

STATUS OF VIRUSES AS BIOCONTROL AGENTS FOR SPRUCE BUDWORMS

J.C. Cunningham

Research Scientist
Forest Pest Management Institute
Canadian Forestry Service
Environment Canada
P.O. Box 490
Sault Ste. Marie, Ontario
P6A 5M7

Abstract

Aerial spray trials with a variety of viruses have been conducted between 1971 and 1983 with 2 656 ha (65 plots) treated to control spruce budworm in Ontario and Quebec and 424 ha (6 plots) treated to control western spruce budworm in British Columbia. Generally, results have been inconsistent and less than satisfactory, but research continues in an effort to develop a viral insecticide for spruce budworm population regulation.

Introduction

Four different types of insect viruses have been isolated from *Choristoneura* spp.; nuclear polyhedrosis viruses (NPV), granulosis viruses (GV), cytoplasmic polyhedrosis viruses (CPV) and entomopoxviruses (EPV). NPVs and GVs are classified as Baculoviruses subgroup A and subgroup B, respectively. All these viruses share one common feature, the virus particles are occluded within proteinaceous bodies. When ingested by the insect host larva, the protein is solubilized in the alkaline gut juices, virus particles are released, penetrate the midgut cells and the infection process is initiated. Viruses isolated from various *Choristoneura* spp. are listed in Table 1. Generally, it has been found that they are cross-infectious within the genus, although all the permutations of cross-infectivity tests have not been conducted.

Some of these isolates may contain one and the same virus, but, to date, ones which have been analysed biochemically and compared are all distinct. Restriction endonuclease enzyme (REN) fragment patterns of *C. fumiferana* and *C. occidentalis* NPVs showed they were distinct, but closely related and *C. murinana* NPV was more distantly related (Rohrmann *et al.*, 1982). The inclusion body proteins (polyhedrins) of these three viruses and *C. diversana* NPV were indistinguishable when analysed by peptide mapping and polyacrylamide gel electrophoresis (PAGE), but, using the same techniques, the patterns of the virus particle proteins were all distinguishable (B.M. Arif, personal communication). *C. fumiferana* EPV and *C. conflictana* EPV were virtually indistinguishable when

analysed by PAGE, but were closely related although distinct using REN. *C. fumiferana* and *C. biennis* EPVs were distinct when analysed by PAGE (B.M. Arif, personal communication).

Table 1: Viruses isolated from *Choristoneura* spp.

SPECIES	VIRUS			
	NPV	GV	CPV	EPV
<i>C. biennis</i>				X
<i>C. conflictana</i>				X
<i>C. diversana</i>	X			X
<i>C. fumiferana</i>	X	X	X	X
<i>C. murinana</i>	X	X		
<i>C. occidentalis</i>	X	X		
<i>C. pinus pinus</i>	X			
<i>C. retiniana</i>		X		
<i>C. rosaceana</i>	X			

The most intensively studied of these budworm viruses is *C. fumiferana* NPV and considerable progress has been made in constructing a physical map of the viral genome (Arif and Doerfler, 1983, 1984). This virus has also undergone extensive safety testing in mammals and birds and no ill effects were observed (Valli *et al.*, 1976). If *C. fumiferana* NPV is ever deemed to be an effective and economical alternative to chemical pesticides for spruce budworm population regulation, little additional research is required to prepare a petition for its registration in Canada.

Naturally occurring virus epizootics have never been observed to date in budworm populations although viruses are quite common at very low levels usually with less than 5% of the population infected (Cunningham, unpublished). Initiating and maintaining a virus epizootic in a budworm population is, therefore, a considerably greater challenge than doing so in populations of such insects as gypsy moth, *Lymantria dispar*, and Douglas-fir tussock moth, *Orgyia pseudotsugata*, which are often decimated by naturally occurring NPV epizootics. Spraying these species with NPV advances the time of a population collapse, thereby reducing defoliation and tree mortality.

Since 1971, NPV, GV, CPV and EPV have all been tested in aerial spray trials either alone or in various combinations. Combinations were not tested by choice, but because contamination with a second, or even another two viruses, occurred during virus production. With rigid quality control procedures, this problem has almost been eliminated, although contamination of our NPV product with CPV continues to be an intermittent occurrence.

Field trials have been conducted with viruses to regulate populations of both spruce budworm *C. fumiferana*, and western spruce budworm, *C. occidentalis*, and such parameters as dosage, timing of application, tank mix and adjuvants, spray equipment, droplet size, impact on different species of host trees and persistence of viruses from year to year have been studied. The life cycles and behavior of both species make them difficult candidates for regulation with viruses. Larvae are virtually

hidden until budflush, by which time they have reached the fourth instar or larger. Smaller instars are more susceptible to virus infection than larger ones; also viruses are relatively slow to kill the host and when larger instars are infected only those which ingest a lethal dose of the actual spray deposit will die. There is no time for secondary infection to occur before the onset of pupation. Secondary infection occurs when larvae die from viral infections, disintegrate and release more infectious inclusion bodies on to the foliage. Sprayed at budflush, viruses are really being used as slow stomach poisons, no epizootic occurs in the year of application and little or no foliage is saved.

It was hoped that, following an application at budflush, a sufficient number of dead larvae would remain overwinter on the foliage to serve as a source of inoculum and initiate a virus epizootic in the next generation. Virus does persist from one year to the next, but subsequent incidence of virus infection has never been spectacular and, at present, this cannot be considered a viable pest management strategy. Budworm larvae make little or no contact with each other unless populations are extremely dense. This situation is not conducive to the transmission of a pathogen.

Application of viruses on second instar larvae, as they emerge from hibernacula, is an attractive concept. These tiny larvae are highly susceptible to virus infection, there is ample time for secondary infection to occur and, if there is a hardwood overstory, leaves have not flushed at this time of year and a good spray deposit on conifers can be obtained. However, timing is critical and there are numerous logistical constraints such as freezing temperatures, snow in the forest and impassable, unpaved roads.

Virus Production

Viruses only grow in living cells, so it is necessary to propagate them either in susceptible insect larvae or susceptible cell cultures. *C. fumiferana* NPV can be grown in cell culture (Arif *et al.*, 1976), but, at present, it is not economically feasible to produce in cell cultures the quantities required for field trials. Hence, insect larvae are used.

C. fumiferana was originally selected as the host for production of EPV, NPV and GV, but in recent years *C. occidentalis* has been used as the host for production of NPV and GV; it is more susceptible to infection with both viruses than *C. fumiferana*; less inoculum is required, there is less loss to pupation and *C. occidentalis* larvae spin less silk webbing facilitating faster harvest of dead and heavily infected larvae.

Budworm larvae are small and not ideal hosts for virus production. A mature, NPV-infected sixth instar larva yields 5×10^8 polyhedral inclusion bodies (PIB) and this yield drops on a production line basis. Virus production is labour intensive

and, therefore, expensive. A search has been made for a larger host larva for production of budworm viruses. Intensive efforts to use saltmarsh caterpillar, *Estigmene acrea*, for this purpose were unsuccessful (Shapiro *et al.*, 1982) and although *C. fumiferana* NPV infects neonate cabbage looper, *Trichoplusia ni*, larvae and wax moth, *Galleria mellonella*, larvae, it does not infect large larvae required for virus production (Stairs *et al.*, 1981). The search for an alternative host continues.

Budworm viruses which are currently being produced in *C. occidentalis* larvae are *C. fumiferana* NPV and *C. occidentalis* GV. Large fifth and early sixth instar larvae are placed on artificial diet which has been sprayed on the surface with virus inoculum. When NPV is used, dead and heavily infected larvae are harvested in 10 days and, with GV, harvest time is 12 days at 22°C and 50% RH. Dead and diseased larvae are frozen, lyophilized and ground to a fine powder by mixing lyophilized material with an equal weight of dry ice and processing it in a Waring blender. Batches of material are tested to determine the number of inclusion bodies per gram. Our NPV product, which is currently being produced, contains 10^{10} PIB/g and our GV product 8×10^{12} capsules/g. A count is made of the total number of aerobic bacteria per gram and a check is made for the presence of mammalian pathogens. This is done by culturing in selective media and by intraperitoneal injection of samples into mice.

Spruce Budworm

The first field trials with spruce budworm viruses were conducted in Ontario in 1959 and 1960 when small trees were sprayed with NPV and GV (Stairs and Bird, 1962). In 1969, a range of dosages of NPV was applied on individual small trees at 2 to 3 day intervals throughout the larval period (Bird and McPhee, 1970). In 1970, an EPV isolated from 2-year cycle spruce budworm, *C. biennis* (Bird *et al.*, 1971), although slow acting, was found to give high levels of mortality in the laboratory at much lower dosages than viruses tested previously. It was thought that the EPV would prove to be a highly effective biocontrol agent and the first aerial spray trials were conducted with EPV in 1971 (Bird *et al.*, 1972). Since then aerial spray trials have been conducted almost every year and a total of 65 plots with a combined area of 2 656 ha have been treated with viruses or chemical insecticide/virus combinations in Ontario and Quebec between 1971 and 1983. A list of these treatments is given in Table 2 and a summary of treatments between 1971 and 1980 and their impacts are described by Cunningham and Howse (in press).

The following methods have been used to assess treatments; (a) deposit was monitored on Kromekote® cards, (b) prespray and pupal counts were made from samples from treated and check plots and population reduction due to treatment calculated using a modified Abbott's formula (Abbott, 1925),

(c) incidence of virus infection was determined either by microscopic examination of samples of larvae from the field or by rearing collections of larvae and examining those which died, (d) adult emergence on treated and check plots was compared and (e) defoliation estimates were made. Follow-up studies were conducted in most of the trials to determine the impact of the virus treatment in the year or years following the year of application.

Table 2: Spray trials with viruses on spruce budworm in Eastern Canada.

YEAR	TREATMENT	NO. OF PLOTS	TOTAL AREA (HA)
1971	EPV (+NPV+CPV) ^a	6	6.5
1971	NPV (+CPV)	2	4.6
1972	EPV	3	508
1972	NPV	3	268
1972	Fenitrothion/ EPV (+NPV)	1	16
1972	EPV (+NPV)	1	16
1972	Fenitrothion/ NPV	1	16
1973	NPV	9	295
1974	NPV	2	526
1975	NPV	5	318
1976	NPV	3	120
1977	NPV	5	108
1978	NPV	7	134
1979	NPV (+CPV)	6	74
1979	GV	1	6
1979	Orthene/NPV	1	40
1980	GV	1	10
1980	NPV (+CPV)	3	42
1981	NPV	3	108
1983	NPV	2	40
Totals		65	2,656

^aviruses in brackets are contaminants in the material applied.

In 1971, the EPV which was applied was found to be contaminated with low levels of both NPV and CPV and the NPV applied was contaminated with CPV. In 1972, pure EPV was applied, but also in this year, it was found that carry-over of NPV from one year to the next was much better than with EPV, and further trials with EPV were suspended. From 1973 to the present, all efforts have concentrated on developing an effective strategy for the use of NPV and only two small plots have been sprayed with GV.

Dosages of NPV have ranged from 2.5×10^{10} PIB/ha to a double treatment with a first application of 3.4×10^{12} followed by a second application of 2.3×10^{12} PIB/ha. The most commonly used dosage has been 7.5×10^{11} which, on some occasions, has given acceptable levels of population reduction (Cunningham and Howse, in press). When populations are monitored on both white spruce and balsam fir hosts, generally better results are obtained on white spruce. A variety of tank mixes and stickers has been used. Until recently, most were aqueous

and contained 25% v/v molasses, but in the last two years an emulsifiable oil (Dipel 88[®] blank carrier vehicle) has been used, with the virus applied in 25% oil and 75% water. Emitted volumes have ranged from 4.5 to 28.2 L/ha, but most applications were made at 9.4 L/ha. Both boom and nozzle and Micronair[®] rotary atomizers have been used. Generally, coarse droplets with a stain diameter of 200 to 400 μ m have given better results than droplets with a diameter less than 100 μ m. This is a very important point which should be clarified before further applications are contemplated.

Reference has already been made to timing of application and most sprays have been applied at budflush when larvae were in the fourth and fifth instars and sometimes even some sixth instar were present. Results from ground spray applications of heavy dosages of NPV on individual trees at, or prior to, second instar emergence have demonstrated that secondary infection can occur (Bird and McPhee, 1970; W.J. Kaupp, personal communication). Four attempts have been made to aerially spray second instar larvae as they emerge from hibernacula and only one was considered to be timed correctly. Our experience in Ontario, near Sault Ste. Marie, has been that weather generally stays warm in late April and that emergence is over a two to three day period; when the first larvae are observed, it is time to spray and the problem has been applying the spray too late, after larvae have mined into needles and buds. In 1983, in Gaspé, Quebec, weather was cool and larvae emerged slowly over a prolonged period. When larvae were observed on the foliage, NPV was applied, but it remained cool after the treatment and results were disappointing (Cadogan, Kaupp and Cunningham, unpublished).

Carry-over of virus from one year to the next has been monitored in most plots. Following application of NPV contaminated with CPV on two isolated stands of mature white spruce in 1971, levels of virus infection were monitored until 1977 (Cunningham *et al.*, 1975; Cunningham and Howse, unpublished). In the year of application, maximum levels of 21% NPV infection and 28% CPV infection were found in weekly samples of larvae from one plot and 46% NPV infection and 35% CPV infection from the second. Population reductions due to treatment were 69 and 80% respectively, but there was no detectable saving of foliage in either plot. In subsequent years, levels of CPV declined; it disappeared from one plot in 1973 and from the other in 1975. Microscopically detectable maximum levels of NPV in weekly samples of larvae from the first plot each season between 1972 and 1977 were 28, 17, 6, 21, 8 and 3% and from the second plot were 24, 14, 6, 9, 0 and 2%. By 1977, the spruce budworm population had declined to a very low level. Although not spectacular, the NPV had a considerable impact on the spruce budworm population over this period, reinvasion of these isolated stands did not occur and the virus probably exerted sufficient control of spruce budworm to reduce defoliation and thereby prevent tree mortality. Subsequent efforts to repeat this experiment and duplicate these results have proved unsuccessful to date.

The problem of CPV contamination of NPV stocks was solved between 1973 and 1978, but returned in 1979 and 1980. Several interesting observations were made on this contaminated material. Firstly, the levels of CPV contamination have been low and the ratio of NPV:CPV PIBs ranged from 178 to 400:1 (W.J. Kaupp, personal communication). In spite of the low levels of CPV, the incidence of CPV was usually greater than NPV when freshly produced material was applied in the field. When lyophilized, CPV contaminated material is stored for more than one year, the CPV virtually loses all activity and is barely detectable after application of the NPV.

Two small trials were conducted with GV; in 1979, a 6 ha plot was sprayed with 2×10^{14} capsules/ha when larvae were in the fifth and sixth instars and, in 1980, a 10 ha plot was sprayed with 6.4×10^{13} capsules/ha when larvae were in the second instar. Results were inconsistent and there was no relationship between population reduction due to treatment and foliage protection (Cunningham and Howse, in press).

There has been considerable interest in using combinations of chemical insecticides and various pathogens as integrated pest management strategies and this topic has recently been reviewed by Jaques and Morris (1981). Two attempts have been made to use this strategy for spruce budworm control. In 1972, the following treatments were compared: a low dosage of fenitrothion (18g AI/ha) alone, NPV (7.2×10^{11} PIB/ha) alone, EPV contaminated with NPV (1.8×10^{11} inclusion bodies/ha) alone, fenitrothion followed by NPV, and fenitrothion followed by EPV (+NPV). Generally, better results were obtained with the chemical/virus combinations than either chemical or virus alone. There was better foliage protection in the year of application in the plots treated with virus alone or fenitrothion/virus combinations than with fenitrothion alone and this foliage protection was also recorded the following year (Morris *et al.*, 1972, 1974). In 1979, a 40 ha plantation containing both black and white spruce trees was treated with acephate (Orthene®) at 560g AI/ha when larvae on white spruce were in the fourth instar and with NPV at 7.5×10^{11} PIB/ha 8 days later when larvae were in the fifth and sixth instars. Significant foliage protection was obtained in the year of application and in the following year (Cunningham and Howse, in press).

Although these two trials showed promising results, this is a line of research which is not being actively pursued. In attempts to initiate and maintain a virus epizootic, the aim has been to kill as many larvae as possible with virus so as to introduce a large virus inoculum pool into the environment. When used in forestry, it is felt that the addition of even low dosages of chemical insecticides reduces this virus inoculum pool and detracts from the appeal of using an environmentally acceptable biocontrol agent.

Western Spruce Budworm

When the pathogenicity of NPV and GV were compared in spruce budworm and western spruce budworm, the western species was considerably more susceptible to both viruses (Cunningham *et al.*, 1983a) and it appeared that western spruce budworm is a better candidate for regulation with viruses than the eastern species. Aerial spray trials were conducted in 1976, 1978 and 1982; 6 plots have been treated with a combined area of 424 ha (Table 3).

Table 3: Spray trials with viruses on western spruce budworm in British Columbia.

YEAR	TREATMENT	NO. OF PLOTS	TOTAL AREA (HA)
1976	NPV	1	20
1978	NPV	3	60
1982	NPV	1	172
1982	GV	1	172

In 1976, an application of 2.5×10^{11} PIB/ha on Douglas-fir trees gave disappointing results (Shepherd and Cunningham, unpublished). In 1978, three plots containing Douglas-fir trees were treated with a dosage of 7.5×10^{11} PIB/ha. Fifteen days post-spray, levels of NPV infection, determined microscopically, were 55, 87 and 25% and population reductions due to treatment were 0, 26 and 48% respectively (Hodgkinson *et al.*, 1979). In 1979 and 1980, surveys were conducted on two of the plots, the third being abandoned because of a local population collapse. In 1979, NPV infection levels were recorded as 18 and 45% and in 1980 as 7 and 17%. In 1979, population declines, compared to 1978 levels, were recorded at 51 and 62% compared to increases of 46 and 201% in corresponding check plots. Unfortunately, this trend was reversed in 1980 with populations more than doubling in the treated plots compared to substantial declines in the check plots (Shepherd *et al.*, 1982). It was felt that small plots may be unsatisfactory when long-term studies are contemplated because immigration of moths may have a profound effect on population densities.

In 1981, small Douglas-fir trees were sprayed with 3 dosages (10 fold differences) each of NPV and GV when larvae were at the peak of the third instar. The lowest dosage of GV at 1.5×10^{12} capsules/ha had a marked impact, but the lowest dosage of NPV at 2.5×10^9 PIB/ha had none, and it was considered that GV may be a better candidate as a biocontrol agent than NPV (Cunningham *et al.*, 1983b). A similar test was conducted in the US in 1981 when individual grand fir trees were treated with NPV and GV at 3 dosages (2 fold differences) at two application dates, when larvae were 50% second instar and 50% third and also when they were 10% second, 53% third and 37% fourth instar (M.J.

Stelzer and D.W. Scott, personal communication). They found no significant differences between the two viruses or the three dosages or any interaction between virus type and dosage. However, the GV provided better control when applied at the early timing compared to the late timing which probably reflects the longer incubation period of the GV compared to the NPV. They do not feel that either virus warrants further development since commercial preparations of *Bacillus thuringiensis* (B.t.) are available.

Following the results of the NPV aerial spray trials in 1978 and results of the ground spray applications in 1981 in B.C., it was decided to treat two large plots, one with NPV and one with GV to determine the long-term impact of these two viruses. This was accomplished in 1982 and two 172 ha plots containing Douglas-fir trees were sprayed at budflush when larvae were at the peak of the fourth instar. The two viruses were compared on the basis of weight of lyophilized material and 9 kg was applied to each plot in a volume of 9.4 L/ha using a tank mix containing 25% emulsifiable oil (Dipel 88® blank carrier vehicle) and 75% water. The actual dosage of NPV was 5.4×10^{11} PIB/ha and the dosage of GV was 1.7×10^{14} capsules/ha. A high level of control was not anticipated in the year of application, but it was hoped that dead larvae remaining overwinter on the foliage would release sufficient viable virus to initiate an epizootic in 1983.

Preliminary analysis of data shows that the NPV had a greater impact than the GV on western spruce budworm in 1982 with 52% population reduction due to treatment compared to 35%. In samples of larvae reared individually in the laboratory, 66 to 80% died from NPV and 59 to 62% from GV; successful adult emergence was 53% in the check plots, ranged from 6 to 13% in the NPV-treated plot and from 12 to 24% in the GV-treated plot. Both viruses had an impact in 1983, but it was less than in the year of application. Population reduction attributed to NPV carry-over was 35% and attributed to GV carry-over was 15%. Two samples from treated and check plots were reared until death or adult emergence. Successful adult emergence was 46 and 46% from the NPV-treated plot, 50 and 42% from the GV-treated plot and 61 and 73% from the check plots (Otvos, Cunningham and Kaupp, unpublished). These results certainly cannot be regarded as dramatic, and it appears that relying on virus carry-over from one year to the next to regulate the insect population is not a viable pest management strategy.

Conclusions

Presently, lack of commercial interest in producing viruses is a major stumbling block in their development. All virus production in North America is at government establishments and the one company, Sandoz Inc., which marketed a viral insecticide, has discontinued production. If production in cell cultures becomes a viable alternative to

using insect larvae, several pharmaceutical companies, with expertise in production of viral vaccines, would probably be interested in this market. The cost of producing budworm viruses in insect larvae at the pilot plant level is extremely high. It is difficult to quote an accurate figure, but an NPV dosage of 7.5×10^{11} PIB/ha probably costs in excess of \$300/ha. This could be justified economically if virus persisted sufficiently well in the environment so as to hold the population below an economic threshold for several years or if it spread from treated to untreated areas allowing zebra stripe applications (widely spaced swaths) to be made. NPV and GV have persisted from one year to the next following some, but not all, applications. However, the incidence of infection has not been, except in rare circumstances, sufficiently high to regulate the spruce budworm population. To date, applications of viruses at budflush have virtually been applications of a very slow acting, expensive, stomach poison which sometimes gives a high level of population reduction with little or no foliage protection.

If one wants to use a non-chemical agent for spruce budworm population regulation, an obvious choice is B.t. which is readily available commercially at a competitive price. Numerous trials and operational applications have shown B.t. to be effective against spruce budworm. Considerably fewer trials with B.t. have been conducted on western spruce budworm, but satisfactory results have also been obtained on that species (M.J. Stelzer and D.W. Scott, personal communication).

Viruses are extremely efficient pest management tools when they act in a truly biological manner, and are probably superior to applications of B.t. By truly biological, one means that the virus treatment initiates an epizootic, secondary infection occurs, virus is spread from treated to untreated areas by such agents as predaceous, parasitic and scavenging insects and also, perhaps, by birds and the virus may persist from one year to the next if the insect population is not completely controlled in the year of application. The most spectacular viruses studied to date are those infecting European spruce sawfly, *Gilpinia hercyniae*, European pine sawfly, *Neodiprion sertifer*, and redheaded pine sawfly, *N. lecontei* (Cunningham and Entwistle, 1981). B.t. is not effective against sawflies. Douglas-fir tussock moth NPV is another outstanding virus which initiates an epizootic in the year of application. Applied on first and second instar larvae, only 10 to 30% may become infected by ingesting the spray deposit, but secondary infection causes collapse of the population (Cunningham, unpublished). Unless an excellent deposit is obtained with B.t., it is not as effective as NPV for regulation of Douglas-fir tussock moth.

However, it would be most unwise to discontinue research on alternative methods of spruce budworm control and rely entirely on B.t. Pest managers should have a variety of options from which to choose. Presently, there are three companies in North America producing B.t. and problems of availability seem unlikely. There is neither evidence

of insects becoming resistant to *B.t.* nor reports of any undesirable side-effects following the use of *B.t.* Currently, viruses cannot be recommended for spruce budworm population regulation and can only be regarded as being at the experimental stage of development.

Improved methods of controlling spruce budworm with viruses are constantly being considered, although the life-cycle and behavior of spruce budworms are major insoluble problems. A continuing search is being made for more virulent virus isolates. If viruses could be kept in an infectious state on the foliage for prolonged periods, their efficacy would be greatly enhanced. Also spraying before second instar larvae emerge from hibernacula may then become a feasible strategy. A search for such tank mix adjuvants as stickers and UV protectants is being made for other viruses and other microbial pathogens and a breakthrough in this field could revitalise research on budworm viruses currently on hand. The epizootiology of spruce budworm viruses is not well studied and a better understanding of the virus - insect host - forest environment could give the answer to why applications of viruses are less than satisfactory. Perhaps the answer is spruce budworm behavior, but it may be a factor which can be modified or manipulated with relative ease.

Lastly, by far the most exciting aspect of current spruce budworm virus research is that of recombinant DNA technology. It is conceivable that the virulence, host range and resistance to UV light can be altered once the genes controlling the various viral functions are identified. Considerable progress has been made in constructing a physical map of the spruce budworm NPV genome. The next step is to produce a transcriptional map which ascribes a function to different regions of the DNA (B.M. Arif, personal communication). Genetic studies have revealed that alfalfa looper, *Autographa californica*, NPV is very closely related to spruce budworm NPV and that these two viruses probably evolved from a common ancestor. A recombinant of these viruses may yield a virus which can be propagated in cabbage looper larvae, a large, easily reared Noctuid, but which still retains its infectivity to spruce budworm (B.M. Arif, personal communication). Speculation on the potential use of genetically manipulated Baculoviruses is limitless, but it will probably be several years until such viruses reach the stage of field testing. Such hurdles as public opinion, safety testing protocols and regulatory agencies will have to be overcome.

Acknowledgments

I wish to thank Dr. M.J. Stelzer, USDA Forest Service, Corvallis, Oregon, Dr. F.B. Lewis, USDA Forest Service, Hamden, Connecticut and Dr. I.S. Otvos, Pacific Forest Research Centre, Victoria, B.C. for advice, access to unpublished data and for reviewing the manuscript; also my colleagues at the Forest Pest Management Institute, Drs. B.M. Arif and W.J. Kaupp for similar help. Thanks to Miss J.A. Novick for typing this manuscript.

Literature Cited

- Abbott, W.S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267; 1925.
- Arif, B.M.; Sohi, S.S.; Krywienczyk, J. Replication of alkali-released NPV in a cell line of *Choristoneura fumiferana*. Pages 108-112 in *Proceedings 1st International Colloquium on Invertebrate Pathology and 9th Annual Meeting Society for Invertebrate Pathology*; Queen's University, Kingston, Canada; 1976.
- Arif, B.M.; Doerfler, W. Characterization of the DNA from *Choristoneura fumiferana* nuclear polyhedrosis virus. *Zbl. Bakt. Hyg. Abt. Orig. A* 254: 147-148; 1983.
- Arif, B.M.; Doerfler, W. Identification and localization of reiterated sequences in the *Choristoneura fumiferana* MNPV genome. *EMBO J.* 3: in press; 1984.
- Bird, F.T.; McPhee, J.R. Susceptibility of spruce budworm to pure nuclear polyhedrosis virus (NPV) sprays. *Can. Dept. Fish. For. Bi-Mon. Res. Notes.* 26: 35; 1970.
- Bird, F.T.; Sanders, C.J.; Burke, J.M. A newly discovered virus disease of the spruce budworm *Choristoneura biennis*, (Lepidoptera: Tortricidae). *J. Invertebr. Pathol.* 18: 159-161; 1971.
- Bird, F.T.; Cunningham, J.C.; Howse, G.M. Possible use of viruses in the control of spruce budworm. *Proc. Entomol. Soc. Ont.* 103: 69-75; 1972.
- Cunningham, J.C.; Howse, G.M.; Kaupp, W.J.; McPhee, J.R.; Harnden, A.A.; White, M.B.E. The persistence of nuclear polyhedrosis virus and entomopoxvirus in populations of spruce budworm. *Can. For. Serv., Sault Ste. Marie, Inf. Rep. IP-X-10*; 1975. 32 p.
- Cunningham, J.C.; Entwistle, P.F. Control of sawflies by baculovirus. Pages 379-409 in *Microbial control of pests and plant diseases 1970 - 1980*. (H.D. Burges, ed.) Academic Press, London; 1981.
- Cunningham, J.C.; Kaupp, W.J.; McPhee, J.R. A comparison of the pathogenicity of two baculoviruses to the spruce budworm and the western spruce budworm. *Can. For. Serv. Res. Notes* 3(2): 9-10; 1983a.
- Cunningham, J.C.; Kaupp, W.J.; McPhee, J.R.; Shepherd, R.F. Ground spray trials with two baculoviruses on western spruce budworm. *Can. For. Serv. Res. Notes.* 3(2): 10-11; 1983b.
- Cunningham, J.C.; Howse, G.M. Eastern spruce budworm viruses: application and assessment. In "Biological control of insects and weeds in Canada, 1969-1980". Commonwealth Institute of Biological Control. Technical Communication, in press.

- Hodgkinson, R.S.; Finnis, M.; Shepherd, R.F.; Cunningham, J.C. Aerial application of nuclear polyhedrosis virus and *Bacillus thuringiensis* against western spruce budworm. British Columbia Ministry of Forests/Canadian Forestry Service Joint Rep. No. 10; 1979. 19 p.
- Jaques, R.P.; Morris, O.N. Compatibility of pathogens with other methods of pest control and with different crops. Pages 695-715 in Microbial control of pests and plant diseases 1970-1980. (H.D. Burges, ed.) Academic Press, London; 1981.
- Morris, O.N.; Armstrong, J.A.; Hildebrand, M.J.; Howse, G.M.; Cunningham, J.C.; McPhee, J.R. Aerial applications of virus-insecticide combinations against spruce budworm *Choristoneura fumiferana* (Clem.) (Tortricidae: Lepidoptera) at Rankin, Ontario, 1972. Can. For. Serv., Ottawa, Inf. Rep. CC-X-37; 1972. 65 p.
- Morris, O.N.; Armstrong, J.A.; Howse, G.M.; Cunningham, J.C. A 2-year study of virus-chemical insecticide combination in the integrated control of the spruce budworm, *Choristoneura fumiferana* (Tortricidae: Lepidoptera). Can. Entomol. 106: 813-824; 1974.
- Rohrmann, G.F.; Melgaard, S.; Beaudreau, G.S.; Martignoni, M.E. Characterization of DNA from three nuclear polyhedrosis viruses pathogenic for *Choristoneura* sp. J. Invertebr. Pathol. 40: 237-241; 1982.
- Shapiro, M.; Martignoni, M.E.; Cunningham, J.C.; Goodwin, R.H. Potential use of the saltmarsh caterpillar as a production host for nucleopolyhedrosis viruses. J. Econ. Entomol. 75: 69-71; 1982.
- Shepherd, R.F.; Gray, R.G.; Cunningham, J.C. Effects of nuclear polyhedrosis and *Bacillus thuringiensis* on western spruce budworm (Lepidoptera: Tortricidae) 1 and 2 years after aerial application. Can. Entomol. 114: 281-282; 1982.
- Stairs, G.R.; Bird, F.T. Dissemination of viruses against the spruce budworm, *Choristoneura fumiferana* (Clemens). Can. Entomol. 94: 966-969; 1962.
- Stairs, G.R.; Fraser, T.; Fraser, M. Changes in growth and virulence of a nuclear polyhedrosis virus from *Choristoneura fumiferana* after passage in *Trichoplusia ni* and *Galleria mellonella*. J. Invertebr. Pathol. 38: 230-235; 1981.
- Valli, V.E.; Cunningham, J.C.; Arif, B.M. Tests demonstrating the safety of a baculovirus of the spruce budworm to mammals and birds. Pages 445-446 in Proceedings 1st International Colloquium on Invertebrate Pathology and 9th Annual Meeting Society for Invertebrate Pathology; Queen's University, Kingston, Canada; 1976.