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AERIAL APPLICATION OF SPRUCE BUDWORM BACULOVIRUS: REPLICATED TESTS WITH AN AQUEOUS FORMULATION AND A TRIAL USING AN OIL FORMULATION IN 1978.

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ABSTRACT

Six 20-ha plots and one 14-ha plot, located north of Thessalon, Ontario, containing predominantly white spruce trees, *Picea glauca* (Moench) Voss, with some balsam fir, *Abies balsamea* (L.) Mill., were aerially sprayed with nuclear polyhedrosis virus to control spruce budworm, *Choristoneura fumiferana* (Clem.). Larvae were mainly in the fifth and sixth instars at the time of application. A dosage of 750 billion polyhedral inclusion bodies/ha was applied with boom and nozzle spray equipment on all plots in an emitted volume of 9.4 ℓ/ha. Six of the plots were treated with an aqueous formulation containing 250 ml/ℓ molasses, 250 ml/ℓ Sandoz Shade® and 0.5 ml/ℓ Triton B-1956® spreader sticker. The seventh plot was treated with an emulsifiable oil formulation containing 650 ml/ℓ Sunoco Sunspray 11E® oil and 350 ml/ℓ water, with rhodamine B dye as a tracer.

The population reduction of spruce budworm larvae due to the treatment was monitored on white spruce hosts in all 7 plots and on balsam fir in 4 plots. In the 6 plots treated with the aqueous formulation, population reduction due to treatment ranged from 33% to 92% and in the 3 plots where balsam fir hosts were studied, population reduction ranged from 37% to 76%. In the plot sprayed with the emulsifiable oil formulation, population reduction was 33% on white spruce hosts and 41% on balsam fir. As larval development was more advanced in this plot with 82% in the sixth instar, these lower population reduction figures do not necessarily mean that the oil formulation was inferior to the aqueous.

In addition to the population studies, the impact of the virus was monitored (i) by sampling larvae 5 to 9 days post-spray, rearing them in the laboratory and determining mortality, (ii) by collecting insects in treated and check plots, dissecting them and examining them microscopically for the presence of pathogens, (iii) by rearing pupae in the laboratory and determining emergence and (iv) by estimating current year's defoliation in treated and check plots.

RESUME

Six places-échantillons de 20-ha et une autre de 14-ha situées au nor de Thessalon (Ontario), dont les arbres prédominants étaient des épinettes blanches, *Picea glauca* (Moench) Voss, et quelques sapins baumiers, *Abies balsamea* (L.) Mill., ont été arrosées par voie aérienne avec un virus à polyèdres nucléaires pour lutter contre la tordeuse des bourgeons de l'épinette, *Choristoneura fumiferana* (Clem.). Au moment du traitement, les larves étaient surtout du cinquième et du sixième instars. On a appliqué une dose de 750 milliards de virus polyèdres/ha avec un dispositif d'arrosage à tangon et à lance sur toutes les places-échantillons à raison de 9.4 ℓ/ha. On en a traité 6 avec une solution aqueuse contenant 250 ml/ℓ de mélasse, 250 ml/ℓ de gommant Sandoz Shade® et 0.5 ml/ℓ de Triton B-1956®; pour la septième, on a utilisé une solution huileuse émulsifiable contenant 650 ml/ℓ d'huile Sunoco Sunspray 11E® et 350 ml/ℓ d'eau avec teinture traçante rhodamine B.

On a surveillé de près la diminution des populations de larves de la tordeuse due au traitement sur les épinettes blanches hôtes dans les 7 places-échantillons et sur les sapins baumiers dans 4. Dans les 6 places-échantillons traitées avec la solution aqueuse, les diminutions de populations étudiées dont les arbres hôtes étaient des sapins baumiers les diminutions de populations ont varié de 37 à 76%. Dans la place-échantillon arrosée avec la solution huileuse émulsifiable, la diminution des populations de larves atteignait 33% sur l'épinette blanche et 41% sur le sapin baumier. Etant donné que le développement larvaire était plus avancé dans cette place-échantillon (82% au sixième instar), ces chiffres indiquant une diminution moindre des populations ne signifient pas nécessairement que la solution huileuse était inférieure à la solution aqueuse.

En plus des études de populations, on a surveillé l'influence du virus (i) par échantillonnage des larves 5 à 9 jours après le traitement en les élevant en laboratoire et en déterminant la mortalité, (ii) par la cueillette des insectes dans les places-échantillons traitées et non traitées, en les disséquant pour examiner les souillures pathogènes au microscope, (iii) par l'élevage des pupes en laboratoire pour noter l'apparition des insectes adultes et (iv) par l'évaluation de la défoliation durant l'année en cours dans les places-échantillons traitées et non traitées.

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INTRODUCTION

The genus *Baculovirus* includes nuclear polyhedrosis viruses (NPV) and granulosis viruses (GV) (Wildy 1971). Such viruses bear no morphological resemblance to any known plant or vertebrate viruses and are considered safe to vertebrates and non-target invertebrates when used as biocontrol agents (Anon. 1973). Several different kinds of viruses are known to infect the eastern spruce budworm, *Choristoneura fumiferana* (Clem.). Most of the attention has been focussed on NPVs and isolates from eastern spruce budworm, western spruce budworm, *C. occidentalis* Free., and a species from Japan, *C. diversana* Hbn., have been compared in eastern spruce budworm larvae in laboratory tests. All three isolates appeared to be equally pathogenic and it would take more detailed biochemical studies to ascertain if they are, in fact, one and the same virus. When the *C. occidentalis* and *C. fumiferana* isolates were compared in a field trial in 1977, their efficacy was similar (Cunningham *et al.* 1978).

Aerial spray trials have been conducted with spruce budworm NPV every year since 1971 and by 1978 a total of 1,656 ha were treated with this virus. These spray trials were generally conducted on an *ad hoc* basis and such parameters as dosage, volume emitted, timing of application, formulation and spray delivery equipment have been evaluated (Howse *et al.* 1973, Cunningham and McPhee 1973, Cunningham *et al.* 1974, 1975*a*, 1975*b*, 1978, Kaupp *et al.* 1978). Little or no foliage protection has been obtained in the year of NPV application but the long-term effects of the treatment are considered to be of major importance. Following the trials in 1971, two stands of white spruce were closely monitored for several years. The NPV persisted well and significant levels of infection were found in the spruce budworm population until 1975 when the virus virtually disappeared. There was no foliage saved in the year of application but in subsequent years the foliage saved, although not spectacular, was sufficient to prevent tree mortality (Cunningham *et al.* 1975*c*).

Following the tests conducted in 1977, it was concluded that best results were obtained with the highest dosage tested, 750 billion polyhedral inclusion bodies (PIB)/ha, and the best formulation tested contained 25% molasses and 60 g/ℓ Sandoz Shade® (Cunningham *et al.* 1978). Trials in previous years indicated that boom and nozzle spray equipment gave better NPV infection than did Micronair (Cunningham *et al.* 1975*b*) and a recent attempt to infect highly susceptible, needle-mining second instar larvae gave very disappointing results (Kaupp *et al.* 1978). Generally, higher infection levels and greater mortality rates have been recorded in spruce budworm larvae on white spruce hosts than on balsam fir hosts. It is postulated that the effect of the NPV is density dependent and the higher insect populations on white spruce are more susceptible to a virus epizootic.

Following 7 years of *ad hoc* tests, it was decided to consolidate these results by replicating on 6 plots the best treatment studied to date. In addition to these replicated tests a further test to determine the efficacy of an emulsifiable oil formulation was added.

As in previous years, Forest Pest Management Institute staff selected the plots, conducted the spray application and determined levels of virus infection. Dr. G.M. Howse of the Great Lakes Forest Research Centre calculated the spruce budworm population reduction due to treatment, determined pupal survival rates and estimated current year's defoliation.

MATERIALS AND METHODS

Virus Production

During the winter months of 1977-78, a total of 1,646,000 late fifth and early sixth instar budworm larvae were infected by contaminating the surface of diet in cups with 0.3 ml of virus suspension containing 5 x 10^7 PIB/ml. Diseased larvae were picked off after 8 days; those which still appeared healthy and active were left for a further 2 days and then harvested. When lyophilised and processed, these virus-infected larvae yielded 15.7 kg of material containing 1.2 x 10^{10} PIB/g.

Experimental Plots

The experimental plots were located in Kirkwood, Bridgland and Rose townships, north of the town of Thessalon, Ontario. The plots in Bridgland Twp. had been treated with NPV in 1976 (Kaupp *et al.* 1978). A low dosage of virus was applied on second instar larvae. Poor initial infection was obtained and virus carry-over in 1977 was negligible. The one plot in Kirkwood Twp. had been treated with a juvenile hormone analogue RO_{10}- 3108 in 1976 (Retnakaran, Howse and Kaupp 1978). The location of the 7 treated plots and 7 check areas are shown in Figure 1 and a description of the stand composition of the treated plots is given in Table 1. All plots were 20 ha in size except plot 7 which was 14 ha. White spruce, *Picea glauca* (Moench) Voss, was the dominant species in all the plots and where balsam fir, *Abies balsamea* (L.) Mill., was sufficiently abundant, spruce budworm larvae were also monitored on this species. There was insufficient balsam fir for study in plots 1, 3 and 5. Details of the age and height of the white spruce trees are also given in Table 1. There was no problem of hardwood overstory except in plot 7 where the poplar did present a slight obstruction to the spray deposit.

Virus Formulation and Dosage

The dosage of virus on all 7 plots was 750 billion PIB/ha in a volume of 9.4 ℓ/ha. The same aqueous formulation was applied on plots 1 to 6. It contained 25% v/v animal feed grade molasses, 60 g/ℓ Sandoz

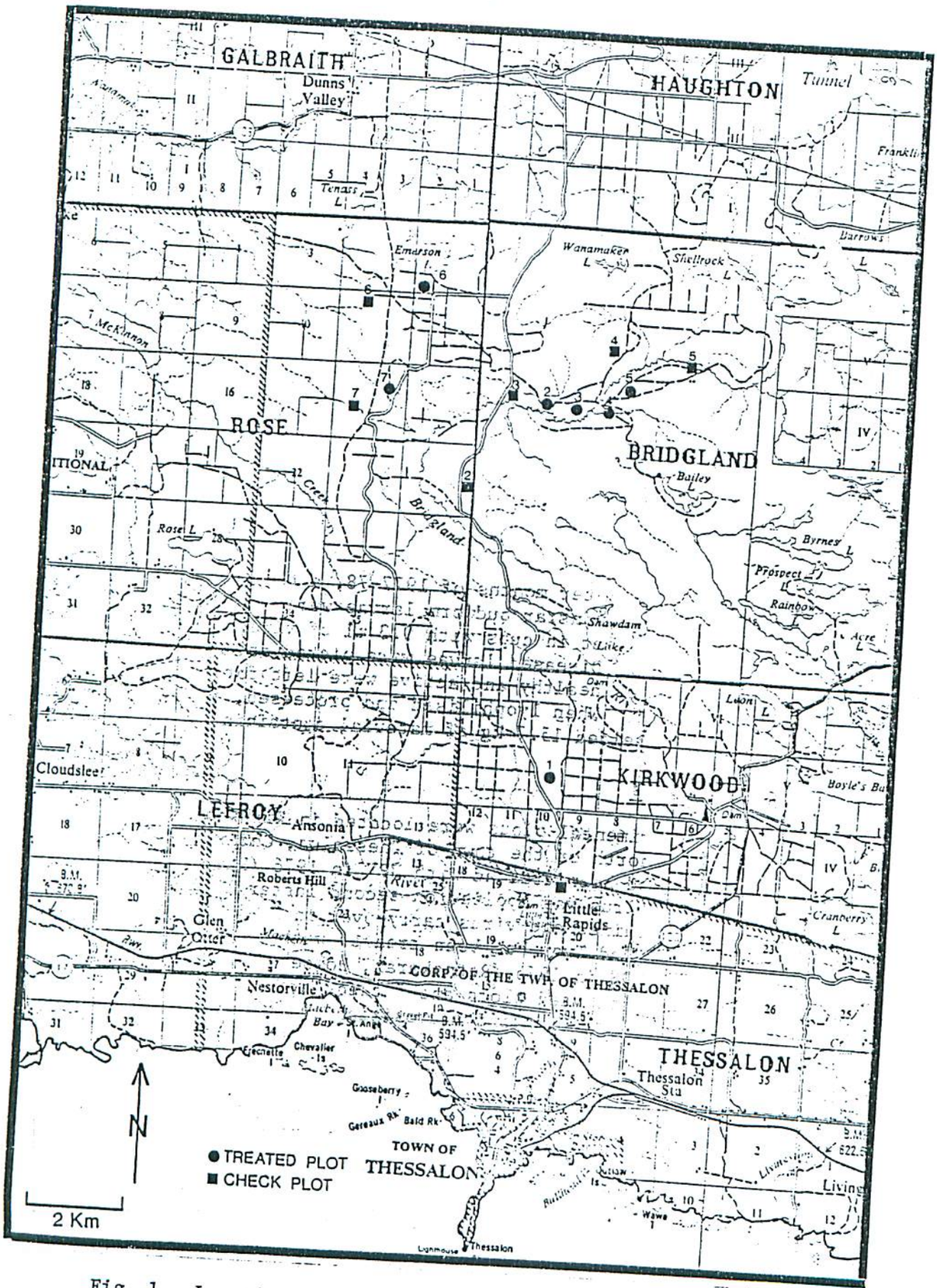

Fig. 1. Location of NPV-treated plots and check plots in Thessalon, Bridgland and Rose townships, Ontario.

Table 1. Description of plots treated with NPV in 1978.

Plot no.	Location (Twp.)	Size (ha)	Stand composition (percent species)							Age of white spruce (yr)	Height of white spruce (m)
			White spruce	Balsam fir	Poplar	Jack pine	White pine	Black spruce	White birch		
1	Kirkwood	20	30		30		40			40	15
2	Bridgland	20	80	5	15					42	16
3	Bridgland	20	100							42	16
4	Bridgland	20	60	10	10	10		10		44	17
5	Bridgland	20	70	10		20				49	17
6	Rose	20	50	10			40			55	14–17
7	Rose	14	30	10	20			20	20	20–50	10–15

Shade® sunlight protectant and 1% Triton B-1956® spreader sticker. Plot 7 was treated with an emulsifiable oil formulation containing 65% v/v Sunoco Sunspray 11E® oil and 35% water with rhodamine B dye added as a tracer.

Spray Application and Larval Development

The Forest Pest Management Institute Cessna 185E fitted with a boom and 20 8010 Tee jet nozzles flying a 30 m swath width at 176 km/hr delivered 9.4 ℓ/ha. Inclement weather delayed the spray application by over a week and the larvae were mainly in the fifth and sixth instars when treated. It was intended to spray the NPV when they were in the fourth and fifth instars. The dates of the applications and the insect development at that time are given in Table 2.

Table 2. Date of application of NPV on spruce budworm larvae and insect development on that date.

Plot number	Date of application	Insect Development (percent instars) IV	V	VI	Pre-pupae
1	June 3rd evening	11	55	34	0
2	June 4th morning	4	61	35	0
3	June 5th evening	19	42	39	0
4	June 6th morning	25	39	36	0
5	June 6th morning	18	36	46	0
6	June 4th morning	21	62	17	0
7	June 6th morning	2	5	82	2

Spray application commenced on plot 1 at 9:20 p.m. on June 3rd and was completed by 9:56 p.m. The temperature was 11°C and the R.H. 93%. On June 4th, spraying commenced at 6:20 a.m. on plot 6 and was abandoned at 6:35 a.m. because chips of fiberglass from a new spray tank and brass filings from a new boom blocked the nozzles. The

nozzles were cleaned at the airport and spraying recommenced at 7:05 a.m. and by 7:15 a.m. the treatment on plot 6 was completed. The temperature was 7°C and the R.H. 100%. Plot 2 was then treated between 7:55 a.m. and 8:23 a.m. Because problems were still encountered with blocked nozzles, the emission rate was greatly reduced and many extra passes had to be made in order to deliver the load on the plot. There was a slight east to west wind at less than 5 km/hr, temperature was 9°C and R.H. at the start of the spray was 100% falling to 86% at the finish. About 11 a.m. on June 4th, it started to rain and 7.5 mm fell.

Plot 3 was sprayed between 9:30 and 9:52 p.m. on June 5th. The temperature was 11°C and the R.H. 93%. On June 6th, the remaining 3 plots were sprayed in the morning. Operations commenced at 5:55 a.m. on plot 5 which was sprayed by 6:16 a.m. The temperature was 6°C and the R.H. 100%. Then plot 4 was treated between 6:30 a.m. and 6:41 a.m. The temperature and R.H. remained the same. Finally plot 7 was sprayed with the oil formulation between 7:11 and 7:23 a.m. The temperature rose to 9°C; the R.H. was 100% at the start, dropping to 95% at the finish.

Monitoring the Deposit

Plots were located so that roads running through them were at right angles to the flight lines. Prior to the application, Kromekote® spray cards on aluminum backings were placed at 15-m intervals within the plots and to a distance of 50 m outside the plot boundaries to monitor drift.

To analyse the droplet spectrum the number of droplets/cm^2 were counted on 5 cm^2 of each card and were measured on 1 cm^2. A microfilm reader with a calibrated screen was used for the counting and the same device was used for measuring droplets. A plastic sheet with circles inscribed on it was used to measure the diameter of the magnified droplets on the screen and they were recorded in 10 size categories. This equipment and instructions on its use were kindly supplied by Mr. A.P. Randall, FPMI.

Meteorological Data

Temperature and rainfall were recorded from May 24th to June 26th in plots 1 and 2 using a recording thermograph and a standard rain gauge.

Assessment

(a) Laboratory Rearing of Larvae to Determine the Initial Impact of the Spray Deposit

Between 5 days and 9 days post-spray 46-cm branch tip samples were collected at mid-crown in the treated and check plots. Three branches were taken at mid-crown from 20 white spruce in each of the treated plots and from 20 balsam fir in plots 2, 4, 6 and 7. From the 7 check areas, samples were collected from 15 white spruce and 15 balsam fir. Larvae were picked off the foliage and placed individually in 20 ml plastic cream cups containing artificial diet (McMorran 1965). They were reared until pupation or death occurred and dead larvae were examined microscopically to determine the cause of death.

(b) Microscopic Examination of Samples of Spruce Budworm to Determine Levels of Infection with NPV and Other Pathogens

The NPV-treated plots were sampled 3 times, on June 15th, 20th and 26th-27th and the check areas once, on June 20th. Two 46-cm branch tips were taken at mid-crown; 10 white spruce trees were sampled in all plots and 10 balsam fir in plots 2, 4, 6 and 7. In the 7 check areas, 5 white spruce and 5 balsam fir trees were sampled the same way. Larvae were removed from the foliage and squash preparations of the fat body and gut tissue were examined microscopically using phase contrast optics. All pathogens observed were recorded.

(c) Sampling for Population Reduction Studies

Pre-spray samples were collected on June 1st, 2nd and 3rd from the 7 NPV-treated plots and the 7 check areas. Two 46-cm branch tips were taken at mid-crown and either 20 or 25 marked white spruce trees were sampled in all treated plots and either 20 or 25 balsam fir trees were sampled in plots 2, 4, 6 and 7. In the check areas, 15 white spruce and 15 balsam fir trees were sampled the same way. Post-spray samples were taken from the same marked trees on July 4th, 5th and 6th by which time most of the spruce budworm larvae had pupated.

Larvae were removed from the foliage using the "drum method" (DeBoo, Campbell and Copeman 1973, Martineau and Benoit 1973) and counted. Pupal samples were picked by hand. Abbott's formula was used to calculate the population reduction of spruce budworm due to NPV treatments (Abbott 1925).

(d) Pupal Emergence

Pupae from the post-spray sample were kept and maintained at room temperature until adult emergence occurred. Percent successful pupal emergence was calculated as follows:

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

(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 the time it was collected, all surviving larvae had pupated and feeding had ceased.

RESULTS

Deposit Assessment

The spray deposit on all 7 plots was considered good to very good. The mean number of droplets/cm^2 for each plot is given in Table 3. For the aqueous formulation, the range was from 37 to 73 drops/cm^2 and the oil formulation had the lowest number of droplets with 28/cm^2. The droplet spectra of the plots treated with the aqueous formulation are shown in Figure 2. In all the plots more than 60% of the droplets were smaller than 150μ and in plot 3, 80% were less than 150μ. No droplets exceeded a diameter of 550μ. The droplet spectrum in plot 7 treated with the emulsifiable oil formulation is shown in Figure 3. Here only 44% of the droplets had a diameter of less than 150μ and large droplets ranged up to 1150μ.

Table 3. Mean number of droplets/cm^2 recorded on Kromekote® cards following application of NPV formulations in 1978.

Plot	Mean number of droplets/cm^2	Standard deviation
1	47	11
2	66	22
3	47	15
4	53	14
5	73	21
6	37	12
7	28	6

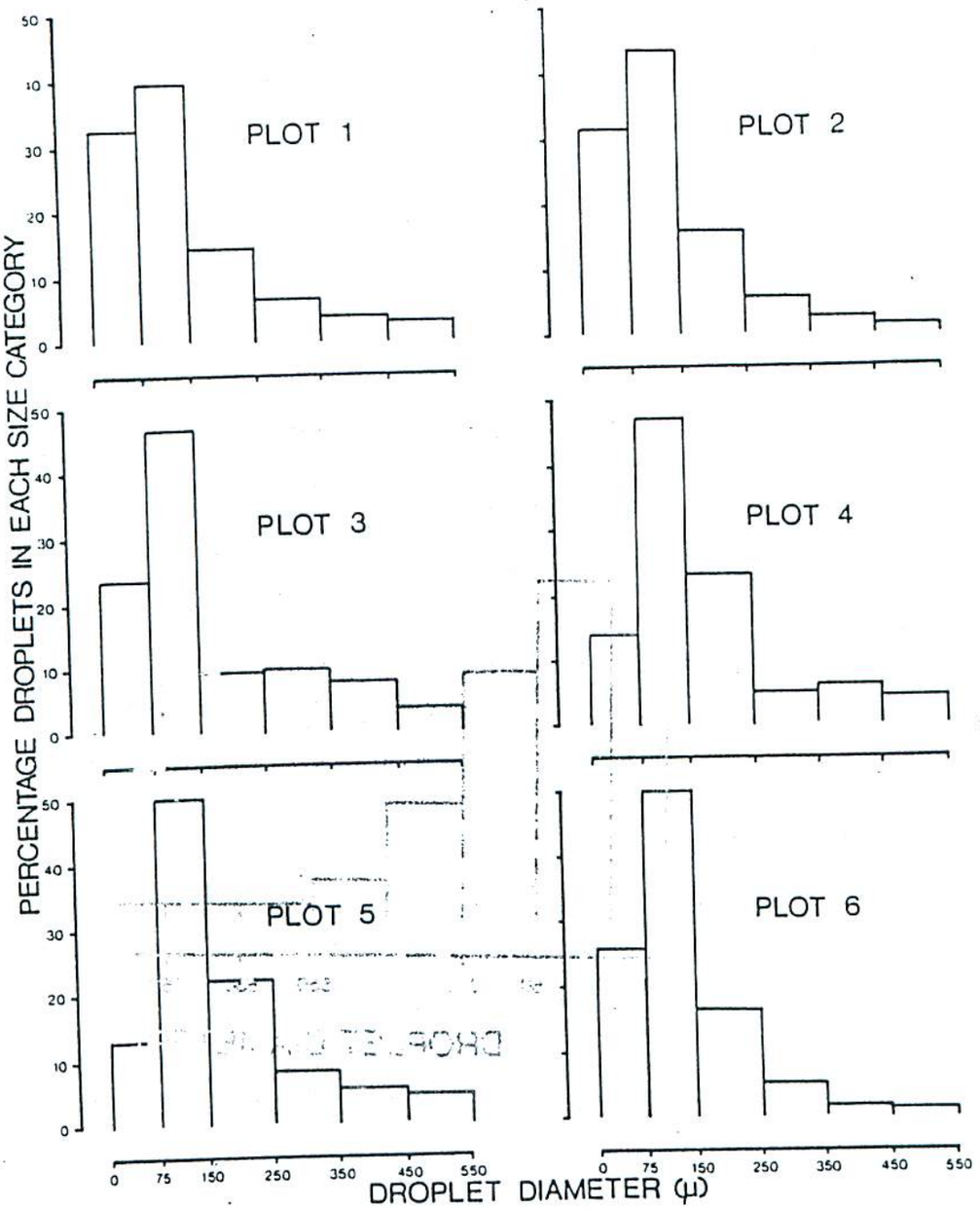

Fig. 2. Diameter of spray droplets on Kromekote® cards from plots treated with the aqueous formulation.

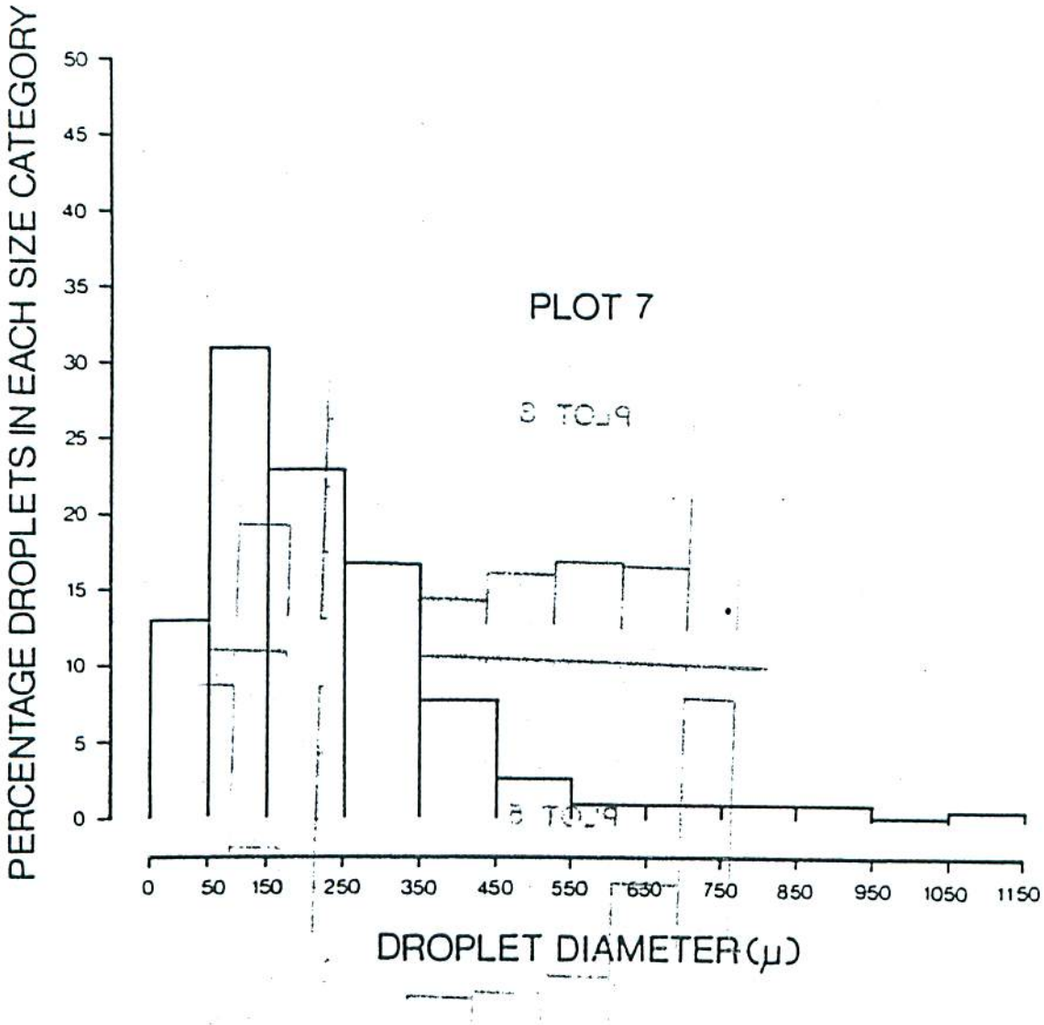

Fig. 3. Diameter of spray droplets on Kromekote® cards from the plot treated with the emulsifiable oil formulation.

Meteorological Data

Temperature and rainfall were recorded in plot 1 and plot 2 from May 24th to June 26th. On plot 1 the mean minimum temperature was 5.6°C and the mean maximum temperature 23.1°C with temperatures below freezing point on 4 nights. The total precipitation was 5.68 cm. On plot 2, the mean minimum and maximum temperatures were 4.5°C and 22.8°C with temperatures below 0°C on 5 nights. The total precipitation was 6.26 cm.

Mortality in Larvae Reared Individually in the Laboratory

Individually reared larvae collected 5 to 9 days post-spray which subsequently died in the laboratory were examined microscopically to determine the cause of death. The only pathogen found was NPV. The number of insects reared from the treated and check plots is given in Table 4 along with the percent mortality due to NPV. The only mortality in the check areas was in check plot 5 where 0.6% NPV mortality was recorded in larvae from balsam fir hosts.

Table 4. Mortality from NPV recorded in spruce budworm collected on June 9th, 13th and 14th from treated and check plots and reared on synthetic diet.

		Treated Plots		Check Plots	
Plot number	Tree species	Number of insects reared	Percent NPV mortality	Number of insects reared	Percent NPV mortality
1	bF	-	-	95	0
	wS	314	20.1	301	0
2	bF	275	34.2	180	0
	wS	391	32.2	359	0
3	bF	-	-	156	0
	wS	319	25.1	261	0
4	bF	180	32.8	130	0
	wS	300	29.3	326	0
5	bF	-	-	157	0.6
	wS	383	29.2	266	0
6	bF	126	37.3	233	0
	wS	350	24.6	307	0
7	bF	293	39.9	70	0
	wS	397	22.2	259	0

In the plots treated with the aqueous formulation, NPV mortality in budworm larvae from white spruce hosts ranged from 20.1% to 32.2% and from balsam fir hosts NPV mortality ranged from 32.8% to 37.3%. In plot 7, treated with the emulsifiable oil formulation, 39.9% NPV mortality was found in budworm larvae from balsam fir hosts and 22.2% in budworm larvae from white spruce hosts.

Levels of Virus Infection and Other Pathogens Determined by Microscopic Diagnosis

Other than the NPV which was disseminated in the spray application, the only pathogen present in this spruce budworm population was the microsporidian, *Nosema fumiferanae* (Thom.). Some parasites were found during dissection of the larvae and were also recorded. The levels of NPV, microsporidia and parasites recorded from the collections made at 3 different dates from the treated plots are presented in Table 5. When the highest recorded levels of NPV in each plot were compared, levels on white spruce hosts treated with the aqueous formulation ranged from a low of 28.1% on plot 1 to a high of 57.1% on plot 4. On balsam fir hosts, levels ranged from 16.4% on plot 4 to 25.5% on plot 6. On plot 7, treated with the oil formulation, the highest recorded level of NPV infection in larvae on white spruce hosts was 52.1% and on balsam fir hosts was 25.9%

The results of the microscopic examination of insects from the one sample from the check plots are given in Table 6. No NPV infection was found in any of the larvae. Very high levels of microsporidial infection were found in larvae from both the treated and check plots. From larvae on white spruce hosts, maximum levels of microsporidial infection in individual plots ranged from 38.6% to 78.6%, and on balsam fir hosts from 16.7% to 52.8% (Tables 5 and 6).

Population Reduction, Pupal Survival and Defoliation Studies

In order to calculate the population reduction due to the NPV treatment using Abbott's formula, check plots with pre-spray spruce budworm population counts nearest to the pre-spray counts on treated plots were compared. However, it was necessary to deviate from this rule in calculating the population reduction on white spruce in plot 1. Originally a population reduction of 0% was determined, but it was obvious from rearing and microscopical studies that the virus has had some impact. Hence, the survival in the check plot with the next closest pre-spray count was compared to treatment plot 1. The pre-spray and post-spray population counts per 46-cm branch tip and the calculated population reductions due to treatments are given in Table 7.

Table 5. Incidence of NPV, microsporidia (microsp.) and parasites in spruce budworm following application of NPV at 750 billion PIB/ha. Insects examined microscopically to determine infection.

Plot	Sample date	Tree species	Number of insects examined	Percent infection		
				NPV	Microsp.	Parasites
1	15 June	wS	191	12.6	48.7	0
	20 June	wS	175	18.9	48.0	1.1
	26 June	wS	89	28.1	46.1	4.5
2	15 June	bF	126	4.0	44.4	0
	15 June	wS	173	9.3	57.2	5.2
	20 June	bF	64	15.6	40.6	0
	20 June	wS	88	23.9	47.7	5.7
	26 June	bF	48	20.8	39.6	0
	26 June	wS	26	11.5	42.3	0
3	15 June	wS	188	5.9	56.4	2.7
	20 June	wS	102	35.3	71.6	2.9
	26 June	wS	14	28.6	50.0	0
4	15 June	bF	107	0	41.1	0
	15 June	wS	157	4.5	44.6	2.6
	20 June	bF	121	5.8	29.8	1.7
	20 June	wS	139	31.7	59.0	2.9
	26 June	bF	67	16.4	47.8	0
	26 June	wS	14	57.1	78.6	0
5	15 June	wS	189	4.2	46.6	0.5
	20 June	wS	88	28.4	54.6	0
	26 June	wS	16	37.5	37.5	6.3
6	15 June	bF	108	7.4	51.9	0
	15 June	wS	171	7.6	55.0	2.9
	20 June	bF	68	25.0	50.0	0
	20 June	wS	77	45.5	46.8	0
	26 June	bF	51	25.5	49.0	2.0
	26 June	wS	35	31.4	28.6	2.9
7	15 June	bF	170	2.9	49.4	1.8
	15 June	wS	198	1.5	60.1	0
	20 June	bF	126	23.8	46.0	0.8
	20 June	wS	158	16.5	47.5	1.9
	26 June	bF	85	25.9	43.5	1.2

Table 6. Incidence of NPV, microsporidia and parasites in spruce budworm larvae collected from check plots in Kirkwood, Rose and Bridgland townships on June 20, 1978 and examined microscopically.

Check plot number	Tree species	Number of insects examined	Percent Infection		
			NPV	Microsp.	Parasites
1	bF	61	0	36.1	1.6
	wS	22	0	68.2	0
2	bF	57	0	35.1	0
	wS	78	0	46.2	0
3	bF	63	0	25.4	1.6
	wS	83	0	38.6	1.2
4	bF	91	0	52.8	0
	wS	49	0	61.2	0
5	bF	70	0	32.9	0
	wS	64	0	50.0	4.7
6	bF	63	0	30.2	0
	wS	67	0	55.2	1.5
7	bF	24	0	16.7	0
	wS	49	0	49.0	0

In the 6 plots sprayed with the aqueous formulation, population reduction due to treatment ranged from 33% to 92% on white spruce hosts, and in the 3 plots where balsam fir hosts were sampled from 37% to 76% on that species. On plot 7 population reduction was 41% on balsam fir hosts and 33% on white spruce hosts.

Successful pupal emergence was generally lower in the treated plots. Survival was greater on white spruce in plot 1 than in the corresponding check plot and in plot 7 the same was true for balsam fir (Table 7). The most marked reduction in successful pupal emergence was on white spruce hosts in plot 3 where 60% of the adult budworm emerged in the check plot and only 27% in the treated plot.

When the defoliation estimates from the treated and check plots were compared the NPV treatment did not save any of the current year's foliage.

Table 7. Population reduction, pupal survival and current defoliation in seven plots sprayed with NPV in 1978.

Plot	Pre-spray larvae/ 46-cm branch tip		Surviving pupae/ 46-cm branch tip		% Population reduction due to treatment		% Successful pupal emergence		% 1978 Defoliation	
	bF	wS	bF	wS	bF	wS	bF	wS	bF	wS
1		24.4		1.85		33		68		40
Check		28.3		3.23				58		50
2	9.7	48.6	.38	.58	76	57	40	46	58	75
Check	6.2	42.4	1.00	1.20			65	72	6	72
3		34.8		1.20		92		27		72
Check		31.8		2.31				60		61
4	17.3	36.7	1.10	1.30	37	89	40	33	58	66
Check	17.1	31.8	1.73	2.31			53	60	69	61
5		36.0		.22		92		46		76
Check		31.8		2.31				60		61
6	9.6	29.0	.55	.52	64	83	45	55	29	68
Check	6.2	29.8	1.00	3.11			65	76	6	45
7	22.6	63.9	1.56	3.10	41	33	68	62	87	87
Check	21.6	52.9	2.53	3.87			54	76	57	78

DISCUSSION

The trials described in this report are the culmination of 7 years of *ad hoc* testing and the 6 replicates of the aqueous formulation applied at 750 billion PIB/ha represent the optimum dosage and formulation tested to date. The application date was considered to have been 7 to 10 days later than desirable, a fact which was unavoidable due to a prolonged spell of wet and windy weather. Ideally, the application should have been made as soon as the white spruce and balsam fir buds flushed and before any sixth instar larvae were present.

In spite of problems with blocked nozzles the spray deposit was very good on all the plots treated with the aqueous formulation. Boom and nozzle equipment has been favoured over Micronair, as slightly better results were obtained when both types of equipment were compared in 1975 (Cunningham *et al.* 1975*a*). However, it is uncertain if these conclusions were fully justified and the use of Micronair equipment will be considered in future trials. There was a fair proportion of large droplets, 350μ+, when the emulsifiable oil formulation was used; hence, the number of droplets/cm^2 was lower. Sunoco Sunspry 11E® is a fairly viscous oil which may have had a bearing on the droplet spectrum.

Although the treatment with the aqueous formulation was replicated 6 times, it was considered pointless to subject the data to statistical analysis in this type of biological experiment where so many unknown and unrecorded variables played a role. In the laboratory rearing of larvae collected post-spray, the mortality figures from the 6 plots were in a fairly narrow range (20.1% to 29.5%) with consistently greater NPV mortality in budworm from balsam fir hosts than from white spruce hosts (Table 4). These figures reflect the initial infection obtained from direct ingestion of the spray deposit. This situation was reversed when figures obtained from the diagnosis of field-collected larvae were compared; higher levels of NPV infection were found in larvae from white spruce hosts (Table 5). Again the range in maximum levels of NPV infection in larvae from white spruce hosts was relatively narrow (23.9% to 57.1%). The range in population reduction figures between the 6 plots was wider (33% to 92%), but the treatments were considered satisfactory on all of the replicates except plot 1 where only a 33% population reduction on white spruce hosts was recorded (Table 7). The figure of 92% population reduction on white spruce hosts on plots 3 and 5 is the highest recorded to date. The same figure, 92% reduction, was recorded on white spruce hosts when this dosage and formulation was used in the same locality in 1977 (Cunningham *et al.* 1978).

The lower population reduction figures obtained with the oil formulation do not necessarily indicate that it is inferior to the aqueous formulation because insect development in plot 7 was more

advanced than in the other plots with 82% of the larvae in the sixth instar (Table 2). Furthermore, to obtain infection and mortality at this very late application time is unusual. Oil formulations have been used as carriers for other baculoviruses, but this is the first attempt to use oil with spruce budworm NPV. Clearly, the oil had no detrimental effect on the virus and the use of an oil opens new avenues for formulation research. Many UV screening agents are soluble only in oil. An oil formulation is also advantageous in areas, such as British Columbia, where long spells of low humidity are sometimes encountered.

Very high levels of the microsporidia parasite, *Nosema fumiferanae*, were detected throughout the experimental area and are typical in an aging spruce budworm population. Microsporidians are chronic, debilitating pathogens which are seldom lethal to their insect hosts. Antagonism between NPV and this microsporidian have been noted when attempts to propagate NPV in microsporidia-infected budworm are made in the laboratory. We found it necessary to suppress the microsporidia with sodium benlate in order to obtain satisfactory virus production (McPhee and Cunningham, unpublished). It is not known if microsporidia in budworm larvae in the field reduce NPV infection, but it is strongly suspected that these pathogens are antagonistic. It was also noted this year that microsporidia were not observed in larvae reared until pupation or death had occurred in the laboratory but were frequently encountered in larvae which were dissected and examined; this confirms an observation made last year (Cunningham *et al.* 1978).

No foliage was saved in the plots treated with NPV this year, a fact which is not surprising because of the late timing of the application. NPV takes 14 days or more to kill spruce budworm fifth or sixth instar larvae under field conditions. The long-term impact of the virus is considered to be of major importance. Following an application of 750 billion PIB/ha in 1971, NPV was found to persist for 4 years in 2 white spruce stands and to prevent defoliation by spruce budworm to an extent where tree mortality was avoided (Cunningham *et al.* 1975*c*). In subsequent years, because lower dosages of virus were used, this long-term effect could not be demonstrated. The plot treated in Kirkwood Township with 750 billion PIB/ha in 1977 was re-examined in 1978 (Cunningham and Howse, unpublished). They found a much reduced population level at fifth instar, 8.0 larvae per 46-cm branch tip as compared to 36.0 in 1977. The population in the corresponding check plot had dropped from 33.1 larvae per 46-cm branch tip in 1977 to 20.3 in 1978. Current year's defoliation in the treated plot was 21% in 1978 as compared to 97% in 1977. When a sample of 249 larvae collected from this plot was reared on diet, 4.8% NPV was recorded. A maximum level of 9.3% NPV was found in samples of field-collected, dissected and microscopically examined larvae. However, no population reduction due to NPV carry-over could be demonstrated when compared to a check plot. The principal criterion for the evaluation of the efficacy of the 6 replicated treatments reported here is to determine the impact of the virus over the next few years.

The dosage of 750 billion PIB/ha compared to dosages recommended for other baculoviruses was discussed in a previous report (Cunningham *et al.* 1978). For some insects, this dosage is considered to be economical, but spruce budworm larve are relatively small compared to the larvae of several other economically important species of Lepidoptera. For example, 7,500 budworm larvae are required to produce a 1-ha dosage of NPV and, because virus production is highly labour intensive using the methods presently available, the cost per ha currently runs about $125-$250. An intensive search is being made for an alternative host insect with larger larvae for virus production. It is possible that the salt marsh caterpillar, *Estigmene acrea* (Drury), may be used for this purpose (Shapiro, pers. comm.). Yielding from 10 to 100 times more PIBs per larva, the cost of treatment could be reduced proportionately, making NPV an economically feasible alternative to chemical pesticides for spruce budworm population regulation.

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