AERIAL APPLICATION OF A NUCLEAR POLYHEDROSIS VIRUS AGAINST SPRUCE BUDWORM NEAR MASSEY AND AUBREY FALLS ONTARIO IN 1973 AND A SURVEY OF THE IMPACT OF THE VIRUS IN 1974

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ABSTRACT

North of Massey, Ontario, four plots containing white spruce and balsam fir with a total area of 385 acres were sprayed with nuclear polyhedrosis virus (NPV) when larvae were in the second and third instar. The application was made using a Grumman Agcat aeroplane fitted with Micronair spray equipment. The emission rate was 1 U.S. gal/acre and dosages of 50 billion, 25 billion and 10 billion polyhedra/acre were tested; a double application of 10 billion/acre was also tested.

Near Aubrey Falls, Ontario, five plots with a total area of 353 acres were sprayed with the same virus when larvae were mainly fifth instar. The following additives were tested:- IMC Shade® UV protectant, Biofilm® wetter/sticker and Chevron® sticker.

The efficacy of the spray was evaluated by population reduction studies, defoliation estimates and microscopic diagnosis of insects to determine the level of virus infection. Samples were again taken in the year following application (1974) to determine if the virus had any carryover effect.

Generally the virus had more impact on larvae feeding on white spruce than balsam fir and the level of infection was directly proportional to dosage and deposit. Chevron® sticker and Biofilm® appeared to enhance deposit of the virus preparation. There was some carryover of NPV from one year to the next and, although at a rather low level, it did provide moderate protection to white spruce in three plots.

RESUME

Au nord de Massey, Ontario, on arrosa quatre lopins contenant des Épinettes blanches et des Sapins baumiers (au total 385 acres) avec un virus de polyédrose nucléaire (NPV) lorsque les larves étaient à leur deuxième et troisième stades. A cette fin on utilisa un avion Grumman Agcat équippé d'arroseurs Micronair. Le taux d'arrosage: 1 gal U.S.A./acre contenant soit 50, 25 ou 10 milliards de polyèdres/acre; on fit aussi un double arrosage de 10 milliards/acre.

Près d'Aubrey Falls, Ontario, on arrosa cinq lopins (au total 353 acres) avec le même virus lorsque les larves avaient atteint leur cinquième stade. On essaya aussi les additifs suivants: le protecteur IMC Shade® UV, le mouilleur/collant Biofilm® et le collant Chevron®.

L'efficacité de l'arrosage fut évaluée par des études de réduction de population, des estimations des défoliations et par le diagnostic du niveau d'infection chez les insectes. On échantillonna à nouveau dans l'année qui suivit l'arrosage (1974) pour savoir si le virus avait produit quelque séquelle.

En général, le virus produisit plus d'effet sur les larves se nourrissant d'Épinettes blanches que de Sapins baumiers et le niveau d'infection se révéla directement proportionnel aux doses et aux virus déposés. Le Chevron et le Biofilm semblèrent favoriser la déposition de virus. Le NPV se propagea un peu d'une année à l'autre, et bien que peu abondant, il protégea modérément l'Épinette blanche dans trois parcelles.

INTRODUCTION

Field trials were undertaken in 1971 and 1972 using a nuclear polyhedrosis virus (NPV) and an entomopoxvirus (EPV) against spruce budworm. The 1971 trials were conducted near Petawawa, Ontario and it was found that the NPV persisted well in the spruce budworm population when samples were taken in 1972 (Bird et al, 1972; Howse et al, 1973). Unfortunately, the NPV preparation was contaminated with cytoplasmic polyhedrosis virus (CPV) and the EPV preparation with both NPV and CPV making it impossible to establish the impact of these viruses applied singly. EPV and NPV, free from other contaminating viruses, were produced in the winter of 1972 and trials were conducted at Chapleau, Ontario in 1972 (Cunningham and McPhee, 1973). Two square miles were sprayed with EPV and one square mile with NPV. Six days after the application snow fell on the plots, decimated the population of spruce budworm larvae and killed the current year's foliage on balsam fir. A few results were obtained but it was considered necessary to repeat the experiment.

Stairs and Bird (1962) conducted small scale ground spray trials with NPV and granulosis virus (GV) and they concluded that the practical use of viruses depended on

1) the development of efficient methods of virus production,
2) a knowledge of the most efficient concentration of virus to apply,
3) determination of the most vulnerable stage of the life cycle of the insect, and
4) whether once established the virus will persist from year to year.

The problem of virus production was solved before the 1971 trials were conducted and methods are reported by Cunningham et al, (1972). The criterion, "A knowledge of the most efficient concentration of virus to apply" should be reworded, "A knowledge of the minimum dosage and deposit of virus required to establish the virus in the population". Very heavy dosages of NPV used in 1971 gave excellent results. Applications were made on both second instar larvae and fourth instar larvae in 1971 and the best results were obtained with the late application of NPV. It has been confirmed also that NPV persists very well from one year to the next (Howse et al, 1973). The virus can be detected only in late instar larvae and it is postulated that transmission is from virus-infected cadavers remaining on the foliage from one season to the next.

In 1972 a formulation of 10% (volume) molasses, 0.2% Biofilm® and 2.5% (weight) IMC sunlight protectant appeared to have an adverse effect on EPV in the field

although none of the ingredients inhibited the virus in the laboratory. No tests of unformulated NPV were conducted in the field. This same formulation was used with both EPV and NPV at Rankin, Ontario with excellent results (Morris et al, 1972; 1974).

The good carryover of NPV from one year to the next in the plots near Pembroke prompted intensification of the study of this virus and it was decided to perform field trials with NPV alone in 1973. It was hoped that data could be obtained on 1) the minimum dosage and deposit which would initiate an epizootic, 2) the optimal time to apply the spray: on second instar when they are most susceptible to virus or on fourth instar which are more exposed to the spray deposit due to flushing of the buds, 3) to confirm if there is a difference in susceptibility of larvae on white spruce and balsam fir as noted by Howse et al (1973) and, 4) to test some formulations in the hope that they enhance the efficacy of the virus.

With these aims in view two areas were selected and a total of nine plots were sprayed with NPV. This report describes the spray operation and impact of the viruses in the year of application and in the following year. The spray application and determination of the level of virus infection were performed by IPRI staff and population reduction and defoliation estimates by GLFRC staff.

MATERIALS AND METHODS

Virus production

In the winter of 1972-73, 817,824 fifth instar larvae were infected with NPV and a total of 8,036 gm of freeze-dried virus-infected material produced. The number of inclusion bodies/gm was calculated to be 5 billion. Batches of virus were bioassayed to establish the absence of foreign viruses.

Experimental plots

The first series of plots were located in Salter and Tennyson Twps. on or near Highway 553 about 8 miles North of Massey, Ontario. They were designated plots #1 to #4 with areas of 100, 70, 140 and 75 acres respectively. Their location is shown in fig. 1. The plots were located where there were suitable white spruce-balsam fir stands and hence the acreage varied. The terrain was flat with balsam fir (40-50 ft. tall) being the dominant species. The percentage composition of tree species on the basis of stems per acre in these plots is given in Table I. White spruce and balsam fir were both well represented but there was a considerable overstory of poplar. Hence it was decided to spray these plots before the poplar leafed out. The ground cover consisted of hazel, pin cherry, suppressed balsam fir

and suppressed poplar.

The second series of plots was located in the Mashagama Lake-Aubrey Falls area which is approximately half way between Thessalon and Chapleau, Ont. There was a total of 5 plots and 3 of them designated #5, 6, and 7 were located along highway 129 as shown in fig. 2. Plot #8 was located at the junction of the Ranger Lake Rd. and the road to Mashagama Lodge and is shown in fig. 3. Plot #9 was located 3 miles from plot #8 and was close to the Aubinadong River as shown in fig. 3.

The terrain was fairly hilly with hardwoods on the ridges. The dominant tree species was balsam fir and there were low levels of white spruce. The percentages of different species on the basis of stems per acre and their height are given in table II. The poplar and white birch were co-dominant along with the conifers and there was no overstory.

Plot marking

Most of the plots were arranged with a road running through them and plot corners were established by compassing and measuring them from the roadway. Plot #2 had a bush road running through it and plot #9 had a clearing cut through it. Stakes were driven along these roads or clearings at 100' intervals and numbered. Trails were marked to the plot corners using red flagging tape.

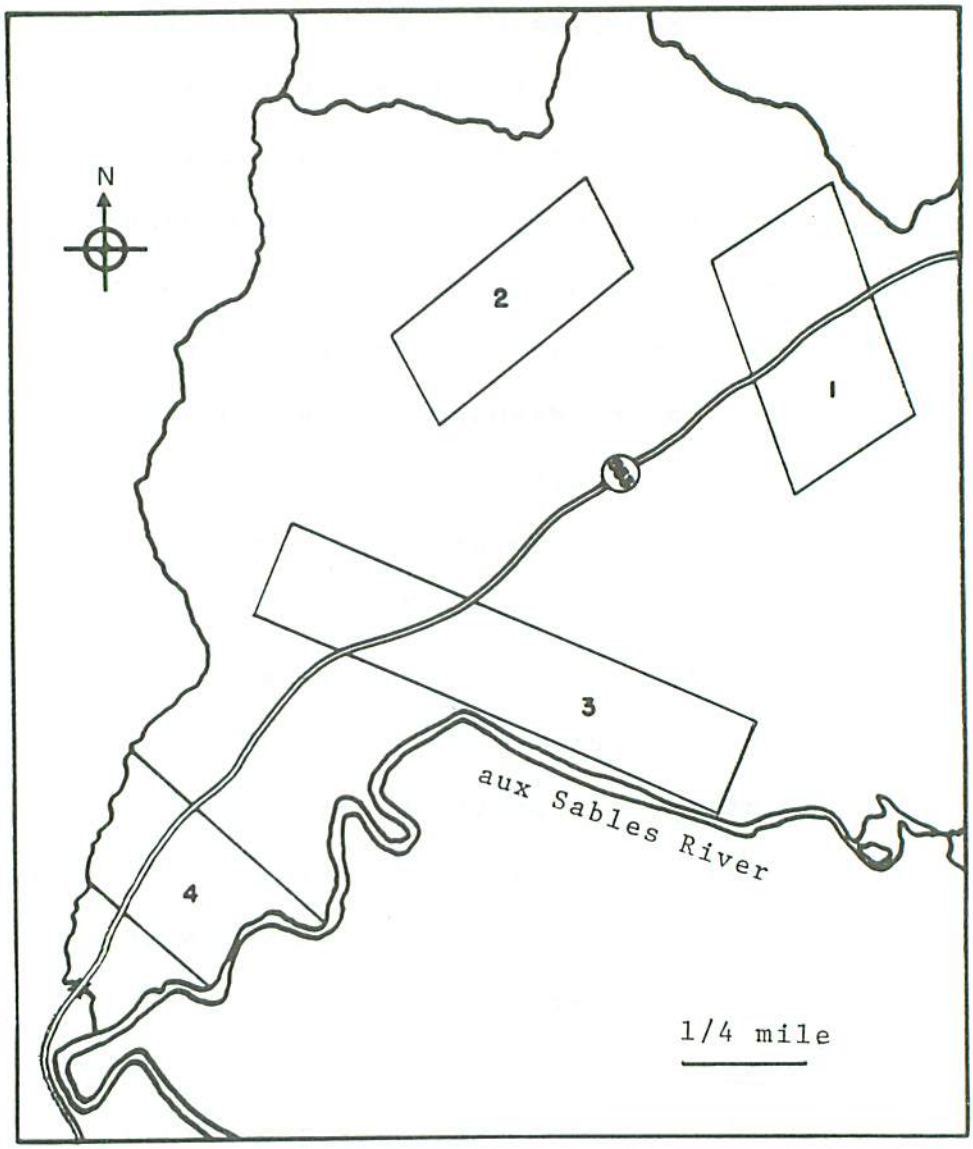

Fig. 1. Plots sprayed with NPV in 1973 located about 8 miles north of Massey, Ontario on Hwy. 553.

Table I

Percentage of different tree species based on stems per acre and height of dominant species on plots located about 8 miles north of Massey, Ontario.

Plot	White spruce	Balsam fir	Poplar	White birch	White pine	Red pine	Jack pine	Black spruce	Larch tamarack
1	25 (60')	25 (40-50')	40 (40-60')	2	2	2	2	1	1
2	50 (60')	20 (30-40')	24 (40-60')	1	1	1	1	1	1
3	25 (40-50')	35 (40-50')	30 (40-60')	10	-	-	-	-	-
4	25 (40-50')	35 (40-50')	30 (40-60')	10	-	-	-	-	-

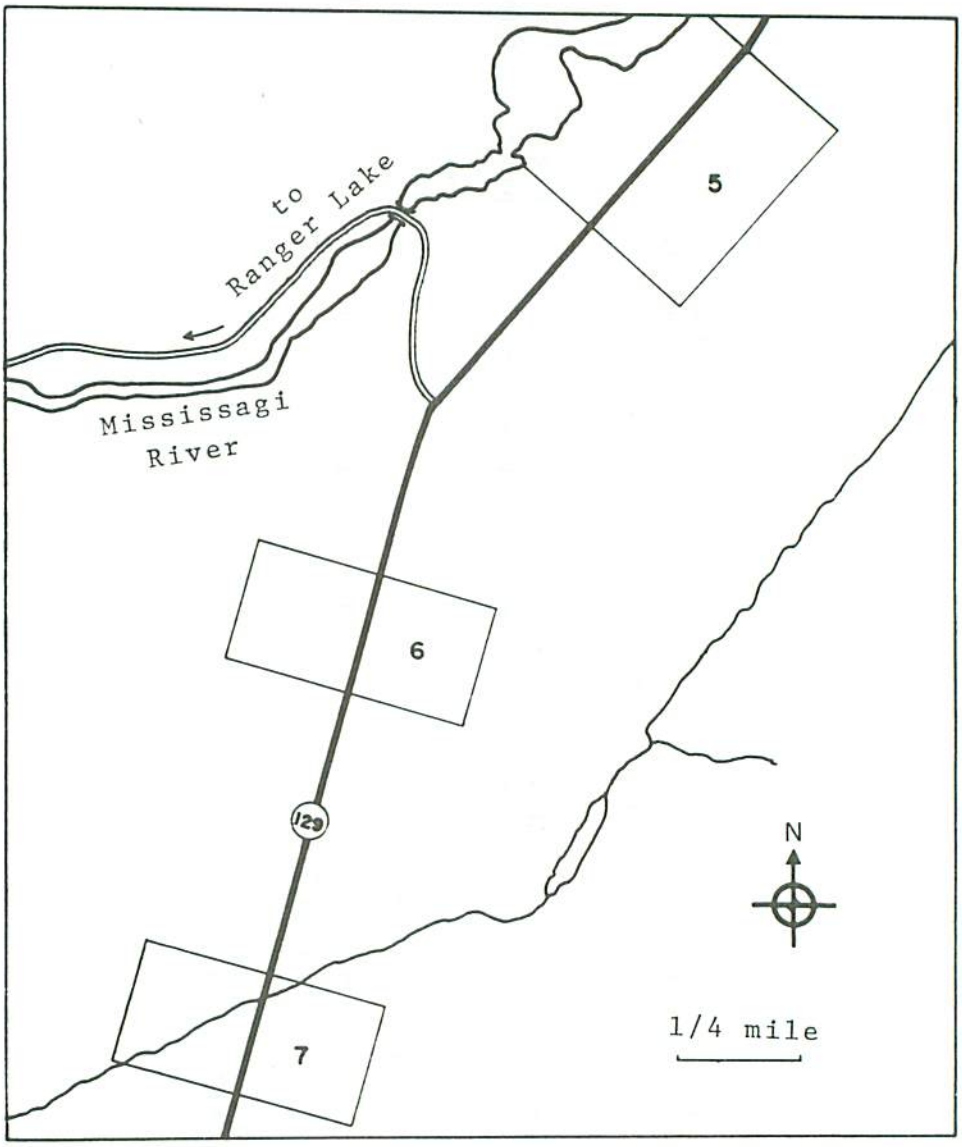

Fig. 2. Plots sprayed with NPV in 1973 located near Aubrey Falls, Ontario on Hwy 129.

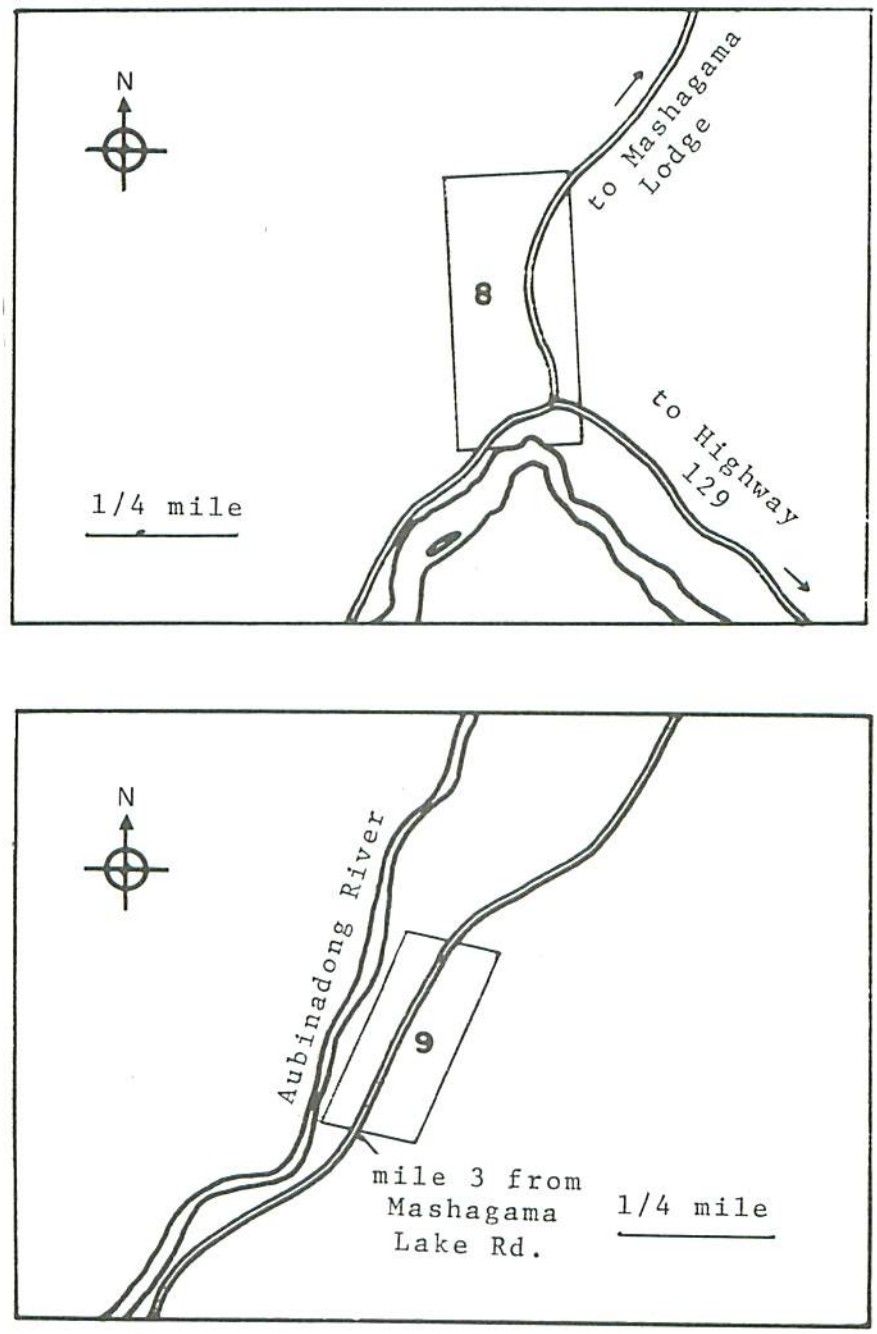

Fig. 3. Plots sprayed with NPV in 1973 located in the Mashagama Lake, Ontario area.

Table II

Percentage of different tree species based on stems per acre and height of dominant species on plots located in the Aubrey Falls-Mashagama Lake area, Ontario.

Plot	Balsam fir	White spruce	Poplar	White birch	White cedar	White pine	Yellow birch	Red maple	Black spruce	Jack pine
5	55 (40-50')	5 (40-50')	25 (50')	10 (30-40')	5	3	2	-	-	-
6	50 (40-50')	10 (40-50')	2	25 (30-40')	2	2	2	2	5	
7	50	5	2	25	2	1	-	-	5	-
8	60 (35-40')	25 (50-60')	5	10	-	-	-	-	-	-
9	50 (40-50')	5 (50-60')	2	20 (25-30')	2	5	-	-	1	5

Red meteorological balloons were used to guide the aircraft. Small cylinders filled with helium were carried to the plot corners and balloons put 30-40 ft. above the canopy prior to spraying. A balloon was also put up on the road and was moved from one stake to the next to guide the aircraft on each pass it made.

Formulation and application

At the Massey location the only additive used was IMC sunlight protectant at 1/4 lb./gal. in the aqueous suspension. Plots #1, #2 and #3 were sprayed once only and the concentration of virus per acre is given in table III. Plot #4 was sprayed twice with the same concentration of virus.

At the Mashagama location two concentrations of virus and 3 formulations were tested. Plot 9 was sprayed with virus in water only to determine the efficacy of the IMC sunlight protectant. The acreage of the plots, concentration of virus and formulation are given in table IV.

Aircraft

A Grumman Agcat biplane fitted with 4 Micronair AU 3000 units and owned by General Airspray Ltd. was contracted for the application. It was found necessary

Table III

The size of plots and concentration of active ingredient applied at 1 U.S. gal/acre at Massey, Ontario, 1974.

Plot Number	Acreage	Freeze-dried NPV-infected material (gm/gal)	PIB/ acre x 10^9
1	100	2	10
2	70	5	25
3	140	10	50
4	75	4 (double application)	20

Table IV

The size of plots, concentration of active ingredient applied at 1 U.S. gal/acre and formulation at Aubrey Falls-Mashagama Lake, Ontario, 1973.

Plot Number	Acreage	PIB/ acre x 10^9	Formulation
5	110	25	2.5% IMC Shade®
6	80	50	2.5% IMC Shade®
7	80	50	2.5% IMC Shade® 0.13% Chevron® sticker
8	50	50	2.5% Shade® 0.2% Biofilm®
9	33	50	None

to remove the pump and line filters in 1972 (Cunningham and McPhee, 1973) and this procedure was again followed. The application rate was calibrated to 1 U.S. gal./acre assuming a 100 ft. swath width. The aircraft was loaded with the gallonage to spray the plot plus 10% extra to allow for overrun.

Communications

Johnson portable transceivers were used to communicate between the plots and air strip; the radio in the aircraft was tuned to the same channel.

Monitoring the deposit

Kromekote spray cards on 1/4" plywood backings were placed along the shoulder of roads running through the plots with the exception of plot #9 where they were placed in the middle of a clearing running through the plot. The cards were placed at 25' intervals within the plots at right angles to the flight path and to a distance of 200 ft. outside the plot boundaries to record drift.

Mixing the formulations

The freeze-dried infected insect powder was weighed and put into an aqueous suspension using a Kalish homogeniser. The amount required for each plot was put into 10 U.S. gal of water and filtered through a 20 mesh sieve. This was done one or two days before the application. IMC Shade®

was added to water at 10 times the final concentration in 40 gal. drums and it went into suspension without stirring over a period of 2-3 hrs. The final mixing was done in a 400 gallon animal feeding trough with the correct volume of water. Chevron® sticker was added to the formulation for plot #7 and Biofilm® was added to the formulation for plot #8. The mixtures were stirred with a paddle.

Spray operation and larval development

Spraying commenced at Massey on May 18 in the evening. Plot #1 was sprayed and the first application put on plot #4. The stage of the larvae at this time is given in table V. On the morning of May 19, plots #2 and #3 were sprayed and on the evening of May 19, plot #4 was given a second application.

At the Aubrey Falls-Mashagama Lake location spraying was delayed due to the use of the aircraft for another experimental spray application and to bad weather conditions. On the morning of June 9, plot #8 was sprayed. The larval development is given in table VI.

On the evening of June 9, plot #9 was sprayed. As there was no marker in the suspension, the deposit was not monitored but there was too much wind and the deposit was probably poor. Bad weather with fog and rain prevented further spraying until the morning of June 12

Table V

Spruce budworm larval development when spraying commenced at Massey, Ontario on May 18, 1973.

On white spruce (40 larvae)

II in buds	17%
III in buds	5%
II in needle mines	54%
III in needle mines	14%
II wandering	5%
III wandering	5%

On balsam fir (40 larvae)

III in buds	4%
II in needle mines	80%
III in needle mines	8%
II wandering	8%

Table VI

Spruce budworm larval development at the commencement of spraying at Mashagama-Aubrey Falls, Ontario on June 9, 1973 and at the termination on June 12.

June 9

On white spruce (50)		On balsam fir (40)	
III	8%	III	15%
IV	36%	IV	37%
V	56%	V	48%

June 12

On white spruce (50)		On balsam fir (50)	
IV	24%	IV	32%
V	54%	V	64%
VI	22%	VI	4%

when conditions were excellent. Spraying commenced on plot #5 but the pilot misjudged the incline of the plot and was forced to dump the load on the second pass. Following this incident plots #6 and #7 were sprayed. On the evening of June 12, another attempt was made to spray plot #5 but considerable air movement resulted in a poor deposit. By this time the spruce budworm larvae had developed considerably and some sixth instar were present. The development of the larvae on June 12 is given in Table VI.

Sampling for population reduction studies in 1973 and 1974 and insect counting techniques

As NPV takes at least 10 days to kill spruce budworm larvae in the field, pre-spray samples were taken 1 week after the start of the spray operation on May 27 at Massey to allow larvae to grow in order to facilitate easier counting. Random samples of 25 white spruce 18" branch tips and 25 balsam fir 18" branch tips were taken from each of the 4 plots at Massey and from 2 untreated check areas, one about a mile north of the plots and one about a mile south of the plots.

Prespray samples were taken from plots #5 and #6 in the Aubrey Falls and Mashagama area on June 9. Random samples of 25 balsam fir 18" branch tips and 25 white spruce branch tips were taken from each plot and from a

check area located midway between the plots (check #2). Unfortunately, examination of these samples was unduly delayed in the laboratory and consequently there are no reliable pre-spray data for Aubrey Falls for 1973.

The same number of post spray samples were collected from the plots. The Massey locality was sampled on July 3 and the Aubrey Falls-Mashagama Lake locality on July 5. All samples were stored at 34° F until they were examined.

In 1974, samples were again taken to determine if the virus had any impact during the development of the spruce budworm larvae. At Massey, plots #1, #2 and #3 were sampled along with the North and South check areas on June 7 when larvae were mainly in the fourth instar. Random samples of 25 white spruce and 25 balsam fir 18" branch tips were taken. A pupal sample was taken from the same number of trees on July 12. At the Aubrey Falls locality in 1974 plots #6 and #7 and a check area in between them (check #3) were sampled. Again 25 samples were taken from each tree species. The early sample was collected on June 10 and the pupal sample on July 3.

Larvae were removed from the foliage and counted using the "drum method" described by DeBoo et al (1973) and Martineau and Benoit (1973). Pupal samples were hand

picked. Abbott's formula (Abbott, 1925) was used to calculate the effectiveness of each treatment with the exception of plots #5 and #6 at Aubrey Falls in 1973. There population reduction was calculated by comparing surviving pupal values in the check area to those in each treatment, due to the lack of adequate pre-spray data.

Sampling larvae for microscopic examination to determine the level of virus infection in 1973 and 1974

Samples of 18" branch tips were collected from Massey on June 15, 27 days after the application and on June 22, 34 days after the application. Random samples were taken from each of the four plots, 10 each from white spruce and balsam fir in plots #1, 2, and 4 and 20 of each species from plot #3. On June 25, the checks to the North and South of the spray plot area were sampled and 10 random samples of each species taken from each area.

Samples were collected from the Mashagama Lake-Aubrey Falls locality on June 26 and 27, 14 days after the application. All the plots were sampled along with two check areas and 10 random samples of each species taken from each area. The first check area was about 2 miles from plot #9 in the direction of Mashagama Lake and the second check area located between Plots #5 and #6. All the plots were sampled again on July 5 and 6 but the samples from plot #6 were lost in transit.

In 1974, most of these plots were sampled again to determine if any carry-over of virus had occurred. At Massey, 10 branch tips (18") were collected from white spruce and 10 from balsam fir from plots #1, #2 and #3 on June 21 and from the North and South checks on July 7. At the Aubrey Falls locality the same number of samples were collected from plots #6 and #7 on June 26 and 29 and a control area in between these plots (check #3) was sampled on July 3.

Microscopic diagnosis

All spruce budworm (living or dead; larvae or pupae) were removed from the foliage samples. Living larvae were dissected and squash preparations were made of the gut and portions of the fat tissue. Dead larvae were smeared on a microscope slide; if they were desiccated they were ground up in a drop of water using a glass rod. The slides were observed under a Leitz Ortholux microscope using phase contrast optics and EPV, NPV, CPV and microsporida were recorded.

Estimates of defoliation

The per cent current defoliation was obtained by detailed examination and estimates of the degree of defoliation sustained by the 18" branch tips collected for the pupal sample from treated areas and check plots after budworm had ceased feeding.

Deposit Analysis

Spray deposit was extremely poor on plot #5 and there was no marker dye in the formulation sprayed on plot #9 (it was suspected that deposit was also poor). Hence no analysis was made of the cards from these plots. The cards from the other plots were analysed by Dr. W.E. Slack of the National Aeronautic Establishment of the National Research Council of Canada.

For this analysis the centre part of each Kromokote spray card was photographed on 35 mm Kodalith Ortho Film type 3 using a Nikon F camera at a 1:1 magnification. The film was processed at N.A.E. and the droplets analysed using the Flying Spot Analyser. The droplets were divided into 14 categories, 9 by size, one of conglomerates and one category of each of the four edges. From the computer print-outs the percentage distribution of the droplet sizes in each plot was calculated along with the mean number of droplets per sq. cm. No correction was made for spread and the size measurements are for the actual spots on the cards.

RESULTS

Deposit analysis

The mean number of droplets per cm^2 recorded on spray cards from the plots which were analyzed is given in Table VII. There was considerable variation between the plots, the best deposit being on plot #7 with 39 droplets per cm^2 and the worst on the first application on plot #4 with 7 droplets per cm^2. The percentage distribution of the droplets in 9 size categories is given in Table VIII. There was a tremendous range in droplet size in all the plots but two peaks can be distinguished, one at the 40-120 μ size and the other at the 200-400 μ size. Plot #1 and the second application on plot #4 had only one peak at the smaller size range and plots #8 and the second application on plot #4 had one peak at the larger size range. The remaining plots had peaks at both size ranges.

Incidence of viruses in the year of application - 1973

The results of the impact of the NPV at the Massey location along with the level of microsporida in the population are given in Table IX. A total of 3,193 insects were examined from the test plots and two check areas. Higher levels of infection were found in the second sample taken 34 days after the application than in the first sample and higher levels of infection were found in larvae on white spruce than on balsam fir.

Table VII

Mean number of droplets per cm^2 on the spray cards

Plot	Mean number drops/cm^2	Standard deviation
1	16	8
2	17	7
3	23	7
4 (1st applic.)	7	4
4 (2nd applic.)	14	8
6	14	4
7	39	13
8	32	16

Table VIII

Percentage of droplets on spray cards in different size categories

Plot	Size of droplets in microns								
	up to 40	41-80	81-120	121-160	161-200	201-400	401-600	601-800	801-1000
1	15.4	29.2	19.9	9.2	4.8	13.1	6.3	1.6	0.6
2	3.4	11.8	14.8	14.0	18.0	34.8	3.2	0.2	0
3	6.2	19.0	24.0	14.2	9.5	23.2	3.6	0.2	0
4 (1st applic.)	10.7	14.8	13.3	8.4	4.1	26.5	17.0	4.0	0.9
4 (2nd applic.)	8.6	24.3	31.9	16.5	8.8	8.3	1.4	0.2	0
6	5.5	13.6	21.7	22.1	14.0	21.3	1.7	0.1	0
7	5.5	18.1	24.7	16.9	14.9	19.5	0.5	0	0
8	5.5	10.8	16.7	17.5	14.6	32.5	2.4	0	0

The relationship between dosage and deposit and resulting infection can be clearly seen with the best results in plot #3 with 37.2% of larvae infected on white spruce and 12.4% on balsam fir. No viruses were found in either of the check areas and only 0.5% and 0.4% CPV was found in plots #3 and #4 respectively.

At the Aubrey Falls-Mashagama Lake locality a higher incidence of viruses was found in the first sample taken 15 days after the application. A total of 5,028 insects were examined and results are shown in Table X. The highest level of infection was found in plot #7 with 17.1% of the larvae infected on balsam fir and 25.8% on white spruce. This was also the plot which had the best deposit. A very high level of virus infection was found in check #1 located near Mashagama Lake with 4.7% infection in larvae on balsam fir and 6.2% on white spruce. In check #2 located between plots #5 and #6 only 0.6% of NPV was detected in larvae on balsam fir and 4.1% natural CPV. Natural CPV was also detected in plots #5 and #9.

Estimates of population reduction and current defoliation in 1973

All four plots at Massey were studied and the results given in table XI. The levels of population reduction followed the dosage administered for plots #1, #2 and #3 for larvae on white spruce hosts, but it is peculiar that the lowest population reduction was recorded for larvae on balsam fir hosts in plot #3 which received the highest dosage and best deposit. Some foliage protection was re-

Table IX

Incidence of viruses and microsporidia in plots sprayed with NPV near Massey, Ont. in 1973

Plot	Sample date	Tree species	Number Insects Examined	Percent Virus Infection		Percent Microsp.
				NPV	CPV	
1	15.6.73	b.F.	152	0	0	3.9
	15.6.73	w.S.	238	3.8	0	6.7
	22.6.73	b.F.	213	1.9	0	7.5
	22.7.73	w.S.	237	8.4	0	3.4
2	15.6.73	b.F.	87	4.6	0	5.7
	15.6.73	w.S.	161	8.1	0	6.2
	22.6.73	b.F.	141	2.8	0	3.5
	22.6.73	w.S.	166	16.9	0	3.6
3	15.6.73	b.F.	189	7.9	0.5	5.3
	15.6.73	w.S.	355	18.3	0	7.3
	22.6.73	b.F.	405	12.4	0	1.2
	22.6.73	w.S.	296	37.2	0	3.0
4	15.6.73	b.F.	142	2.1	0	9.9
	15.6.73	w.S.	208	3.4	0	7.2
	22.6.73	b.F.	210	3.3.	0	4.8
	22.6.73	w.S.	230	10.9	0.4	7.4
Check North	25.6.73	b.F.	167	0	0	2.4
	25.6.73	w.S.	191	0	0	9.4
Check South	25.6.73	b.F.	152	0	0	4.6
	25.6.73	w.S.	149	0	0	6.7

Table X

Incidence of viruses and microsporidia in plots sprayed with NPV in the Aubrey Falls-Mashagama Lake, Ont. locality in 1973

Plot	Sample date	Tree species	Number insects examined	Percent Virus Infection		Percent Microsp.
				NPV	CPV	
5	26.6.73	bF	288	2.4		1.7
	26.6.73	wS	188	4.8	1.1	1.1
	5.7.73	bF	459	0.7	0.2	5.2
	5.7.73	wS	177	9.6	0	7.9
6	26.6.73	bF	318	9.8	0	5.7
	26.6.73	wS	170	12.9	0	2.4
7	26.7.73	bF	269	17.1	0	4.5
	26.7.73	wS	182	25.8	0	2.2
	5.7.73	bF	273	1.8	0	3.3
	5.7.73	wS	135	7.4	0	2.2
8	27.6.73	bF	185	7.0	0	2.2
	27.6.73	wS	165	12.7	0	4.2
	6.7.73	bF	150	0.7	0	4.0
	6.7.73	wS	113	3.5	0	6.2
9	27.6.73	bF	182	6.0	1.6	1.6
	27.6.73	wS	149	6.7	0.7	1.3
	6.7.73	bF	179	1.1	0	7.3
	6.7.73	wS	105	0	0	5.7
Check 1	26.6.73	bF	212	4.7	0	3.3
	26.6.73	wS	177	6.2	0	5.1
	5.7.73	bF	159	1.3	0	4.4
	5.7.73	wS	43	0	0	11.6
Check 2	27.6.73	bF	169	0.6	4.1	4.7
	27.6.73	wS	200	0	0	5.0
	6.7.73	bF	190	0	0	5.6
	6.7.73	wS	191	0	0	2.1

Table XI

Population reduction, pupal survival and current defoliation in four plots at Massey in the year of application of NPV sprays

Plot	Pre-spray larvae/18" branch tip		Surviving pupae/18" branch tip		% Population reduction due to NPV spray		% Successful pupal emergence[a]		% 1973 Defoliation	
	bF	wS	bF	wS	bF	wS	bF	wS	bF	wS
1	27.1	58.9	7.2	15.0	33	17	69	86	66	73
2	15.2	42.9	3.8	6.6	37	50	77	93	69	61
3	16.7	50.1	5.7	6.2	14	59	74	94	64	44
4	13.6	52.8	3.7	8.5	31	47	74	86	53	76
Check	10.8	29.8	4.3	9.1	-	-	68	80	58	51

a % successful pupal emergence = $\frac{\text{emerged budworm}}{\text{budworm alive on sample date}} \times 100$

corded on white spruce in plot #3.

At the Aubrey Falls-Mashagama Lake location there were good levels of population reduction on both host species in Plot #6 which were comparable to the best results at Massey. Results are given in Table XII. It was known that plot #5 had a poor deposit and the results should be ignored. In plot #6 a 45% population reduction of insects on balsam fir and a 43% reduction on white spruce were recorded. There was no measurable amount of foliage saved at this location but larvae were at the peak of the fifth instar when the spray was applied. Pupal survival was apparently not seriously adversely affected at either location in any treatment plot by the NPV sprays.

Incidence of viruses in the year following application (1974)

At the Massey locality a total of 2,092 insects were examined from plots #1, #2, #3 and the two controls. The results are shown in Table XIII. Levels of virus infection were extremely low compared to the previous year and there were no marked differences between the three plots. An EPV was detected at low level in the North check area.

At the Aubrey Falls locality a total of 1,184 insects were examined from plots #6 and #7 and from a check area in between these plots. The results are shown

Table XII

Population reduction, pupal survival and current defoliation in two plots at Aubrey Falls in the year of application of NPV sprays

Plot	Pre-spray larvae/18" branch tip		Surviving pupae/18" branch tip		% Population reduction due to NPV spray		% Successful pupal emergence[a]		% 1973 defoliation	
	bF	wS	bF	wS	bF	wS	bF	wS	bF	wS
5			13.7	19.9	21	0	72	81	89	92
6			9.5	10.1	45	43	80	96	93	94
Check			17.3	17.7	-	-	79	85	93	96

a % successful pupal emergence = $\frac{\text{Emerged budworm}}{\text{budworm alive on sample date}} \times 100$

in Table XIV. Levels of virus infection were lower in these plots than in the year of application. The level of virus infection in the check was surprisingly high. This check area was not sampled in 1973.

Population reduction due to virus and current defoliation in the year following application (1974)

Population reduction, pupal survival and current defoliation for three plots at Massey and two plots at Aubrey Falls are presented in Table XV. In the three plots at Massey, carryover was better on white spruce than balsam fir and manifested itself in population reductions on white spruce of 48% in plot #1, 62% in plot #2 and 26% in plot #3. Moderate protection of white spruce foliage was also evident.

At Aubrey Falls, population reduction was determined to have occurred only in plot #7. However, since the check plot was found to be contaminated with NPV, the population reduction data for both plots #6 and #7 at Aubrey Falls are not reliable. Significant population reduction due to NPV carryover probably occurred in both plots #6 and #7 and the check area as well. There was no foliage protection but this is not surprising in view of the extremely high larval populations that were present.

There is no conclusive evidence that pupal survival was affected by NPV carryover in any of the plots. The

very poor pupal success at Aubrey Falls is interesting and may be indicative of populations that are probably severely stressed due to the competition for food and space caused by high larval numbers.

Table XIII

Incidence of viruses and natural microsporida in plots sprayed with NPV in 1973 in the year following application at Massey, Ontario.

Plot	Sample Date	Tree Species	Number Insects Examined	Percent Virus Infection EPV	NPV	CPV	Percent Microsp.
1	21.6.74	b.F.	285	0	1.4	0	13.0
	21.6.74	w.S.	581	0	1.4	0	16.2
2	21.6.74	b.F.	233	0	1.3	0	7.7
	21.6.74	w.S.	445	0	3.6	0	9.7
3	21.6.74	b.F.	203	0	1.5	0.5	9.4
	21.6.74	w.S.	157	0	2.5	0	5.7
Check North	7.7.74	b.F.	73	1.4	0	0	5.5
	7.7.74	w.S.	57	1.8	0	0	14.0
Check South	7.7.74	b.F.	21	0	0	0	4.8
	7.7.74	w.S.	37	0	0	0	0

Table XIV

Incidence of viruses and microsporida in plots sprayed with NPV in 1973 in the year following the application at Aubrey Falls-Mashagama Lake, Ontario.

Plot	Sample Date	Tree Species	Number Insects Examined	Percent Virus Infection			Percent Microsp.
				EPV	NPV	CPV	
6	26.6.74	b.F.	306	0	2.9	0.3	6.9
	26.6.74	w.S.	389	0	6.2	0.8	8.5
7	29.6.74	b.F.	165	0	1.8	0.6	5.5
	29.6.74	w.S.	217	0	2.8	0.9	10.1
Check 3	3.7.74	b.F.	61	0	13.1	6.6	0
	3.7.74	w.S.	46	0	2.2	2.2	8.7

TABLE XV

Population reduction, pupal survival and current defoliation in three plots at Massey and two plots at Aubrey Falls in the year following application of NPV sprays

Plot	Early Sample Larvae/18" Branch Tip		Surviving Pupae/18" Branch Tip		% Population Reduction due to NPV Carryover		% Successful Pupal Emergence[a]		% 1974 Defoliation	
	bf	ws	bf	ws	bf	ws	bf	ws	bf	ws
Massey										
1	18.6	47.4	7.0	3.8	0	48	72	84	45	30
2	11.1	54.2	3.9	3.2	7	62	78	86	40	30
Check	16.2	32.2	6.1	5.0	-	-	92	93	47	44
3	14.5	12.8	5.2	3.2	4	26	87	94	48	17
Check	16.2	28.2	6.1	9.4	-	-	92	92	47	33
Aubrey Falls										
6	66.4	92.0	6.1	3.8	0	0	28	17	100	96
7	99.6	159.1	4.4	3.8	42	37	25	26	100	97
Check[b]	75.2	181.0	5.7	6.8	-	-	27	42	100	99

a % successful pupal emergence = $\frac{\text{Emerged Budworm}}{\text{Budworm Alive on Sample Date}} \times 100$

b Contaminated with NPV-probably drift from 1973 spray and carryover

DISCUSSION

When the deposit analysis for the 1972 spray trials at Chapleau was compared to deposit analysis reported here it can be seen that a better deposit was obtained at Chapleau with a considerably higher number of droplets per cm^2 and most of the droplets in 40-80 μ size category. Exactly the same equipment was used in 1972 and 1973 and it is not known why more of the drops were in the larger categories in 1973. Radically different methods of analysis were used which could have given small differences in the results; also, molasses was used in all but one of the plots in 1972 and could have influenced the droplet spectrum, and different meteorological conditions could have had an effect.

At Massey, the difference in levels of NPV-infection and population reduction of spruce budworm on white spruce and balsam fir hosts were clearly demonstrated following the early spray application on second and third instar larvae. This confirms results reported previously (Bird et al. 1972; Howse et al, 1973; Cunningham and McPhee, 1973). A possible explanation for this phenomenon was given by Cunningham and McPhee who suggested that because spruce budworm larvae mine several needles on white spruce and usually only one on balsam fir, larvae on white spruce have a greater chance of ingesting polyhedra following an early virus application. Needle mining habits of spruce budworm larvae have been reported by McGugan (1954).

Thus, when spruce plantations or stands which contain predominantly white spruce are sprayed with NPV against spruce budworm, an early application is recommended, unless a massive dosage such as that used at Petawawa in 1971 is applied to fourth instar larvae. The optimal time of application on balsam fir is still in doubt although the evidence indicates that a later application on fourth or even small fifth instar, after the buds have flushed, may give the best results.

At Massey on plot #3, 37% infection was recorded in larvae on white spruce and 12% on balsam fir with an application of 50 billion PIB/acre. In 1971, 300 billion PIB/acre sprayed on second and third instar larvae in a white spruce plantation near Petawawa, Ont. resulted in 34% NPV and CPV infection. The same concentration sprayed when larvae were at the peak of the fourth instar gave 71% NPV and CPV infection (Howse et al, 1973). The early spray operation at Massey, therefore, compares favourably with the 1971 early spray operation. There was little current defoliation in 1974 on white spruce in plot #3 and there is little doubt that this situation is due to the effect of the NPV. The double application on plot #4 was an inadequate test as the deposit was poor and the applications were only 24 hours apart. A more suitable time interval would have been 3 to 4 days.

At the Mashagama Lake-Aubrey Falls locality the best level of infection was obtained in plot #7. It should be

noted that this plot, where the Chevron® sticker was used, also had the best coverage, although plot #8, where Biofilm® was tested, had almost as good coverage but about half the number of infected budworm larvae. These results indicate that Chevron® sticker may be a desirable spray additive for use with aqueous suspensions of NPV although more thorough testing is required. As compared to the late spray application on fourth instar larvae near Petawawa in 1971, the results were disappointing. Of course, in 1973, due to unavoidable delays the larvae were at the peak of the fifth instar at the time of the application and this is considered too mature for a satisfactory virus introduction.

Unexpectedly high levels of NPV were recorded in larvae in check #1 near Mashagama Lake in 1973 and in check #3 on Hwy 129 near Aubrey Falls in 1974. Check #3 was not sampled in 1973 and it is not known if the NPV infection of larvae in this area was due to natural virus, spread of the virus from plots #6 and #7 or spray drift at the time of the application. Check #1 was far removed from the nearest spray plot, thus it is unlikely that spray drift or spread of virus from a spray area caused NPV infection of larvae in this plot.

It is interesting to note that EPV was recorded in the North check at Massey in 1974. This virus has also

been found near Chapleau in unsprayed areas and in Lake Superior Park (unpublished data). EPV is probably more widespread in the spruce budworm population than was supposed. Low levels of CPV were found in both the Massey and the Aubrey Falls-Mashagama Lake localities in 1973 and 1974. It can be seen by comparing Tables IX and X and Tables XIII and XIV that the levels of microsporida in the spruce budworm population increased from 1973 to '74 and this is to be expected as a spruce budworm population ages.

Generally, the initial introduction of NPV in 1973 was successful but the levels of virus infection and population reduction in the following year were considered disappointing when compared to the results obtained from plots sprayed in 1971. In that year two isolated white spruce plantations at Deluthier Rd. near Petawawa were sprayed with NPV (contaminated with CPV) and a series of plots were sprayed with EPV (contaminated with NPV and CPV) at Achray in Algonquin Park. The NPV in these plots in both localities is still having a marked effect on the spruce budworm population in 1974 which is 3 years after the application.

How a virus epizootic is established is not clearly understood. The white spruce plantations near Petawawa

were small and isolated and it is probable that there was little movement of moths in and out of them. In contrast, the EPV plots at Achray were located in a heavy spruce budworm infestation and the situation was comparable to the Massey location. It is interesting to speculate that the mixtures of viruses used in 1971 may have been more efficient for the initiation of a virus epizootic than the non-contaminated NPV used in 1972 and 1973!

Further trials should be conducted in isolated plantations, or over large areas, to reduce the possibility of the movement of moths in and out of the sprayed areas and facilitate the studies of the persistence of the virus in the spruce budworm population.

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