# AERIAL APPLICATION OF NUCLEAR POLYHEDROSIS VIRUS ON SPRUCE BUDWORM ON MANITOULIN ISLAND, ONTARIO IN 1975.

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#### ABSTRACT

Five plots infested with spruce budworm and totaling 318 ha in size located in Robinson and Dawson Twps. on Manitoulin Island, Ontario were sprayed with a nuclear polyhedrosis virus (NPV) when larvae were at the peak of the fourth instar. A Grumman Agcat biplane was used for the application and a comparison was made between boom and nozzle and Micronair spray equipment. All applications were at 9.4 <u>1</u>/ha and the dosage of virus ranged from 44 billion to 250 billion polyhedra/ha. Three formulations were compared including a commercial preparation, Sandoz adjuvant V, and a molasses based formulation.

The efficacy of the virus was evaluated by population reduction estimates, defoliation estimates and microscopic diagnosis of insects to determine the level of virus infection. Excellent results were obtained on 3 of the plots with 91%, 91% and 84% population reduction due to the virus application on white spruce hosts and 21%, 53% and 65% on balsam fir hosts. This follows a pattern noted in previous years with a greater impact on white spruce than balsam fir hosts. There was no measurable reduction in defoliation in the sprayed areas. The results indicated that boom and nozzle spray equipment was as good as Micronair for high volume applications of aqueous formulations and the molasses based formulation appeared as good as the more expensive commercial formulation.

Two of the plots were located close to a 520 ha block sprayed with NPV in 1974. Now that this virus has been introduced into a large area on Manitoulin Island, long term studies of the interaction of the NPV with the spruce budworm population will be conducted.

#### Résumé

Cinq parcelles d'une superficie totale de 318 ha infestées par la Tordeuse des bourgeons de l'Epinette dans les comtés de Robinson et de Dawson sur l'Ile Manitoulin (Ont.) ont été arrosées de virus de la polyédrose nucléaire (VPN), alors que les larves se trouvaient à l'apogée du quatrième stade de développement. On a utilisé à cette fin un biplan Grumman Agcat, en comparant les systèmes de pulvérisation Micronair et par buses et ajutage. Toutes les applications ont été faites à raison de 9.4  $\ell/h$  et à la dose de 44 à 250 billions de polyèdres de virus/ha. Trois formules de préparation ont pu être ainsi comparées, dont une en vente sur le marché, l'adjuvant V de Sandoz et une autre à base de mélasse.

L'efficacité du virus a été évaluée en fonction de la réduction de population, de la défoliation des arbres et du niveau de l'infection virale selon examen microscopique des insectes consécutivement à l'arrosage. L'application du virus a produit d'excellents résultats dans 3 parcelles en y réduisant la population d'insectes de 91%, 91% et 84% sur l'Epinette blanche et de 21%, 53% et 65% sur le Sapin baumier, respectivement. Ces résultats sont en harmonie avec ceux obtenus au cours des années précédentes, avec un impact plus grand sur l'Epinette blanche que sur le Sapin baumier. Il n'y avait pas de réduction mesurable de la défoliation dans les zones arrosées. Les résultats ont montré que le système de pulvérisation par buses et ajutage était aussi bon que le système Micronair pour les applications à fort volume de solutions aqueuses et que la formule à base de mélasse équivalait à la formule commerciale plus coûteuse.

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Deux de ces parcelles se situaient au voisinage d'un bloc de 520 ha arrosé au VPN en 1974. Maintenant que ce virus a été introduit sur une grande superficie dans l'Ile Manitoulin, des études de longue haleine seront conduites sur son interaction avec la population de la Tordeuse des bourgeons de l'Epinette.

#### INTRODUCTION

Plans were made in 1974 to spray a large plot on Manitoulin Island, Ontario with nuclear polyhedrosis virus (NPV) to control spruce budworm and study the long term impact of the virus. A 520 ha block was sprayed in 1974 (Cunningham <u>et al</u>, 1975a) and, at that time, it was decided to extend the plot with further spraying in 1975. With this aim in view, an intensive ground and aerial survey was made of the area and it was found that there was insufficient forest of the correct type to merit spraying a large block. Two areas close to the 1974 spray block were considered suitable for virus application and a further 3 plots were selected about 5 km from this general area.

Balsam fir is the dominant species in this area and virus introductions have always been less successful on this species than on white spruce. This year, 1975, is the fifth year of aerial spray trials using NPV on spruce budworm. Previous trials have been reported fully (Howse et al., 1973, Cunningham and McPhee, 1973, Cunningham et al., 1974 and Cunningham et al., 1975a).

An entirely satisfactory formulation for the aerial application of insect viruses on forests has not yet

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been developed. Inactivation of virus by the ultraviolet in sunlight greatly reduces the persistence of viruses deposited on foliage and an adequate protectant is of prime importance. Also stickers are required to prevent wash-off by rain and anti-evaporants are desirable to enhance the deposit of the aqueous suspensions. Sandoz Wander Inc. had just developed and were marketing a virus adjuvant, Sandoz adjuvant V and it was decided to test this material and compare it with the formulation used in 1974 and a molasses-based formulation. Molasses based formulations have been used previously in spruce budworm virus spray trials (Morris <u>et al</u>. 1972; Cunningham and McPhee, 1973) and with Douglas fir tussock moth NPV (Stelzer, Neisess and Thompson, 1975).

When the first aerial application of NPV was made in 1971, a helicopter fitted with a boom and nozzle spray system was used (Howse <u>et al</u>. 1973) and since then a fixed wing aircraft fitted with Micronair spray equipment has been used for all applications. Spray card analysis of the 1973 and 1974 spray trials (Cunningham <u>et al</u>., 1974; Cunningham <u>et al</u>., 1975a) showed that a large proportion of the droplets were in the 200-400 $\mu$  size category and it is possible that

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an application rate of 9.4 <u>l</u>/ha, which is routinely used, overloads the Micronair system and a very small droplet spectrum is not obtained. Boom and nozzle spray equipment was used at Sandbanks Provincial Park in May 1975 for the control of European pine sawfly with NPV and excellent results were obtained (Cunningham <u>et al</u>., 1975b). It was decided to compare applications made by boom and nozzle and Micronair equipment in the 1975 trials and determine the difference in deposit and the resulting infection and mortality of spruce budworm larvae.

The virus introductions would, of course, be compared to the application made in the same general area in 1974 and any improvements in application technique or formulation noted.

With these goals, a total of 5 plots were sprayed using different equipment and formulations. This report describes the spray operation and assessment of the impact of the NPV on the spruce budworm population. As in previous years, the spray operation and determination of levels of virus infection in the spruce budworm population were performed by personnel from the Insect Pathology Research Institute and population reduction studies and defoliation estimates by staff of the Great Lakes Forest Research Centre.

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#### MATERIALS AND METHODS

#### Virus production

During the winter season of 1974-75, an estimated total of 4,929,500 second instar budworm larvae were reared and 2,966,300 of these reached the fifth instar. They were placed on diet sprayed with NPV. The diseased larvae were harvested after 8 days, freezedried and pulverized. A total of 12,567 g of this powder containing 5.5 billion polyhedral inclusion bodies (PIB) per gram was obtained.

As an additional experiment 4,000 larvae were infected with NPV and left for 15 days until they all died. The entire contents of the cups, including unused synthetic diet, frass and webbing were collected, freeze-dried and pulverized, and this yielded 1,850 g of material with a count of 2 billion PIB/g.

## Experimental plots

Two plots, designated No. 1 and No. 3 of 120 and 80 ha respectively were located close to the 520 ha block sprayed in 1974 in Robinson Twp., Manitoulin Island. A further 3 plots, No. 2, No. 4 and No. 5 of 80, 60 and 18 ha were near Highway 540, No. 2 and No. 4 in Robinson Twp. and No. 5 in Dawson Twp. about 5 km from plots No. 1 and No. 3. The plot locations are illustrated in Figs. 1 and 2. Fig. 3 shows the location of the plot sprayed in 1974.

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The terrain was level with a limestone base. The stand composition was similar in plots No. 1, No. 3 and No. 5 with balsam fir the dominant species comprising 60% of the stand, the remainder being 25% white spruce stems, 5% white, red and jack pine stems and 10% white birch, poplar and cedar stems. The composition of plots No. 2 and No. 4 consisted of 35% balsam fir stems, 15% white spruce stems, 35% white birch, poplar and maple stems, 10% white cedar stems and 5% white, red and jack pine. The height of the balsam fir and white spruce in all the plots was 10 to 15 m. Ground cover in all the plots consisted of juniper, choke cherry, and suppressed poplar and white birch. With the exception of plot No. 2 there was no deciduous overstory; in plot No. 2 the white birch and poplar were 15 to 18 m tall and formed an overstory.

Ten check plots were also selected in locations which were comparable in stand composition and spruce budworm population density to the treated plots. Five of these plots were close to the treated areas and are illustrated in Figs. 1 and 2. The remaining check areas were located east of these treated areas. Plots were laid out and marked using the same methods as in previous years (Cunningham <u>et al</u>. 1974).

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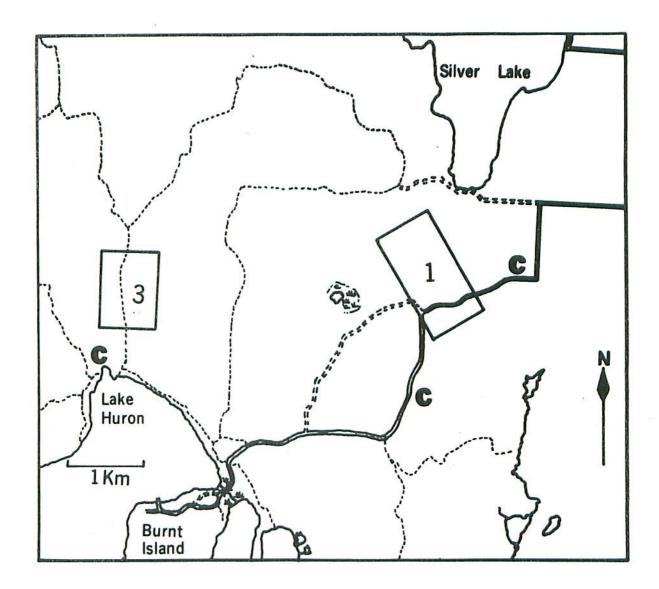


Fig. 1. Locations of Plots No. 1 and No. 3 in Robinson Twp. Manitoulin Island sprayed with NPV in 1975. Check areas (C) from east to west are No. 1, No. 2 and No. 3 respectively.

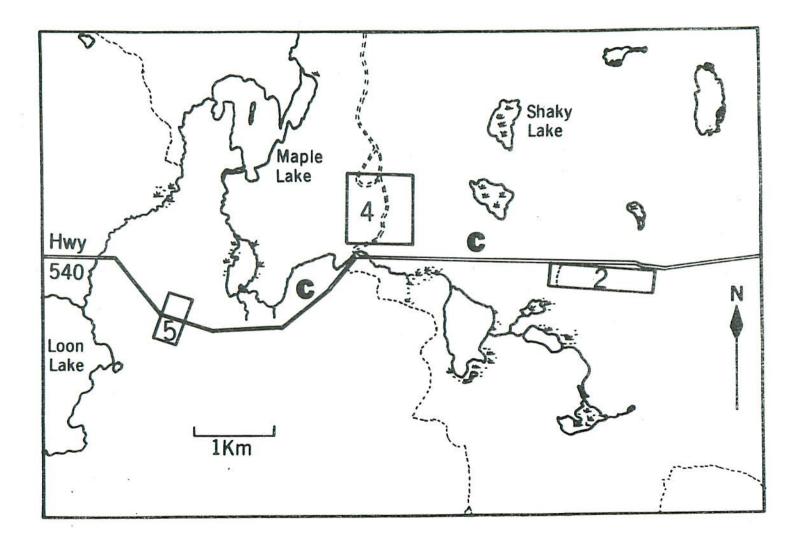


Fig. 2. Locations of plots no. 2 and no. 4 in Robinson Twp. and plot no. 5 in Dawson Tqp. sprayed with NPV in 1975. Check areas (C) from west to east are no. 4 and no. 6.

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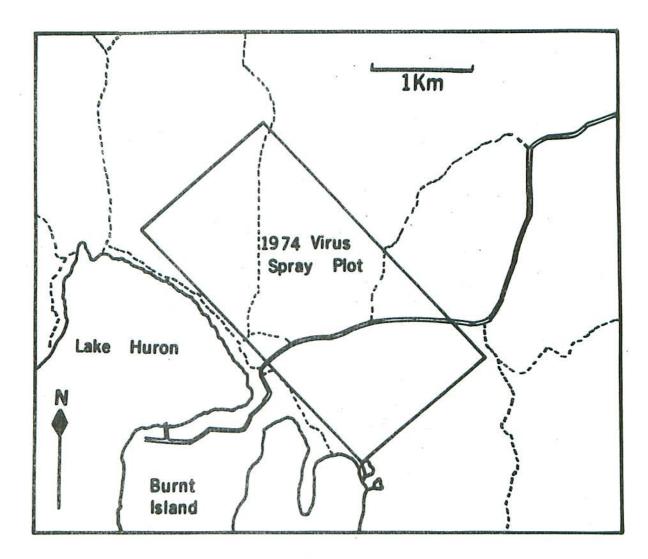


Fig. 3. Location of the 520 ha block in Robinson Twp., Manitoulin Island.

# Treatments and formulations

The different treatments, formulations and plot sizes are listed in Table I. Plots No. 1 and No. 2 were sprayed with Micronair equipment and plots No. 3, No. 4 and No. 5 with boom and nozzle equipment. In plots No. 1, No. 4 and No. 5 Sandoz adjuvant V<sup>1</sup> was added to an equal volume of virus in water. On plot No. 3 a molasses formulation was used with 250 ml/1 animal feed grade molasses, 60 g/1 IMC 90-001 UV protectant<sup>1</sup> and 1.25 ml/1 Chevron<sup>R</sup> sticker<sup>2</sup>. On plot No. 2, the formulation and dosage used in 1974 was replicated -30 g/1 IMC 90-001 and 1.25 m/1 Chevron<sup>R</sup> sticker at a dosage of 250 billion PIB/ha. Plots No. 1, No. 3 and No. 4 were sprayed with half that dosage, 125 billion PIB/ha. The virus prepared by processing diet ingredients as well as the dead larvae (see virus production) was used on plot No. 5.

A Kalish<sup>R</sup> turbo homogenizer was used to put the freeze-dried virus infected larval powder into suspension. Batches of 1000 g were homogenized in 20 <u>1</u> of water (non-chlorinated, obtained from a well) and the resulting suspension then filtered through a 20 mesh sieve. This operation was performed one or two days before the spray application and the suspension stored in plastic gasolene cans. IMC 90-001 was added to water the day

Sandoz-Wander Inc. Homestead, Florida 33030
Chevron Chemical Co. Ortho Division, San Francisco,

2 Chevron Chemical Co. Ortho Division, San Flancisco, Calif. 94119. before spraying and allowed to go into solution without stirring. Formulations without the virus were prepared in 1,600 <u>1</u> animal water troughs; the concentrated virus suspensions were poured directly into the aircraft spray tank and the correct volume of formulated water pumped in for each application.

Some difficulties were encountered with mixing the material for plot No. 5. The diet ingredients formed a heavy sludge which would not pass through the 20 mesh sieve and the supernatant had to be poured off after this sludge was allowed to sediment. Unfortunately, about half the polyhedra were lost in this operation. To compound the problems encountered when treating this plot, 245  $\underline{1}$  and not 170  $\underline{1}$  were accidentally loaded on the aircraft and hence the application rate which should have been 125 billion PIB/ha was, in fact, 44 billion PIB/ha.

# Spray application and larval development

A Grumman Agcat biplane owned by General Airspray Ltd. was contracted for the spray operation as in the previous 4 years. Spray mixing and loading was conducted at Gore Bay airport. The aircraft was fitted with 4 Micronair AU 3000 units and was also equipped with a boom and nozzle spray system. Delivery from the two systems could easily be interchanged in about 30 min.

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# Table I

# Formulation, Application and Area of Experimental Plots

Plot	Area in hectares	Application	PIB/ha x10 <sup>9</sup>	Formulation
1	120	Micronair	125	Sandoz adjuvant V (500 ml/ <u>l</u> )
2	40	Micronair	250	IMC 90-001 (30g/ <u>1</u> ) Chevron sticker (1.25 ml/ <u>1</u> )
3	80	Boom and nozzle	125	IMC 90-001 (60g/1) Molasses (250 m17 <u>1</u> ) Chevron Sticker (1.25m1/ <u>1</u> )
4	60	Boom and nozzle	125	Sandoz adjuvant V (500 m1/ <u>1</u> )
5	18	Boom and nozzle	44	Sandoz adjuvant V (500 ml/ <u>l</u> )

The Micronair system was calibrated to 9.4 <u>1</u>/ha assuming a 30 m swathe width and the boom and nozzle system, using 15 D8 nozzles, was calibrated to the same flow rate by increasing the pump pressure. Line and pump filters were removed from the Micronair system (Cunningham and McPhee, 1973). Filters were removed from the nozzles but the swirl plates left in position.

Since the plots selected were mainly composed of balsam fir it was decided to commence spraying when the buds on this species began to elongate and flush open. In 1975, the spring was unusually warm and spraying commenced on the morning of June 3 when plot No. 1 was sprayed under ideal conditions. The following morning, plots No. 4 and No. 5 were sprayed using boom and nozzle equipment. Conditions remained ideal throughout the spray operation on plot No. 4 but deteriorated as spraying on plot No. 5 was terminated. On these plots, larval development had reached the peak of the fourth instar as shown in Table II.

The aircraft was loaded with the formulation to spray plot No. 2 on the morning of June 5 but due to high winds, attempts to spray were abandoned. A spell of bad weather with rain and high winds set in and no more attempts were made until June 8 in the evening. The material remained in the aircraft during this period. Only about one third of the plot was sprayed on June 8 due to deteriorating weather conditions and it was noted that, on the return to the airport, there was heavy foam on the formulation. The application, made with Micronair, was completed on the morning of June 9. Meanwhile larval development on this plot had passed the peak of the fourth instar and larvae were mainly in the sixth instar on white spruce hosts (Table II).

On the evening of June 9, plot No. 3 was sprayed with boom and nozzle equipment. Larval development was slower on this plot due to its proximity to Lake Huron and the spruce budworm were still at the peak of the fourth instar. Weather conditions were most favourable for the application.

#### Spray Deposit Monitoring and Analysis

Spray deposit was monitored using Kromekote<sup>R</sup> spray cards on 6mm plywood backings. Cards were placed at 15 m intervals along the road transversing each plot, and to a distance of 46 m outside plot boundaries to monitor drift.

The cards were analyzed to determine the mean number of droplets per cm<sup>2</sup> and percentage of droplets in 9 size categories. The number of droplets were counted in five lcm<sup>2</sup> on each card and measured in

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#### Budworm larval development when plots were sprayed

				Nc. of	Percen	Instar		
Plot No.	Date Spray	yed	Tree Species	larvae examined	III	IV	V	VI
1	June	3	bF	236	8	75	16	1
			wS	104	6	56	35	3
2	June	9	bF	57		42	26	32
			wS	41		24	10	66
3	June	9	t F	100		70	27	3
			wS	100	3	62	32	3
4 *	June	4						
5*	June	4						

parable to plot No. 1.

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lcm<sup>2</sup> only. The halos around some droplets due to spread, were disregarded when measurements were made. A Wilde M5 binocular microscope fitted with a calibrated eyepiece was used for the observations. <u>Insect sampling 1) to determine the level of virus</u> infection and 2) to study population reduction

1) To obtain insects for microscopic diagnosis of viral and protozoan infection, samples of 20 white spruce 46 cm branch tips and 20 balsam fir 46 cm branch tips from mid-crown were collected from plots No. 1, No. 4 and No. 5 on June 11, June 19 and June 25, 26. June 11 was one week after the spray application on these plots. The same number of samples were taken from plots No. 2 and No. 3 on June 19 and June 25. Trees were selected so that each set of samples transversed that particular plot. Ten samples of each species were taken from three check areas, No. 1, No. 2 and No. 3, near plots No. 1 and No. 3 and from one check area, No. 6, near plots No. 4 and No. 5 on June 18,19 and June 25. Larvae and pupae from these samples were dissected and examined microscopically to determine the presence of NPV, cytoplasmic polyhedrosis virus (CPV), entomopoxvirus (EPV) and microsporidia as in previous years (Cunningham et al., 1974).

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2) Population reduction studies required a prespray and post-spray sample. The pre-spray sample was taken on June 8, and consisted of 25 white spruce and 25 balsam fir 46 cm branch tips collected at mid-crown. This was 5 days after the application on plot No. 1, however, NPV takes at least 10 days to kill spruce budworm larvae and no virus-associated mortality would occur in this time interval. Ten check plots were sampled at the same time and 15 samples of each species collected from each plot.

The same number of branch tips were collected for the post-spray (pupal) sample on July 1. Foliage examination was conducted in the same manner as in previous operations (Cunningham <u>et al</u>, 1974) and Abbott's formula (Abbott, 1925) was used to calculate the effectiveness of each application.

#### Defoliation estimates

Estimates of the degree of current year's defoliation were obtained by examination of the 46 cm branch tips collected for the pupal samples from treated plots and check areas after spruce budworm larvae had ceased feeding.

#### RESULTS

#### Deposit Analysis

The mean number of droplets/cm<sup>2</sup> recorded on spray cards for each of the experimental plots is shown on Table III. The largest number of droplets was found in plot No. 1 where Micronair equipment was used. Micronair equipment was also used on plot No. 2 but the number of droplets on the cards probably does not reflect the true deposit as the road where the cards were placed was narrow and there was a heavy overstory of poplar. The number of droplets/cm<sup>2</sup> was lower on the plots sprayed with boom and nozzle equipment.

The size distribution of the droplets for each plot is shown on Table IV. On plots No. 1 and No. 2 where Micronair was used 85% of the droplets were less than 160  $\mu$ . There was more variation in size in the plots sprayed with boom and nozzle equipment. The droplet spectrum in plot No. 3 was very similar to those plots sprayed with the Micronair equipment with 77% of the droplets less than 160  $\mu$ . In plots No. 4 and No. 5 only 41% and 59% respectively of the droplets were less than 160  $\mu$ .

# Incidence of viruses in spruce budworm in sprayed and untreated areas.

The results of the impact of the virus on plots sprayed with Micronair are given in Table V and plots sprayed with boom and nozzle in Table VI. A total of 4,714 larvae were examined from the treated plots and 1,514 insects from the four check areas. Results for the check areas are given in Table VII. The level of infection was rather low in plot No. 1 in spite of the excellent coverage with maximum levels of virus infection of 12.0% of the insects on balsam fir and 12.7% on white spruce. When samples were taken from this plot on June 19, a sample of larvae, which appeared to be diseased, was hand picked at eye level. Microscopic examination showed that these larvae were all infected with NPV and it is possible that diseased larvae are found mainly on the lower branches. The levels of infection on plot No. 2 were also low, a maximum level of virus infection of 3.8% was found in insects on balsam fir and 5.6% on white spruce.

The results from the plots sprayed with boom and nozzle equipment were much more encouraging. On plot No. 3, maximum levels of virus infection reached were 16.8% of the insects on balsam fir and 40.4% of the insects on white spruce. On plot No. 4 the situation was reversed with a higher level of infection on balsam fir than white spruce, 30.8% of insects on the former species and 17.1% on the latter. In spite of the greatly reduced dosage on plot No. 5 maximum levels of virus infection recorded were 14.5% of insects on balsam fir and 10.1% on white spruce. Traces of CPV were recorded in plots No. 2, No. 4 and No. 5 with 1.2%, 2.0% and 0.6% respectively.

Unexpectedly high levels of virus infection were found in three of the four check plots. On check No. 1, a maximum level of 4.5% of insects on balsam fir were found to be infected with NPV and 9.9% on white spruce; in check No. 2, 3.5% on balsam fir and 12.5% on white spruce and in check No. 3, 1.1% on balsam fir and 9.2% on white spruce. These plots were in close proximity to the 520 ha block sprayed with NPV in 1974 and possible reasons for the high levels of NPV in these check areas are discussed later. Check No. 6 was about 5 km from this area and only a trace (1.1%) of NPV infection was found in insects on balsam fir.

Levels of microsporidia ranged from 4.3 to 26.3% infected insects with a mean of 11.8% when the sprayed plots and checks were analysed together. When analysed separately the mean in the checks was 10.2% and in the sprayed plots 12.8%, so it is concluded that spraying NPV has no effect on the level of natural microsporidia in the spruce budworm population. Population reduction, pupal survival and current defoliation

The results of larval mortality, pupal mortality and foliage protection attributed to the NPV spray application are given in Table VIII. Three of the ten check areas could not be used to compute the population reduction figures due to the high levels of virus infected insects on them. It can be seen that the results from plots No. 1, No. 3 and No. 4 were excellent with very heavy mortality on white spruce, 91%, 91% and 84% respectively. Plots No. 3 and No. 4 also had good spruce budworm kill on balsam fir with 53% and 65% death due to virus. It is not known why plot No. 1 had only 21% kill on balsam fir.

The NPV did not appear to have any effect on pupal emergence and there was no detectable foliage protection which could be attributed to the NPV. This is to be expected since larvae were not sprayed until the fourth or fifth instar and they continued feeding for ten days or longer.

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# Table III

# Mean number of droplets per cm<sup>2</sup> on spray cards

Plot	Application	Mean Number Drops/cm <sup>2</sup>	Standard Deviation
1	Micronair	167	91
*2	Micronair	38	26
3	Boom and Nozzle	32	15
4	Boom and Nozzle	22	8
5	Boom and Nozzle	29	8

\*Problem with deciduous overstory.

## Table IV

# Percentage of droplets on spray cards in different size categories

Size of droplets in microns

Plot	Up to 40	41-80	81-120	121-160	161-200	201-400	401-600	601-800	801-1000	1
1	10.2	23.8	36.7	18.0	8.6	2.7	0	0	0	- 24 -
2	19.1	27.3	20.8	17.5	9.9	5.4	0	0	0	
3	14.7	32.5	21.1	8.9	6.5	12.1	3.5	0.7	0.3	
4	1,3	6.9	16.3	16.5	11.9	31.0	13.4	2.2	0.5	
5	2.8	15.8	23.0	17.5	14.0	18.5	5.6	2.5	0.3	

## Table V

## Incidence of viruses and microsporidia in plots sprayed with NPV using Micronair equipment on Manitoulin Island, Ontario in 1975

Plot No,	Sample date	Tree species	Number of insects examined	Percent viru NPV	is infection CPV	Percent microsp
1	June 11	bF	344	2.9		9.9
1000		wS	373	4.0		18.0
	June 19	bF	200	12.0		8.0
		WS	267	5.2		16.1
	June 25	ЪF	164	4.9		6.7
		wŞ	110	12.7		25.5
2	June 19	bF	161	2.5	1.2	11.8
		wS	171	0.6		11.7
	June 25	bF	159	3.8		4.4
		wS	179	5.6		26.3

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# Table VI

Incidence of viruses and microsporidia in plots sprayed with NPV using boom and nozzle equipment on Manitoulin Island, Ontario in 1975

Plot No.	Sample date	Tree species	Number of insects examined	Percent vir NPV	us infection CPV	Percent microsp.
3	June 19 June 25	bF wS bF wS	245 <b>309</b> 119 109	1.6 9.4 16.8 40.4		17.1 17.8 10.1 29.4
4	June 11 June 19 June 26	wS bF wS	117 146 120 88 104 49	4.3 1.4 30.8 17.1 4.8 10.2	2.0	7.7 14.4 5.8 3.4 9.6 10.2
5	June 11 June 18 June 26	bF wS bF wS	180 244 159 227 176 194	1.7 2.5 14.5 10.1 7.4 4.1	0.6	7.2 14.3 8.8 13.2 9.1 16.0

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# Table VII

# Incidence of viruses and microsporidia in check areas on Manitoulin Island, Ontario in 1975

Check No.	Sample date	Tree species	Number of insects examined	Percent virus in NPV	nfection. Percent Microsp.
	June 19	bF	80	0	10.0
1	June 19	wS	109	1.8	5.5
	X	bF	111	4.5	11.7
	June 25	wS	71	9.9	8.5
				0	6.2
2	June 19	bF	81	0.6	10.1
		wS	159	3.5	11.8
	June 25	ЪF	85	12.5	6.3
		wS	32	12.5	
3	June 19	bF	172	0.6	11.2
2	June 17	wS	187	1.1	
	June 25	bF	94	1.1	4.3
	June 25	wS	76	9.2	21.1
	1		48	0	6.3
6	June 18	bF	85	0	20.0
	- 05	wS	88	1.1	5.7
	June 25	bF	36	0	25.0
		wS	20		

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Table VIII

Population reduction (adjusted for natural mortality), pupal survival and current defoliation in five plots sprayed with NPV on Manitoulin Island, Ontario in 1975

	Pre-spray larvae/46cm branch típ		Surviving pupae/46cm branch tip		% Population reduction due to NPV spray		% Successful Pupal Emgernce <sup>a)</sup>		% Current Defoliation in 1975	
Plot	bF	wS	bF	wS	bF	wS	ЪF	wS	bF	wS
1	33.8	43.4	7.48	. 52	21	91	90	76	98	70
Check	28.65	39.9	8.00	5.62			68	83	92	79
2	11.4	16.6	3.40	4.16	31	18	89	88	18	81
Check	11.0	17.3	4.76	5.31			70	89	75	68
3	38.9	69.1	3.40	.38	53	91	62	38	96	96
Check	30.6	41.5	5.73	2.67			62	74	85	98
4	13.9	28.7	2.31	1.44	65	84	81	95	68	72
Check	15.7	31.9	7.47	9.87			64	95	65	80
5	11.1	32.0	6.44	6.72	0	32	87	95	76	79
Check	15.7	31.9	7.47	9.87			64	95	65	80

a) % Successful Pupal Emergence = Emerged Budworm

Budworm Alive on Sample Date x 100

#### DISCUSSION

The results from three of the plots in the 1975 NPV spruce budworm spray trials are considered most encouraging. The population reductions and levels of virus infection obtained were the highest recorded in five years of field testing of NPV. In 1971, 80% population reduction was recorded on white spruce hosts with 71% virus infection (combined NPV and CPV) (Howse et al., 1973). Here the dosage was six times higher than used on Manitoulin Island and the application rate was 28 <u>1</u>/ha.

The lower levels of population reduction and virus infection on plot No. 2 can be attributed to a combination of factors. Firstly, there was considerable deciduous overstory on the plot which had leafed out by the time of the spray application and impeded the deposit. Secondly, due to unfavourable weather conditions, the application was delayed and 66% of the larvae had reached sixth instar on white spruce and 32% on balsam fir when the spray was eventually applied. Thirdly, the spray formulation was left for 3 days in the aircraft spray tank; heavy foaming was noted when it was circulated with the aircraft pump and a strong smell in the tank indicated that some

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chemical reaction was underway possibly between the aluminum baffles in the tank and the formulation. The levels of virus infection recorded from plot No. 5 led one to believe that population reduction would be satisfactory, but this was not the case. This plot was sprayed with a much reduced dosage of virus, 44 billion PIB/ha instead of the 125 billion/ha applied in plots No. 1, No. 3 and No. 4.

In plot No. 1 the population reduction on white spruce was excellent at 91%, but only 12.7% maximum virus infection was recorded on this species. The reason for this anomaly is not understood although numerous dead larvae in which NPV virus infection was confirmed were observed on lower branches in this plot. It is suggested that diseased larvae may drop to the lower branches and that mid-crown sampling for virus infection gives misleading results.

The reasons for the high levels of virus infection in check areas No. 1, No. 2 and No. 3 are at the moment not known. They were close to plots No. 1 and No. 3 and to the area sprayed in 1974 (figs. 1 and 2) (Cunningham <u>et al</u>, 1975b). The check area used for infectivity studies in 1974 was located in the centre of 1975 spray plot No. 1 and no virus was recorded there in 1974. These high levels of virus infection in check areas cast some doubt on the results obtained in spray plots No. 1 and No. 3. The question arises as to whether there was virus present in them prior to the spray application. Similar results were found in spray plots No. 3 and No. 4 and only a trace of virus was found in check area No. 6 which was close to spray plot No. 4, a point which helps dispel this doubt. Possible explanations for the presence of virus in these check areas are discussed by Cunningham et al., 1975a) and are:- 1) A natural virus in the spruce budworm population. This is unlikely as it was not detected in 1974 2) Spread of virus from the large plot sprayed in 1974. Spread of virus from sprayed areas has not been conclusively proved in previous years and, if this has indeed occurred, the potential of NPV for spruce budworm control is greatly increased. 3) Drift from the plot sprayed in 1974.4) Drift from the plots sprayed in 1975. Spray deposits were closely monitored in both years with spray cards in the plots and beyond the plot boundaries and no detectable drift occurred.

Although there were no replicates of the treatments, two inferences can be drawn from the 1975 spray trial

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results. Firstly, boom and nozzle spray equipment appears to give as good as, if not better results in NPV applications at 9.4 <u>1</u>/ha than Micronair and, secondly, the molasses formulation appears to be as good as the Sandoz adjuvant V and is considerably less expensive. The Sandoz adjuvant cost 2.34/1 and was used at  $4.7 \underline{1}/ha$ Hence the cost per hectare for the additive was 11.00. In contrast the estimated cost of the molasses formulation was about 1.50/ha.

One of the aims of this operation was to introduce NPV into a large area and follow its persistence in the environment and impact on the spruce budworm population. This has now been achieved, 850 ha were sprayed in 1974 and 1975 and the presence of virus in check areas No. 1, No. 2 and No. 3 would indicate that virus is present now throughout at least 1,000 ha in the area morth of Burnt Island in Robinson Township. No further spray applications are planned in this area and the interaction of the virus and the spruce budworm population will be followed for the next few years.

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#### REFERENCES

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. <u>18</u>, 265-267.
- Cunningham, J. C. and J. R. McPhee. 1973. Aerial application of entomopoxvirus and nuclear polyhedrosis virus against spruce budworm at Chapleau, Ontario, 1972. Can. For. Serv. Sault Ste. Marie. Inf. Rep. IP-X-3.
- Cunningham, J. C., J. R. McPhee, G. M. Howse and A. A. Harnden. 1974. 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. Can. For. Serv. Sault Ste. Marie. Inf. Rep. IP-X-4.
- Cunningham, J. C., W. J. Kaupp, J. R. McPhee, G. M. Howse and A. A. Harnden. 1975a. Aerial application of nuclear polyhedrosis virus against spruce budworm on Manitoulin Island, Ontario in 1974 and a survey of the impact of the virus in 1975. Can. For. Serv. Sault Ste. Marie, Inf. Rep. IP-X-8. Cunningham, J. C., W. J. Kaupp, J. R. McPhee, W. L. Sippell and C. A. BArnes. 1975b. Aerial

application of a nuclear polyhedrosis virus to control

European pine sawfly, <u>Neodiprion sertifer</u> (Geoff.), at SAndbanks Provincial Park, Quinte Island, Ontario in 1975. Can. For. Serv. Sault Ste. Marie Inf. Rep. IP-X-7.

- Howse, G. M., C. J. Sanders, A. A. Harnden, J. C. Cunningham, F. T. Bird and J. R. McPhee. 1973. Aerial application of viruses against spruce budworm, 1971. Part A. Impact in the year of application (1971). Part B. Impact in the year following application (1972). Can. For. Serv. Sault Ste. Marie Inf. Rep. O-X-189.
- Morris, O. N., J. A. Armstrong, M. J. Hildebrand, G. M. Howse, A. A. Harnden, J. C. Cunningham and J. R. McPhee. 1972. Aerial applications of virusinsecticide combinations against spruce budworm, <u>Choristoneura fumiferana</u> (Clem.) (Tortricidae: Lepidoptera) at Rankin, Ontario, 1972. Can. For. Serv. Ottawa Inf. Rep. CC-X-37.

Stelzer, M. J., J. Neisess and C. Y. Thompson. 1975. Aerial application of a nucleopolyhedrosis virus and <u>Bacillus</u> thuringiensis against Douglas fir tussock moth. J. Econ. Ent. <u>68</u>, 269-272.