-forming facultative soil bacterium (Dulmage and Aizawa, 1982, p. 211; Falcon, 1971, p. 73-74). However, the similarities between Ishiwata's *B. sotto*, Berliner's *B. thuringiensis* and a newly discovered variety originally named *B. cereus* var. *alesti*, and the fact that these bacteria possessed a proteinaceous crystal that was absent from *B. cereus* prompted bacteriologists to re-name and classify *B. sotto*, *B. thuringiensis*, and *B.c. alesti* under the name *Bacillus thuringiensis*. *Bacillus thuringiensis* Berliner was re-named as *Bacillus thuringiensis* var. *thuringiensis* (Fishner and Rosner, 1959, p. 686; Ghassemi *et al.*, 1981, p. A-399). *B. cereus* var. *alesti* was placed within the *Bt* group as *Bacillus thuringiensis* var. *alesti* (Heimpel, 1967a, p. 291), and *Bacillus sotto* was renamed as *Bt* var. *sotto* (Heimpel and Angus, 1960, p. 270-271).

## Table 1:
## History of the Discoveries of Varieties of Bacillus thuringiensis[1]

| Variety | Place of Discovery | Year | H-serotype | Discoverer |
|---|---|---|---|---|
| sotto | Japan | 1901 | 4a,4b | Ishiwata |
| thuringiensis | Germany | 1911 | 1 | Berliner |
| entomocidus | United States | 1950 | 6 | Steinhaus |
| alesti | France | 1951 | 3a | Toumanoff and Vago |
| finitimus | Canada | 1956 | 2 | MacNamee |
| galleriae | USSR | 1956 | 5a,5b | Isakova |
| toumanoffi | France | 1956 | 11a,11b | Toumanoff |
| darmstadiensis | Germany | 1961 | 10 | Krieg |
| aizawai | Japan | 1961 | 7 | Aizawa |
| kenyae | England | 1961 | 4a,4c | Norris and Burges |
| kurstaki | France | 1962 | 3a,3b | Kurstak |
| morrisoni | Scotland | 1963 | 8a,8b | Norris |
| tolworthi | England | 1963 | 9 | Norris |
| thompsoni | United States | 1969 | 12 | Thompson |
| canadensis | Canada | 1972 | 5a,5c | Morris |
| ostriniae | China | 1975 | 8a,8c | Ren et al. |
| pakistani | Pakistan | 1975 | 13 | Shaikh |
| israelensis | Israel | 1977 | 14 | Goldberg and Margalit |
| indiana | United States | 1978 | 16 | DeLucca and Larson |
| dakota | United States | 1978 | 15 | DeLucca and Larson |
| kyushuensis | Japan | 1979 | 11a,11c | Ohba and Aizawa |
| yunnanensis | China | 1979 | 20a,20b | Wan-yu, et al. |
| tohokuensis | Japan | 1981 | 17 | Ohba, et al. |
| kumamotoensis | Japan | 1981 | 18 | Ohba, et al. |
| tochigiensis | Japan | 1981 | 19 | Ohba, et al. |
| colmeri | United States | 1984 | 21 | DeLucca, et al. |
| shandongiensis | China | 1986 | 22 | Ying, et al. |
| japonensis | Japan | 1988 | 23 | Ohba and Aizawa |
| neoleonensis | ? | 1988 | 24 | Rodriguez-Padilla, et al. |
| mexicanbensis | Mexico | 1988 | 27 | Rodriguez-Padilla and Gallan-Wong |
| nigeriensis | ? | ? | 8b,8d | De Barjac, et al. |
| ponderichieriensis | United States | ? | 20a,20c | DeLucca, et al. |
| coreanensis | ? | ? | 25 | de Barjac and Lee |
| silo | ? | ? | 26 | de Barjac and Lecadet |

| Pathovar[2] | Place of Discovery | Year | H-serotype | Discoverer |
|---|---|---|---|---|
| thuringiensis, Mattes isolate | Germany | 1927 | 1 | Mattes |
| kurstaki, HD-1 | United States | 1967 | 3a,3b | Dulmage |
| dendrolimus | USSR | 1956 | 4a,4b | Talalaev |
| subtoxicus | United States | 1945 | 6 | Steinhaus |
| tenebrionis | Germany | 1982 | 8a,8b | Krieg et al. |
| san diego | United States | 1986 | 8a,8b | Herrnstadt et al. |
| wuhanensis | China | 1976 | ? | Hubei Institute |

[1] From Dubois and Lewis, 1981, p. 236; Dulmage and Aizawa, 1982, p.214-215; Krieg et al., 1983, p. 500; Krieg et al., 1987, p. 417; Melin and Cozzi, 1990, p. 151; De Barjac and Frachon, 1990, p. 236; Ellis, 1991, p. 59.

[2] These varietal names reflect unique biochemistry and insecticidal qualities rather than a different serotype. For example, although both Bt var. tenebrionis and Bt var. san diego are reported as serotype 8a,8b (like morrisoni), both are given varietal names other than morrisoni to indicate the different pathogenic characterisitics exhibited by these isolates.

Varieties of *Bt* were further subdivided into serotypes, based upon flagellar characteristics called "H" antigens, such that *thuringiensis* was classified as H-serotype 1, *kurstaki* as 3a,3b, and *israelensis* as 14 (Bonnefoi and de Barjac, 1963, p. 229; Heimpel, 1967b, p. 365-366; Melin and Cozzi, 1990, p. 151; Dulmage and Aizawa, 1982, p. 212, 226).

At present, 34 serotypes have been classified based upon the H antigen. In addition, there are numerous "pathovars", varieties identified solely from their unique biochemical and pathogenic characteristics (De Barjac and Frachon, 1990, p. 236-237).

## C) DISTRIBUTION IN NATURE

*Bt* has been isolated from diseased insects in a variety of countries from Canada and the United States to Great Britain, Germany, Israel, Commonwealth of Independent States (formerly USSR), Pakistan, China and Japan (Dulmage and Aizawa, 1982, p. 214-215).

A common thread runs through all but one of the situations in which the various stains of *Bt* have been discovered: the insects infected with the disease were confined to a closed environment such as a rearing laboratory or a grain storage unit. The one exception is var. *dendrolimus*, which was apparently isolated from an epizootic in the Siberian silkworm, *Dendrolimus sibiricus* (Led.) (Lepidoptera: Lymantriidae), on conifers (DeLucca *et al.*, 1981, p. 866). Epizootics, or widespread dispersion of *Bt*, are rare, occurring only in or around areas where lepidopterous insects are in confined environments. Although the occasional individual insect infected with *Bt* has been discovered in open areas, epizootics do not normally occur and have not been observed under field conditions in nature (DeLucca *et al.*, 1981, p. 865-866; Burges and Hurst, 1977, p. 131). Furthermore, infected hosts produce relatively few vegetative cells and spores, which are readily destroyed or inactivated by sunlight and other elements (Dimond and Morris, 1984, p. 105; Morris, 1982, p. 240).

One case of a natural epizootic was discovered in a conifer forest in East Siberia in the crown of a single spruce tree (Talalayeva, 1967, p. 191). The tree, containing 175 dead larvae and 200 dead pupae of *Selenephera lunigera* Esp. (Lepidoptera: Lasiocampidae), was discovered in 1963 by a pathologist inspecting a plantation (p. 191). Dead larvae and pupae were collected and analyzed; most of the cultures yielded bacteria that produced an inclusion body (crystal). The larvicidal activity of the cultures was verified on *S. lunigera* and *Dendrolimus sibiricus* by feeding larvae cedar needles treated with the isolated cultures (p. 191). Mortality of the tested larvae of these two species was 95% and 75%, respectively, while mortality was only 10% and 0% in the controls (p. 192). The isolated bacterium was identified as belonging to the *thuringiensis* group, although the variety was not determined (p. 192).

Several other documented cases of natural *Bt* epizootics were discovered in old watermills in Yugoslavia (Vankova and Purrini, 1979, p. 217). *Btt* (var. *thuringiensis*), *Btk*, and *Bt* var. *morrisoni* were isolated from various host larvae in 10 localities, the majority of the isolates being *Btk* (p. 218). The epizootics were present in larvae of *Ephestia kuehniella*, *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae), and *Plutella maculipennis* Curtis (Lepidoptera: Plutellidae), and in most cases multiple varieties were present at any one location (p. 219).

*Bt* occurs naturally in the environment, especially in soils, even though it does not usually cause widespread infection in insects. Spores of *Bt* have been shown to exist in soils of the United States in areas previously untreated with any *Bt* products (DeLucca *et al.*, 1981, p. 866-867). Field tests conducted over a two-year period involved the sampling of 32 soils the first year and 63 soils the second, including such areas as cultivated soils, grass, rocky soil, and soils from virgin woods. Results showed that *Bt* was present in 17% of the soils tested, and that only 0.05% and 0.75% of the isolates of *Bacillus* were *Bt* for the first and second year of sampling, respectively (p. 866-867). In the second year of sampling, 50% of the isolates were variety *kurstaki* and 43% were variety *galleriae*, while the remaining isolates were *indiana*, *darmstadiensis*, *dakota*, and a few unknowns (p. 869). The authors suggest that the isolates of *Bt*, including var. *kurstaki*, were present in the soils naturally, and not as a result of a previous insecticide spray or drift from sprays in other areas (p. 869).

In Japan, 24 varieties of *Bt* were isolated from soils and 30 varieties from cadavers of *Arctia caja phaeosoma* Linneaus (Lepidoptera: Arctiidae) larvae in natural environments, presumably areas never treated with any *Bt* product (Kikuta and Iizuka, 1990, p. 258). The majority of isolates from soils were identified as subspecies *indiana*, a variety that was non-pathogenic when assayed against silkworm larvae. From the insect cadavers, the majority of isolates were identified as subspecies *kurstaki* (p. 258). Such discoveries of naturally occurring *Bt* populations confirms the fact that *Bt* is present in the environment as an integral part of many forest ecosystems.

*Bt* is also considered to be a natural component of the common leaf flora of deciduous and conifer trees (Smith and Couche, 1991, p. 311). Several vari-

eties of *Bt* were isolated from leaf samples of temperate-climate trees in the United States, including basswood, Norway maple, burr oak, red elm, rock elm, black cherry, brittle willow, white pine, red pine, jack pine, balsam fir, northern white cedar, lodgepole pine, and spruce (p. 312-13). Twenty-one isolates were recovered from foliage samples, including *Btk*, *Bti*, and *Bt* var. *tenebrionis* (p. 313-314). The gel electrophoresis patterns of a few of the isolates resembled those of either *Btk*, *Bti*, or *Bt* var. *tenebrionis*, while other isolates gave unique patterns (p. 313). Tests to determine the taxonomy of the other varieties were not performed. The authors suggest that varieties of *Bt* recovered from tree foliage represent natural populations of the organism, and not residues from insecticidal treatments. The relatively large amount of *Bt* var. *tenebrionis* (16%) found on sampled foliage supports the idea that these are naturally occurring populations because of the minimal use of this variety in pest control to date. For this reason, the presence of *Bt* on the phylloplane may contribute a small degree of insect mortality in forest stands (p. 314).

Other occurrences of *Bt* have also been shown to be a natural element of the environment. In New Orleans, *Bt* colonies isolated from settled and respirable dust samples from grain storage elevators were determined to be primarily variety *aizawai* (DeLucca *et al.*, 1982, p. 453). The storage site was chosen because it had not been previously contaminated with *Bt* for the control of storage pests; therefore, it represented an environment in which the presence of *Bt* would be "natural" (p. 452). Of the 255 *Bacillus* colonies isolated from the 73 dust samples taken (20 settled and 53 respirable), 31% were determined to be *Bacillus thuringiensis*. Serological tests showed that 95% of these were of the variety *aizawai*. Other isolates were varieties *kurstaki*, *morrisoni*, *canadensis*, and *indiana* (p. 453). These results are quite different from those of DeLucca *et al.* (1981) who reported that 93% of the *Bt* varieties isolated from soil samples from the United States were *kurstaki* and *galleriae*, while other varieties isolated in low quantities were *indiana*, *darmstadiensis*, and *dakota* (p. 869). This, and the fact that *Bt* var. *aizawai* is not available in commercial formulations, suggests that this variety of *Bt* is a naturally occurring bacterium in stored-product environments (DeLucca *et al.*, 1982, p. 455), just as varieties *kurstaki*, *galleriae*, *darmstadiensis*, *dakota*, and *indiana* are naturally occurring in soil environments (DeLucca *et al.*, 1981, p. 869).

The fact that epizootics of *Bt* occur very rarely in nature indicates that persistence must be relatively low following treatment. Many studies have shown that both the spores and crystals are subject to inactivation by sunlight in water and on foliage, as well as biological degradation in the soil. Wash-off by rain also accounts for a substantial reduction in efficacy (Morris, 1983a, p. 1218-1219; Pozsgay *et al.*, 1987, p. 251; van Frankenhuyzen and Nystrom, 1989, p. 870; Menon and De Mestral, 1985, p. 271-272; West *et al.*, 1984b, p. 151-153). Dimond and Morris (1984, p. 109) state that *Bt* remains viable on foliage for only 3 to 7 days, while other reports suggest that spores can remain dormant in soils for long periods of time, without germinating, due to unfavorable pH conditions (DeLucca *et al.*, 1981, p. 869; Saleh *et al.*, 1970, p. 678-680). Although *Bt* appears to be widespread in soils, epidemics of *Bt* do not occur in nature under normal circumstances, because spores or crystals are present in very small numbers in the environment.

## D) VARIETIES AVAILABLE AND VARIETIES IN USE

Today there are at least 34 varieties of *Bt* (subspecies being identified by properties of the flagellar H-antigens), and perhaps thousands of pathovars, the latter division being based on the pathogenic characterisitics of the bacteria (Table 1). Only a small percentage of the the many *Bt* pathovars have been isolated and identified. Several *Bt* varieties are active against nearly 200 species of lepidopterans, but variety *kurstaki* is the most important subspecies used in the control of lepidopterans (Morris, 1982, p. 2; Thomas and Ellar, 1983, p. 181). Three serologically different isolates of crystals have been discovered within the subspecies *kurstaki*, HD-1, HD-73, and NRD-12, each having their own toxicological efficacy towards various insects (Krywienczyk *et al.*, 1978, p. 372; Moar and Trumble, 1990, p. 196). Early studies initially indicated that NRD-12 is more toxic towards *Spodoptera* species (Lepidoptera: Noctuidae) than the HD-1 isolate (Moar and Trumble, 1990, p. 196). Further studies of the two serotypes used for this experiment revealed that the HD-1 used for the initial experiment was missing the CryIA(b) gene, which expresses the protein with the maximum toxicity to *Spodoptera exigua*. Subsequent experimentation, using purified toxic proteins, indicated that there was no significant difference in the toxicity between HD-1 and NRD-12 (Moar et al., 1990, p. 2482; van Frankenhuyzen *et al.*, 1992, p. 151-152).

Although *Btk*, *Bt* var. *thuringiensis*, *aizawai*, *morrisoni* and *tolworthi*, all have some insecticidal activities against mosquito larvae of the genera *Aedes* and *Culex*, *Bacillus thuringiensis* var. *israelensis* has been shown to be much more effective in the control of these pests (Davidson, 1982, p. 290-291). Other genera of mosquitoes controlled by *Bti* include *Anopheles*, *Culiseta*, *Psorophora*, *Wyeomyia*, and

*Uranotaenia* (Novo Industri A/S, 1988a). The serotype HD-14 is the most potent serotype of variety *israelensis*, and is therefore used in commercial products of *Bti*.

Two "varieties" of *Bt* have recently been investigated for their activity against Coleoptera. *Bt* var. *san diego* and *Bt* var. *tenebrionis* are reportedly effective in controlling some species of beetles such as the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) and the elm leaf beetle, *Pyrrhalta luteola* (Muller) (Coleoptera: Chrysomelidae) (Ferro and Lyon, 1991, p. 806; MacIntosh *et al.*, 1990, p. 261; Poinar *et al.*, 1990, p. 196-197). *Bt* var. *tenebrionis* is currently registered by Novo Laboratories Inc. as BK-100 for use on the Colorado potato beetle and other chrysomelid beetle larvae (Novo Industri A/S, 1988b). Varieties *san diego* and *tenebrionis* have been shown to be identical in their protein structure, and are designated as serotype 8a,8b (MacIntosh *et al.*, 1990, p. 258), although there is dispute over whether *san diego* is a new variety or is really variety *tenebrionis* (Krieg *et al.*, 1987, p. 417). These two new varieties are the same serotype as *Bt* var. *morrisoni*, although varieties *san diego* and *tenebrionis* are unique in their biochemistry and insect host range, and therefore have been given distinct names (Ellis, 1991, p. 59). *Bt* var. *morrisoni* is also toxic to the Colorado potato beetle, although its toxicity is associated with a new toxin tentatively named sigma-exotoxin (Argauer *et al.*, 1991, p. 205). This toxin, found in the HD-116 serotype of *Btm*, differs from beta-exotoxin found in *Btt* both chemically and structurally (p. 212).

*Btk*, *Bti*, *Bt* var. *morrisoni*, and several others have been shown to possess a toxin lethal to eggs of the ruminant nematode *Trichostrongylus colubriformis*, and therefore have potential for use in nematode control (Bone *et al.*, 1988, p. 102; Meadows *et al.*, 1989, p. 159). The toxicity towards this nematode does not appear to be related to the insecticidal activity of the delta-endotoxin, but instead may affect eggshell permeability (Bone *et al.*, 1988, p. 102, 107). A few important species of insects susceptible to *Bt* are listed in Appendix I.

In Canada, only *Bt* var. *kurstaki* (HD-1), *Bt* var. *israelensis* (HD-14) and *Bt* var. *tenebrionis* (HD-8a8b) are registered for use against agricultural, forestry, public health, and home-garden insect pests (Morris *et al.*, 1986, p. 6) (Tables 2 and 3). *Btk* is applied aerially or by ground sprayers directly to plant foliage to be consumed by target Lepidoptera, while *Bti* is released directly into waterways for control of certain Diptera. Large-scale use of *Btk* in Canadian forests is mainly for the control of the eastern and western spruce budworm and the gypsy moth, as mentioned previously. The major use of *Btk* in the United States is in agriculture where it is used mainly in the control of the cabbage looper, *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae), the cabbageworm, *Artogeia rapae* (Linnaeus) (Lepidoptera: Pieridae), and the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (Morris *et al.*, 1986, p. 7,15). Other uses include control of tobacco pests, stored grain pests, and pests of domestic and ornamental plants (Faust and Bulla, 1982, p. 177-187). Only 3% of the *Btk* applied in the United States is against forest pests; most of this is applied in the eastern U.S. (Dubois and Lewis, 1981, p. 238).

It should be noted that each variety of *Bt* is highly specific towards a certain small group of insects, and therefore *Bti*, for example, would not be employed for control of a forest defoliating insect, while *Btk* would not be released into waterways for control of mosquitoe larvae.

# III
# LEPIDOPTERAN-ACTIVE VARIETIES

## A) TOXINS

### 1) Beta-Exotoxin

Prior to 1971, various formulations of *Bt*, specifically varieties *thuringiensis, galleriae, aizawai, morrisoni, tolworthi, galleriae,* and *darmstadiensis,* contained a specific substance known to be toxic not only to the target lepidopterans, but also to many non-target insects and to vertebrates as well (Faust and Bulla, 1982, p. 82-83; Flexner *et al.*, 1986, p. 228). This substance, called beta-exotoxin (sometimes referred to as "thuringiensin", or "fly knock-down factor"), is a powerful inhibitor of RNA polymerase enzymes, preventing the synthesis of RNA in many animals, both vertebrates and invertebrates (Ellis, 1991, p. 15; Melin and Cozzi, 1990, p. 157; Beebee *et al.*, 1972, p. 619; Mackedonski *et al.*, 1972, p. 65). Beta-exotoxin was banned from use in North America in 1971, and has not been present in either *Btk* HD-1, *Bti* HD-14, or *Bt* var. *tenebrionis* HD-8a8b since 1971. Therefore, it is necessary to keep in mind that data obtained on *Bt* varieties studied and published prior to 1971 cannot be directly compared with data collected since then on *Btk* and *Bti*. It is very probable that any side effects reported for some *Bt* applications prior to 1971 were due to the presence of beta-exotoxin in the product (Ellis, 1991, p. 2, 15; Forsberg *et al.*, 1976, p. 22).

Beta-exotoxin is a water-soluble heat-stable secondary metabolite of some varieties of *Bt*, the growth of which can be selected for or against. It is produced during the vegetative phase of *Bt* growth, but the presence, and amount, of beta-exotoxin in *Bt* can be altered depending on the medium employed in the growth of the bacterium (Herbert and Harper, 1987, p. 592; Hoy and Ouyang, 1987, p. 507; Dubois and Lewis, 1981, p. 234; Melin and Cozzi, 1990, p. 157-158). It is highly toxic to insects in the order Diptera. Although formulations containing this toxin were banned from use in 1971 in North America, products containing beta-exotoxin, such as Abbott's Di-Beta, are still available in some European countries, and in the Commonwealth of Independent States (formerly the USSR) (Ellis, 1991, p. 15). Germany, like North America, does not allow the use of products containing this toxin (Krieg, 1978, p. 177).

In mammals, beta-exotoxin blocks the synthesis of ribosomal RNA, the site of action most likely being the liver. It is also weakly mutagenic to mammals (Burges, 1982, p. 100). Injection of a lethal dose causes death only after a latent period, the length of the delay depends upon the strength of the dose (Melin and Cozzi, 1990, p. 157). The toxin, however, is poorly absorbed through the mammalian gut and hence has low oral toxicity (Dulmage and Aizawa, 1982, p. 213).

In insects, beta-exotoxin causes mortality, reduces fecundity and longevity, inhibits larval development, and has sublethal teratogenic effects. Unfortunately, this toxin is highly unselective towards insects and other arthropods, and may have contact as well as oral toxicity towards these organisms (Melin and Cozzi, 1990, p. 157-158; Hoy and Ouyang, 1987, p. 507). Beta-exotoxin also causes the aforementioned deleterious effects among many species of beneficial, as well as non-target organisms, including species of Lepidoptera, Coleoptera, Diptera, Hymenoptera, Isoptera, and Orthoptera (Heimpel, 1967a, p. 296; Herbert and Harper, 1987, p. 592). Toxicity of exotoxin has been documented for *Scolothrips sexmaculatus* (Pergande) (Thysanoptera: Thripidae), *Lygus* spp. (Heteroptera: Miridae), *Apis mellifera* Linnaeus (Hymenoptera: Apidae), *Neoseiulus fallacis* (Garman) (Acarina: Phytoseiidae), *Typhlodromus occidentalis* Nesbitt (Acari: Phytoseiidae), *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae), and several species of spiders (Araneae) (Melin and Cozzi, 1990, p. 159-161; Herbert and Harper, 1987, p. 594; Hoy and Ouyang, 1987, p. 507).

Beta-exotoxin is known to be toxic to all animals, but the effects of the toxin are related to the method of exposure. Contact toxicity is relatively low in all insects, and ingestion is necessary to cause lethal or sublethal effects. Many non-target insects may not be affected simply due to the type of feeding activity they employ. However, insect parasitoids and

**Table 2:**
**Bt Products Currently Registered For Use In Insect Control In Canada[1]**

| Variety | Product | Potency | Registrant |
|---|---|---|---|
| *kurstaki* HD-1 | | | |
| | Bactospeine Suspension | 9.7 BIU/L | Biochem |
| | Bactospeine Powder | 80,000 ITU/mg | Biochem |
| | Bactospeine Liquid | 20,000 ITU/mg | Biochem |
| | CIL Organic Insect Killer (Thuricide) | 4.2 BIU/L | Chipman |
| | Dipel WP | 16.1 BIU/kg | Abbott |
| | Dipel 48AF | 12.7 BIU/L | Abbott |
| | Dipel 88 | 8.5 BIU/L | Abbott |
| | Dipel 132 | 12.7 BIU/L | Abbott |
| | Dipel 176 | 16.9 BIU/L | Abbott |
| | Dipel Technical Powder | | Abbot |
| | Envirobac WP | 16.0 BIU/kg | Pfizer |
| | Foray 48B | 12.7 BIU/L | NOVO Nordisk |
| | Foray Technical | | NOVO Nordisk |
| | Futura XLV | 14.4 BIU/L | Biochem |
| | Novabac-3 | 8.6 BIU/L | Cyanamid |
| | Pfizer Envirobac ES | 8.5 BIU/L | Pfizer |
| | Thuricide R-HPC | 4.2 BIU/L | Sandoz |
| | Thuricide 48 LV | 12.7 BIU/L | Zoecon |
| *israelensis*[2] H-14 | Bactimos Wettable Powder | 3.5 BIU/L | Biochem |
| | Bactimos Granules | 0.2 BIU/L | Biochem |
| | Bactimos Primary Powder | 7.0 BIU/L | Biochem |
| | Teknar (R) Tech. Conc. | 0.5 BIU/L | Zoecon |
| | Vectobac 200 G | 200 ITU/mg | Abbott |
| | Vectobac 200 G (granule) | 200 ITU/mg | Abbott |
| | Vectobac Technical Powder | 5000 ITU/mg | Abbott |
| *tenebrionsis* H-8a8b | Trident Biological *Bt* spp. *tenebrionsis* Strain Sa-10 | 3.3 BIU/L | Sandoz |

[1] D. Guindon, 1993 personal communication. List subject to frequent change.

predators affected by the exotoxin are at risk through direct ingestion of infected prey, or when the death of the host terminates the development of the parasitoid (Melin and Cozzi, 1990, p. 161-162).

### 2) Delta-Endotoxin

The toxin of interest in commercial formulations of *Btk* is the proteinaceous delta-endotoxin, contained in the crystal portion of the bacterium, which is released upon dissolution of the crystal by proteases within an alkaline environment found in the gut of some lepidopterans (Burges, 1982, p. 87). The crystals are composed of polypeptide components or subunits, and are produced at the time of sporulation, which is a defensive mechanism induced by a lack of nutrients. The reason for the formation of the proteinaceous crystal is still not understood. Generally one crystal is produced for every spore in most varieties of *Bt*, but on average, there are three crystals for every spore of *Bti* (Luthy *et al.*, 1982, p. 42, 47, 51; Faust and Bulla, 1982, p. 96).

The protoxin is present in the crystal as a heat-labile protoxin that is converted to the toxin by midgut proteases once solubilized in an alkaline environment (Burges, 1982, p. 87; Luthy *et al.*, 1982, p. 53; Faust and Bulla, 1982, p. 106). "The toxin acts at the surface of gut epithelial cells to cause a rapid loss of ATP from the cells, stimulating respiration and glucose uptake; soon the microvilli swell and the cell apices begin to swell into the gut lumen" (Burges, 1982, p. 87). Feeding inhibition occurs at this time, and ionic imbalance in the haemolymph leads to paralysis and death (p. 87).

The effects of the toxin upon susceptible insects are extremely rapid in most cases. Delta-endotoxin fragments have been observed in the larval haemolymph of two important target species within one minute of consumption of *Btk* crystals (Fast and Videnova, 1974, p. 280). Last-instar larvae of *Choristoneura fumiferana* and *Malacosoma disstria* (Hübner) that consumed purified, radioactive-labelled *Btk* crystals showed rapid uptake of the pro-

**Table 3:**
**Bt Products Previously Registered[1] For Use In Insect Control In Canada[2]**

| Variety | Product | Potency | Registrant |
|---|---|---|---|
| kurstaki HD-1 | Bactospeine A | 9.7 BIU/L | Biochem |
| | Bactospeine F | 9.7 BIU/L | Biochem |
| | Dipel F | 16.0 BIU/mg | Abbott |
| | Dipel SC | 9.9 BIU/kg | Abbott |
| | Dipel 45B | 9.9 BIU/L | Abbott |
| | Dipel 64AF | 16.9 BIU/L | Abbott |
| | Futura XLV-HP | 33.0 BIU/L | Duphar |
| | Marquette Organic Biological | 4.0 BIU/kg | Marquette |
| | Novabac-3 | 8.6 BIU/L | Biochem |
| | Thuricide 16B (aerial) | 4.2 BIU/L | Sandoz |
| | Thuricide 16B (ground) | 4.2 BIU/L | Sandoz |
| | Thuricide 32B | 8.5 BIU/L | Sandoz |
| | Thuricide HPC | 4.2 BIU/L | Zoecon |
| | Thuricide 32 LV | 8.5 BIU/L | Zoecon |
| | Thuricide 32 F | 8.5 BIU/L | Zoecon |
| | Thuricide 38 LV | 12.7 BIU/L | Zoecon |
| israelensis[3] H-14 | Bactimos Wettable Powder | 3.5 BIU/L | Biochem |
| | Bactimos Granules | 0.2 BIU/L | Biochem |
| | Vectobac | 2000 ITU/mg | Abbott |
| | Vectobac 600L | 600 ITU/mg | Abbott |
| | Vectobac 1200L | 1200 ITU/mg | Abbott |

[1] Products no longer registered because registration has been allowed to lapse. Most of these products have been replaced by more potent forms of Btk or Bti.
[2] Morris et al., 1986, p. 6; Guidon, D., 1993, pers. comm.
3 Potency is given in Aedes aegypti international toxic units (ITU)/mg.

tein endotoxin in haemolymph within 1 minute of consumption (p. 281).

The crystal, either alone or in combination with the spore, is responsible for the important insecticidal activity of *Bt* against insect pests (Dulmage and Aizawa, 1982, p. 213-216; Forsberg *et al.*, 1976, p. 94-101). The efficacy of delta-endotoxin differs with the variety and serotype of *Bt* applied, because in most cases, each serotype of *Bt* produces a crystal which is biochemically unique (Dulmage and Aizawa, 1982, p. 216; Burges, 1982, p. 87; Faust and Bulla, 1982, p. 146). Even within the variety *kurstaki*, two serologically different groups of crystals have been identified (HD-1 and HD-73), each having its own toxicological efficacy towards larvae of *Trichoplusia ni* (Hübner) and *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) (Krywienczyk *et al.*, 1978, p. 372). Other factors may contribute to the efficacy of the endotoxin such as the "ability of the gut juice to dissolve the crystals, and the intrinsic susceptibility of the insect to the toxin" (Jaquet *et al.*, 1987, p. 503).

The specific mode of action of the endotoxin is poorly understood. Some species of insects suffer from gut paralysis only and die from starvation, while others suffer from general paralysis and die from septicemia caused by the spores, which are present in all *Btk* products. No matter what the ultimate cause of death may be, the delta-endotoxin is necessary to initiate gut epithelial degradation, which provides the entry sites for spores into the haemolymph (Forsberg *et al.*, 1976, p. 95).

## B) MODE OF ACTION

In general, it is known that toxicity of the *Btk* endotoxin towards target insects involves the solubilization of the parasporal crystal in the alkaline gut environment of susceptible species, followed by activation of the delta-endotoxin. The range of pH levels required to activate *Btk* and *Bti* delta-endotoxin,

both in isolated cell assays and in direct insect feeding experiments, have been reported to be between pH 7.5 and 11.5; although optimum pH for activation of *Btk* reportedly ranges between pH 9.5 and 11.5 (Johnson *et al.*, 1990 p. 237, 241, 242; Thomas and Ellar, 1983, p. 192; Burges, 1982, p. 87; Harvey and Wolfersberger, 1979, p. 293; Yunovitz *et al.*, 1987, p. 321). The bacterium must be ingested to be effective because the endotoxin does not possess any contact activity. For this reason, only the larval instars of target Lepidoptera, Diptera and Coleoptera are susceptible to *Bt* infection, having the appropriate gut environment to dissolve the crystal toxins of *Btk*, *Bti* and *Bt* var. *tenebrionis*, respectively (Burges, 1982, p. 80; Harper, 1974, p. 3; Davidson, 1982, p. 290-291). Following ingestion of spores and crystals, gut paralysis ensues, and eventually septicemia occurs within the host insect. Larval septicemia and death induced by the delta-endotoxin of *Bt* begins with the binding of the endotoxin molecule to a binding receptor on the cell surfaces. This is followed by the insertion of the alpha-helices of the endotoxin into the cellular membrane (Gill *et al.*, 1992, p. 615). It is postulated that several endotoxin molecules (probably six) form a non-selective ion port in the cell membrane, which permits the free movement of potassioum ions into the cell (Gill *et al.*, 1992, p. 625-626).

This is followed by an increase in oxygen uptake by midgut cells, indicating that uncoupling of oxidative phosphorylation has occurred. This causes ATP levels to fall, and the rate of NADH use to increase dramatically, the latter of which causes stimulation of the Krebs TCA cycle. Glycolysis is then stimulated, and glucose uptake increases as well, but the lack of ATP eventually causes the cessation of NADH production. As a result, midgut cells swell and lyse due to a breakdown in transport and osmoregulatory mechanisms. Feeding inibition may occur within 5 minutes following ingestion of *Btk*. The degradation of the midgut lining allows ions to leak into the haemolymph, causing a change in pH, and allowing spore germination and cell growth in the gut. Vegetative cells may enter the haemocoel causing a full-scale infection within the host insect (septicemia). The change in gut pH, in combination with septicemia and gut paralysis, ultimately causes the death of the insect (Forsberg *et al.*, 1976, p. 23; Travers *et al.*, 1976, p. 408-409; Morris, 1982, p. 241; Sacchi *et al.*, 1986, p. 213; Gill *et al.*, 1992, p. 622).

The mode of action of *Btk* is still not fully understood. The current knowledge suggests that, due to differing proteolytic processes, some insects may be killed by spores, others by the toxic crystals, and still other insects require both crystals and spores for mortality to occur. In most cases, the crystals alone are the insecticidal element necessary for control of Lepidoptera (Faust and Bulla, 1982, p. 107; Dulmage and Aizawa, 1982, p. 222). However, some studies have shown that the spore-crystal complex is necessary for the most effective control of some target insects such as the spruce budworm. McGaughey (1978) has shown that *Btk* crystals are 30 times more toxic to the almond moth larvae, *Ephestia cautella* (Walker), than the spores. Conversely, crystals were only 3 times more toxic than pores to the Indianmeal moth larvae, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), but a 50:50 mixture of spores and crystals was even more toxic than crystals alone (p. 687).

Larvae of the spruce budworm, *Choristoneura fumiferana* (Clemens), are virtually unaffected by spores of *Btk* serotype HD-1 (Dipel at 16,000 IU, supplied by Abbott Laboratories) (Fast, 1977, p. 1515). The $LD_{50}$ of the crystals was determined to be 0.094 g/larva, while that for the spore/crystal complex (50:50) was 0.096 g/larva. The $LD_{50}$ of the spores alone was $1.04 \times 10^8$ g/larva (p. 1516). The author suggests that the spores act in the same fashion towards budworm larvae as the crystals, but the amount of toxic material in the spores is so minimal as to have almost no effect on toxicity (p. 1518).

The mode of action of *Btk* in the greater wax moth *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae), is completely different from that in the budworm. A 1:1 mixture of spores and crystals of *Btk* (Thuricide) was found to be 10 times more potent than spores alone, and $10^4$ times more potent than crystals alone (Burges *et al.*, 1976, p. 87). The $LC_{50}$ of crystals only was $3.33 \times 10^{10}$ crystals/g of food, the $LC_{50}$ of spores was only $2.0 \times 10^7$ spores/g of food, and for a 1:1 ratio of spores and crystals the $LC_{50}$ was $1.74 \times 10^6$ spores + crystals/g of food (p. 88). A mix of spores and crystals is necessary to ensure the most efficient control of the greater wax moth in spray programs. Perhaps the effects of the spore-crystal complex also vary with the target insect. In summary, in some cases the crystal is sufficient to cause mortality, while in others, the spore may be the ultimate toxic element. Still other insects may require both the spores and crystals to cause a substantial infection, or the effects of the infection are more rapid when both elements are present.

These results suggest that the mode of action of *Btk* is different for various target insects, and therefore toxicity and mode of action must be studied individually for each insect when implementing spray programs. The potency of the delta-endotoxin is variable depending upon the variety of *Bt* applied to the insect, the degree of solubility of the crystal in the gut enzymes, and the susceptibility of the target insect to the toxin, which may be a factor of age and biomass of the insect, and of temperature during and following applications of *Bt* (van Frankenhuyzen, 1990b, p. 71-74; Jaquet *et al.*, 1987, p. 503). It has recently been demonstrated that insect susceptability to the delta-endotoxin is related to the concentration of binding sites on the cell surface. At least three receptors are suspected to be involved, each with a different binding affinity for each of the various delta-endotoxin molecules (van Rie, 1989, p. 243).

Delta-endotoxin may affect target insects in a variety of ways. Heimpel and Angus (1959) categorize

insects by the symptoms they exhibit as a result of *Bt* ingestion into Type I and Type II. Type I insects suffer from mid-gut paralysis and general paralysis a few minutes after ingestion, and die within 7 hours. This is accompanied by an increased pH level in the blood due to leakage of the alkalne gut contents into the blood stream. Type II insects experience only gut paralysis and no change in blood pH; they die in 2 to 4 days from starvation and/or bacteriaremia (p. 152). Most Lepidoptera are Type II. Heimpel and Angus (1959) found that a third type of insect differed from the others in that both spores and crystals were essential to cause mortality. *Ephestia kuehniella* specimens died in 2 to 4 days only if injected with a solution of both spores and crystals (p. 152).

Several studies have shown that some insects are susceptible to *Btk* in that they experience disruption of the gut which leads only to a temporary reduction or cessation of feeding. These insects often recover from infection in eight hours or less and resume feeding, but larval and pupal development is affected by the lack of nutrition. This results in adults that are undersized, have a poorer rate of survival, and are unable to lay as many eggs as normal adults (Dimond and Morris, 1984, p. 105; van Frankenhuyzen and Nystrom, 1987, p. 941; Retnakaran *et al.*, 1983, p. 233; Fast and Regniere, 1984, p. 123).

Yang *et al.* (1985) showed that in addition to direct mortality of *Spodoptera litura*, *Btk* caused important secondary effects in the larvae (p. 21). Infected third-instar larvae experienced a loss of appetite, became sluggish in movement, and had reduced pupation and emergence rates. Females that did emerge from pupae did not produce eggs. Smirnoff (1983) reports that *Btk* significantly reduces vigour of surviving *Choristoneura fumiferana* populations, in terms of pupal weight, total protein content, and other biochemical parameters (p. 225). Surviving pupae of both field and laboratory populations treated with *Btk* had average pupal weights of 46.9 mg compared with an average weight of 69.5 mg for untreated populations, and 82.0 mg for populations treated with organophosphates (fenitrothion or phosphamidon) (p. 227). Surviving pupae from *Btk*-treated, untreated, and organophosphate-treated populations gave the following results, respectively: total calcium ion, 54.0, 80.0, and 114.5 mg/kg; total proteins, 19.2, 27.6, and 36.3 g/kg; alkaline phosphatase, 251.0, 541.0, and 580.0 mU/g (p. 227). The results indicate that *Btk* has a detrimental effect on survivability on *C. fumiferana* populations, "involving a marked decline in the energy potential which is seen in practice as a reduction in the reproductive ability of the pest population" (p. 229).

These secondary effects of *Btk* infection may offer an explanation for the 1- and 2-year carry-over effects that have been observed and reported in some treatment areas (Morris, 1977b, p. 1239; Dimond and Spies, 1981, p. 661).

## C) COMBINATIONS OF *Btk* AND CHEMICAL INSECTICIDES

Integrated Pest Management (IPM) involves a variety of approaches to pest control in agriculture, forestry, and the home environment. It integrates chemical, biological, cultural, genetic (the use of resistant plant varieties), and other control methods to prevent large-scale pest outbreaks while releasing as little chemical insecticide as possible into the environment. During the "insecticide era", approximately 1940-1962, chemical pesticides were often applied in orchards and in agriculture more according to a calendar schedule than the status of the pest or the condition of the crops, and with little concern or lack of knowledge concerning the potential side effects on the environment. IPM focuses on a school of thought completely different from tactics used in agriculture during the insecticide era, and instead reverts back to traditional crop rotation and "planned" planting dates (Pedigo, 1989, p. 275). This traditional "crop protection" in forest management would be of sanitation and/or the avoidance of single-species, single-age-class plantings over a large area. IPM is based on three main principles: employing multiple tactics for pest suppression, maintaining pest populations below economic levels, and conserving the quality of the environment (Pedigo, 1989, p. 276-278).

The use of *Btk* in combination with chemical insecticides is an alternative to exclusive use of chemicals, and some combinations may result in synergistic effects. Also, combinations of insecticides and *Btk* allow for lower volumes of either of the pesticides released into the environment, and this may even lessen the possibility of insects developing resistance to chemical insecticides as well as *Btk*. However, the use of *Btk* alone is significantly and environmentally safer than the use of chemical-*Bt* mixtures because of the hazardous effects of wide-spectrum chemical insecticides.

Before any chemical can be successfully used in combination with *Btk*, mixtures of insecticides must be tested for physical compatability, as well as compatibility in terms of the effect the chemical may have on spore germination, vegetative cell replication, and crystal formation and viability (Morris, 1981, p. 1). Considerations must also be made for any side effects resulting from emulsifiers and adjuvants in the chemical formulations (Morris, 1977a, p. 855).

The *Btk* product Biobit, a wettable powder formulation registered in the United States, is reportedly compatible with a number of chemical insecticides. These include acephate, azinphos-methyl, carbofuran, captan, chlordimeform, diazinon, endosulfan, fenitrothion, malathion, maleic hydrazide, methyl parathion, permethrin, tricholfon, and several others (Novo Industri A/S, 1988c). Whether or not combinations of *Btk* with these various chemicals provide better protection from pest insects is unknown at this time, except for acephate, fenitrothion, and methyl parathion which are discussed below. Successful control of the spruce budworm, *C. fumiferana*, has been achieved in field trials using formulations of *Btk* (Dipel 36B) and Orthene (acephate) mix at a ratio of 10 BIU *Btk* to 21 grams Orthene in white spruce, *Picea glauca* Moench, and balsam fir, *Abies balsamea* L., forests in Ontario (Morris *et al.*, 1975, p. 10-12).

From this experiment Morris *et al.* (1975) concluded that combinations of *Btk* and Orthene were substantially more effective in controlling spruce budworm larvae than applications of *Btk* alone. Moth emergence and oviposition of the budworm were also reduced significantly (p. 14). Furthermore, no deleterious effects of the *Btk*-Orthene treatments were observed among parasitoids, indicating that *Btk* plus Orthene has potential in IPM programs (p. 15). *Btk*-Orthene and *Btk*-only treatments also caused high mortality (69 - 80%) of spruce coneworm, *Dioryctria reniculella* (Grote) (Lepidoptera: Pyralidae), sometimes associated with spruce budworm (p. 10). Further investigations by Morris *et al.* (1980) confirmed these findings (p. 2).

During the spray trials of Orthene and *Btk* in Ontario, trials of *Btk* plus fenitrothion were simultaneously conducted to determine the efficacy of this combination towards the spruce budworm, and the possible side-effects to parasites (Morris and Armstrong, 1975). Dipel WP (16,000 IU/mg) and Thuricide 16B (4.2 BIU/L), at concentrations of 10 BIU, were each mixed with 17.5 grams active ingredient of fenitrothion and applied at 4.7 L/ha over a mixed forest of white spruce and balsam fir (p. 1281-1282). In contrast with the results obtained from *Btk*-Orthene applications, neither Dipel-fenitrothion nor Thuricide-fenitrothion applications significantly protected the trees from defoliation in the year of the treatment (p. 1284-1285). This may have been partly due to the low application and deposit rates of *Btk*-fenitrothion mixtures (p. 1284).

*Btk* was shown to be compatible with methyl parathion and with pyrethroid when tested against the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), and in addition, synergism between methyl parathion and the pathogen was observed (Habib and Garcia, 1981, p. 7, 10). Thuricide HP (16,000 IU/mg) combined with methyl parathion reduced the LD50 from 15.7 ppm (methyl parathion alone) to 10.5 ppm (p. 11). Synergism was not apparent with pyrethroid, but inhibition of *Btk*-action did not occur either (p. 11).

Morris (1977a) investigated the compatibility of 27 chemical insecticides, including organophosphates, carbamates and pyrethrins, with *Btk* for use against pest insects in forestry and agriculture. In general, the technical formulations were less harmful than wettable powders, which were less harmful than emulsifiable concentrates. Of the insecticides tested, those most compatible with *Btk* were Dylox, Orthene, Lannate and Dimilin (Morris, 1977a, p. 863). Morris (1981) has also shown that technical grade acephate (94% active ingredient), the compound used in Orthene, is compatible with *Btk* at concentrations up to 10,000 ppm (p. 5). However, other considerations include toxicity of the various chemicals towards non-target organisms, and the efficacy of the combinations. All of the tested chemical insecticides have half-lives ranging from 2 to 14 days, and Lannate is highly toxic to mammals, birds, and bees (Morris, 1977a, p. 863). Furthermore, if a combination of *Btk* and a chemical insecticide does not provide significantly greater protection from defoliation by lepidopterous pests than applications of *Btk* alone, the use of *Btk* alone would be more publicly acceptable and environmentally sound than employing mixtures of *Btk* and chemical pesticides.

## D) OPERATIONAL USE AND APPLICATION RATES OF *Btk*

In Canada, large-scale operational use of *Btk* is directed toward the control of the eastern spruce budworm, *C. fumiferana*. In 1986, the planned aerial application of *Btk* was to account for 74% of the total area treated for spruce budworm and Jack pine budworm control in Canada, 1,426,000 ha of the 1,932,000 total ha to be treated (Morris *et al.*, 1986, p.7). Despite changes, over 45% of the operational treatments against spruce budworm were conducted using *Btk* in 1986. In combination with other control programs, 941,653 ha were sprayed with *Bt* (van Frankenhuyzen, 1990a, p. 501). This is a dramatic increase over the figures from 1979, at which time *Btk* use accounted for only 1% of the total area treated for spruce budworm control (27,115 ha were treated with *Btk* out of 2,210,641 ha treated in total) (Morris *et al.*, 1986, p. 7). In comparison, the use of biological insecticides (including bacteria and viruses) accounted for only 0.04% of the forest area aerially treated for pest control in Canada from 1944 to 1973. DDT, phosphamidon, and fenitrothion ac-

counted for 98.8% of the total area treated during this time (Prebble, 1975, p. v).

*Bt* has been in use in Canada and in the United States since the late 1950s, but these initial formulations were much less concentrated, and hence less effective, than those used today. Past commercial products did not specify what serotype, or even what variety of *Bt* was in the product. Modern sprays are 2 to 3 times more concentrated, are specially formulated for aerial application, and contain stickers to reduce the effects of rain (Dimond and Morris, 1984, p. 105). The potency of *Btk* formulations has steadily increased since 1973, from 4.2 BIU/L up to 16.9, 25.9 and even 33 BIU/L (van Frankenhuyzen, 1990a, p. 500).

The cost of *Btk* treatments has steadily decreased since 1980. Increased potency in modern formulations has resulted in decreased volumes required in treatment programs, allowing for reduced shipping and application costs (van Frankenhuyzen, 1990a, p. 499). Unfortunately, an increase in the cost of aerial applications of all pesticides has resulted in an overall increase in the cost of *Btk* applications. During this time chemical insecticide product and application rates have also steadily increased. Figures from 1983 indicated that *Btk* was 1.5 to 3 times more expensive to apply than chemical insecticides, depending on the size of the spray program. In Maine, the total cost of *Bt* applications in control programs against the spruce budworm has decreased from 2.74 to 1.23 times the cost of chemical controls between 1979 and 1985. Between 1979 and 1982, the above figures are for single applications of both *Btk* and chemical insecticides, while for 1983 to 1985, the figures are for double applications of chemical insecticides compared with single applications of *Btk* (Irland and Rumpf, 1987, p. 89). The average cost of *Btk* in 1985 was $8 to $10 for a 20 BIU dosage to cover 1 hectare (Morris *et al.*, 1986, p. 9).

Prior to the 1970s, there was no universal monitoring system for the potency of *Bt*. Some of the earlier literature provides potency levels of *Bt* in counts of spores/pound or spores/gram, such as Thuricide 65B at 65 billion spores/g (Forsberg *et al.*, 1976, p. 87-88). However, this was often an inaccurate and ambiguous expression of potency because of the variation in insecticidal potency between varieties. In 1966, a standardized unit was adopted worldwide employing the International Unit (IU) based on a reference standard containing 1,000 IU/mg of *Bacillus thuringiensis* var. *thuringiensis* (Luthy *et al.*, 1982, p. 60; van Frankenhuyzen, 1990a, p. 499). One IU of a product was that amount which caused mortality equal to that of the reference standard serotype, bioassayed against the cabbage looper, *Trichoplusia ni* (Dulmage *et al.*, 1971, p. 240). A new reference standard was adopted in 1972 and employed the HD-1 serotype of *Btk*, designated HD-1-S-1971 (Dulmage, 1973, p. 200). *Btk* has been shown to be 18 times more potent than *Btt*, and thus its activity corresponded to 18,000 IU/mg (Dulmage, 1973, p. 200; Luthy *et al.*, 1982, p. 60). In 1980, the reference standard was once again updated and designated HD-1-S-1980, and was bioassayed at 16,000 IU/mg (Beegle *et al.*, 1986, p. 44). The new standard is based on *Btk* activity towards both *Trichoplusia ni* and *Heliothis virescens* (p. 44). Modern field dosages of *Btk* are usually expressed as BIU (billion international units) per acre or hectare (Dimond and Morris, 1984, p. 106).

*Bt* is most often applied aerially as liquid formulations, either water or oil-based. Some products are wettable powders and must be mixed with water as well as a sticker to reduce run-off by rain. Other additives include thickening agents to provide uniform suspensions, wetting agents to ensure adequate leaf coverage, anti-evaporants, emulsifiers, and sun screens to reduce the degradation by sunlight (Ellis, 1991, p. 8, 35). Such "formulation enhancements" allow some reduction in the amount of active ingredient needed, which reduces the overall cost of material and applications (Ellis, 1991, p. 35).

Standard rates of application currently range from 20 to 50 BIU/ha (8 to 20 BIU/acre) (Dimond and Morris, 1984, p. 107; Rossiter *et al.*, 1990, p. 2211). In efforts to increase the efficacy and decrease the cost of applications, manufacturers increased the concentrations of *Btk* formulations since 1973. However, efficacy increases with increasing *Btk* concentration up to certain level, at which point higher dosages do not improve insect control. Morris *et al.* (1982) investigated the relationship between efficacy and dosage of *Btk* for control of the spruce budworm, *C. fumiferana*, using Dipel 88 (Abbott Laboratories) and Thuricide 32 BX (Sandoz, Inc.) (p. 2). Results indicated that efficacy of *Btk* (both formulations) increased accordingly as dosages were increased from 10 to 40 BIU/ha in 9.4 L/ha, but at dosages above 40 BIU/ha, budworm control was not significantly greater, 14 or 21 days after treatment (p. 9-10).

Multiple applications of *Btk* may or may not provide better protection to foliage. Morris *et al.* (1982) concluded that double applications of *Btk* did not improve efficacy of Thuricide nor Dipel in controlling the spruce budworm in Ontario. Double applications of Dipel 88 and Thuricide 32 BX (2 x 10 BIU, 2 x 20 BIU) were compared with single applications (20 BIU, 40 BIU) (p. 15). Neither Thuricide nor Dipel were significantly more effective when applied in double dosages, both at 14 and 21 days after treatment (p. 17-18). Conversely, Kettela (1990) found that two applications of Futura XLV-HP (*Btk*) at 15 BIU/ha each application provided better protection from spruce budworm damage in New Brunswick than a single application of 15 or 30 BIU/ha (p. 18). Because of these conflicting results in efficacy, more data is required to determine if double applications provide better control of target insects. Interestingly, the product label for Dipel 176, an emulsifiable suspension of *Btk*, recommends using one to two applications of Dipel undiluted for effective control of the hemlock looper, applied at the peak of the first and second instars (Abbott Laboratories, product label).

Both weather and timing of the application must be carefully considered before spraying. The best time to spray is at the peak of the fourth-instar larval stage, in clear sunny weather, especially, when there is a slight wind (6 km/h or less is considered satisfactory) (Dimond and Morris, 1984, p. 107-109; Smirnoff and Valero, 1984, p. 15). Larvae should be "free feeders", actively feeding on the foliage, and there should be no threat of rain for at least 24 hours after spraying to prevent losses by run-off (Trial, 1984, p. 12). Efficacy of *Btk* has been shown to be greatly hindered by precipitation following aerial applications, and by delaying applications because of poor weather conditions such that treatments are not done at the optimal larval stage (Lewis *et al.*, 1974, p. 353). Furthermore, although *Bt* remains viable for only 3 to 7 days on the foliage in the absence of rain, budworm larvae tend to feed more during warm weather; therefore it is more advisable to spray at these times (Dimond and Morris, 1984, p. 109; Kettela, 1990, p. 19). In Canada, *Btk* is generally used against the younger (fourth) instars with the aim of foliage protection, while in the United States the treatment is applied to older larvae with the aim of higher population reduction in the first year of application (with little or no foliage protection). In the latter case, foliage protection is thought to be achieved in the second year of application.

Smirnoff and Valero (1984) report that the distribution of nozzles on the aircraft boom employed during application of *Btk* may affect deposition which in turn is related to efficacy (p. 15). Three formulations were tested: Futura (applied at 20 BIU/ha or 2.5 L/ha), Thuricide 32LV (applied at 40 BIU/ha or 4.7 L/ha), and Dipel 88 (same dosage as Thuricide) (p. 14). The DC-4G aircraft was equipped with 154 open-type nozzles, 77 on each boom. Ground cover was greatest when Futura was applied using 110 open nozzles (55 on each boom) located close to the fuselage, as opposed to 110 evenly spaced along the booms, or 110 open only at the wing tips (p. 15). It should be noted, however, that only one pass was made by the test aircraft, and no information on the length of the sample line or spray swath was provided. Application of Futura was more efficient than Dipel or Thuricide, even though the volume of Futura sprayed was less than either of the other two products (p. 15). Thus, in addition to such factors as weather and larval instar, the formulation and method of aerial application (number and kind of dispensers) of *Btk* may also be important factors affecting efficacy of spray deposit and control of insect larvae.

## E) *Btk* DEPOSIT ANALYSIS IN FOREST APPLICATIONS

Spray deposit analysis and tree crown penetration testing is important to determine whether enough insecticide contacts the target organisms or their feeding site to ensure the greatest efficacy in insect control. The size of droplets that impact and deposit on forest foliage and target insects directly affects the efficacy of the treatment. In general, spray nozzles calibrated to produce smaller droplet sizes generate a greater number of droplets for a given volume of insecticide; therefore, a higher percentage of the volume of the insecticide is deposited on the insects and the foliage. It is known that smaller droplets of a *Btk* product are more effective at controlling target insects than larger droplets, provided there is a lethal dose of insecticide in the small droplets. For example, the probability of one 400 µm droplet contacting an insect or conifer needle is much less than the probability of one of 8,000 20 µm droplets contacting the target. Therefore, the efficacy of the treatment could be improved upon by increasing the availability of the smaller-sized droplets to the target (Barry and Ekblad, 1978, p. 1-2).

Spray deposit analysis information is also useful for determining minimum dosages of ultra-low-volume applications (ULV) of highly concentrated *Btk* formulations necessary for more cost-effective control of target insects (van Frankenhuyzen *et al.*, 1989, p. 9; Morris, 1982, p. 247). Highly concentrated formulations of *Btk* allow for decreased product volumes, shipping, and application costs, thereby making *Btk* more competitive economically with chemical insecticides (Morris *et al.*, 1986, p. 9).

Deposit analysis of bacterial pathogens has been done by several methods, including an agar plate technique. This involves placing a petri dish filled with agar in plots where sprays are conducted, and then counting the bacterial colonies after 18 hours of incubation. Colony density is used to estimate the deposition rate, but this method greatly underestimates the actual spray dosage (Morris, 1982, p. 249). A better method involves the use of glass plates to collect droplets, which are washed with distilled water and 0.1% peptone water to separate the spores, and then are spread on agar and allowed to colonize (Morris, 1982, p. 250). Kromekote® cards are also used to gather information about droplet size, density, and spectra, as well as volume/unit area. Visible spots on the cards are analyzed using microcard readers or image analyzers (Morris, 1982, p. 250).

Barry and Ekblad (1978) found that deposition of Dipel (*Btk*) in coniferous forests was such that 92% to 94% of the droplets were less than 61 µm in diameter when applied at a rate of 18.70 L/ha (0.436 kg *Btk* in 7.75 L water). More than 67% of the droplets were less than 21 µm in diameter (p. 3). These

droplet sizes are sufficiently small to ensure efficient distribution of the sprayed insecticide. The distribution of *Btk* was significantly more efficient than that of carbaryl and trichlorfon, sprayed on two other blocks, suggesting that more foliage area is covered when *Btk* is sprayed, as compared with these chemical control agents (p. 4). However, direct comparisons cannot be made between contact insecticides such as trichlorfon and carbaryl, and *Btk* which requires ingestion of infected foliage. Dipel has been shown to satisfy the recommended mass median diameter (MMD) of 90 µm for *Btk* spray droplets (Randall *et al.*, 1980, p. 1).

van Frankenhuyzen *et al.* (1989) studied canopy penetration of *Btk* in oak stands to determine the efficacy of undiluted, as compared with diluted, formulations of the insecticide. Leaves from cut branches were examined individually under a microscope to determine the number of droplets in a given area. Droplet size was also estimated. Dipel 8AF was sprayed at 15 BIU in 0.9 L/ha (undiluted), at 30 BIU in 1.8 L/ha (undiluted), and at 30 BIU in 6 L/ha (diluted). Branches were collected from three canopy levels: upper, middle, and lower (p. 2-4). Droplet densities were always greatest in the upper level, but the difference in density between upper and middle levels was greatest in areas treated with the diluted formulation, indicating that low-volume applications achieved the best canopy penetration (p. 6).

Similar results were obtained from spray trials in mixed forests of red oak, white oak, and red maple in Pennsylvania following applications of *Btk* in 1986 and 1987 (Bryant and Yendol, 1991, p. 542). Thuricide 64 LV and SAN415, supplied by Sandoz Inc., were applied to test plots at a concentration of 29.6 BIU/ha in 7.1 L/ha (p. 543). Upper canopy, lower canopy, and ground deposit samples were taken to determine the forest penetration ability of *Btk* (p. 543-544). Results were highly variable, both within and between strata samples; no consistent pattern of deposition could be determined among the plots. In general, however, the upper canopy trapped and retained a greater amount of *Btk* than either the lower canopy, or the ground. Approximately 55% of the spray was recovered from foliage, while only 6% was recovered in ground samples. The remaining spray not accounted for was presumed to have contacted trunks and stems, or may have drifted from the spray area (p. 543). Highly variable deposition results of *Btk* in hardwood forests have been documented by Yendol *et al.* (1990) in similar studies of canopy penetration (p. 173).

## F) EFFICACY OF *Btk* IN PEST CONTROL

The efficacy of *Btk* is variable among target lepidopterans; it provides excellent control of some insects like the spruce budworm (Trial, 1984, p. 7-11), and somewhat less reliable control of the gypsy moth and the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough) (Lepidoptera: Lymantriidae) (Harper, 1974, p. 32-33; Brown *et al.*, 1984, p. 6; Kegg, 1984, p. 33; Schneeberger, 1984, p. 35). Pests of fruit trees such as the codling moth, *Carpocapsa pomonella* Linnaeus (Lepidoptera: Tortricidae), and the summer fruit tortrix moth *Adoxophyes reticulana* Hübner (Lepidoptera: Tortricidae) are highly susceptible to *Btk* in laboratory tests (dosages not provided, although the susceptibility of codling moth is similar to that of the cabbage looper). However, they are not effectively controlled in field trials, due in part to the fruit boring habit of these insects which allows for only a narrow "window" of susceptibility (Falcon, 1971, p. 85; Luthy *et al.*, 1982, p. 64).

Similarly, control of the winter moth, *Operophtera brumata* (Linnaeus) (Lepidoptera: Geometridae), in British Columbia blueberry fields was unsuccessful even though laboratory tests indicated that the pest was susceptible to *Btk* (Sheppard *et al.*, 1990, p. 27). Dipel WP, ground-applied at 1,100 g A.I./ha to blueberry bushes, did not significantly reduce larval populations relative to control bushes (p. 27-28). However, other studies have indicated adequate control of the winter moth with *Btk* on apple trees (Hardman and Gaul, 1990, p. 931; Tonks *et al.*, 1978, p. 6).

Even among highly susceptible pests, efficacy varies from spray to spray and from year to year due to variations in climate, larval instar, and spray deposit (Kettela, 1984, p.22). However, because of increasing potencies and improved spray delivery systems, the efficacy of *Btk* has increased significantly for the control of many important insects since the early 1970s (Dimond and Morris, 1984, p. 105; van Frankenhuyzen, 1990a, p. 500; Trial, 1984, p. 7).

Many factors affect the efficacy of field applications such as weather conditions, temperature, precipitation, direct sunlight, exposure time, larval instar and insect density, dosage of *Bt*, and natural enemy populations. Other factors influencing efficacy include the serotype of *Bt* used in control programs, and the susceptibility of a particular insect to the delta-endotoxin of *Bt* (Jaquet *et al.*, 1987, p. 503; van Frankenhuyzen, 1990b, p. 71-74).

A summary of efficacy tests using various formulations of *Btk* for control of some common forestry pests is given in Table 4.

## Table 4:
## Efficacy of Btk in Experimental Trials for the Control of Forestry Pests

| Pest Species | Product | Dosage (BIU/ha) | Volume (L//ha) | Population Reduction (%)[1] | Defoliation (%) | Foliage Protection (%) | Reference |
|---|---|---|---|---|---|---|---|
| Choristoneura fumiferana | Thuricide 32 BX | 10 | 9.4 | 9 | 69 | 14 | Morris, |
| | | 20 | 9.4 | 68 | 45 | 44 | 1984, p. 987 |
| | | 80 | 9.4 | 95 | 61 | 24 | " |
| | Dipel 88 | 10 | 9.4 | 47 | 71 | 11 | " |
| | | 20 | 9.4 | 84 | 24 | 70 | " |
| | | 40 | 9.4 | 89 | 39 | 51 | " |
| | | 80 | 9.4 | 85 | 37 | 54 | " |
| | Thuricide 48B | 30 | 2.4 | 68 | 45 | 48 | Morris, |
| | | 30 | 4.7 | 72 | 59 | 31 | 1984, p. 989 |
| | | 30 | 9.4 | 76 | 56 | 35 | " |
| | Dipel 48B | 30 | 2.4 | 88 | 68 | 21 | " |
| | Futura | 20 | 2.5 | 93.3 | 10.7 | — | Valero, |
| | Futura | 20 | 2.5 | 100.0 | 14.6 | — | 1989, |
| | Futura | 16.6 | 2.0 | 91.1 | 32.2 | — | p. 10-12 |
| | 64B (Zoecon) | 22 | 2.0 | 97.5 | 3.7 | — | " |
| | 64B (Zoecon) | 22 | 2.0 | 100.0 | 2.3 | — | " |
| | Futura XLV | 25 | 2.0 | 96.9 | 15.8 | — | " |
| | Btk[2] | 15 | 1.18 | 94.6 | 13.8 | — | Carter, |
| | Btk[2] | 30 | 1.01-2.36 | 99.6 | 7.0 | — | 1990, p. 8 |
| | Btk[2] | 15[3] | 1.18 | 96.7 | 6.1 | — | " |
| | Btk[2] | 30[3] | 2.02 | 91.3 | 29.9 | — | " |
| Choristoneura pinus pinus | Futura XLV | 20 | 1.39 | 49 | 56 | 28 | Cadogan |
| | | 30 | 2.08 | 74 | 26 | 64 | 1986, et al., p. 62-63 |
| Lambdina fiscellaria fiscellaria | Thuricide 48LV | 30[3] | 2.36 | 34 | 0 | — | West et al., |
| | Thuricide 64B | 30[3] | 1.78 | 100 | 39 | — | 1987, p. 456 |
| | Futura XLV | 20[3] | 1.4 | 0 | 10 | — | " |
| | Futura XLV | 30[3] | 2.1 | 10 | 0 | — | " |
| L. f. fiscellaria | Dipel 132 | 30[3] | 2.36 | 98.3 | 0 | 100 | West et al., |
| | Dipel 176 | 30[3] | 1.78 | 99.4 | 0 | 100 | p. 59-61 |
| | Dipel 264 | 30[3] | 1.18 | 95.5 | 0 | 100 | 1989, |
| | Dipel 176 | 40 | 2.36 | 85.0 | <5 | 63 | " |
| | Dipel 264 | 40 | 1.58 | 96.8 | 0 | 100 | " |
| Orgyia pseudotsugata | Dipel WP (in 25% molasses) | 7.26 | 2.0 gallons /acre | 95 | 19.4 | — | Harper, 1974, p. 33 |
| | Dipel WP | 7.26 | 2.0 gallons /acre | 73 | — | — | Harper, 1974, p. 34 |
| | Dipel WP + molasses | 20 | unknown | 97 | 19 | 70 | Stelzer et al., 1975[4] |
| Lymantria dispar | Dipel WP | 40 | 19.0 | 99 | 29 | good | Reardon et al., 1979, p. 305 |
| | Dipel LC | 40 | unknown | 91 | 58 | 38 | Hanson et al., |
| | Thuricide 16B (undiluted) | 40 | unknown | 81 | 50 | 42 | 1975[4] |
| Malacosoma disstria | Dipel WP + molasses | 4.4-8.8 | unknown | 95 | — | — | Abrahamson and Harper, 1973[4] |

[1] Larval mortality only
[2] Data was pooled for treatments of Dipel 176 and Futura XLV
[3] Double applications
[4] Cited in Morris, 1982, p. 258-271 (original not available)

# IV
# PERSISTANCE AND ENVIRONMENTAL TOXICOLOGY OF *BACILLUS THURINGIENSIS*

The environmental impact of any pest control agent, whether chemical or biological, must be considered and assessed thoroughly when planning control programs. The ecology of any ecosystem will be affected to some degree by the artificial introduction of predatory and parasitic organisms or foreign substances in efforts to reduce target insect populations. The goal of Environmental Impact Assessment (EIA) programs in forestry is to estimate the severity of the effects upon the environment, and to determine whether the benefits of the treatment exceed the possible side effects. The requirement for the preparation of EIA statements began with large megaprojects and it is slowly filtering down, as a requirement, to other and smaller projects.

An environmental impact assessment statement put forth by the United States Department of Agriculture proposed six possible alternatives or tactics to implement for control of the gypsy moth in Virginia and West Virginia (USDA, 1989). Five of these alternatives included *Btk* as part of the arsenal to be employed in the program. Alternative 5, a program involving biological tactics (including *Btk*, NPV, disparlure (pheromone trapping), natural enemies, and the release of sterile life stages) and a chemical insect growth regulator (diflubenzuron), was chosen as the preferred avenue of gypsy moth control (USDA, 1989, p. 8 of "Record of Decision").

The environmental impact assessment statement concluded that no serious environmental effects of *Btk* treatment of forested areas would result from the spray program (USDA, 1989, p. II-6 - II-7, II-9). Many ecological elements of the treatment areas were assessed, including vegetation, wildlife, non-target Lepidoptera and other insects, aquatic organisms, wetlands, wilderness, water and air quality, soil, and public health. No possible effects of *Btk* were indicated for any of the components of the environment except the following. First, some potential indirect effects on birds and small mammals may result from a decrease in food supply of insects. However, the "effectiveness of *Bt* declines rapidly after application, normally lasting from 7 to 14 days. Lepidoptera larvae emerging after this period would not be affected and would become a food source to insect-eating wildlife" (p. IV-18). The extent of any effects on wildlife would depend on the availability of alternate food sources during the 14 days following the treatment period. Second, non-target lepidopterans may be affected to some degree because of their susceptibility to the insecticidal qualities of *Btk*, but they would have to be actively feeding in the treatment area, and they would only be affected for a short period following treatment (p. IV-18). The effect upon non-target lepidopterans would also be dependent upon the relative size of the sprayed areas compared to the range and flight abilities of the species in question. Predators of the target and non-target insects might also be affected due to a decrease in food supply (p. IV-19). Beneficial effects of the treatment would include foliage protection, decreased tree mortality, and control of gypsy moth populations (p. IV-17), as well as a temporary increase in food supply to fish because of an increase in dead and dying target larvae that fall into streams as a result of *Btk* infection (p. IV-20).

The general conclusions indicate that *Btk* is safe for the environment and its various components. In 18 years of *Btk* use, no scientifically documented cases of human infection have been reported in association with its use in forestry (USDA, 1989, p. D-2). Other studies, involving non-target and beneficial insects, terrestrial invertebrates, birds, small and large mammals, and aquatic fauna have shown *Btk* (and other specific varieties of *Bt*) to be relatively harmless to these organisms, and therefore extremely useful in pest control programs. Furthermore, *Bt* (variety not specified) is non-phytotoxic to over 140 species of plants (Faust and Bulla, 1982, p. 159).

Specific investigations into the safety of *Btk*, *Bti*, and other varieties present quantitative data about the toxicity of *Bt* and the potential risks of spray programs. Many laboratory and field experiments have been conducted, both before and after the ban on beta-exotoxin. An environmental impact assessment of any insecticide is a general statement or conclusion based on the quantitative data obtained

in experiments, and from past experience in the use of this insecticide. Some of the information used by the U.S. Department of Agriculture in compiling the environmental impact assessment of the gypsy moth spray program is presented below in this section on the environmental fate and impact of *Bt*. Many other experiments and reports are also included in an effort to present all available information regarding the safety of *Bt*.

## A) PERSISTENCE OF *BACILLUS THURINGIENSIS*

Knowledge of the persistence of natural entomopathogens is important from both an environmental and an economical point of view. Economically speaking, those concerned with the spraying of natural insecticides such as *Btk* prefer more persistent formulations to achieve the most efficient and inexpensive control of target pests. The environmentalists prefer that insecticides released into our environment be short-lived and present the least hazard to non-target flora and fauna. The persistence of *Bt* in water, soil, and on foliage, therefore, provides useful information about the efficacy and safety of this insecticide to all organisms that may come in contact with it.

Laboratory tests on the persistence of *Bt* generally indicate that both the spores and crystals are susceptible to degradation in natural sunlight and artificial light sources; therefore, insecticidal activity is limited by weathering factors. Morris (1983a) demonstrated that complete inactivation of *Btk* spores (Thuricide 16B and Dipel 36B) occurred when exposed to sunlight in the 300-400 nm range, but no spores were inactivated when exposed to the 400-700 nm range (p. 1216, 1218). He also showed that spore viability decreased much more rapidly and dramatically than insecticidal activity in the presence of UV radiation (95% loss of spore viability but only a 50% loss of insecticidal activity), suggesting that any insecticidal contribution by the spores occurs only initially and is soon lost as exposure to sunlight increases (p. 1219). Lengths of exposure periods were not given for either of the experiments.

Similarly, Panbangred *et al.* (1979) investigated the toxicity of several varieties of *Bt*, including *Btk* (Dipel HD-1 serotype, from Abbott Laboratories), to *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) and discovered that gamma irradiation renders the spores inviable but leaves the crystal proteins intact (p. 343-346). Thus, spore viability is lost or decreased, but insecticidal activity may persist.

Conversely, Pozsgay *et al.* (1987) report that exposure of purified *Btk* crystals to either natural sunlight or to light from a solar simulator causes a loss of toxicity (p. 251). Dried and purified crystals of *Btk* were exposed to light from a sunlight simulator that "delivers light which, in terms of both power and spectral distribution, is identical to that from sunlight at the earth's surface" (p. 247). Toxicity of the irradiated crystals was assayed against fifth-instar silkworm larvae, *Bombyx mori*, to determine $LD_{50}$ values (p. 248). When compared with control samples which were given a potency ratio of 1, crystal potency was reduced to 0.30, 0.03, and 0.0 after exposure to 10, 20, and 40 megaJoules of light/$m^2$, respectively. This corresponds to 4, 8, and 16 hours of exposure, respectively. When the crystals were placed in natural sunlight, the potency ratio was reduced to 0.37, 0.1, and 0.0 after exposure to 7, 26, and 55 megaJoules of light/$m^2$, respectively (p. 251). The length of time for these exposure periods cannot be estimated because the intensity of natural sunlight in this experiment was not provided. The experiment was conducted in Sault Ste. Marie, but no information was given as to the time of year the experiments were conducted, nor the intensity of sunlight on test days. It is only stated that the crystals were exposed only during periods of bright sunlight, not under hazy or misty conditions (p. 248).

Purified crystals of *Btk* (serotypes HD-1 and HD-73) were subject to significant degradation when exposed to light in the 300 to 380 nm range for a 24-hour period (Pusztai *et al.*, 1991, p. 43). This degradation resulted in a loss of toxicity of the crystals when assayed against silkworm larvae (p. 44). The $LD_{50}$ of HD-1 crystals was increased from 0.15 g *Btk*/larva for control (non-irradiated) crystals, to 2.55 g *Btk*/larva when crystals were irradiated at 350 nm (p. 45). Above 380 nm, the inactivation by sunlight was minimal (p. 45). A similar loss of toxicity was observed for the HD-73 serotype (p. 46).

Cohen *et al.* (1991) showed that *Btk* (whole product containing both spores and crystals) was almost completely inactivated after 12 hours of exposure to artificial light in the 300-350 nm range (p. 343-344). Toxicity loss was measured as efficacy against *Heliothis armigera* Hübner (Lepidoptera: Noctuidae). Mortality of the insects dropped to less than 40% after only 6 hours of exposure to irradiation (p. 345-346).

In a report on the efficacy of Dipel 12L, a concentrated oil formulation of *Btk* containing 25.4 BIU/L, and Dipel 16L, a *Btk* formulation containing 33.8 BIU/L, suggests that efficacy of these products may be affected more by initial deposit than by the elements of nature (Nigam and Holmes, 1990, p. 25-26). In 1988, plots were treated with 15 and 30 BIU/ha of Dipel 16L in 0.55 and 1.1 L/ha, respectively, and efficacy and spray deposit were analyzed (p. 24). In 1989, plots were treated with Dipel 16L in

0.4 L/ha once to obtain a volume of 15 BIU/ha, and a second time to obtain a volume of 30 BIU/ha (p. 24). In 1989, Dipel 16L had "sufficient" residual toxicity lasting up to 5 days after application (p. 25). Nigam and Holmes (1990) did not define what "sufficient" meant - presumably it means sufficient to cause mortality in the bioassay. The authors considered the low efficacy of the Dipel 12L to be "more a function of lower deposit than of the effects of weather elements" (rain) (p. 25). Higher deposits render the insecticide effective for longer periods of time (p. 26). The authors also suggest that temperature, relative humidity, and radiation do not affect the residual toxicity to a large extent, but instead may simply modify the efficacy (p. 26). These results are contradictory to those of Morris (1983a), Panbangred *et al.* (1979), and Pozsgay *et al.* (1987). Quantitative data to support the conclusions were not provided in the report by Nigam and Holmes (1990).

In spite of these conflicting conclusions, it is generally accepted that the decline in insecticidal activity of *Btk* in the field is accelerated by UV radiation, heat, humidity and precipitation, and enzymatic activity on the surface of the foliage, as well as microbial degradation, pH, and adsorbance to sediment particles in soils and water bodies (Saleh *et al.*, 1970, p. 677; Ohana *et al.*, 1987, p. 828).

### 1) Persistence on Foliage

Persistence of *Btk* on foliage is affected by exposure to sunlight and the effects of rain run-off. van Frankenhuyzen and Nystrom (1989) tested the residual toxicity of Thuricide 48LV (*Btk*, supplied by Sandoz Inc.) after aerial application on a 60-ha plantation of white spruce trees in Ontario. Thuricide was applied at 30 BIU in 2.4 L/ha. Efficacy was tested by collecting foliage from the spray area at 2 hours, and then 2, 4, 6, and 10 days after treatment. Spruce budworm larvae from a colony at the Forest Pest Management Institute of Forestry Canada in Sault Ste. Marie were put on the foliage for 14 days and mortality was assessed (p. 868). Originally, 50% mortality of spruce budworm was achieved within 2 hours of treatment, but mortality dropped to a level similar to that of organisms on untreated foliage within 2 days. Persistence of Thuricide was, therefore, less than 2 days in this particular investigation (p. 870). The day following treatments was sunny, and the day after that was partly sunny with an accumulation of 6.8 mm rain in 5 hours. This "wash-off" by rain may have been largely responsible for the reduced activity, whereas inactivation by sunlight seems to play a relatively minor role according to the authors (15% loss of activity on treated trees exposed only to sunlight and not to rain, while those exposed to 6 mm rain dropped 50% in efficacy) (p. 870).

Heavy dew on *Btk*-treated foliage apparently does not decrease efficacy to the extent that rain does, although this has not been proven in the field (van Frankenhuyzen, 1987, p. 956). Simulated dew misted onto foliage treated with Thuricide 48LV (8.4 BIU/L) did not affect efficacy of *Btk* towards spruce budworm larvae placed on the foliage and monitored for 14 days, as compared with treated foliage that was not misted (p. 955). A red dye additive in the *Btk* formulation showed that some disintegration of the droplets had occurred as a result of the simulated dew, which presumably resulted in a slight dilution of the insecticide. However, this apparently did not affect efficacy. Natural field conditions likely to be encountered were not explored in this experiment, namely wash-off by rain, and the loss of *Btk* as a result of water droplets being shaken from the trees by wind (p. 956).

Reardon and Haissig (1984) reported on a CAN-USA-sponsored field test comparing *Btk* formulations of Thuricide 16B and Dipel 4L applied against eastern spruce budworm, *Choristoneura fumiferana*, using 20 BIU in 9.35 L/ha on balsam fir and white spruce trees (p. 153). Results showed that persistence decreased with time: no Dipel was detected on white spruce foliage 4 days after spraying, but it lasted 16 days on balsam fir; Thuricide was detectable up to 8 days after treatment on both white spruce and balsam fir (p. 155-156). Furthermore, *Btk* crystalline proteins were detected 6 hours and 2 days after treatment in larvae infected with Thuricide (collected from white spruce). Similar quantities of Dipel crystals were not detected in the larvae (p. 156). Although actual numbers are not given, the authors report that approximately 1 year after treatment, viable *Btk* endospores were recovered from white spruce branch samples that received either Dipel or Thuricide treatment in 1981, although the quantity of spores detected on the foliage is not given (p. 157).

The persistence of various formulations of *Btk* on oak trees in Northern California ranged between 3.9 and 22.1 days following treatment of several areas for California oakworm, *Phryganidia californica* Packard (Lepidoptera: Dioptidae) (Pinnock *et al.*, 1971, p. 409). Biotrol Dustable BTB 183 2.5 D persisted the longest on foliage, while Biotrol Wettable BTB 183 25 W and Thuricide 90TS 950-T persisted between 3.9 and 7.7 days (p. 409). The authors conclude that the spores of *Bt* are subject to continual degradation and mortality in the field (p. 406).

The persistence of *Btk* (Dipel 4L, Abbott Laboratories) on tomato foliage was less than 48 hours when monitored for efficacy against the tomato fruitworm, *Helicoverpa* (= *Heliothis*) *zea* (Boddie) (Lepidoptera: Noctuidae) (Walgenbach *et al.*, 1991, p. 978). Tomato plants were treated with *Btk* at 2.4 L/ha, and leaf tissue was collected at various intervals following treatment to determine the rate of degradation (p. 979). Although larval mortality was 94.4% one day after treatment, mortality declined significantly thereafter indicating a rapid loss in toxicity on the foliage of the plants (p. 982-983).

Beegle *et al.* (1981) report an insecticidal activity half-life for *Btk* (HD-1 serotype) of 1.5 - 2 days, after 2 years of testing the persistence of Dipel (16,000 IU)

**Table 5:**
**Density of colony forming units in *Btk* formulations applied against spruce budworm in Quebec in 1984.[1]**

| Product Used | Dosage applied (BIU/ha) | Volume applied (L/ha) | Colony forming units (c.f.u.x$10^5$/cm) |
|---|---|---|---|
| Thuricide 32LV | 20 | 4.68 | 5.5 |
| Thuricide 48LV | 30 | 2.37 | 4.3 |
| SAN 415 | 30 | 3.55 | 5.9 |

[1] From Cardinal and Marotte, 1987, p.5.

applied to cotton foliage at various concentrations (p. 400-401). Salama *et al.* (1983) report a half-life for *Btk* spores (dosage not given) sprayed on cotton foliage in Egypt ranging between 3.5 and 8.5 days in June and August, respectively. An obvious decrease in spore viability occurred after one day of environmental exposure. UV radiation appears to be the major factor decreasing spore viability (p. 324-325). Dimond and Morris (1984, p. 109) reported that *Btk* remains viable on foliage for 3 - 7 days, and that the major factor responsible for this is sunlight. Morris (1976) documented a decrease in mortality of spruce budworm from 41% 5 days after treatment, to 10% 15 days following treatment with formulations of *Btk* (Dipel WP and Thuricide 16B) (p. 230). A minimal amount of activity persisted up to 42 days after treatment, although this accounted for only 4.2% mortality of budworm (p. 232).

Three preparations of *Bt*, namely Dipel (*Btk*), Bactimos (*Bti*) and Bactucide (*Bti*), each at 16,000 IU potency/mg, were shown to persist for only 8 days on foliage of deciduous and coniferous trees (Niccoli and Pelagatti, 1986, p. 337). The insecticides were sprayed on trees of *Quercus cerris* L., *Crataegus oxyacantha* L., and *Pinus nigra* Arnold., in 1984, and the foliage was analyzed for viable spores immediately following treatment, as well as, 3 and 8 days later. Spore viability was shown to diminish sharply in 8 days following the spray, although persistence was slightly greater for Bactucide (length of time not specified) (p. 337).

In spite of a such short residual periods, carry-over effects of *Btk* have been documented for up to 2 years after treatment. Dipel 36B (Abbott Laboratories), sprayed at 20 BIU/ha to control spruce budworm, resulted in foliage protection and decreased egg-laying by the pest in the year following treatment (Morris, 1977b, p. 1239). Larval population densities were twice as high in the control plot than in the treatment plots two years after treatment (p. 1239). Dimond and Spies (1981) also report that *Btk* sprayed at 8 BIU in 5.83 L/ha provided good control of the spruce budworm in Maine, both in the year of treatment and one year later (p. 661). Protection in the year after treatment equaled that in the year of treatment, even though the area had a very dense budworm population. Mean defoliation in 1979 (treatment year) was 57% for all the *Btk* treatments combined compared to 100% defoliation in the control plots. In 1980, defoliation was 49% in the treatment plots and 100% in the controls (p. 662). Shepherd *et al.* (1982) have also shown that *Btk* may have a significant impact on western spruce budworm populations in treated plots up to one year after treatment (p. 282).

Observed carry-over effects in the field may be related to the mode of action of *Btk* upon some insects whereby developing larvae ingest a sublethal dose that retards their development, reduces their ability to reproduce, and hence diminishes their survival in the following season (Dimond and Morris, 1984, p. 105; van Frankenhuyzen and Nystrom, 1987, p. 941; Retnakaran *et al.*, 1983, p. 233; Fast and Regniere, 1984, p. 123; Dimond and Spies, 1981, p. 662; Yang *et al.*, 1985, p. 19). *Bti* has also been shown to cause delayed mortality among mosquitoes kept in a *Bti*-treated environment, and to cause morphogenic anomalies in treated larvae and pupae (Mulla and Singh, 1991, p. 420). In some instances, predators, parasites and other natural causes may contribute to the decline of the target insect population (which may be cyclic) in years following treatments (Reardon and Haissig, 1983, p. 1142). Reardon and Haissig (1983) investigated the persistence of *Btk* spores 1 year after treatment in Wisconsin balsam fir plots sprayed with Dipel 4L and Thuricide 16B (20 BIU/ha in 9.35 L/ha) for spruce budworm control. They reported that although viable *Btk* spores were detectable in small amounts on foliage, the numbers were insufficient to cause pathogenicity to spruce budworm. Spores were recovered from only 5 trees in a total of 12 plots, 20 ha each, in which 10 three-tree clusters were randomly selected from each plot for foliage samples (p. 1142). Morris (1976) also reports that dead spruce budworm larvae collected from balsam fir plots 1 year after treatment with *Btk* did not contain any spores (p. 232). Thuricide 16B (4 BIU/qt) and Dipel WP (16,000 IU/mg) gave variable results in budworm control in the treatment year, but larval densities were lower in all treated plots than in the control plot the following year. Absence of *Btk* in budworm cadavers indicates other

factors involved in reducing the numbers, such as parasites and post-larval effects (reduced pupal weight and reduced fecundity) (p. 232). Therefore, the benefits of *Btk* in years following treatment are more likely to be due to reduced vigour and fecundity of the surviving insects than to the persistence of sufficient amount of the bacterium to cause mortality the year following treatment. Several authors documented this reduced longevity and fecundity of surviving adult insects as the added beneficial results of *Btk* treatment of larvae (Ali and Watson, 1982; Potter *et al.*, 1982; Salama and Zaki, 1986; Talukder *et al.*, 1989).

### 2) Persistence in Water

Several studies have been conducted on the persistance of *Btk* in the aquatic environment following the aerial application of *Btk* against the eastern spruce budworm, *Choristoneura fumiferana*, in Quebec from 1984 to 1986. Thuricide 32LV, Thuricide 48LV, Futura, Dipel 132 and San 415 were applied to several areas in eastern Quebec in 1984, Futura, Novabac 3 and Thuricide 48LV in 1985, and Thuricide 48LV alone in 1986 (Dostie *et al.*, 1986a, p. 3; Cimon and Marotte, 1987, p. 3; Dostie *et al.*, 1988b, p. 2). The monitoring which was performed at this time followed the procedure outlined in Cardinal and Marotte (1987). The presence of *Btk* was determined by measuring the number of colony forming units (c.f.u.) in a given sample. One c.f.u. represents the presence of a viable *Btk* colony in a culture plate produced by 1 or more vegetative cells or the germination of 1 or more viable spores. Concentrations of c.f.u.'s for each of the treatments applied to Quebec forests in 1984 is given in Table 5. No attempt was made to examine the ecological impact of these applications. The effect that these applications may have on the aquatic environment may be surmised from information in the section on "Effects on Aquatic Fauna".

Dostie *et al.* (1988b) examined the temporal and spatial distribution of *Btk* spores in lotic (running water) environments. Sterile plastic bottles were lowered into streams, one near each of the stream banks and two at the centre of the stream to determine the spatial distribution of the bacterium in the water (p. 5). These samples were taken 20, 30, 40, 60, 90 and 120 minutes following the application of Thuricide 48LV at 30 BIU/ha at a volume of 2.37 L/ha to the area. Five temporal samples were taken over 4-minute periods at 23, 33, 43, 53 63 and 93 minutes following the termination of *Btk* application (p. 6). The results indicated that single samples were as relevant in determining *Btk* concentrations as were multiple samples (p. 8). Concentrations of *Btk* ranged between 16 x $10^4$ and 160 x $10^4$ c.f.u./L for the spatial collections for both single and multiple samples. These data also indicated that the *Btk* was evenly distributed throughout the stream immediately following the application and throughout the sampling period. Temporally, the maximum concentrations of *Btk* in the streams were recorded from 23 to 67 minutes following the application (p. 8, 11). Dispersal of the bacterium occurred rapidly shortly thereafter (p. 14).

In 1984, concentrations up to a maximum of 500 x $10^4$ c.f.u./L (colony forming units) of *Btk* were found in water samples collected from streams in treated areas immediately after aerial application of Futura at 30 BIU/ha (Dugal *et al.*, 1985, iii). These concentrations decreased rapidly soon after the application. In 1985, the maximum concentration of *Btk* in samples collected after treatment from streams in treated areas occurred between 20 and 120 minutes following application and peaked between 58 x $10^4$ c.f.u./L and 860 x $10^4$ c.f.u./L (Delisle *et al.*, 1986b). After 11-50 days, concentrations of *Btk* had decreased to between 0.22 x $10^4$ c.f.u./L and 4.6 x $10^4$ c.f.u./L.

Sampling stations, located outside areas treated with *Btk*, monitored 9 rivers which had regions of their drainage basins treated in 1985. Water samples were collected before and immediately following the application of *Btk* to a portion of the 9 river basins. Samples collected prior to the application of *Btk* indicated that 8 of the 9 rivers contained residual amounts of the bacterium from the 1984 application of *Btk*, ranging from 0.015 c.f.u./L to 0.086 c.f.u./L (Dostie *et al.*, 1986b, p. 23). The persistance of low levels of *Btk* from the 1984 application (less than 1 x $10^4$ c.f.u./L) in streams and rivers is suspected to be caused by leaching of spores from forest soils (Dugal *et al*, 1985, iii). Maximum numbers of colony forming units were recorded between 8 and 127 hours after the applications, and maximum concentrations of *Btk* ranged from 18 x $10^4$ to 250 x $10^4$ c.f.u./L (Dostie *et al.*, 1986b, p. 22). With one exception, *Btk* levels decreased to less than 1 x $10^4$ c.f.u./L after 15 days (p. 24).

In 1986, the persistance of *Btk* was examined in streams and rivers located inside and outside the treated areas, respectively. The 20 streams and 9 rivers which were examined in 1985 were used for this study (Dostie *et al.*, 1988c, p. 4). Water samples were collected 11 and 23 months after the date of the 1985 application. Concentration of *Btk* in all but 1 of the samples, regardless of whether the sample was collected inside or ouside the treated area, were less than 1 x $10^4$ c.f.u./L (Dostie *et al.*, 1988a; Dostie *et al.*, 1988c, p. 11).

A study of 33 lakes and ponds (14 of which were in the treated areas) was conducted in Quebec in 1985. Samples collected prior to the application of *Btk* indicated residual levels of *Btk* not exceeding 0.87 x $10^4$ c.f.u./L for those lakes previously sprayed, and 0.04 x $10^4$ c.f.u./L in lakes which had never been sprayed (Dostie *et al.*, 1986a, p.11). Within the treated areas, maximum concentrations of *Btk*, between 7.6 x $10^4$ and 380 x $10^4$ c.f.u./L, were recovered 2 - 3 days after application; this is comparable with results obtained the previous year (p. 11). Within 11 - 40 days after application, concentrations of *Btk* had diminished to between 0.079 x $10^4$ c.f.u./L and 8.2 x $10^4$ c.f.u./L (p. 11). Lakes out-

side the treated areas had increases in *Btk* concentrations, with maximum levels between 0.11 x $10^4$ c.f.u./L and 0.46 x $10^4$ c.f.u./L being recorded 11 - 40 days after the application, after which levels decreased to less than 0.1 x $10^4$ c.f.u./L (p. 12). All *Btk* collected in the samples were considered to be introduced to the area by the spray program, and did not reflect a natural background level (p. 21).

Delisle *et al.* (1986a) examined the persistance of *Btk* in drinking water collected from areas adjacent to areas treated with either fenitrothion or *Btk* (p. 1). Low levels of *Btk* (0.067 x $10^4$ c.f.u./L and 0.014 x $10^4$ c.f.u./L) were recorded at 2 of the 4 stations prior to the aerial application of *Btk*. After the application, *Btk* was absent from samples collected from 1 of the sample stations (which had previously recorded *Btk*), while the maximum concentration of 0.72 x $10^4$ c.f.u./L *Btk* was recorded (p. 13).

The persistence of *Btk* in water was examined in Nova Scotia by Menon and De Mestral (1985) after a field application of Thuricide 16B (4.2 BIU/$L^3$), at a rate of 4.7 to 9.4 L/ha (0.34% *Btk* in the diluted formulation) to an area containing a watershed (p. 266-267). Water and shellfish samples (oysters and clams) were analyzed for the presence of the insecticide, both before and after aerial application (p. 268). No *Btk* was detected in any of the shellfish samples in the field experiment, but *Btk* was recovered from river water samples 8 days and 13 days after treatment at two different sites (p. 269).

*Btk* persistence in 4 different types of water (deionized, tap, lake, and sea water) was also tested in the laboratory (Menon and De Mestral, 1985, p. 268-269). Each of the tested waters (2,000 mL) was filter sterilized through a 0.2 m membrane filter before being seeded with approximately $10^4$ *Btk* viable cells (isolated from Thuricide 16B) (p. 268). The inoculated flasks of water were incubated at 20°C in the dark under aeration, and samples were drawn daily for up to 70 days (p. 268). Survival of *Btk* in the 4 types of water indicated that it was broken down more quickly in sea water than in fresh water. A 90% reduction of *Btk* in sea water was observed in 30 days while a 50% reduction in distilled and tap waters was evident after 20 days. In lake water, a 50% reduction of *Btk* was achieved in approximately 50 days, its stability most likely being increased by greater nutrient availability which enhances bacterial growth (p. 271-272). However, two major decomposition factors (UV radiation and microbial degradation) were absent during the tests, suggesting that the reported levels are not likely to occur in the field. Furthermore, the pH levels of the 4 types of water tested were not provided in the report.

### 3) Persistence in Soils

Soil is the natural habitat of *Bt*. *Bt* (several varieties) has been isolated from soils in areas previously untreated with the insecticide (DeLucca *et al.*, 1981, p. 866), and *Bt* has been shown to persist for several months when soils were inoculated with *Bt* spores (Harper, 1974, p. 5). Several reports indicate that persistence of *Bt* in soils is directly related to pH. Acidic soils render *Bt* spores incapable of germination, while more alkaline soils actually allow *Bt* to complete its life cycle and multiply.

Cardinal and Marotte (1987) examined the persistance of *Btk* in forest soils during the aerial application against *Choristoneura fumiferana* in Quebec in 1984. Thirteen sample sites were chosen, and three 1m x 3m rectangular sample plots laid out in a triangular pattern (p. 7). Two soil samples were collected from each of the sample plots, each sample measuring 5.2 cm in diameter and 2 - 3 cm deep. The highest concentrations of *Btk* were recovered 1 and 2 days after the application, the maximum being 380 x $10^3$ c.f.u./cm2. Of the remainder, 23% of the samples had less than 50 x $10^3$ c.f.u./$cm^2$. Concentration of the colony forming units 2 - 3 months after the application averaged between 9.54 x $10^3$ c.f.u./cm2 and 50 x $10^3$ c.f.u./$cm^2$ for 48% of the samples (p. 15). After 11 months, *Btk* was still present in the soil, and in 85% of the samples the concentration of *Btk* exceeded pre-application levels (p. 17). It was concluded that continued application of *Btk* could, given several consectutive years of applications, produce increases in the quantity of *Btk* present in the forest soils (p. 29). Cardinal and Marotte (1987) gave no indication of the possible effects which this might have on soil ecology; information concerning the possible impacts that persistance might have on soil ecology may be obtained from the section "Effects on Earthworms".

Several experiments conducted in Japan using soils from mulberry plantations have shown specifically that *Btk* cannot grow in acidic soils (Akiba *et al.*, 1980, p. 13). The authors found that optimal growth of *Btk* occurred in soils ranging from pH 5.5 to pH 8.5, while growth did not occur in soils ranging from pH 4.0 to pH 5.4 (Akiba *et al.*, 1979, p. 220; Akiba *et al.*, 1980, p. 13). Total soil carbon and nitrogen content does not affect *Btk* growth (Akiba *et al.*, 1980, p. 13). Further tests indicated that the spore is the only state in which the *Bt* organisms are able to exist in natural soils (Akiba, 1986a, p. 76). *Bt* varieties *morrisoni* and *thuringiensis* were able to germinate and grow in sterile soils. However, the same species were unable to germinate in nonsterilized natural soils. Vegetative cells rapidly disappeared within 2 days from unsterilized soils, but were able to form spores in the natural soil (p. 76).

West *et al.* (1984b) have also shown that *Bt* does not grow vegetatively under most natural soil conditions. In soils of pH 5.2, 91% of viable *Btk* vegetative cells were degraded in 24 hours as a result of autolysis, while crystals and spores persisted for much longer (p. 151-153). Less than 2% of the crystals were present after 65 days, while numbers of spores remained unchanged. However, no germination of spores occurred in the soil throughout the 3-month testing period (p. 153) indicating that *Bt* is not well adapted to a soil environment, but requires the alkaline conditions available in an insect body to germinate and flourish.

### 4) Persistence of Other Varieties of *Bt*

The persistence of *Bti* in water environments has been tested both in laboratory and field experiments, and results indicate low residual activity of spores due to several environmental factors. Rapid reduction in larvicidal efficacy of *Bti* in waters following field applications has been attributed to adsorption of *Bti*, possibly spores or crystals or both, onto mud particles (Ohana *et al.*, 1987, p. 828-830). Almost 100% of *Bti* sprayed in a fresh water habitat adsorbed onto mud particles within 45 minutes of application and remained viable for up to 22 days in a simulated field situation (p. 828). The spores, however, were unable to germinate in mud conditions. Larvicidal activity was regained when the settled mud particles were vigourously stirred; this released the trapped insecticide and resulted in 100% mortality of *Aedes aegypti* larvae (p. 830). Dupont and Boisvert (1986) indicate similar results using *Bti* (Teknar, supplied by Sandoz Co.), except that the toxicity of *Bti* increased after 43-69 days, and continued for up to 174 days, suggesting that sporulation of *Bti* had occurred in the stream environment (p. 435-436).

Recovered spore counts from a stream environment following application of *Bti* (400-600 ITU/mg, Abbott Laboratories) lend support to the findings of Ohana *et al.* (1987) (Frommer *et al.*, 1981, p. 331). *Bti* sprayed at 3.10 ppm across a stream (124,000 spores/mL) for 35 minutes was recovered in minimal amounts at four sites 37, 91, 152, and 312 m downstream from the treatment site (p. 331). At 37 m downstream, the spore count fell to less than 1.0 ppm after 50 minutes; reduction rates were more dramatic at sites farther down from the treatment site (p. 333-336). Some of the losses of *Bti* can be attributed to settling and attachment of particles to substrates, and dilution of the spore suspension (p. 337).

The persistence of *Bti* in a water environment was examined in a microcosm laboratory test, using a simulated and self-sustaining biological model of an autotrophic pond (Shannon *et al.*, 1989, p. 226). Communities were composed of bacteria, fungi, protozoa, algae, and herbivorous zooplankton and insects (cladocerans, copepods, ostracods, amphipods, and chironomids) in a 1-litre beaker containing 50 mL of sterile soil (p. 226). Vectobac (dosage not given, Abbott Laboratories) and Mosquito Attack (dosage not given, Reuters Laboratories) were added to different microcosms at concentrations of $10^3$, $10^4$, and $10^6$ spores/mL (p. 228-229). Concentrations of *Bti* in the water were maintained over a 6-week period, but little or no germination occurred in the microcosms (p. 230). Potency of *Bti* was maintained for at least 7 days, but it did not last for more than 14 days, as shown in potency bioassays against *Aedes aegypti* larvae (p. 230). Possible reasons for the decline in efficacy are inactivation by UV light, and adsorption of *Bti* onto soil particles; however, potency was not regained upon stirring of the sediments, as shown by Ohana *et al.* (1987) (Shannon *et al.*, 1989, p. 237).

Other reports also indicate a short residual life of *Bti* in water. Balaraman *et al.* (1983a) report a dramatic decrease in efficacy of a cultured population of *Bti* (serotype H-14) after only 9 days in water (p. 36). Efficacy was determined in bioassays against the dipterans *Culex quinquefasciatus* Say and *C. tritaeniorhynchus* (Culicidae), both at 56.6 ITU/250 mL water, and *Anopheles stephensi* (Culicidae), at 226.5 ITU/250 mL water (p. 33-34). The cultured *Bti* was mixed into six formulations using different additives (additive names not provided) (p. 33), one of which lost potency after only 1 day. Five of the six formulations provided less than 30% mortality after 6 days (p. 36). The authors related mosquito larvicidal efficacy to the amount of spore-crystal complex that remains suspended in the top layers of the water column; i.e., the higher the amount of suspended *Bti* in the water column, the greater the efficacy (p. 36).

In California, insecticidal activity of *Bti* was nil 24 hours after treatment in pasture plots experiencing flood-irrigation outbreaks of the culicids *Aedes melanimon* Dyar, *A. nigromaculis* (Ludlow), and *Culex tarsalis* Coquillet (Mulligan *et al.*, 1980, p. 687). Even at the highest dosage of 2.50 kg/ha of *Bti* (700 ITU/mg, Sandoz Inc.), applied by hand sprayer, efficacy fell from 100% mortality of mosquito larvae to 0% in 24 hours; at 1.0 kg/ha, efficacy fell from 98% 4 hours after treatment to 5% at 24 hours (p. 687). In duck ponds, following an aerial application of *Bti* at 1.12 kg/ha, bioassays of treated water yielded 100% mortality of *C. tarsalis* after 2 hours, but no activity after 24 and 48 hours (p. 687). City catch basins in Fresno County were also monitored following applications of *Bti* at 0.1, 1.0, 10, and 100 ppm (p. 685). Mortality of *C. quinquefasciatus* populations was 100% at all levels except 0.1 ppm (87% mortality) immediately following treatment (p. 686). Bioassays showed that, for the highest dosage, efficacy had decreased to 32% by 7 days after treatment, and then to 6% at 14 days. At 10 ppm, efficacy was 5% at 7 days after treatment (p. 687). Results indicated that *Bti* applied at concentrations yielding 98-100% control of pestiferous mosquito larvae do not persist in fresh water habitats for longer than 7 days (p. 687).

The persistence of other varieties of *Bt* has been studied by Cantwell and Franklin (1966) who tested the persistence of *Bacillus thuringiensis* var. *thuringiensis* spores exposed to various sources of light during a test period between August 3 and September 7, 1965 in Beltsville, Maryland (p. 256). Three-week-old spores were placed in water, and then 100 mL aliquots were drawn through Millipore filters (0.45 µm pore size), such that the spores remained on the filters. Filters placed outside under sunlight were not dried (p. 256). After 30 minutes of exposure to direct natural sunlight, the spore count was reduced from 171 to 85, a loss of 50% of the spores. After 60 minutes of exposure, the spores

were reduced by 82%, from 326 spores to 58 (p. 257-258).

In a similar test, Cantwell (1967) investigated the effects of exposure to UV light in the laboratory on the crystals of *B. thuringiensis* var. *sotto*, the variety toxic to silkworm, *Bombyx mori*. The aqueous suspensions of crystals were dried on glass slides and exposed to a UV lamp for 0, 7.5, 15, 30, 60, and 120 minutes (p. 138). Results showed that *Bt* exposed to UV from 0 - 120 minutes still retained 90% of its efficacy (i.e., 90% mortality of third-instar silkworm larvae; less than 10% of control larvae were lost). In general, the author concluded that "prolonged exposure will apparently not alter the toxicity of the parasporal body of *B. thuringiensis* var. *sotto*" (p. 139-140). The results suggest that although the spore may be degraded by UV radiation, the crystals on the other hand do not lose insecticidal quality as readily as the spores.

In a report by Saleh *et al.* (1970) optimum growth of *Bt* var. *thuringiensis* in soil (Thuricide 90T, containing 4 x $10^{10}$ spores/mL) occurred at a pH of 6.4-6.7, while no growth occurred at a pH of 4.4 (p. 678). In sterile soils, spores remained viable for up to 64 days but showed no signs of germination, therefore the effect of pH is most likely one of inhibition of spore germination rather than a reduction of viability (p. 678). Data suggest that *Bt* spores can remain viable for long periods of time in soils, but will not germinate and complete their lifecycle unless exposed to the appropriate soil pH (alkaline) (p. 680).

Viable spore counts of *Bt* var. *galleriae* (Thuricide 90TS Flowable, International Minerals and Chemical Corporation) in clay loam soil of pH 6.8 were shown to decrease by 76%, while pathogenicity decreased by more than 99% over a 135-day period (Pruett *et al.*, 1980, p. 168). Potency of spores recovered from inoculated soil decreased by more than 50% after only 7 days when tested against larvae of the greater wax moth, *Galleria mellonella* (p. 172). Results suggest that the crystals of *Bt* are degraded more rapidly than the spores in a soil environment (p. 173).

The persistence of *Bt* var. *aizawai* (beta-exotoxin free) was shown to be related to the presence of indigenous soil microorganisms (West *et al.*, 1984a, p. 128). Both natural and autoclaved samples of clay loam soil (ph 5.0) were inoculated with spores of *Bt* var. *aizawai*. This pH level of 5.0 was lower than the optimum growth range of pH 6.4-6.7 for *Btt* as reported by Saleh *et al.*, 1970 (p. 678). No germination was detected in either soil sample over the test period, but mortality of *Bta* spores was 2.85 times greater in the natural than in the autoclaved soil sample (West *et al.*, 1984a, p. 123, 125). The combination of the low pH of the soils and the presence of natural microorganisms likely contributed to the degradation of *Bta* in the non-autoclaved soil samples (p. 126).

The results of these many tests support that *Btk* and other varieties of *Bt* may persist for up to 16 days on foliage and up to 3 months or longer in various soils. Carry-over effects have been observed 1 and 2 years following treatment in some areas, although the effects observed may not be attributable only to the presence of *Btk* in the environment. Sunlight has been suggested as the principal factor which reduces efficacy and field persistence of *Bt* sprays, and rain is an important factor when considering persistence on foliage. Spores may remain viable for long periods in soils because of acidity, which prevents germination until appropriate conditions are met. In laboratory tests, *Btk* may persist for up to 30 days in sea water and 50 days in freshwater, while *Bti* often adheres to mud particles in the field and has been shown to remain viable for 22 days following treatment.

## B) FATE IN CADAVERS

Some concern has been expressed about possible side effects of *Btk* to non-target animals, especially birds and fish, via ingestion of dead or infected larvae. When host conditions are appropriate, spore digestion leads to germination and growth of vegetative cells, and ultimately to sporulation of *Btk* within the insect (Burges, 1982, p. 83). It has been suggested that sporulation, and consequently crystal formation, occurs following the death of the host larvae (Akiba, 1986b, p. 99; Burges, 1982, p. 84). The concern arises about the toxicity of these second-generation spores and crystals to non-target animals that may feed on moribund and dead larvae. There is, however, no general agreement on the fate of *Bt* in the host cadavers and the evidence presented by various authors seems contradictory, even when they report on the same variety of *Bt*.

Injection of *Bt* spores into the haemocoel of susceptible insects reportedly results in the germination and growth of vegetative cells within the host. Sporulation ensues when nutrients within the dying host become scarce. When death from septicemia or starvation occurs, the host may contain a large amount of vegetative cells, as well as a few spores and crystals (Angus, 1968, p. 12). Ingestion of viable *Bt* spores from treated vegetation may or may not result in germination within the host because of unfavorable gut conditions. Extremely alkaline environments may inhibit germination while more neutral gut conditions may encourage this. The damage done by the crystal to the gut wall of target

insects creates an appropriate environment for cell replication, the resulting vegetative cells being responsible for complete septicemia occurring within the host (Angus, 1968, p. 12).

Akiba (1986b) has shown that orally administered *Bt* (var. *thuringiensis, aizawai, morrisoni, alesti,* and *sotto*) replicated in last-instar larval cadavers of silkworm, *Bombyx mori,* and fall webworm, *Hyphantria cunea* (p. 99). Vegetative cells increased 15 to 66 times in cadavers, and sporulation occurred within the dead insects. The author suggests that "recycling of *B. thuringiensis* in nature takes place in insect cadavers under the low density of competitive microorganisms" (p. 99).

Prasertphon *et al.* (1973) found several incidents of sporulation and crystal formation of *Btk* in insect host cadavers. Three noctuid lepidopterans - *Heliothis armigera* Hübner, *Prodenia litura* Fabricius, and *Spodoptera exigua* (Hübner) - were fed cotton leaves dipped in suspensions of Dipel (Abbott Laboratories), Thuricide (International Minerals Corporation) or Biotrol (supplied by Nutrilite Corporation), and smear preparations of the cadavers were made 1 - 2 days after the death of the insects (p. 205-206). In the Dipel experiment, 30 larvae of *H. armigera* were fed cotton leaves dipped in 1 of 3 suspensions of 0.1, 0.5 or 1.0 g Dipel/40 mL distilled water. Control larvae were fed leaves dipped in distilled water and the wetting agent (Tween 80) which was added to both the Dipel and control suspensions at a concentration of 1:2,000. All the Dipel-fed larvae died; 12 of these were examined and 7 of the 12 cadavers contained both spores and crystals (p. 205).

In a second test using Dipel at 0.5 g in 50 mL distilled water containing Tween 80 at 1:2,000, 40 larvae of each of *H. armigera* and *P. litura* were fed on cotton leaves dipped in the prepared Dipel suspension (Prasertphon *et al.*, 1973, p. 205). Of the 24 *H. armigera* cadavers examined in smear preparations, five contained spores and one contained both spores and crystals (p. 205). Four of the six examined *P. litura* cadavers contained spores and crystals (p. 205).

Trial three of the same experiment involved larvae of *S. exigua* fed leaves dipped in suspensions of either Dipel, Thuricide, or Biotrol at concentrations of 0.5 g/50 mL distilled water and the same wetting agent. Twenty larvae were used for each formulation of *Btk* (Prasertphon *et al.*, 1973, p. 205-206). Of the 10 Dipel cadavers examined, one contained only spores while six contained both spores and crystals. Of the seven Thuricide cadavers examined, three contained only spores, while none contained any crystals. Finally, of the six Biotrol cadavers examined, none of the smears contained either spores or crystals (p. 206). No information as to the fate of any of the control larvae is provided.

The results of this experiment indicate that germination of the bacterium must occur in the host, which subsequently leads to sporulation and possibly crystal formation. This idea is supported by the fact that the numbers of spores and crystals found in the cadavers were very high, they were often enclosed in sporangia, and that viable bacterial rods were also found in the smear preparations (Prasertphon *et al.*, 1973, p. 205). The data, however, do not adequately indicate whether sporulation and crystal formation occur before or after the insect dies, and this remains somewhat of a mystery. Even though natural epizootics have rarely been reported, and the general opinion is that *Btk* does not germinate in host insect cadavers, the evidence above suggests that a significant number of spores and crystals are formed in insect hosts, either prior to or following their death (p. 205-206).

In spite of the occurrence of vegetative cells, spores, and possibly crystals in infected and dead host insects following treatment with *Btk*, outbreaks of *Btk* infection are rare, and require special conditions such as a closed system (see section entitled "Distribution in Nature"). The amount of *Bt* found in natural environments and dead insects is extremely small, and the persistence of these "agents of mortality" (vegetative cells, spores and crystals); is low; thus *Bt* is unable to accumulate in soils and on vegetation to levels that could cause epizootics (Angus, 1968, p. 12; DeLucca *et al.*, 1981, p. 865-866). Angus (1968) states that vegetative cell growth can occur at low oxygen levels, but that sporulation and crystal formation require "vigourous aeration of liquid cultures" or solid media exposed to atmospheric oxygen (p. 12). These conditions are unavailable in moribund and dead insects; thus, the host insect contains only small numbers of spores and crystals following infection. The vegetative cells are even less persistant than the spores and crystals, and therefore breakdown rapidly (p. 18).

However, the toxicity of dead and moribund *Bt*-infected larvae in aquatic environments is of concern by some. A host of organisms, including certain fish and aquatic invertebrates, prey on larvae of mosquitoes and blackflies, the target insects of *Bt* var. *israelensis,* in streams and ponds. Although an alkaline gut is required for activation of *Bt,* and very few organisms possess this, it may be possible that the previously activated toxin within dead and moribund target insects could adversely affect the organisms that consume such infected insects.

Aly (1985) found that spores of *Bti* germinate and multiply within the host mosquito larvae, *Aedes aegypti* (p. 6). Vegetative cells grew and divided throughout the host body, whether the organism was living, dead, or moribund. Vegetative cells of *Bti* were detected in the esophagus of cadavers 6, 12, and 24 hours following death, but bacterial growth ceased at 24 hours (p. 3). Spore numbers decreased in both moribund and dead larvae, while a large number of vegetative cells were detected in cadavers ($3.7\text{-}4.2 \times 10^4$ cells). This decline in the number of spores in the gut of cadavers (up to 24 hours following death) and the drastic increase in number of vegetative cells indicates that the spores were

germinating into vegetative cells both in moribunds and cadavers (p. 6).

Aly *et al.* (1985) have shown that *Bti* spore counts in infected host insects of *A. aegypti* decrease from 8 x $10^3$ spores/larvae 2 hours after treatment to 2-3 x $10^2$ spores/larvae up to 4 hours after treatment, indicating germination of spores. Twenty-four hours after treatment the number of spores per cadaver increased substantially. At 48 hours, cadavers contained 5.5 x $10^3$ spores and after 72 hours, they contained 7-14 x $10^4$ spores (these would be second generation spores). No increase occurred between 72 and 120 hours (p. 253). The results indicate that the vegetative cells reproducing in the cadaver begin to sporulate as host conditions change. Thus, the mosquito larvae provide the necessary environment for vegetative growth, sporulation, and crystal production if cadavers remain intact for more than 24 hours, because sporulation does not begin in the cadaver until 24 hours have past (p. 255-257). This is in contrast to the opinion of Angus (1968) who suggested that sporulation and crystal formation were inhibited in moribund and dead insects because of low oxygen levels (p. 18). Data provided by Angus (1968), however, were likely obtained from the *thuringiensis* variety, and direct parallels between *Bti* and *Btt* cannot necessarily be drawn.

Other authors have shown that the cells of *Btk* and *Bti* do not germinate and multiply in the host cadavers of *A. aegypti* larvae (Panbangred *et al.*, 1979, p. 346; Silapanuntakul *et al.*, 1983, p. 389-390). Silapanuntakul *et al.* (1983) showed that the *Bti* did not grow in the presence of dead larval material in an experiment conducted in Bangkok. Larvae of *A. aegypti* and *C. quinquefasciatus* were kept in tap water treated with *Bti* at 1.33 x $10^4$ and 6.67 x $10^4$ spores/mL, respectively, and mortality was monitored daily for 7 days at which time the procedure was repeated. The population of *Bti* present in jars of *A. aegypti* cadavers decreased significantly from 1 x $10^4$ spores/mL to 1 x $10^3$ spores/mL over 128 days. *Bti* decreased from near 1 x $10^5$ to less than 1 x $10^3$ spores/mL over 112 days in tests using *C. quinquefasciatus* (p. 389-390). The results suggest that the bacterium was unable to germinate and grow in the dead larval tissue of these species (p. 389). Panbangred *et al.* (1979) sobtained similar results in another test performed in Bangkok; *Bti* did not germinate and multiply in *A. aegypti* larvae following treatment (p. 346).

Because of conflicting evidence, the potential exists that *Bt* may multiply in infected hosts and thereby become a possible hazard to fish, birds, and other animals that feed on moribund insect larvae, provided that the organism would have an alkaline stomach (which these do not!). Although this potential may exist, it is not very probable and there is no evidence or data to support it.

## C) EFFECTS ON HUMANS

*Bacillus thuringiensis* was first produced in the United States commercially as Thuricide in 1957 by Pacific Yeast Products Inc. It was registered with a partial exemption for use on food and forage crops in 1958, and with a full exemption in 1960. Full exemption indicates that no waiting period is required between crop treatment and harvest time. In Canada, Thuricide was registered in 1961. Biotrol-BTB, produced by Nutrilite Products Inc., was registered in 1959, and Dipel, produced by Abbott Laboratories, was registered in 1970 (Ignoffo, 1973; p. 143).

No human health problems have proven to be directly attributable to the application of *Btk* over its 35 years of use since registration. During these years, *Bt* has been used extensively on fruit and vegetable crops, including maize, broccoli, cabbage, lettuce, apple and tomato (Burges, 1980, p. 86). The U.S. Environmental Protection Agency approves the use of *Bt* products (beta-exotoxin free) on products destined for human consumption up to and including the very day that these products are harvested, as well as on stored food products (USEPA, 1984). The absence of a "waiting period" clearly indicates the safety of *Bt* to consumers. Most likely, there have been instances where spores or crystals were present on treated produce sold at grocery stores, and the consumer did not wash, or inadequately washed, the purchased goods. The absence of human infection by *Bt*, as well as the fact that alkaline conditions are required for *Bt* infection and cell multiplication (the human gut environment is acidic), confirms the safety of this pesticide towards humans (Harper, 1974, p. 4).

Several studies were conducted on human volunteers and other vertebrates in the late 1950s and early 1960s without any indication of serious human health effects. Only one incident of possible *Btk* infection has been recorded over the past 35 years - an agricultural worker suffered a corneal ulcer after accidently splashing the insecticide into his eye (Samples and Beuttner, 1983, p. 259). There have been no other human infections during this time that have been proven to be the result of *Btk* exposure.

In a series of tests to initially register *Bt* as an insecticide, Fisher and Rosner (1959) for the first time tested human volunteers using Thuricide (9 x $10^9$ spores/g, supplied by Bioferm Corporation), a product containing *Bt* var. *thuringiensis*, and therefore also containing beta-exotoxin. In spite of the presence of beta-exotoxin in the formulation, those tested showed no adverse effects resulting from ei-

ther ingestion or inhalation of *Bt* ($3 \times 10^9$ spores/g) over a 5-day period. Eighteen volunteers consumed 1 g of Thuricide daily for 5 days, and five of these volunteers also inhaled 100 mg of the powder daily for 5 days (p. 687). Complete physical examinations were conducted before and after administration of the insecticide, involving measurements of weight, height, blood pressure, respiratory rate, and pulse rate, as well as evaluations of genitourinary, gastrointestinal, cardiorespiratory and nervous systems. All volunteers remained well throughout the test period and afterward (p. 687). In addition, the 8 employees of Bioferm Inc. who are continuously exposed to *Bt* during the manufacturing process were observed over a 7-month period. No health problems arose in any of the employees, and medical examinations of these people did not reveal any adverse effects resulting from their exposure to *Bt* (p. 688). Table 6 summarizes the data of the human volunteer tests, as well as data on mammals, birds, and fish, cited by Ignoffo (1973, p. 152), but originally given by Fisher and Rosner (1959) (Table 6 is found in the section entitled "Effects on Mammals").

A study conducted in 1984 in southeastern Quebec to determine quantities of *Btk* spores present in the air following aerial spraying showed that the levels of *Btk* present in these situations were lower than amounts inhaled and ingested by the human volunteers reported by Fisher and Rosner (1959), and lower than quantities administered during many animal toxicological tests (Major *et al.*, 1985, p. 19-20). Furthermore, the human volunteers in the 1959 experiment were administered a *Bt* product containing beta-exotoxin, while varieties used today in North America *do not* contain this potentially dangerous toxin.

The levels of *Btk* in the air were monitored at two municipalities between June 11 and July 7, 1984, during the peak of the *Btk* application period for control of spruce budworm in Quebec (Major *et al.*, 1985, p. 2). *Btk* spores were collected by drawing air through a filter using a vacuum pump, and the number of spores collected/minute/L (spores-min/L) of air sampled was calculated. They found that levels of *Btk* in the air ranged between 0 and 132.59 spores-min/L (94.0 spores/$m^3$), but most measurements showed levels below 2 spores-min/L (1.4 spores/$m^3$) (p. 12-14). Insecticides used on the various blocks in the area were Thuricide 48LV, Thuricide 32LV, Futura, Dipel 88, and Dipel 132; dosages ranged between 2.34 and 5.85 L/ha (p. 5). In general, the concentration of spores present in air samples was higher following aerial treatment of a nearby area (within 20 km) with *Btk*, and the farther away that spraying was conducted, the lower the level of *Btk* in the air (p. 15-17). Factors, such as wind speed and direction, humidity, local topography, and dosage of *Btk* sprayed also affected the concentration of *Btk* in air samples (p. 18).

The important point this investigation in Quebec makes is that the highest level of *Btk* present in air samples, following nearby spraying, was determined to be 132.59 spores-min/L. Assuming that this quantity of *Btk* in the air remained constant for a day and a respiration rate of 30 L/min, the authors suggest that over a 24-hour period a person in the area could inhale a total of 4,000 spores. Over a 25-day period, this person could inhale a total of $10^5$ *Btk* spores (Major *et al.*, 1985, p. 19). In contrast, the human volunteers in Fisher and Rosner's experiment (1959) ingested 33,000 times ($3.3 \times 10^9$ spores) this number of spores daily, and inhaled 3,300 times ($3.3 \times 10^8$ spores) this number of spores daily for 5 days without experiencing any ill effects (Major *et al.*, 1985, p. 19). Workers in the treated areas in Quebec in 1985 were exposed to concentrations of $4.506 \times 10^3$ to $2.776 \times 10^5$ spores-min/L without health problems (Dugal *et al.*, 1986, iv). Furthermore, several animal toxicological studies have shown that mice and rats have ingested, inhaled, and been injected with *Bt* spores at levels between $10^7$ and $10^{12}$ for up to 14 days and not suffered any observable effects (Major *et al.*, 1985, p. 20). The authors state that "the *Bt* concentrations detected in the two municipalities monitored represent only a very minimal hazard for the populations concerned" (p. 21).

Several other tests (Major *et al.*, 1986; Dugal and Major, 1987; Dugal, 1988), very similar to the one conducted by Major *et al.* (1985) were conducted in the same area in 1985, 1986, and at other municipalities in Quebec in 1987 during peak spray periods confirmed the findings of Major *et al.* (1985). As with the first study, *Btk* levels in air samples were generally low except for a few times when levels were elevated in areas adjacent to spray regions. However, the levels of *Btk* measured in air samples taken in Quebec were lower than those experienced by human volunteers and laboratory animals in previous experiments (Fisher and Rosner, 1985), and were not considered to be hazardous to the health of those living in the areas.

There is one case when occupational exposure to *Btk* resulted an infection. An 18-year-old male agricultural worker accidentally splashed the commercial product Dipel into his right eye while working with the product (Samples and Buettner, 1983, p. 259). The affected person washed his eye with water and then applied an antibiotic ointment. Three days later a corticosteroid ointment was applied, but a corneal ulcer had developed over the next week and medical treatment was necessary. Cultures of the corneal ulcer produced *Btk* cells, the same as those in the formulation of Dipel used (p. 259). Samples and Beuttner (1983) suggest that this is the first reported case of a human infection resulting from exposure to *Bt* (p. 260). However, this incident would have been avoided had the individual worn safety glasses, as recommended on the product labels (labels for Dipel 176, Foray 48B, Biobit, and Skeetal). No other information, published or unpublished, suggests any "harmful" effects of *Btk* (or other varieties) towards humans as a result of their occupational exposure during manufacture, mixing, or

spraying of the insecticide. The absence of such information is an eloquent indication of the relative safety of *Btk* use in pest management.

Only two epidemiological studies have been conducted during aerial application of *Btk* in, around, or over human habitation. One was conducted in Oregon (Green *et al.*, 1990), the other in B.C. (Noble *et al.*, 1992) where a special effort was made to look for signs of human infection by *Btk*. Both surveillance studies were conducted by medical doctors and the inhabitants in the treated areas to identify any possible effects which *Btk* application might have on humans. As this is a major concern, these two studies will be discussed in some detail. The epidemiological study was conducted in Oregon, during two seasons of the aerial spray program (1985 and 1986) of *Btk* for control of the gypsy moth (Green *et al.*, 1990; p. 848). A total of 55 *Bt*-positive cultures were obtained from patients from three hospitals and one outpatient setting, all within the spray area. Over the 2-year period, a total of 120,000 people, living within the boundaries of the spray plot, would have come into contact with *Btk* at some time. Of the 55 cultures collected, 52 were determined to be the result of contamination. One of these cultures was collected from a spray project worker who received an accidental splash of *Btk* to his face, including the eyes; after treatment with a steroid cream he completely recovered. In the case of the remaining three cases of possible *Btk* infection, it could not be proven whether the three remaining cultures were or were not the result of an epidemiological infection by *Btk* (p. 848, 850). However, in-depth case studies of the 3 affected revealed that all three were immunocompromised as a result of some other health condition (p. 848). All three cases occurred in 1985 when the *Btk* treatment was applied from May 1 to mid-June.

The first patient was a 77-year-old male with an underlying lung disease. He developed a fever and pneumonia in July of 1985, following the first spray. Thirteen days later he died; the family refused autopsy (Green *et al.*, 1990, p. 850). *Btk* was cultured from one out of four blood samples taken from the patient, but he did not respond to antibiotics to which *Btk* is susceptible, indicating that the pneumonia was most likely caused by a different organism (p. 851).

The second patient was a 31-year-old mentally retarded female who suffered spastic hemiplegia (paralysis of one side of the body caused by a disease in the opposite hemisphere of the brain) and seizure disorder in addition to bilateral subdural hemorrhages (bleeding just below the dura mater, the thickest and outermost of the three meninges or connective tissues surrounding the brain and spinal column) (Laurence Urdang Associates, 1981, p. 125, 187, 189, 252, and 399). The conditions were the result of a car accident that occurred 10 years previously (Green *et al.*, 1990, p. 850). She was diagnosed in April of 1985 as having multiple gallstones, after complaining of chronic abdominal pain, which was relieved by surgery, after which the patient recovered "uneventfully". No *Btk* was cultured from gall bladder tissue, and only 1 of the 8 fluid specimens showed *Btk* growth after 5 days of incubation (Green *et al.*, 1990, p. 850). These observations, and the patient's lack of fever, argue against infection by *Btk* (p. 851).

The third culture was isolated from an abscess on the right forearm of a 25-year-old female who had a history of IV drug abuse (Green *et al.*, 1990, p. 850). Twenty colonies of *Btk* grew from the abscess sample taken from the injection site in June of 1985, but by July 1 the wound did not produce any *Btk* cultures (p. 850). The infection may have been caused by another organism, or the *Btk* cultured from this area could also have been a contaminant (p. 851).

Green *et al.* (1990) state that many of the complaints from the public were related to skin rashes and eye irritation, and agree that these symptoms could have been caused by the gypsy moth itself rather than by the *Btk* application. Both dermatitis and eye irritation have been documented by large numbers of people from the northeastern U.S. and has been attributed to exposure to the "hairs" of gypsy moth larvae.

Although *Btk* is considered to be non-pathogenic to humans and other animals, Green *et al.* (1990) state that in the past 30 years there has been an increase in the number of people immunocompromised, as well as a change in the perspective taken towards the pathogenicity of bacteria. "The medical community has become more reluctant to label any bacterium as absolutely non-pathogenic to humans... These [pest control] microorganisms may have potential for causing disease in immunocompromised persons. Therefore, such individuals should be advised on how to use biopesticides and how to protect themselves from undue exposure in areas where they are used" (p. 852).

All these statements are prudent, even though a few paragraphs earlier the authors concluded that "*Bacillus thuringiensis* var. *kurstaki* has a remarkable safety record in view of its wide use by gardeners, in agriculture, and for major pest eradication projects such as the one undertaken in Lane County." (p. 852 - the site of the Oregon study).

The other, much larger epidemiological study "examined the records of more than 26,000 telephone calls, 1,140 family practice patients, 3,500 admissions to hospital emergency departments, and closely monitored 120 workers with occupational exposure to *Btk* spray. In addition, the study examined more than 400 bacterial cultures that had been referred in from 10 participating laboratories. In addition the study samples of air exposed to general concentrations and occupational concentrations of *Btk*, and samples of food from a variety of times and sources." (Noble *et al.*, 1992, 'Summary' p. 1). This study was conducted by the University of British Columbia, contemporaneously with a spray program for control of the Asian and European gypsy

moths in the lower mainland and Greater Vancouver areas (Noble *et al.*, 1992). The objective of the study was to monitor any changes in health that may have occurred during or after the combined aerial and ground spray of Foray 48B. The study was multi-faceted, including food sampling studies during and after spraying, monitoring of health effects on workers who experience occupational exposure to *Btk*, monitoring the frequency of visits to physicians offices and hospital emergency wards during and after the spray, and examination of isolates of *Btk* collected from patients visiting hospitals and physicians during the test period ('Background' p. 2). The results of this study indicate that the large-scale spray program of *Btk* in the lower mainland for control of the Asian and European gypsy moth did not cause any "measurable increase in serious community unwellness that could be attributed to the spray"('Summary' p. 3).

The food sampling studies showed that *Btk* was repeatedly cultured from commercially available vegetables during and after the spray program; therefore, the general public was "readily exposed to sources of *Btk* other than either the aerial or ground sprays" (Noble *et al.*, 1992, 'Conclusions' p. 1). In spite of this, the general health of individuals living in the spray area and exposed to such produce was not adversely affected by this insecticide. Among individuals who attended physician's offices throughout the lower mainland, complaints of respiratory and eye symptoms were not more frequent among those living within the spray zone than those living outside of the spray zone. Also, complaints of such symptoms were not more frequent among individuals who had evidence of having been directly exposed to the insecticide. "While symptoms may have been attributable to the spray, it is not possible to distinquish these from the identical complaints that regularly occur during spring due to environmental factors such as dust and pollen" ('Summary', p. 1). Furthermore "There was no evidence to suggest that the number of visits, and reasons for visits, to emergency departments were different as a result of the spray program ('Summary', p. 1 and 2).

"Concern that HIV-positive people or individuals with other immunosuppressive diseases may be vulnerable to infection by *Btk* was considered and examined." ('Summary', p. 2). Although the bacterium was recovered from a broad range of body sites (blood, body fluids and tissues), the authors "... were unable to find a single case where Btk was a pathogen causing infection" ('Summary', p. 2). Examination of all significant cultures collected during the test period showed that there were no cases of infection in immunosuppressed persons (including immunosuppressed HIV-positive patients) as a result of exposure to *Btk* (Noble *et al.*, 1992, 'Summary' p. 2).

The results of the study on workers exposed to the bacterium during the actual spraying of *Btk* indicated that symptoms of eye, nose, and throat irritation, dry skin, and chapped lips were more common among those exposed to *Btk* compared to the control workers (Noble *et al.*, 1992, 'Summary' p. 2). These symptoms were most prevalent among workers who had a previous history of allergies. However, there were no days of work loss attributable to *Btk* exposure in spite of the fact that the ground spray workers were exposed to *Btk* at rates as high as 500 times that which the general public would have encountered ('Summary', p. 2).

Perhaps the extensive microbiological and epidemiological surveillance study conducted by Noble *et al.* (1992) during the combined aerial and ground spray of Foray 48B, the bioinsecticide containing *Btk*, can be best summarized by the lines of their report: "Nonetheless it can be said with confidence that no cases of infection were detected in any hospitalized patients, or in any patients studied with bacterial cultures. Finally, we found no evidence by examining individuals attending emergency departments that there was any measurable increase in serious community unwellness that could be attributed to the spray" ('Summary' p. 3).

Another concern expressed by some is the effect of *Btk* spray on honey bees, honey, and subsequently on people. This may originate from a paper by Hoover (1987). A brief article published in a Pennsylvania pest management periodical outlined a possible case of human illness in Missouri resulting from ingestion of honey (Hoover, 1987, p. 3). Three members of a family ate honey that was received as a gift from a relative who ordered the honey from a retail outlet in Maine. All three who ate the honey suffered from diarrhea and vomiting approximately 5-10 hours after ingesting it, but recovered within 18 hours (p. 3). The Centre for Disease Control (CDC) in Atlanta determined that *Bacillus thuringiensis* was present in the honey. However, the epidemiologist at the CDC in Atlanta stated that presently there is no evidence that implicates *Bt* as the cause of the illness suffered by those who ate the honey. He also stated that there was no evidence to stop the use of *Bt* in the gypsy moth control program being conducted in Pennsylvania (p. 3).

A recent article focused on the safety of *Btk* endotoxins in transgenic plants (plants that have been genetically engineered to express the gene of the *Btk* delta-endotoxin). Some *Btk* transgenic plants have already been developed, and may provide an extremely useful strategy for insect control in agriculture. However, toxicity tests on humans, animals, and other non-target organisms have involved feeding, inhalation, and injection tests using the *Btk* product itself, not the activated toxin expressed by genetically manipulated plants such as tomatoes and tobacco.

The issue of transgenic plants is a completely separate issue from that of spray programs in which *Btk* is used; in spray programs only naturally occurring *Btk* is used and not any recombinant forms of the bacterium. Some, like Goldburg and Tjaden

(1990), even suggest that the existing data on *Btk* safety are invalid for transgenic plants because the recombinant toxin differs chemically from the native form used currently in spray programs (p. 1012). Studies into this area of *Btk* safety are lacking, except for a few on the toxicity of *Btk* towards mammalian cells (Hofmann *et al.*, 1988; Thomas and Ellar, 1983). Although these reports suggest that mammalian cells are not damaged by activated delta-endotoxin, this may not be sufficient evidence to conclude that *Btk* plants are harmless to those mammals which consume fruits and other products of these plants (Goldburg and Tjaden, 1990, p. 1013). The safety of these plants towards humans and other non-target animals, including pollinators, must still be investigated (p. 1014).

# D) EFFECTS ON MAMMALS

Modern formulations of *Btk*, as mentioned previously, are free of beta-exotoxin, a substance toxic to mammals and many other non-target animals. Prior to 1971 the presence of beta-exotoxin in formulations of *Bt* did not hinder the registration of this product as an insecticide. However, several subsequent studies have indicated that beta-exotoxin is toxic to mammals because it inhibits all RNA polymerase activity (Beebee *et al.*, 1972, p. 619; Mackedonski *et al.*, 1972, p. 56). Tests done prior to 1971, and since that time, have provided satisfactory results in terms of the risk of *Bt* infection to mammals. The $LD_{50}$ of *Btk* to rats, mice, rabbits, and guinea pigs via oral, subcutaneous, dermal, inhalation, and intraperitonial administration is greater than 2,000 mg/kg body weight (Novo Industri A/S, 1988a). The safety of *Bt* formulations containing the beta-exotoxin demonstrates even more profoundly, although indirectly, the safety of those without beta-exotoxin.

Other factors contributing to the safety of *Bt* towards mammals include an unfavorable acidic gut environment, and the lack of specific enzymes to activate the toxin. Lepidopteran larvae possess the appropriate enzymes able to degrade the protoxin into smaller chemical units which act upon the gut wall. Vertebrates not only lack the alkaline gut pH and enzymes necessary to activate delta-endotoxin, they do not possess the particular cellular structures to which the toxin binds itself. "The biochemical mechanisms involved in the infectious process are extremely complicated, but they are the basis for differences between susceptible organisms and immune organisms" (Harper, 1974, p. 4).

A series of tests were conducted, as required, to evaluate the safety of *Bt* to vertebrates and other non-target organisms before *Bt* was registered for commercial use in Canada and the United States in 1960. Acute toxicity, sub-acute toxicity, sensitivity-irritation, and persistence tests on rainbow trout, salmon, chickens, mice, rats, swine, rabbits, and humans were conducted using *Bt* var. *thuringiensis*, *sotto*, *alesti*, *entomocidus*, and *subtoxicus*, *Btt* being the only variety possessing beta-exotoxin (Ignoffo, 1973; p. 141-143, p. 152). The results of these tests are summarized in table 6. There is no differentiation between those results obtained using *Btt* and any of the other varieties of *Bt* which do not possess beta-exotoxin. When commercial preparations of *Bt* (which do not possess beta-exotoxin) were used, the toxic manifestations of *Bt* were "not observed when chicks were fed commercial preparations of *B. thuringiensis* at a rate of 2.7 - 432 x $10^6$ spores/chick" (Ignoffo, 1973, p. 152). Ignoffo (1973) concludes from these data that commercial microbial insecticides are "virtually harmless to vertebrates and forms of life other than target pests" (p. 159), but that this safety to vertebrates is relative and cannot be guaranteed in all living systems at all times (p. 160). Ignoffo (1973) also goes on to state that interpretation of all test results must take into account the dose administered, method of administration and the manner in which the safety concerns were evaluated.

### 1) Laboratory Tests on Small Mammals

Fisher and Rosner (1959) summarized the results of some of the earlier tests conducted on mammals to register *Bt* Berliner (now called *Bt* var. *thuringiensis*) in 1958 in the United States. No mortality was observed in these tests when a 1.0 mL preparation of Thuricide containing 3 x $10^8$ organisms/mL (spores and crystals) was injected intraperitoneally into white mice. Blood samples of the mice injected with *Bt* contained the bacterium (it is not specified whether this means spores, crystals or vegetative cells) 48 hours after injection, but *Bt* could not be found 72 hours after injection (p. 687). No mortality resulted from intraperitoneal injections of a *Bt* glucose broth into guinea pigs, nor was any mortality or observable abnormal behavior detected in mice subjected to repeated inhalation tests, nor was any mortality or symptoms of toxicity observed as a result of oral administration of *Bt* to rats (p. 687-688). Allergenicity tests of *Bt* to guinea pigs caused slight erythema (abnormal flushing of the skin caused by dilation of the blood capillaries; usually a sign of infection) and edema (excessive local accumulation of fluid in the tissues) when applied on abraded skin, indicating local irritation. This did not, however, occur on intact skin (Fisher and Rosner, 1987, p. 687; Laurence Urdang Associates, 1981, p. 130 and 144). Siegel *et al.* (1987) performed intracerebral injections on rats using both *Bti* and *Btk* at concentrations ranging between $10^4$ c.f.u./mL and $10^7$ c.f.u./mL

## Table 6:
## Summary of In Vivo Tests Conducted to Evaluate the Safety of Bt Preparations to Fishes, Birds, and Mammals[1]

| Species | # of animals | Inoculum[2] (billion/kg) | Route[3] | Results |
|---|---|---|---|---|
| Human | 18 | 0.2 | po | negative |
| | 5 | 0.02 | ih | negative |
| Mouse | 48 | 0.1-0.3 cells | ip | lethal dose, 50% |
| | 48 | 0.04-2 cells | sc | lethal dose, 50% |
| | 48 | 0.8-7.8 spores | ip | lethal dose, 50% |
| | 48 | 20-160 spores | sc | lethal dose, 50% |
| | 10 | 77 | po | negative |
| | 10 | 15 | ip | abdominal irritation; some death |
| | 10 | 20,000 | ih | negative |
| Rat | 10 | 2000 | po | negative |
| | 100 | 7700 | diet | negative |
| | 20 | 0.01-1.0 | ip | negative |
| Guinea pig | 10 | 77 | diet | negative |
| | 10 | 40 | ip | negative |
| | 10 | 4000 | ip | negative |
| | 10 | 0.3 | sc | localized reaction at injection site |
| | 10 | 16 | DA | slight erythema on abraded skin |
| Swine, duroc | 3 | 185 | diet | negative |
| Wild pheasant | 9 | 3600 | diet | negative |
| Partridge | 2 | 5800 | diet | negative |
| Cornish chick | 190 | 0.2 | diet | negative |
| Chick | 48 | 300-1900 | diet | negative |
| New Hampshire laying hen | 16 | 1000-3000 | diet | negative |
| Cockeral, Vantress cross | 60 | 480-15,800 | diet | negative |
| Rainbow trout, black bullhead, yellow perch, mosquito fish | 40 | <0.01-4.5 | TE | negative |
| Coho salmon, juvenile | 20 | 300-1800 | TE | toxicity at higher doses |

[1] After Ignoffo, 1973, p. 160.
[2] Viable spore count of whole culture preparation that included spores, vegetative cells, crystals, and whole culture broth, unless otherwise specified.
[3] po-per os; ip-intraperitoneal; ih-inhalation, sc-subcutaneous; TE-topical exposure; DA-dermal application.

(Siegel and Shadduck, 1990, p. 207). Both autoclaved and viable inocula were administered, and mortality was evaluated over 1 week. Mortality of rats resulting from injections of autoclaved *Bti* or *Btk* was insignificant at all concentrations; however, at this level, mortality due to viable *Btk* and *Bti* cells ranged between 67% and 83%. At a concentration of $10^6$ c.f.u/mL, mortality resulting from either variety was insignificant (p. 207). The intracerebral toxicity of *Bti* and *Btk* may have been the result of "bacterial metabolites and/or heat-labile toxins at high concentrations" (p. 208). *Bt* remained in the brain of injected animals for up to 3 weeks, but its continual decline in brain tissue indicated that replication did not take place (p. 208). The results suggest that *Bti* and *Btk* are similar in their toxicity to mammals when injected intracerebrally; therefore, the long history of safe *Btk* use provides a sense of assurance about the safety of *Bti*. Furthermore, the testing of unconventional methods of exposure - intracerebral injection and intraocular irritancy - are part of a testing philosophy called "maximum challenge" because the exposure routes severely compromise the test animal's natural defenses. These tests represent

the worst possible scenario. Interpretation of the results may pose difficulties because "even nonpathogenic bacteria can produce mortality when injected in massive quantities into a vulnerable site such as the brain" (p. 202-203).

Siegel and Shadduck (1990) also performed tests on immunocompromised mice to determine the importance of an intact immune system in preventing infection of *Bti* (p. 209). Forty mice were immunosuppressed by injections of cortisone acetate twice weekly beginning 1 week prior to treatment, at which time they were administered *Bti* for a total of 49 days. Forty-two mice (euthymic) were used as control animals. Immunodeficient mice (athymic), lacking T-lymphocytes, were also administered *Bti* for 36 days. Mice were sacrificed at intervals throughout the test periods, and their spleens were analyzed for counts of bacteria/g of spleen. *Bti* in the spleens declined over time for both test groups, although athymic mice had higher levels in their spleens than euthymic mice (p.209). Successful clearance of *Bti* from the spleen occurred in spite of the immunosuppression of the mice (p. 215). In light of these results, Siegel and Shadduck (1990) concluded that the *Bti* preparations did not pose a significant risk to mammals, and that they were avirulent and noninvasive to mammals when given by conventional methods of exposure. For these reasons *Bti* may be used safely in environments in which human exposure may occur (p. 214-215). Since *Bti* is considered slightly more toxic to mammals than *Btk*, these tests also infer that *Btk* is safe to use in situations where small mammals may be affected.

Earlier, Thomas and Ellar (1983) had studied the effects of *Btk* (HD1) (dosages not given) when administered through intravenous and subcutaneous injection, as well as orally, to suckling and adult Balb.c mice (p. 184). Two formulations were administered: solubilized *Bt* crystal delta-endotoxin (0.1, 0.5, and 1.0 mg toxin) in 0.2-0.4 mL buffer, and native crystal delta-endotoxin (0.5 and 1.0 mg toxin); a control was administered using 50 mM of the buffer only (p. 184). The buffer used was $Na_2CO_3$-HCl at pH 9.5 (p. 192). All tests of *Btk* resulted in zero mortality of test mice (suckling and adult) at all concentrations and for all methods of exposure. Controls, using buffer solutions via all methods of administration, also resulted in zero mortality (p. 192).

Various varieties of *Bt* (not specified) resistant to the antibiotics tetracycline, streptomycin and bacitracin were administered to male Swiss mice following antibiotic treatment, a procedure which renders the mice more susceptible to infection by these *Bt* varieties (Som *et al.*, 1986, p. 102). Each animal was fed 1 mL of a particular suspension containing a *Bt* variety resistant to one of the antibiotics (concentrations of insecticide were 4-7 x $10^9$ c.f.u./mL) (p. 103). Autopsies showed that spores of *Bt* were unable to multiply in the gastrointestinal tract of the mice, and that the spore population became "negligibly low" 24 hours after treatment. No mortality resulted from administration of *Bt*, even though the mice were fed antibiotic-resistant strains of *Bt*, followed by antibiotics to kill the intestinal fauna thereby favoring the reproduction of *Bt*. Furthermore, the population of *Bt* in the gut constituted less than 0.1% of the total population of microorganisms. Hence, the ingestion of *Bt* by mice does not appear to cause any deleterious effects (p. 107).

### 2) Laboratory Tests on Large Mammals

Several pre-1971 reports (Harvey and Brethour, 1960; Dunn, 1960; Ode and Matthyse, 1964; Gingrich, 1965; Gingrich and Escle, 1966) and one recent article (Hadley *et al.*, 1987) outlines feeding experiments involving sheep and cows. The test results indicate that the spores and crystals survive passage through the gut of these animals and remain intact, even varieties containing beta-exotoxin.

A lengthy and detailed study of the effects of *Btk* on sheep was conducted using Dipel and Thuricide. Chronic feeding tests, involving castrated male mixed rambouillet/merino sheep over a 5-month period, did not result in any serious adverse effects to the test animals (Hadley *et al.*, 1987). Eight sheep were assigned to each of four test groups: a control group, a placebo diet group, test substance D (Dipel supplied by Abbott Laboratories), and test substance T (Thuricide-HP supplied by Sandoz, Inc.). The animals were fed on a diet containing 1 pound of crushed corn containing 80 g of granular molasses mixed with 100 mL of tap water just prior to feeding. "Placebo animals" received 500 mg/kg of a mixture similar to the test substance T carrier (as prepared by Sandoz, Inc.). Animals in the D and T test groups received 500 mg/kg of diet per day of Dipel or Thuricide, respectively (approximately $10^{12}$ spores) (p. 237). The sheep were observed daily while feeding for any changes in eating or behaviour; they were weighed weekly, and fecal analyses for intestinal parasites were done monthly (p. 237). Tissue samples were taken 5 times at regular intervals during the 24-week test period (4 weeks before treatment began and 20 weeks during treatment) and analyzed at 24, 48, and 72 hours of incubation (p. 238).

Results of this experiment suggest that *Bt* is an avirulent bacterium in sheep when administered orally (Hadley *et al.*, 1987, p. 242). The control and placebo groups showed no observable significant clinical changes in feeding, behaviour, or body weight throughout the test period (p. 240). Periodic significant increases in food consumption in the placebo and two test substance groups were observed, but the authors suggested that this was biologically insignificant (p. 240). Several animals in both test groups suffered diarrhea and occasional loose stools during the test, but symptoms did not persist for more than 1 week. As well, 2 sheep (1 from each test group) suffered from indigestion which was self-corrected within a week in each case. Body weights of the test animals did not differ significantly from the control animals (p. 240). Results of

the blood and body fluid tests performed throughout the test period showed no compound-related changes, although the data were not provided (p. 240). Tissue samples taken at intervals during the test showed the presence of *Bt* in all 4 groups of animals. Three blood samples and four tissue samples taken from control group sheep were positive for *Bt* cultures in low numbers (p. 241). In the placebo group, four rumen, three blood and one tissue sample tested positive for the presence of *Bt* (p. 241). The fact that *Bt* was present in the diet of the placebo and control groups may have been the result of contamination during feeding, although the spore counts in the control animals were extremely low (1 to 2 x $10^4$ spores/g) compared to those of the test groups ($10^8$ to $10^9$ spores/g). In the D test group, *Bt* was cultured from all blood and rumen samples (from all 8 test animals), from six tissue samples, and from one spleen sample (p. 241). In the T group, *Bt* was present in 8 blood and 8 rumen samples, as well as 1 spleen culture. One abnormal tissue lesion was cultured and proved positive for *Bt* (p. 241). Tissue samples analyzed at the time of death revealed lymphoid hyperplasia in Peyer's patches (oval masses of lymphoid tissue) of the cecum and colon in two of the D sheep and 1 of the T sheep, but the authors stated that this cannot be definitively linked to the test agents (i.e. to *Btk*)(Hadley *et al.*, 1987, p. 241, 242; Laurence Urdang Associates, 1981, p. 315).

Hadley *et al.* (1987) suggested that in spite of the tissue culture results, that *Bt* is avirulent to sheep when administered orally. The absence of lung lesions, which could have resulted because of the feeding habits of the animal, suggested that *Bt* does not cause disease in sheep when inhaled (p. 242). Of all the cultures taken from abnormal tissue lesions, only one proved positive for *Bt*, and it was considered an aseptic lesion (p. 242).

Experiments conducted by Harvey and Brethour (1960), Dunn (1960), Ode and Matthyse (1964), Gingrich (1965) and Gingrich and Escle (1966) to test the efficacy of insecticides in controlling house fly, *Musca domestica* (L.), face fly, *Musca autumnalis* De Geer, horn fly, *Haematobia irritans* (L.) and stable fly, *Stomyx calcitrans* (L.) in cattle feces involved feeding various concentrations of *Bt* to cows. In tests conducted by Harvey and Brethour (1960), *Bt* (variety not specified) was administered at 20 g/head/day in the feed for 5 days, at which time a feces sample was taken to test for the presence of larvae. No mention is made by the authors of toxicity or side effects observed in the tested animals; thus, it is assumed that *Bt* had no detrimental effects on the animals. At the concentrations tested, 100% control of *M. domestica* was achieved in steer feces; thus, *Bt* must have survived passage through the digestive tract to be effective for larval control in feces (p. 775). Dunn (1960) fed *Btt* to 2 steers at a rate of 6 x $10^9$ spores/g of bran additive, 1 pound of which was incorporated into 10 pounds daily feed for 10 days, achieving 92% control of *M. domestica* (p. 13-14). No ill effects were observed in the 2 test animals (p. 16).

Ode and Matthyse (1964) administered *Bt* Berliner (*Btt*) to dairy cows in doses ranging from 8.6 to 35.1 mg/kg body weight. The authors reported that "no symptoms of toxicity to the cows were observed at any time" (p. 638). At the highest dosage, 100% mortality of *M. autumnalis* larvae and pupae was achieved, indicating that the spores and/or crystals survived passage through the gut of the cows and remained viable in the feces (p. 638).

Gingrich (1965) documents the efficacy of 3 formulations of *Btt* in controlling *M. domestica*, *H. irritans*, and *S. calcitrans* emergence in cattle feces. Bakthane L-69, Biotrol BTB 5% and S2-97 were fed, each to 1 animal, at dosages ranging from 4 to 120 g/animal/day for 7 days. Control of dipterans ranged from 80 to 100% for *H. irritans*, from 15 to 100% for *S. calcitrans*, and from 37 to 100% for *H domestica* during treatment, but fly emergence began to return to normal levels 2 to 3 days after treatment was stopped for most dosages, indicating that the larvicide did not persist in the digestive tract of the animals (p. 363-364). Gingrich and Eschle (1966) reported that control of *H. irritans* emergence reached almost 100% in treated Angus steers when administered *Btt* daily in water (p. 286). No apparent ill effects (health or behaviour) of the test animals were reported when Bakthane L-69 was given at a dosage of 10 g whole product (spores and crystals) for a total of 68 hours (p. 287).

The fact that some success of face fly, house fly, horn fly, and stable fly control in feces has been documented by Harvey and Brethour (1960), Dunn (1960), Ode and Matthyse (1964), Gingrich (1965) and by Gingrich and Eschle (1966), suggests that spores and/or crystals most likely survive passage through the gut of mammals, and thus are viable in the feces. This statement is supported by Adams and Hartman (1965) through *in vitro* tests on survival of *Bt* in the rumen environment of cows. The variety of *Bt* used is not specified, but the results showed that "spores of *Bt* are very resistant to the rumen environment... There is probably no germination of spores in the rumen" and thus most of the spores ingested would survive passage through the gut (p. 246). All of these tests conducted on cattle were done prior to the ban on *Bt* formulations containing beta-exotoxin in 1971, and thus prove even further the safety of current *Bt* formulations (since they do not contain this toxin) to mammals and other non-target animals (Ellis, 1991; p. 15).

### 3) Laboratory Tests on Mammalian Cell Lines

Thomas and Ellar (1983) studied the toxicity of *Btk* (HD1) (dosages not given) to various mammalian cell lines through *in vitro* tests. Mouse fibroblasts and epithelial carcinoma cell lines, primary pig lymphocytes, as well as horse, human, sheep, rat, mouse and rabbit erythrocytes were tested (p. 184). The crystalline delta-endotoxin was separated from the spores of *Bt* in order to test the toxicity of

the crystals only (p. 185). None of the solutions of *Btk* and/or buffer ($Na_2CO_3$-HCl buffer at a pH of 9.5) caused haemolysis of mammalian erythrocytes (p. 191).

Prepared brush border membrane vesicles from rabbit small intestine were tested for sensitivity to activated toxin of *Btk* (Sacchi *et al.*, 1986, p. 214, 217). The permeability of the vesicle membranes was not altered such that ion transport was affected. Both sodium and potassium ions were taken up by the cells at rates similar to those prior to exposure to toxin (p. 217). In susceptible lepidopterans, potassium ion permeability is severely affected in vesicles, which leads to swelling of the cells and a loss of midgut integrity (p. 213). Binding of the toxin of *Bt* var. *thuringiensis* was shown to occur in vitro in rat intestinal cells, although this was determined to be non-specific as opposed to the specific binding of toxin in target insects (Hofmann *et al.*, 1988, p. 90). The delta-endotoxin of *Btt* was isolated, activated with trypsin, and labelled with iodine-125. Isolated vesicles of the small intestine of rat were treated with the toxin at various concentrations and then analyzed using gel electrophoresis (p. 86-87). The rat vesicles were able to bind the labelled iodine, even though these cells are considered non-target tissue for delta-endotoxin (p. 87). However, the amount of bound toxin was 3.2 times less in the rat cells than in isolated gut cells of cabbage butterfly, *Pieris brassicae*, and the toxin was bound non-specifically in the rat cells (p. 88). This non-specific binding was considered to be caused by an attachment of the toxin to the membrane, not a true binding. In the case of the rat cells, the transport of amino acids and sugars was not affected by the toxin, while in the cells of *P. brassicae* inhibition of these functions occurred (p. 90-91).

*Bt* var. *aizawai* toxin, when activated using midgut juice from silkworm larvae in a buffer of pH 9.6, did not cause any morphological changes to various mammalian cell lines, while the same suspensions caused swelling and bursting of cells from a mosquito, a cockroach, and from silkworm and cabbage looper larvae (Nishiitsutsuji-Uwo *et al.*, 1980, p. 133-134). The swelling of the isolated insect cells began within 15 minutes for the cabbage looper, within 30 minutes for the mosquito, within 60 minutes for the silkworm, and within 1 hour for the cockroach (p. 136). Mammalian cells tested were HeLa and KB developed from human epithelial cancer cells, human erythrocytes (group O), mouse connective tissue cells and erythrocytes, and mouse sarcoma 180 ascites cells (S-180) (p. 134, 136). None of the mammalian cells exhibited any swelling when exposed to the activated toxin for up to 5 hours (p. 136-137). The authors concluded that the dissolved delta-endotoxin of *Bt* had no cytotoxic effect on isolated mammalian cell lines *in vitro* (p. 139).

The high degree of safety and selectivity of *Bt* delta-endotoxin towards mammalian cells has been demonstrated for this particular variety of *Bt*, as well as for *Btk*, *Bti* and *Btt*. Even activated toxin does not cause adverse effects to various cells, except in the case previously mentioned where activated *Bti* caused swelling and cell lysis of mammalian cells (Thomas and Ellar, 1983, p. 188). However, exposure of humans and other mammals to *Bt* would most likely involve the inactive whole product containing spores and intact crystals which cannot be activated in the mammalian gut.

### 4) Field Tests on Small Mammals

Buckner *et al.* (1974) investigated the effects of *Btk*, applied against spruce budworm, *Choristoneura fumiferana*, in Ontario and Manitoba, on non-target small mammals - 53 specimens representing six species. The species observed were the woodland jumping mouse, *Napaeozapus insignis*, the deer mouse, *Peromyscus maniculatus*, short-tailed shrew, *Blarina brevicauda*, common shrew, *Sorex cinereus*, red-backed vole, *Clethrionomys gapperi*, and the eastern chipmunk, *Tamias striatus* (p. F26). Four plots were sprayed using formulations of Thuricide 16B (4 BIU/quart) and Dipel WP (16,000 IU/mg), and two control blocks were monitored (p. F26). Animals of various age groups (juvenile, sub-adult, and adult) were trapped, their numbers recorded, and females were checked for placental scars indicating the recent birth of a litter (p. F36). Results showed that approximately 80% of all adult females inspected bore placental scars indicating that normal breeding continued throughout the *Btk* treatment period (p. F36). As well, statistical tests revealed no significant differences in small mammal populations between control and treatment blocks (p. F36).

An operational spray trial was conducted in 1985 in which *Btk* was sprayed against jack pine budworm, *Choristoneura pinus pinus* Freeman, in northern Ontario (Innes and Bendell, 1989, p. 1318). Small mammal populations in sprayed areas were monitored and compared with those of the control area to determine if any effects were evident as a result of the spray. Two areas were studied, 1 young stand (20 years old) and another which contained an older stand (40 years old). Thuricide 48 LV (20 BIU potency/ha) was applied at 1.8 L/ha to the young stand, while Futura (30 BIU potency/ha) was sprayed at 1.5 L/ha to the older stand. Animal populations were monitored using baited live traps, snap traps and pitfall traps. Animals captured in the live traps were released after identifications were made, while animals from the other traps were preserved and identified later (p. 1319). Observed animals were either shrews or rodents (species names listed in Appendix II), the most numerous of which was the shrew, *Sorex cinereus*. Results showed that small population increases were monitored over the summer season in both treated and control plots by the live traps and snap traps, while in pitfall traps, the numbers were not significantly different. There were no significant differences in population numbers between the sprayed and control plots at either concentration (p. 1320). Sprayed at normal field dos-

ages, *Btk* did not affect the rodent and shrew populations of northern Ontario (p. 1322).

# E) EFFECTS ON BIRDS

Several studies on the efficacy of *Bt* fed to chickens in daily diet to control house fly, *Musca domestica*, suggest that *Bt* passes through the alimentary tract and, as in cattle administered similar feed additives, the digestive tract of chickens is resistant to spores and crystals of the insecticide and no deleterious health effects are suffered by the birds.

Briggs (1960) fed both *Btt* and *Bts* (var. *sotto*) to chickens at dosages ranging from 0.5 to 20 g/day/animal for 3 days and achieved 99% control of house fly emergence when hens consumed 2 to 3 g of *Bt* per day (p. 425). Results also showed that 79% to 93% of spores fed to the birds were recovered in feces (p. 427). Autopsies on "replaced" hens (the author gives on indication of what is meant by "replaced") revealed no abnormalities in organs or tissues, and there was no significant difference in egg production between treated and control hens (p. 429). These observations, in addition to the fact that tested animals readily accepted treated feed, suggest that no ill effects were suffered by the chickens (p. 432).

*Bt* was administered to egg-laying hens at a dosage of 7.8 mg/hen/day for 18 days in a similar test conducted to determine the efficacy of *Bt* (variety not specified) in controlling house fly in livestock feces (Harvey and Brethour, 1960, p. 774-775). No mention is made throughout the report of any side effects suffered by the hens; therefore, it is assumed that none was observed. Excellent control (92% mortality) of house fly was achieved in feces of tested birds; thus, *Bt* must have survived passage through the gut to be viable in the feces (p. 775).

In another study, some deleterious effects of administering certain formulations of *Bt* to caged laying hens in feed has been documented. Burns *et al.* (1961) reported a reduction in food consumption, body weight, and egg production of hens when two formulations of *Bt* (variety not specified) was incorporated into the feed at a concentration of $53 \times 10^6$ viable spores per gram of feed for 58 days. Food consumption dropped from an average of 89.8 g/bird/day, to 76.2 g/bird/day. Egg production dropped from 63.3%/day to 39.4%/day (p. 914). These effects, however, were not observed at lower concentrations of *Bt* (p. 914). At a concentration of $35.2 \times 10^6$ spores/g feed, 62% and 84% control of house fly in feces was achieved in two different feeding trials (p. 914). Although negative effects were observed at the higher concentrations, control of house fly at lower concentrations suggests some survival of *Bt* spores and/or crystals through the digestive tract of hens. In light of the fact that only one of the two formulations caused the observed side effects, it is highly probable that the inert formulation, rather than the strain of *Bt* itself, was responsible for observed side effects in the test animals.

Control of house fly in feces was also achieved by Borgatti and Guyer (1963) by incorporating *Btt* into the feed of Japanese quail, *Coturnix coturnix japonica* (p. 377). Thuricide WP containing $42 \times 10^9$ spores/g (supplied by Bioferm Corp.), Biotrol Feed Additive containing $10 \times 10^9$ spores/g (Nutrilite Products, Inc.), and Agritrol WP containing $70 \times 10^9$ spores/g (Merck and Co.) were fed to caged quail for 70 days, while feces samples were collected and analyzed at 2-week intervals (p. 378). Insecticides were administered in feed at dosages ranging from 1 to 28 g/lb of food, and control of house fly emergence ranged from 52% to 94% (p. 380). Abrupt reductions in fly control occurred immediately following cessation of treatments, suggesting that specimens did not retain the spores in their digestive tracts (p. 383). Furthermore, no mention is made of any ill effects or changes in behaviour of tested birds.

Results of impact studies on forest inhabiting birds by Buckner *et al.* (1974) in spray plots in Ontario and Manitoba showed no detrimental effects to bird populations when *Btk* (Dipel WP, 16,000 IU/mg; Thuricide 16B, 4 BIU/quart) was aerially applied to control spruce budworm (p. F10, F26). Populations in most treatment blocks paralleled those in the control blocks. Some populations of sparrows and warbler deviated from the norm after treatment, but these were shown to be statistically insignificant (p. F11). Warblers, sparrows, robins, woodpeckers, wrens, ravens, grouse, and finches were among the species monitored.

Morris (1982, p. 280), citing Stephens *et al.* (1970) (original report could not be obtained) reports that bowhite quail were unaffected by heavy doses of *Btk*. Forsberg *et al.* (1976), also citing Stephens *et al.* (1970), states that bobwhite quail were administered 10 g of *Bt*/kg of body weight (variety not specified) without any apparent ill effects, except that body weight increase in treated birds was 17.4% while that of control birds was 25.3% at 21 days after treatment (Forsberg *et al.*, 1976, p. 53).

In a review of spruce grouse management in Ontario, Alison (1989) refers to a study conducted by Bendall from the University of Toronto, that implies that *Btk* spraying for jack-pine budworm control is responsible for a decline in spruce grouse populations. Bendall states that *Bt* spraying "reduces growth and survival of grouse chicks" because it di-

minishes larval populations of local butterflies and moths which the chicks feed upon (Alison 1989, p. 15). This statement is not supported with any scientific data on proportions of these insects in the grouse diet, nor information on actual numbers of grouse before and after tretment of the area with *Btk*. This is currently under investigation in Ontario.

Rodenhouse and Holmes (1992) conducted a 4-year study on the effects which aerial application of *Btk* has upon the breeding population of black-throated blue warblers, *Dendroica caerulescens*, a foliage gleaning species. Four 30-ha plots were established in White Mountains National Park in New Hampshire, adjacent to areas previously used for studying *D. caerulescens* populations. Forty randomly selected grid points were chosen as sample locations within each of the plots, and two 50-leaf samples from each sample point were examined bi-weekly throughout the breeding season (late May to early August) for arthropod populations. Two malaise traps were also established in each plot to collect flying insects during this same time period (p. 359). Thuricide 32LV (with Rhoplex sticker) was applied at a rate of 3.5 L/ha to 1 plot each year (p. 359). No significant reductions in adult arthropods were noted throughout the duration of the study as a result of the *Btk* application. Neither was there a significant difference in egg production, nestling mortality, nor fledgling success of *D. caerulescens* between treated and control plots (p. 362-364). The only statistically significant difference between the treated and control plots was the lower number of pairs attempting second broods in the treated plots (p. 365). The authors concluded that this may have been the result result of the overall availability of food rather than a direct effect of the *Btk* application on any one group of insects.

# F) EFFECTS ON NON-TARGET TERRESTRIAL INVERTEBRATES

*Btk* has been shown to be highly toxic to many lepidopterans because of their alkaline gut environment which is necessary to digest the toxic crystals and release the toxin. However, some non-target invertebrates, especially non-target lepidopterans, may also be susceptible to *Btk* and are therefore at risk when sprays are conducted. Some studies have shown that populations of earthworms, arthropods, honey bees, and certain other insects have been affected both by *Bt* in the laboratory and in the field, while later reports present contradictory data. It must be noted, however, that in the earlier studies beta-exotoxin was present in the *Bt*, while in later studies *Bt* products used in pest control were free of this toxin. No *Bt* product registered for insect control has contained beta-exotoxin since 1971.

### 1) Effects on Non-target Lepidopterans

Miller (1990) monitored the effects of *Btk* on non-target lepidopterans feeding on Garry oak trees, *Quercus garryana*, following treatment for control of the gypsy moth, *Lymantria dispar*, in 1986. *Btk* (product not given) was applied by helicopter at a rate of 16 BIU/2.8 L of water/0.4 ha at three different times - May 15, May 26, and June 5 (p. 136). The area had never been sprayed with *Btk* before 1986, and was not sprayed again (p. 136). Species diversity, and population size were monitored for 1986, 1987, and 1988. Lepidopterans observed were Geometridae, Noctuidae, Tortricidae, Arctiidae, Gelechiidae, Gracillariidae, Lasiocampidae, Lymantriidae, and Notodontidae (p. 136).

Results of this experiment suggested that rare non-target species of Lepidoptera may be "ecologically 'at risk' in large-scale pest control programs based on *B. thuringiensis kurstaki*" (Miller, 1990, p. 135). The study showed that both numbers of non-target insects over the test period and species richness were depressed for 3 years following treatment (p. 135). In the year of pesticide application, the number of larval lepidopterans in the treated plots was significantly lower than that of the control plots for three months following application of *Btk* (p. 137). Species richness in the treated plots decreased by 56%, while that in the control plots showed a 1.7-fold increase; this decrease in species richness was evident for 60 to 80 days after treatment (p. 137). Results for 1987, one year after treatment, also indicated significantly lower levels of lepidopteran numbers and diversity. Numbers of larvae were 50% lower than those of the untreated plots, while diversity was 38% lower in treated plots than in control areas (p. 137). In 1988, larval density in treated plots had recuperated to levels similar to the untreated plots. Species diversity in 1988, although it equalled the pre-spray diversity of treatment plots in 1986, was still significantly lower in treated plots compared with untreated plots. "These data indicated species of Lepidoptera were recolonizing, but the negative effect on spring species richness extended for at least 760 days" (p. 138).

The effects of *Btk* spraying on non-target lepidopteran larvae may only be a concern when endangered species of insects are in the spray area; however, repeated treatments could eventually change the balance of non-target insect populations. The relative 'value' of the endangered species should be compared to the potential damage in case of non-treatment against the target species.

### 2) Effects on Honeybees

One of the major concerns associated with the use of *Bt* to control pest insects is the potential risk towards honeybees, *Apis mellifera*. This concern is reasonable when one considers both the social living environment and the gathering activities of this insect. A major concern is that *Bt* sprayed on floral parts of plants may be ingested by or transferred to the foraging worker bee, which in turn may deliver these spores to the colony, possibly contaminating honey stores, other workers, developing larvae, and possibly the queen. It is feared by some that such infection could affect communication pheromone (kairomone) and wax production, as well as the quality and quantity of eggs laid (Wilson, 1962, p. 269; Cantwell *et al.*, 1966, p. 228).

Hamed (1979) studied the possibility of *Btk* spore germination while feeding 50:50 honey and water mixtures containing large doses of *Btk* to parasites of the small ermine moth, *Yponomeuta evonymellus*. No germination of *Btk* spores took place during the 6 days that the mixture was fed to the parasites (p. 296).

Buckner *et al.* (1974) recorded adult honeybee flight activity, mortality, weight of pollen collected daily, and weight gain following treatment of areas in Manitoba and Ontario with Thuricide 16B (4 BIU/quart) and Dipel WP (16,000 IU/mg) (*Btk*) (p. F43-45). Flight activity of bees in treated blocks did not decrease after treatment, but paralleled that of bees in the control areas (p. F43). Mortality of adult and young brood bees in treated areas was not significantly different from controls (p. F45). **Furthermore, the weight of pollen collected and weight gain of the colonies was unaffected by *Btk* treatments** (p. F45).

Wilson (1962) studied some of the potential side effects of *Bt* to disease-free honeybee colonies by administering both dust and wet preparations of *Bt* Berliner - *Btt* (Thuricide) incorporated into feed (p. 269-270). Four colonies were administered *Btt*, both as wet and dust preparations. Colony 1 was administered *Btt* through direct sprinkling of the dust over the top bars of the hive and on the combs in the area of the brood nest. Five grams of dust were sprinkled for each of six treatments, on each of 11 days, for a total of 30 g of *Bt* (p. 269). Colony 2 received 5 g of *Bt* dust mixed with 15 g of powdered sugar applied in a similar manner as in colony 1 for a total of 30 g (p. 269). Colonies 1 and 2 were monitored for side effects at each application of *Bt*, and then once 6 weeks following the final treatment. Colony 3 was fed 15 g of wettable powder *Bt* in a 1:1 solution of sugar and water, which was consumed in a little less than 4 days (p. 270). In colony 4, the combs were sprinkled with a 500 mL solution (it is not mentioned what this solution is composed of) containing 5 g wettable powder. Colonies 3 and 4 were observed 1 and 2 weeks after treatment (p. 270). No detrimental effects, nor any symptoms of disease were observed in any of the colonies during the after treatment period. Colony "morale" was maintained throughout the treatments and honey production was normal (p. 269-270). The authors stated that *Btt* appeared to be completely safe for use in areas where honeybees are foraging for pollen (p. 270).

Ali *et al.* (1973) investigated the potential side effects of *Bt* (Thuricide-HP, variety unspecified) to honeybees when used to control the greater wax moth, *Galleria mellonella*, a serious pest of honeybee combs (p. 117). The *Bt* product, supplied by the International Mineral and Chemical Corporation, contained $5.5 \times 10^9$ IU/lb ($50 \times 10^9$ viable spores/g) (p. 118). It was fed to 1-2 day old worker bees in a prepared food of sugar and honey at concentrations of 25, 50, 100, 150, 200, and $250 \times 10^8$ spores/g of food; mortality counts were made at 24-hour intervals after treatment up to 168 hours (p. 118, 121). At the lowest concentration, only 6% mortality of honeybees resulted after 168 hours of feeding on *Bt* infected food; 100% mortality, however, was obtained in 96 hours at a concentration of $150 \times 10^8$ spores/g food (p. 121). Comparatively, in similar tests using the target insect *G. mellonella*, much higher concentrations of *Bt* were required to kill the honeybees than were required to kill *G. mellonella*. The 48-hour $LC_{50}$ for *G. mellonella* was $9 \times 10^8$ spores/g (for 3rd-4th instar larvae) while that of honeybees was $120 \times 10^8$ spores/g (p. 120-121). Reasonable control of the greater wax moth was achieved using *Bt* (at the lowest dosage) on honeybee combs without serious side effects to the bees themselves.

On the other hand, in feeding tests of the honeybee using *Btt*, it was shown that the insecticide caused significant mortality within bee colonies (Martouret and Euverte, 1964, p. 198). Two formulations of *Btt* were tested, one containing only the spore-crystal complex, and the other having the complex as well as a thermostable soluble toxin (the toxin was not specified it is assumed to be beta-exotoxin). It must be noted that at the time of this experiment by Martouret and Euverte (1964), the ban on beta-exotoxin was not in effect and therefore these tested formulations likely contained the toxin. *Bt* was administered in a prepared solution of one part honey and three parts sugar, at concentrations of 1, 2.5, and 5% (proportions by weight). Mortality was high for both treatments at all concentrations by the sixth day of testing, and reaching 98-100% by the 14th day (p. 201). In spite of these findings, the authors suggest that it is "unlikely that in nature a working bee would ingest such a large quantity of bacterial substance and over such a long period, as was the case in our experiments" (p. 201-202). Cantwell *et al.* (1972) in a later study also suggested that under normal field applications the rates of beta-exotoxin (or delta-endotoxin) do not reach sufficient levels to harm foraging honey bees (p. 257).

Cantwell *et al.* (1966) lend support to the idea that the beta-exotoxin is the element responsible for mortalities to non-target organisms, especially honey bees, in *Bt* formulations used prior to 1971.

They tested various concentrations of *Bt* var. *thuringiensis*, *sotto*, and *alesti*, using cultures of spores only or crystals only, freeze-dried exotoxin only (presumably beta-exotoxin), and total product (spores and crystals), both in the laboratory and in hive tests. Bees were fed 1:1 solutions of sugar and water containing various concentrations of *Bt* (p. 228). Results of the laboratory tests showed that formulations containing exotoxin caused 100% mortality 7 days and 11 days after treatment in the exotoxin-only and total-product tests, respectively (p. 231). The crystal-only treatment did not cause any significant mortality 1 week after *Bt* was administered, nor any paralysis or cessation of feeding. However, high doses ($1.67 \times 10^9$) of pure spore solutions caused significant mortality 8 days after treatment (p. 231). In hive tests, significant mortality occurred only in treatments containing the exotoxin, where 100% mortality of adults was observed as well as discoloured pupae and discoloured and dried up larvae within 2 weeks (p. 231). The concentrations used in these tests were much higher than those that would be expected to occur in treated areas in the field. As well, the exotoxin, which is detrimental to many non-target organisms including the honeybee, is not present in current *Bt* products. The authors concluded, therefore, that ***Bt*, as currently used, would not be injurious to bees when applied against target insects, including *G. mellonella* under normal circumstances** (p. 232).

### 3) Effects on Other Non-target Arthropods

Toxicity of *Bt* (varieties *entomocidus*, *sotto*, and Bakthane varieties) to the Indian stick insect, *Carausius morosus* Br. (Phasmida: Phasmitidae), was investigated by ingestion and injection of crystals or crystal digests (Pendelton, 1970, p. 287). Digests were prepared using 0.2M carbonate-bicarbonate buffer at pH 9.5 and gut juice from *Philosamia ricini* (Boisduval) larvae (Lepidoptera: Saturniidae). Some insects were injected dorsally with 6 g of crystal digest per insect, while others were fed leaf discs covered with 10 g of the crystal protein per insect (p. 287). Seven days after treatment, no mortality resulted in the feeding tests for any of the *Bt* preparations; however, mortalities of 60% and 48% were observed in test organisms injected with *entomicidus* and Bakthane, respectively, while the *sotto* variety caused no more mortality than occurred in the controls (12% mortality). However, it should be noted that injection is a rather "unnatural" way to get *Bt* into test organisms, which may be acceptable in laboratory "challenging" experiments, but is not useful for extrapolating to field situations. "Tests showed that *C. morosus* gut juice did not release any soluble toxin from the crystals *in vitro*" because the insect has a gut pH of approximately 7, which is not alkaline enough to allow dissolution and release of the proteinaceous crystal toxins (p. 287).

Buckner *et al.* (1974) observed no detrimental effects among populations of non-target insects in areas treated with Thuricide 16B and Dipel WP in Ontario and Manitoba (p. F67). Although some decline was observed in populations of spiders (Araneae), ground beetles (Coleoptera: Carabidae) and ants (Hymenoptera: Formicidae), this was paralleled in the check plots and thus considered to be the result of natural causes (p. F50). Non-target lepidopterans were apparently unaffected by treatments (p. F50). It should be noted, however, that these data were not analysed statistically.

MacPhee and Sanford (1961) conducted several studies on the effects of various insecticides on non-target arthropods used in apple orchards in Nova Scotia. *Bt* Berliner (*Btt*), at $75 \times 10^9$ spores/g, caused little or no reduction in numbers of most test species. Of the 20 species tested, only six were affected such that some reduction of numbers was observed in the orchard spray plots (see Appendix III for species names). The *Bt* used in 1961 probably contained beta-exotoxin which is toxic to many non-target organisms.

A study conducted by the Newfoundland and Labrador governments on an experimental block treated with *Btk* found that the insecticide did not have any adverse effects on the terrestrial arthropod community in the spray block (Government Newfoundland and Labrador, 1980, p. 10). The block was in a regeneration forest (15-20 years growth of fir and spruce), and constituted an area of 800 ha treated with *Btk* at a rate of 20 BIU/ha in June of 1979 (p. 3). Arthropods were sampled by methods of pitfall trapping and foliage sampling twice weekly between May 23 and August 15 of that year (p. 8-9). Results indicated that there were no significant differences between mean pre-spray and mean post-spray arthropod catches at the treatment site (p. 10). Soil samples taken from the treatment site prior to treatment, and 2, 9, 18, and 44 days post-spray indicated that *Btk* spores did not accumulate in the soil environment. In fact, post-spray samples indicated lower numbers of spores in soil than in pre-spray samples, which suggests that *Btk* was naturally occuring in the soil of the block before treatment with *Btk* (p. 14).

An experiment conducted in Hungary in 1980 and 1981, showed that *Bt* (Thuricide HP) was effective in reducing populations of *Euproctis chrysorrhoea* and *Lymantria dispar*, the target pests, but did not harm non-target arthropods in the spray area (Lesko *et al.*, 1982, p. 406). The application of *Bt* reduced target pest numbers, but "saved other members of the forest fauna" (p. 406).

Two species of coccinellids were shown to be slightly susceptible to the effects of *Btk* (Dipel) when in the larval stage (Olszak, 1982). *Adalia bipunctata* (L.) and *Coccinella septempunctata* (L.), two species of benefical ladybird beetles found in apple orchards, are predaceous on aphids and may be at risk when *Btk* formulations are sprayed for the control of various organisms. Five newly hatched larvae of these beetles were placed in plastic containers with two apple leaves collected from an orchard sprayed with a 0.2% solution of Dipel (potency not given), and

mortality checks were made daily for 7 days. These tests were conducted at 2 and 24 hours, and at 3, 7, 14, and 21 days following field treatment of the apple orchard (p. 143). Egg masses were tested by dipping them into aqueous solutions of Dipel for 5 seconds at the same concentration, and mortality of eggs was checked 3 days following the treatment (p. 143). Toxicity of Dipel to coccinelid eggs was insignificant, but newly hatched larvae were somewhat affected. Of the eggs tested, 76.6% survived to the larval stage. However, only 6.5% of these newly hatched larvae survived 3 days after hatching (p. 142). It is not specified from which experiment the data have been collected (i.e., how much time has elapsed between spraying the trees and testing the beetles).

#### 4) Effects on Earthworms

Smirnoff and Heimpel (1961) documented sensitivity of the earthworm, *Lumbricus terrestris* Linnaeus, to *Btt* (Thuricide, supplied by Bioferm) at dosages of 30 x $10^9$ and 50 x $10^9$ spores/g (p. 404). Jars with a diameter of 100 mm were filled with 300 g of granular clay soil which was covered with rotting leaves of birch, maple, or elm. Ten worms were placed in each jar, and after a short adaptation period, one of the Thuricide preparations was added to each jar at a concentration of 3, 15, 30, or 60 g respectively (p. 403-405). These high concentrations can only be expected to occur at spill sites. In addition, this *Bt* formulation contained beta-exotoxin which is known to be toxic to many non-target animals. Experiments were repeated three or four times, and control jars were also set up for comparison. A mortality rate of 100% was observed after a 2-month exposure period of test worms to the smallest dose of *Btt* (3 mg *Btt* at 30 x $10^9$ spores/g in 300 mg soil) (p. 406). At a concentration of 60 mg Thuricide (30 x $10^9$ spores/g) per 300 mg soil, 100% mortality was observed in as little as 10 days. Dissection of the cadavers revealed that the bacteria had penetrated the gut wall, entered the body cavity, and undergone sporulation and crystal-formation (p. 407). This laboratory experiment suggests that *Btt* is pathogenic to the earthworm. However, the authors feel that "it is unlikely that the earthworms will be seriously affected by the amount of living insecticide to be used in normal control operations against any insect pest" (p. 408). In addition, Benz and Altwegg (1975) claim that the ammounts used for this experiment were **10,000 to 100,000 times** the amount which would occur in the soil after an application of *Bt*. The EPA also dismissed this report because of the extremely high levels of exposure to the earthworms, and concluded that no adverse effects to annelids would be expected to result from normal field use of the insecticide (Rogoff, 1982, p. 650).

**Tests using *Btk* on earthworms have shown that current formulations (beta-exotoxin-free) are safe to these invertebrates.** Benz and Altwegg (1975) applied Dipel (*Btk* serotype H3; 16,000 IU/mg) at concentrations of 60, 600, and 6000 mg/$m_2$ on plots of soil measuring 3m x 3m. The objective was to test the toxicity of *Btk* to earthworms in a field experiment in an ash and maple forest. Earthworms were counted before and after treatment, and trials were run on a cloudy day to avoid inactivation of *Btk* by sunlight (p. 125). **Even at the highest concentration, a dose 100 times higher than would be applied during a field treatment, adverse effects were not observed** in any of the plots up to 9 weeks after treatment. No significant differences were observed between control and treatment plots before and after spray (p. 126). The authors concluded that normal application rates of *Btk* would not harm earthworms (p. 126).

## G) EFFECTS ON NATURAL ENEMIES OF TARGET INSECTS

Many studies have been conducted to assess the risk of *Btk* spraying to parasites and predators of target insects. Such non-target beneficials may be at risk to side effects of *Btk* directly from the insecticidal spray, or indirectly due to residue-contaminated foliage surfaces. In such cases, internal contamination could occur as a result of ingestion of spores during grooming, consumption of infected food, or direct penetration of *Btk* through an integumental wound (Flexner *et al.*, 1986, p. 228). The alteration of the food sources of natural enemies may also account for some of the observed detrimental effects of *Btk* in field studies. If host insects die before parasite larvae are fully developed, or if *Btk* drastically reduces the availability and quality of a predator's food source, then the survival of the beneficials may be hindered (Flexner *et al.*, 1986, p. 229).

Apart from the possibility of direct toxicity of the insecticide to beneficial insects there also exists the potential for detrimental sublethal effects to parasite egg deposition and viability, larval and pupal development, sex ratios, adult longevity, and adult emergence of parasites that attack infected hosts (Elzen, 1989, p. 129; Flexner *et al.*, 1986, p. 229, 232). Other sublethal effects may include changes in behaviour such as alterations in foraging patterns, disruption of sexual communication, and lack of host recognition (Elzen, 1989, p. 129). Although some authors report or, like the ones above, postulate the potential side-effects of *Btk* towards natural enemies, **most experiments have shown that at recommended dosages, *Bt* (both *Btk* and *Bti*) do not adversely affect the activities of parasites and predators of the target insects, either acutely or sublethally.**

### 1) Natural Enemies of the Eastern and Western Spruce Budworms

Buckner *et al.* (1974) concluded that Dipel WP (16,000 IU/mg) and Thuricide 16B (4 BIU/quart) spray treatments did not adversely affect parasitism of the eastern spruce budworm by hymenopterous parasites in Ontario and Manitoba (species names not given). In some blocks, parasitism actually increased, while results were variable in other plots. Parasitism decreased in the control block, which may have been the result of variations in sample size (p. F55).

Otvos and Raske (1980) reported the effects of *Bt* spraying on the two most important parasites of the eastern spruce budworm, *Choristoneura fumiferana*: *Apanteles fumiferanae* (Viereck) (Hymenoptera: Braconidae) and *Glypta fumiferanae* (Viereck) (Hymenoptera: Ichneumonidae). *Bt* Berliner (*Btt*) plus Orthene was aerially applied to a region in Newfoundland in 1977, along with several other insecticides, in an effort to control an outbreak of the eastern spruce budworm (p. 1). Parasitism for both parasites combined increased significantly from 1.1% and 3.2% in the treatment and control blocks respectively (pre-treatment), to 18.5% and 8.6% respectively after treatment (p. 8). No mortality of adult parasites was observed following treatments (p. 10). This apparent increase was explained by the authors as a result of the behavior of the parasitized larvae. It is a well known fact that parasitized larvae are smaller and slower in development than non-parasitized larvae. Parasitized eastern spruce budworm larvae emerge later from their hibernacula than non-parasitized larvae, thus if treatment is applied to earlier instars, not all the parasitized larvae may be feeding at the time of treatment, and thus would not be exposed to the insecticide. Therefore, proportionately more non-parasitized larvae would be affected by the spray, resulting in an apparent higher level of parasitism.

This was indirectly confirmed by Nealis and van Frankenhuyzen (1990) during their investigations on the effects of *Btk* on *Apanteles fumiferanae*, and have shown that deleterious effects of *Btk* on this parasitoid are dependent upon the larval stage of the host, the eastern spruce budworm (p. 585). Thuricide 48LV (supplied by Sandoz) was diluted to 8.4 BIU/L and applied to foliage of balsam fir shoots in a spray chamber; parasitized or unparasitized larvae of either peak third or peak fourth instar were placed on each shoot (p. 587). Results showed that *Btk* was more effective on unparasitized larvae, possibly because parasitized larvae are less likely to ingest a lethal dose of *Btk* due to reduced feeding as compared to unparasitized larvae (p. 589). However, the parasitoid populations were reduced by 50-60% because of premature death of the host (p. 589). The parasitoids were affected because their host was infected with *Btk*, but the insecticide did not affect the parasitoid directly. Parasitoid survival was incresed to 64.4% when host larvae were infected with *Btk* at the peak fourth instar stage instead of at the third instar stage (p. 591). Furthermore, there were no adverse effects of *Btk* on the development of *A. fumiferanae* that emerged from infected hosts (p. 591). The results indicate that timing of application of *Btk* is not only important in terms of efficacy, but may also be a factor in preventing deleterious effects on natural enemies. The activities of natural enemies can contribute greatly to the success of a *Btk* treatment, but only if survival of the enemies is sufficient. To prevent significant mortality of *A. fumiferanae*, treatment of an area with *Btk* should be delayed until parasites have emerged or are advanced enough that their hosts will not be feeding, at least until the peak fourth instar. In this way, premature death of hosts will not result in resurgent populations of budworm in the spray season or subsequent seasons (p. 591-592).

Morris (1983b) reported no deleterious effects on parasites of the eastern spruce budworm when *Btk* (Dipel 88) was used at concentrations of 10, 20, 40, and 80 BIU/ha. Larvae were collected 14 and 21 days after treatment and fed an artificial diet, while pupae were collected at peak pupation and reared until parasites or adult moths emerged (p. 1002). Rates of parasitism in the collected insects (Hymenoptera and Diptera - species names not given) were not significantly different from controls when ratios of larval density to percent parasitism were calculated and compared, even for the highest dosage (p. 1006). At normal field application rates, the author suggested that, Dipel 88 is not detrimental to parasites of the eastern spruce budworm (p. 1006).

Parasitism of the eastern spruce budworm was unaffected by *Btk* treatments in Wisconsin forests of balsam fir, aspen, and paper birch (Reardon *et al.*, 1982, p. 509). Parasites recovered from budworm collected from the sprayed areas and reared on an artificial diet in the laboratory did not differ significantly in number from those collected from check plots (p. 512-513). Dipel 4L (an oil invert emulsion) and Thuricide 16B (a water-based emulsifiable concentrate), applied to treatment plots at 20 BIU/ha in 9.35 L/ha, did not adversely affect parasites of the eastern spruce budworm (species names not provided) (p. 510, 513).

Niwa *et al.* (1987) reported no adverse effects to parasites of the western spruce budworm (WSBW), *Choristoneura occidentalis* Freeman, resulting from a field spray test of Thuricide 32LV at 20 and 30 BIU/ha (*Btk*) (p. 750). Insects were collected from sprayed trees at intervals for up to 16 days after treatment and reared to determine mortality rates. Only 1.2% of the parasites collected, the majority of which were *Glypta fumiferanae* and *Apanteles fumiferanae*, contained *Btk* spores and crystals. "These parasites matured sufficiently to exit from their hosts but were not able to complete development." The authors state that "We were unable to determine whether the cause of this premature mortality was microbial infection or insufficient nutrition of these parasites because of the early deaths of

their hosts" (p. 751-752). Percentage of parasitism was analyzed for *G. fumiferanae*, *A. fumiferanae*, and other species. "There were no significant differences (P 0.05) between treatments for any single species or group of parasites within a sample period... Fewer than 2% of emerging parasites showed [*Btk*] symptoms, indicating that the number of infected parasites must be very small" (p. 752). These symptoms of *Bt* infection include cessation of feeding, paralysis, and ultimately death. "Neither total parasitism nor the species distribution of the parasite complex differed between the control and the sprayed plots during any of the sample periods. Thus, although *B. thuringiensis* may affect a small number of parasites developing within infected hosts, we conclude that field application of *B. thuringiensis* for control of WSBW would not be detrimental to the associated parasite complex" (p. 752).Conversely, Hamel (1977) reported that parasite populations were altered as a result of *Btk* treatment for western spruce budworm control in Montana forests of Douglas-fir, engelmann spruce, and subalpine fir (p. 1409-1410). The effects of Dipel WP, applied at 453 g in 7.57 L water per 0.4 ha, were monitored for parasites of both the western spruce budworm and the spruce coneworm, *Dioryctria reniculelloides* Mutuura and Munroe (Lepidoptera: Pyralidae), a spruce defoliator often associated with the budworm (p. 1409, 1411). The majority of budworm parasites were *Apanteles fumiferanae*, *Glypta fumiferanae*, and *Phaeogenes hariolus* (Cresson) (Hymenoptera: Ichneumonidae), *Ceromasia auricaudata* Townsend (Diptera: Tachinidae) and *Madremyia saundersii* (Williston) (Diptera: Tachinidae); other parasites from the families Sarcophagidae, Chalcididae, Trichogrammatidae, and Ichneumonidae were also monitored (p. 1410-1411). Parasitism varied greatly both for individual species and the combined parasitism, which ranged between 7 and 25% (p. 1412). The only difference in parasite populations between *Btk* plots and the check plot before spraying was a significantly greater number of *G. fumiferanae* in the check plot (p. 1413). At 7, 14, and 21 days after treatment, the *A. fumiferanae* population was significantly greater in the *Btk* than the check plots. *G. fumiferanae* numbers were greater in the check plots up until 21 days after treatment, at which time the reverse situation occurred in this population (p. 1413). Parasitism by all other parasites was significantly lower in the treated plots than in the check plots at all sample periods. *P. hariolus*, *C. auricaudata*, and *M. saundersii*, parasites that attack late instars or pupae, were significantly reduced in the treatment plots following application of *Btk* (p. 1413). Total parasitism was not significantly lower in the treatment blocks at 21 days after treatment, but the natural balance of parasite populations was somewhat altered as compared with the control blocks. However, the authors did not attribute these differences to the insecticidal action of *Btk* on the parasitoids per se; rather, they were "a function of host response prior to and after spraying" (p. 1414). These differences in percent parasitism by the pupal parasites could also have been a result of the density-dependent behaviour response of the parasitoids. Similar results were obtained for *D. reniculelloides*, where total parasitism was significantly reduced in the treatment plots at 21 days after treatment (p. 1413).

### 2) Natural Enemies of the European Gypsy Moth

A 'synergistic' relationship seems to exists between *Btk* and natural enemies of the gypsy moth. The insecticidal action of *Btk* is enhanced by the presence of parasites because *Btk* allows for an increase in parasitism in some cases. Sublethal doses of *Btk* retard larval development thereby increasing the amount of time that the parasite, which attacks only a certain size of host, can successfully parasitize the larvae (Weseloh, 1984, p. 1371). However, in some cases, other species of parasites are adversely affected by *Btk* treatments.

The most common parasitoid of gypsy moth is *Apanteles* (=*Cotesia*) *melanoscelus* (Ratzberg) (Hymenoptera: Braconidae), having two generations per year, the second of which overwinters in diapause. The first generation emerges in early May and parasitizes the very young and relatively small larvae available at that time. The progeny of this generation do not diapause, but emerge in mid-June to parasitize the comparatively larger gypsy moth larvae (Weseloh *et al.*, 1983, p. 100). It is parasitism by this second generation of *A. melanoscelus* that is enhanced as a result of *Btk* aerial sprays, because the sublethal effects retard the development of the larvae long enough for the parasitoids to successfully attack and oviposit on more hosts than normally possible (Weseloh, 1984, p. 1371).

In addition to enhanced parasitism, Weseloh *et al.* (1983) found that resulting cocoons of the second generation of *A. melanoscelus* were more abundant in *Btk*-treated plots than in control plots, suggesting that the parasitoid does not experience side effects from *Btk* sprays. Mixed hardwood forest plots in Connecticut were treated with 20 BIU/ha of *Btk* (HD-1, HD-243, and HD-263 serotypes, supplied by Abbott Laboratories) in 9.4 L water/ha in 1981 to control the gypsy moth. Each plot was sprayed twice, on May 21st and 28th, except for three additional plots of HD-1 serotype (Dipel 4L) which received only single treatments (p. 100). Target larvae developed more rapidly in all treatment plots such that 5.2% of the larvae in the control plots were in third instar or smaller, as compared to 21.6% to 51.5% in the sprayed areas by June 11th (p. 101). Percent parasitism was 6 to 12 times higher in treatment than in control plots, with the greatest differences recorded on June 11th, 18th, and 25th (2, 3, and 4 weeks after treatment). On June 18th and 25th, parasitism by *A. melanoscelus* was 0%, while in the treatment plots it ranged from 8.9% to 21.2% on the 18th, and from 5.9% to 23.2% on the 25th (p. 101). This increase was most likely due to enhanced parasitism in the second generation of parasitoids at

a time when host larvae had already reached fourth instar in the control plots (p. 101). Numbers of *A. melanoscelus* cocoons (parasitizing host larvae) in the treatment plots were 3 to 5 times higher than in the controls, suggesting that the growth/development retardant effect of *Btk* on the host, and not the effect of parasitoid:host ratios, was the cause of increased parasitism (p. 102). The authors emphasize the potential of *Btk* in pest management programs involving low doses of *Btk* for use as a feeding deterrent in conjunction with introduced populations of *A. melanoscelus* (p. 103).

Dunbar *et al.* (1973) studied the effects of aerial test sprays of *Btk* (Thuricide HPC; 16,000 IU/gal) on parasitoids of the gypsy moth, *Lymantria dispar* (p. 6). *A. melanoscelus*, the most abundant parasite, was not significantly affected in terms of number of overwintering cocoons (p. 20). Furthermore, parasitism was elevated in *Btk*-treated plots as compared to untreated plots, which was most likely due to a greater proportion of parasites with respect to hosts following insecticidal treatment (p. 22). There were no significant differences in parasitism between treated and untreated blocks for the two other tachinid parasites, *Blepheripa scutellata* (Robineau-Desvoidy) and *Parasitigena agilis* (Robineau-Desvoidy) (p. 22). The study indicated that there were no apparent detrimental effects resulting from aerial treatment of forest stands with *Btk* for gypsy moth control (p. 22).

Weseloh and Andreadis (1982) documented a developmental lag in larvae of the gypsy moth resulting in greater parasitism of these insects by *Apanteles melanoscelus* when *Btk* was used as a method of insect control (p. 435). *Apanteles melanoscelus* females preferentially parasitized treated larvae 10 days after treatment, yet the number of offspring reared from the treated and untreated larvae did not differ significantly (p. 437). "Gut paralysis evidently led to the retarded development of at least some gypsy moth larvae that ingested a sublethal dose. Such delayed development explains the high degree of ovipositional activity and parasitization by *A. melanoscelus* on treated caterpillars 10 days after infection" (p. 437). Contributing to this increase in parasitism is the greater proportion of parasites to hosts, which increased the effectiveness of the parasite (p. 437).

Ticehurst *et al.* (1982) showed that although enhancement of parasitism of gypsy moth larvae by *A. melanoscelus* occurred as a result of *Btk* treatments, some subtle side effects were evident in other species of parasites (p. 1058). Dipel 4L was applied in 1980 at a concentration of 19.8 BIU/ha in 9.35 L/ha, a dosage one-half the commonly used rate (p. 1058). Insect data was collected and documented for 1980 and 1981. In 1980, parasitism by *A. melanoscelus* was enhanced from 4.4% in control blocks to 32.3% in Dipel-treated blocks; *Phobocampe unicincta* (Gravenhorst) (Hymenoptera: Ichneumonidae) and *Parasetigena silvestris* (Robineau-Desvoidy) (Diptera: Tachinidae) were unaffected by *Btk*. However, parasitism was depressed in *Compsilura concinnata* (Meigen) (Diptera: Tachinidae) from 2.5% to 0.2% in 1980 (p. 1061). Parasitism by *Blepharipa pratensis* (Meigen) (Diptera: Tachinidae) decreased from 15.5% to 0.1% during that same year. In 1981, parasitism by *P. unicincta*, *C. concinnata*, and *B. pratensis*, and *P. silvestris* was unaffected, while a slight increase was again observed in *A. melanoscelus* (p. 1061).

The observed enhancement of parasitism in *A. melanoscelus* was most likely the result of a developmental lag in infected gypsy moth larvae, allowing more time for the appropriate instar larvae to be parasitized (Ticehurst *et al.*, 1982). Reduction of parasitism in *B. pratensis* may have been the result of the feeding suppressant action of Dipel which could have limited the ingestion of parasite eggs by the host. An unfavorable parasite to host ratio for *C. concinnata* could have been responsible for the decrease in parasitism by this insect (p. 1061). When used according to recommended dosages, taking into account the control benefits of parasites, Dipel may actually enhance the activity of some gypsy moth parasites and thus be more effective than parasite or insecicide alone in pest control (p. 1061-1062). However, its subtle effects on other parasite species may upset the population balance of parasite and host, thereby depressing parasitism in some of the less common natural enemies.

Other authors have documented the sensitivity of several enemies of the gypsy moth to sprays of *Btk*. Ahmad *et al.* (1978) showed that at all concentrations of Dipel HG (supplied by Abbott Laboratories, 4320 IU/mg) parasitism by *A. melanoscelus* effectively increased mortality of gypsy moth larvae, although the differences in mortality between parasitized and unparasitized larvae were greater at lower concentrations (p. 74-75). At 9.375 IU/mg of diet, mortality of parasitized larvae was 90.0%, while that of the unparasitized larvae was 54.0%. At 75.0 IU/mg, respective mortality values were 98.7% and 94.0%. Mortality of untreated larvae was increased from 26.0% to 68.7% (p. 74). The mortality of the combined treatments was less than the sum of that for individual treatments, due in part to the overlap of effects of *Btk* and the parasite. Mortality of gypsy moth larvae due to *Btk* began as soon as the insecticide had been ingested and continued for 28 days, while the effects of the parasite, *A. melanoscelus*, began approximately 15 days after treatment and continued for a total of 28 days, during which time larvae could have been attacked by either *Btk* or parasites (p. 74-75). $LC_{50}$s and $LC_{95}$s, both 7 and 14 days after treatment, were not significantly different for parasitized and unparasitized larvae (p. 74).

The impact of spraying Dipel in a mixed forest of chestnut and white oak in New Jersey was evident in counts of benefical parasites monitored for 2 years following the treatment (Reardon *et al.*, 1979). The wettable formulation of Dipel was applied at a dosage of 8 BIU/0.4 ha on May 30th and June 6th in

1973, and several species of beneficials were collected in 1973 and 1974 (p. 306-307). Dipteran parasites collected were the tachinids *Blepharipa pratensis*, *Parasetigena silvestris*, *Compsilura concinnata*, and species from the family Sarcophagidae. Hymenopteran parasites were the ichneumonids *Phobocampe disparis* (Viereck), *Coccygomimus pedalis* (Cresson), *Theronia atalantae fulvescens* (Cresson), and *Itoplectis conquisitor* (Say); the chalcid *Brachymeria intermedia* (Nees); and the braconid *Apanteles melanoscelus* (p. 308). In 1973, the number of *B. intermedia* collected after treatment was significantly lower in the Dipel block than in the control area (p. 308). In 1974, *P. silvestris*, *C. concinnata*, *A. melanoscelus*, Sarcophagidae spp., and other species of braconids (not attacking the gypsy moth) were significantly lower in number in the Dipel than the control block (p. 309). The drastic effect of *Btk* on the host population (99% reduction) may have been responsible for the changes in parasite populations (p. 309). Some parasites are known to act in a density-dependent fashion and the high population reduction of hosts, as the authors themselves suggest, may have been responsible for this change.

Application of the gypsy moth NPV (nuclear polyhedrosis virus) causes epizootics resulting in a population collapse, and often suppresses subsequent outbreaks for several generations as a result of treatment (Webb *et al.*, 1989, p. 1695). The interaction between aerially applied NPV and natural enemies of the gypsy moth will determine the extent to which the target insect population is controlled, both in the treatment year and subsequent years. Naturally occurring NPV populations (not a result of NPV applications) have been considered as part of a natural gypsy moth control complex with *Apanteles melanoscelus*, and therefore a non-target beneficial potentially at risk when other control methods are implemented (Webb *et al.*, 1989, p. 1695). In a mixed hardwood forest in Maryland, application of Dipel to plots containing both naturally-occurring NPV and *A. melanoscelus* resulted in significantly greater parasitism by *A. melanoscelus*, but a significant decrease in the incidence of NPV, as compared to control plots (p. 1697). The application of Dipel 8L (40 BIU/ha in 9.5 L water/ha) may have reduced the host population to such a level that the natural NPV incidence decreased from a lack of available hosts (p. 1698). The incidence of viral infection is influenced by host density.

Conversely, Andreadis *et al.* (1983) do not consider reductions in the natural NPV populations as a result of *Btk* aerial applications indicative of an upset in the balance of natural controls. The authors compared the efficacy of highly concentrated single doses of Dipel 4L (32 BIU/3.8 L) with double applications at recommended rates. The single applications were at 12 BIU/0.4 ha and 16 BIU/0.4 ha, while the double application was two sprays of 8 BIU/0.4 ha eight days apart (p. 1417). Target larvae were collected weekly following application and reared in the laboratory to determine parasite emergence and the incidence of NPV (p. 1418). NPV incidence was significantly lower in the plot that received a double application of *Btk* than in the control block at 2 and 9 weeks after treatment, but did not differ from the control from 16 to 30 weeks after treatment. The other treatments did not differ from the control plot except the 12 BIU dosage which had a significantly lower NPV incidence at 9 weeks after treatment only (p. 1418). A correlation was drawn between the incidence of NPV and the availability of target larvae in the plots such that "untreated plots with the highest larval densities had the highest virus loads, whereas treated plots with small numbers of larvae also had fewer numbers infected" (p. 1420). Parasitism by *A. melanoscelus* increased from 0.6% in the control block to 12.6 in the double application block; all treatment plots had a significantly greater incidence of parasitism by this insect than the control block. No significant differences were observed in parasitism by *Parasetigena silvestris*, *Blepharipa pratensis*, or *Compsilura concinnata*, but significantly fewer *Brachymeria intermedia* were found in larvae from all treated areas than in the control plot, which was attributed to the fact that *B. intermedia* is not an effective parasitizer at low larval densities (p. 1420-1421). Because the reduced incidence of NPV was also attributed to a reduction in larval density and not to *Btk* itself, the authors suggest implementation of the virus with *Btk* in pest control programs (p. 1421).

### 3) Natural Enemies of the Tobacco Budworm

Dunbar and Johnson (1975) showed that a braconid wasp parasite,

*Cardiochiles nigriceps* Viereck, of the tobacco budworm, *Heliothis virescens*, was adversely affected, while the hemipteran predator *Jalysus spinosus* Say was unffected by *Btk*. The insects were exposed to Dipel WP (16,000 IU/mg) and Biotrol XK WP (7500 IU/mg) in food, through direct spraying, and through treated leaves (p. 352-353). Survival of Dipel-fed wasps was reduced from 2.4 to 1.5 days in one test, and from 3.5 to 1.3 days in a second test; Biotrol decreased survival from 3.5 to 1.8 days. Topical treatment of wasps with Dipel using a hand sprayer resulted in decreased life-span from 2.0 to 1.4 days in 1971, and from 6.0 to 4.4 days in 1972 (p. 353-354). The results of the topical treatment were not significant at a 5% probability level. Exposure of wasps to tobacco leaves treated with Dipel did not affect survival (p. 354). Numbers of *J. spinosus* exposed to Dipel-treated leaves showed no significant differences between untreated and treated trials 20 days after treatment, and it was concluded that Dipel on tobacco leaves did not affect this predator (p. 354). The experiment did not consider the possible effects which the oil carrier in the Dipel formulation might have on the insects.

Thoms and Watson (1986) found that Dipel (16,000 IU/mg) was detrimental to the immature endoparasite *Hyposoter exiguae* (Viereck) (Hymenoptera: Ichneumonidae) because of premature host

death (p. 178). Using tobacco budworm as a test host, it was shown that "parasitoids developing in infected *H. virescens* larvae demonstrated trends of decreased mean egg-larval developmental time and percentage pupation as the interval between parasitization and Dipel exposure was decreased. These trends indicate that premature host death was the probable cause of death for parasitoids developing in infected hosts" (p. 180-181). Durations of pupal and adult parasite stages did not differ significantly from controls, and therefore *Btk* was apparently non-pathogenic to the parasitoids (p. 181). Mortality of male *H. exiguae* fed Dipel (2 mg/mL sugar and water feed) for 7 days increased from 37.9% to 97.4% (p. 181). Mortality of male wasps fed autoclaved Dipel formulations did not differ significantly from controls, therefore the authors feel that the component causing mortality is thermolabile (p. 182). "The results of this study indicate that *H. exiguae* can survive exposure to Dipel. Immature parasitoids complete their development in [*Btk*]-infected hosts if hosts do not die prematurely. Adult parasitoids can ingest low concentrations of Dipel without significant mortality. Applications of [*Btk*] in field conditions would probably adversely affect immature *H. exiguae* more than adults, due to premature host death" (Thoms and Watson, 1986, p. 182-183).

Parasitism of the tobacco budworm, *Heliothis virescens*, by *Diadegma* species was apparently unaffected by treatment with *Btk* in field trials conducted in Cuba (Jimenez and Fernandez, 1985, p. 76). Preparations of *Btk*, called bitoxibacillin (BTB-202), dendrobacillin, and Dipel, were applied alone and in combination with Endosulfan 50 WP against the tobacco budworm. BTB-202 was applied at 1, 1.5, 2.5, and 4 kg/ha, as well as at 3 kg/ha combined with 0.48 kg/ha endosulfan. Dipel was applied at 1.5 kg/ha, as well as at 1 kg/ha with 0.48 kg/ha endosulfan. Dendrobacillin was applied at 3 kg/ha (p. 69). Parasitism in the treatment plots, which ranged between 31% and 80%, did not differ significantly from parasitism in the control plots. At recommended rates, endosulfan did not affect parasitism either (p. 76).

### 4) Natural Enemies of the Cabbage Looper

Yousten (1973) studied the effect of *Btk* (18,000 IU/mg) on the Chinese praying mantis, *Tenodera aridifolia sinensis* Saussure (Orthoptera: Mantidae), via ingestion of infected cabbage looper larvae, *Trichoplusia ni*. Looper larvae that had been fed a diet containing *Btk* (150 g *Btk*/mL of diet) were fed to the mantids in both 1-day and 5-day feeding trials (p. 312-313). "The results of these experiments indicate that the young praying mantis is not susceptible to the *Btk* spore-delta-endotoxin mixture when that material is consumed as part of the intestinal contents of cabbage looper larvae... the mantids ate about as many larvae as could be forced upon them and larger feedings would probably represent an unnatural situation, unlikely to be encountered in the field" (p. 313).

The striped earwig, one of the most important predators of the cabbage looper in crucifer crops, was shown to be unaffected by the presence of *Bt* Berliner (*Btt*) in the soil (Workman, 1977, p. 401). Field collected adult specimens of *Labidura riparia* (Pallas) (Dermaptera: Labiduridae) were placed in pint Mason jars with 113 mL (4 oz) of loamy sand common to areas in Florida where crucifers are grown. *Bt* was applied to the soil at a dosage of 10 lb A.I. in 100 gal of spray/acre (379 L/ha). Mortality was recorded 1, 2, and 5 days after the initiation of the experiment, but only the data for the second day is provided. After 2 days, no mortality of the earwigs had occurred as a result of *Btt* (p. 401). However, the authors suggested that the need for 6-8 seasonal insecticide treatments on cabbage may cause unexpected side effects to the earwig and other non-target insects in the spray area (p. 401).

In a field test on lettuce crops in Arizona, the use of *Btk* (HD-1 serotype, wettable powder supplied by Abbott Laboratories) sprayed alone at 11 BIU/acre, or in combination with a nuclear polyhedrosis virus (NPV) for *T. ni* at 5.4 BIU/acre, resulted in decreased parasitism of the looper as compared to untreated plots (Vail *et al.*, 1972, p. 783). In the *Btk*-NPV treatment, parasitism of collected target larvae was 15.4%, and that of the *Btk* only treatment was 0%. The control plots averaged 28.9% parasitism of larvae (p. 783). However, the insecticide treatments were sprayed weekly for a total of nine applications on one lettuce crop (p. 781). As well, this experiment was conducted in 1969, and although the variety used should have been free of beta-exotoxin, the authors suggest that "non-detectable amounts of exotoxin" may have been responsible for the reduction in parasitism (p. 784). Parasites reared from collected larvae were hymenopterans and dipterans, although species names were not given (p. 781).

### 5) Natural Enemies of the Cotton Leafworm

The cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), is a serious pest of cotton in Egypt with an important natural enemy complex that contributes to its control (Salama and Zaki, 1984, p. 485). Applications of Dipel (supplied by Abbott's in Belgium), at 500 g/feddan (1 feddan = 1.038 acres), did not adversely affect populations of cotton leafworm natural predators following treatment (p. 488). Adult *Coccinella undecimpunctata* Linnaeus (Coleoptera: Coccinellidae), a predator of both the leafworm and an aphid pest of cotton in Egypt, *Aphis gossypii* Glover (Homoptera: Aphididae), was found in lower numbers on treated plants than untreated plants following application. However, populations both in the treated and untreated plots were greater than pre-spray counts. Treated and untreated plots averaged 4.2 and 3.3 adults of *C. undecimpunctata* per hill (2 plants per hill) before *Btk* application, while averages were 4.0

and 7.1, respectively after treatment (p. 488). Differences between numbers of this parasite after treatment were attributed to the reduction in host populations (cotton leafworm larvae only) in the treated plot (p. 487). At four weeks after treatment, differences were not significant between treated and untreated areas. *C. undecimpunctata* larvae were unaffected by the treatments, most likely because they are predators of cotton leafworm eggs, which the authors showed were unaffected by *Btk* (p. 488). Other predators monitored were *Chrysopa carnea*, *Orius albidipennis* Reut, and *O. laevigatus* Fieb. (Hemiptera: Anthocoridae); *Scymnus interruptus* Goeze and *S. syriacus* Mars. (Coleoptera: Coccinellidae); *Paederus alfierii* Koch (Coleoptera: Staphylinidae) (p. 486). Populations of all of these predators were significantly lower in the treated than in the control plot up to 3 weeks after treatment in most counts. However, all predators were found in greater numbers after treatment than in pre-spray plots. Therefore, although the differences were significant between treated and untreated plots, the application of *Btk* did not reduce numbers below what they were before treatment. By 4 weeks after treatment, no significant differences existed between treatment and control populations (p. 488).

Salama *et al.* (1982) studied the effects of *Bt* var. *entomocidus*, a pathogen used in the control of the lepidopterous cotton leafworm, on one parasite and two predators. The parasite tested was *Microplitis demolitor* Wilk (Hymenoptera: Braconidae); the predators were *Chrysopa carnea* and *Coccinella undecimpunctata* (p. 498). Both survival of the parasites (cocoons) and adult parasite emergence were decreased in larvae fed on a diet containing *Bte* for 24 hours (concentration was 500 g/mL diet). Parasite emergence decreased from 92.4% in controls to 87.0% in treated larvae (it is unknown whether this is due to pre-mature host death or to toxic effects of the bacterium); cocoons of the parasite were collected from 87.1% of the control larvae while cocoons were collected from only 1.2% of the treated larvae. Furthermore, reproductive potential of surviving parasites was depressed from 84.0% to 62.0% in treated insects (p. 500). The data indicate that the parasite, *M. demolitor*, is adversely affected when the host larvae are fed on a diet containing *Bte*.

*Bte* prolonged the larval duration and depressed food consumption of the predator, *C. carnea*, when fed infected larvae of *S. littoralis* (Salama *et al.*, 1982). These changes in feeding and developmental behaviour were significantly different from the control parasites which were fed untreated larvae (p. 501-502). Similar results were observed when the predator *C. undecimpunctata* was fed treated aphids (*Aphis durantae*) (p. 502-503).

Salama and Zaki (1983), in a follow-up, studied the effects of *Bt* var. *entomodicus* on the parasite *Zele chlorophthalma* Nees (Hymenoptera: Ichneumonidae), and the predator *Paederus alferii* (Coleoptera: Staphylinidae) of the cotton leafworm, *Spodoptera littoralis* (p. 425). Results showed a significant decrease in parasitism of the host insect by *Z. chlorophthalma*, from 78.6% in controls to 19.8% in larvae fed on a diet containing 500 ug *Bte* per mL diet (p. 427). Egg incubation, and larval and pupal duration periods were increased in the parasite; reproductive potential was decreased from 56.8% to 41.4% in emerged adults (p. 427). The data suggest that *Bte* significantly affects parasitism of the cotton leafworm by *Z. chlorophthalma* (p. 426). Conversely, differences in feeding habit and longevity between *P. alferii* fed on the *Bte* diet and those fed control cotton leafworm, were statistically insignificant; thus, the authors concluded that this predator was not adversely affected when fed *Bte*-infected larvae.

The golden eyed fly, or green lacewing, *Chrysopa carnea*, a predator of the cotton leafworm and other lepidopterans, was tested for its sensitivity to *Bt* in the former USSR The English summary provided limited details about the methods of the experiment. However, in the summary, the authors reported that three preparations of *Bt* were non-toxic to larvae of *C. carnea*, but were toxic to the adult stage (Babrikova *et al.*, 1982, p. 45). *Bt* var. *galleria*, *Btk*, and *Btt* (dosages not given) caused up to 20% mortality among larvae of this predator, but mortality in the adults ("aged individuals") was as high as 75% (p. 45).

### 6) Natural Enemies of Other Lepidopterans

*Apanteles plutellae* Kurdj (Hymenoptera: Braconidae), an important parasite used to control the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), was found in higher ratios in an area treated with Dipel (IU not given) than in a control area in Malaysia (Lim *et al.*, 1986, p. 195). The parasitoid to host ratio was 3.22:1 in the treatment area (2.5 g/4.5 L was applied eight times at weekly intervals), and 0.64:1 in the control block, indicating that the use of *Btk* encouraged parasitism of diamondback moth (p. 195-196). In addition to increased parasitism, the population of adult parasitoids in the Dipel-treated cabbage field was greater than in the control field, while the population of diamondback moth was significantly lower in the treated field than in the control, indicating efficient control of the target pest without side effects to the natural parasitoid (p. 200).

Natural predators of the imported cabbageworm, *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae), and of the cabbage moth, *Plutella maculipennis* were similarly affected by field treatments of *Btk* (serotype HD-1) applied to control vegetable aphids in China (Lu *et al.*, 1986, p. 71). Chemical control methods employed in past efforts to control cabbage pests had deleterious effects on the predator populations while the target insects increased greatly in numbers. The application of *Btk* on affected areas, however, actually increased the numbers of natural enemies, including *Aphidius gizueusis*, *A. avenae*, *Calathus halersis*, *Pheropsophus jessoersis*, *Epistrophe balteata*, *Stenus cicindela*, and *Enigonidium gramincola*. Furthermore, the treatment did not affect the natu-

ral relationship between each predator and its preferred host and niche (p. 71). Specific data and results of this report are given in Chinese while the summary is given in English, therefore, only qualitative results are available.

A hymenopterous parasite, *Cotesia rubecula* Marshall (Coleoptera: Braconidae), of the imported cabbageworm, *Pieris rapae*, was detrimentally affected when its hosts were fed *Btk* (ABG-6167; 16.9 BIU/L) at a concentration of 850 IU/mL (McDonald *et al.*, 1990). This dosage is only one-tenth of the recommended field dosage, but successful pupation of the parasite was reduced by 75% as compared to controls. The lower tested concentrations of 85 IU/mL and 8.5 IU/ml reduced pupation by 31% and 24%, respectively (p. 423). Tipping and Burbutis (1983) tested the effects of *Btk* (Thuricide; 4,000 IU/mg) on *Trichogramma nubilale* Ertle and Davis (Hymenoptera: Trichogrammitidae), an egg parasite of the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae) (p. 892). Greenhouse tests were conducted, the results of which indicated no detrimental effects on parasitism by *T. nubilale*; in fact, parasitism was significantly higher in treated than untreated eggs in the 14-day and 21-day post-spray tests, which may also be related to a developmental-lag effect caused by *Btk* which allows for a greater time period in which eggs may be parasitized (p. 894).

*Bti*, *Btk*, and *Btt* were shown to be harmless to *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) when fed to adults at $5 \times 10^7$ spores and crystals/mL of solution over a 7-day period (Krieg *et al.*, 1980, p. 81-82). The capacity of *T. cacoeciae* to parasitize host eggs of the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Gelechiidae) was not significantly affected. The percent of parasitism by adults fed on *Btt*, *Btk*, and *Bti* was 89.3%, 98.7%, and 97.1%, respectively, as compared with 100% parasitism by control insects (p. 82).

Treatment of apple trees in Connecticut for control of the elm spanworm, *Ennomos subsignarius* (Hübner) (Lepidoptera: Geometridae), did not affect an important egg parasite, *Telenomus alsophilae* Viereck (Hymenoptera: Scelionidae) (Kaya and Dunbar, 1972, p. 1132). Dipel Wettable and Biotrol XK (dosages unknown) were applied at concentrations ranging from 0.10 to 0.64 lb/gallon of water. Thuricide HPC was applied at 0.40 and 0.80 pt/gallon water (p. 1133). All treatments were ground (knapsack) applied such that a total of 1.5 gallons of finished spray were applied to each tree (p. 1132). Egg masses heavily parasitized by *T. alsophilae* were collected from sprayed and control trees 5 days after treatment to determine parasite emergence and adult longevity. Parasite emergence in the control egg masses was 93%; emergence in all the *Btk* treatments was 95% or greater except for Biotrol at 0.64 lb/gal (81.2%) and Dipel at .20 lb/gal (60.7%). The reason for this is unknown, although the treatment concentrations were 10 to more than 20 times the recommended dosages for a hydraulic sprayer (p. 1132). In the 10 day monitoring period following adult emergence from treated egg masses, "little mortality" occurred, although this is not quantified by the authors (p. 1133). Egg masses were also collected from unsprayed trees 7 days after the experiment, were dipped in a solution of Dipel at 0.20 lb/gal or tap water (control), and were monitored for parasite emergence (p. 1132-1133). Emergence from the Dipel-dipped egg masses was 85.5% as compared with 84.1% in the control egg masses, suggesting that even submersion of eggs into *Btk* does not harm egg parasites. No mortality of adults was observed in the 10-day observation period following emergence (p. 1133).

Hamed (1979) found adverse effects on some parasites of the small ermine moth, *Yponomeuta evonymellus* Linnaeus (Lepidoptera: Yponomeutidae), when the parasite larvae were fed on a 1:1 honey and water mixture containing high dosages of *Btk*. Germination of the *Btk* spores did not occur in the honey and water mixture during the 6 days that it was administered to the parasite larvae. Two tachinids, *Bessa fugax* (Rond.) and *Zenillia dolosa* (Meigen), as well as one ichneumonid, *Trichionotus* sp., were not sensitive to *Btk* at a dosage of $10^8$ to $5 \times 10^8$ spores/mL. However, the hymenopteran parasites, *Diadegma armillata* (Gravenhorst), *Pimpla turionellae* (Bouche), *Ageniaspis fuscicollis* Dahlbom and *Tetrastichus evonymellae* (Bouche), were sensitive to *Btk* if they took up the spores with food sap (p. 294). Mortality of *D. armillata* larvae fed on Dipel at $5 \times 10^8$ spores/mL reached 96.97% after 8 days. Thuricide fed to *D. armillata* larvae at $5 \times 10^7$ and higher caused mortality at rates between 73.3% and 100.0% (p. 300). Male *D. armillata* were more susceptible to *Btk* than were females (p. 296).

Larval mortality for *P. turionellae* fed Dipel at day 6 was 30%, 37% and 85% for concentrations of $10^7$ spores/mL, $10^8$ spores/mL and $5 \times 10^8$ spores/mL, respectively. By the termination of the experiment at day 20, the cumulative mortalities had reached 66.6%, 92.6% and 95%, respectively (p. 303). Vegetative cells were found inside many cadavers of *D. armillata*, *P. turionellae*, *T. evonymellae* and *A. fuscicollis* larvae, indicating that replication of the bacterium had taken place. Adult *D. armillata* midguts were innoculated *in vivo* with *Btk* spores, the adults sacrificed at 18, 24 and 48 hours after innoculation and fixed in alcohol-Bouin's fluid. Sectioning of the adults showed that spore germination and midgut damage had occurred as a result of the introduction of *Btk* into the adult parasites (p. 306-307). Experiments conducted by feeding Btk to parasitized *Y. evonymellus* larvae showed that the number of parasites that emerged from the treated larvae was reduced (p. 304). A similar test done on the heteropteran predator, *Picromerus bidens* Linnaeus, resulted in no observable adverse effects (p. 294).

Two important hymenopteran parasites, *Apanteles glomeratus* Linnaeus (Braconidae) and *Pimpla*

*turionellae* (Ichneumonidae), experienced significant mortality in *Btk* feeding tests, but only at exceptionally high concentrations, and only after 14 to 18 days of continual feeding (Muck *et al.*, 1981, p. 303). Dipel (dosage not given) was orally administered at concentrations of $10^7$, $10^8$, and $10^9$ spores/mL to both parasites. The braconid suffered mortality of 39% and 100%, respectively at the two higher concentrations within 14 days of feeding, as compared with 9% in the controls. At the lowest concentration, mortality was 6% and 43% after 14 and 18 days respectively, compared to 9% and 23% in the control population. The authors suggest that this increase in mortality would not be important in field situations because the period of reproduction was finished at the end of the 18-day period (p. 303). In addition, the parasite is unlikely to encounter *Btk* in such high quantities in the field. *P. turionellae* was less severely affected by *Btk*: mortality was 13%, 18%, and 26% only after 18 days of continuous feeding of the three dosages, respectively. The control mortality was 2%. Normal field applications of *Btk*, constituting significantly shorter exposure periods and natural feeding habits, would be sufficient to avoid any deleterious effects of the insecticide on these parasites (p. 303). Moreover, *Btk* generally deteriorates naturally in 3 to 7 days in the environment, a time period much shorter than those used in the above feeding tests.

Temerak (1980) found that *Bt* (variety not specified) had deleterious effects on the survival of an ectoparasite of the pink borer larvae, *Sesamia cretica* (Led.) (Lepidoptera: Noctuidae) (p. 315). *Bracon brevicornis* Wesmael (Hymenoptera: Braconidae) (two females and one male) were kept in a vial with a single host larva injected with *Bt* (20 replicates of the host were used for each concentration), at a concentration of $3 \times 10^{16}$, $3 \times 10^{10}$, or $3 \times 10^4$ viable bacteria/mL. Data were collected on the number of immobilized larvae after 6 and 9 hours of association with the parasite, the number of deposited eggs during the first 24 hours, the number of formed cocoons, the number of emerged adults, and longevity of the parental parasitoid (p. 316). The numbers of immobilized larvae after 6 and 9 hours of contact increased with increasing concentration of *Bt*; thus, the bacterium enhanced parasitization of the larvae (p. 317). However, number of deposited eggs, number of cocoons, number of emerged parasite adults, and longevity of the adult parasite were all significantly reduced in *Bt* trials as compared with controls (p. 317-318).

Similar tests were conducted using a predator and a parasite of the Indianmeal moth, *Plodia interpunctella*, to determine the effects of *Btk* on these beneficial insects (Salama *et al.*, 1991, p. 245). Parasitic *Bracon brevicornis* was monitored for changes in egg deposition by adult females, larval duration period, number of formed cocoons, adult emergence, and adult longevity. The predator, *Xylocoris flavipes* (Reuter) (Heteroptera: Anthocoridae), was monitored for changes in nymphal duration, adult longevity and fecundity, and consumption of host larvae. In all tests, *Btk* (Dipel) was administered to host larvae, or to the beneficial insects at concentrations of 16, 32, 63, 125, 250, and 500 µg/g of diet, each insect being fed for 24 hours on the diet before beginning each experiment (p. 245). When two adult males and one adult female *B. brevicornis* were placed in vials with ten *Btk*-fed host larvae (parasitized hosts were removed every 24 hours and replaced with unparasitized *Btk*-fed hosts), some effects of *Btk* were observed. *Btk*, at 63 g/g diet or higher, resulted in significantly lower numbers of eggs deposited by the adult female as compared with the control and with the treatments at 16 and 32 g/g diet (p. 247). Percentage of egg hatching, percentage of formed cocoons, and adult longevity (both males and females) was signficantly lower for all treatments over the control (p. 247). Emergence of adults was significantly reduced at 250 and 500 g/g diet, while larval duration was significantly lower only at the highest concentration (p. 247).

Newly emerged adults of *B. brevicornis*, fed on a diet of honey containing *Btk* at the various dosages, were also adversely affected (Salama *et al.*, 1991, p. 248). The treated adults were fed on the diet for 24 hours and then placed in vials (two males and one female) with 10 untreated host larvae, similar to the previous experiment (p. 245). At all concentrations of *Btk*, egg deposition, egg hatching, emergence of adults, and male adult longevity were significantly reduced as compared with the control (p. 248). Percentage of formed cocoons was significantly lower at levels of 125, 250, and 500 µg/g diet, while no significant differences were observed in larval duration or female adult longevity as compared with the control (p. 248).

*Btk* also had observable effects on adult and nymph stages of the predator *X. flavipes* (Salama *et al.*, 1991, p. 249). When newly hatched nymphs were provided with *Btk*-fed host larvae as their only food source until the adult stage was reached, only female adult longevity was not affected at any level of *Btk* (p. 249). The number of consumed host larvae/nymph, percentage of hatching of deposited eggs, and male adult longevity were significantly reduced at all treatment levels. Nymph duration was reduced at dosages above 16 µg/g diet, and number of eggs produced per one female was affected only at the highest dosage (p. 249). Similarly, when adult predators were provided with *Btk*-fed host larvae as their only food source for the duration of their lives, the number of eggs produced per female was significantly reduced at all treatment levels (p. 251). Percentage of egg hatching was reduced at dosages above 16 µg/g diet, while adult female longevity was reduced only at the highest level (p. 251). Adult male longevity was unaffected by *Btk* (p. 251).

Wilkinson *et al.* (1975) investigated the effects of several insecticides, including *Btk* (Thuricide HPC) at a dosage of $8 \times 10^9$ IU/L, on parasites and predators of four lepidopterans (p. 114-115). The non-target insects were from the orders of Hymenoptera,

Diptera, Neuroptera, and Coleoptera; the host lepidopterans were from the families Gelechiidae and Noctuidae (see Appendix IV for species names of hosts and non-target insects) (p. 113). Organisms were exposed to *Btk* in laboratory tests at a concentration of approximately 5 L/ha, which is the minimum recommended field dose, and mortality readings were taken at 1, 2, 4, 16, 24, 48, 72, 96, and 120 hours after treatment (p. 115-116). The following parasites were tested: *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae), *Meteorus leviventris* Wesmael (Hymenoptera: Braconidae), *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcidae), *Voria ruralis* Fallen (Diptera: Tachinidae), and *Chelonus blackburni* Cameron (Hymenoptera: Braconidae). No mortality of any of the parasites resulted from exposure to *Btk*. Mortality was only 3.3% for each of the predator insects (*C. carnea* and *H. convergens*) (p. 115-116). The authors concluded that *Btk* had "little or no effect on beneficial insects" (p. 117).

No significant differences in longevity, egg deposition, and fecundity were observed in adults of *Pimpla instigator* (Fabricius) (Hymenoptera: Ichneumonidae), a general parasite of lepidopterans, when fed *Bt* Berliner (*Btt*; 4,500 IU/mg). Pupae resulting from infected lepidopteran hosts treated with *Btt* were not abnormally developed or adversely affected (Biache, 1975, p. 616).

### 7) Natural Enemies of Other Insects

Franz *et al.* (1980) gave *Btk* (Dipel, dosage not given) a rating of "harmless" in initial contact toxicity tests on some beneficial predators and parasites (p. 234). Dipel was applied at the highest concentration registered (0.10%) to the following test organisms: *Pales pavida* Meigen (Diptera: Tachinidae), *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae), Phygadeuon *trichops* Thomson (Hymenoptera: Ichneumonidae), *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae), *Coccygomimus turionellae* (Linnaeus) (Hymenoptera: Ichneumonidae), and *Chrysopa carnea* (p. 232). Qualitative ratings were based on an observable reduction of beneficial performance in comparison to untreated controls; a rating of "harmless" constituted a reduction in performance of less than 50% (p. 232). Pesticides which caused any mortality were rated as "harmful" (p. 233). All six tested species of non-target beneficials were affected minimally, if at all, and therefore Dipel was considered to be harmless to all of them (p. 234). Naton (1978) also showed that Dipel was harmless to the beneficial insect *Phygadeuon trichops* in laboratory assays (p. 136-137).

A study conducted in the Commonwealth of Independent States (formerly the Soviet Union) showed that some natural predators were susceptible to *Btk* formulations, while others were not (Kiselek, 1975, p. 23). Five *Bt* products (variety not given, although Dipel is *Btk*) were tested on beneficial insects: entobacterin, toxobacterin, dipel, exotoxin, and boverin. Insects tested were the predators *Cryptolaemus montrouzieri*, *Chrysopa carnea*, and *Coccinella septempunctata*, and the parasitoid *Trichogramma pallida*. Predators were provided food mixed with 1% of one of the *Bt* products: Sitotroga eggs to *Chrysopa*, coccidae to *Cryptolaemus*, and aphids to *Coccinella*. Entobacterin (1%) was sprayed on eggs of *Grapholitha molesta* parasitized by *Trichogramma*. According to the author, entobacterin and Dipel were "non-toxic" to larval *Cryptolaemus*, while exotoxin caused 1.7% mortality and toxobacterin caused 10% mortality of the predators. Boverin caused 50% mortality in the larvae. Control mortality was zero. Adult *Cryptolaemus* were unaffected by any of the *Bt* formulations over the 15-day feeding period (p. 23). Adult *Chrysopa* did not experience any mortality from feeding on infected prey, although the larvae were adversely affected by toxobacterin and exotoxin, which both had teratogenic effects. Boverin was harmless, while Dipel caused 20% and exotoxin caused 18% mortality, adjusted to account for natural mortality among control insects (p. 23). Larvae of *Coccinella* suffered 28% and 90% mortality among those insects exposed to entobacterin and exotoxin, respectively. No mortality was observed among adults. Entobacterin did not adversely affect emergence of *Trichogramma* from treated *Grapholitha* eggs (p. 23). It is likely that the preparations that caused high mortality among beneficial insects contained the exotoxin no longer found in North American *Bt* products, because the author states that "biopreparations containing exotoxin need further research" while the others do not cause any toxin effects on the studied predators and parasitoid (p. 23).

Horn (1983) documented the effects of *Btk* (Dipel, dosage not specified) on selected parasites and predators of the green peach aphid, *Myzus persicae* Sulzer (Homoptera: Aphididae). Dipel was sprayed onto collard plants weekly at a concentration of approximately $10^6$ spores/plant, and larvae from the families Chrysopidae, Coccinellidae, and Syrphidae were counted on each plant weekly (p. 209). Syrphidae larvae were significantly reduced on *Btk* treated plants as compared with control plants beginning in the sixth week of the experiment and continuing for 3 weeks (p. 210). No significant differences were observed in numbers of Coccinellidae and Chrysopidae between control and Dipel-treated plants throughout the experiment (p. 210). *Diaertiella rapae* (McIntosh) (Hymenoptera: Aphidiidae) showed no significant differences in parasitism between control and treated plants, nor were there any significant differences in parasitism of *D. rapae* by the secondary parasites *Asaphes lucens* (Provancher) (Hymenoptera: Pteromalidae) and *Aphidencyrtus aphidivorus* (Mayr) (Hymenoptera: Encyrtidae) between control and Dipel-treated collards (p. 210).

Applications of *Btk* alone or in combination with pyrethroids to apple orchards in Nova Scotia for control of the winter moth, *Operophtera brumata*, did not cause outbreaks of pestiferous mites, as did applications of pyrethroids alone at recommended dosages (Hardman and Gaul, 1990, p. 920). Dipel

(16,000 IU/mg, supplied by Abbott Laboratories) was mixed at concentrations of 16.0 and 33.0 g/100 L with low dosages of cypermethrin, deltamethrin, fenvalerate, and permethrin (10% of the 67.5 g A.I./ha recommended rate for winter moth control) (p. 921). Dipel alone was applied at 33.0 g/100 L for efficacy comparisons. Various pyrethroids were also applied alone at half-rate and full-rate dosages, concentrations that have been shown to decimate *Typhlodromus pyri* Sheuten (Acari: Phytoseiidae) populations, a natural predator of the European red mite, *Panonychus ulmi* Koch (Acari: Tetranychidae), and the apple rust mite, *Aculus schlechtendali* Nalepa), (Acari: Eriophyidae) (p. 920). The *Btk*-pyrethroid combinations effectively controlled the winter moth while preserving populations of *T. pyri* (p. 931). As a result, populations of the red mite and the apple rust mite were lower in these treatment plots than in plots treated with pyrethroids alone (p. 933).

Stewart *et al.* (1983) found no detrimental effects to predators of the mosquito larvae *Culex tarsalis* from *Bti* following aerial application of 1.1 kg/ha in 9.36 L water/ha for control of the target insect (p. 91). Control of *C. tarsalis* was achieved without any noticeable reduction of predator populations (p. 92-93). Selected test organisms included mayfly, damselfly, dragonfly, chironomids, corixids, notonectids, cladocerans, copepods, ostracods, *Tropisternus lateralis* (Fabricius) (Coleoptera: Hydrophilidae), several species of beetles from the family Dytiscidae (*Hygrotus* spp., *Laccophilus* spp., *Cybister* spp.), and mosquitofish, *Gambusia affinis* (p. 93).

Rhabditoid nematodes that infect a variety of insects, both soil-dwelling and stem/branch-inhabiting, are an effective biological control mechanism used in pest management programs (Poinar *et al.*, 1990, p. 195). The artichoke plume moth, *Platyptilia carduidactyla*, (Riley), for example, is effectively controlled by the nematode *Neoaplectana carpocapsae* (Bari and Kaya, 1984, p. 225). Infective nematodes are also naturally occurring organisms in the soil of many habitats, and therefore contribute to the control of various organisms. *Heterorhabditis* and *Neoaplectana* species of nematodes are symbiotically associated with a bacterium, *Xenorhabdus* spp., which causes a fatal septicemia in certain insects when released by the infective nematode in the host (Poinar *et al.*, 1990, p. 196). Compatability of the nematodes with commercial varieties of *Bt* is necessary if the two control methods are to be used together in integrated pest management programs.

Poinar *et al.* (1990) tested the compatability of three varieties of *Bt* with two species of nematodes, both at the infective and the developing stages (p. 196). *Btk* (Dipel at 32,000 IU/mg, from Abbott Laboratories), *Bti* (ABG-6193 at 1200 ITU/mg, from Abbott Laboratories), and *Bt* var. *san diego* (M-ONE at 22,500 Colorado Potato Beetle IU/mg) were each mixed with the A11 strain of *Neoaplectana carpocapsae* (300,000 infective juveniles/mL) and the HP88 strain of *Heterorhabditis heliothidis* (120,000 infective juveniles/mL) (P. 196). *Bt* var. *san diego* is a subspecies of *Bt* with insecticidal qualities for certain Coleoptera. All *Bt* formulations were diluted with sterile water, *Btk* at 1:170 (which gave $3.12 \times 10^9$ spores/mL), *Bti* at 1:80 ($21.2 \times 10^8$ spores/ml), and *Bt* var. *san diego* at 1:50 ($4.8 \times 10^8$ spores/mL). Approximately 1,000 infective stage juveniles of each species of nematode were placed in 4 mL of each of the *Bt* solutions at room temperature for 48 hours, at which time mortality counts were taken and nematode efficacy was tested (p. 196-197). Control mortality (nematodes placed in tap water) ranged between 0% and 11%, while mortality of all of the *Bt* formulations ranged between 0% and 13%. All surviving nematodes, when tested against larvae of the greater wax moth, *Galleria mellonella*, were infective (p. 198).

Poinar *et al.* (1990) also tested the effects of *Bt* on developing nematodes in host larvae by infecting susceptible hosts with *Bt* and nematodes simultaneously (p. 197). *G. mellonella* larvae, susceptible to both *Btk* and the nematodes, were injected with 10 L of the Dipel solution ($3.12 \times 10^7$ spores/injection) and placed on filter paper containing 2 mL suspensions of either the A11 or the HP88 strain of nematode (600,000 and 240,000 infective stages, respectively) for 5 days. For comparison, host larvae were placed in contact with each nematode species for 24 hours before injecting them with *Btk*. Elm-leaf beetle larvae, *Pyrrhalta luteola*, were injected with 5 L of *Bt* var. *san diego* ($2.4 \times 10^6$ spores/injection) and placed on 2 mL solutions of each of the nematodes (same concentrations as with *G. mellonella*) for 5 days (p. 197). *Culex tarsalis* larvae were placed in a 30 mL solution of *Bti* containing $1.3 \times 10^8$ spores with the A11 strain or the HP88 strain at 17,700 and 7100 infectives, respectively, for 5 days (p. 197).

Mortality of hosts in all controls was 100%; nematode development, along with the associated *Xenorhabdus* sp., was normal in all infected control larvae except in *C. tarsalis*. Both organisms were present in the beetle and the lepidopteran hosts (Poinar *et al.*, 1990, p. 201). Only 25% of the greater wax moth larvae showed signs of nematode development of the A11 strain (although no *Xenorhabdus* was present), and no nematode development was detected in larvae infected with the HP88 strain. Those nematodes that did develop were smaller than normal, had fewer food reserves stored in intestinal cells, and had *Btk* spores in the intestinal lumen. Normal nematode development occurred when *Btk* injection was delayed 24 hours after infection of hosts with nematodes (p. 201). Nematodes were found in only 10% of the *C. tarsalis* larvae kept in the *Bti* solution. In both the control larvae and those infected with nematodes in the *Bti* solution, nematode development was terminated prematurely because of host disintegration. Nematode development, and the presence of *Xenorhabdus* bacteria, was evident in 6 of the 10 beetle host larvae injected with *Bt* var. *san diego* (p. 201). The results indicate that the use of *Bt* and nematodes in

integrated pest management programs must be carefully planned to ensure that competition for host larvae between *Bt* and *Xenorhabdus* bacteria does not occur. To avoid this situation, *Bt* could either be sprayed at least 24 hours after the application of nematodes to a population of insects susceptible to both controls, or non-competing varieties of *Bt* and nematodes could be applied to the same ecosystem but against different target insects (p. 202). These results are consistent with Bari and Kaya (1984) who report that efficacy of *N. carpocapsae* applied in combination with *Btk* to control the artichoke plume moth was decreased in comparison to treatments with the nematode alone (p. 227). Efficacy was reduced by as much as 14% for mortality rates of II, III, and IV instar larvae treated with both biocontrol agents (p. 228).

Other species of nematodes were effectively mixed with *Btk* in a field trial to control the spruce budmoth, *Zeiraphera canadensis* Mutuura and Freeman (Lepidoptera: Tortricidae) (Eidt and Dunphy, 1991, p. 379). Although the *Btk* itself did not contribute to the control of the pest insect, the formulation used (Futura XLV) contained the appropriate antidesiccants and wetting agents needed to ensure the effectiveness of the nematode (p. 381). The larvae of *Z. canadensis* are difficult to control because they are not reduced significantly by contact insecticides or by ones that must be ingested such as *Btk* due to the cryptic nature of the insects (p. 379). However, soil and foliage treatments with *Steinernema feltiae* and *S. carpocapsae* were effective in reducing larval populations when mixed with *Btk*, yet the *Btk* itself did not interfere with the ability of the nematodes to infect the larvae (p. 382-383).

*Btk* has also been mixed with a naturally occurring ubiquitous fungus, *Beauveria bassiana* (Balsamo) Vuillimen, to suppress populations of the corn borer, *Ostrinia nubilalis* (Lewis and Bing, 1991, p. 387). Both *Btk* and the fungus are toxic towards the corn borer, *Btk* being effective for immediate suppression, and the fungus being effective for long-term control (p. 388). When combined and applied to a field corn, no interactions occurred between *Btk* (Dipel WP at 16,000 IU/mg) and *B. bassiana* (AGB-6178). Efficacy of the combined insecticides was not enhanced, nor was it inhibited in any way as a result of interactions (p. 392). For this reason, the authors suggested that these two control methods can be used simultaneously for effective control of the corn borer over the growing season (p. 392).

# H) EFFECTS ON AQUATIC AND MARINE FAUNA

Concern has been expressed by some members of various environmental and other civic groups that the use of *Bacillus thuringiensis* var. *kurstaki* in forest insect control may present a potential hazard to aquatic fauna. The main concern is that many areas treated for control of various defoliators contain streams and lakes into which *Btk* droplets may, and probably do, fall during aerial spraying. Another concern is that precipitation run-off from treated areas that do not contain streams may still contribute to the presence of *Bt* in fresh and salt water. The question of the environmental impact and risk of *Btk* application for forest insect control to marine and aquatic organisms was considered and investigated by a number of studies (Kreutzweiser *et al.*, 1992; Perrin and Richardson, 1993; Eidt, 1985; Morris, 1982; Forsberg *et al.*, 1976; Alizieu, 1975; Kingsbury and Sarrazin, 1975; Buckner *et al.*, 1974; Mastri *et al.*, 1970; Stephens *et al.*, 1970; Feng, 1966; Todd and Jackson, 1961).

### 1) Effects on Aquatic Vertebrates

The potential danger of *Btk* spray treatments to fresh water fish species pertains to the ingestion of moribund or dead larvae of aquatic insects, or those larvae which may drop from treated trees overhanging streams and lakes. There is concern that the ingestion of vegetative *Btk* cells in this manner could cause infection or side effects in fish and could result in fish kills in spray areas. Although this may be a possibility, no fish kills have ever been documented for any of the major spray programs during the past three decades of *Btk* use either in forestry or in agriculture in either the United States or Canada (Ellis, 1991, p. 21). Several million hectares of forestry land across North America have been sprayed for spruce budworm, gypsy moth, and jack pine budworm control since the mid-1970s without any reports of side effects to fish, either directly through contact with the insecticide and carrier ingredients in waterways, or indirectly through ingestion of infected larvae (Ellis, 1991, p. 21).

The impact of aerially applied *Btk* upon fish populations was studied by Buckner *et al.* (1974) in an area of Algonquin Park, Ontario that contained a portion of the Opeongo River that was treated with Thuricide 16B (4 BIU/quart) to control spruce budworm, *Choristoneura fumiferana* (p. F3). SCUBA divers made observations and counted individuals of several species of fish during pre-spray sampling procedures at two stations along the river, and populations were monitored for 4 weeks by the same method following treatment (p. F9). No abnormalities in fish behaviour or populations of brook trout, *Salvelinus fontinalis*, white suckers, *Catostomus commersoni*, and smallmouth bass, *Micropterus dolomieui*, were observed 3 days and 1 month after treatment (p. F65). Buckner *et al.* (1974) concluded that no ad-

verse effects of the *Btk* spraying were exhibited by the fish populations in the Opeongo River (p. F67).

During a field application of *Bt* (presumably *Btk*) over a Quebec stream to control spruce budworm, fish eggs and fry were observed and monitored to assess the impact of the treatment (Kingsbury and Sarrazin, 1975, p. 81). The *Bt* formulation is not given, but the product was applied at a dosage of 7.13 BIU/ha, which is equivalent to 280 g/ha. Although specific data are not provided, the authors stated that fish eggs present in the treated stream developed normally, and many fry were observed in the samples taken 6 days after treatment (p. 82).

Morris (1982, p. 280) cited reports by two authors (the original reports could not be obtained) on the effects of *Bt* (variety not specified) towards several vertebrates. Morris (1982), citing Mastri *et al.*, 1970 and Stephens *et al.*, 1970 stated that rainbow trout, *Salmo gairdnerii*, and bluegills, *Lepomis macrochirus*, were "not affected by heavy doses of *B. thuringiensis* in laboratory tests".

Todd and Jackson (1961) conducted an experiment on the safety of *Bt* (variety unspecified) to coho salmon in a stream in an untreated area following the chemical insecticidal treatment on Moresby Island for blackheaded budworm, *Acleris variana*, in 1959 (p. 15). Sixty native coho fry were placed in each of two liveboxes within two small streams prior to treatment, and bottom samples were taken (p. 20). Two areas were sprayed with *Bt*, one at a rate of 2.7 lb in 1.8 U.S. gallons of fuel oil per acre, and the other at 4.0 lb of Thuricide in 2.7 gallons of fuel oil per acre (p. 26). No mortality or distressed behavior was observed in the tested fry, nor any difference in dry weight of bottom organisms sampled 1 week before and 1 week after treatment (species names of bottom organisms are not provided). Furthermore, no observable effects were noticed in resident fry (p. 26). The authors concluded that "*Bacillus thuringiensis* [applied] in two concentrations to plots of immature hemlock apparently caused no mortality to resident coho fry or to aquatic insects upon which they feed" (p. 28). It is worth noting that the two doses of *Btk* were applied in 1.8 U.S. or 2.7 U.S. gallons of fuel oil per hectare, respectively, and even with this oil carrier and the high probability that the *Bt* used contained beta-exotoxin, bottom organisms (unspecified) were not affected.

### 2) Effects on Aquatic Insects and Other Invertebrates

Several species of aquatic insects were tested to determine if *Btk* would have any adverse effect on these organisms when applied near water ways in insect abatement programs (Kreutzweiser *et al.*, 1992, p. 252). A total of 16 insect species from the orders Ephemeroptera, Trichoptera, Plecoptera and Odonata (Appendix V) were tested, and both direct mortality and drift effects of *Btk* on these insects were monitored. Dipel 8AF (16.9 BIU/L; Abbott Laboratories) was added to circulating water in test aquaria at a concentration of 600 IU/mL, a dose estimated to be 100 times the expected environmental concentration in 50 cm of water as a result of direct aerial spraying of *Btk* at a rate of 30 BIU/ha (p. 253). Mortality of the non-target organisms was determined by exposing the test insects to *Btk* for a 24-hour period, after which the tanks were flushed of the treated water and recirculated with fresh water. Drift effects of *Btk* were investigated at a streamside test system constructed at Icewater Creek Research Area near Sault Ste. Marie, Ontario. Water from Icewater Creek was diverted through artificial channels containing natural substrate, and *Btk* was dripped into the lower portions of these channels. The upper regions of the artificial channels served as control units.

In this test, *Btk* was administered to the stream for a 24-hour period, which "more closely resembles the transient nature of pesticide contamination in streams than conventional 48 or 96-hr exposures" (Kreutzweiser *et al.*, 1992, p. 254). No significant mortality was observed among most of the insects treated within the aquaria up to 9 days after treatment (most showed less than 5% mortality when corrected for control mortality). However, one species of Plecoptera, *Taeniopteryx nivalis*, showed an average of 30% mortality, significantly higher than the mortality in the control, at the end of the 9-day observation period (p. 255). No adverse effects of *Btk* treatment in the diversion channels was observed. Both levels of drift, and direct mortality were not significantly different from those levels within the control areas (p. 255-257). Although mortality of *T. nivalis* was significant in the laboratory tests, the level of *Btk* applied in the water was 100 times higher than what would normally be found in water ways following aerial treatment of an area for control of insect pests. A similar study was conducted at Muir Creek near Sooke on Vancouver Island, B.C., in August of 1992. Fifteen independent flow-through flumes were constructed and a gravel substrate placed in the bottom of each. Water from Muir Creek was diverted into the flumes for 6 weeks prior to the beginning of the experiment to allow for colonization of the gravel by benthic insects (Perrin and Richardson, 1993, p.1). *Btk* was administered to the flumes to investigate both the mortality and the drift effects this application would cause within an aquatic insect community comprised of *Baetis* sp., *Paraleptophleba* sp., *Corynoneura* sp., Chironomini, Orthocladiinae and Tanypodinae (p. 2). Five flumes were treated with Foray 48B at 50 BIU/ha, the maximum recommended rate for aerial application against gypsy moth (and almost double the rate recommended for spruce budworm), five were treated with Foray 48B at a dose greater than 100 times (5,000 BIU/ha) the maximum recommended rate, and five served as controls.

*Btk* was released directly into the flumes continuously over a 2.5 hour period to mimic a "worse case" scenario of *Btk* contamination, even at the lower level (50 BIU/ha) of application (Perrin and

Richardson, 1993, p. 2). Within the 3 hours following the start of the application, there was a significant increase in the drift of *Baetis* sp. (5-15 individuals in 3 hours). This represented 3.7% of the individuals, over double the numbers in the control (1.19%). The authors concluded that the drift was a response of *Baetis* sp. to increased turbidity following the application, not to any adverse effects caused by the *Btk* applications. Results of the adult emergence and benthic community studies showed that *Btk* treatment of the streams, even at the higher level of 5,000 BIU/ha (which mimics an accidental spill), did not adversely affect the abundance and composition of the benthic insect community (p. 2).

In an earlier study, Buckner *et al.* (1974) conducted an environmental impact study to determine whether any mortality of non-target aquatic organisms was attributable to the aerial application of *Btk* against spruce budworm, *Choristoneura fumiferana*, in Algonquin Park, Ontario, and Spruce Woods, Manitoba (p. F1). Thuricide 16B (4 BIU/quart) was sprayed over a 2,500 acre plot in Algonquin Park that contained a portion of the Opeongo River (p. F3). Mortality of aquatic invertebrate populations were monitored at two different stations along the river (p. F9). A variety of aquatic insects were collected in the pre-spray samples including midge larvae (Diptera: Chironomidae), caddisfly larvae (Trichoptera), mayfly nymphs (Ephemeroptera), stonefly (Plecoptera), and beetles (Coleoptera). Other aquatic invertebrates collected during the pre-spray samples included Turbellaria, Nematode, Oligochaete, Hirudinea, Amphipoda, Hydracarina, Gastropoda, Arthropoda, and Pelecypoda.

Buckner *et al.* (1974) found "There were no immediate effects of the bacterial spray on the bottom fauna populations at Station 1 or 2. Over the next four weeks the total number of benthic organisms per square foot at Station 1 declined steadily but this was directly attributable to the emergence of adult caddisflies, mayflies, and stoneflies" (p. F58). One result of the spray was a striking increase in the number of adult mayflies and midges captured in the drift net during the 2-hour treatment, and this was considered to be a direct result of physical knockdown by the spray (p. F59, F64). The authors feel that this effect was not much different from that of heavy rains; within one-half hour following the completion of the spraying, these insects were once again observed in swarms above the river (p. F65). Collections made by SCUBA divers of sponges, planarians, hydras, crayfish, clams, Trichoptera, Ephemeroptera, Plecoptera, Odonata, Coleoptera and Diptera showed no abnormalities in invertebrate populations both 3 days and 1 month after treatment (Buckner *et al.*, 1974, p. F65).

Water samples and examination of clams collected 30 minutes and 2 days after treatment in the same study revealed the presence of viable *Bt* spores; however, no spores were found when similar samples were tested 1 month after treatment. Crayfish specimens examined after treatment contained no spores, and survival times of crayfish in control blocks and treatment blocks were not significantly different (p. F65-66). A bucket of water exposed to the aerial application contained 22,800 spores/mL water, but this number was reduced to 7,800 spores/mL 2 months later after the water had been kept refrigerated in darkness (p. F66).

Overall, Buckner *et al.* (1974) concluded that the bottom fauna (including insects) suffered no adverse effects as a result of the *Bt* spray trial in the Opeongo River (p. F67).

Kingsbury and Sarrazin (1975) monitored aquatic fauna populations in a Quebec stream before and after an aerial application of *Bt* (variety not specified, but it is assumed to be *Btk*) conducted to control spruce budworm, *Choristoneura fumiferana*. *Bt* was sprayed at a low rate of 280 g/ha (7.13 BIU/ha) over a 1,600 ha area through which a fast-moving shallow stream flowed (p. 81). A control area, containing a similar stream, was also monitored for comparison (p. 80). Of those organisms monitored 6 days before and 6 days after the spray, only caddisfly larvae (Trichoptera) populations exhibited a significant decline. However, since this decline paralleled one which occurred in the control stream, it was determined that the decline represented a natural, rather than *Btk*-induced population reduction. Kingsbury and Sarrazin (1975) suggested the decline may be attributable to the emergence of adult caddisflies. Bottom fauna monitored are listed in Appendix VI (p. 86).

Eidt (1985) speculated that the application of *Bt* over large areas in the forest environment may present "a potential hazard to fish in lakes and streams through effects on their food organisms, most important of which are aquatic insects" (p. 829). To examine this potential threat, Eidt (1985) conducted tests to assess the impact of *Btk* on insect populations. Using Thuricide 32LV (*Btk*) at concentrations of 4.3, 43, and 430 IU/mL of water, where the lowest concentration represents the "worst case field situation", insects from the orders Trichoptera, Plecoptera, Ephemeroptera, and Diptera were placed in 300 mL of treated water for varying amounts of time (p. 830). The only test species that was visibly affected by the *Btk* application was *Simulium vittatum* (blackfly larvae) and only at the highest concentration. Based on the results, Eidt (1985) concluded that *Btk* poses no hazard towards fish-food organisms when used for the purpose of spruce budworm control at the label dose, i.e. 30 BIU/ha (p. 836).

### 3) Effects on Marine Vertebrates

Alzieu *et al.* (1975), in a study on the effects of *Btk* upon aquatic fauna, exposed young eels (elvers), *Anguilla anguilla*, to varying concentrations of Dipel (formulation not given, toxicity of 16,000 IU/mg) in seawater (p. 12). The young eels were placed in aquaria containing suspensions with concentrations of 0, 10, 25, 50, 100, 200, and 400 mg Dipel/L seawater, and mortality was monitored at 48 and 96 hours

(p. 12). A second series of experiments were run using the same concentrations of Dipel which had been filtered through high porosity paper (p. 13). At the highest concentration of unfiltered suspension, 10% of the eels were dead after 96 hours. No mortality was observed in any of the other treatments (p. 13). The authors considered this mortality insignificant because of the extreme level of exposure to *Btk*, levels that greatly exceed those encountered during field sprays (p. 20).

The excessive levels of *Btk* used for this experiment can be illustrated by the following calculation. 400 mg of Dipel (at a toxicity of 16,000 IU/mg) was added to each litre of seawater, or 0.4 mg of Dipel/ml. This equals 6400 IU/ml, a toxicity which is about 1488 times higher than the "worst case field situation", estimated by Eidt (1985) to be 4.3 IU/ml. This "worst case scenario" may represent the effects of a large spill in a small tidal pool. Wave action accompanying the rising tide would quickly dilute any such spill below the concentration which would have any effect on the fish. In addition, the Dipel used was a wettable powder; therefore, at 400 mg/L the particulate matter in the unfiltered suspension itself would have contributed to the 10% mortality rate of the eels. This was confirmed by the absence of mortality of eels exposed to a filtered suspension containing the same concentration of Dipel.

**4) Effects on Marine Invertebrates**

The effects of *Btk* spraying for control of lepidopterous pests on marine fauna was evaluated in the laboratory using mussels, *Mytilus edulis*, oysters, *Crassostrea gigas*, winkles, *Littorina littorea*, brine shrimp, *Artemia salina*, and brown shrimp, *Crangon crangon* (Alzieu *et al.*, 1975). Acute toxicity was determined for lots of 14 animals immersed for 48 and 96 hours in solutions containing 0, 10, 25, 50, 100, 200, and 400 mg Dipel WP (wettable powder, 16,000 IU/mg) per litre of seawater (p. 12). At the highest concentration (1,488 times the amount in "worst case" scenario), no mortality was recorded for the winkles, brown shrimp and the oysters. However, after 48 hours at this concentration, 50% of the mussels lost their fixation ability, and after 96 hours, 29% of the mussels and 90% of the brine shrimp had died (p. 13).

Similar tests were performed using solutions that had been filtered through high porosity paper to remove insoluble particles (Alzieu *et al.*, 1975, p. 12). The Dipel filtrate (at highest concentration) did not cause mortality to any of the animals except the brine shrimp (10%) after 96 hours. At this level, 14% of the mussels had lost their fixation ability at 48 hours, but no mortality recorded (p. 13). No abnormal mortality rates were observed for 77 days following the removal of the animals from the suspensions and placement into clean seawater. The effects of *Btk* on brine shrimp was attributed to a "mechanical effect" of the insoluble fraction of Dipel (p. 13).

Alzieu *et al.* (1975) conducted further tests on the sublethal physiological effects of *Btk*, specifically, the effects on the valvular activity of oysters (p. 13). The oysters were placed in suspensions containing 100 or 200 mg Dipel WP/L seawater, doses that are 372 and 744 times greater than those used during aerial sprays (see previous calculation)(p. 13-14). Valvular activity was monitored for 48 hours for any changes indicating accelerated opening and closing of the valves, or a cessation of valve movement, presumably to avoid the suspension. The presence of Dipel did not affect the valvular activity of the test animals in any way, indicating no short-term effects of *Btk* to oysters (p. 14).

Bioaccumulation of *Btk* in oysters resulting from a severely polluted environment was investigated to determine the ability of the contaminated oysters to remove *Btk* once returned to an unpolluted habitat (Alzieu *et al.*, 1975, p. 14). Young oysters were immersed in seawater containing 20 mg Dipel WP per litre of water for 24, 48, 72, and 96 hours, corresponding to an oyster bed in 0.5 m water following aerial treatment with 100 kg Dipel/ha (normal application rate varies between 0.5 and 1 kg/ha) (p. 14). Oysters were removed to clean water, and tissue samples were analyzed after 1, 2, 3, 4, 7, and 10 days in clean water (p. 15). Oysters subjected to Dipel for 24 hours were able to remove 94% of the organism within 3 days, while those immersed for 96 hours removed 82% of *Btk* within 10 days (p. 15). The longer the oysters were exposed to the contaminant, the more time they required to eliminate it from their systems, but successful elimination occurred when the oysters were only exposed for 48 hours or less (p. 16).

In the conclusion, the authors suggested that the effects of *Btk* on marine organisms are minimal (Alzieu *et al.*, 1975, p. 16-17). *Artemia salina* appeared to be sensitive to *Btk* at very high concentrations; however, it must be noted that the $LD_{50}$s for *A. salina* at 48 and 96 hour are 85 and 65 mg/L, respectively. These quantities of Dipel used were determined to be 300 to 400 times higher than those found in a 0.5-m-deep oyster bed accidently treated with 1 kg/ha of Dipel (p. 13, 16). Comparatively, DDT is 1,000 times more toxic to crustaceans and 10,000 times more toxic to molluscs than *Btk* (p. 17).

Feng (1966) studied the effects of *Bt* (variety not specified) on the marine oyster, *Crassostrea virginica*. Spores and vegetative cells ($2.5 \times 10^7$ *Bt* in a 1:1 ratio of spores and cells) were injected intracardially, and blood and tissue samples were monitored at intervals for the next 10 days. The bacterium was rendered non-viable by the oysters, the *Bt* was rapidly removed from the organism's system, and none of the specimens suffered any adverse effects of the insecticide (p. 505, 509).

# I) EFFECTS OF INERT INGREDIENTS

Few investigations have been conducted to determine the potential side effects of the inert ingredients or carriers used in the various *Btk* formulations. Specific information about adjuvants of *Btk* products is not required on product labels because it pertains to the formulation and not the active ingredient, yet some toxicity has been associated with the use of these carriers (Orton, 1987, p. 30). Concerns have been expressed over the potential health and environmental problems that may be associated with *Btk* sprays stemming solely from the inert ingredients (p. 31). The safety of *Btk* to the environment and its inhabitants cannot be fully assessed unless the potential toxicity of the whole product, not just the active ingredient, is thoroughly investigated.

Ellis (1991) in his review states that although information pertaining to the toxicity of standard inert carriers is proprietary, and therefore not publicly available. However, Federal agencies such as Health and Welfare Canada, Agriculture Canada, and Environment Canada, agencies responsible for reviewing toxicological data, are provided with this proprietary information by the companies submitting a new product for registration (p. 9). Presumably, if a product has attained registration through the Pest Control Products Act of Canada, the potential side effects of the inert ingredients are either nil or must be at levels low enough to satisfy the federal standards. Similar safety standards must be met by products registered through the United States Environmental Protection Act, although specific levels of allowable toxicity differ from Canadian standards (Melin and Cozzi, 1990, p. 161).

The safety of carrier ingredients has been supported by a Canadian study involving the product Dipel 88 (Morris, 1983b). The Dipel vehicle (Abbott Laboratories), applied at a concentration of 9.4 L/ha, was diluted to 25% to approximate the amount present in Dipel 88 when applied at 20 BIU in 9.4 L/ha (p. 1002). The carrier had no effect on spruce budworm, *C. fumiferana*, larval density. Pupal emergence was not significantly different from the control plot, and larval reduction following the spray was nil for both the control and the carrier-treated plot (p. 1003). No toxicity towards spruce budworm parasites was observed as a result of the carrier (p. 1006).

In another study, a Moellman spray chamber was used to test the toxicity of the oil carrier found in Dipel 4L against the predatory green lacewing, *Chrysopa carnea* Stephens (Neuroptera: Chrysopidae) and the lady beetle, *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae), as well as a parasitic aphelinid, *Aphytis melinus* De Bach (Hymenoptera: Encyrtidae) (Haverty, 1982, p. 337). Three aerosol spray treatments were applied: treatment 1 was water only, treatment 2 was carrier and water (1:3), and treatment 3 was carrier and water (1:3) at twice the volume of treatment 2. Treatment 2 replicated an application rate of 9.4 L/ha (the optimal rate of Dipel 4L applied aerially over forested areas) while 18.7 L/ha were applied in treatment 2 (represented the maximum rate of application) (p. 338). Twenty larvae of each of the non-target insects were exposed to the treatments in the spray chamber for 60 seconds, and up to 20 replicates were used for each treatment (p. 337-338). In the spray chamber, the amount of solution that reached the insects at the optimal application rate (9.4 L/ha) was more than four times that which would be expected to reach the insects under normal field application. At the maximum application rate (18 L/ha), this quantity was double the amount expected to reach the insects in the field (p. 338). In spite of this, mortality rates for all tested organisms, including larvae and adults, were generally low. Corrected mortality rates (i.e., mortality that resulted solely from the carrier), using Abbott's formula, ranged between 0.0 and 2.1% for treatment 1, 7 days after spraying. In treatment 2, corrected mortality ranged between 2.1 and 6.8%, except for adult *H. convergens* which had a corrected rate of 13.4% (p. 338). Significant differences from the control mortalities were present only in treatment 2 for *C. carnea* and *H. convergens* adults, both 3 and 7 days after spraying. The results suggest that the oil carrier in Dipel 4L may be potentially harmful to non-target insects *only* when sprayed at concentrations greatly exceeding the recommended levels (p. 338). Fortin *et al.* (1986), working with a different variety of *Bt* (*Bti*), found that brook trout became stressed when exposed to Teknar (p. 1669). Through experimentation it was discovered that the carrier xylene, not the *Bti* itself, which was causing the symptoms (p. 1669). Fortin *et al.* (1986) concluded that the sensitivity of organisms to various carriers depends upon the composition of the carrier and its concentration in each formulation. Another important factor is the method of exposure and possible route of entry into the body (p. 1670).

However, it should be noted that the carrier xylene is no longer used in Teknar.

# J) RESISTANCE AMONG TARGET INSECTS

Until 1990, *Bt* had been used extensively for over 30 years without any apparent signs of resistance among target insects in the field (van Rie *et al*, 1990, p. 72). Many of the people involved in the research and use of *Btk* (and other *Bt* varieties) assumed that if development of resistance were to occur, it would have become apparent and widespread by now (resistance to chemical insecticides became evident over a much shorter period of use).

Field application rates of *Btk* have increased from 8 to 20 BIU/acre (in most cases 20 to 30 BIU/ha, and up to 50 BIU/ha in some cases) between 1978 and 1990 in order to provide more repeatable results and more effective control of certain pests. This change, in part, was the result of more concentrated products and new, improved formulations. In spite of the increased dosages, some failures in the field (i.e. partial control) and inconsistant results occurred. It is not known if the reason for the partial control is due to delivery of an insufficient amount (sublethal dose) of *Btk* to the target insects, or to some other factor. Most researchers and operational users of *Btk* feel that it is the former.

Variations in the efficacy of *Btk* to some lepidoterans has been shown to exist in the laboratory where populations have been repeatedly treated with *Btk* (Rossiter *et al.*, 1990, p. 2211-2212). Resistance outside the laboratory has been documented for a few stored-product insect pests like the Indianmeal moth (McGaughey, 1985a, 1990), and the almond moth (McGaughey, 1990). Resistance has also been documented where there has been extensive use of *Btk* in a short period, like the case of the diamondback moth which was treated 50-100 times in 4 years (Tabashnik *et al.*, 1990), or in the laboratory when the researcher deliberately sets out to examine and document whether resistance to *Btk* can develop (Brewer, 1991).

Somewhat more obvious signs of resistance have surfaced in laboratory tests as a result of selection pressure on target insects. Recently, resistance towards *Btk* has been confirmed in experimental populations of Indianmeal moth, *Plodia interpunctella*, almond moth, *Cadra cautella* (McGaughey and Beeman, 1988, p. 28), and was also observed in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (McGaughey and Whalon, 1992), and in tobacco budworm, *Heliothis virescens*, towards *Pseudomonas fluorescens* (a soil bacterium) cells expressing the *Btk* delta-endotoxin (Stone *et al.*, 1989, p. 229-231). Resistance can be bred for in the laboratory. Practical examples of this are the development of honey bee and silkworm stocks resistant to economically damaging diseases (Burges, 1971, p. 450). Such evidence suggests that resistance is a possible potential problem associated with the heavy repeated and long-term use of microbial pesticides.

The mechanism of resistance among normally susceptible insects is not fully understood. Johnson *et al.* (1990) report that the ability to activate the *Bt* delta-endotoxin does not differ between susceptible and resistant populations of Indianmeal moth, but rather changes in the midgut itself may be the cause of resistance (p. 242). These authors suggested that the specific binding sites on insect cell surfaces no longer recognize and bind the toxin molecules "with the same proficiency as those present in susceptible strains" (p. 242-243). Ferre *et al.* (1991) have also suggested that resistant strains of diamondback moths, *Plutella xylostella*, differ from susceptible strains in a membrane receptor that recognizes and binds of one of the crystal proteins of *Btk* (p. 5119). The protein is unable to bind to the brush border membrane of midgut epithelial cells, because of either a reduced binding affinity, or because the receptor molecule was absent (p. 5119). Further investigation into the development and mechanism of resistance to *Btk* is continuing. Examples of pest resistance to *Btk* which have been published to date are summarized below.

### 1) Resistance in the Indianmeal Moth and Almond Moth

Recent concerns about signs of resistance have focused on the use of *Btk* to control larvae in grain storage facilities. In these confined environments the repeated application of *Btk* provides the selection pressure required for the development of resistant insects. Grain storage bins provide for "continuous breeding of isolated pest populations with all stages in constant contact with stable residual deposits of the insecticide " (McGaughey, 1990, p. 594). Of greatest concern is the increasing resistance to *Btk* observed in populations of Indianmeal moth, *Plodia interpunctella*, and almond moth, *Cadra cautella* Walker (Lepidoptera: Pyralidae) under laboratory selection pressure.

McGaughey (1985a, p. 1093) found variation in levels of control of Indianmeal moth populations: 50-60% of the Indianmeal moth in wheat and over 80% control in corn. McGaughey (1985a) attributed this variation in levels of control "...to the wide range of *Bt* susceptibility among moth populations and to the development of *Bt* resistance in populations in the treated bins". A small population continually survives to reproduce in the confines of storage bins, thus reducing the heterogeneity of future generations (McGaughey, 1985b, p. 194). Some resistance has been shown to appear in target insects within two or three generations when fed on diet containing large amounts of *Btk* (McGaughey and Beeman, 1988, p. 29; McGaughey, 1985b, p. 193).

Resistance to date is most evident in the Indianmeal moth collected and reared from native populations in treated and untreated grain storage facilities throughout the United States. It was shown that the $LC_{50}$s of *Btk* (Dipel wettable powder) were 25.1 2.0 mg/kg of diet and 20.7 1.7 mg/kg of diet

for insects taken from previously treated and untreated areas, respectively (McGaughey, 1985b, p. 193). The author stated that although this difference is insignificant, *Btk* appears to have had a selective effect on these insect populations. A laboratory experiment was conducted to determine whether resistance to *Btk* in the colony could be selected through rearing. During the course of the experiment, the survival of test insects was increased from 19% in the first generation to 82% in the fourth generation when larvae were fed a diet containing 62.5 mg (1,000,000 IU) of *Btk*/kg of diet, a dose expected to control larvae by 70-90%. After four generations, the survival rate was identical for the treated colony (68-89% survival) and the untreated colony (71-89% survival). After treatment for 15 generations, however, the amount of *Btk* required to achieve an $LC_{50}$ for the treated *P. interpunctella* colony was 97 times that of the average level of $LC_{50}$ for the untreated colony (p. 193). Similar results were obtained when the *Btk* dose was increased 8-fold to 500 mg/kg of diet. Survival increased from 32% in the first generation to 51% in the second and third, after which it fluctuated between 64% and 81% (p. 193-194). Three other colonies of *P. interpunctella* were similarly tested, and resistance among the insects appeared within two or three generations (p. 194). "The speed at which *P. interpunctella* developed resistance to *Bt* in this study suggests that it could do so within a single storage season in bins of treated grain" (p. 194).

McGaughey (1985b) reported that colonies of *P. interpunctella* selected through nine generations for resistance to *Btk* in the laboratory, when fed untreated diet for seven generations, showed no decrease in resistance to *Btk*. Furthermore, when virgin males and females of both the resistant (R) and susceptible (S) strains were crossed, progeny from both crosses (male R with female S, and female R with male S) were susceptible to normal doses of *Btk*, indicating that resistance to *Btk* is controlled by a single recessive gene (p. 194). $LC_{50}$'s of the progeny were 26.2 and 43.1 mg/kg of diet for the crosses of R females with S males and S females with R males, respectively. The $LC_{50}$ for the untreated susceptible laboratory colony was 27 mg/kg of diet (p. 194).

McGaughey and Beeman (1988) conducted similar tests, showing that resistance occurs in Indianmeal moth and almond moth when these insects are reared on *Btk*-infected food for several generations (p. 28). Five colonies of Indianmeal moth (from Iowa, Nebraska, Oklahoma and Illinois) and one colony of almond moth were fed diet containing 62.5 mg of *Btk* (Dipel WP at 16,000 IU/mg) per kg of diet (p. 29). Within four to seven generations survival of Indianmeal moth was greater than 70%, similar to the average survival rate for untreated or control specimens (i.e. reared on *Btk*-free diet). When the dosage was increased by 8-fold, survival decreased to 30-40%, but reached 70-80% again within seven generations (p. 29). $LC_{50}$s for one colony increased 100-fold in 16 generations, from 16.6 mg/kg to 1,433 mg/kg of diet (p. 29-30). McGaughey and Beeman (1988) also found that resistance was recessive in Indianmeal moth, because progeny of susceptible and resistant parent insects were found to be susceptible (p. 31).

Resistance among specimens of almond moth is less pronounced than for Indianmeal moth (McGaughey and Beeman, 1988, p. 31-32). *Btk* selection pressure achieved only a 7-fold resistance level, and $LC_{50}$s increased from 48.0 mg/kg diet after 10 generations to 121.2 mg/kg diet after 23 generations. Non-treated insects had $LC_{50}$s ranging from 15.2 to 31.2 mg/kg diet.

In all colonies of *P. interpunctella* and *C. cautella* tested by McGaughey and Beeman (1988), resistance appeared within two or three generations, but it progressed at different rates for each colony. Although native populations are thought to be naturally polymorphic for resistance genotypes, the rapid development of resistance within *small populations* of multivoltine insects (eg. the almond moth has 13.5 generations per year) in the laboratory suggests that resistance could occur within a relatively short time in the grain-storage bins, thus rendering the recommended application dose of *Btk* less effective against these important grain pests. Moreover, even though the trait is recessive, continual immigration of susceptible adults is necessary to ensure that these small populations do not become completely resistant to *Btk* (McGaughey and Beeman, 1988, p. 32-33).

This apparent potential buildup of resistance clearly indicates that the recommended dose and use of *Btk* for control should be carefully adhered to and the increase in resistance closely monitored. Also, it would be highly desirable, wherever possible, to alternate the use of *Btk* with some other environmentally safe control agent(s) to minimize or delay the development of resistance.

### 2) Resistance in the Diamondback Moth

The development of resistance in the field to *Btk*, as a result of extensive and repetitive pest control treatment, has been documented for the diamondback moth, *Plutella xylostella* (Tabashnik *et al.*, 1990, p. 1671). Sample populations of 50-300 insects were collected from six fields on a watercress farm in Hawaii that had been treated 50-100 times with *Btk* between 1978 and 1982, after which time treatment was suspended by the farmer. During 1986 and 1987, these repeatedly treated population were compared with five other populations, also in Hawaii, collected from areas treated less than 10 times prior to the comparison to determine if susceptibility of the insects to *Btk* had been altered due to the repeated treatment. In addition, both heavily (50-100 times) and lightly ( 10 times) treated populations were compared with laboratory strains that had been reared for 13 to 60 generations without exposure to any biological or chemical insecticide (p. 1672). Two formulations of Dipel (unspecified formulation at 16,000 IU/mg in 1986 and Dipel 2X at

32,000 IU/mg in 1987) were feed to test insects by dipping cabbage leaves in water formulations containing 0.256, 2.56, 25.6, 256, and 2,560 mg of *Btk*/L of distilled water. Recommended field rate is equivalent to 25.6 mg of *Btk*/L water (p. 1672).

The results showed that the heavily treated field population was significantly more resistant to *Btk* than the laboratory strains in that the $LC_{50}$s and $LC_{95}$s were approximately 6 times greater in the field population (Tabashnik *et al.*, 1990, p. 1673). The $LC_{50}$s for the laboratory strains ranged from 1.76 to 2.57 mg/L while the $LC_{50}$ for the resistant strain was 10.2 mg/L. $LC_{50}$s for the heavily treated population were significantly higher than those for the minimally (fewer times) treated field populations, which ranged from 1.56 to 6.72 mg/L; one of these minimally treated populations had a $LC_{50}$ of 11.9 mg/L, no explanation for this anomaly is given by the authors (p. 1673). Mortality rates of the populations were tested using the recommended field dosage of *Btk* (25.6 mg/L) applied onto cabbage leaves, the results showed that the heavily treated field pest population was reduced by only 60%, while the minimally treated populations were reduced by 60-90% (most were reduced by at least 74%). The laboratory populations were reduced by 95-100% (p. 1674).

In the same report, the authors referred to subsequent tests conducted in 1989 to determine if resistance among *P. xylostella* larvae had increased as a result of additional 15 *Btk* applications over a 2-year period (Tabashnik *et al.*, 1990, p. 1672). The $LC_{50}$ of the heavily treated population increased from 10.2 to 24.1 mg/L, while for one of the minimally treated populations, the $LC_{50}$ increased from 3.67 to 6.33 mg/L. The $LC_{50}$s for the laboratory strains did not significantly change over the two year period (p. 1673). Mortality rates for the laboratory populations and the one tested minimally treated population did not significantly change from 1987 to 1989, while for the heavily treated population, control of the diamondback moth decreased from 60% to 35% (p. 1674). The results of these tests suggest that the diamondback moth has the potential to develop resistance to *Btk* given the appropriate circumstances, i.e. selective pressure.

Kirsch and Schmutterer (1988) examined *P. xylostella* resistance to *Btk* in the Philippines. Cabbages were treated five times with a variety of pesticides (the *Btk* formulation Thuricide HP, two Neem extracts, mixtures of Thuricide HP with each of the Neem extracts, an insect growth hormone and the organophosphate profenofos), starting 11-14 days after the cabbages were transplanted. Ten randomly selected plants were examined weekly for insect infestation (p. 250). Two trials were conducted, one at "high *P. xylostella* infestation pressure", and the other during "low *P. xylostella* infestation pressure". At the end of the experiment the *Btk* treated plants did not differ in pest population or damage levels from that of the control plants. This apparent lack of control suggests resistance (p. 250-252). Kirsch and Schmutterer (1988) hypothesize that because of the extensive use of *Btk* alone or in combination with chemical insecticides in the area for many years, there could be "... decreased susceptibility or, to a greater extent, resistance" to *Btk* (p. 254).

### 3) Resistance in the Sunflower Moth

Brewer (1991) created a resistant population of sunflower moth, *Homeosoma electellum* Hulst (Lepidoptera: Pyralidae), from a "parent" colony by rearing larvae on diet treated with *Btk* (Dipel WP) for 12 generations. The dosage applied was 16,000 IU/mg of formulation, which was calculated to have a 3-day $LD_5$, i.e. 5% of the population died by day 3. Mortality was not followed beyond 3 days. Dipel was applied topically to the diet in individual cups at a concentration of 0.031 g per diet cup (2.5 mL diet) for the first generation, and was continually increased each successive generation to ensure a mortality rate between 40% and 70%. Larvae from generation 8 were used to test differences in mortality, developmental time, pupal weight, and fecundity between the "resistant colony" and the "parent colony" from which the resistant population was derived. A solution containing 60 µg of Dipel was topically applied to the surface of diet as "treatment", and 100 "parent" larvae and 95 "resistant" larvae from generation 8 were placed on the diet. Controls were also set up by placing 20 larvae from the parent colony and 20 larvae from the resistant colony on diet sprayed with distilled water. Specimens were checked daily for mortality until adult emergence (p. 317).

The results showed that resistant larvae fed on Dipel WP-treated diet had lower mortality than the parent colony larvae. Larval development time was prolonged by exposure to *Btk* in the diet; however, larvae from the resistant colony developed at a faster rate than those larvae from the non-resistant parent colony. *Btk* treatment did not influence the time spent by the insects in the pupal stage, however, *Btk* did have an interesting effect on the sex ratio. The sex ratio of male to female remained unchanged (1:1) in the treated resistant colony, but was altered in favor of males in the treated non-resistant (i.e. parent) colony (ratio of 3.3 males for every female)(p. 317-318). The treated resistant colony had significantly greater fecundity (P) than the treated parent colony, which had the lowest fecundity of all four groups (control and treated) (p. 319).

### 4) Possibility for Development of Resistance in the Gypsy Moth

In a laboratory study, Rossiter *et al.* (1990) investigated the variation of susceptibility of the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae) to *Btk*. In their paper, the authors stated that "... variation in $LC_{50}$s suggested the potential for resistance development through natural selection". Moreover, they hypothesized "that variation in susceptibility to *B. thuringiensis* in the gypsy moth is based on vigor differences in growth and develop-

mental capability, attributes that are the product of both genotype and the maternally determined nutritional status of the egg" (p. 2211). However, no evidence of existing resistance to *Btk* was found in gypsy moth.

### 5) Resistance Towards Genetically Engineered Bacteria and Crops Containing the Delta-Endotoxin of *Bacillus thuringiensis* var. *kurstaki*

The genetic engineering technology in the area of bioinsecticides has allowed for the development of recombinant organisms that express the delta-endotoxin of *Btk*. The cloning of the toxin genes, and subsequent introduction of these genes into bacterial cells or important plant crops, allows manipulation of the gene to increase toxin yields or to expand the insecticidal range of the toxin (Wilcox *et al.*, 1986, p. 397). The use of transgenic plants in agriculture may also eliminate some of the problems associated with *Btk* applications, such as, the high cost of product and application, inconsistent control, low persistence of the insecticide in the environment, and the concern over exact timing of applications for efficient pest control (Perlak and Fischhoff, 1990, p. 461).

The gene that produces delta-endotoxin has been successfully incorporated into the bacteria *Escherichia coli*, *Pseudomonas fluorescens*, and other *Bacillus* species, as well as into tobacco, tomato, potato, and cotton plants (Sims and Stone, 1991, p. 206; Meeusen and Warren, 1989, p. 373; Wilcox *et al.*, 1986, p. 397-398; Lindow *et al.*, 1989, p. 1300-1302; Perlak and Fischhoff, 1990, p. 462). However, if plants genetically engineered to contain delta-endotoxin become widely-used in the field, this may lead to greater problems than those it was meant to solve; namely, it may speed up the development of resistance to *Btk*. Tomato plants expressing this toxin have been shown to be resistant to tobacco hornworm, *Manduca sexta* (Linnaeus) (Lepidoptera: Shingidae), tobacco budworm, *Heliothis virescens*, tomato fruitworm, *Helicoverpa zea*, and tomato pinworm, *Keiferia lycopersicella* (Walsingham) (Lepidoptera: Gelechiidae), over plants not expressing this toxin (i.e. non-transgenic plants) (Delannay *et al.*, 1989, p.1265; Perlak and Fischhoff, 1990, p. 462). Transgenic cotton plants have been effectively protected from square (unopened cotton flowers) and boll damage caused by the cotton bollworm, *Heliothis zea*, and other lepidopteran pests in laboratory and field assays (Perlak *et al.*, 1990, p. 939).

Although the future of transgenic plants and bacteria appears bright, the potential for the development of resistance to these organisms may be even greater than for the traditional formulations of *Btk*. Current use of *Btk* in the field involves intermittent spraying, often with inconsistent coverage of affected areas. This, with the short residual effect of *Btk* and natural insect dispersal has limited the extent to which insects contact the insecticide (Stone *et al.*, 1989, p. 228; Meeusen and Warren, 1989, p. 377). Transgenic plants and microorganisms would subject the target insect populations to continual exposure to the toxin in food supplies throughout the growing season. Dosages cannot be altered or modified to specifically control the pest, or allow new strains of *Bt* into the control program on short notice (Meeusen and Warren, 1989, p. 377; Stone *et al.*, 1989, p. 228-229; Cutler, 1991, p. 7; Sims and Stone, 1991, p. 206). Such an environment encourages the development of resistance to the *Btk* toxin, as shown by McGaughey (1985b) in feeding tests of the Indianmeal moth, *Plodia interpunctella*, and by Tabashnik *et al.* (1990) for *Plutella xylostella* after extensive treatments (50-100 times) in the field. This concern was addressed by researchers participating in a colloquium on *Btk* resistance in Washington D.C., in 1991 (sponsored by the National Audubon Society). The scientists suggested that seeds of transgenic plants should be sold only as mixtures with normal seeds when they are introduced to the market in the mid-1990s. If this is not done, the monoculture of transgenic crops will only encourage and hasten the development of resistance among target insects (Gibbons, 1991, p. 646).

This possibility is illuminated by the results of two studies which have focussed on resistance of tobacco budworm, *Heliothis virescens*, to a genetically engineered *Pseudomonas fluorescens* expressing the delta-endotoxin of *Btk* (HD-1 serotype). Stone *et al.*, (1989), document dramatic increases in $LC_{50}$ values in populations subjected to moderately high selection pressures (mean = 33.9% survivors/generation). The insects were maintained for three generations prior to the experiment on diet containing 150 µg *P. fluorescens*/mL of diet. Selection for resistance was accomplished by continually increasing the dosage of *Btk* in each generation during the experiment to ensure a 10-50% survival. By the thirteenth generation, dosage had increased to 10 mg/mL diet (p. 229-231). The $LC_{50}$ of the selected population increased from 1.05 mg/mL diet in the F3 generation, to 4.53 mg/ml in the F14 generation (p. 232).

In an effort to determine the genetic basis of resistance to the endotoxin-containing *P. fluorescens*, Sims and Stone (1991) crossed resistant and susceptible strains of *H. virescens*. The authors suggested that resistance is autosomally inherited, it is incompletely dominant, and that it is under the control of several genes (p. 208). This is in contrast to McGaughey (1985b) who suggested that resistance to *Btk* was due to a single recessive gene (p. 194). Genetic variability within the resistant colony, however, was initially low due to small sample size, a factor which appears to be necessary for resistance to develop (p. 209). In large populations under typical field conditions, genetic variability is relatively large and resistance may develop more slowly. Furthermore, the development of resistance in many of the above experiments has occurred as a result of severe selective pressure placed on a small population of insects. In the field, *Btk* (or other varieties) is applied one to three times yearly for control of large

populations of insects, therefore the development of resistance is less likely.

### 6) Strategies to Prevent Resistance to *Btk*

Gibbons (1991) reported that evidence of resistance to *Btk* has surfaced in other areas including New York, Florida, Japan, Thailand, and the Philippines since the discovery in 1986 of the resistant diamondback moth population feeding on watercress in Hawaii (p. 646). Gibbons (1991) states that all these cases involved frequent applications of high dosages of *Btk* within one growing season - as many as 15 treatments per year (p. 646). Studies at the University of Hawaii, Cornell University, and North Carolina State University suggested that successful control of target insects (60% to 90% mortality) created extremely high pressures on the pest to adapt and develop resistance to survive, consequently the offspring of these insects became more resistant than their parents (p. 646).

Well-planned crop management (eg. crop rotation, alternating applications of different biological control measures) may be the best solution at this time for preventing, or at least delaying, the development of resistance among *Btk*-treated insects. Crop rotation in agriculture has been used widely not only as a method of pest control, but also as a means to minimize nutrient depletion of the soil. Both Gibbons (1991) and McGaughey and Whalon (1992) suggest rotating *Btk*-treated crops with crops that do not require such treatments, and reducing the dosage and the frequency of applications. Alternating applications of *Btk* with other control measures may provide a challenge to insects each time an area is treated (McGaughey and Whalon, 1992, p. 1453). Another idea is to leave a few plants in each field unsprayed so that susceptible and resistant insects are given a food source that does not put selective pressure on them, and the chance to breed to reduce the expression of the recessive resistant gene (Gibbons, 1991, p. 646). Gould and Anderson (1991) have shown that target insects provided with no alternative to *Btk*-infected diet adapt much more quickly (develop resistance) than insects provided a choice between treated and untreated diets (p. 37).

Cutler (1991) suggests that the best means of reducing or avoiding resistance among target insects to *Btk* and other varieties is to rotate and/or combine different varieties of *Bt* to provide a "different biological challenge every year..." (p. 7). The large-scale use of transgenic plants, expressing only one of the varieties of *Bt*, say *Btk*, would eliminate this latter option, thereby hastening the development of resistance in the target pest. Further variation may be attained by employing other control methods in conjuction with *Btk*, such as small doses of other chemical or biological pesticides (pyrethroids, pheromones, viruses, carbamate, etc.) (Cutler, 1991, p. 7; Perlak and Fischhoff, 1990, p. 462).

# V
# SUMMARY AND CONCLUSIONS

***Bacillus thuringiensis* (*Bt*) is a naturally occurring bacterium** isolated in many countries around the world. **Its natural habitat is in soils,** but it has also been found naturally on the foliage of conifer and deciduous trees. **Widespread outbreaks of *Bt* infection among insects are rare** because the bacterium is present in very low numbers. Biological degradation by soil microorganisms and unfavorable pH conditions in soil also prevent *Bt* from flourishing. **Natural epizootics generally occur in confined spaces such as grain storage units, or insect rearing facilities.**

***Btk* has low persistence in the environment because of the natural degradation processes that act on it.** Sunlight has been shown to inactivate the spores while leaving the crystals (the main insecticidal component) intact. Other experiments report contradictory results, suggesting that only the crystals of *Btk* are inactivated in sunlight. **Generally, in the field, *Btk* insecticidal qualities are diminished by sunlight, heat, acidic pH, and microbial degradation in 3 to 7 days on foliage,** although longer persistence periods have also been reported, especially in soils. Humidity, precipitation and run-off accentuate this loss of activity by removing spores and crystals from foliage where most target insects normally contact *Btk* by eating the contaminated foliage.

**The beta-exotoxin, which has been implicated in some cases of toxicity to non-target organisms, has been eliminated from all *Bt* products used in North America since 1971.** The safety of the delta-endotoxin found in *Btk* to non-target organisms has been investigated repeatedly since the initial registration of *Btk* products for pest control.

Before registration is granted, information on the carriers and/or inert materials in the *Btk* formulations must be provided by the manufacturers to the regulatory agencies (Agriculture Canada in Canada and the Environmental Protection Agency in the United States). This proprietary information and data is reviewed by the regulatory agencies, and **the carriers and inert materials must be documented to be safe before the product is granted registration.**

Concerns have been raised that animals which feed on insects infected by *Btk* may themselves become infected with *Btk*. However, the anaerobic conditions and declining food supply within dead and moribund insects prevents excessive sporulation from occurring. As well, **very few organisms possess the necessary alkaline gut environment to activate *Btk* toxins that may be ingested with infected food.**

Concern has also been expressed by some that the application of *Btk* might have unforseen effects on non-target terrestrial invertebrates, including parasites, predators, and other beneficial insects via direct toxicity of *Btk*. **No documented cases of direct toxicity of *Btk* to parasites, predators and other beneficial insects have been found in the published literature.** However, non-target lepidopterans may be at risk when treatments are conducted when the feeding, or larval, stage of these insects are present in the treatment areas. Many of these insects possess the necessary gut environment to activate the toxins. However, this does not pose a serious problem unless the particular insect is considered to be a rare or endangered species. Knowledge of the ecology and lepidopteran diversity of an infested area is necessary when developing and planning insect abatement programs in an area, including those that involve *Btk*. After considering the possible potential for negative effects on these rare and endangered species of Lepidoptera (should they be in stage of development susceptible to *Btk*), then these should be balanced against the benefits of controlling the target pest organism and the expected damage caused by of the unchecked pest outbreak.

**It is conceivable, although extremely unlikely, for natural enemies of economically important pests to be harmed by *Btk* treatments, either directly or indirectly.** Indirect effects, although less obvious than direct mortality, could be detrimental if they allow for a rebounding target insect population in subsequent seasons. **No evidence of indirect effects has been reported to date.** Often, the effect of *Btk* treatments on parasite populations is an increase in parasitism due to a lengthening of the period when the host is available for attack by parasites. In addition, the development and commencement of activity by parasitized larvae is delayed; thus, parasitized larvae emerge later from

their overwintering sites than the non-parasitized larvae, and subsequently the parasitized larvae may not be affected by early applications of *Btk*. This has been documented for parasites of the eastern and western spruce budworms, as well as several other insects.

Other possible side effects of *Btk* application that occasionally occur include reduced pupation rates, decreased longevity, and lower adult emergence. In most cases, the exceptional benefits of controlling the target pest insect greatly outweigh the indirect effects of *Btk* on some beneficials. **Field studies to date seem to indicate that *Btk* does not have a significant detrimental effect on parasite populations. Indeed, in several studies "synergistic" effects were observed between *Btk* application and parasitism.** However, as mentioned earlier, a good knowledge of the ecology of natural populations in any spray area is vital to ensure safety of *Btk* spraying to non-target organisms.

Concerns have been raised by some environmentalists and owners of apiaries that the application of *Btk* may affect honeybee populations. It has been conclusively proven that *Btk* is incapable of germinating and growing in honey. Indeed, all information to date suggests that ***Btk* does not have harmful effects to honeybees unless administered in doses at least 100 times the concentrations recommended for control of the pest insects.**

A laboratory study indicated that the earthworm, *Lumbricus terrestris*, was significantly affected by extremely high doses of *Btt*, such as those that might occur at spill sites. **Further tests, using *Btk*, have demonstrated that this variety of *Bt* is safe to earthworms.**

Aquatic fauna have been monitored in spray programs of *Btk*, and several laboratory tests have also been conducted. At recommended field application rates, the safety of *Btk* has been supported by the findings of these experiments. Mussels and brine shrimp experienced mortality resulting from total immersion into *Btk*-suspensions for 96 hours, however the **mortality was attributed to the concentration of particulate matter in the water, not to the *Btk* itself.** In another test, populations of aquatic insects were altered following *Btk* application. The aquatic insect populations increased and decreased both in the treatment and the control blocks, but the overall population levels were not significantly different from pre-treatment population levels, and all changes could be attributed to natural phenomena. In another study, conducted in B.C., **a "worse case" scenario of *Btk* contamination (mimicking an accidental spill of ca. 5,000 BIU/ha) did not adversely affect the abundance and composition of the benthic insect community.**

Fish and other vertebrates exposed to *Btk* within stream and lake environments showed no side effects. Following aerial application against spruce budworm in Ontario, no alteration in fish behavior or populations was observed. Indeed, **there are no records of any fish kills caused by *Btk* anywhere in North America during the 35 years of use in insect control programs.**

***Btk* has been shown to be harmless to populations of wild birds as well as chickens.** Both in laboratory feeding tests and in field spray trials, birds were generally unaffected by the insecticide. Chickens have been shown to pass *Bt* through their gut such that spores are recovered in feces and some control of house fly in feces is achieved. One report showed that hens fed high concentrations of *Bt* (variety unspecified) experienced a reduction in food consumption, body weight, and egg production. The variety of *Bt* used for the experiment most likely contained beta-exotoxin, which could have been responsible for the negative effects.

**Both laboratory and field tests on mammals have shown *Btk* to be harmless to these animals, even under extreme cases and drastic testing conditions.** In laboratory tests on small animals, the occurrence of **abscesses at injection sites** have been reported, along with conjunctival congestion following **eye irritancy tests. Intracerebral injections of *Btk* into rats caused high levels of mortality. However, it should be noted that this type of testing is considered unconventional and extrapolations from these results are questionable. Since even non-pathogenic bacteria will cause high mortality when tested in this fashion, intracerebral injections are not a good indicator of *Bt* safety.** Tests using large animals such as sheep and cows have shown that the spores of *Btk* and other varieties survive passage through the gut and are found in large numbers in feces. The crystals have no apparent deleterious effects on these tested animals, and even provide a degree of control over house, horn, stable and face fly in livestock feces.

**Populations of small mammals inhabiting forest plots where *Btk* has been sprayed in field trials have not been adversely affected by such treatments. *Btk* sprayed at recommended field dosages did not result in any significant changes in small mammal populations, nor in the breeding activities of these animals following field applications.**

Another concern associated with the use of *Btk* to control pest organisms is the development of resistant insect populations. This was first observed where the target insects received multiple applications of *Btk* per year for several years (diamondback moth larvae in Hawaii received 15 treatments per year for 5 years). Another example is the reported build-up of resistance of two stored product pests, the Indianmeal moth and the almond moth to *Btk*. The infestations by the insects occur in storage bins, a confined environment, where *Btk* in the treatment would not be inactivated readily, as it would be in open field applications. The "continuous" exposure of the target organisms in this confined environment provides a selection process which favours the build-up of resistance in the surviving insects.

There are other concerns associated with the development of transgenic agricultural crops that possess the gene to express *Btk* delta-endotoxin for

insect control. Tests on the safety of *Btk* towards mammalian cell lines indicate that these cells are unharmed by activated toxin. However, there is a need for thorough research into the safety of transgenic plants, both to humans and to non-target organisims.

Since the bacteria must be ingested to be effective, the organism must possess an alkaline gut environment to activate the toxin and possess the appropriate protein receptors on the cell membranes of the intestinal vesicles for the toxin to have any effect at all, **the safety of *Btk* towards humans (and other mammals) is accepted. No medical or experimental data exists which shows any health risks associated with the use of *Btk*.**

**No harmful effects or symptoms of bacterial infection have occurred in human volunteers as a result of ingestion or inhalation of *Btt* (which contained beta-exotoxin). Over the past 35 years of its use, incidents of human health problems associated with the manufacture and application of *Bt* (*Btt* and *Btk*) have been infinitesimally low.** One case of a corneal ulcer following accidental splashing of *Btk* into the eye of an agricultural worker has been reported, although this condition was remedied and cleared up by medical treatment.

The use of *Btk* and other varieties of *Bt* to control pest insects involves many aspects of environmental health and safety. **The relationship between immunocompromised individuals and *Btk* was recently examined in Oregon and investigated in Vancouver, B.C., and *Btk* was not shown to be related to any of the health problems of these individuals.** In Oregon, three incidents of health problems, including one death, arising in immunocompromised individuals, were reported in an area treated over a 2-year period with *Btk*. All three patients had serious underlying medical conditions prior to the spraying, and **there was insufficient evidence from tissue cultures to adequately conclude that *Btk* was responsible for the health problems or that it contributed to the death. Indeed, during the Vancouver spray, Noble *et al.* (1992) "were unable to find a single case where Btk was a pathogen causing infection. Since all significant cultures collected during the period were examined, we conclude that no cases of infection in immunocompromised persons have occurred during the time of the spray" ('Summary', p.2). In light of the absence of any medical complications arising from the recent application of *Btk* over Greater Vancouver in 1992, it is extremely improbable that *Btk* poses any health risk, even to people who are immunocompromised.**

**During the more than 35 years that *Bt* has been in use in forestry, agriculture, and domestic situations, no cases of harmful side effects to humans, mammals, or vertebrates, including fish, have emerged.** In conclusion, *Bt*, like any other pest control product, should always be used with care according to the label, and in accordance with recommended precautionary measures. **When applied according to label instructions, *Btk* is considered one of the safest pesticides available today for operational use in agriculture and forestry, and by organic growers and home gardeners.**

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# VII APPENDICES

## APPENDIX I
## Economically Important Insects Susceptible to the Larvicidal Effects of *Btk*[1,2]

| *Scientific Name* | *Common Name* |
|---|---|
| *Achroia grisella* (Fabricius) | lesser wax moth |
| *Acleris variana* (Fernald) | eastern blackheaded budworm |
| *Acleris gloverana* (Walsingham) | western blackheaded budworm |
| *Agrostis ipsilon* (Hufnagel) | black cutworm |
| *Alabama argillacea* (Hübner) | cotton leafworm |
| *Alsophila pometaria* (Harris) | fall cankerworm |
| *Anarsia lineatella* Zeller | peach twig borer |
| *Anisota senatoria* (J.E. Smith) | orange-striped oakworm |
| *Antheraea pernyi* (Guerin) | sinjyu-silkworm |
| *Anticarsia gemmatalis* Hübner | velvetbean caterpillar |
| *Archips argyrospilus* (Walker) | fruit tree leafroller |
| *Archips fervidana* (Clemens) | oak webworm |
| *Argyrotaenia juglandana* (Fernald) | hickory leafroller |
| *Argyrotaenia velutinana* (Walker) | redbanded leafroller |
| *Artogeia rapae* (Linnaeus) | imported cabbageworm |
| *Boarmia selenaria* Schiffermuller | giant looper |
| *Bombyx mori* (Linnaeus) | silkworm |
| *Bucculatrix thurberiella* Busck | cotton leaf perforator |
| *Bupalus piniarius* Linnaeus | pine moth |
| *Cadra cautella* (Walker) | almond moth |
| *Carpocapsa pomonella* Linnaeus | codling moth |
| *Ceramica picta* Harris | Zebra caterpillar |
| *Choristoneura fumiferana* (Clemens) | eastern spruce budworm |
| *Choristoneura occidentalis* Freeman | western spruce budworm |
| *Choristoneura pinus pinus* Freeman | jack pine worm |
| *Choristoneura rosaceana* (Harris) | oblique-banded leafroller |
| *Colias eurytheme* Boisduval | alfalfa caterpillar |
| *Crambus sperryillus* (Waler) Klots | lawn moth |
| *Cremona cotoneastri* Busck | cotoneaster webworm |
| *Cryptoblabes gnidiella* (Milliere) | Christmas berry webworm |
| *Datana integerrima* Grote & Robinson | walnut caterpillar |
| *Dendrolimus sibiricus* (Tschetw) | Siberian silkworm |
| *Desmia funeralis* (Hübner) | grape leaf folder |
| *Diacrisia obliqua* Walker | jute hairy caterpillar |
| *Diatraea saccharalis* (Fabricius) | sugarcane borer |
| *Ennomos subsignarius* (Hübner) | Elm spanworm |
| *Ephestia elutella* (Hübner) | tobacco moth |
| *Ephestia* (=*Anagasta*) *kuehniella* (Zeller) | Mediterranean flour moth |
| *Erranis tiliaria* (Harris) | linden looper |

*Estigmene acrea* (Drury) . . . . . salt marsh caterpillar
*Euproctis chrysorrhoea* (Linnaeus) . . . . . browntail moth
*Galleria mellonella* (Linnaeus) . . . . . greater wax moth
*Helicoverpa* (= *Heliothis*) *zea* (Boddie) . . . . . corn earworm-tomato fruitworm
*Heliothis armigera* Hübner . . . . . cotton bollworm
*Heliothis virescens* (Fabricius) . . . . . tobacco budworm
*Hemileuca oliviae* Cockerell . . . . . range caterpillar
*Homoeosoma electellum* (Hulst) . . . . . sunflower moth
*Hyphantria cunea* (Drury) . . . . . fall webworm
*Keiferia lycopersicella* (Walsingham) . . . . . tomato pinworm
*Lambdina fiscellaria lugubrosa* Hulst . . . . . western hemlock looper
*Lambdina f. fiscellaria* (Guenee) . . . . . eastern hemlock looper
*Loxostege sticticalis* (Linnaeus) . . . . . beet webworm
*Lymantria dispar* (Linnaeus) . . . . . gypsy moth
*Macalla thyrsisalis* Walker . . . . . mahogany webworm
*Malacosoma americanum* (Fabricius) . . . . . eastern tent caterpillar
*Malacosoma disstria* Hübner . . . . . forest tent caterpillar
*Malacosoma fragile* (Stretch) . . . . . Great basin tent caterpillar
*Malacosoma pluviale* (Packard) . . . . . western tent caterpillar
*Mamestra configurata* Walker . . . . . bertha armyworm
*Manduca sexta* (Linnaeus) . . . . . tobacco hornworm
*Manduca quinquemaculata* (Haworth) . . . . . tomato hornworm
*Nygmia phaeorrhoea* (Donovan) . . . . . brown tail moth
*Operophtera brumata* (Linnaeus) . . . . . winter moth
*Orgyia pseudotsugata* (McDunnough) . . . . . Douglas-fir tussock moth
*Orgyia vetusta* (Boisduval) . . . . . Western tussock moth
*Ostrinia nubilialis* (Hübner) . . . . . European corn borer
*Paleacrita vernata* (Peck) . . . . . spring cankerworm
*Papilio cresphontes* Cramer . . . . . orange dog
*Pectinophora gossypiella* (Saunders) . . . . . pink bollworm
*Phalonia hospes* Walsingham . . . . . banded sunflower moth
*Phryganidia californica* Packard . . . . . California oakworm
*Pieris brassicae* (Linnaeus) . . . . . large white butterfly
*Pieris protodice* Boisduval & Leconte . . . . . Southern cabbage worm
*Pieris rapae* (Linnaeus) . . . . . imported cabbage worm
*Plathypena scabra* (Fabricius) . . . . . green clover worm
*Platyptilia carduidactyla* (Riley) . . . . . artichoke plume moth
*Plodia interpunctella* (Hübner) . . . . . Indianmeal moth
*Plutella xylostella* (Linnaeus) . . . . . diamondback moth
*Plutella maculipennis* (Curtis) . . . . . cabbage moth
*Prodenia litura* . . . . . tobacco caterpillar
*Proxenus mindara* Barnes & McDonnough . . . . . rough-skinned cutworm
*Pseudaletia unipuncta* (Haworth) . . . . . armyworm
*Pseudoplusia includens* (Walker) . . . . . soybean looper
*Pyrausta nubilalis* Hübner . . . . . European corn borer
*Pyrrhalta luteola* (Muller) . . . . . elm leaf beetle
*Sesamia cretica* (Led.) . . . . . pink borer
*Schizura concinna* (J.E. Smith) . . . . . redhumped caterpillar
*Simulium vittatum* Zetterstedt . . . . . blackfly
*Spilonota ocellana* (Denis & Schiffermuller) . . . . . eye-spotted bud moth
*Spodoptera exigua* (Hübner) . . . . . beet army worm
*Spodoptera frugiperda* (J.E. Smith) . . . . . fall army worm
*Spodoptera litura* Fabricius . . . . . tobacco caterpillar
*Spodoptera mauritia* (Boisduval) . . . . . lawn armyworm
*Spodoptera praefica* (Grote) . . . . . western striped armyworm
*Stilpnotia salicis* (Linnaeus) . . . . . satin moth
*Thaumetopoea wilkinsoni* Tams . . . . . processionary pine moth
*Thymelicus lineola* (Ochsenheimer) . . . . . european skipper
*Thyridopteryx ephemeraeformis* . . . . . (Haworth)bagworm
*Tineola bisselliella* (Hummel) . . . . . webbing clothes moth
*Trichoplusia ni* (Hübner) . . . . . cabbage looper

*Udea rubigalis* (Guenee) . . . . . celery leaf tier
*Vanessa cardui* (Linnaeus) . . . . . painted lady butterfly
*Yponomeuta evonymellus* Chambers . . . . . small ermine moth

[1] Taken from Ellis, 1990, p. 52-53; Dulmage and Aizawa, 1982, p. 215; Forsberg, 1976, p. 18 and 93; Hamed, 1979, p. 294; Tipping and Burbutis, 1983, p. 892; Temerak, 1980, p. 315; Van Rie *et al.*, 1990, p. 72; McGaughey, 1978, p. 687; Prasertphon *et al.*, 1973, p. 205; Niwa *et al.*, 1987, p. 750; Innes and Bendell, 1989, p. 1318; Lu *et al.*, 1986, p. 71; Perlak and Fischhoff, 1990, p. 462; Morris *et al.*, 1986, p. 7; Poinar *et al.*, 1990, p. 196-197; Vankova and Purrini, 1979, p. 218; Faust and Bulla, 1982, p. 174-175; West *et al.*, 1989, p. 59; Brewer and Anderson, 1990, p. 2219; Lutwama and Matanmi, 1988, p. 173; Morris, 1973; Lacey and Mulla, 1977, p. 47-48; Talukder *et al.*, 1989, p. 587; Howard, 1990, p. 225. Novo Industri A/S, 1988c.

[2] N.B. This list is based on experiments conducted on the various insects included, and is in no way meant to be an exhaustive and complete list.

## APPENDIX II
## List of Small Mammals Monitored in Northern Ontario Following an Aerial Spray of *Btk*[1]

Shrews
*Blarina brevicauda*
*Sorex cinereus*
*Sorex fumeus*
*Sorex hoyii*
Rodents
*Clethrionomys gapperi*
*Eutamius minimus*
*Microtus chrotorrhinus*
*Napaeozapus insignis*
*Peromyscus maniculatus*
*Phenacomys intermedius*
*Tamia striatus*
*Zapus hudsonius*

[1] Innes and Bendell, 1989.

## APPENDIX III
## List of Non-target Insect Species Studied in Conjunction with a Spray Trial of *Bt* in Apple Orchards of Nova Scotia[1]

Acarina
*Anystis agilis* Banks
*Mediolata novaescotiae* Nes.
*Phytoseius macropilis* Banks
*Typhlodromus finlandicus* (Oudms.)
*Typhlodromus rhenanus* (Oudms.)
*Typhlodromus pyri* Scheuten
Miridae
*Atractotomus mali* (Meyer)
*Campylomma verbasci* (Meyer)[2]
*Deraeocoris fasciolus* Knight
*Deraeocoris nebulosus* (Uhl.)
*Diaphnidia* spp.
*Hyaliodes harti* Knight[2]
*Phytocoris* spp.[2]
*Pilophorus perplexus* D. & S.
*Plagiognathus obscurus* Uhl.
*Psallus* sp.[2]
Anthocoridae
*Anthocoris musculus* (Say)[2]
Thysanoptera
*Haplothrips faurei* Hood[2]
*Leptothrips mali* (Fitch)
Parasitic hymenoptera
*Aphytis mytilaspidis* (LeB.)

[1] MacPhee and Sanford, 1961, p. 672.

[2] Species affected such that a reduction in numbers was observed, although quantitative data is not presented (p. 672).

# APPENDIX IV
## Hosts, Parasites and Predators Exposed to Btk in Laboratory Tests to Determine the Non-target Effects of *Btk* Spraying[1]

Lepidopteran hosts of parasites and predators
*Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) - cabbage looper
*Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae) - black cutworm
*Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) - pink bollworm
*Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) - Angoumois grain moth

Tested species of parasites
*Brachymeria intermedia* (*Hymenoptera*: *Chalcididae*)
*Campoletis sonorensis* (*Hymenoptera*: *Ichneumonidae*)
*Chelonus blackburni* (*Hymenoptera*: *Braconidae*)
*Meteorus leviventris* (*Hymenoptera*: *Braconidae*)
*Voria ruralis* (*Diptera*: *Tachinidae*)

Tested species of predators
*Chrysopa carnea* (*Neuroptera*: *Chrysopidae*)
*Hippodamia convergens* (*Coleoptera*: *Coccinelidae*

[1] Wilkinson *et al.*, 1975, p. 114.

# APPENDIX V
## Aquatic Insect Species Monitored during Controlled Application of *Btk* to Aquaria and Simulated Stream Beds[1]

Epemeroptera
*Ephemerella* sp.
*Eporus vitrea*
*Heptagenia flavescens*
*Isonychia* sp.
*Paraleptophlebia ontario*
*Rithogena* sp.
*Stenonema vicarium*

Plecoptera
*Acroneuria abnormis*
*Isogenoides* sp.
*Pteronarcys* sp.
*Taeniopteryx nivalis*

Trichoptera
*Hydropsyche* sp.
*Hesperophylax designatus*
*Lepidostoma* sp.

Odonata
*Boyeria grafiana*
*Ophiogomphus carolus*

[1] Kreutzweiser *et al.*, 1992, p. 255, 256.

# APPENDIX VI
## Stream Bottom Fauna Monitored in a Quebec Stream Prior to and Following a Field Spray of *Btk*[1]

Ephemeroptera
Plecoptera
Trichoptera
Diptera-Tipulidae
Diptera-Heleidae
Diptera-Rhagionidae
Nematoda
Hydracarina
Mollusca-Gastropoda
Odonata
Megaloptera
Coleoptera
Diptera-Chironomidae
Diptera-Simuliidae
Platyhelminthes
Oligochaeta
Mollusca-Sphaeriidae

[1] Kingsbury and Sarrazin, 1975, p. 86.