AERIAL APPLICATION OF SPRUCE BUDWORM BACULOVIRUS: TESTS OF VIRUS STRAINS, DOSAGES AND FORMULATIONS IN 1977

by

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Abstract

Five plots with a total area of 148 ha were treated with spruce budworm, *Choristoneura fumiferana*, nuclear polyhedrosis virus to test 3 formulations, 2 dosages and 2 strains of virus. The application was made with a Grumman Agcat equipped with boom and nozzle spray equipment calibrated to deliver 9.4 l/ha. Larvae were mainly in the fourth and fifth instar in 4 of the plots and fifth instar with some sixth instar present in the other plot.

The efficacy of the treatments was assessed by 5 methods. (i) Larvae collected from the plots 5 days after the application were reared in the laboratory until death or pupation occurred. (ii) Samples of larvae from treated and check plots, taken at weekly intervals, were examined microscopically to determine if they were infected with virus. (iii) Pupal survival in treated areas was compared to check areas. (iv) Population reduction estimates due to the treatment were made by comparing prespray larval and postspray pupal samples from treated and check areas. (v) Defoliation estimates were made in treated and check areas.

Generally the results indicated that a formulation containing 250 ml/l molasses, 60 g/l IMC 90-001 UV protectant and 1.25 ml/l Chevron® sticker was superior to a polymer bound carbon formulation or a virus adjuvant manufactured by Sandoz Inc., SAN 285 wettable powder. Subsequent laboratory tests showed that considerable inactivation of the virus had occurred as a result of preparing the polymer bound carbon formulation. The SAN 285 wettable powder was compatible with the virus.

The efficacy of virus isolates obtained from *Choristoneura fumiferana* and *C. occidentalis* were similar. When two dosages of the *C. fumiferana* nuclear polyhedrosis virus were compared, 750 billion polyhedra/ha gave considerably better results than 250 billion. The former dosage gave a population reduction of 92% which is the best result obtained to date with a nuclear polyhedrosis virus treatment on spruce budworm.

Résumé

Cinq places-échantillons couvrant une superficie totale de 148 ha furent traitées avec un virus de la polyédrose nucléaire de la Tordeuse des Bourgeons de l'Epinette, *Choristoneura fumiferana*, afin de mettre à l'essai 3 formules, 2 dosages et 2 lignées de virus. On procéda à l'arrosage avec l'appareil "Grumman Agcat" muni d'un dispositif à tangon et à lance, calibré pour éjecter 9.4 ℓ/ha. Les larves étaient surtout aux 4^{e} et 5^{e} stades dans 4 des placettes et aux 5^{e} et 6^{e} stades dans la dernière placette d'échantillonnage.

L'efficacité des traitements fut évalueé via cinq méthodes. (i) Les larves recueillies dans les placettes 5 jours après l'arrosage furent élevées en laboratoire jusqu'à ce que survienne la mort ou la pupation. (ii) Des échantillons de larves cueillies à intervalles d'une semaine, furent examinés au microscope pour déterminer si le virus les avait infectées. (iii) L'apparition des pupes dans les superficies traitées fut comparée aux secteurs témoins. (iv) On évalua la réduction de la population due au traitement en comparant des échantillons

de larves avant l'arrosage et de pupe après l'arrosage, provenant des aires traitées et des aires témoins. (v) On évalua la défoliation dans les aires traitées et les aires témoins.

Généralement, les résultats indiquèrent qu'un liquide contenant 250 ml/ℓ de mélasse, 60 g/ℓ IMC 90-001 UV (un agent protecteur) et 1.25 ml/ℓ de gommant Chevron®, était supérieur à un liquide polymérisé au carbone ou à un adjuvant à virus fabriqué par Sandoz Inc. (la poudre mouillable SAN 285). Des tests subséquents en laboratoire montrèrent une considérable inactivité du virus, suite à l'utilisation du liquide polymérisé au carbone. La poudre mouillable SAN 285 était compatible avec le virus.

L'efficacité des isolats de virus de *Choristoneura fumiferana* et de *C. occidentalis* fut semblable. En comparant deux doses de polyédrose nucléaire de *C. fumiferana*, 750 milliards de virus polyèdres/ha donnèrent des résultats de beaucoup supérieurs à 250 milliards. La première dose réduisit la population de 92%, soit le meilleur résultat obtenu à ce jour d'un traitement avec un virus de la polyédrose nucléaire contre la Tordeuse des bourgeons de l'Épinette.

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Introduction

Aerial spray trials have been conducted every year since 1971 with the nuclear polyhedrosis virus (NPV) of the spruce budworm, *Choristoneura fumiferana* (Clem.). Nuclear polyhedrosis viruses and granulosis viruses are classified in the genus "baculovirus" (Wildy, 1971). Since 1971, 24 plots with a combined area of 1,508 ha have been sprayed. Dosages ranging from 25 to 750 billion polyhedral inclusion bodies (PIBs)/ha were tested. In the first two years of trials volumes of 18.8 and 28.2 l/ha were applied, but since then 9.4 l/ha has been used routinely. In 1971 a helicopter fitted with boom and nozzle equipment was used; in 1972 a fixed wing aircraft fitted with Micronair spray units was employed and this was continued until 1975 when boom and nozzle and Micronair equipment were compared. As marginally better results were obtained with the boom and nozzle equipment, it has been used to date.

A total of 7 different aqueous formulations have been tested and the best results were obtained with one which contained 250 ml/l molasses, 60 gm/l IMC 90-001 (a UV protectant) and 1.25 ml/l Chevron® sticker. Timing of the application has been tested at two stages of spruce budworm development, firstly on highly susceptible needle-mining second instar larvae and secondly on fourth and fifth instar larvae which are exposed to the spray deposit when buds flush on white spruce and balsam fir. Results of early applications on second instar larvae have been generally disappointing, although better virus infection has been found in larvae feeding on white spruce than on balsam fir hosts. Previous spray trials have been reported in detail (Howse

et al., 1973; Cunningham and McPhee, 1973; Cunningham et al., 1974; Cunningham et al., 1975a; Cunningham et al., 1975b; Kaupp et al., 1978).

The best results obtained to date were those following the application of 750 billion PIB/ha in 1971 (Howse et al., 1973) when the NPV (with a cytoplasmic polyhedrosis virus (CPV) contaminant) gave 34% virus-infected larvae following an application on second instar and 71% on fourth instar. The above infection rates resulted in 69% and 80% population reduction due to treatment in these plots respectively. The virus persisted well from year to year in these plots and significant levels of NPV were recorded in the spruce budworm populations until 1975 when it declined drastically. There was no foliage protection observed in the year of application but, in subsequent years, the saving of foliage, although not dramatic, was sufficient to prevent tree mortality (Cunningham et al., 1975c). The mode of virus transmission from one year to the next may be due to virus-killed larvae and pupae which remain trapped over winter in webbing on the foliage.

The dosage of 750 billion PIBs/ha applied in 1971 was considered to be economically unacceptable and, in subsequent years, dosages ranging from 25 to 250 billion PIB/ha were tested in a variety of formulations. Ultraviolet in sunlight rapidly inactivates NPV on foliage and attempts have been made to find a suitable UV screening compound. Such a compound should keep the virus in a viable state on the foliage over a longer period of time thereby making lower dosages possible. Since the trials in 1971, mediocre results have been obtained. The molasses formulation described above is the best to date,

but there is still room for a great deal of improvement. Carryover of virus from one year to the next in plots sprayed in 1972, 1973, 1974 and 1975 has been poor (unpublished data).

The testing of budworm NPV has been on an ad hoc basis to date and it was decided to continue this policy until a promising treatment was found. Such parameters as dosage per hectare, volume of spray per hectare, formulation, timing of application and spray distribution equipment have been studied. It was decided to test the following treatments in 1977:

1. An NPV isolate was obtained from western spruce budworm, *Choristoneura occidentalis* Freeman, in 1976. This virus was propagated in eastern spruce budworm larvae in the laboratory and when compared to the extensively studied *C. fumiferana* isolate, it was found to have similar virulence. However, it was added to the list of candidates for testing to determine if its behavior is different from the original NPV under field conditions.
2. A reference was found to a new, polymer-bound carbon formulation which gave excellent results when used with *Heliothis* NPV on cotton (Bull et al., 1976). The carbon is used as an ultraviolet screen and the polymer dissolves in the alkaline insect gut juice. This formulation was prepared using spruce budworm NPV and was field tested.
3. Dr. T.R. Sheih of Sandoz Inc. developed an adjuvant for *Heliothis* virus formulations sprayed on cotton. It is called SAN 285 WP 76. He kindly supplied us with a sample and it was tested with spruce budworm NPV.

4. In order to ascertain any benefit from the new virus strain and new formulations a plot was sprayed with the formulation developed over the last few years (i.e. 250 ml/l molasses, 60 g/l IMC 90-001 and 1.25 ml/l Chevron® sticker).

5. A dosage similar to that used in the 1971 trials, 750 billion PIB/ha, was repeated in order to determine if the results could be reproduced, or even improved using the molasses formulation listed in treatment 4, and to determine if the same carryover from one year to the next could be obtained as that following the 1971 application.

With the exception of treatment 5, the same dosage of virus, 250 billion PIBs/ha, was used on all the plots so that comparisons could be made. As in previous years Forest Pest Management Institute staff selected the plots, conducted the spray applications and determined the levels of virus infection and Dr. G.M. Howse of the Great Lakes Forest Research Centre calculated the spruce budworm population reduction due to the treatments, recorded the pupal survival and estimated the foliage protection obtained.

Materials and Methods

Virus production

In the winter of 1976-77, a total of 1,567,000 budworm larvae were reared on synthetic diet and infected with NPV. Of these 957,000 were infected with the *C. fumiferana* strain of NPV and they yielded 10.7 kg of freeze-dried virus infected material containing 10 billion PIBs/g.

The remaining 610,000 larvae were infected with the *C. occidentalis* strain of NPV and they yielded 7.2 kg containing 8 billion PIB/g.

Experimental plots

Plot no. 1 was located in Kirkwood Twp., north of Thessalon, Ont. and was the same 40 ha block which was sprayed with NPV in the 1976 operation (Kaupp et al., 1978). In this plot about 80% of the trees were white spruce. Approximately 8 ha of this plot was a white spruce plantation with trees about 14 m high and in the remainder of the plot there were small amounts of balsam fir, poplar, white pine and white birch. Two check areas were selected in the Kirkwood area which were designated check no. 1 and check no. 5 and were the same locations as used in the 1976 operation.

The remaining 4 treated plots were located southeast of Hearst, Ontario. No. 2 plot was in Kendall Twp. and had an area of 20 ha and nos. 3, 4 and 5 were in Shetland Twp. with areas of 8 ha, 40 ha and 40 ha respectively. In plot no. 2, white spruce (14 to 17 m high) was the dominant species with some balsam fir and poplar. In the other 3 plots about 60% of the stand was composed of balsam fir (8 to 10 m high), about 30% was black spruce and the remaining 10% was white spruce, poplar and white birch. There was no overstory problem with hardwoods in any of the plots. A total of 6 check areas were selected in the same general locality as the treated plots.

Virus formulation and dosage

The plot areas, location, virus strain, dosage applied and formula-

tion are given in Table 1. In plot 1, the standard molasses, IMC 90-001 UV protectant and Chevron^{R} sticker formulation was used and the virus applied at 750 billion PIB/ha. This was the dosage of virus used in the 1971 trials which has produced the best results to date. The *C. fumiferana* strain of NPV was used. Plot no. 5 was treated with the same formulation and virus isolate but the dosage was 250 billion PIB/ha. Plot no. 4 received the same dosage and formulation as plot no. 5 but the *C. occidentalis* strain of NPV was used.

Plot no. 2 was treated with a *C. fumiferana* NPV polymer-bound carbon formulation at 250 billion PIB/ha. This formulation was prepared by the Southwest Research Institute, San Antonio, Texas using the methods described by Bull et al., 1976. In order to prepare the polymer-bound carbon formulation, NPV was purified by suspending mascerated, virus-infected larvae in 0.5% SDS (sodium dodecyl sulphate) and stirring the mixture occasionally over a period of 10 days. The mixture was strained through a double layer of cheesecloth and the polyhedra precipitated by centrifugation at 1,000 rpm for 10 min. A total of 1 l of virus suspension containing 6.5×10^{12} PIBs was shipped to the Southwest Research Institute.

In the preparation of the polymer-bound particles, individual batches of 600 g of 10% SMA 2625 (styrene-maleic anhydride copolymer half ester) in acetone, 128 g of Sterling R carbon black and 21.1 ml of NPV suspension (6.5×10^{9} PIB/ml) were blended and spray dried. This mixture of ingredients yielded 188.4 g plus the weight of virus in the dried particles and contained approximately 7.3×10^{8} PIB/gm. The total yield of formulated virus was 8.9 kg (L.M. Adams, personal communication).

Table 1

Plots sprayed in 1977 and the treatments applied

Plot No.	Location	Area (ha)	Virus isolate	Virus dosage PIB/ha	Formulation
1	Kirkwood Twp. (near Thessalon)	40	C. fumiferana	750 billion	Molasses 250 ml/l IMC 90-001 60 g/l Chevron sticker 1.25 ml/l
2	Kendall Twp. (near Hearst)	20	C. fumiferana	250 billion	Polymer bound carbon formulation Triton X-100 1 ml/l
3	Shetland Twp. (near Hearst)	8	C. fumiferana	250 billion	San 285 WP adjuvant (76 manufacture) 1 kg/l Rhodamine B dye
4	Shetland Twp. (near Hearst)	40	C. occidentalis	250 billion	Molasses 250 ml/l IMC 90-001 60 g/l Chevron sticker 1.25 ml/l
5	Shetland Twp. (near Hearst)	40	C. fumiferana	250 billion	Molasses 250 ml/l IMC 90-001 60 g/l Chevron sticker 1.25 ml/l

The polymer-bound carbon formulation proved difficult to wet and put into suspension. This problem was solved by the addition of 1 ml/l Triton X-100® wetting agent.

Plot no. 3 was treated with the *C. fumiferana* isolate at 250 billion PIB/ha formulated with an adjuvant supplied by Sandoz Inc. This adjuvant, SAN 285 wettable powder (1976 manufacture), was added at a rate of 1 kg/l. As the colour of this formulation was pale, rhodamine B dye was added so that the deposit could be monitored. Aqueous formulations were applied on all 5 plots at the rate of 9.4 l/ha.

Spray application and larval development

A Grumman Agcat biplane was contracted from General Airspray Ltd. for the applications. It was fitted with boom and nozzle equipment and had 22 nozzles with 45° swirl plates and no. 8 orifices. Flying a 30 m swath the delivery rate was 9.4 l/ha. Spraying commenced at 6 a.m. on May 27th on the plot at Kirkwood with 80% of the larvae in the fifth instar and 20% in the sixth. No temperature and humidity recordings were made. There was a slight breeze during the application but the deposit was not affected.

On June 2nd at 5.45 a.m. spraying commenced on plot no. 4 near Hearst. The spray drifted about 30 m to the south. The mean temperature during the application was 1.4°C and the humidity was 100%. Plot no. 5 was sprayed with the next load between 6.45 a.m. and 7.05 a.m. Again there was slight drift to the south, the mean temperature had risen to 2.4°C and the relative humidity remained at 100%. The remaining

2 plots were sprayed on the evening of June 2nd. Spraying of plot no. 3 commenced at 8.30 p.m. and finished at 9.00 p.m. There was very slight drift, the mean temperature was 13.2°C and the relative humidity was 81%. Then plot no. 2 was sprayed between 9.25 and 9.35 p.m. There was no drift, mean temperature was 10.6°C and relative humidity 91%.

Larval samples from the Hearst area were collected and analysed for development on May 29th and on June 6th so there are no figures for the day of the application. On May 29th there were 1.8% second instar, 9.7% third, 54.9% fourth, 31.0% fifth and 2.6% sixth. On June 6th there were 22.6% fourth instar, 51.6% fifth and 25.8% sixth. One can deduce that on June 2nd there were probably about equal numbers of fourth and fifth instar with some sixth instar present.

Monitoring the deposit

Plots were laid out either with a road running through the centre or along one end. Prior to the application, Kromekote® spray cards on aluminum backings were placed at 15 m intervals at right angles to the flight lines. In order to analyse the droplet spectrum, the number of droplets on 5 cm^2 of each card were counted and the diameter of each droplet on 1 cm^2 was measured using a calibrated eyepiece in a dissecting microscope.

Meteorological data

The temperature and rainfall were monitored for the duration of the experiment in the Hearst area but no measurements were made at Kirkwood.

Assessment

(a) Laboratory rearing of larvae to determine (i) the levels of natural virus and (ii) the impact of the spray application.

Random samples of larvae were collected from 46 cm branch tips taken at mid-crown from Hearst and Kirkwood before and 5 days after the spray application. These larvae were reared individually in plastic cream cups on synthetic diet (McMorran, 1965) until pupation or death occurred. Dead larvae were examined microscopically to determine the cause.

(b) Microscopic examination of samples of larvae to determine levels of infection with viruses and other pathogens.

Random samples of larvae were obtained from 46 cm branch tips collected from the mid-crown of trees at Kirkwood and at Hearst. Following the spray application, the treated plots and check areas were sampled at approximately one week intervals until pupation occurred. Squash preparations were made of larval and pupal gut and fat-body tissue. These were examined microscopically under phase contrast for the presence of nuclear polyhedrosis virus and other pathogens such as cytoplasmic polyhedrosis virus and microsporidia.

(c) Sampling for population reduction studies.

In plot no. 1 at Kirkwood 50 white spruce 46 cm branch tips were randomly selected from the mid-crown of trees for prespray counts (taken on the day of application) and postspray counts (taken when 80% of the insects were in the pupal stage). At the same time 25 white spruce 46 cm branch tips were collected from the 2 check areas.

At the Hearst site, ten subplots were selected in each of the 4

treated plots with 5 balsam fir in each giving a total of 50 balsam fir per plot. In each of these 4 plots 20 white spruce trees were selected throughout the plot. Branch tips (46 cm) were collected from the mid-crown of the sample trees 3 days after the spray application as a pre-spray sample (no virus mortality occurs before 7 days postspray) and again when most of the larvae had pupated as a postspray sample. Six check plots were located in the same general area and 46 cm mid-crown branch tips were collected from them. There was some difficulty in obtaining a sufficient number of white spruce sample trees and the check areas, sampled at the same time as the sprayed plots, contained the following numbers of trees: no. 1 had 50 balsam fir, no. 2 had 50 balsam fir and 15 white spruce, no. 3 had 20 white spruce, no. 4 had 50 balsam fir, no. 5 had 50 balsam fir and no. 6 had 15 white spruce. This gave a total of 200 branch tips (46 cm) from balsam fir and 50 from white spruce.

Larvae were removed from the foliage using the "drum method" (DeBoo et al., 1973; Martineau and Benoit, 1973) and then counted. Pupal samples were hand picked. Abbott's formula (Abbott, 1925) was used to calculate the population reduction of spruce budworm attributable to the virus treatments.

(d) Pupal emergence

Pupae from the postspray sample were maintained at room temperature and adult emergence recorded. In addition to this sample an extra collection of 300 pupae was made from plot no. 1 at Kirkwood and 300 from the 2 Kirkwood check areas. They were also reared to determine the successful emergence rate.

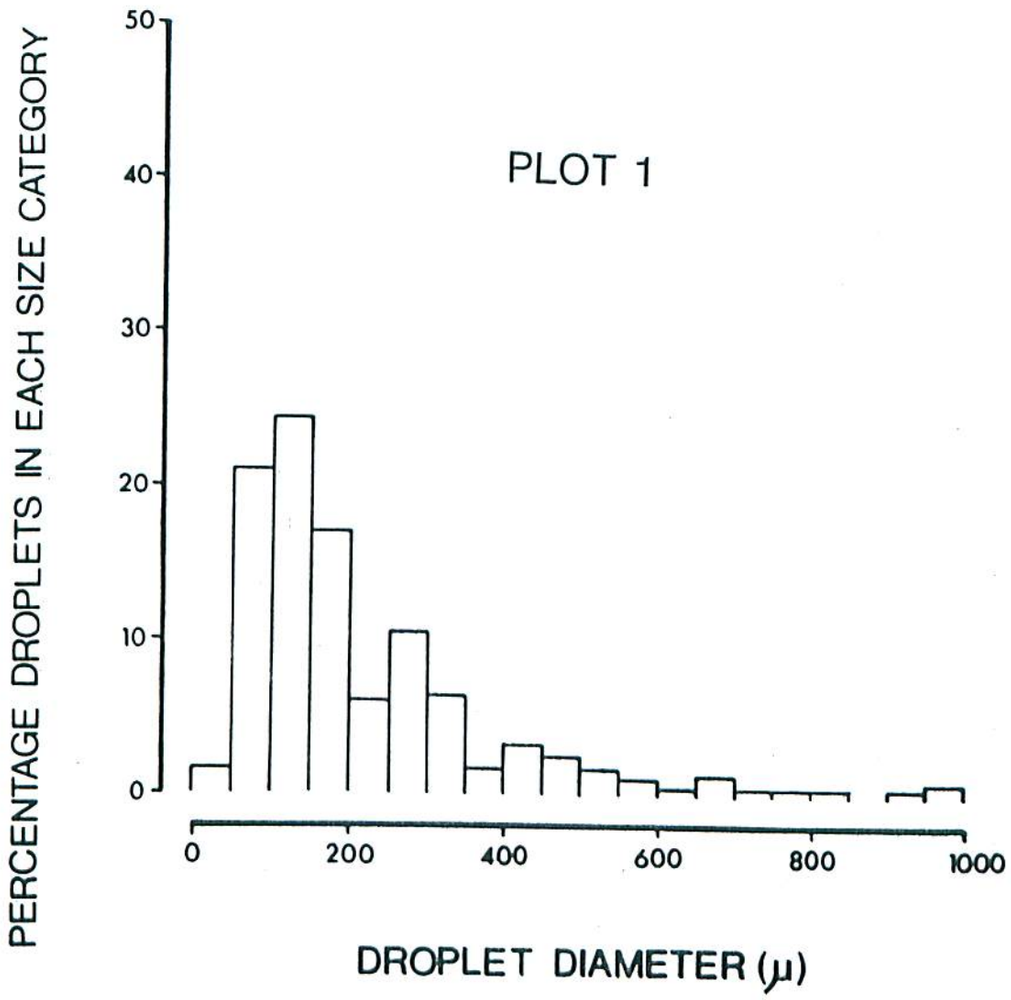

Fig. 1. Analysis of spray droplets on Kromekote® cards in plot no. 1.

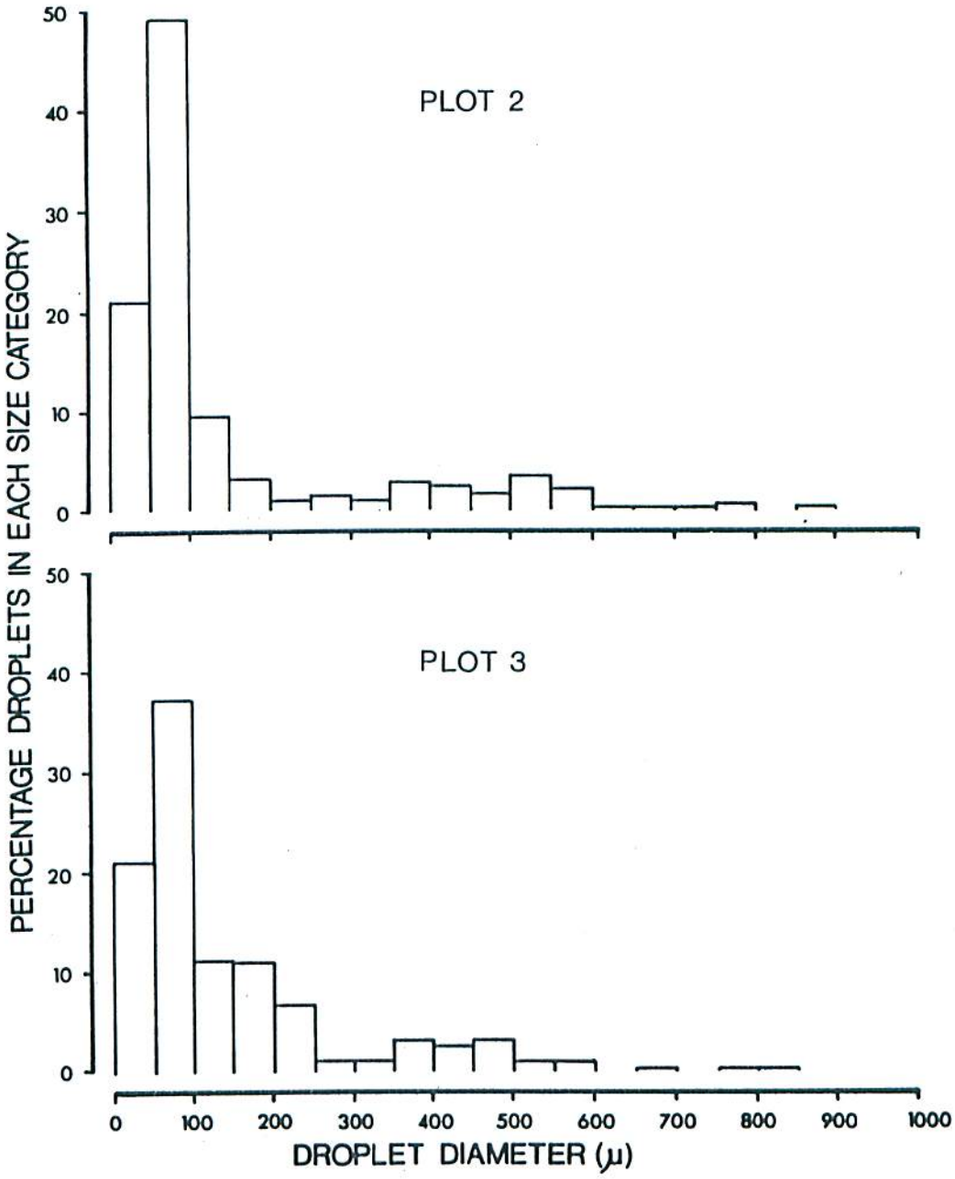

Fig. 2. Analysis of spray droplets on Kromekote® cards in plots no. 2 and no. 3.

Percent successful pupal emergence was calculated as follows:

$$\text{Percent successful pupal emergence} = \frac{\text{emerged budworm X 100}}{\text{budworm alive on sample date}}$$

(e) Estimates of current year's defoliation.

The percent current defoliation was obtained by detailed examination of the 46 cm branch tips collected for the post-spray sample from the treated plots and check areas. By this time all surviving larvae had pupated and feeding ceased.

Results

Deposit Analysis

The mean number of droplets per cm^2 (with standard deviation), as recorded on the Kromekote® cards, was 22 ± 15, 14 ± 4, 39 ± 13, 40 ± 15 and 50 ± 17 from plots no. 1 to no. 5 respectively. The percentage of droplets in different size categories is given in Figs. 1 to 3. As can be seen from these histograms, more than 50% of the droplets were less than 100 μ except in plot no. 1 where the spectrum was slightly larger. The deposit assessment in plot no. 1 may be rather misleading as the spray cards were located on a narrow trail across the plot and the smaller droplets were probably filtered out by heavy foliage. When observed from the ground, the spray cloud was seen drifting into the treetops and the figure of 22 droplets/cm^2 for this plot is probably low.

Meteorology

At Hearst the mean maximum daytime temperature was 28.4°C and the

mean minimum night temperature was 1.7°C for the duration of the experiment. Between May 30th and June 30th, the temperature fell below 0°C on 16 of the 32 nights. The mean precipitation recorded at 5 stations was 7.59 cm for the duration of the experiment.

Mortality in Laboratory Reared Larvae

The mortality in prespray samples of larvae reared individually is shown in table 2. Results from plot no. 1 at Kirkwood (treated with NPV in 1976), check areas no. 1 and no. 5 at Kirkwood and a general sample from the Hearst area are given. Only 4.3% mortality from NPV was found in Kirkwood plot no. 1 prior to the spray operation and only 1 NPV killed larva (0.8%) was found on the balsam fir sample from Hearst.

The mortality in samples collected 5 days after the spray application is shown in table 3. Plot no. 1 gave encouraging results with 60.3% NPV in larvae collected from white spruce and plots no. 4 and no. 5 at Hearst also gave satisfactory levels of virus infection. In plot no. 4 there was 60.9% NPV mortality in larvae from balsam fir hosts and 31.6% from white spruce hosts and in no. 5, 36.5% NPV from balsam fir and 39.5% from white spruce. Larval mortality due to NPV was low in plots no. 2 and 3 at Hearst with 7.4% and 4.4% NPV mortality in larvae from balsam fir and white spruce hosts in plot no. 2 and 10.5% and 13.3% NPV mortality in plot no. 3. Several of the samples from the Hearst area contained less than 50 larvae and were considered too small to be significant.

The level of virus mortality of larvae from the samples taken 5

days after the spray application can be directly attributed to ingestion of the spray deposit. In this period of time there is no secondary infection caused by death of NPV-infected larvae and spread of polyhedra from the cadavers on to the host tree.

Levels of virus infection diagnosed microscopically

Plot no. 1 at Kirkwood was sampled 3 times and the larvae examined microscopically to determine the level of NPV infection and to detect other pathogens present. White spruce hosts were sampled on all 3 occasions and small samples of larvae were also taken from balsam fir hosts on the last two occasions. White spruce were sampled in Kirkwood check no. 1 twice and once in check no. 5 and the results are given in table 4.

The level of NPV infection increased in larvae on white spruce hosts from 43% on June 6th to 60.9% on June 16th. On balsam fir, 42.9% larvae were found to be infected with NPV on June 10th and 32.7% on June 16th. Only 1 NPV infected larva was found in the check areas. Levels of microsporidia occurring naturally in the spruce budworm population were high and ranged from 18.9% to 56.1%.

At Hearst, 2 samples of spruce budworm were taken from each of the 4 treated plots and results are shown in table 5. All plots received the same dosage of virus - 250 billion PIB/ha. In plot 2, treated with the polymer-bound carbon formulation extremely low levels of NPV infection were recorded with the maximum being 2.0%. In plot 3, treated with the Sandoz SAN 285 wettable powder formulation, 11.5% infection

was recorded in larvae on white spruce and 9.1% on balsam fir. The insect population was low in plot 4 and none of the samples were considered to be large enough to give significant results. However, figures of 25% NPV (1 out of 4 larvae) and 22.7% were recorded in larvae from balsam fir and white spruce hosts respectively. This plot was treated with the *C. occidentalis* virus strain in the molasses formulation. Plot no. 5 was treated with the *C. fumiferana* virus strain in the molasses formulation. Maximum levels of NPV infection were recorded as 16.1% in larvae from balsam fir hosts and 29.6% in larvae from white spruce hosts. One larva infected with CPV was found in samples from plot no. 5.

Samples taken from the Hearst check areas and examined microscopically are recorded in table 5. Of the 11 samples, 4 were considered too small to be significant. Only 1 NPV-infected larva was found and it came from check area 5.

Levels of microsporidia were much lower at Hearst than at Kirkwood. Levels at Hearst ranged from zero to 14.3% in individual samples. Levels of microsporidia build up as the spruce budworm population in an area ages. The spruce budworm infestation in the Hearst area is much more recent than in Kirkwood.

Population reduction, pupal survival and current defoliation studies.

The population reduction due to treatment, percent successful pupal emergence and percent 1977 defoliation for plot no. 1 at Kirkwood are given in table 7. A figure of 92% population reduction was achieved

but there was 97% defoliation compared to 63% defoliation in the check area. This high defoliation figure was attributed to the heavy population of defoliating species of insects which were not spruce budworm and not susceptible to spruce budworm NPV. There were 4 times more of these insects on the treated plot than the check.

At Kirkwood, 46% pupal emergence was recorded from plot no. 1 and 62% from the check using the insects taken for the postspray sample. In a large pupal collection (300) made independently from this sample, pupal emergence was found to be 41% from plot no. 1 and 55% from the check areas. Hence, an overall difference of about 16% pupal mortality attributable to NPV was calculated from the first collection and 14% from the second.

The results of the same studies in the Hearst area are given in table 8. It can be seen that the insect population densities were very low in plots no. 3 and no. 4 which makes it difficult to deduce meaningful results. There was some frost damage in this locality which is also recorded in table 8. The frost probably killed spruce budworm larvae thus contributing to the lower than normal survival of insects in parts of this area. The frost damage to the trees and 1977 defoliation recorded in table 8 are separate estimates. The percent damage due to frost was estimated first and then the 1977 spruce budworm defoliation was estimated on the remaining current foliage not damaged by frost.

The NPV polymer-bound carbon formulation gave 45% population reduction on balsam fir hosts and 11% on white spruce hosts. There was

Table 2

Mortality in prespray samples of larvae reared in the laboratory.

Plot	Tree Species	No. of larvae reared	Percent Pupation	Percent Mortality		
				NPV	Micros.*	Other
Kirkwood Check No. 1	wS	223	97.3	0	0	2.7
Kirkwood Check No. 5	wS	255	97.2	0	1.2	1.6
Kirkwood Plot No. 1	wS	232	92.7	4.3	0.4	2.6
Hearst Area	bF	119	96.6	0.8	0	2.5
Hearst Area	wS	91	93.4	0	1.1	5.5

*Micros = level of natural microsporidia in the spruce budworm population.

Table 3

Mortality in samples of larvae taken 5 days post-spray and reared in the laboratory.

Plot	Tree Species	No. of Larvae	Percent Pupation	Percent Mortality		
				NPV	Micros.	Other
1	wS	224	27.2	60.3	0	12.5
2	bF	95	90.5	7.4	1.1	1.1
	wS	113	92.0	4.4	0.9	2.7
3	bF	19	79.0	10.5	10.5	0
	wS	30	73.3	13.3	0	3.3
4	bF	23	39.1	60.9	0	0
	wS	19	68.4	31.6	0	0
5	bF	63	58.7	36.5	0	4.8
	wS	76	57.9	39.5	1.3	1.3

* Micros = level of natural microsporidia in the spruce budworm population.

Table 4

Incidence of NPV, microsporidia and parasites as determined by microscopic diagnosis, in larvae from a plot sprayed with NPV at the rate of 750 billion PIB/ha and in check areas at Kirkwood, Ontario in 1977.

Plot	Sample Date	Tree Species	No. of Samples	No. of Positive Samples	No. of Insects Examined	Percent Diseased Insects		
						NPV	Micros.**	Parasites
1	6.6.77	wS	20	19	237	43.0	43.5	1.3
	10.6.77	bF	5	5	98	42.9	27.6	3.1
	10.6.77	wS	20	19	410	54.4	35.1	0.7
	16.6.77	bF	5	5	55	32.7	20.0	1.8
	16.6.77	wS	20	20	233	60.9	18.9	1.3
Check 1	10.6.77	wS	5	1	82	1.2	56.1	1.2
	16.6.77	wS	6	0	32*	0	50.0	0
Check 5	16.6.77	wS bF	5	0	49	0	42.9	0

* Sample too small to be significant

** Micros = level of natural microsporidia in the spruce budworm population.

Table 5

Incidence of NPV, microsporidia and parasites as determined by microscopic examination of larvae collected from plots sprayed with NPV near Hearst, Ontario in 1977.

Plot	Sample Date	Tree Species	No. of Samples	No. of Positive Samples	No. of Insects Examined	Percent Diseased Insects			
						NPV	CPV	Micros.**	Parasites
2	19.6.77	bF	10	1	154	0.7	0	9.7	0.7
	19.6.77	wS	10	2	100	2.0	0	11.0	2.0
	30.6.77	bF	20	1	111	0.9	0	11.7	0
	30.6.77	wS	20	1	125	0.8	0	7.2	0
3	19.6.77	bF	10	1	58	3.5	0	5.2	1.7
	19.6.77	wS	10	2	26*	11.5	0	11.5	7.7
	30.6.77	bF	20	4	66	9.1	0	7.6	0
	30.6.77	wS	20	0	24*	0	0	4.2	4.2
4	19.6.77	bF	10	3	22*	22.7	0	0	4.6
	19.6.77	wS	10	4	21*	19.1	0	14.3	0
	30.6.77	bF	20	1	4*	25.0	0	0	0
	30.6.77	wS	19	0	6*	0	0	0	0
5	19.6.77	bF	10	7	112	16.1	0	9.8	0
	19.6.77	wS	10	7	71	29.6	1.4	2.8	0
	30.6.77	bF	20	5	86	8.1	0	3.5	1.2
	30.6.77	wS	20	4	58	8.6	0	3.5	1.7

*Sample too small to be significant.
**Micros - level of natural microsporidia in the spruce budworm population.

Table 6

Incidence of pathogens and parasites, as determined by microscopic diagnosis, in larvae collected from check areas near Hearst, Ontario in 1977.

Plot	Sample Date	Tree Species	No. of Samples	No. of Positive Samples	No. of Insects Examined	Percent Diseased Insects NPV	Micros.**	Parasites
Check 1	19.6.77	bF	5	0	17*	0	0	0
Check 2	19.6.77	bF	5	0	88	0	4.6	0
	19.6.77	wS	5	0	47	0	6.4	2.1
	30.6.77	bF	20	0	254	0	8.3	1.6
	30.6.77	wS	14	0	83	0	9.6	0
Check 3	19.6.77	wS	5	0	11*	0	0	0
	30.6.77	wS	20	0	10*	0	0	0
Check 4	19.6.77	bF	5	0	67	0	3.0	0
	30.6.77	bF	20	0	132	0	3.0	0
Check 5	19.6.77	bF	5	1	22*	4.6	0	0
Check 6	19.6.77	wS	5	0	90	0	1.1	0

*Sample too small to be significant.
**Micros = level of natural microsporidia present in the spruce budworm population.

Table 7

Population reduction, pupal survival and current defoliation on white spruce sprayed with NPV at the rate of 750 PIB/ha at Kirkwood, Ontario in 1977.

Plot	Prespray larvae per 46 cm tip	Surviving pupae per 46 cm tip	% Population reduction due to treatment	% Successful pupal emergence	% 1977 Defoliation
1	36.0	.40	92	46	97
Check	33.1	4.88		62	63

Table 8

Population reduction, pupal survival and current defoliation in four plots sprayed with NPV near Hearst, Ontario in 1977. Dosage of virus was 250 billion PIB/ha and virus strains and formulations are listed in Table 1.

Plot	Prespray larvae per 46 cm tip		Surviving pupae per 46 cm tip		% Population reduction due to treatment		% Successful pupal emergence		% Frost damage		% 1977 Defoliation	
	bF	wS	bF	wS	bF	wS	bF	wS	bF	wS	bF	wS
2	14.8	13.4	2.02	2.90	45	11	56	59	2	4	84	63
Check	18.6	17.7	4.60	4.28			65	65	0	0	87	56
3	5.9	5.4	.92	.30	0	0	69	48	2	0	27	12
Check	11.1	3.9	1.44	.10			50	50	11	1	39	2
4	3.5	4.9	.23	.21	58	22	70	38	7	0	3	4
Check	5.9	5.4	.92	.30			69	48	2	0	27	12
5	19.6	19.6	1.94	.92	60	81	51	46	6	1	78	76
Check	18.6	17.7	4.60	4.28			65	65	0	0	87	56

a small effect on pupal emergence on both tree species but no significant saving of foliage was found. No larval population reduction could be demonstrated in plot no. 3 on either white spruce or balsam fir hosts. This plot was treated with NPV in the Sandoz SAN 285 wettable powder formulation.

In plot no. 4, the *C. occidentalis* virus strain in the molasses formulation gave 58% population reduction on balsam fir hosts and 22% on white spruce. There was no pupal mortality attributable to NPV in insects on balsam fir hosts but on white spruce there was only 38% emergence as compared to 48% in the check. At the low population density in this plot a significant amount of foliage was saved. In plot no. 5, the *C. fumiferana* virus isolate in the molasses formulation gave 60% population reduction of larvae on balsam fir hosts and 81% on white spruce. There was a marked effect on pupal survival from this treatment with 51% emergence of insects on balsam fir hosts compared to 65% in the check and 46% emergence on white spruce compared to 65% in the check. Some foliage was saved on balsam fir with 78% defoliation in the plot compared to 87% in the check, but none was saved on white spruce.

Discussion

The deposit analysis showed results similar to those obtained with boom and nozzle equipment calibrated to deliver 9.4 l/ha in 1975 (Cunningham et al., 1975) and in 1976 (Kaupp et al., 1978) with numbers of droplets per cm^2 ranging from 14 to 50. The size of the droplets

was, on the whole, smaller in 1977 with more of them classed as less than 100 μ.

In previous years, samples of larvae from the treated and check plots were not reared individually on diet. This appears to be a useful method of getting a fast evaluation of the efficacy of the application. There was also a good correlation between the figures obtained by this method and the population reduction estimates. Low mortality from virus was obtained in plots no. 2 and no. 3 and this fact is clearly illustrated by both methods of analysis. Where high levels of population reduction were achieved, the rearing method did not show the full impact. This is due to the fact that samples were taken 5 days after spray application and mortality relates directly to the dosage of NPV ingested following the spray. It does not take into account any spread and secondary infection.

It is interesting to compare the levels of microsporidia detected in samples of reared larvae and levels found in samples of larvae which were dissected and examined microscopically (tables 2, 3, 4, 5 and 6). It can be seen that the levels are much lower in the reared samples which is a further demonstration of the well documented fact that the principal microsporidian parasite present in spruce budworm populations in Ontario, *Nosema fumiferanae*, is a chronic, debilitating pathogen which is seldom lethal to its host (Thomson, 1958).

The level of spruce budworm control achieved in Kirkwood plot no. 1 is most encouraging and it yielded the best results obtained to date. At Deluthier Rd. in 1971, using 750 billion PIB/ha, 69% population

reduction was achieved in one white spruce plantation sprayed with NPV when larvae were in the second instar and 80% in another when larvae were in the fourth instar. At Kirkwood, larvae were mainly in the fifth instar and 92% population reduction was achieved with this dosage. The maximum level of virus infection recorded at Kirkwood was 61%. The situation at Deluthier Rd. was complicated by the fact that there was a mixture of two viruses, NPV and CPV. When both viruses were considered, maximum levels of 34% and 71% were reached but, when the NPV alone was analyzed, these figures were reduced to 21% and 46% (Howse et al., 1973).

The heavy defoliation in Kirkwood plot no. 1, 97% as opposed to 63% in the check, was disappointing but can be attributed to an unforeseen factor - the population of defoliating insects which were not spruce budworm was four times higher in the sprayed plot than in the check. This illustrates one of the drawbacks of viruses for insect control. NPVs are very specific in their host range and only a few closely related Tortricids are susceptible to spruce budworm NPV. Had *Bacillus thuringiensis* instead of NPV been applied, most leaf eating Lepidoptera would have been controlled.

In the Hearst area, the results were marred by the varying population densities of spruce budworm larvae in the different plots, but there was a clear indication that both of the new formulations, the polymer-bound carbon formulation and the Sandoz SAN 285 wettable powder formulation were inferior to the molasses and IMC 90-001 formulation which has been used routinely for the last 3 years.

The polymer-bound carbon formulation was supplied a few days prior to the spray application and there was no time to test it in the laboratory. Following the disappointing results in the field, laboratory tests were performed to check the activity of SDS purified virus and polymer-bound carbon formulated virus against fresh, untreated virus. On the basis of the polyhedral count given for the carbon formulated material (this could not be checked), there was considerable loss of activity. The SDS purification had no effect, so inactivation must have occurred during the formulation process (Appendix 1). Laboratory tests showed that SAN 285 WP was compatible with spruce budworm NPV. No simple explanation can be given for its poor performance in the field, although it must be noted that this formulation was intended as a feeding attractant for *Heliothis zea* larvae on cotton and not spruce budworm on white spruce and balsam fir.

The finding that no population reduction attributable to NPV could be demonstrated in plot no. 3 treated with the SAN 285 wettable powder formulation contradicts the data collected by laboratory rearing and microscopic diagnosis. This anomaly is probably due to large sampling error associated with the low spruce budworm population in this plot. A further factor contributing to this error is the abnormally low larval survival in the check plot which was probably due to frost. Laboratory reared larvae from white spruce from plot no. 3 showed 13.3% mortality from NPV and larvae from balsam fir showed 9.1% mortality from NPV. Hence, it is concluded that there must have been some population reduction due to the NPV treatment on both tree species in this plot.

When all factors, including the different population densities in the two plots are considered, there appears to be little difference in the efficacy of the *C. occidentalis* virus strain compared to the *C. fumiferana* virus strain. They may well be one and the same virus but confirmation of this point must await detailed biochemical analysis.

The results from plot no 5 are similar to those obtained in 1974 and 1975 when 250 billion PIB/ha and 125 billion PIB/ha were applied on Manitoulin Island using the same formulation and equipment (Cunningham et al, 1975a; Cunningham et al, 1975b). In 1974 maximum levels of NPV infection in larvae on white spruce and balsam fir hosts were 11.6% and 19% and this resulted in 58% and 5% population reduction. In 1975, 125 billion PIB/ha gave maximum levels of NPV infection in larvae on white spruce and balsam fir hosts of 40.4% and 16.8% resulting in 91% and 53% population reduction. At Hearst, maximum levels of virus infection using 250 billion PIB/ha on white spruce were 16.1% on balsam fir and 29.6% on white spruce which resulted in 60% and 81% reduction of spruce budworm on these species.

The follow-up studies of the Manitoulin Island trials in 1974 and 1975 indicated that following these successful introductions of NPV there was very little carryover of virus to the following year and by 1977 only minute traces of NPV could be found in the spruce budworm population (unpublished data). Followup studies for the years after the 1971 trial at Deluthier Rd. showed that in 1975 the NPV was still present in the spruce budworm population (21.3% in one plot and 9% in the other) and acting as a control factor.

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APPENDIX 1

Laboratory experiment - to test the activity of SDS purified and polymer-bound carbon formulated spruce budworm NPV.

Suspensions of fresh untreated virus, SDS purified virus and polymer-bound carbon formulated NPV were standardised to a concentration of 10^8 PIB/ml from which dilutions of 10^7, 10^6 and 10^5 were prepared. Samples (0.2 ml) were pipetted on to the surface of synthetic diet in plastic cream cups and 7 fourth instar larvae were placed in each cup. Cups with no NPV applied provided a control. Dead larvae were examined microscopically to determine the cause of death.

The results are shown in table A1 and it can be seen that the SDS had no effect on the viability of the virus but the carbon binding process caused drastic inactivation. No deaths occurred in the control group.

Table A1

Concentration of NPV PIB/ml	Mortality from NPV		
	Fresh Virus	SDS treated virus	Polymer bound carbon formulation
10^8	35/42 (83%)	40/43 (93%)	6/44 (14%)
10^7	37/43 (86%)	37/42 (88%)	4/45 (9%)
10^6	35/39 (90%)	38/42 (90%)	0/44 (0%)
10^5	21/40 (52%)	30/45 (67%)	1/46 (2%)