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AERIAL APPLICATION OF SPRUCE BUDWORM BACULOVIRUS: TESTS ON SECOND INSTAR LARVAE IN 1976

by

W.J. Kaupp, J.C. Cunningham, G.M. Howse

J.R. McPhee and P. de Groot

Forest Pest Management Institute
and
Great Lakes Forest Research Centre
Sault Ste. Marie, Ontario

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Director
Forest Pest Management Institute
Canadian Forestry Service
Department of Fisheries and the Environment
Box 490, Sault Ste. Marie, Ontario
P6A 5M7

ABSTRACT

Three plots, containing mainly white spruce, *Picea glauca* (Moench) Voss, with a total area of 120 ha, were sprayed with a nuclear polyhedrosis virus when larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.), were in the needle-mining second instar. Aerial applications were made at 9.4 ℓ/ha with a virus dosage of 125 billion polyhedra/ha. The aqueous formulation contained 250 ml/ℓ molasses, 60 g/ℓ IMC 90-001 sunlight protectant and 1.25 ml/ℓ Chevron® sticker. Two of the plots received a second application 6 days later with the same dosage and formulation.

Levels of virus infection and subsequent mortality were lower than those recorded following virus application on later instars in previous years. The double application gave better larval infection than the single but was still unsatisfactory. It is concluded that the second instar, although highly susceptible to nuclear polyhedrosis virus, is not the best stage of insect development for virus application with the formulation currently available.

RESUME

On a arrosé trois placettes échantillons contenant surtout des Epinettes blanches, *Picea glauca* (Moench) Voss, sur une superficie totale de 120 ha avec un virus de la polyédrose nucléaire, au moment où les larves de la Tordeuse des bourgeons de l'Epinette, *Choristoneura fumiferana* (Clem.), étaient à leur deuxième stade, celui où elles minent les aiguilles. Des arrosages aériens furent effectúes à raison de 9.4 ℓ/ha d'une dose de 125 milliards de virus polyèdres/ha. Le liquide aqueux contenait 250 ml/ℓ de mélasse, 60 g/ℓ de protecteur IMC 90-001 contre la lumière solaire, puis 1.25 ml/ℓ de gommant Chevron®. Six jours plus tard, une seconde application eut lieu dans deux des placettes, avec la même dose et la même solution.

Les niveaux d'infection due au virus et la mortalité subséquente furent moindres que ceux observés après une application du virus sur des larves à un stade plus avancé au cours d'années antérieures. La double application produisit une meilleure infection larvaire que l'application unique, mais elle fut encore insatisfaisante. Les auteurs concluent que le deuxième stade, bien que hautement vulnérable au virus de la polyédrose nucléaire, n'est pas le stade idéal de développement de l'insecte pour une application du virus avec la formule actuellement disponible.

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INTRODUCTION

Experimental aerial spray trials using viruses have been conducted against the spruce budworm, *Choristoneura fumiferana* (Clem.), every year since 1971. In 1971 and 1972 an entomopoxvirus was tested, but trials with this virus were abandoned in favour of a nuclear polyhedrosis virus. Nuclear polyhedrosis viruses (NPV) and granulosis viruses have been classified in the genus "Baculovirus" (Wildy, 1971). Baculoviruses have no morphological resemblance to any known plant or vertebrate viruses and are considered safe and ecologically acceptable for insect control provided sufficient safety testing has been undertaken (Summers et al., 1975).

Previous aerial spray trials on spruce budworm have been reported in detail (Howse et al., 1973; Cunningham and McPhee, 1973; Cunningham et al., 1974; Cunningham et al., 1975a; Cunningham et al., 1975b). To date a total of 1,414 ha have been treated with this virus. The best results were obtained in 1971 when a dosage of 750 billion polyhedral inclusion bodies (PIB) per hectare was applied (Howse et al., 1973). Following this treatment the virus was found to persist well in the spruce budworm population for 4 years (Cunningham et al., 1975c).

In laboratory tests, second instar budworm larvae are about tenfold more susceptible to NPV than fourth instar and 100 fold more susceptible than fifth instar (Bird, personal communication). Attempts were made to infect early instar larvae (second and third instar) in 1971, 1972 and 1973 but these applications were generally not as successful as the ones on fourth and fifth instar after white spruce

and balsam fir buds had flushed.

In 1974 and 1975 an improved formulation containing molasses, IMC 90-001 sunlight protectant and Chevron® sticker was developed. It was hoped that this formulation would keep the virus in a viable state on the foliage over a longer period and infect a large proportion of needle-mining second instar larvae. These larvae would die and cause secondary infection among the survivors resulting in substantial mortality. As emergence of second instar larvae from hibernacula occurs over a period of 5 to 10 days, it was decided to apply a double as well as a single application of NPV to determine if the double application would significantly increase population reduction.

Second instar spruce budworm larvae can ingest a deposit of NPV while starting a mine into a needle or into a bud. Due to the high susceptibility of these small larvae it was expected that an early application of NPV should be an efficient and economical method of initiating an epizootic. With these goals in mind, plans were made to spray 3 plots with NPV using the improved formulation and compare the single and double applications.

This project was a collaborative effort between the Forest Pest Management Institute and the Great Lakes Forest Research Centre. FPMI staff selected the plots, conducted the spray applications and determined the levels of virus in the spruce budworm population. Dr. G.M. Howse of GLFRC calculated the population reduction due to treatment, recorded the pupal survival and estimated the foliage protection obtained.

MATERIALS AND METHODS

Virus Production

During the winter months of 1975-76, a total of 7,208 g of freeze-dried NPV-infected spruce budworm larvae was produced. This material contained 1.5 billion PIB/g which is a considerably lower count than in previous years. Major problems were encountered with the quality of the insect synthetic diet and with bacterial contamination of the virus inoculum. Both these problems were rectified near the end of the production season but not in time to substantially increase the yield.

Experimental Plots

Spray plots were located in Kirkwood Forest Management Unit north of Thessalon, Ontario in Kirkwood and Bridgeland Twps. The plots were located in plantations 40 ha in size and containing mainly white spruce, *Picea glauca* (Moench) Voss, 15 m high. A total of 6 check areas were selected in Rose Twp, Bridgeland Twp, Kirkwood Twp and Thessalon Twp. The location of these plots and check areas is shown in Fig. 1.

Virus Formulation and Dosage

The powder prepared from the freeze-dried virus-infected larvae was suspended in water using a Kalish® turbo-homogeniser at a concentration of 250 g/ℓ. This operation was performed the day before the spray application to avoid excessive growth of bacteria. Other ingredients in the formulation were mixed in a large tank and the virus concentrate added just prior to loading the aircraft. The aqueous formulation contained 250 ml/ℓ animal feed grade molasses, 60 g/ℓ

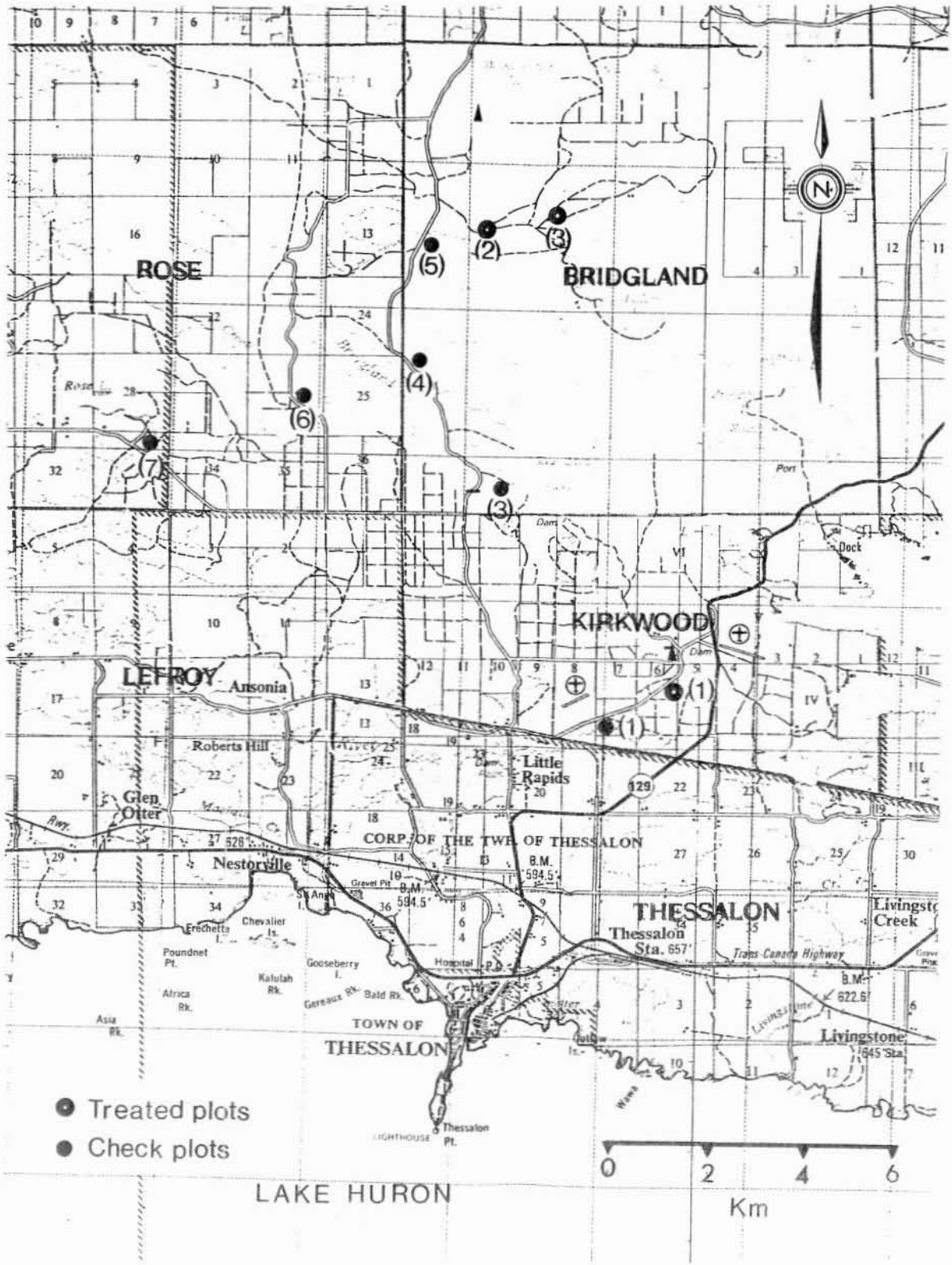

Fig. 1. Map showing the location of the three plots treated with virus and six check plots. (There is no #2 check plot in the series.)

IMC 90-001 sunlight protectant[1/] and 1.25 ml/ℓ Chevron® sticker[2/]. The emitted dosage was 125 billion PIB/ha applied at a rate of 9.4 ℓ/ha. Plots #1 and #3 received double applications giving a total virus dosage of 250 billion PIB/ha.

Meteorological Observations

Temperatures and precipitation were monitored on plots #1 and #2 for the duration of the experiment using a hydrothermograph and standard rain gauge.

Monitoring the Deposit

Plots were laid out so that they were transected by a road running at right angles to the flight lines. Prior to the application, Kromekote® spray cards on aluminum backings were placed along the roads at 15 m intervals and to a distance of 45 m on both sides of the plot to monitor drift. The number of droplets was counted in five 1 cm^2 on each card and the diameter of each droplet was measured in 1 cm^2 only on each card. Droplet diameter was measured using a calibrated eyepiece in a dissecting microscope.

Spray Application

A Grumman Agcat biplane with boom and nozzle spray equipment was contracted from General Airspray Ltd. There were 30 nozzles on the boom with #8 orifices. Flying a swath of 30 m, it was calibrated to deliver 9.4 ℓ/ha. Spraying commenced on May 9th on plot #3 at 6:30 a.m. and was finished at 6:55 a.m. Temperature during the application

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1/ Sandoz Wander Inc., Homestead, Florida.

2/ Chevron Chemical Co., Ortho Division, San Francisco, California.

was 1.0°C and relative humidity was 90%. At 7:27 a.m. spraying began on plot #2 and finished at 7:46 a.m. The temperature was 7.3°C and the relative humidity 89%. On May 10th spraying of plot #1 began at 8:25 a.m. and finished at 8:45 a.m. The temperature was 1.5°C and the relative humidity was 75.7%. This completed the first application on all three plots.

On May 14th, plots #1 and #3 were given a second application. Spraying commenced at 8:25 p.m. on plot #1 and finished at 8:30 p.m. The temperature was 13.8°C and the relative humidity was 73%. Spraying of plot #2 commenced at 8:55 p.m. and finished at 9:15 p.m. The temperature was 11.5°C and the relative humidity was 86%.

Larval Development

On May 9th second instar were observed to be emerging from their hibernacula and beginning to mine their first needle. On May 14th larvae were still in the second instar and were mining their second or third needle. Spruce budworm larvae mine several needles on white spruce and observations were made on this tree species only.

Assessment

(i) Sampling for population reduction studies

For population reduction studies 25 white spruce 46 cm branch tips collected at mid crown were taken from each of the three treated plots for prespray and postspray population counts. From the 6 check plots 15 white spruce 46 cm branch tips were collected at the same time. For the prespray count the needles and buds were torn apart and the larvae removed. After the first examination the foliage was

carefully re-checked for more larvae. For the postspray count larvae and pupae were also hand picked. Abbott's formula (Abbott, 1925) was used to calculate the population reduction of the spruce budworm attributable to the virus treatments.

(ii) Microscopic examination of samples of larvae to determine levels of virus and other pathogens

Samples of larvae were also collected to determine the levels of virus infection in the spruce budworm population. The first sample was collected on June 1st about 3 weeks after the first application and 2 more samples were taken on June 11th and June 17th. Thirty samples of 46 cm white spruce branch tips were collected from each of the treated plots and 8 to 15 samples from each of the check areas. Living and dead larvae and pupae were removed from these samples in the laboratory. Squash preparations were made of gut tissue and examined microscopically using phase contrast optics to determine the presence of polyhedra.

(iii) Pupal emergence

Pupae from the postspray samples were maintained at room temperature and adult emergence recorded. Percent successful pupal emergence was recorded as follows:

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

(iv) Estimates of Current Year's Defoliation

The percent current defoliation was estimated by detailed examination of the 46 cm branch tips collected for the postspray sample from the treated and check areas.

RESULTS

Deposit Analysis

The mean number of droplets per cm^2 with standard deviation as determined from the Kromekote® cards is given in Table 1. It can be seen that the best deposits were obtained from the two applications which were made in the morning. The droplet spectrum is shown in Figs. 2, 3 and 4. It can be seen that most of the droplets are less than 300 μ and the largest category is the 50 μ to 100 μ class for all applications with the exception of the first application on plot #3 where the under 50 μ class is the largest.

Meteorological Observations

For the duration of the experiment 1.68 cm of rain fell on plot #2. The mean maximum daytime temperature was 26.1°C and the mean minimum night temperature was 1°C. Between May 9th and June 3rd the night temperature dropped below freezing on 12 occasions.

Microscopic Diagnosis of Larvae

Three samples of larvae were collected from the treated plots and check plots on June 1st, June 11th and June 17th. The results are shown in Tables 2 and 3. In plot #1 a maximum level of 12.1% of larvae with NPV infection was recorded, in plot #2 a maximum of 5.4% and in plot #3 a maximum of 9.0%. In the check plots only traces of natural NPV were found with 0.4% in check #1 on 17th June and 0.8% in check #2 on 11th June. Traces of cytoplasmic polyhedrosis virus were found in plot #3 and in check area #1. High levels of microsporidia were found in all treated and check plots. In the first

sample taken on 1st June only low levels were observed microscopically which ranged from 0.6% to 4.2%. In the final sample, microsporidial infection ranged from 26.4% to 35.2%.

Population Reduction, Successful Pupal Emergence and 1976 Defoliation

Population reduction, successful pupal emergence and 1976 defoliation estimates as compared to the check areas are given in Table 4.

Population reduction was calculated to be 52%, 34% and 0% in plots #1, #2 and #3 respectively. The virus treatment had a slight effect on successful pupal emergence with all the treated plots showing lower emergence than the checks. No significant foliage protection could be demonstrated in any of the treated plots.

DISCUSSION

The results of these trials in which NPV was applied on second instar spruce budworm larvae are considered to be unsatisfactory. Higher levels of infection and mortality have been recorded in previous trials (Howse et al., 1973; Cunningham et al., 1975a; Cunningham et al., 1975b). It appears that the formulation currently available does not keep the virus in a viable state on the foliage for a sufficient period of time. Ideally, if an NPV is used to treat second instar budworm larvae, the formulation should protect this virus against inactivation by the ultraviolet component of sunlight for 7 days or more. Until such a protectant is developed, spruce budworm control operations with NPV should be limited to applications on later instars after budflush.

The data from microscopic diagnosis of larvae indicate that the double application gave slightly better results than the single, although the percent infection figures are so low in all plots that the difference is not significant. These data conflict with the figures obtained from the population reduction studies in which reductions of 52% and 0% were obtained from the plots #1 and #3 which received the double application, and 34% from plot #2 which received the single application. Saving of foliage was negligible in this test although this situation is usually observed in the year of NPV application. It is only in subsequent years that saving of foliage is significant.

The best results obtained to date were in 1971 when 750 billion PIB/ha were applied on white spruce at a rate of 29.2 ℓ/ha in water alone. This NPV preparation had a cytoplasmic polyhedrosis virus

contaminant. When applied on second instar larvae a maximum level of 34% virus infected larvae was observed which resulted in 69% spruce budworm population reduction. When applied on fourth instar larvae a maximum level of 71% infection resulted in 80% population reduction (Howse et al., 1973). This dosage was considered to be economically unacceptable and was reduced in subsequent years. In 1975 plots were treated with NPV on Manitoulin Island with a variety of molasses-based formulations and dosages of 125 billion PIB/ha and 250 billion PIB/ha. Maximum levels of virus infection in larvae in the plot yielding the highest results were 16.8% of larvae on balsam fir and 40.4% on white spruce. These levels of infection resulted in 53% population reduction due to treatment on balsam fir and 91% on white spruce (Cunningham et al., 1975b). Generally better results are obtained when NPV is used for control of spruce budworm on white spruce hosts than on balsam fir hosts. Unfortunately follow-up studies of the Manitoulin Island trials showed very little carry-over of NPV from one year to the next.

To initiate a virus epizootic in a spruce budworm population presents many difficulties, but, until a marked improvement in formulation is achieved, spray applications should be made after budflush when the larvae are more exposed to a spray deposit.

Table 1

Mean number of droplets per cm^2 on spray cards

Plot	Spray Date	Application	Mean Number Drops/cm^2	Standard Deviation
1	May 10th a.m.	first	23	17
	May 14th p.m.	second	23	18
2	May 9th a.m.	one only	43	17
3	May 9th a.m.	first	48	11
	May 14th p.m.	second	20	8

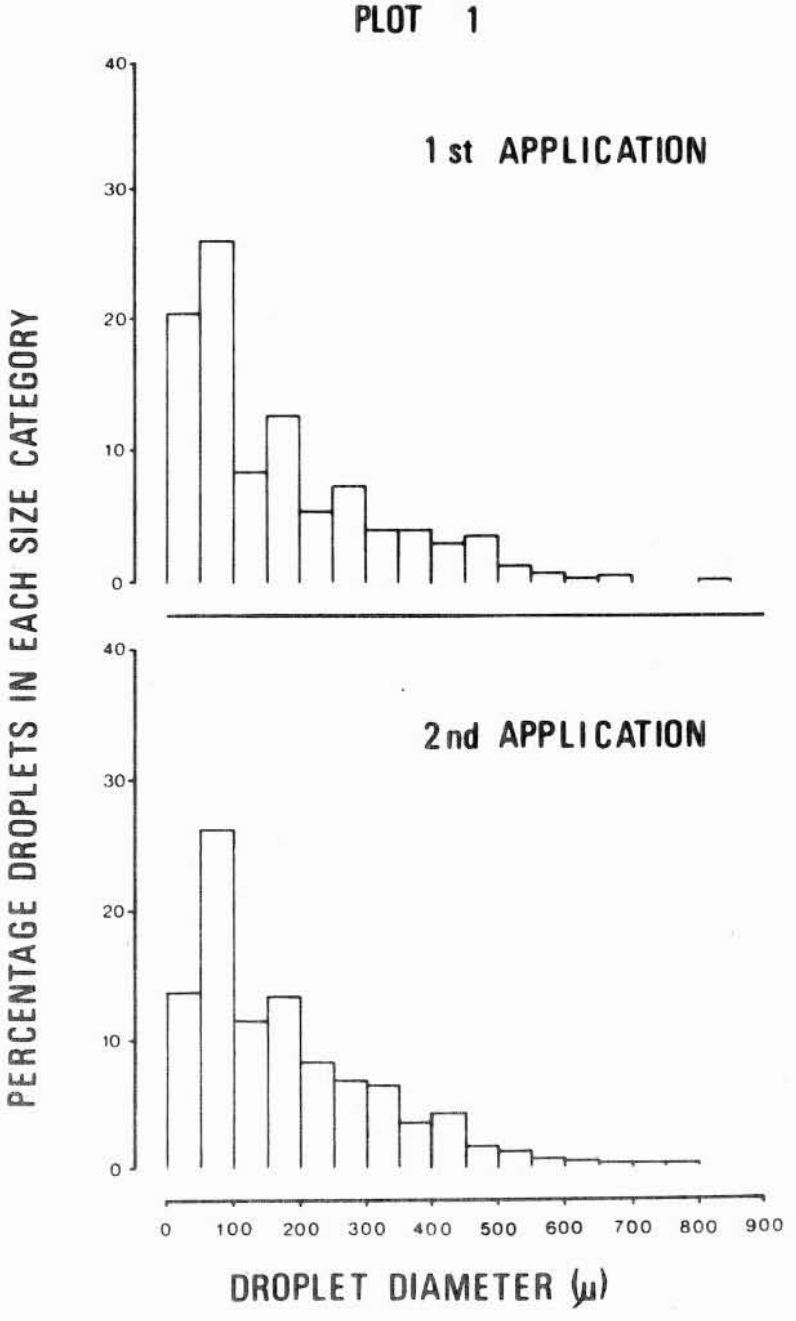

Fig. 2. Analysis of the spray drops on Kromekote® cards in plot #1.

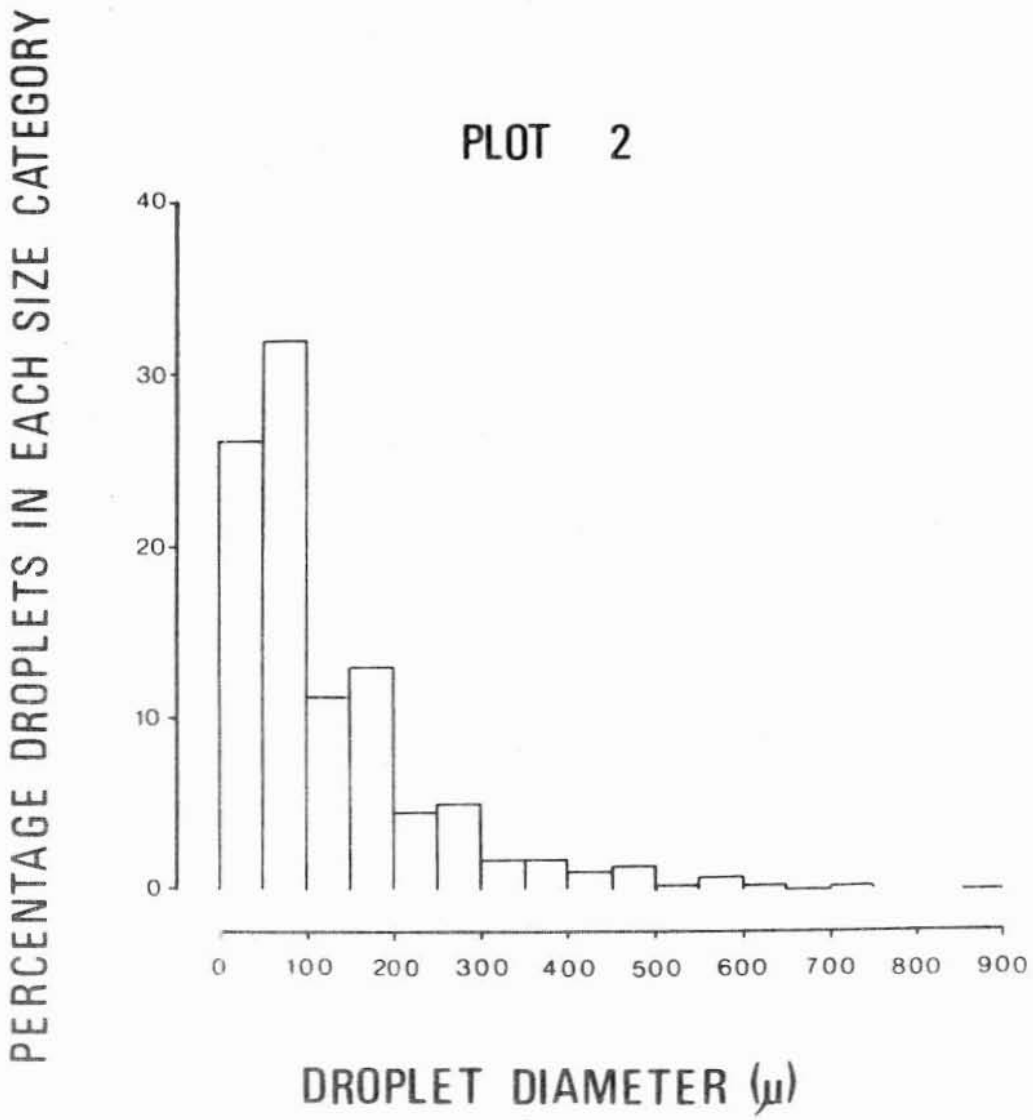

Fig. 3. Analysis of spray droplets on Kromekote® cards in plot #2.

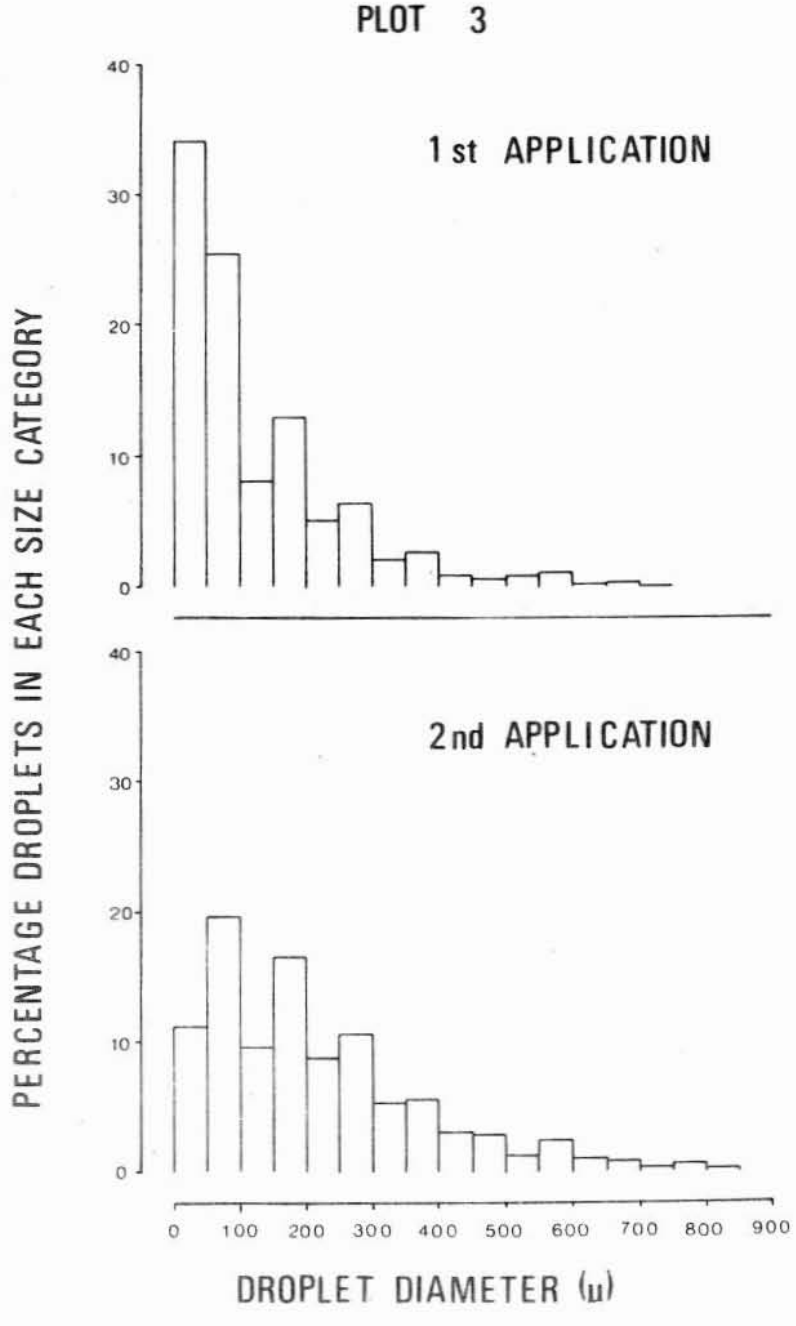

Fig. 4. Analysis of spray droplets on Kromekote® cards in plot #3.

Table 2

Incidence of viruses, microsporidia and parasites as determined by microscopic diagnosis, in larvae on white spruce trees from plots sprayed with single and double applications of NPV in 1976 at a rate of 125 billion PIB/ha when they were in the second instar

Plot	Number of applications	Sample date	No. of larvae examined	Percent virus infection		Percent other organisms	
				NPV	CPV	Microsporidia	Parasites
1	2	1 June	328	2.1		0.6	2.4
		11 June	451	4.2		30.2	0.7
		17 June	356	12.1		34.6	0.6
2	1	1 June	230	3.9		1.3	3.0
		11 June	666	2.4		19.1	0.3
		17 June	390	5.4		26.4	1.3
3	2	1 June	335	2.1		3.0	1.5
		11 June	617	1.5	0.3	20.1	1.0
		17 June	421	9.0		31.4	1.7

Table 3

Incidence of viruses, microsporidia and parasites in spruce budworm larvae on white spruce trees in check plots located north of Thessalon, Ontario in 1976

Check Plot Number	Date of sample	No. of samples	No. of larvae examined	Percent of virus infection		Percent other organisms	
				NPV	CPV	Microsporidia	Parasites
1	1 June	15	143			4.2	3.5
	11 June	10	211			34.6	
	17 June	15	233	0.4	0.9	35.2	1.3
3	1 June	15	52			1.9	3.9
	17 June	15	103			27.2	
4	1 June	15	71			4.3	1.4
	11 June	8	136			21.3	
	17 June	15	241			32.0	1.2
5	1 June	15	98			4.1	
	11 June	10	123	0.8		27.6	
	17 June	15	147			26.5	

Table 4

Population reduction, pupal survival and current defoliation on white spruce in three plots sprayed with NPV near Thessalon, Ontario, 1976. Budworm larvae were in the second instar at the time of application.

Plot	Prespray larvae/ 46 cm branch tip	Surviving pupae/ 46 cm branch tip	Percent population reduction due to treatment	Percent successful pupal emergence	Percent 1976 defoliation
1	37.8	2.36	52	52	51
Check	41.9	5.40		56	46
2	14.1	4.33	34	60	43
Check	18.7	8.66		70	29
3	39.6	5.10	0	53	40
Check	41.9	5.40		56	46

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