# MICROBIAL INSECTICIDES IN CANADA: THEIR REGISTRATION AND USE IN AGRICULTURE, FORESTRY AND PUBLIC AND ANIMAL HEALTH




A report prepared by the Special Committee of the Science Policy Committee, Entomological Society of Canada

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see inside front cover

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**NOTE:** This report was printed in Ottawa from a computer disk and, in order to expedite it, the proofs were not sent to the committee chairman. Unfortunately two pages of galleys were reversed, which in effect placed the List of Tables and part of the Introduction in the middle of the most important item, the Conclusions and Recommendations.

This copy is the corrected version. The incorrect copies were mailed to all members and students in Canada and the United States. These persons will be sent the corrected first and last three sheets so they can replace the incorrect ones.

The last paragraph of the report (on page 34) should be ignored as it is also included in the correct position on page 16.

Special Committee

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## 1. CONCLUSIONS AND RECOMMENDATIONS

Entomopathogenic microorganisms, including bacteria, viruses, fungi, protozoa and nematodes, contribute significantly to regulation of populations of several species of pest insects of importance in agriculture, forestry, and public and animal health in Canada. Research, in which Canadian scientists have been important pioneers, has indicated that these entomopathogens have the potential for a much greater role in management of pest insects by exploitation of naturally occurring entomopathogens and by application of entomopathogens as microbial insecticides.

This Special Committee of the Society has reviewed the status of entomopathogens in Canada and has evaluated their potential role in management of pest insects. The following recommendations for support of research and development of entomopathogens are presented because of the obvious potential of these biological agents for playing a major role in integrated management systems of insect pests of agricultural crops, forests, and public and animal health.

A. Research is required to identify and evaluate the effectiveness of naturally occurring entomopathogens against pest insects and to determine the feasibility of developing entomopathogens as applied microbial insecticides against significant species of insect pests of agriculture, forestry, and public and animal health.

This research should include studies on short- and long-term effects of entomopathogens, including bacteria, viruses, fungi, protozoa, and nematodes, on populations of target and non-target insects and other non-target fauna, studies on epizootiology of diseases, including host-pathogen relationships and environmental factors that influence entomopathogens in host populations, and evaluation of the role of entomopathogens in management systems.

The effectiveness of applied entomopathogens relative to other control practices must be increased to enhance the competitive status of microbial insecticides. It is recommended that increased research should include genetic engineering and similar biotechnological techniques and strain selection to develop entomopathogens that are more active against target species of insects but safe for non-target species in the habitat, and to develop strains that are more suitable as components of integrated management systems by, for example, increasing tolerance to pesticides and by reducing susceptibility to inactivation by adverse environmental factors. Improved formulations that are more suited to a specific use and that enhance effectiveness of entomopathogens, and improved equipment for application of microbial insecticides, must be developed. In addition, more efficient techniques for mass-propagation of entomopathogens are required, as are improved techniques for detection and identification of entomopathogens.

Host-pathogen systems of particular interest include: spruce budworm/(*Bacillus thuringiensis* (*B.t.*)), nuclear polyhedrosis virus (NPV); bertha armyworm and redbacked cutworm/viruses, nematodes; cabbage looper/NPV; cabbageworm/granulosis virus (GV); cabbage root maggot, carrot weevil and other root maggots/nematodes; codling moth/GV; corn borer/microsporidia; greenhouse whitefly/*Verticillium* and *Aschersonia* spp.; gypsy moth/NPV, *B.t*; hemlock looper/NPV, *B.t.*; Swaine's jack pine sawfly/NPV; migratory grasshopper/*Entomophaga* spp., *Nosema* spp.; mosquitoes and blackflies/*Bacillus thuringiensis* var *israelensis* and *Bacillus sphaericus*; and pathogens of insect pests of stored products, and bark beetles and boring beetles.

It is recommended that this research should be supported more generously by public funding of in-house research and by support through research grants.

B. It is recommended that governments should provide a more favorable climate for participation of private industry in development of entomopathogens by financial support and other incentives to encourage development, propagation, formulation and distribution of entomopathogens for use in management of insect pests of agricultural crops, forests, and public and animal health. Toxicological assessments and registration should be facilitated and supported by public funding to encourage development of entomopathogens.

The development and use of entomopathogens and other biological control agents to their full potential as components of pest management systems should be encouraged by technology transfer, emphasizing to the agricultural and forest industries and the public, the advantageous features of biological control agents for regulation of pest insects. Those microbial agents that are already registered in Canada should be actively promoted for use in forestry, agriculture and public and animal health.

C. Establishment of a course of instruction in insect pathology and microbial control in at least one Canadian university is recommended. This should be developed as a significant component of a program of instruction and research in biological control and integrated pest management. Coordination of research and development of microbial control by government agencies, industry and universities and the establishment of an expert committee on the use of biological agents in insect control are highly desirable.

A study of the distribution of scientists world-wide and in Canada currently working in insect pathology and microbiol control indicates that Canada has fallen well behind other industrialized countries in this type of research. The committee concluded that the number of scientists engaged in this research in Canada is extremely small compared to the problems existing in this country and that greater funding for manpower and operations in both government and university establishments is critical to bringing more microbiol agents to the level of registration.

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## 3. INTRODUCTION

In this report the term microbial insecticide refers to a disease-causing agent used either by itself, or with other control methods in the management of an insect pest. These disease-causing agents include entomopathogenic viruses, bacteria, protozoa and fungi. Entomogenous nematodes, some of which are vectors of entomopathogenic bacteria, are also included even though they are parasitic helminths.

The recorded history of insect diseases originated with Aristotle's description of certain diseases of honey bees in his *Historia animalium*, but insect pathology as a science began with Agostino Bassi who, in 1834, showed experimentally that a microorganism (viz. the fungus *Beauveria bassiana*) was the cause of an infectious disease in the silkworm (*Bombyx mori*). This was the first hint that a microbial agent might be used to destroy harmful insects (Steinhaus 1963). The first significant experiments using a pathogen, the fungus *Metarhizium anisopliae*, were performed by Metchnikoff in 1879 in Russia.

Fungal pathogens of insect pests of agricultural crops, including apples and potates, and of forests were studied as early as the 1920's in Canada. This early research led to the establishment by the Department of Agriculture of the Laboratory of Insect Pathology at Sault Ste. Marie and of smaller laboratories for studies on insect pathology at Belleville and at other locations in Canada. This research emphasized the role of viruses, fungi and bacteria in natural control of insects with the aim of developing entomopathogens as agents to serve as alternatives to chemical pesticides. The commercial development of the bacterium, *Bacillus thuringiensis*, as a microbial insecticide for use in forestry and agriculture stimulated interest in the potential of microorganisms as an important element in pest management. This enhanced interest came at a time when widespread use of chemical insecticides was being questioned because of environmental contamination, pest resistance and resurgence, and their toxicity to natural biotic control agents.

Microorganisms may be used for pest control in Canada in a variety of ways depending on the characteristics of the entomopathogen, the pest insect and the crop:

(a) *Introduction.* An exotic organism is introduced into and becomes established in a new area or insect population and maintains the density of that population below the economic threshold. An example is the control of the European spruce sawfly in Canada by the introduction of a virus (Bird and Burk 1961).

(b) *Augmentation.* This is the strategy of increasing the density of a native entomopathogen within an insect population on the assumption that the natural density of the pathogen is insufficient to maintain the pest density at an acceptable level. (e.g. nuclear polyhedrosis virus vs the cabbage looper, Jaques 1977a).

(c) *Inundation.* Large numbers of native or exotic microoganisms are propagated in a laboratory or the field and applied in the same manner as an insecticide to pests at critical periods of the life cycle of the pests for short-term suppression of pest numbers (Greathead and Waage 1983). The most obvious example of this strategy is the use of *Bacillus thuringiensis* against the spruce budworm.

(d) *Integrated Pest Management (IPM).* This strategy entails the simultaneous or sequential use of several control methods in managing insect pest populations. Microbial insecticides have considerable potential as components of IPM systems. Unfortunately, very little research has been done in Canada to optimize the joint use of microbial agents and other control methods. Potentially useful strategies of this type could involve the interaction of entomopathogenic

microorganisms with pest resistant cultivars of crops, cultural control, insecticide-resistant entomophagous parasites and predators, parasitic nematodes, low risk systemic chemical insecticides such as demeton and oxydemeton-methyl, autocidal control, genetic control, insect attractants and repellants (including pheromones), insect growth regulators and plant growth regulators (Glass 1975; Morris 1980a; Klassan 1981; Jaques 1983).

At present, various commercial formulation of *B. thuringiensis* are widely used in Canada against forest insect pests and against pests of agricultural crop. Two viruses are registered for use against forest insects, but they are not yet widely used. Current research in this country is mainly oriented toward improving application technology of microbial agents in forestry, and the development of potentially useful agents in pest control, such as viruses, *B. thuringiensis* var. *israelensis* (H-14), entomopathogenic fungi and microsporidia, and the nematode-bacteria complexes of *Steinernema* (= *Neoaplectana*) and *Heterorhabditis* spp.

This report summarizes the present status of operational use of microbial insecticides in Canada and research and development being conducted.

## 4. ENTOMOPATHOGENS IN THE CONTROL OF INSECT PESTS OF FORESTS

### 4.1 *Bacillus thuringiensis*

*Bacillus thuringiensis* (*B.t.*) is a rod-shaped, gram-positive crystalliferous, spore-forming bacterium. When cultured under the appropriate conditions, *B.t.* sporulates and forms a protein parasporal body called the delta-endotoxin which is often referred to as the crystal. When sporulation is complete, the bacterial cell lyses and the spore and crystal are released into the surrounding medium. Ingestion of the crystal or spore-crystal complex destroys the gut epithelium of many species of lepidopterous larvae; this may kill larvae directly or they may die from septicaemia following germination of the spore and growth of vegetative cells in the haemocoele. *B.t.* is a naturally occurring pathogen which has been isolated from a wide variety of lepidopterous insects (Morris 1983a). Naturally occurring epizootics of *B.t.* do not occur, therefore, *B.t.* must be produced in large quantities in a fermentor on a suitable substrate and applied in the same manner as a conventional stomach-poison type of insecticide in order to control pest populations.

Presently, three commercial manufacturers of *B.t.* in North America supply the Canadian market: Abbott Laboratories, Zoecon Industries Ltd. (Subsidiary of Sandoz Ltd.) and Biochem Products (Divison of Salsbury Laboratories). Fourteen formulations are registered for use in forestry and agriculture (Table 1). The spore-crystal complex of *B.t.* var. *kurstaki* strain HD1 forms the basis of all commercial preparations used against forest and agricultural insects in Canada. Potency of batches of *B.t.* is determined by bioassay on cabbage looper (*Trichoplusia ni*) larvae to determine an LD50. A standard preparation is compared to the test batch and potency determined in International Units per milligram. Dosages applied for insect control on a per hectare basis are quoted in Billion International Units (BIU) per ha. *B.t.* is, in fact, the only microbial insecticide which has been successfully commercialized on a large scale and generates multi-million dollar annual sales.

It has been found that the gene for the *B.t.* toxin is on a plasmid (Gonzalez *et al.* 1982). This gene has been cloned and its DNA sequence determined. Because of this finding, *B.t.* is an excellent candidate for genetic manipulation and there is considerable commercial interest in exploiting this new technology. Genetic engineering techniques may lead to the development of strains with higher toxin yields,

Table I. Commercial products of *Bacillus thuringiensis* var. *kurstaki* (*B.t.*)that are registered for forestry and agricultural use in Canada and products of *B.t.* var. *israelensis* registered for biting fly control as of October 1985.

| Variety | Product | Registrant | Potency | Label[1] |
|---|---|---|---|---|
| *kurstaki* | Dipel F | Abbott | 16.0 BIU/mg | R |
| | Dipel SC | Abbott | 9.9 BIU/kg | R |
| | Dipel 45B | Abbott | 9.9 BIU/L | C |
| | Dipel WP | Abbott | 16.1 BIU/kg | C |
| | Dipel 88 | Abbott | 8.5 BIU/L | R |
| | Dipel 132 | Abbott | 12.7 BIU/L | R |
| | Novabac-3 | Biochem | 8.6 BIU/L | R |
| | Futura | Biochem | 14.4 BIU/L | R |
| | Bactospeine A | Biochem | 9.7 BIU/L | C |
| | Bactospeine F | Biochem | 9.7 BIU/L | R |
| | C.I.L. Organic Insect Killer | Chipman | 4.2 BIU/L | D |
| | Novabac-3 | Cyanamid | 8.6 BIU/L | R |
| | Marquette Organic Biological[2] | Marquette | 4.0 BIU/kg | D |
| | Envirobac WP | Pfizer | 16.0 BIU/kg | C |
| | Pfizer Envirobac ES | Pfizer | 9.7 BIU/L | R |
| | Thuricide 16B (aerial) | Sandoz | 4.2 BIU/L | R |
| | Thuricide R-HPC | Sandoz | 4.2 BIU/L | D |
| | Thuricide 16B (ground) | Sandoz | 4.2 BIU/L | C |
| | Thuricide 32B | Sandoz | 8.5 BIU/L | R |
| | Thuricide HPC | Zoecon | 4.2 BIU/L | C |
| | Thuricide 32 LV | Zoecon | 8.5 BIU/L | R |
| | Thuricide 32 F | Zoecon | 8.5 BIU/L | R |
| | Thuricide 38 LV | Zoecon | 12.7 BIU/L | R |
| *israelensis*[3] | Vectobac | Abbott | 2000 ITU/mg | R |
| | Vectobac 200 G | Abbott | 200 ITU/mg | R |
| | Vectobac Technical Powder | Abbott | 5000 ITU/mg | R |

[1]R = Restricted - forestry and public health
C = Commercial - agriculture, woodlots, etc
D = Domestic - homeowner

[2]Contains Thuricide

[3]Potency stated in *Aedes aegypti* international toxic units (ITU) per mg

enhanced specific activity, hybrid delta-endotoxins with altered host spectra or multipurpose microbial insecticides having activity against two or more distinct targets (Luthy *et al.*, 1984; Carlton, 1984). Genetic manipulation of *B.t.* is discussed in greater detail in Section 10.

**4.2. Operational use of *B.t.***

The first aerial applications of *B.t.* in Canada were carried out against spruce budworm (*Choristoneura fumiferana*) in New Brunswick and black headed budworm (*Acleris variana*) in British Columbia (Prebble *et al.* 1961; Morris *et al.* 1975). Early attempts with wettable powders were not particularly successful, but technology slowly improved. Major advances have been made in the last five years and high potency oil-based and aqueous formulations are available which can be applied undiluted on the forest. This has eliminated the need for mixing at an airport and reduced volumes have reduced application costs. In 1979, the first Canada-USA (CANUSA) guidelines for the operational use of *B.t.* against spruce budworm were formulated (Morris, 1980b). Morris (1982) and Smirnoff and Morris (1984) reviewed the use of *B.t.* against forest insects in Canada up to 1981. The most recent CANUSA guidelines highlight critical points to be considered when applying *B.t.* against spruce budworm (Morris *et al.* 1984).

Table II. Areas of forests (ha) operationally treated for spruce budworm control with chemical insecticides and *B.t.*in Canada, 1979-1985.*

| Year | Total | *B.t.* | Chemical | % *B.t.* |
|---|---|---|---|---|
| 1979 | 2,210,641 | 27,115 | 2,183,526 | 1 |
| 1980 | 1,849,890 | 74,485 | 1,775,405 | 4 |
| 1981 | 2,886,142 | 54,671 | 2,831,471 | 2 |
| 1982 | 3,061,911 | 62,832 | 2,999,159 | 2 |
| 1983 | 2,844,873 | 79,416 | 2,765,457 | 3 |
| 1984 | 1,788,679 | 387,345 | 1,401,334 | 22 |
| 1985 | 1,681,552 | 689,528 | 812,024 | 52 |
| 1986** | 1,932,000 | 1,426,000 | 506,000 | 74 |

* Includes jack pine budworm in Ontario
** Planned

Areas treated with *B.t.* to control spruce budworm in eastern Canada in the 7-year period between 1979 and 1985 are given in Table II. It can be seen that *B.t.* usage was limited to between 1% and 4% of the total area treated between 1979 and 1983, but rose dramatically to 22% in 1984 and 52% in 1985. A breakdown by individual provinces is given for 1985 in Table III with Ontario and Nova Scotia using 100% *B.t.*, Quebec 72%, Newfoundland 37% and New Brunswick 12%. The province of Quebec plans a 95% conversion to *B.t.* in 1987.

*Bacillus thuringiensis* is used operationally on a large scale in Canadian forests only against spruce budworm. It is used occasionally against urban tree insect pests such as western tent caterpillar (*Malacosoma pluviale*), tent caterpillar (*M. disstria*), spring cankerworm (*Paleacrita vernata*), fall cankerworm (*Alsophila pometaria*) and gypsy moth (*Lymantria dispar*). The most frequent use of *B.t.* in agriculture is to control the cabbageworm (*Artogeia rapae*), the cabbage looper (*Trichoplusia ni*) and other foliage-eating insects on cruciferous crops, cankerworms and other leaf-eating insects on applies, the tobacco hornworm (*Manduca sexta*) on tobacco, and the bertha armyworm (*Mamestra configurata*) on canola (See Section 5).

The use of *B.t.* in forestry has grown steadily (Table II) and this growth is expected to continue. On the other hand, use in agriculture was nearly static between 1980 and 1984, but increased dramatically in 1985 (Table IV). The static use pattern in agriculture during the early 1980's is surprising considering that it is recommended by most provinces for several pests of field and horticultural crops. Half of the *B.t.* manufactured in North America is used in agriculture, but in Canada, only 5.7%, 1.2% and 3.6% of the total *B.t.* imported in 1983, 1984, and 1985 respectively, was for agricultural use (Table IV).

There are several possible reasons for the greater use of *B.t.* in forestry in Canada compared to its use in agriculture. The most important reason is that an insecticide of any kind is not required, or *B.t.* is not effective or *B.t.* is not competitive

Table III. Area (ha) of forests in each province sprayed for spruce budworm control in eastern Canada in 1985.

| Province | Total | *B.t.* | Chemical | % *B.t.*† |
|---|---|---|---|---|
| Ontario* | 250,715 | 250,715 | 0 | 100 |
| Quebec | 672,497 | 486,363 | 186,134 | 72 |
| New Brunswick | 701,000 | 81,000 | 620,000 | 12 |
| Nova Scotia | 48,000 | 48,000 | 0 | 100 |
| Newfoundland | 9,340 | 3,450 | 5,890 | 37 |
| Totals | 1,681,552 | 869,528 | 812,024 | 52 |

* Includes jack pine budworm

† Percentages of *B.t.* planned for 1986 are: Ontario 100, Quebec 91, New Brunswick 18, Nova Scotia 100, Newfoundland (no sprays), total 74.

Table IV. Units[1] of *B.t.* shipped to Canada between 1980 and 1985 for use in agriculture and forestry.

| Year | Forestry Use | Agricultural Use | % for Agriculture |
|---|---|---|---|
| 1980 | 86,000 | 8000 | 8.5 |
| 1981 | 6,800 | 7350 | 51.9 |
| 1982 | 71,300 | 8240 | 10.3 |
| 1983 | 134,000 | 8100 | 5.7 |
| 1984 | 633,000 | 8000 | 1.2 |
| 1985 | 1,546,376 | 58224 | 3.6 |

[1]1 Unit = 16 billion international units of potency. Data provided by Zoecon Corporation, Palo Alto, California, Biochem Products, Monchanin, Delaware and Abbott Laboratories, Chicago, Illinois.

with chemical insecticides for control of significant pests of several major Canadian agricultural crops such as wheat and other cereals, corn, alfalfa, soybeans, potatoes and tomatoes. Furthermore, although *B.t.* is effective against several pests of agricultural crops, for example, against foliage-eating lepidopterous insects on cruciferous crops and apples, tobacco cutworm on tobacco and bertha armyworm on canola, the areas of land treated for control of these pests is relatively small. By comparison, aerial application of chemical insecticides to extensive areas of forests is required to control major pests. The application of chemical insecticides to such vast areas of forest is an emotional and political issue, for which a feasible alternative is application of *B.t.*, which has a minimum impact on non-target organisms in the forest habitat. Furthermore, in forestry usually one species of insect is the target for regulation, whereas in agriculture it is frequently necessary to control several species at a time and some of these may not be affected by *B.t.*. In addition, *B.t.* usually acts more slowly than do chemical insecticides, a feature that is generally less important in forestry than in agriculture.

Public pressure to reduce use of chemical insecticides may be greater in forestry than in Canadian agriculture and this pressure is heeded more readily by the forestry industry, partly because of the political lobby and partly because the choice of an insecticidal agent is made by a relatively few people in forestry, whereas in agriculture each grower makes the decisions based on his need, its cost and its history of effectiveness.

**4.3. Cost consideration of *B.t.* usage**

The cost of *B.t.* products has declined significantly since 1980. Competitive bidding by suppliers to fulfill contracts with provincial governments for spruce budworm control have forced prices down. Recently, more concentrated products, lower shipping costs and lower finished application volumes have reduced application costs as well as product costs. The average cost of a dosage of 20 BIU/ha has declined from $13.18 in 1980 to $6.96 in 1983. However, over this period aerial application costs for all pesticides have risen and the average applied cost of *B.t.*/ha in 1980 was $27.13 compared to $26.73 in 1983. During this period the cost of chemical insecticides has increased steadily.

*Bacillus thuringiensis* is still more expensive than chemical insecticides when most recent figures (1983) are compared, but these figures vary by province, the greatest differences being found in provinces with the largest spray operations: Ontario 1.5 x more than chemicals, New Brunswick 2.5x and Quebec 3.0x (Carrow 1983). In 1985, *B.t.* product cost $8 to $10 for a 1 ha dosage of 20 BIU.

**4.4 Viruses**

Since about 1940, a considerable amount of experimental work has been conducted in Canada on viruses and, in a few instances, viruses have been applied operationally against insect pests of forests. Some of the most notable successes have been the regulation of several species of sawflies, although lepidopterous insects have also been studied.

The outstanding example of biological control in Canada is the termination of a severe outbreak of European spruce sawfly (*Gilpinia hercyniae*) by a nuclear polyhedrosis virus (NPV) and introduced parasites (Dowden 1940; Balch and Bird 1944; Bird and Elgee 1957). In the 1930's and 1940's, European spruce sawfly was as important a forest insect in eastern Canada and the northeastern United States as spruce budworm is today. Parasite introductions were made from Europe and it is thought that the virus was accidentally introduced along with parasites. European spruce sawfly is presently at an endemic level throughout its North American range

and is held in check by the NPV and parasites. It is ironic that this perfect example of biological control was due to a fortuituous incident and not planned. However, it was observed and documented. The outcome of this incident was the establishment of the Insect Pathology Research Institute in Sault Ste. Marie in 1948 with a mandate to develop pathogens for the regulation of other major forest insect pests. There have been other successes, but none as dramatic, spectacular and economically significant as regulation of the European spruce sawfly.

A sample of virus-killed European pine sawfly (*Neodiprion sertifer*) was received from Sweden by Bird in 1949 and he conducted field trials in Ontario in 1950, 1951, and 1952 (Bird 1953). At this time, European pine sawfly was a major pest of Christmas tree plantations and parasite releases were also being made. Operational use of the NPV by Christmas tree growers in the 1950's and 1960's has not been documented. However, during the 1970's, this insect has been only a minor pest due to use of the NPV and the parasite introductions (Griffiths *et al.* 1984). European pine sawfly NPV was registered by USDA Forest Service staff in the USA under the name Neochek-S in 1983 and by MicroGeneSys Inc. under the name Preserve® in 1985. It is registered by Microbial Resources in the UK under the name Virox® and it is also commercially available in Finland. A Canadian registration petition for a product called Sertifervirus, utilizing American safety testing data, was submitted for evaluation in March 1985. European pine sawfly NPV is the most widely used insect virus and at least 10,000 ha have been treated in 12 different countries over the last 25 years (Cunningham and Entwistle 1981).

Following 8 years of experimental work, the NPV of redheaded pine sawfly (*Neodiprion lecontei*) was temporarily registered in Canada in 1983 under the name Lecontvirus. All the safety testing prior to registration was conducted in Canada. Red pine (*Pinus resinosa*) is a popular species of tree when planting abandoned farm land and in recent years redheaded pine sawfly has been a severe pest in plantations in Ontario and Quebec (Cunningham and deGroot 1984). In these provinces, 388 plantations with a total area of 3,262 ha have been sprayed from the ground or air between 1976 and 1985 with encouraging results.

Some tests have been conducted with Swaine's jack pine sawfly (*Neodiprion swainei*) NPV. A small aerial spray in 1960 gave satifactory results (Smirnoff *et al.* 1962) and in 1964, 1,600 ha were treated. Cold weather followed the application and the concentration of virus may have been too low. The sawfly population was not decimated (Smirnoff and McLeod 1975). Smirnoff (1972) has suggested disseminating virus-infected cocoons in order to initiate an epizootic. This has not been investigated sufficiently to determine if it is, in fact, a feasible pest management strategy. No work has been published recently on this virus. Swaine's jack pine sawfly is a serious pest in both Ontario and Quebec and it is suggested that efforts be continued to develop a viral product for its regulation.

An NPV for the control of Douglas-fir tussock moth (*Orgyia pseudotsugata*) was registered by EPA in the USA in 1976 under the name TM BioControl-1 and a similar product, Virtuss, received temporary registration in Canada in 1983. Douglas-fir tussock moth populations usually collapse in 4 to 5 years due to a naturally occurring virus epizootic. However, during this period severe damage and tree mortality occur and application of virus accelerates termination of the outbreak. Virus has never been used for operational control of Douglas-fir tussock moth in Canada and only experimental trials have been conducted to date. Generally, population reduction has been excellent in the year of application of the virus, but the epizootic has often been too slow to develop and severe defoliation has occurred when insect

populations were heavy (Stelzer *et al.* 1977; Shepherd *et al.* 1984). This virus could probably be used to control whitemarked tussock moth (*O. leucostigma*) and rusty tussock moth (*O. antiqua*) and, if efficacy tests give satisfactory results, these species should be added to the Virtuss label.

Considerable effort has been made to develop a virus for the control of spruce budworm and between 1971 and 1983 a total of 65 plots, with a combined area of 2,656 ha, has been experimentally treated with viruses. Most attention has been focussed on an NPV which has also been extensively safety tested. However, a granulosis virus (GV), entomopoxvirus (EPV) and cytoplasmic polyhedrosis virus (CPV) have also been field tested (Cunningham and Howse 1984; Cunningham 1985). Naturally occurring virus epizootics have never been observed to control spruce budworm populations and success in initiating an epizootic has been limited. High mortality can result from application of the virus at budflush but there is not time for secondary infection to occur, little or no foliage is saved, virus carry-over from one year to the next is not enough to regulate the population and virus is too expensive to apply in the same manner as *B.t.* or a chemical insecticide. The same viruses which infect spruce budworm also infect western spruce budworm (*C. occidentalis*) and jackpine budworm (*C. pinus pinus*) but field trials on these species also gave unsatisfactory results (Cunningham 1985; Cunningham and Cadogan, unpublished).

Gypsy moth NPV was registered by USDA Forest Service staff in 1978 under the name Gypchek. Material supplied by American colleagues was tested on two plots with a combined area of 63 ha in Ontario in 1982 (Meating *et al.* 1983). High population reductions were obtained, but unlike application of Douglas-fir tussock moth NPV, survivors of the epizootic gave rise to moderate populations of gypsy moth the following year (Cunningham, unpublished). Safety testing data are available for this virus and a Canadian registration petition could be prepared; this virus should receive more attention in Canada.

Other viruses have been used in small-scale trials with mixed results. Meriting further investigation are NPVs of the balsam fir sawfly (*N. abietis*) (Olofsson 1973), jack pine sawfly (*N. pratti banksianae*) (Bird 1955; 1961), red pine sawfly (*N. nanulus nanulus*) (D.G. Embree, personal communication), and eastern hemlock looper (*Lambdina fiscellaria fiscellaria*) (Cunningham, unpublished). Less encouraging results have been obtained with NPVs of forest tent caterpillar (Ives 1984), Bruce spanworm (*Operophtera bruceata*) (Ives and Cunningham 1980) and winter moth (*O. brumata*) (Cunningham *et al.* 1981). Preliminary investigations are being made on the possible use of the NPV of the alfalfa looper (*Autographa californica*) for control of black army cutworm (*Actebia fennica*) a pest of newly planted conifers in burned-over areas. Alfalfa looper NPV is the best studied of all insect viruses and is atypical in that it has a relatively wide host range of lepidopterous species.

Insect viruses are also being studied from an entirely different perspective. Females of ichneumonid and braconid wasps, which are parasites of lepidopterous larvae, contain non-occluded baculoviruses (also called polydnaviruses) that replicate in the reproductive system of the parasite. They are injected into host larvae along with eggs and these viruses prevent encapsulation of the wasp eggs (Stoltz and Vinson 1979). The viruses affect the insect defence mechanism and enhance the effectiveness of the parasites. Studies on these viruses are being conducted at Dalhousie University in Halifax and, from the standpoint of forest insect pests, parasites of satin moth (*Stilpnotia salicis*) and gypsy moth are being investigated.

**4.5 Protozoa and fungi**

Far less research has been conducted on application of protozoa or fungi

than on to *B.t.* or viruses. Protozoa and fungi are common naturally occurring pathogens of several major forest insect pests (Morris 1983a; Smirnoff and Juneau 1973). The microsporidian parasite (*Nosema fumiferanae*) is particularly prevalent in long established outbreaks of spruce budworm and can reach a level at which 80% of the insects are infected (Wilson, 1982). Chronic rather than acute disease results from infection with *N. fumiferanae*; it retards larval and pupal development, fecundity and longevity of adult moths. In small-scale tests using single trees, the microsporidia *N. fumiferanae* and *Pleistophora schubergi*, have been applied against spruce budworm and *N. disstriae* against forest tent caterpillar (Wilson, 1982). The protozoans were successfully introduced into the spruce budworm population. *P. schubergi* has a wide host range and infects a large number of forest insect pests (Wilson 1981). There are presently no plans for developing a protozoan for forestry use in Canada.

Spectacular epizootics of fungal diseases have been observed to decimate populations of eastern hemlock looper in Newfoundland (Otvos *et al.* 1973) and spruce budworm in Newfoundland (Otvos and Moody 1978) and in Ontario (Harvey and Burke 1974; D. Tyrrell and D.F. Perry, personal communication). These epizootics were caused by fungi of the order Entomophthorales, but there are presently problems in the mass production of either conidia or resting spores of such fungi in culture. Small scale individual tree trials have been conducted in Ontario and Newfoundland with limited success (K.P. Lim and D.F. Perry, personal communication). Entomophthorales may possibly be used to initiate and maintain an epizootic, whereas other fungi such as imperfect fungi may be applied as a biological insecticide in the same manner as *B.t.* treatments. Currently, there is interest in modelling the potential impacts of fungal treatments on spruce budworm (Perry and Whitfield 1984). Fungi which are not natural pathogens of spruce budworm are also being evaluated. Several imperfect fungi are registered around the world as mycoinsecticides and are commercially available in some countries. A large screening program has been established for species of fungi which can readily be mass produced. This screening, in conjunction with developmental studies and process modelling, will serve as a basis for well planned field trials in the future (D.F. Perry, personal communication).

**4.6 Nematodes**

Steinernematid nematodes can be easily and economically mass produced (Bedding 1981; Hara *et al.* 1981) and are now available commercially. Laboratory tests have shown that a wide variety of forest pests, notably the spruce budworm and hemlock looper, are susceptible to them (Schmiege 1963; Poinar 1979; Finney and Bennet 1983, 1984a, b; Finney *et al.* 1982). However, the most successful applications of these nematodes has been against insect pests dwelling in cryptic habitats: larvae in cocooning sites on tree bark (Dutky 1959) and bark beetles in their tunnels (Moore 1970; Finney and Walker 1977) and it is against such pests that the use of these specialized nematodes may prove highly beneficial. Feasibility studies are presently underway in British Columbia and Newfoundland. Cold tolerant strains of steinernematids which can withstand the temperatures normally encountered in Canada have been isolated (Finney 1984). Although they have been used in trials conducted under semi-natural conditions, further research and development is necessary before they can be successfully applied in the field.

Recently a rhabditid nematode, *Heterorhabditis heliothidis*, has been tested on spruce budworm, larch casebearer (*Coleophora laricella*) and eastern hemlock looper in both petri dish experiments in the laboratory and in greenhouse trials. Encouraging results were obtained and single tree tests will be the next stage of experimentation (Finney-Crawley, unpublished).

### 4.7 Status of integrated pest management (IPM)

The forest ecosystem is an ideal environment for IPM for several reasons: 1) Forests are usually long lasting ecosystems with a high degree of stability, a long evolutionary history and a great diversity of plant and animal life; 2) They often extend over large areas, so that their pest, parasite, and predator complexes usually exhibit only minor regional differences; and 3) The natural enemy complex of forest insects has a high degree of predictability upon which pest management strategies can be based.

To successfully manage forest insect pests, such as the spruce budworm and the gypsy moth, a variety of silvicultural practices and the optimization of natural controls must be systematically integrated and *B.t.* should play an important role in such a system. The development of such an approach to the management of those two insect pests is an urgent need in Canada and is likely to succeed. In both cases, the required knowledge of the biotic and abiotic factors affecting the population dynamics is already available, methods of predicting population trends have been established, a variety of tools to reduce population levels are on hand and computer modelling capability is also available. Some success in managing these and other forest insects has been achieved with the integrated use of *B.t.*, chemical insecticides, the enzyme chitinase, entomopathogenic viruses and the parasite, *Apanteles melanoscelus* (Morris 1982).

Other established techniques may eventually be combined with *B.t.* and other microorganisms in managing our forest insect pests. For example, the breeding and use of pest-resistant trees is a sound crop-protection tool which could be incorporated into a pest management scheme. Tolerant cultivars could raise the pest insect population density level necessary to justify control action, thereby reducing the need for chemical insecticide treatments. Sex pheromones show promise for use with microbial insecticides. Pathogens could be used initially to reduce pest population density, followed by pheromones to disrupt the normal communication of adult survivors. The development of strategies such as these could replace the traditional short-term management strategies such as broad-spectrum applications which are sometimes ineffective and counter-productive (Blais 1976).

### 4.8 Research and development needs in forestry

The committee believes that further research in the following areas would improve the efficacy of microbial agents used in forestry and enhance the technology currently available:

1. A method of accurately assessing deposits of entomopathogenic microorganisms on coniferous and deciduous foliage as opposed to measurement of deposits at ground level should be developed. Some progress has been made on studying *B.t.* droplet density on balsam fir needles related to mortality of spruce budworm larvae (P.G. Fast, personal communication).
2. Tank mixes containing effective sunlight screens and stickers should be developed for all microbial agents. Chevron spray sticker (Chevron Canada) which was widely used with *B.t.* and other microbial agents is considered ineffective by American scientists and it is no longer on the market. Other stickers should be investigated. The use of certain chemical sunscreens has been shown to enhance the effectiveness of *B.t.* against spruce budworm (Morris 1983b). The same additives used for *B.t.* tank mixes would probably also enhance the effectiveness of virus applications. If nematodes are to be applied as foliar sprays, a tank mix must be developed with an appropriate humectant to prevent desiccation.

3. Further studies should be conducted on the long-term effects of *B.t.* applications on forest insect populations. Studies with *B.t.* on spruce budworm indicate that this pathogen can have a long-term suppressive effect on populations (Morris 1977; Dimond and Spies 1981). Smirnoff (1983) demonstrated that a *B.t.* treatment on spruce budworm in Quebec had a debilitating effect on survivors. Survivors of chemical insecticide treatments were more vigorous and had greater energy reserves than *B.t.*-treated survivors or untreated populations. If such a phenomenon can be confirmed by further research, the full cost-benefit of *B.t.* applications in forestry and agriculture may be more fully appreciated.
4. There is a need to increase the efficacy of *B.t.* by selecting more toxic strains. Members of the family Tortricidae, such as the spruce budworm, are highly susceptible to variety *kurstaki* which is the commercially produced strain of *B.t.* Members of other families such as Noctuidae and Geometridae vary widely in their susceptibility to variety *kurstaki.* There are more than 1,000 strains or isolates of *B.t.* held in an international depository in the USA. The committee recognizes a need to test these strains against refractory insect species with a view to selecting strains which are more effective than *B.t.* var. *kurstaki.* Screening of other pathogens, such as virus and fungi, from around the world against spruce budworm, hemlock looper and other important forest insect pests has been conducted in a rather haphazard manner in the past. Such research could have rewarding results and efforts should be intensified to screen exotic pathogens.
5. Only a few viruses are obvious candidates for development and registration in Canada. Foremost are the NPVs of gypsy moth and Swaine's jack pine sawfly. American safety testing data are available for the former virus, but none are available for the latter. A few other viruses are at an early stage of development and merit further research to determine if they are potentially useful microbial control agents. There are NPVs of balsam fir sawfly, jack pine sawfly, red pine sawfly, and eastern hemlock looper; also included in this list is alfalfa looper NPV for control of black army cutworm. Efficacy tests should be conducted so that whitemarked tussock moth and rusty tussock moth can be added to the Virtuss label.
6. Theoretically, fungi could be useful for management of forest insect pests and research on this group of pathogens should be continued and intensified. Several imperfect fungi are available commercially and can be produced relatively inexpensively; their use pattern would be similar to that of *B.t.* Use of Entomophthorales to initiate and maintain fungal epizootics is a much more attractive strategy and further research should be conducted to overcome the technical problems involved in mass production of this group of fungi.
7. Nearly all attention in forestry has been focussed on leaf-eating Lepidoptera and Hymenoptera. Research on pathogens of bark beetles, boring beetles, aphids and seed and cone insects has been virtually negligible other than a few small scale experiments involving the use of fungi. Research on microbial control of these important groups of forest insect pests should either be initiated or intensified.
8. Nematodes have generally been dismissed as practical agents for forestry application. However, they are the only agent described in this report with the ability to search out the host and hence the only agent with any capability of controlling insects which live in cryptic habitats. The committee feels that research on entomopathogenic nematodes should be encouraged.
9. There is an excellent opportunity to apply IPM systems in forestry for the regulation of such well-studied species as spruce budworm or gypsy moth. IPM is

discussed frequently from a theoretical standpoint and rarely, if ever, implemented from a practical standpoint. The committee feels that most, if not all of the mechanisms are in place which enable forest managers to practice IPM for these few major pest species and the committee urges that decision makers be advised better on the courses of action open to them.

10. The advent of recombinant DNA technology offers the possibility of genetically manipulating bacteria, viruses and other microorganisms and enhancing their effectiveness as pest control agents. The committee is of the opinion that this new technology should be exploited quickly and put to use in the improved management of forest insect pests. Research on genetic manipulation of *B.t.* has already reached an advanced stage and some research has been conducted with NPVs. Speculation as to the successful outcome of genetic manipulation of pathogens is limitless and this aspect is a leading research priority. This is discussed in greater detail in Section 10.

## 5. ENTOMOPATHOGENS IN THE CONTROL OF PESTS OF AGRICULTURAL CROPS

### 5.1 Effectiveness of entomopathogens in the control of pests of agricultural crops.

Entomopathogens are known to cause mortality or other detrimental effects in field populations of many insect pests of agricultural crops. Many of these are microorganisms that cause a pathological condition only under exceptional circumstances and, as such, are not predictable regulatory agents. A lesser number of naturally occurring entomopathogens, on the other hand, do exert a consistent effect on populations of some pests in certain circumstances and locations and have potential for a substantial role in management of insect pests. A relatively small number of entomopathogens have been tested against pests of agricultural crops; some of these (Table V) appear to have potential for a role in integrated management systems. Only one entomopathogen, the bacterium, *Bacillus thuringiensis* (*B.t.*), is registered for use on agricultural crops in Canada. Several formulations of *B.t.* are registered in Canada for commerical and domestic agricultural use (Table I).

Although the effectiveness of formulations of *B.t.* for control of several pests of agricultural crops in Canada was demonstrated in the 1960's and 1970's (Fox and Jaques 1966; Jaques 1961, 1965, 1973, 1977a), use of the microbial insecticide remained rather static during 1980-84, increasing substantially in 1985 (Table IV). Competition in cost and effectiveness with new chemical insecticides, particularly the synthetic pyrethroid compounds, is considered partly responsibile for the lack of growth in use of *B.t.* in this period. Furthermore, the development of resistance of *B.t.*-susceptible pests to chemical insecticides was limited, not providing incentive to utilize the microbial insecticide. In addition, the need to reduce use of chemical insecticides in production of agricultural crops to avoid pollution of the field habitat has not been sufficiently well accepted by agriculturalists to provide incentive to use *B.t.* and other biological control agents instead of chemicals.

Formulations of *B.t.* are recommended among the insecticidal materials listed in the Ontario Vegetable Production Recommendations for control of the cabbage looper (*Trichoplusia ni*), cabbageworm (*Artogeia* (=*Pieris*) *rapae*) and diamondback caterpiller (*Plutella xylostella*) on cruciferous crops and rutabaga and for control of the cabbage looper on celery, lettuce, spinach and tomato. The extent of the use of *B.t.* is indicated by the estimate that more than half of the cruciferous crops in Ontario (ca 4000 ha in 1983) was sprayed at least once, but usually more frequently, with a formulation of *B.t.* in 1984. Formulations of *B.t.* are also recommended for

control of lepidopterous leaf-eating insects on cabbage and other crucifers in Nova Scotia, Prince Edward Island, Quebec and British Columbia. Although the effectiveness of *B.t.* against these pests is competitive with that of chemical insecticides, formulations of the bacterium are not used as extensively in other provinces as they are in Ontario.

Formulations of *B.t.* are registered for use on tree fruits, including apples and pears, being particularly effective against foliage-eating lepidopterans. However, *B.t.* is not used extensively against these pests. For example, *B.t.* is not included in the pesticide recommendations for tree fruits in British Columbia, but it is included in the B.C. Tree Fruit Production Guide for Interior Districts as an alternate material for control of leafrollers. It is particularly useful against leafrollers that have developed resistance to organophosphates. In Nova Scotia, formulations of *B.t.* are used on apple orchards to control the winter moth (*Operophtera brumata*) as an alternate to pyrethroids and other chemicals.

*Bacillus thuringiensis* is a very effective insecticide for control of the tobacco hornworm (*Manduca sexta*) on tobacco and is recommended for this use in Ontario. A majority of Ontario tobacco growers apply the microbial insecticide for control of this pest with consistently good results. It is noteworthy that inclusion of the beta exotoxin in formulations of *B.t.* has been found to broaden the host spectrum of *B.t.* to include the Colorado potato beetle (*Leptinotarsa decemlineata*) and other species of pests. Formulations of *B.t.* containing the exotoxin are not registered in Canada.

Several entomoviruses that are not registered for use on agricultural crops have been found to have particular potential as introduced components of pest management systems. The granulosis virus of the codling moth (*Cydia pomonella* GV), is of vital interest as a candidate for commercial development as a microbial insecticide for management of the codling moth, an important pest of apples and pears. The virus is very active against neonate *C. pomonella* in the laboratory (Laing and Jaques 1980), but it has given varible results in trials in apple orchards in several locations in Canada (Jaques and Laing 1984; Jaques *et al.* 1977, 1981), partly because the larva feeds on exposed surfaces for only a short time before entering the apple. Timing of application has significant influence on effectiveness. Extensive assessments of entomoviruses for control of cole crop insects in Ontario (Jaques 1973, 1977a; Jaques and Laing 1978) and in the Atlantic provinces (Fox and Jaques 1966, 1977; Fox *et al.* 1972) demonstrated that applications of the granulosis virus of *Artogeia* (= *Pieris*) *rapae* (*A. rapae* GV) and the nuclear polyhedrosis viruses of *Trichoplusia ni* (*T. ni* NPV and *Autographa californica* NPV) are highly effective in control of the imported cabbageworm (*A. rapae*) and the cabbage looper (*T. ni*) respectively. The potential of these viruses as components of a treatment regime for cole crops integrating microbial and chemical insecticides is discussed in Section 5.4.

The fungus, *Verticillium lecanii*, has been tested quite extensively in Ontario, Alberta, and British Columbia for control of the greenhouse whitefly and aphids on cucumbers, tomatoes and chrysanthemums grown in the greenhouse. The fungus is registered for these uses in the United Kingdom. Results of tests in Canada have been variable, with some tests giving indications of acceptable effectiveness of the fungus for control of aphids on greenhouse crops, whereas other tests of the fungus, notably tests in Ontario against the greenhouse whitefly, have yielded less favorable indications of effectiveness.

Several other pathogens, including the NPV of *Euxoa messoria*, the fungi *Beauveria bassiana* and *Entomophthora* (*Zoophthora*) *phytonomi*, the microsporidia *Nosema locustae*, *Nosema pyrausta*, and *Vairimorpha necatrix* and the nematode

*Steinernema feltiae* (=*Neoplectana carpocapsae*) have been evaluated against species of insects that are important pests of agricultural crops. Discussion later in this report (Section 5.4) indicates that *E. messoria* NPV, *N. pyrausta* and *V. necatrix* were effective when applied to control the dark-sided cutworm, the European corn borer and the cabbage looper, respectively, in field tests. On the other hand, preparations of *Z. phytonomi* did not kill the alfalfa weevil in plot studies. The nematode *S. feltiae* was not effective against most foliage-eating lepidopterous larvae, probably because the nematodes were killed by desiccation, but application of the nematode to soil appeared to be promising for suppression of some soil-inhabiting insects (Section 5.4).

**5.2 The role of microbial agents in IPM**

Entomopathogens are considered to have the potential for a significant role as introduced and applied components of integrated management systems for a number of insect pests of agricultural crops (Jaques 1983). In addition, entomopathogens that occur naturally in the field habitat may have substantial impact on populations of some species of pest insects if conditions are suitable for development of natural epizootics.

Some entomopathogens have been found to be quite competitive with chemical insecticides as applied control agents not only in effectiveness in protecting the crop, but also in cost. Furthermore, entomopathogens are especially attractive because of their high degree of specificity for particular target species, or groups of target species, resulting in negligible environmental impact with little or no direct effect on parasitic or predaceous arthropods and other animals in the habitat, and with little or no detectable hazard to applicators of the pesticide or to consumers of the agricultural produce (Burges 1981). Entomopathogens are also attractive as components of management systems for agricultural crops because pest species do not develop resistance to pathogens, most pathogens of interest are naturally occurring mortality agents, and because many of the entomopathogens are compatible with other techniques and materials used in crop protection (Jaques and Morris 1981).

**5.3 The role of naturally occurring entomopathogens**

Naturally occurring entomopathogens may contribute substantially to suppression of some pest insects (Table V), particularly when crop management practices that are compatible with development of diseases are followed. Indigenous entomopathogens may be enzootic in populations, usually killing a small proportion of the pest population, or they may cause epizootics resulting in high mortality and dramatic declines in populations, in some cases contributing substantially to cyclical occurrence of pest species. For example, mortality from naturally occurring nuclear polyhedrosis virus (NPV) may be a major cause of rapid declines in populations of the armyworm (*Pseudeletia unipuncta*). Similarly, a naturally occurring NPV infected as many as 65%, but usually less than 10%, of larvae of the bertha armyworm (*Mamestra configurata*) collected in a survey of canola fields in Manitoba (Wylie and Bucher 1977) probably contributing to regulation of field populations of this important pest. Granulosis virus (GV) and NPV are known to cause high mortality in populations of *A. rapae* and *T. ni*, respectively (Jaques 1973, 1975), reducing pest populations especially late in the growing season in Ontario, but the epizootics appear to have little direct influence on populations in the subsequent year unless there has been a substantial accumulation of the viruses in the soil (Jaques 1985).

Entomogenous fungi occur naturally in field populations of several species of pest insects, in some cases causing substantial mortality. For example, the fungus *E. phytonomi* and other fungi cause high mortality in larval populations of the alfalfa weevil (*Hypera postica*) and contribute to suppression of the pest in some localities in

Table V. Entomopathogens which are potentially useful in integrated management of insect pests of Canadian agriculture crops

| Entomopathogen | Target Insect | Crop |
|---|---|---|
| **Bacteria** | | |
| *Bacillus thuringiensis* | Cabbage looper | Crucifers, rutabaga |
| | Imported cabbageworm | |
| | Diamondback moth | |
| | Cabbage looper | Tomato, celery, spinach |
| | Leafrollers, etc. | Apple |
| | Tobacco hornworm | Tobacco |
| | Corn borer | Sweet corn |
| | Indian meal moth | Stored wheat, potato |
| *Bacillus thuringiensis* with Beta-exotoxin | Colorado potato beetle | Potato, tomato |
| **Viruses** | | |
| *Cydia pomonella* GV | Codling moth | Apple |
| *Euxoa messoria* NPV | Dark-sided cutworm | Tobacco |
| *Mamestra configurata* NPV | Bertha armyworm | Canola |
| *Pieris rapae* GV | Imported cabbageworm | Crucifers |
| *Pseudaletia unipuncta* NPV | Fall armyworm | Cereals |
| *Trichoplusia ni* NPV (and *Autographa californica* NPV) | Cabbage looper | Crucifers |
| **Fungi** | | |
| *Aschersonia* spp. | Greenhouse whitefly | Greenhouse tomato, cucumber |
| *Beauveria bassiana* | Codling moth | Apple |
| | Colorado potato beetle | Potato |
| *Entomophthora grylli* | Grasshoppers | Cereals, rangeland |
| *Metarrhizium anisopliae* | Various insects | |
| *Verticillium lecanii* | Greenhouse whitefly, aphids | Greenhouse tomato, cucumber |
| *Zoophthora phytonomi* | Alfalfa weevil | Alfalfa, clover |
| *Zoophthora aphidis* | Aphids | Potato, peas, etc. |
| **Microsporidia** | | |
| *Nosema locustae* | Grasshoppers | Cereals, rangeland |
| *Nosema pyraustae* | Corn borer | Corn |
| *Vairimorpha necatrix* | Cabbage looper, corn borer, etc. | Crucifers, corn, etc. |
| **Nematodes** | | |
| *Steinernema feltiae* | Corn rootworms | Corn |
| | Root maggots | Crucifers, carrot |
| | Cutworms | Cereals, crucifers |

Ontario (Harcourt *et al.* 1974, 1977). Similarly, various species of aphids are infected and killed by naturally occurring entomofungi, principally, *Entomophthora* (=*Zoophthora*) species. For example, the high mortality of the pea aphid by

entomofungi in fields of peas in Nova Scotia and Prince Edward Island (H.B. Specht, personal communication) caused significant reductions in populations of the aphid. Likewise, populations of the apple sucker (*Psylla mali*) in some Nova Scotia apple orchards were maintained at economically acceptable densities by the entomogenous fungus *Entomophthora sphaerosperma* (Jaques and Patterson 1962). In addition, populations of grasshoppers in western Canada have been reduced by epizootics of naturally occurring fungi, particularly *Entomophaga grylli*. On the other hand, a long-term study showed that *B. bassiana* killed only a small portion of larvae of the codling moth overwintering on apple trees in Nova Scotia, apparently having little impact on population survival (Jaques and MacLellan 1965).

Microsporidia, an order of protozoa that infects insects, occur naturally in many species of agricultural pests. For example, up to 10% of grasshoppers collected in a survey of southern and central Saskatchewan fields were infected by *N. locustae* (Ewen 1983), a microsporidium that is found in populations of grasshoppers in other locations as well. Microsporidia, principally *N. pyraustae*, were found in as many as 20% of adults of the corn borer (*Ostrinia* (=*Pyrausta*) *nubilalis*) trapped in southern Ontario by Laing and Jaques (1985). In addition, infection by microsporidia is found in Canadian field populations of several species of noctuids including the white cutworm (*Euxoa scandens*) (Hannay and McLeod 1981) and the cabbage looper.

**5.4 The role of applied or introduced entomopathogens**

Formulations of the bacterium, *B. thuringiensis*, the only entomopathogen registered for use on agricultural crops in Canada, as well as entomopathogens that have been effective experimentally, are recognized to have the potential for a major role as applied biological components of integrated systems for management of agricultural crops. *B.t.* is used by a substantial proportion of growers, particularly in Ontario, to protect cruciferous crops against foliage-eating lepidopterous pests, particularly imported cabbageworm, cabbage looper, and diamondback moth larvae. It is suggested that this use should be expanded as should the use of *B.t.* for management of cabbage loopers and other leaf-eating lepidopterous insects on tomatoes, celery, lettuce and rutabaga. The extensive use of *B.t.* for control of the tobacco hornworm indicates that the microbial insecticide is competitive with chemical insecticides for control of this important pest of tobacco. The use of *B.t.* on cabbage, cauliflower, lettuce, tobacco and other crops is attractive because *B.t.* may be applied up to and during the harvest period, because *B.t.* has little or no impact on non-target arthropods in the field habitat (Jaques 1965), because its use is not hazardous to the applicator or to other workers who handle the crop, and because residues of the bacterium are not harmful to the consumer. Several stored product moth larvae are naturally infected by many varieties of *B.t.* (Subramanyam and Cutkamb, 1985), but research and development of these pathogens for the control of insect pests of stored products have not been pursued in Canada.

The viruses of *T. ni* and *A. rapae* are attractive for development as microbial insecticides because they are effective under field conditions (Jaques 1973, 1977a; Jaques and Laing 1978). In addition, the viruses persist and accumulate in the field habitat, principally in soil (Jaques 1975, 1977b, 1985) and, therefore, application of the viruses early in the season may have an extended effect on populations of the pest insect or natural epizootics may be initiated by viruses persisting in the field habitat. Furthermore, the viruses of *T. ni* and *A. rapae* are compatible with chemical insecticides and may be used alone, combined as spray mixtures, or combined sequentially in spray programs with *B.t.* or chemical insecticides to enhance protection of the crop and to reduce the quantity of chemical insecticide applied to the field. It is

noteworthy that *A. rapae* GV has less potential for development as a microbial insecticide because *A. rapae* larvae are readily controlled by application of *B.t.*, reducing the need for development of a second effective biological control agent (Jaques 1983). On the other hand, viruses of *T. ni*, particularly *A. californica* NPV, are attractive for commercial development not only because they are more effective than *B.t.* against *T. ni*, but also because *A. californica* NPV is also effective against other species of pest insects, mostly noctuids, and thus would have broad usage.

The granulosis virus of the codling moth has particular potential in management of the codling moth in apple and pear orchards not only because it is effective against the codling moth which is a world-wide pest of apples, pears and nuts, but also because use of the virus is specific for the target insect and does not affect the arthropod balance in the orchard (Jaques *et al.* 1977, 1981). In contrast, applications of some broad-spectrum chemical insecticides decimate populations of predaceous and parasitic arthropods resulting in increases in secondary pest species. Economical mass-propagation of the codling moth GV is a significant hindrance in commercial development.

Other viruses have potential for use in management of insect pests of agricultural crops. For example, damage to young tobacco plants by the dark-sided cutworm was reduced by applications of an NPV to the cover crop before the tobacco was planted in field tests (Cheng and Jaques 1973). Other pest insects, including the variegated cutworm (*Peridroma saucia*), the fall armyworm (*Laphygma frugiperda*) and the bertha armyworm, are susceptible to viruses that may have potential for development.

Several fungi are effective as introduced components of integrated management systems. Strains of *Verticillium lecanii* have been developed for control of aphids and the greenhouse whitefly on cucumbers and tomatoes grown in greenhouses. Effectiveness of the fungus against the whitefly has been variable probably because of difficulties in integrating conditions for control of fungal diseases of crop plants with the conditions required for development of the entomofungus. The entomofungi, *B. bassiana* and *Metarrhizium anisopliae*, are infectious for a variety of agricultural insects. Use of *B. bassiana* against the Colorado potato beetle on potatoes and tomatoes and against lepidopterous pests of other crops is of particular interest. Development of satisfactory methods for propagating species such as *E. phytonomi*, *E. aphidis*, and *E. grylli*, would greatly enhance realization of the potential of these entomofungi in management of the alfalfa weevil, aphids, and grasshoppers, respectively.

Microsporidia, especially *N. pyraustae*, *N. locustae*, and *V. necatrix*, applied as baits have been found to be effective against grasshoppers on rangelands in Montana (Henry and Onsager 1982) and in western Canada (Ewen 1983). Development of these protozoans as a component of rangeland management systems is considered to be feasible. *N. pyraustae*, a microsporidian that infects a substantial proportion of population of adults of the corn borer (*O. nubilalis*), in the north-central U.S.A. and in southwestern Ontario, was moderately effective as an insecticide applied to plots of sweet corn, reducing damage by the borer by at least 50% (Laing and Jaques 1985). The microsporidium was less effective than were recommended chemical insecticides in these trials.

*Verticillium necatrix* infects several lepidopterous insects. For example, in tests at Harrow, Ontario, *V. necatrix* was as effective as *N. locustae* against the corn borer in sweet corn and was as effective as *T. ni* NPV or *A. californica* NPV against the cabbage looper on cabbage when applied in field trials. Microsporidia, like

entomoviruses, are difficult to mass-propagate because they are obligate parasites, multiplying only in living insect tissue. It is noteworthy however, that some microsporidia, such as *V. necatrix* are not specific, and can be propagated in insects, like *T. ni*, that are readily mass-reared enhancing potential for development as microbial insecticides.

Nematodes of the family Steinernematidae and, to a less extent, of the family Mermithidae, parasitize various species of insects. *Steinernema feltiae* (=*Neoaplectana carpocapsae* = DD136) is the only species that has been studied to any extent as an applied biological control agent. Although laboratory tests have indicated that *S. feltiae* has a wide host range among species of insects that are pests in Canada (Morris 1985), field applications of the nematode have caused mortality of relatively few species of pest insects, mostly species inhabiting soil or other habitats protected against desiccation (Finney 1984). For example, Welch and Briand (1960) found that applications of *S. feltiae* reduced damage to cabbage, radish and rutabaga by the cabbage root maggot (*H. brassicae*). Similarly, applications of *S. feltiae* in transplanting water protected tobacco plants from root damage by *Hylemya* spp. as well as applications of the recommended chemical insecticide (Cheng and Bucher 1972). On the other hand, foliar applications of the nematode to potatoes had little effect on populations of the Colorado potato beetle (Welch 1958; Welch and Briand 1961). Likewise, populations of the cabbageworm on young cruciferous plants were not reduced by foliar applications of the nematode in tests by Welch (1971), but applications of *S. feltiae* to older plants which retained moisture in the heads and leaves were effective against *A. rapae* in other field tests (Fox and Jaques 1966). Similarly, damage to sweet corn by the European corn borer (*O. nubilalis*) was reduced by application of *S. feltiae* because moisture was retained in leaf whorls (Welch and Briand 1960). It is noteworthy that although foliar applications of *S. feltiae* to apple trees did not reduce populations of larvae of the winter moth (*O. brumata*) probably due to rapid desiccation of the nematode (Jaques 1967), drenching soil under apple trees with suspensions of the nematode killed a substantial proportion of *O. brumata* and the leafroller (*P. mali*) pupating in the soil (Jaques *et al.* 1968). Significant mortality of the carrot weevil (*Listronotus oregonensis*) and the cabbage root maggot in the field and the chrysanthemum leaf miner (*Phytomyza syngenesiae*) in the greenhouse by naturally occurring nematodes suggests the potential of entomophilic nematodes in management of certain species of pest insects in habitats in which desiccation can be retarded.

It is evident that desiccation of nematodes is a major factor limiting their effectiveness in control of pest insects. It is noteworthy, in addition, that species of steinernematids differ in virulence, tolerance to adverse environmental conditions, ability to seek a host, and behavior in soil (Finney 1984). The establishment of a profile based on their characteristics is suggested in order to exploit their characteristics in selection of a strain or species to be used in a particular habitat or against a particular species of insect.

It should be emphasized that entomopathogens are particularly attractive as applied or introduced components of integrated management systems because they have little or no impact on predaceous and parasitic arthropods and other fauna that influence populations of pest species. This feature of entomopathogens is manifested, for example, by the relatively low populations of aphids found on cruciferous crops sprayed with *B.t.* or viruses and by the low populations of mites in orchards treated with *B.t.* to control leaf-feeding lepidopterous insects (Jaques 1965). The minimum impact on beneficial arthropods in the fauna of apple orchards is a very significant

Table VI. List of insect-pathogen-crop systems requiring priority research in Canada.

| Pest species | Crop | Entomopathogen |
|---|---|---|
| **Forestry** | | |
| Spruce budworm, Western spruce budworm | | |
| Jackpine budworm, *Choristoneura* spp. | Balsam fir, spruces | NPV, *B.t.* |
| Swaine's Jackpine sawfly, *Neodiprion swainei* | Pine | NPV |
| Gypsy moth, *Lymantria dispar* L. | Oaks, willow, aspens birches, basswood, apple, larch, hemlock, pines, spruces, elms, maples, | NPV, *B.t.* |
| Hemlock looper, *Lambdina fiscellaria fiscellaria* | Hemlock, balsam fir | NPV, *B.t.* |
| Black army cutworm, *Actebia fennica* | Coniferous seedlings | NPV |
| Bark beetles and boring beetles | Coniferous and deciduous trees | Fungi, Nematodes |
| **Agriculture** | | |
| Codling moth, *Cydia pomonella* | Apples, pears | GV |
| Cabbage looper, *Trichoplusia ni* | Cole crops, tomatoes | NPV |
| Corn borer, *Ostrinia nubilalis* | Field corn, sweet corn | Microsporidia |
| Imported cabbage worm, *Pieris rapae* | Cole crops | GV |
| Cutworms (climbing and subterranean) eg. *Actebia fennica* | Vegetables, tobacco forage crops | Viruses, *B.t.* Nematode-bacteria complexes, fungi |
| Armyworms, eg. *Mamestra configurata* | Canola | Viruses, *B.t.* |
| Aphids | Various field crops | Fungus, *Zoophthora aphidis* |
| Leafrollers and fruitworm | Apple | *B.t.* |
| Grasshoppers | Rangeland and various field crops | Fungus (*Entomophthora grylli*); Microsporidia (*Nosema locustae*) |
| Greenhouse whitefly and aphids | Greenhouse crops | Fungi (*Verticillium lecanii*) |
| Stored products insects, eg. Rusty grain beetles and Indian meal moth | Stored products | Bacteria, Microsporidia, nematode-bacteria complexes |
| Cabbage root maggot | Cole crops | Nematode-bacteria complexes |
| **Public and animal health** | | |
| Mosquitoes and blackflies | | *B.t.i.*-H14 *Bacillus sphaericus* |

NPV = nuclear polyhedrosis virus; B.t. = *Bacillus thuringiensis*; GV = granulosis virus

feature favoring use of the granulosis virus of the codling moth as a component of a management system for apple orchards. In addition, the entomopathogens that are considered for development are safe for humans, higher animals, and fish. This is a particularly important consideration in regard to the safety of the applicator of pesticides and of handlers of the crop and in regard to residues on the crop immediately prior to harvest.

Compatibility with chemical pesticides applied to crops has a significant influence on effectiveness of introduced and naturally occurring entomopathogens in management of insect pests. Compatibility of entomopathogens with chemicals in tank mixes and with deposits of chemicals on the crop are important considerations for applied pathogens, whereas only the latter is of concern to naturally occurring pathogens. Entomoviruses and *B.t.* are generally influenced less by chemicals than are entomofungi or microsporidia and most insecticides have less effect than fungicidal chemicals (Jaques and Morris 1981). Some chemical insecticides have been observed to interfere with entomopathogens apparently because the chemical insecticide kills more quickly than does the pathogen, thus reducing mortality by the pathogen. Some insecticides are directly toxic to entomopathogens, e.g. parathion and phorate are toxic to some entomofungi (Ignoffo 1981; Jaques and Morris 1981). On the other hand, mixtures of some chemical and microbial insecticides are additive or synergistic in action, with mixtures being more effective than the components used alone. For example, low-dosage mixtures of *B.t.*, *T. ni* NPV, and *A. rapae* GV with chlordimeform (Jaques and Laing 1978) or permethrin (Jaques and Laing, unpublished) were as effective against *T. ni* and *A. rapae* in field tests as were the components used alone at the full rate.

Deposits of some fungicides have reduced effectiveness of certain entomopathogens, particularly entomofungi, indicating the need to use selective chemicals in pest management systems. For example, Soper *et al.* (1974) found that benomyl was more toxic to species of *Entomophthora* which are pathogenic to aphids than it was to *Alternaria solani*, a pathogen of potatoes, whereas chlorothalonil inhibited the potato pathogen and had little effect on the aphid pathogen. Similarly, certain fungicides used to control apple scab reduced mortality of the pest insect, *P. mali*, by the fungus, *E. sphaerosperma*, whereas other fungicides were less detrimental to the entomofungus (Jaques and Patterson 1962). In addition, additives may prolong persistance of entomopathogens (Jaques 1977b, 1985). For example, materials that protect from the ultraviolet light of sunlight may extend the effectiveness of entomoviruses and of *B.t.* substantially (Jaques 1972, 1975).

Crop management practices may influence establishment and dissemination of entomopathogens in the field habitat. For instance, it is evident that relative humidity has a substantial effect on development of epizootics of *V. lecanii* to populations of the greenhouse whitefly. In regard to the effect of humidity on fungal diseases, it is interesting to note that humidity in the microhabitat around plants such as soybeans was increased by closed-canopy growth, thus promoting mortality by fungi (Allen *et al.* 1978). In addition, accumulation of entomoviruses in surface soil may be increased by reduced tillage, thus increasing the probability of viral contamination of foliage of crop plants and initiation of disease epizootics (Jaques 1970).

**5.5 Research and development needs in agriculture**

1. Research is required to identify and evaluate effects of naturally occurring entomopathogens on pest insects and to determine feasibility of developing entomopathogens as applied microbial insecticides against species of pests.

   This research should include studies on effectiveness and on

environmental factors that influence effectiveness including impact on non-target fauna. These studies should emphasize epizootiology and dynamics of disease development in populations and host-pathogen relationships. Crop management practices, particularly practices and selective pesticides that may influence effectiveness of indigenous and applied entomopathogens, should be identified and use of those that are compatible with entomopathogens should be integrated into management systems.

Host-pathogen systems of particular interest in management of agricultural insect pests include:

- *B.t.* for control of various foliar insects and strains of *B.t.* effective against coleopterous insects;
- Granulosis virus of the codling moth on apple and pear.
- Viruses of the cabbage looper and the cabbageworm on cruciferous crops.
- Viruses of various noctuids, particularly the bertha armyworm.
- Fungi against the greenhouse whitefly, alfalfa weevil, aphids, the potato beetle and grasshoppers.
- Microsporidia infections for grasshoppers, the corn borer, and various lepidopterous species.
- Nematodes for control of root maggots of cruciferous crops, subterranean cutworms, carrot rust fly, *Psila rosae*, carrot weevil, and chrysanthemum leaf miner.

2. The effectiveness of applied entomopathogens relative to chemical insecticides must be increased to enhance the competitive status of microbial insecticides. Research, including genetic manipulation techniques, is required to develop more active strains of entomopathogens and to increase suitability of entomopathogens as components of IPM systems. (For example, increased tolerance of pesticides and increased resistance to inactivation by ultraviolet light). Improved formulations and improved application techniques should be developed. In addition, more efficient techniques for mass-propagation are required. This research should be well supported by public funding.
3. Government should provide a more favorable climate for participation of private industry in development of entomopathogens by financial support and other incentives to encourage development, propagation, formulation and distribution of these valuable components of integrated pest management systems. Toxicological assessment and registration should be facilitated and supported by public funding to encourage development of entomopathogens.
4. The exploitation and use of entomopathogens and other biological control agents to their full potential as components of an integrated management system should be encouraged by technology transfer, emphasizing to the agribusiness community the advantageous features of biological control agents in crop production.

## 6. ENTOMOPATHOGENS IN THE CONTROL OF INSECTS OF PUBLIC AND ANIMAL HEALTH

### 6.1 Review of previous uses of bacteria

The most important pests concerned with animal and human health in Canada are blood-feeding Diptera. Blackflies primarily pose a nuisance problem, but mosquitoes are both a nuisance and important disease vectors. Both are associated with lack of productivity. These flies are pests because of irritation of a host through their activity around it, through allergic reactions of the host to bites and through

transmission of viruses and parasites from host to host. The isolation of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) from mosquito breeding sites in Israel (Goldberg and Margalit 1977) provided the first biological control agent that has proved to be feasible for mosquito and blackfly control in Canada. *B.t.i.*, the H-14 serotype of *B. thuringiensis*, is an aerobic, gram-positive, spore-forming bacterium which causes rapid insect mortality following its ingestion. Because of this relationship, *B.t.i.* was easily mass produced following the protocols laid down for the commercial production of *B. thuringiensis* var. *kurstaki* and was available for testing soon after its isolation (Couch and Ross 1980).

Most of the development work for mosquito control utilizing *B.t.i.* was conducted in the United States. A number of field tests were run across Canada to test the efficacy of various formulations. These trials were generally successful and indicated that granular formulations would be most efficacious under Canadian conditions. These formulations helped to avoid loss of activity of the bacterium due to inactivation of the crystal toxin or binding of the crystal with organic materials in habitats containing sediment (Ignoffo *et al.* 1981). The effectiveness of *B.t.i.* against larval mosquitoes in the field depends to a great extent on host habitat and on the length of time the bacterium remains available to the target host.

Using unformulated *B.t.i.*, Undeen and Nagel (1978) first showed its effectiveness against several species of blackflies in Newfoundland. Undeen and Colbo (1980) showed that the bacterium was equally effective in the field. Four streams in which temperatures varied from 3-22°C were treated with an unformulated aqueous suspension of the bacterium. The bacterium produced up to 100% larval kill in some streams. The results of this test and those undertaken in the next few years elsewhere (Gaugler and Finney 1982) indicated that low volume application of the product for periods of short duration could be highly effective for control of all larval instars of a range of blackfly species. Changes in formulation were needed to prevent the inoculum of bacteria from dropping out of suspension particularly in areas where pools interrupted stream flow. Downstream carry was directly correlated with the stream flow rate and any stream characteristics such as rock formations or vegetation that would hinder the flow would also reduce the carry and therefore the effectiveness of the product measured in distance along a stream.

Recent research has been oriented towards the correlation of information of the effects of stream depth, width, length and characteristics on carry so that efficacy of predetermined doses of *B.t.i.* in this environment can be reliably predicted (Colbo 1984). In addition to Newfoundland and Labrador where the thrust of these investigations have been focussed, field research is also being carried out in Quebec and at the Canadian Biting Fly Centre, Winnipeg.

So far *B.t.i.* has proven to be the most effective microbial insecticide available for use against larval mosquitoes and blackflies. Present research is focussed on refining field protocols to facilitate more widespread use of the bacterium for biting fly control.

**6.2 Review of previous uses of nematodes**

The potential of aquatic mermithids for control of mosquitoes and blackflies lies in their ability to kill their hosts at the larval stage or cause sterility in the adults. Of the many mosquito-parasitic nematodes under consideration as control agents only one, *Romanomermis culicivorax*, has been mass produced and utilized extensively in field tests in a variety of mosquito habitats worldwide (Finney 1981a; Platzer 1981; Poinar 1979). In the only Canadian field test which used a mermithid for mosquito control, *R. culicivorax* was applied to snow-melt pools in Manitoba in an attempt to control *Aedes* spp. (Galloway and Brust 1976), but significant reduction in

larval numbers was not achieved. The lack of effectiveness in this habitat was attributed to the temperature which was below the threshold for infectivity by this particular nematode. Until mermithids which are infective at temperatures experienced here can be produced in sufficient quantities for field testing, the role of mermithids in the reduction of Canadian mosquito populations in their natural habitat cannot be assessed.

Although mermithid parasites of blackflies can be maintained in the laboratory, a method for their mass production has not yet been developed. Their use in the field has, therefore, been limited. In Newfoundland, an attempt was made to enhance natural populations of nematodes in streams by the addition of laboratory-maintained parasites. However, these tests had only limited success. Effective use of blackfly mermithids in the field will only be achieved when the complex relationships between parasite and host in the natural habitat are understood (Finney 1981b).

The aquatic habitat is one of the few environments where water is not a limiting factor in the use of steinernematids. However, the use of *Steinernema* spp. for mosquito control has not yet been exploited. Worldwide there is only one report of a field test and this was carried out in Canada by Briand and Welch (1963). They found that the application of *S. feltiae* resulted in reduced larval density and adult emergence, but they gave few details about the experiment. This absence of data makes it difficult to determine how effective a control agent *S. feltiae* or other steinernematids could be in this environment.

**6.3 The role of bacterial agents in IPM**

Although several microbial agents including viruses, fungi and nematodes with larvicidal and/or adulticidal potential have been considered for use against pests of importance in human and animal health, only *B.t.i.* has been used against these pests. This bacterium has proved highly effective in the field against mosquitoes and blackflies while having minimal impact on aquatic non-target organisms (Colbo and Undeen 1980; Ali 1981). It is being used as a larvicidal agent for source reduction of these pests as a safer alternative to more environmentally harmful chemical insecticides. The Vectobac 200G granular formulation is registered in Canada for mosquito control under certain conditions, and a liquid Vectobac formulation is currently registered as a blackfly larviciding agent.

In 1984, *B.t.i.* was used in the mosquito control program in Winnipeg, Manitoba; temephos and chlorpyriphos are generally used as larvicidal agents in the spring and summer. The choice of larvicide is made depending on its residual activity and by temperature and precipitation conditions under which it will be used. In 1985, a formulation of *B.t.i.* was successfully used as a mosquito larvicidal agent in Ontario.

Against mixed populations of mosquito larvae and pupae, *B.t.i.* acts as a larvicidal agent only and is of limited use. The use of FLIT MLO, a light mineral oil, as a pupicide has been an integral part of the Winnipeg program. As indicated by Ellis (1984), Exxon has ceased production of this product, but recent research by Levy *et al.* (1984) has shown that Arosurf MSF (Monomolecular Surface Film) is safe and effective in controlling mosquito larvae, pupae and emerging adults. Field tests in which Arosurf MSF was applied with *B.t.i.* at recommended application rates resulted in 100% mortality of mixed larval and pupal populations. This integrated approach may be considered for future use in the Winnipeg control program.

The results of a pilot study carried out in a small stream in St. John's, Newfoundland, in 1980-81, showed that *B.t.i.* could control larval blackfly populations effectively when applied at low dosages over an entire season (Colbo and O'Brien 1984). As a result, adult populations were significantly reduced. Subsequently, a large scale trial carried out over a 2-year period in the Labrador City-Wabush area

achieved effective control of the larval stage. Data from these trials enabled a procedural protocol to be defined and utilized in a full scale program in 1984. This program showed that 82% adult control could be achieved at an economically feasible cost (Colbo 1984). Thus *B.t.i.* appears to have provided the first effective pesticide for blackfly control in Canada since the development of DDT and methoxychlor.

**6.4 Research and development needs in public and animal health**

*Bacillus thuringiensis* var. *israelensis* is a microbial insecticide available for use in Canada for mosquito and blackfly control. However, there are several field related parameters which need investigation. Of particular importance is research into the persistence of *B.t.i.* after application for mosquito and blackfly control. From the practical standpoint, an understanding of persistence in the mosquito habitat would help define application time frames. The persistence of *B.t.i.*in pools and lakes after stream application against blackflies and its effects on non-target organisms over the long-term in the environment is not known. As application of *B.t.i.* increases for biting fly control, it is possible that persistence and/or repeated exposure to sub-lethal doses could initiate resistance to the product in the species treated. Such a possibility should be tested in the laboratory and monitored in the field.

Research should be oriented toward improving the virulence of *B.t.i.* to decrease the amount of product applied in the field. This may be achieved in either of two ways. Firstly, genetic manipulation of the available strains should be considered. Although this technology has been applied to other microbial agents, its role in increasing the effectiveness of this bacterium needs to be explored. Secondly, we should continue to survey for the presence of naturally occurring bacteria. It was only through such diligent efforts and selective testing that *B.t.i.* was first isolated, as was *B. sphaericus*. There are numerous strains of *B. sphaericus*, another spore forming bacterium, some of which are highly pathogenic to mosquito larvae. These have proved to be highly effective and persistent in the field over an extended period of time. It is important that the virulence of these strains continues to be investigated and compared under Canadian conditions with the currently available *B.t.i.* formulations.

Aquatic mermithid nematodes, which have the potential for control of mosquitoes and blackflies under Canadian conditions, are not yet available in sufficient quantities for intensive research into their development as biological control agents. Attempts to culture these nematodes *in vivo* have proved unsuccessful, basically because possible laboratory hosts are difficult to rear. Therefore, research should be directed towards *in vitro* culture of these nematodes. Although long term and initially capital intensive, such research, if successful, will prove economical and provide sufficient nematodes for development and eventual implementation of a biting fly control program.

## 7. PRODUCTION OF MICROBIAL INSECTICIDES IN CANADA

Viruses and protozoa can only be propagated in living cells, either in the host insect larvae or in cultured cells. Bacteria and some fungi can be propagated in liquid culture in fermenters. Nematodes can be propagated either in insect larvae or on artificial protein based substances. The use of cell cultures for production of nuclear polyhedrosis viruses has received considerable attention, but technical problems exist (Stockdale and Priston 1981). Some pharmaceutical companies with expertise in vaccine production may be interested in commercial production of viruses if problems are eventually resolved. From the standpoint of quality control, it is much more attractive to produce viruses *in vitro* than *in vivo*.

There is a small-scale production plant for viruses at the Forest Pest Management Institute in Sault Ste Marie. Production of viruses in living insects in the

laboratory is labour-intensive and expensive. The basic cost factor is the number of larvae which has to be reared, infected and harvested to produce a 1 ha dosage of virus. Sufficient Virtuss (Douglas-fir tussock moth NPV produced in whitemarked tussock moth larvae) to treat about 400 ha is produced annually. About 250 larvae are required to produce a 1-ha dosage costing about $50.00. On the other hand, viruses of colonial species of sawflies can be produced in the field by treating heavy populations at the correct stage of insect development and harvesting diseased and dead colonies. Redheaded pine sawfly NPV (Lecontvirus) and European pine sawfly NPV (Sertifervirus) have been produced by this method. About 50 larvae are required to produce 1 ha dosage at a cost of less than $2.50. Presently, no viruses are produced commercially in Canada and at the time of writing, there are only two viral insecticides[1] marketed in the U.S.A. Efforts should be made to obtain Canadian registrations for potentially useful U.S. products and import them for pest control operations.

Mass production of the nematode, *Steinernema feltiae*, on artificial diet has recently been reviewed by Hara *et al.* (1981) and by Gaugler (1981). Bedding (1981) has been able to produce *S. feltiae* and *H. heliothidis* on a homogenate of pig kidney and beef fat on crumbled polyurethane sponge at a cost of 2¢/million nematodes. At an application rate of 50,000 nematodes/$m^2$ the cost of material would be U.S. $10/ha. Several companies in the U.S. now produce steinernematids on a commercial basis. The Nematode Farm Inc. of California produces 3 strains of *S. feltiae* under the trade name Seek. Importation of these products into Canada requires a permit from Agriculture Canada. These nematodes are also available in Canada from a small production facility at Memorial University, St. John's, Newfoundland.

*Romanomermis culicivorax* has been produced *in vivo* in its mosquito host at a cost of about U.S. 90 ¢/million preparasites (Chapman and Finney 1982). In the past this mermithid nematode was mass produced by both Fairfax Biological Laboratories and Nutrilite Products, but both companies encountered difficulties in production and shipment and are no longer in this business. There is, obviously, considerable scope for establishing production facilities for nematodes as small scale commercial enterprises in government laboratories or in universities. There is worldwide interest in nematodes, particularly neoaplectanids for insect pest control and the development of production facilites in Canada should be encouraged in order to meet the growing demand for these biocontrol agents.

*Bacillus thuringiensis* is not produced in Canada although facilities exist for formulating *B.t.* products. *B.t.* used in Canada is produced in the U.S. or Europe. The actual cost of manufacturing and formulating *B.t.* is considered company-confidential information. A large percentage of the market for *B.t.* in Canada is by tender and therefore the companies involved do not want information such as manufacturing, formulating and packaging costs, and production methods to become common knowledge.

## 8. REGISTRATION OF MICROBIAL INSECTICIDES IN CANADA

The development of guidelines for the registration of microbial pesticides in various countries has been reviewed by Engler and Rogoff (1980), Hascoet and Hurpin (1980), Burges *et al.* (1980a), Harrap (1982) and Rogoff (1982). Guidelines are available including those developed by the European Economic Community (EEC,

[1] Two European pine sawfly NPV products, one called Preserve®, produced by MicroGeneSys. Inc., and the other called Virox®, produced by Microbial Resources Ltd., U.K.

1980), the International Organization for Biological Control of Noxious Animals and Plants, Western Paleartic Region (10BC/WPRS) regarding baculoviruses (Burges *et al.* 1980a, b), bacteria (Burges *et al.* 1982) and fungi (Hall *et al.* 1982), the UK Ministry of Agriculture, Fisheries and Food (Papworth 1980), the World Health Organization (WHO 1981), and the US EPA (Betz 1981). The WHO and EPA guidelines involve a tier testing approach. The first tier provides a maximum challenge and progression to a further tier is necessary only if a hazard is detected in the first tier. Canadian guidelines for registration of naturally occurring microbial pesticides have been drafted and these are similar to the EPA guidelines (W.E. Stewart, personal communication).

Twenty-three formulations of *B.t.* var. *kurstaki*, 3 formulations of *B.t.*var. *israelensis* and 2 virus preparations are registered under the Pest Control Products Act (Canada). Registrations for additional microbial agents are pending. A submission was made for registration of the European pine sawfly NPV in March 1985.

## 9. SAFETY OF MICROBIAL INSECTICIDES

The safety of insect pathogens applied as biocontrol agents has been the subject of considerable heated argument and debate. Safety is a relative term; it is virtually impossible to prove a negative finding in a biological system, so safety should really be termed risk or hazard assessment. It is the duty of regulatory authorities to evaluate the benefits of microbial agents compared to the potential health and environmental hazards of using them.

Philosophies of safety testing for microbial insecticides have evolved considerably over the past 15 years. In early tests, microbial insecticides were treated in the same manner as chemical insecticides and the criterion for safety was lack of mammalian toxicity. Some scientists argued that the mere fact that insect pathogens are naturally present in the environment and do not appear to affect animals other than invertebrates is reason enough to presume they are safe. Added to this was the vast amount of circumstantial evidence which has accumulated from laboratory work and field trials with pathogens before any serious consideration was given to safety testing. It is beyond the scope of this report to discuss all safety aspects of a variety of microbial insecticides, but this subject has been reviewed recently by Burges (1981) and, with special reference to viruses, by Harrap (1982).

Recent safety testing has progressed beyond toxicity testing and infection giving rise to either overt or latent disease in non-target organisms has been examined. When materials are sprayed, aerosols are produced and the potential hazard of inhalation of such materials has been scrutinized closely. This is a potential route of infection to which man and other animals are not normally exposed. There is the possibility that immunosuppressed people or animals may be more susceptible to infection with insect pathogens than healthy ones, but more studies are needed before definite conclusions can be drawn. Cell cultures can be studied in greater detail than whole animals and much safety testing is now done in *in vitro* systems. The question as to whether insect viruses can transform mammalian cells has been raised and tests to date indicate that baculovirus genomes do not integrate with mammalian cell DNA.

A major concern with microbial insecticides is mutation of the pathogen. By mutation, concerned citizens envisage mutation of an insect pathogen to a mammalian pathogen, such as *B. thuringiensis* mutating to *B. cereus* or *B. anthracis*. Mutations continually occur in all organisms, micro-organisms and viruses and these changes are minute and subtle. Scientists are aware that one species cannot instantly mutate to another species nor will an invertebrate pathogen suddenly mutate to become a vertebrate pathogen, but it is extremely difficult to educate the public on this particular topic.

None of the safety testing conducted to date indicates that there is a hazard from insect pathogens and nematodes which are being developed as microbial insecticides. Extensive safety testing has been conducted on products of *B. thuringiensis* of both var. *kurstaki* and var. *israelensis*. Several baculoviruses have been safety tested, but less attention has been paid to other groups of insect viruses. A limited amount of testing of protozoans, fungi and nematodes has also yielded negative results (Burges 1981). Entomophilic nematodes are not subject to registration by the Environmental Protection Agency in the U.S. and as such E.P.A. guidelines for safety testing are not available. However, some safety tests have been carried out with *Steinernema* sp. against rats, mice and earthworms with negative effects (Burges 1981; Gaugler and Finney 1982). It has also been shown that because the growth of nematodes and their associated bacteria is inhibited at temperatures above 30° C, parasitism of homoiothermic animals is highly unlikely. Protocols for the registration of nematodes in Canada are not yet formulated.

Currently, it is considered that there are two principal dangers in the use of microbial insecticides. The first is the possibility of hazardous contaminants in the biological preparation. This problem can be avoided with rigorous quality control procedures. The second problem is the risk of allergies due to repeated exposures to protein and polysaccharide antigens. This is particularly important in a production facility where there is daily exposure to a pathogen over prolonged periods of time. In such a facility, suitable precautions such as containment chambers, protective clothing and face masks are necessary.

Considerable time and effort have been devoted to compiling the EPA guidelines for biorational pesticides. Biochemicals such as pheromones and insect growth regulators as well as microbial agents are included in the term "biorationals". The EPA published in the Federal Register of Nov. 24, 1982: "Biorational pesticides are a distinct group, inherently different from conventional pesticides. Some of the characterisitics that typically distinguish biorational from conventional pesticides are their unique non-toxic mode of action, low use volume, target species specificity and natural occurrence. Based on these characteristics, the agency expects that many biorational pesticides pose lower potential risks than convential pesticides. Therefore these pesticides are subject to a different set of data requirements".

It is unlikely that any microbial agent being developed as an insecticide will require testing beyond tier 1. Tier 1 tests involve 1) acute oral, dermal, and inhalation exposure; 2) intravenous, intracerebral and intraperitoneal injection; 3) primary eye and primary dermal irritation; 4) hypersensitivity; 5) cellular immune response and 6) tissue culture studies (viruses only) (Betz 1981). An American committee priced this set of tests at $141,000 U.S. in 1982. Based on previous experience in Canada, it appears certain that wildlife studies and studies on fish and aquatic invertebrates will also be mandatory. A cost estimate of the safety testing of a microbial agent in Canada is about $250,000 Can.

## 10. GENETIC ENGINEERING APPLIED TO MICROBIAL INSECTICIDES

The advent of recombinant DNA technology has opened several avenues of research which can be applied directly to the enhancement of insect pathogens and the development of a new generation of insect control agents. In the last five years, many new companies with interests in biotechnology, and often funded with venture capital, have appeared in North America and Europe, and major pesticide manufacturers are also taking an active interest in current developments. Most of the attention is focussed on *B.t.*, but research is also being conducted on baculoviruses and on *Bacillus*

*sphaericus* (Luthy and Arif 1985; Kirschbaum 1985).

The gene for the *B.t.* delta-endotoxin is on a plasmid and it has been cloned in *Escherichia coli* (Schnepf and Whiteley 1981) and in *B. subtilis* (Klier *et al.* 1982). The nucleotide sequences of the toxin gene have been determined for var. *kurstaki* (HD1) (Schnepf *et al.* 1985), var. *kurstaki* (HD73) (Adang *et al.* in press) and var. *sotto* (Shibano *et al.*1985) and this gene consists of 3.3 to 3.6 kilobase pairs. There are many varieties of *B.t.* which differ in the specific activity of the delta-endotoxin and gene recombination may produce a toxin with increased toxicity or a different host spectrum. A *B.t.* strain, toxic to both lepidopterous and dipterous larvae, has already been engineered (Klier *et al.* 1983). Much of the research on genetic manipulation of *B.t.k.* and *B.t.i.* has been conducted by industry and is proprietary information. However, in Canada, a consortium of scientists has been formed to work on all aspects of *B.t.* crystal biotechnology and it has been called the BIOCIDE network.

Scientists in the BIOCIDE network are studying molecular genetics, protein chemistry, receptor chemistry and activation chemistry. The consortium has the skills necessary to conduct high quality research in all areas of biotechnology and has access to fermentation and other techniques required for the development of new *B.t.* products. The BIOCIDE network is being jointly funded by the Canadian Forestry Service and the National Research Council of Canada. Of particular note is the molecular genetics research spearheaded by Dr. R. Brousseau of the National Research Council's Biotechnology Research Institute in Montreal. A crystal gene, differing from those already published, has been cloned and is being sequenced in collaboration with Dr. Peter Lau of NRC, Ottawa. There are currently three postdoctoral fellows associated with this aspect of the work. Expertise in immunology and bioassay to support this genetic research is being supplied by the Forest Pest Management Institute of the Canadian Forestry Service. A further three postdoctoral associates are involved in protein chemistry, receptor studies, bioassay and host spectrum studies. Thus a Canadian contribution to the development of second generation *B.t.*s is assured and, hopefully, a made-in-Canada *B.t.* product will result (P.G. Fast, personal communcation).

Genetic studies have been conducted on a few baculoviruses (nuclear polyhedrosis viruses). The situation with these viruses is much more complex than studies on the toxin gene of *B.t.* These viruses have a large, double-stranded DNA genome containing 100 to 200 kilobase pairs which code for about 30 proteins. Several nuclear polyhedrosis viruses have been characterized and mapped using restriction enzyme endonucleases including alfalfa looper (*Autographa californica* NPV) (Vlak and Smith 1982) and spruce budworm NPV (Arif *et al.* 1984). Alfalfa looper NPV genome is by far the most extensively studied. Having elucidated a physical map of the DNA, the next phase of the research is to ascribe functions to various nucleotide sequences. The sequences for the polyhedrin gene (inclusion body protein) (Hooft van Iddekinge *et al.* 1983) and the p10 gene (a protein of unknown function) (Kuzio *et al.* 1984) have been determined for alfalfa looper NPV. It is possible to remove and replace these genes with cloned genes from other organisms and use baculoviruses as expression vectors. Alfalfa looper NPV, grown in cell culture, has been used successfully as an expression vector for human beta-interferon, betagalactosidase (Kirschbaum 1985) and the human oncogene protein c-*myc* (Miyamoto *et al.* 1985).An NPV of silkworm has been used as a vector to produce human alpha-interferon in silkworm larvae (Maeda *et al.* 1985). To date, this research has been aimed primarily at pharmaceutical products, but it may well lead to the development of improved viral insecticides.

In the near future it should be possible to transfer genes between different

baculoviruses and thus engineer hybrid strains with altered biological properties, such as virulence and host specificity. It has been suggested that insect neurotoxins can be transferred into baculoviruses, the aim being to use the baculovirus to target cells within an insect and allow expression of the toxin from within the insect host. Of particular interest for this purpose is the gene for the p10 protein which is produced late in the viral cycle and is of unknown function. It has a strong promotor which permits an abundant synthesis of the product (B.M. Arif, personal communication). Recently, a virus envelope protein essential for infectivity, has been identified (Volkman *et al.* 1984); current work on sequencing its gene will make it possible to manipulate it into other baculoviruses (P. Faulkner, personal communication). Currently research on baculoviruses is being conducted in Canada at the Forest Pest Management Institute and at the Dept. of Bacteriology and Immunology, Queen's University, Kingston, Ontario.

The field of genetic manipulation of insect pathogens is being addressed by a few scientists in Canada, but it is a vast field with extraordinary potential. Identifying and sequencing individual genes is slow and exacting work and progress in Canada will be hampered unless increased manpower and resources are channeled into this area of research on microbial insecticides.

## 11. RESEARCH MANPOWER

The report of the Canadian Grains Council on agricultural research in Canada dated October 1985 drew the following conclusions: 1) The total manpower commitment to research in Canada is below world levels. 2) There has been no substantial increase in research manpower in 12 years. 3) Unlike other industrialized nations, the number of research scientists and engineers in Canada has been static since 1969. These statements also reflect the current state of research manpower in insect pathology and microbial control in Canada.

A comparison of world-wide and Canadian distribution of scientists working primarily in insect bacteria, fungi, nematodes, viruses, protozoa and integrated control shows a massive increase in manpower world-wide, but insignificant changes in Canada between 1970 and 1986 (Table VII). These data show a total of 236% increase in manpower world-wide compared with a 2% loss of manpower in Canada during the same period. The largest manpower increase world-wide was in the study of entomophilic nematodes as microbial control agents (487%), but Canada registered the largest manpower loss in this discipline. Canada has a 3% increase in manpower in integrated control compared with 258% increase world-wide.

The ratio of gross domestic product (billion $US) to the number of scientists working primarily in invertebrate pathology in selected industrialized countries were as follows: Canada 3.4, Great Britain 3.6, USSR 4.6, U.S.A. 5.6, France 10.3, West Germany 13.9, and Japan 14.1. Canada has the least committment to this type of research among these nations. It is evident that Canada, in spite of its historical reputation as a pioneer in the research and development of microbial control, has fallen well behind other industrialized countries in this area. If we are serious about the search for alternative means of pest control and about environmental protection, this erosion of research effort in a proven productive area of biological control must be rectified immediately.

The committee concludes that the number of research scientists in microbial control in Canada is extremely small compared to the size of the pest management problems existing in this country. The need for greater funding for manpower and operations in both government and university establishments is critical to bringing more microbial agents to the level of registration.

Table VII. World-wide and Canadian distribution of scientists in selected areas of research in insect pathology and microbial control in 1970 and 1986[1].

| Research areas | 1970 | | | 1986 | | | Changes | |
|---|---|---|---|---|---|---|---|---|
| | Worldwide | Canada | Canada % of World | Worldwide | Canada | Canada % of World | World % | Canada % of World |
| **Etiology** | | | | | | | | |
| Bacteria | 85 | 7 | 8 | 154 | 4 | 3 | +118 | -5 |
| Fungi | 63 | 3 | 5 | 175 | 12 | 7 | +269 | +2 |
| Nematodes | 31 | 4 | 13 | 151 | 5 | 3 | +487 | -10 |
| Viruses | 182 | 7 | 5 | 332 | 11 | 3 | +182 | -2 |
| Protozoa | 44 | 2 | 5 | 116 | 2 | 2 | +264 | -3 |
| **Integrated and microbial control** | | | | | | | | |
| Insects of public health and veterinary importance | 30 | 2 | 7 | 58 | 8 | 14 | +193 | +7 |
| Insect pests of forestry and agriculture crops | 88 | 9 | 10 | 246 | 11 | 4 | +280 | -6 |
| TOTALS | 523 | 34 | 6 | 1232 | 53 | 4 | +236 | -2 |

[1]Data from 1970 and 1986 issues of The Directory for Invertebrate Pathology, Dept. of Entomology, Ohio State University, Columbus, Ohio. Published by the Society for Invertebrate Pathology.

## 12. ACADEMIC INSTRUCTION IN INSECT PATHOLOGY AND MICROBIAL CONTROL IN CANADA

Courses of instruction in insect pathology are offered in universities in 17 countries. Such instruction is not offered in any Canadian university. Considering that the scientific and intellectual sophistication of this country is of the highest calibre, that agriculture and forestry are the 2 largest industries in Canada, that Canadian scientists have pioneered this field, that the environmental implications of the large-scale use of broad-spectrum chemical insecticides are well known and appreciated by the scientific and public communities and that insect diseases are recognized world-wide as having significant regulatory effects on the population dynamics of numerous species of pest insects, the complete lack of formal instruction in insect pathology in Canadian universities is appalling. The committee feels that an effort should be made soon by at least one Canadian university to correct this rather obvious deficiency in the training of Canadian entomologists. Preferably, the university should have a well established faculty of agriculture and/or forestry and should have a large body of graduate entomology students. It should be located in an area of the country in which agriculture and forestry are major industries and in which microbial insecticides are already known and appreciated by farmers and landowners.

The aim of the course we have in mind would be to teach students the principles of insect pathology and develop the students' interest in microbial control as a discipline within entomology. It should develop the ability to identify diseases of insects and their causative agents; to understand the life-cycle of pathogens and mechanisms of infection; to use insect pathogens for insect control; to control disease in cultured or beneficial insects; to understand the epizootiology of infectious diseases. At least one university in Canada should give a full lecture/laboratory course. Other universities may consider combining insect pathology with their courses in classical biological control. The course should be offered at the advanced undergraduate or graduate level and should make use of Canadian specialists as visiting lecturers wherever possible.

Topics which should be covered include: history; importance, nonmicrobial pathology (eg. metabolic/nutritional, neoplastic, genetic); microbial flora of normal insects; immunity and resistance; histopathology; coverage of all entomopathogens including taxonomic considerations (bacteria, viruses, fungi, protozoa, nematodes, rickettsiae), predisposition to disease, pathogen/pathogen and pathogen/non-pathogen interactions, IPM, application technology, diagnosis and identification, diseases in laboratory colonies, bee diseases, biotechnology, safety and economics of microbial control. There are course outlines available from the Society for Invertebrate Pathology (SIP) which could form the basis of a Canadian course. There is also available from SIP a color slide atlas i.e. a teaching slide set consisting of 200 -2x2 color transparencies of invertebrate pathogens and diseases. Topics illustrated include pathogen life stages, gross pathology, histopathology, and symptomatology.

*Bacillus thuringiensis* is a very effective insecticide for control of the tobacco hornworm (*Manduca sexta*) on tobacco and is recommended for this use in Ontario. A majority of Ontario tobacco growers apply the microbial insecticide for control of this pest with consistently good results. It is noteworthy that inclusion of the beta exotoxin in formulations of *B.t.* has been found to broaden the host spectrum of *B.t.* to include the Colorado potato beetle (*Leptinotarsa decemlineata*) and other species of pests. Formulations of *B.t.* containing the exotoxin are not registered in Canada.

## References


Adang, M.J., M.J. Staver, T.A. Rocheleau, J. Leighton, R.F. Barker, and D.V. Thompson. 1985. Characterized full-length and truncated plasmid clones of the crystal protein of *Bacillus thuringiensis* subsp. *kurstaki* HD-73 and their toxicity to *Manduca sexta*. Gene **36:** 289-300.

Ali, A. 1981. *Bacillus thuringiensis* serovar. *israelensis* (ABG-6103) against chironomids and some nontarget aquatic invertebrates. J. Invertebr. Pathol. **38:** 264-72.

Allen, G.E., C.M. Ignoffo, and R.P. Jaques. (Eds.) 1978. Microbial Control of Insect Pests: Future Strategies in Pest Management Systems. Proc. NSF-USDA - Univ. of Florida Workshop, Jan. 1978, 290 pp.

Arif, B.M., J. Kuzio, P. Faulkner, and W. Doerfler. 1984. The genome of *Chriostoneura fumiferana* nuclear polyhedrosis virus: Molecular cloning and mapping of the EcoRI, BamHI, SmaI, XbaI and BgIII resistriction sites. Virus Res. **1:** 605-614

Balch, R.E., and F.T. Bird. 1944. A disease of the European spruce sawfly, *Gilpinia hercyniae* (Htg.), and its place in natural control. Sci. Agric. **25:** 65-80.

Bedding, R.A. 1981. Low cost *in vitro* mass production of *Neoaplectana* and *Heterorhabditis* species (Nematoda) for field control of insect pests. Nematologica **27:** 109-114.

Betz, F.T. 1981. Human safety evaluation for microbial pest control agents. Summary proceedings. Cotton Biological Control Conference, Dallas, Texas. E.P.A. Washington, D.C.

Bird, F.T. 1953. The use of a virus disease in the biological control of the European pine sawfly, *Neodiprion sertifer* (Geoffr.). Can. Ent. **85:** 437-446.

Bird, F.T. 1955. Virus disease of sawflies. Can. Ent. **87:** 124-127.

Bird, F.T., and J.M. Burk. 1961. Artificially disseminated virus as a factor controlling the European spruce sawfly, *Diprion hercyniae* (Htg.), in the absence of introduced parasites. Can. Ent. **93:** 228-238.

Bird, F.T., and D.E. Elgee. 1957. A virus disease and introduced parasites as factors controlling the European spruce sawfly, *Diprion hercyniae* (Htg.), in central New Brunswick. Can. Ent. **89:** 371-378.

Bird, F.T. 1961. Transmission of some insect viruses with particular reference to ovarial transmission and its importance in the development of epizootics. J. Insect Pathol. **3:** 351-380.

Blais, J.R. 1976. Can *Bacillus thuringiensis* replace chemical insecticides in the control of spruce budworm? For. Chron. **52:** 57-60.

Briand, L.J., and H.E. Welch. 1963. Use of entomophilic nematodes for insect pest control. Phytoprotection **44:** 37-41.

Burges, H.D. 1981. Safety, safety testing and quality control of microbial pesticides. pp. 737-767 in H.D. Burges (Ed.). Microbial Control of Pests and Plant Diseases 1970-1980. Academic Press, London.

Burges, H.D., G. Crozier, and J. Huber. 1980a. A review of safety tests on baculoviruses. Entomophaga **25:** 329-340.

Burges, H.D., J. Huber, and G. Croizier. 1980b. Guidelines for safety testing on insect viruses. Entomophaga **25:** 341-348.

Burges, H.D., A. Krieg, P. Luthy, and H. de Barjac. 1982. Guidelines for safety tests and registration of bacterial pesticides. Entomophaga **27:** 225-236.

Carlton, B. 1984. Biological pesticides: safety and specificity. ECN Fertilizers and Agrochemicals Supp., Feb. 20, 1984. pp. 39-40.

Carrow, J.R. (Ed.). 1983. *B.t.* and the spruce budworm 1983. Proc. Seminar, Fredericton, N.B. N.B. Dept. Nat. Res. 90pp.

Chapman, H.C., and J.R. Finney. 1982. Mass production of mermithid and steinernematid nematodes with vector control potential. pp. 358-362 in Proc. 3rd Int. Coll. Invertebr. Pathol. and 15th Ann. Meeting Soc. Invertebr. Pathol., Brighton, U.K. Sept. 1982.

Cheng, H.H., and G.E. Bucher. 1972. Field comparison of the neoaplectanid nematode DD-136 with diazinon for control of *Hylemya* spp. on tobacco. J. Econ. Entomol. **65:** 1761-1763.

Cheng, H.H., and R.P. Jaques. 1973. Comparison of microbial preparations with chlorpyrifos for control of the dark-sided cutworm. Pest. Res. Rep., Can. Comm. Pest. Use Agr. pp. 159-160.

Colbo, M.H. 1984. Development of a field protocol for ground application of *Bacillus thuringiensis* var. *israelensis* as a blackfly larvicide in Northern Canada. Report DSS Contract No. 8SU82-00283.

Colbo, M.H., and H. O'Brien. 1984. A pilot black fly (Diptera:Simuliidae) control program using *Bacillus thuringiensis* var. *israelensis* in Newfoundland. Can. Ent. **116:** 1085-1096.

Colbo, M.H., and A.H. Undeen. 1980. Effect of *Bacillus thuringiensis* var. *israelensis* on non-target insects in stream trials for control of Simuliidae. Mosq. News **40:** 368-71.

Couch, T.L., and D.A. Ross. 1980. Production and utilization of *Bacillus thuringiensis*. Biotech. Bioeng. **22:** 1297-1304.

Cunningham, J.C. 1985. Status of viruses as biocontrol agents for spruce budworms. Proc. Symp. Microbial Control of Spruce Budworms and Gypsy Moths. Windsor Locks, CT. USDA-GTR 100, 175pp.

Cunningham, J.C., and P.F. Entwistle. 1981. Control of sawflies by baculovirus. pp. 379-407 in H.D. Burges (Ed.), Microbial Control of Pests and Plant Diseases 1970-1980. Academic Press, London.

Cunningham, J.C., and P. deGroot. 1984. *Neodiprion lecontei* (Fitch), redheaded pine sawfly (Hymenoptera:Diprionidae). pp. 323-329 in J.S. Kelleher and M.A. Hulme, (Eds.) Biological Control Programmes against Insects and Weeds in Canada 1969-1980. Commonw. Agr. Bureaux, Slough, U.K.

Cunningham, J.C., and G.M. Howse. 1984. *Choristoneura fumiferana* (Clemens), spruce budworm (Lepidoptera:Tortricidae). B. Viruses: Application and assessment. pp. 248-259 in J.S. Kelleher and M.A. Hulme (Eds.) Biological Control Programmes against Insects and Weeds in Canada 1969-1980. Commonw. Agric. Bureaux. Slough, U.K.

Cunningham, J.C., N.V. Tonks, and W.J. Kaupp. 1981. Viruses to control winter moth, *Operophtera brumata* (Lepidoptera:Geometridae). J. Ent. Soc. B.C. 78:17-24.

Dimond, J.B., and C.J. Spies III. 1981. Two year effects of a *Bacillus thuringiensis* treatment on spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Can. Ent. **113:** 661-663.

Dowden, P.B. 1940. Larval disease prevalent in heavy infestations of the European spruce sawfly in southern New Hampshire and Vermont. J. For. **38:** 970-972.

Dutky, S.R. 1959. Insect Microbiology. Adv. Appl. Microbiol. **1:** 175-200.

EEC 1980. Meeting of group experts on safety and regulation of biological pesticides. Commission of European Communities, Director-General for Agriculture: DG VI Directorate F-Division 4, Programme on integrated pest control. Brussels.

Ellis, R.A. 1984. Annual report on mosquito surveillance and control in Winnipeg. 1984.

Engler, R., and M.H. Rogoff. 1980. Registration and regulation of microbial pesticides. Biotech. Bioeng. **22:** 1441-1448.

Ewen, A.B. 1983. Extension of the geographic range of *Nosema locustae* (Microsporidia) in grasshoppers (Orthoptera:Acrididae). Can. Ent. **115:** 1049-1050.

Finney, J.R. 1981a. Potential of nematodes for pest control. pp. 603-620 in H.D. Burges (Ed.) Microbial Control of Pests and Plant Disease. Academic Press, London.

Finney, J.R. 1981b. Potential of mermithids for control and *in vitro* culture. pp. 325-333 in M. Laird (Ed.) Blackflies: The future for biological methods in integrated control. Academic Press, N.Y.

Finney, J.R. 1984. Alternative sources of Steinernematid nematodes for use as biocontrol agents against insect pests in Newfoundland. CANUSA Newsletter **34:** 5.

Finney, J.R., and G.F Bennett. 1983. The susceptibility of some sawflies (Hymenoptera:Tenthredinidae) to *Heterorhabditis heliothidis* (Nematoda: Rhabditidae) under laboratory conditions. Can. J. Zool. **61:** 1177-1180.

Finney, J.R., and G.F. Bennett. 1984a. *Heterorhabditis heliothidis* (Nematoda): A potential biocontrol agent of agricultural and forest pests in Newfoundland. J. Agric. Ent. **1:** 287-295.

Finney, J.R., and G.F. Bennett. 1984b. Susceptibility of early instars of the spruce budworm (Lepidoptera:Tortricidae) to *Heterorhabditis heliothidis* (Nematoda: Rhabditidae). Can. Ent. **116:** 285-286.

Finney, J.R., K.P. Lim, and G.F. Bennett 1982. The susceptibility of the spruce budworm *Choristoneura fumiferana* (Lepidoptera:Tortricidae) to *Heterorhabditis heliothidis* (Nematoda:Heterorhabditidae) in the laboratory. Can. J. Zool. **60:** 958-961.

Finney, J.R., and C. Walker. 1977. The DD-136 *Neoaplectana* sp. as a potential control agent for the European elm bark beetle, *Scolytus scolytus*. J. Invertebr. Pathol. **29:** 7-9.

Fox, C.J.S., T.H. Haliburton, K.P. Butler, and F. Huston. 1972. Control of caterpillers on cabbage with chemical and biological insecticides. Phytoprot. **53:** 82-86.

Fox, C.J.S., and R.P. Jaques. 1966. Preliminary observations on biological insecticides against the imported cabbageworm. Can. J. Plt. Sci. **46:** 497-499.

Fox, C.J.S., and R.P. Jaques. 1977. Biological control of leaf-eating caterpillars on cabbage. Pest Res. Rep., Can. Comm. Pest. Use Agr. p. 83.

Galloway, T.D., and R.A. Brust. 1976. Field application of the mermithid nematode, *Romanomermis culicivorax* Ross and Smith, for the control of mosquitoes, *Aedes* spp., in spring in Manitoba. Manitoba Entomologist. **10:** 18-25.

Gaugler, R. 1981. Biological control potential of neoaplectanid nematodes. J. Nematol. **13:** 241-249.

Gaugler, R., and J.R. Finney. 1982. A review of *Bacillus thuringiensis* var. *israelensis* (Serotype 14) as a biological control agent of black flies (Simuliidae). Misc. Publ. Entomol. Soc. Am. **12:** 1-17.

Glass, E.H. 1975. Integrated pest management: rationale, potential, needs and implementation. Ent. Soc. Amer. Special Pub. 75-2, 141 pp.

Goldberg, L.J., and J. Margalit. 1977. A bacterial spore demonstrating rapid larvicidal

activity against *Anopheles sergentii*, *Uranotaenia unguiculata*, *Culex univitattus*, *Aedes aegypti* and *Culex pipiens*. Mosq. News **37**:355-8.

Gonzalez, Jr. J.M., B.J. Brown, and B.C. Carlton. 1982. Transfer of *Bacillus thuringiensis* plasmids coding for delta-endotoxin among strains of *Bacillus thuringiensis* and *B. cereus*. Proc. Natl. Acad. Sci. **79:** 6951-6955.

Greathead, D.J., and J.K. Waage. 1983. Opportunities for biological control of agricultural pests in developing countries. World Bank Tech. Pap. #11. Washington, D.C.

Griffiths, K.J., J.C. Cunningham, and I.S. Otvos. 1984. *Neodiprion sertifer* (Geoffroy), European pine sawfly (Hymenoptera:Diprionidae). pp. 331-340 in Kelleher, J.S. and M.A. Hulme (Eds.). Biological Control Programmes against insects and weeds in Canada 1969-1980. Commonw. Agric. Bureaux, Slough, U.K.

Hall, R.A., G. Zimmerman, and A. Vey. 1982. Guidelines for the registration of entomogenous fungi as insecticides. Entomophaga **27:** 121-127.

Hannay, C.L., and D.G.R. McLeod. 1981. A microsporidian infecting the white cutworm, *Euxoa scandens* (Lepidoptera:Noctuidae). Can. Ent. **113:** 173-175.

Hara, A.H., J.E. Lindegren, and H.K. Kaya. 1981. Monozenic mass production of the entomogenous nematode, *Neoaplectana carpocapsae*, Weiser on dog food/agar medium. USDA, SEA. Adv. Agric. Technol., Western Series AAT-W-16. 8 pp.

Harcourt, D.G., J.C. Guppy, D.M. MacLeod, and D. Tyrrell. 1974. The fungus *Entomophthora phytonomi* pathogenic to the alfalfa weevil, *Hypera postica*. Can. Ent. **106:** 1295-1300.

Harcourt, D.G., J.G. Guppy, and M.R. Binns. 1977. The analysis of intrageneration change in eastern Ontario populations of the alfalfa weevil, *Hypera postica* (Coleoptera: Curculionidae). Can. Ent. **109:** 1521-1534.

Harrap, K.A. 1982. Assessment of the human and ecological hazards of microbial insecticides. Parasitology **84:** 269-296.

Harvey, G.T., and J.M. Burk. 1974. Mortality of the spruce budworm on white spruce caused by *Entomophthora spaerosperma* Fresenius. Environ. Can., For. Serv., Bi-monthly Res. Notes. **30:** 23-24.

Hascoet, M., and B. Hurpin. 1980. Proposed French registration guidelines for unconventional pesticides. pp. 145-154. in B. Lundholm and M. Stackerud (Eds.). Environmental Protection and Biological Forms of Control of Pest Organisms. Ecol. Bull, (Stockholm) 31.

Henry, J.E., and J.A. Onsager. 1982. Large-scale test of control of grasshoppers on rangeland with *Nosema locustae*. J. Econ. Entomol. **75:** 31-35.

Hooft van Iddekinge, B.J.L., G.E. Smith, and M.D. Summers. 1983. Nucleotide sequence of the polyhedrin gene of *Autographa californica* nuclear polyhedrosis virus. Virology 131:562-565.

Ignoffo, C.M. 1981. The fungus *Nomuraea rileyi* as a microbial insecticide. pp. 513-538 in H.D. Burges (Ed.), Microbial Control of Pests and Plant Diseases 1970-1980. Academic Press, London.

Ignoffo, C.M., C. Garcia, M.J. Kroha, T. Fukuda, and T.L. Couch. 1981. Laboratory tests to evaluate the potential efficacy of *Bacillus thuringiensis* var. *israelensis* for use against mosquitoes. Mosq. News. **41:** 85-93.

Ives, W.G.H. 1984. *Malacosoma disstria* Hubner, Forest tent caterpillar (Lepidoptera:Lasiocampidae). pp. 311-319 in J.S. Kelleher and M.A. Hulme (Eds.). Biological control Programmes agsinst Insects and Weeds in Canada 1969-1980. Commw. Agric. Bureaux, Slough, U.K.

Ives, W.G.H., and J.C. Cunningham. 1980. Application of nuclear polyhedrosis virus to control Bruce spanworm (Lepidoptera:Geometridae). Can. Ent. **112:** 741-744.

Jaques, R.P. 1961. Control of some lepidopterous pests of apple with commercial preparations of *Bacillus thuringi ensis* Berliner. J. Invertebr. Pathol. **3:** 167-182.

Jaques, R.P. 1965. The effect of *Bacillus thuringiensis* Berliner on the fauna of an apple orchard. Can. Ent. **97:** 795-902.

Jaques, R.P. 1967. Mortality of five apple insects induced by the nematode DD-136. J. Econ. Entomol. **60:** 741-743.

Jaques, R.P. 1970. Application of viruses to soil and foliage for control of the cabbage looper and imported cabbageworm. J. Invertebr. Pathol **15:** 328-340.

Jaques, R.P. 1972. The inactivation of foliar deposits of viruses of *Trichoplusia ni* (Lepidoptera: Noctuidae) and *Pieris rapae* (Lepidoptera: Pieridae) and tests on protectant additives. Can. Ent. **104:** 1985-1994.

Jaques, R.P. 1973. Tests on microbial and chemical insecticides for control of *Trichoplusia ni* and *Pieris rapae* on cabbage. Can. Ent. 105:21-27.

Jaques, R.P. 1975. Persistance, accumulation, and denaturation of nuclear polyhedrosis and granulosis virus. pp. 90-99 in M.D. Summers, R. Engler, L.A. Falcon, and P.V. Vail (Eds.), Baculoviruses in Insect Pest Control: Safety Considerations. Monogr. Am. Soc. Microbiol.

Jaques, R.P. 1977a. Field efficacy of virus infections to the cabbage looper and imported cabbageworm on late cabbage. J. Econ. Entomol. **70:** 111-118.

Jaques, R.P. 1977b. Stability of entomopathogenic viruses. pp. 99-117 in D.L. Hostetter and C.M. Ignoffo (Eds.), Environmental Stability of Microbial Insecticides. Misc. Publ., Entomol. Soc. Am. 10.

Jaques, R.P. 1983. The potential of pathogens for pest control. Agric. Ecosystems Envir. **10:** 101-126.

Jaques, R.P. 1985. Stability of insect viruses in the environment. pp. 285-360, in K. Maramorosch and K.E. Sherman (Eds.), Viral Insecticides for Biological Control. Academic Press, New York.

Jaques, R.P., and D.R. Laing. 1978. Efficacy of mixtures of *Bacillus thuringiensis*, viruses, and chlordimeform against insects on cabbage. Can. Ent. **110:** 443-448.

Jaques, R.P., and J.E. Laing. 1984. *Cydia pomonella* (L.), Codling Moth (Lepidoptera:Tortricidae). pp. 25-27 in J.S. Kelleher and M.A. Hulme (Eds.). Biological Control Programmes Against Insects and Weeds and Canada 1969-1980. Commonw. Agric. Bureaux, Slough, U.K.

Jaques, R.P., J.E. Laing, C.R. McLellan, M.D. Proverbs, K.H. Sanford, and R. Trottier. 1981. Apple orchard tests on the efficacy of the granulosis virus of the codling moth, *Laspeyresia pomenella* (Lepidoptera:Olethreutidae). Entomophaga, **26:** 111-118.

Jaques, R.P., and C.R. MacLellan. 1965. Fungal mortality of overwintering larvae of the codling moth in apple orchards 96.

Jaques, R.P., C.R. MacLellan, K.H. Sanford, M.D. Proverbs, and E.A.C. Hagley. 1977. Preliminary orchard tests on control of codling moth larvae by a granulosis virus. Can. Ent. **109:** 1079-1981.

Jaques, R.P., and O.N. Morris. 1981. Compatibility with other methods of pest control and with different crops. pp. 695-715 in H.D. Burges (Ed.) Microbial Control of Pests and Plant Disease 1970-1980. Academic Press, London.

Jaques, R.P., and N.A. Patterson. 1962. Control of the apple sucker, *Psylla mali* Schmid., by the fungus *Entomophthora sphaerosperma* (Fresenius). Can. Ent. **94:** 818-825.

Jaques, R.P., H.T. Stultz, and F. Huston. 1968. The mortality of the pale apple leafroller and winter moth by fungi and nematodes applied to soil. Can. Ent. **100:** 813:818.

Kirschbaum, J.B. 1985. Potential implication of genetic engineering and other biotechnologies to insect control. Ann. Rev. Entomol. **30:** 51-70.

Klassen, W. 1981. The role of biological control in integrated pest management system. pp. 433-445 in G.C. Papavisas (Ed.) Biol. Control Crop Prod., BARC Symposium #5.

Klier, A., F. Fargette, J. Ribier, and G. Rapoport. 1982. Cloning and expression of the crystal protein genes from *Bacillus thuringiensis* strain *Berliner* 1715. EMBO J. **1:** 791-799.

Klier, A., C. Bourgourin, and G. Rapoport. 1983. Mating between *Bacillus subtilis* and *Bacillus thuringiensis* and transfer of cloned crystal genes. Mol. Gen. Genet. **191:** 257-262.

Kuzio, J., D.Z. Rohel, C.J. Curry, A. Krebs, E.B. Carstens, and P. Faulkner. 1984. Nucleotide sequence of the p10 polypeptide gene of *Autographa* **californica** nuclear polyhedrosis virus. Virology **139:** 414-418.

Laing, D.R., and R.P. Jaques. 1980. Codling moth: Techniques for rearing and bioassaying granulosis virus. J. Econ. Entomol. **73:** 851-853.

Laing, D.R., and R.P. Jaques. 1984. Microsporidia of the European corn borer (Lepidoptera: Pyralidae) in southwestern Ontario: Natural occurrence and effectiveness as microbial insecticides. Proc. Ent. Soc. Ont. **115:** 13-17.

Levy, R., C.M. Powell, B.C. Hertlein, and T.W. Miller Jr. 1984. Efficacy of Arosurf MSF (Monomolecular Surface Film) base formulations of **Bacillus** *thuringiensis* var. *israelensis* against mixed populations of mosquito larvae and pupae: Bioassay and preliminary field evaluations. Mosq. News **44:** 537-543.

Luthy, R., and B.M. Arif. 1985. Designing microorganisms for insect control. BioEssays **2:** 22-25.

Luthy, P., H.M. Fischer, and R. Hutter. 1984. Deliberate release of microorganisms for insect control. pp. 55-65 in W. Arber, K. Illmensee, W.J. Peacock and P. Starlinger Eds.) Genetic manipulation: impact on man and society. Garden City Press, Harfordshire, U.S.

Maeda, S., T. Kawai, M. Obinate, H. Fujiwara, T. Horiuchi, Y. Saeki, Y.Soto, and M. Furusawa. 1985. Production of human interferon in silkworm using a baculovirus vector. Nature **315:** 592-594.

Meating, J.H., H.D. Lawrence, J.C. Cunningham, and G.M. Howse. 1983. The 1982 gypsy moth situation in Ontario: general surveys, spray trials and forecasts for 1983. Can. For. Serv. Sault Ste. Marie. Inf. Rep. 0-X-352. 14pp.

Miyamoto, C., G.E.Smith, J. Farrell-Towt, R. Chizzonite, M.D. Summers, and G. Ju. 1985. Production of human c-*myc* protein in insect cells infected with a baculovirus expression vector. Mol. Cell. Bio. **5:** 2960-1965.

Moore, G.E. 1970. *Dendroctonus frontalis* infection by the DD-136 strain of *Neoaplectana carpocapsae* and its bacterium complex. J. Nematol. **2:** 341-344.

Morris, O.N., T.A. Angus, and W.A. Smirnoff. 1975. Field trials of *Bacillus thuringiensis* against the spruce budworm, 1960-1973. pp. 129-133 in M.L. Prebble (Ed.). Aerial Control of Forest Insects in Canada. Dept. of the Environment, Ottawa.

Morris, O.N. 1977. Long term study of the effectiveness of aerial application of *Bacillus thuringiensis*-acephate combination against the spruce budworm, *fumiferana* (Lepidoptera:Torticidae). Can. Ent. **19:** 1239-1248.

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

Morris, O.N. 1980b. Report of the 1979 CANUSA cooperative *Bacillus thuringiensis* (*B.t.*) spray trials. Can. For. Serv. Rept. FPM-X-40. Sault Ste. Marie, Ontario. 75p.

Morris, O.N. 1982. Bacteria as pesticides: forest-applications. pp. 239-287 in E. Marcel Dekker, N.Y.

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

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

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

Morris, O.N., J.B. Dimond, and F.B. Lewis. 1984. Guidelines for the operational use of *Bacillus thuringiensis* against the spruce budworm. USDA Handbook #621, 26 pp.

Olofsson, E. 1973. Evaluation of a nuclear polyhedrosis virus as an agent for the control of balsam fir sawfly, *Neodiprion abietis* (Harr.). Can. For. Serv. Sault Ste. Marie Inf. Rep. 1P-X-2. 30pp.

Otvos, I.S., D.M. MacLeod, and D. Tyrrell. 1973. Two species of *Entomophthora* pathogenic to eastern hemlock looper (Lepidoptera: in Newfoundland. Can. Ent. **105:** 1435-1441.

Otvos, I.S, and B.H. Moody. 1978. The spruce budworm in Newfoundland: history status and control. Can. For. Ser., St. John's. Inf. Rep. N-X-150. 76pp.

Papworth, D.S. 1980. Registration requirements in the UK for bacteria, fungi and viruses used as pesticides. pp. 135-143 in B. Lundholm and M. Stackerund (Eds.) Environmental Protection and Biological Forms of Control for Pest Organisms. Ecol. Bull. (Stockholm) 31.

Platzer, E.G.1981. Biological control of mosquitoes with mermithids. J. Nematol. **13:** 257-262.

Perry, D.F. and G.H. Whitfield. 1984. The interrelationships between microbial entomopathogens and insect hosts: a system study approach with particular reference to the entomophthorale s and the eastern spruce budworm. pp. 307-331 in J.M. Anderson, A.D.M. Rayner and D.W.H. Walton (Eds.) Proc. Joint Brit. Mycol. Soc./Brit. Ecol. Soc. Meet. Exeter, UK. Sept. 1982.

Poinar, G.O. Jr. 1979. Nematodes for biological control of insects. CRC Press. Boca Raton, Florida. 277 pp.

Prebble, M.L., T.A. Angus, A.M. Heimpel, R.A. Fisher, O.N. Morris, and J.M. Kinghorn. 1961. Tests of a microbial insecticide against forest defoliators. Can. Dept. Forestry, Bi-monthly Prog. Rept. **17:** 1-4.

Rogoff, M.H. 1982. Regulatory safety data requirements for registration of microbial pesticides. Pp. 645-679 in E. Kurstak (Ed.) Microbial and Viral Pesticides, Marcel Dekker, N.Y.

Schnepf, H.E., and H.R. Whiteley. 1981. Cloning and expression of the *Bacillus thuringiensis* crystal protein gene in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78:** 2893-2897.

Schnepf, H.E., H.C. Wong, and H.R. Whiteley. 1985. The amino acid sequence of a crystal protein from *Bacillus thuringiensis* deduced from the DNA base sequence. J. Biol. Chem. **10:**6254-6272.

Schmiege, D.C. 1963. The feasibility of using a neoaplectanid nematode for control of some forest insect pests. J. Econ. Entomol. **56:** 427-431.

Shepherd, R.F., I.S. Otvos, R.J. Chorney, and J.C. Cunnningham. 1984. Pest management of Douglas-fir tussock moth (Lepidoptera:Lymantriidae); prevention of an outbreak through early treatment with a nuclear polyhedrosis virus by ground and aerial application. Can. Ent. **116:** 1533-1542.

Shibano, Y., A. Yamagata, N. Nakamura, T. Iizuka, H. Sugisaki, and M. Takanami. 1985. Nucleotide sequence coding for the insecticidal fragment of the *Bacillus thuringiensis* crystal protein. Gene **34:** 234-251.

Smirnoff, W.A. 1972. Promoting virus epizootics in populations of the Swaine jack pine sawfly by infected adults. Bio Science **22:** 662-663.

Smirnoff, W.A. 1983. Residual effects of *Bacillus thuringiensis* and chemical insecticide treatments on spruce budworm, *Choristoneura fumiferana (Clem.). Crop Prot.* **2:** 25-230.

Smirnoff, W.A., and A. Juneau. 1973. Quinze annees de recherches sur les micro-organismes des insectes forestieres de la province de Quebec. Ann. Soc. Ent. Quebec **18:** 147-181.

Smirnoff, W.A., J.J. Fettes, and W. Haliburton. 1962. A virus disease of Swaine's jack pine sawfly, *Neodiprion swainei* Midd., sprayed from an aircraft. Can. Ent. **94:** 477-486.

Smirnoff, W.A., and J.M. McLeod. 1975. Swaine jack-pine sawfly, *Neodiprion swainei* Middleton. pp. 235-240 in M.L. Prebble (Ed.) Aerial Control of Forest Insects in Canada, Dept. of the Environment, Ottawa.

Smirnoff, W.A., and O.N. Morris, 1984. Field development of *Bacillus thuringiensis* Berliner in eastern Canada, 1970-1980. pp. 238-247 in J.S. Kelleher and M.A. Hulme (Eds.), Biological Control Programmes against Insects and Weeds in Canada 1969-1980, Commonw. Agric. Bureaux, Slough, U.K.

Soper, R.S., F.R. Holbrook, and C.C. Gordon. 1974. Comparative pesticide effects on *Entomophthora* and the phytopathogen *Alternaria solani.* Envir. Entomol. **3:** 560-562.

Steinhaus, E.A. 1963. Insect pathology, an advanced treatise. Vol. 1. Academic Press, New York, N.Y. 661pp.

Stelzer, M., J. Neisess, J.C. Cunningham, and J.R. McPhee. 1977. Field evaluation of baculovirus stocks against Douglas-fir tussock moth in British Columbia. J. Econ. Ent. **70:** 243-246.

Stockdale, H., and R.A.J. Priston. 1981. Production of insect viruses in cell culture. pp. 313-328 in H.D. Burges (Ed.). Microbial Control of Pests and Plant Diseases 1970-1980. Academic Press, London.

Stoltz, D.B., and S.B. Vinson. 1979. Viruses and parasitism in insects. Adv. Virus Res. **24:** 125-171.

Subramanyam, B.H., and L.K. Cutkomp. 1985. Moth control in stored grain and the rate of *Bacillus thuringiensis*: An overview. Res. Revs. **94:** 1-47.

Undeen, A.H., and M.H. Colbo. 1980. The efficacy of *Bacillus thuringiensis* var. *israelensis* against blackfly larvae (Diptera:Simuliidae) in their natural habitat. Mosq. News. **40:** 181-4.

Undeen, A.H., and W.L. Nagel. 1978. The effect of *Bacillus thuringiensis* ONR-60A strain (Goldberg) on Simulium larvae in the laboratory. Mosq. News. **38:** 524-7.

Vlak, J., and G.E. Smith. 1982. Orientation of the genome of *Autographa californica* nulcear polyhedrosis virus: a proposal. J. Virol. **41:** 1118-1121.

Volkman, L.E., P.A. Goldsmith, R.T. Hess, and P. Faulkner. 1984. Neutralization of

budded *Autographa californica* NPV by a monoclonal antibody: identification of the target antigen. Virology **133:** 354-362.WH0 1981. Mammalian safety of microbial agents for vector control: A WHO memorandum. Bull. WHO **59:** 857-863.

Welch, H.E. 1958. Test of a nematode and its associated bacterium for control of the potato beetle, *Leptinotarsa decemlineata* (Say). Ann. Rept. Ent. Soc. Ont. **88:** 53-54.

Welch, H.E. 1971. Various target species: Attempts with DD-136. Pp. 62-66 in Biological Control Programmes Against Insects and Weeds in Canada, 1959-1968. CIBC Tech. Comm. 4, Commw. Agric. Bureaux, Slough, U.K.

Welch, H.E., and L.J. Briand. 1960. Field experiment on the use of a nematode for the control of vegetable crop insects. Proc. Ent. Soc. Ont. **91:** 197-202.

Welch, H.E., and L.J. Briand. 1961. Tests of the nematode DD-136 and an associated bacterium for control of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Can. Ent. **93:** 759-863.

Wilson, G.G. 1981. 1981. The potential of *Pleistophora schubergi* in control of forest insects. Can. For. Serv. Sault Ste. Marie, Inf. Rep. FPM-X-49. 7pp.

Wilson, G.G. 1982. Protozoans for insect control. pp. 587-600 in E. Kurstak (Ed.) Microbial and Viral Pesticides. Marcel Dekker, N.Y.

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

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