THE STATUS OF VIRUSES FOR SPRUCE BUDWORM POPULATION REGULATION

File Report No. 7 January 1981

John C. Cunningham, Basil M. Arif and Jean Percy

Forest Pest Management Institute
Canadian Forestry Service
Sault Ste. Marie, Ontario

Collapse of a spruce budworm population due to a virus epizootic, or due to any other pathogen for that matter, has never been observed. However, four different types of viruses have been found in populations of either eastern spruce budworm, *Choristoneura fumiferana*, or western spruce budworm, *C. occidentalis*. All four have one feature in common: virus particles are occluded within proteinaceous inclusion bodies. These have a macromolecular, crystalline structure and are sufficiently large to be visible through a light microscope, making them easy to recognise. When these inclusion bodies are ingested by a spruce budworm larva, the matrix protein dissolves in the alkaline gut juices, liberating the virus particles. Some of these particles attach to gut cell membranes, and a cylce of penetration, replication and maturation commences.

The four types of viruses are illustrated in plate 1. Nuclear polyhedrosis virus (NPV) inclusion bodies, which are 1.0 to 1.5 µm in diameter, contain bundles of rod-shaped virus particles (Fig. A). Their nucleic acid is DNA and they replicate in the nuclei of gut, fat body, blood, muscle and hypodermal cells. Granulosis viruses (GV) (Fig. B) replicate in the same tissues as NPV, but in the cell cytoplasm as well as the nucleus. Like NPV, the virus particles are rod-shaped and the nucleic acid is DNA. However, there is only one virus particle per inclusion body which is about 0.5 x 0.2 µm and is called a capsule. Cytoplasmic polyhedrosis virus (CPV) has inclusion bodies which are slightly smaller than NPV (Fig. C). The virus particles are spherical, contain segmented RNA and replicate in the cytoplasm of gut cells. Larvae which are heavily infected with this virus die from starvation. Entomopoxvirus (EPV) inclusion bodies (Fig. D) are oval and larger than the other viruses, ranging in size from about 3.0 µm to 12.0 µm x 2.0 µm to 8.0 µm. The virus particles are also oval, have a mulberry-like surface structure, contain DNA and replicate in the cell cytoplasm of all tissues except the germ cells. Adult moths cannot become infected with any of these viruses which must be ingested by larvae in order to initiate the

infection process. However, if larvae are only lightly infected, they can develop into pupae and then adults and virus replication can occur in both these stages.

Viruses found in other species of budworm such as the two-year cycle spruce budworm, *C. biennis*, the Modoc budworm, *C. viridis*, the jack pine budworm, *C. pinus*, and the large aspen tortrix, *C. conflictana*, are generally cross-infectious to both eastern and western spruce budworm larvae, and a considerable collection of virus isolates has been amassed. The highest levels of naturally occurring virus disease (20% to 30% of larvae) found in the field, were EPV in two-year cycle spruce budworm and GV in western spruce budworm. In the eastern species, extensive surveys have shown levels of NPV and CPV usually at less than 1%.

In collaboration with Dr. Gordon Howse, Great Lakes Forest Research Centre, NPV, GV, CPV and EPV have, at some time, been tested in the field in Ontario on eastern spruce budworm either alone or in combinations. Only NPV, to date, has been tested on western spruce budworm. Virus treatments applied from the air have generally been at budflush at the peak of the fifth larval instar. However, applications on highly-susceptible, second instar larvae have also been tested. Most research efforts have been concentrated on NPV and over 2000 ha have been sprayed with this virus. Extensive safety testing and biochemical studies have been undertaken with NPV and little additional information is required should registration of this virus as a biocontrol agent be considered.

The first aerial spray trial using viruses against eastern spruce budworm was conducted in 1971 when a mixture of NPV and CPV (ratio 400:1) at a dosage of 7.5×10^{11} polyhedra/ha was applied. An EPV was also tested in 1971. Good population reduction was recorded with the NPV-CPV mixture, and the NPV persisted in the budworm population for over 5 years. It is thought that carry-over of viruses from year-to-year is in cadavers which remain overwinter, webbed-up on foliage. Transmission of virus inside

eggs on or their surface has not been demonstrated with any budworm viruses.

The EPV was tested again in 1972 with 512 ha treated. As better carry-over from year-to-year was recorded with NPV, tests with EPV were suspended at this time. Between 1972 and 1977, reduced dosages of NPV were tested, and such parameters as timing of application, volume emitted, tank-mix and spray equipment were evaluated. Although some satisfactory initial levels of population reduction were achieved, the persistence of virus from one year to the next was low and levels of foliage protection were disappointing. In 1977, the high dosage used in 1971 was retested, and 92% population reduction due to treatment (modified Abbott's formula) was recorded on white spruce trees. In 1978, the 7.5×10^{11} polyhedra/ha dosage was applied on 6 plots at the peak of the fifth instar. Population reductions due to treatment on white spruce ranged from 33% to 92% and on balsam fir trees from 37% to 76%. Generally, a higher virus kill has been found on white spruce than on balsam fir. In all trials conducted between 1971 and 1978, foliage protection was negligible during the year of application although some foliage protection was recorded in a few of the plots in subsequent years.

Viruses only replicate in living cells. A cell culture system is available in which spruce budworm NPV will replicate, but the medium required to maintain these cells is extremely expensive. Therefore, living insect larvae are routinely used for virus production. A spruce budworm larva which is heavily infected with NPV will yield 5×10^8 polyhedra but, in mass production, this figure falls to a mean of 1×10^8. Hence, 7500 larvae were required to produce the dosage used to treat 1 ha in the 1971, 1977 and 1978 trials. Production of virus in insect larvae is labour-intensive and the cost is therefore high.

In 1979, a mixture of NPV and CPV (178:1) was retested on two plots at a dosage of 7.5×10^{11} polyhedra/ha, this time on second instar larvae as they emerged from hibernacula. GV was also tested for the first time in an aerial application, but was

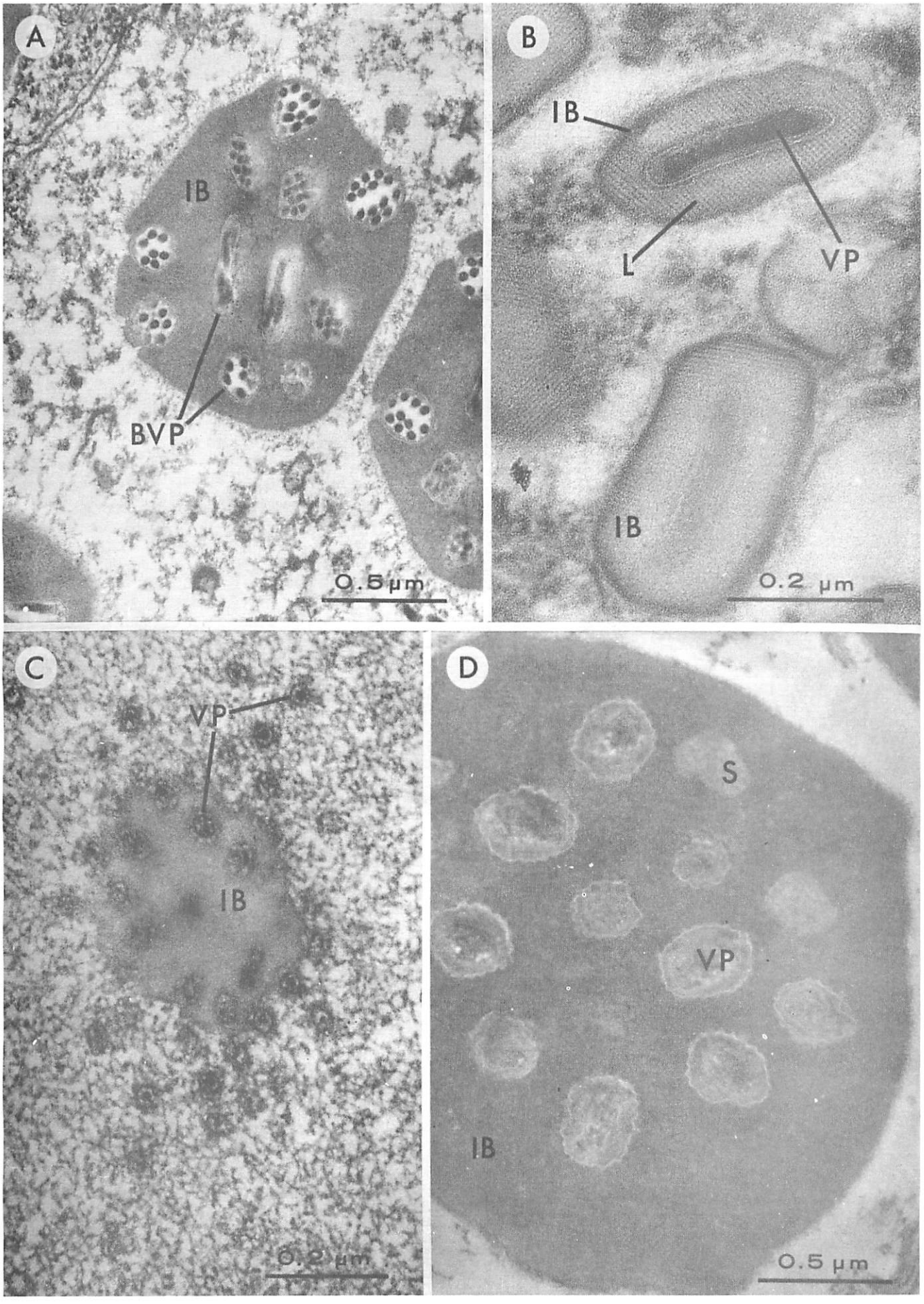
applied on fifth instar larvae at budflush. In spite of the low level of CPV in the mixture, this virus was more prevalent than NPV in treated larvae. Population reduction due to treatment were recorded at 50% and 85% on balsam fir and 45% and 81% on white spruce trees. For the first time, significant foliage protection was recorded during the year of virus application, with 78% foliage saved on balsam fir and 68% on white spruce in one of the plots. In the other 43% of foliage was saved on balsam fir and 39% on white spruce. GV at 2×10^{14} capsules/ha (7500 GV-infected larvae) gave 74% population reduction due to treatment on white spruce hosts, with 17% foliage saved. GV is much slower in killing budworm larvae in the laboratory than NPV and has not received as much attention as the latter virus. There is a great lack of information on the ecology of all these budworm viruses and, when the interaction of virus, host and forest ecosystem is considered, it may be that the best biocontrol agent is not necessarily the most virulent virus.

In collaboration with Dr. Roy Shepherd, Pacific Forest Research Centre and BC Forest Service staff, trials were conducted in 1977 and 1978 in British Columbia using NPV against western spruce budworm on Douglas-fir. The trial in 1977 was a failure due to too low a dosage of virus being applied too late. However, in three, 20-ha plots treated at budflush with 7.5×10^{11} polyhedra/ha in 1978, 48% population reduction due to treatment was recorded in one plot 15 days post-spray, 26% in the second and none in the third which had a low population density and was abandoned for follow-up studies. In 1979, high levels of virus disease were found in these two plots and budworm population densities were about half those recorded in check plots. In 1980, NPV was still present in the budworm population, but larval numbers had increased greatly and exceeded those in the check plots. This was probably due to immigration from the high populations which surrounded these small plots.

Unless more virulent viruses are found, it may be unrealistic to expect viruses which we are currently testing to

provide a complete solution to the spruce budworm problem. Viruses have been used successfully for control of Douglas-fir tussock moth, gypsy moth and two species of sawflies but, in all these cases, virus production is economically feasible and efficient year-to-year virus transmission mechanisms exist. Concerning the viruses currently available, the best we can expect, in terms of spruce budworm control, is their development for use on high value stands and ecologically sensitive areas. Research continues with the following priorities: 1) a search for new, more virulent viruses, 2) a search for an alternative insect host, larger than spruce budworm, for virus production, 3) a study of improved tank-mixes which will maintain viruses in a viable state on foliage for a longer period of time and 4) a detailed investigation of viral nucleic acids with a long-term goal of genetically manipulating them in order to enhance virulence.

Figs. A-D: Electron micrographs of ultrathin sections of viruses found in spruce budworms. When virus development is complete, virus particles (VP) are occluded in proteinaceous inclusion bodies (IB). Fig. A. Nuclear polyhedrosis viruses contain bundles of rod-shaped virus particles (BVP). 48,000X. Fig. B. Granulosis viruses contain only one virus particle per inclusion body. At high magnification, the macromolecular crystalline lattice (L) of the inclusion body protein can be seen. 150,000X. Fig. C. Cytoplasmic polyhedrosis virus with spherical virus particles in and around a developing inclusion body. 116,000X. Fig. D. Entomopoxvirus. The virus particles have a mulberry-like surface structure. The inclusions also contain spindle-shaped protein bodies (S) which are not infectious. 51,000X.


A
IB
BVP
0.5 µm
B
IB
L
VP
IB
0.2 µm
C
VP
IB
0.2 µm
D
S
VP
IB
0.5 µm