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Aerial Applications of Nuclear Polyhedrosis Virus and Bacillus Thuringensis Against Western Spruce Budworm

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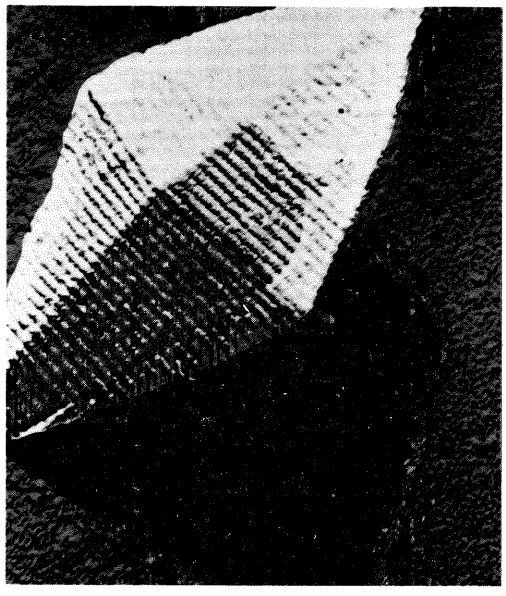

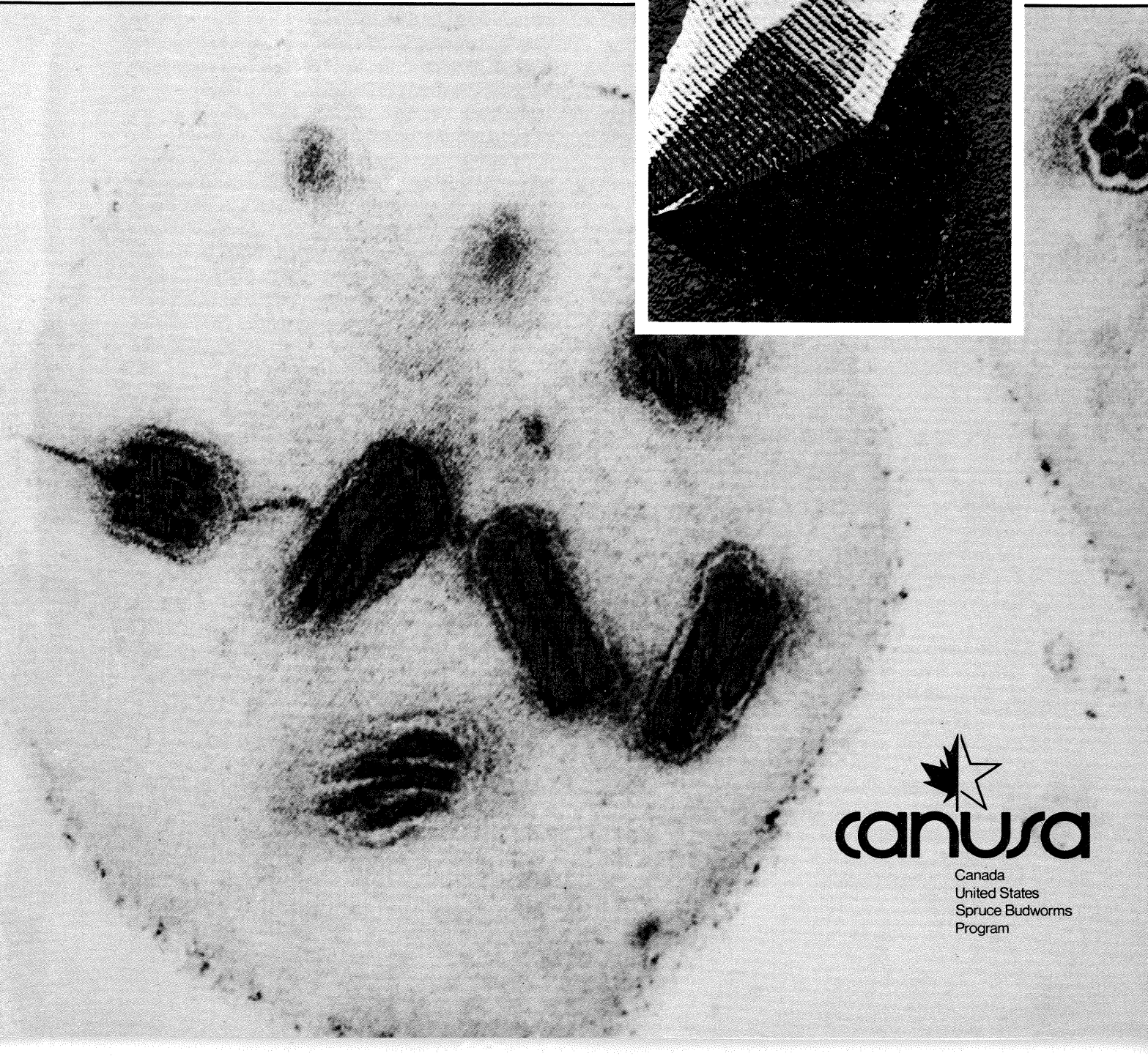

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ABSTRACT

Three 20 ha plots of Douglas-fir trees infested with fifth instar larvae of *Choristoneura occidentalis* were aerially treated at a rate of 750 billion polyhedra/ha with a nuclear polyhedrosis virus which had been propagated in *C. fumiferana.* At 15 days post-spray, NPV infection levels were 25, 55 and 87% and population reductions owing to treatment were 0, 26 and 48%. Moth emergence was lower in treated plots and the male:female ratio was 2:1 in treated plots compared to 1:1 in check plots.

Four 40 ha plots were similarly treated with *Bacillus thuringiensis* at a rate of 20 BIU/ha. At 15 days post-spray, population reductions owing to treatment were 32, 66, 79 and 91%. The proportion of moths emerging and the number of eggs laid were similar to check plots. No consistent foliage protection was obtained with either treatment.

RÉSUMÉ

Trois parcelles de 20 hectares de Douglas taxifolié infestées de larves au cinquième stade de *Choristoneura occidentalis* ont fait l'objet d'un traitement aérien avec une suspension du virus de la polyédrose nucléaire propagé sur *C. fumiferana,* à raison de 750 milliards de polyèdres/ha. Au bout de 15 jours les niveaux d'infection par le VPN y étaient de 0, 55 et 87% et les réductions de population déterminées par le traitement de 0, 26 et 48%, respectivement. L'envol des papillons était moindre dans les parcelles traitées et le rapport mâles:femelles était de 2:1 dans les parcelles traitées, comparé à 1:1 dans les parcelles témoins.

Quatre parcelles de quarante ha ont de même été traitées avec une suspension de *Bacillus thuringiensis* à raison de 20 UIB/ha. Au bout de 15 jours les réductions de population obtenues par ce traitement se chiffraient à 32, 66, 79 et 91%. Les proportions de papillons prenant leur envol ainsi que le nombre d'oeufs pondus étaient identiques à ceux relevés dans les parcelles témoins. Aucune protection consistante du feuillage n'a été obtenue avec l'un ou l'autre traitement.

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INTRODUCTION

The western spruce budworm, *Choristoneura occidentalis* (Free.), has been a major defoliator of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, in British Columbia for many years. Promising developments in the use of microbial insecticides led to an evaluation of a nuclear polyhedrosis virus and a bacterium, *Bacillus thuringiensis* Berliner, for the control of this pest.

Experimental aerial applications of nuclear polyhedrosis virus (NPV) on eastern spruce budworm, *Choristoneura fumiferana* (Clem.), in Ontario have been conducted every year since 1971 (Howse et al. 1973; Cunningham and McPhee 1973; Cunningham et al. 1974, 1975a, 1975b, 1978, 1979; Kaupp et al. 1978). To date, 36 plots with a total area of 1790 ha have been treated and parameters such as dosage, formulation, time of application and spray equipment have been studied. The best results were obtained with an aqueous formulation containing 25% molasses v/v and 60 g/l of a commercial ultraviolet screening agent, Sandoz Shade®. The dosage was 750 billion polyhedral inclusion bodies (PIB) per ha applied in a volume of 9.4 l/ha at the time when buds flushed (Cunningham et al. 1978). The virus persisted for as long as 5 years following the application and during this time exerted some control on the spruce budworm population (Cunningham et al. 1975).

The NPV used in this field test was found originally in eastern spruce budworm. An NPV has also been isolated from western spruce budworm. These viruses are both cross-infectious to the other species and are of the same order of virulence. Detailed biochemical studies will be required to determine if they can be distinguished.

A trial was conducted using eastern NPV on western spruce budworm feeding on Douglas-fir in 1976. A 20.5 ha plot near Mission Mt., B.C. was treated (Shepherd and Cunningham, unpublished) with a dosage of 250 billion PIB/ha in an aqueous formulation containing 250 ml/l molasses, 60 g/l IMC 90-001 (now renamed Sandoz Shade®) and 10 ml/l Chevron sticker from a fixed wing aircraft at 9.4 l/ha when larvae were in the fourth, fifth and sixth instars. The resulting population reduction owing to treatment, calculated by Abbott's formula (Abbott 1925), was 36%. The incidence of virus, determined microscopically, in samples of over 300 larvae from the treated plot and a check plot was 6.8 and 1.6%, respectively. In 1977, the incidence of virus in the treated plot dropped to 1.3%. These results were considered unsatisfactory and were attributed to late application of an insufficient dosage of virus.

Trials with *Bacillus thuringiensis* (B.t.) on eastern spruce budworm have been on a much larger scale than trials with NPV. Early laboratory experiments and field trials using B.t. gave variable results during the 1960s (Mott et al. 1961; Smirnoff 1963; Klein and Lewis 1966; Angus et al. 1970). The availability of an improved strain of the bacterium, HD1, coupled with new spruce budworm outbreaks in eastern Canada and the northeastern United States (Harper 1974) led to renewed testing. Tripp (1972) achieved high larval mortality (80-90%) with two commercial preparations of B.t., but results were obscured by frost damage following the application. In 1970, Smirnoff indicated that the addition of the enzyme chitinase may enhance the efficacy of B.t. formulations (Smirnoff 1971) and field trials of B.t. plus chitinase, were carried out in eastern Canada and Maine from 1971 to 1976 (Dimond 1972, 1973, 1974; Morris and Hildebrand 1974; Smirnoff 1974; Auger et al. 1975; Pelletier 1976; Smirnoff 1976). Thompson et al. (1977), compared three rates of application, two spray droplet spectra and five formulations against western spruce budworm and Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough). The most effective formulation included 25% sorbitol and 50 g/l Shade® applied in coarse droplets (vmd > 300 μm).

In a continuing effort to develop acceptable control methods against forest defoliators, a commercial B.t. formulation, Thuricide 16B®, was field tested against western spruce budworm for the first time in British Columbia in 1978. Also, the nuclear polyhedrosis virus, propagated in eastern spruce budworm larvae at the Forest Pest Management Institute, was retested using three times the dosage applied in 1976.

MATERIALS AND METHODS

Plot Description and Preparation

Test plots of open-grown Douglas-fir, *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco, were established in three regions at elevations between 800 and 1100 m, west of Cache Creek, southwest of Ashcroft and east of Lillooet, British Columbia

(Fig. 1). The 9-to 15-m-high trees had been under light attack from the western spruce budworm for 1-2 years. Forecasts of moderate larval densities were made in these regions for 1978, based on egg-mass sampling in August 1977.

One 20 ha block was selected for treatment with NPV and one check area was selected in each of the three regions. Two 40 ha blocks were chosen for B.t. treatment in Cache Creek and Ashcroft (Fig. 1). Fifteen sample trees were selected in each plot and two branch tips (46 cm) were taken from the mid-crown of each tree. Trees in treatment blocks were chosen in a transect at right-angles to the aircraft spray swath. Plot corners were marked with fluorescent plastic markers, shot into the tree tops with a line gun (Collis and Harris 1973) and with helium filled balloons put up just prior to aerial application.

Spray formulation and application

The NPV formulation contained 7.1 x 10^7 PIB/ml, 30 g/l Sandoz Shade[2], 250 ml/l molasses and 0.5 ml/l Triton B-1956® spreader-sticker. With an emission rate of 9.4 l/ha, this gave a dosage of 750 billion PIB/ha. The water used for mixing had a pH of 8.9, but the acidity of the molasses lowered the pH of the final formulation to 5.3.

The commercial preparation of B.t. used was Thuricide 16B®(lot no. 2W04816). It was formulated by adding equal volumes of water (pH 8.9) and Thuricide plus 0.01% Erio Red dye as a marker. With a delivery rate of 9.4 l/ha, the application rate should have been 20 Billion International Units (BIU)/ha. Potency of the tank batches was determined by Dr. W.A. Smirnoff, who calculated that they were 17.3 BIU/ha for plot 1, 16.1 BIU/ha for plot 2, 12.9 BIU/ha for plot 3 and 13.1 BIU/ha for plot 4 (Smirnoff, pers. comm.).

Applications were made with a Cessna 'Agtruck' aircraft equipped with booms fitted with T8015 flat fan nozzles[3] at a pump pressure of 275 k Pa. The aircraft was calibrated to deliver 9.4 1/ha while flying 30 m above tree tops at 175 km/h, with a spray swath of 30 m. A private airstrip 2 miles south of Ashcroft, B.C. was used for storage, mixing and loading of the formulation[4]. Radio communications between aircraft, ground crews and airstrip were maintained throughout the treatment period.

Applications were made as soon as possible after bud flush. Before bud flush, larvae mine the interior of buds and are not exposed to a spray deposit. Careful monitoring of buds indicated that a sudden hot period caused the flush to occur in all areas within 2 or 3 days. Population densities were measured and aerial application of virus commenced on the morning of June 12. Plot 2 received a brief rain shower before treatment and similar precipitation fell on plot 3 shortly after spray application. Aerial application of B.t. occurred on the mornings of June 16 and 17 under ideal meteorological conditions.

Meteorological Monitoring

A recording pyrheliometer, hygrothermograph and a rain gauge were set up in each of the three spray areas. Conditions in the spray plots were also monitored by ground crews with portable instruments on the morning of application.

Deposit Analysis

Kromekote®[5] cards, 10 cm x 10 cm, were placed 50 cm above the ground on clearings, one near each of the 15 sample trees per plot. Cards were collected approximately 30 min after virus application, except those on virus plot 1 which were retrieved sooner because of rain showers. Average spray droplet size and density were determined for each plot but precipitation prevented interpretation of results on virus plot 1.

Biological Assessment

In the virus-treated and check plots, two branch tips, about 46 cm long, were collected from the mid-crown of each of 15 sample trees per plot, 1 day prior to treatment and 8 and 15 days after treatment. In the B.t. plots, samples were collected

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2 Sandoz Inc., 480 Camino Del Rio So, San Diego, Calif.

3 Spray System Co., Wheaton, Ill.

4 Application Contractor: Conair Aviation Ltd. Abbotsford, B.C.

5 Kruger Paper Co., Montreal, Que.

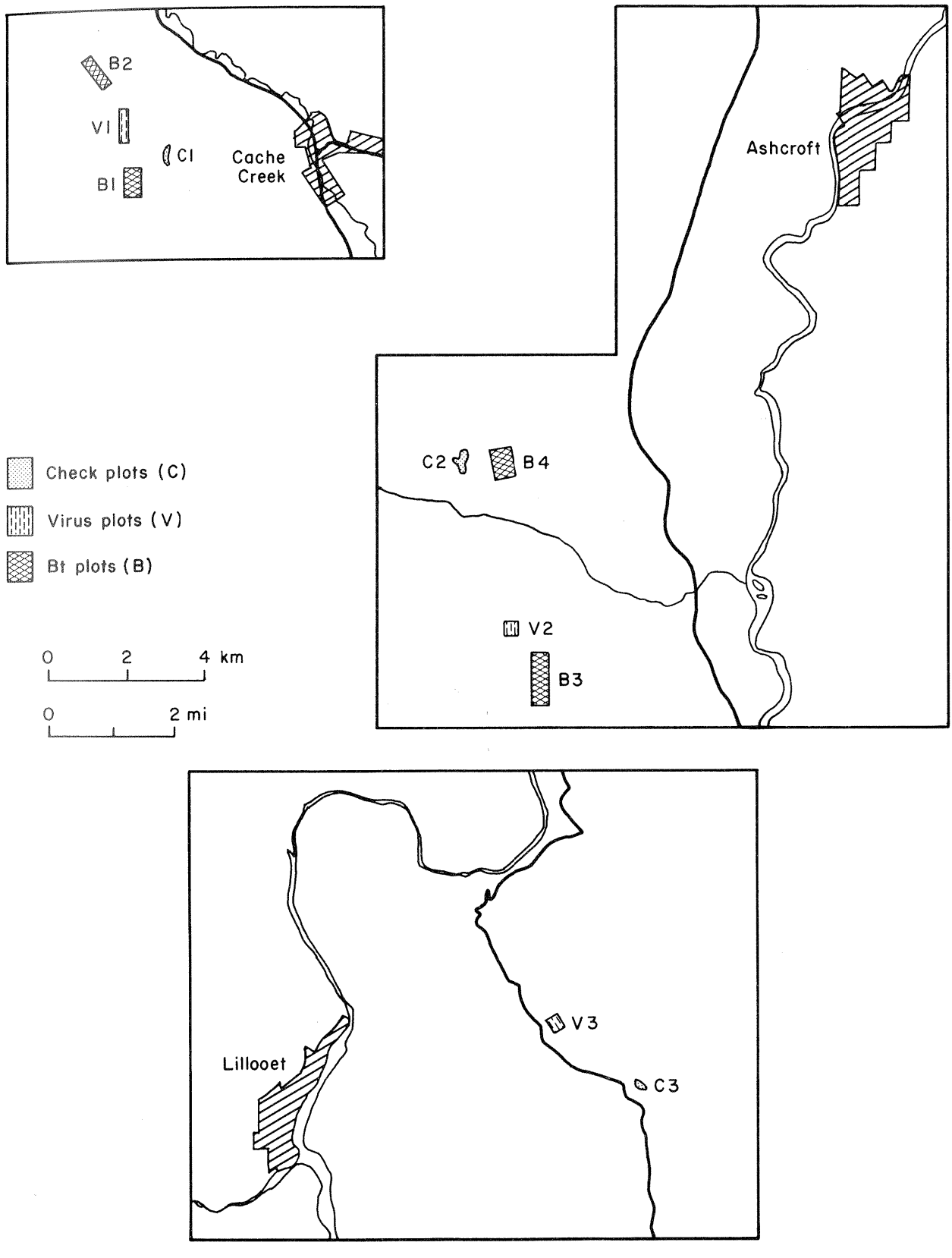

Fig. 1. Locations of B.t. and NPV-treated and check plots in British Columbia, 1978.

2 to 4 days prior to treatment and at 8 and 15 days post-spray. Branch length and width at the mid-point were tallied and, in the B.t. study, the number of new buds was also counted. Larvae were removed from foliage in the field by shaking branches inside the sampling bag which was attached to the head of the pole pruners. Larvae were counted and preserved for determination of instar.

Two additional post-spray larval collections were made from the virus-treated plots and check plots and shipped to the Forest Pest Management Institute (F.P.M.I.), Sault Ste. Marie, Ontario. Insects from the first collection were reared in plastic cream cups on synthetic diet until death or pupation occurred. The cause of death was determined by examination of smear preparations using a phase contrast microscope. Insects from the second collection were all sacrificed and squash preparations of their gut and fat tissue were examined to estimate amount of infection with NPV, other viruses and microsporidia.

Pupae collected at 3 weeks' post-spray from both virus- and B.t.-treated plots were sexed and the emerging adults allowed to mate and lay eggs on foliage in 70 x 50 x 50-cm screened cages located at the B.C. Forest Service, Ashcroft Ranger Station. Data were obtained on moth emergence, fecundity, egg viability and parasitism. Dead pupae were examined at F.P.M.I. to estimate amount of NPV infection and presence of other pathogens. Parasites recovered from pupae reared at Ashcroft were identified at Simon Fraser University, Burnaby, B.C. and at the Biosystematics Research Institute in Ottawa, Ontario.

A visual assessment was made of the loss of the current year's foliage on all sample trees in treated and check plots after moth emergence. Tree crowns were divided into upper, middle and lower crown levels, and the defoliation for each level was individually recorded.

DATA ANALYSIS AND TESTS OF SIGNIFICANCE

Data were collected in the B.t. study on both the size of each branch and the number of buds or current shoots per branch; insect density was calculated on both bases and compared. Coefficient of variation of insect population densities calculated on the basis of density/100 buds was about 75% of that calculated on the basis of density/m^2 (1550 in^2) of branch area. The number of insects per branch was better correlated to number of buds per branch ($r^2 = 0.27$) than branch size ($r^2 = 0.06$). This was particularly noticeable in the later larval stages and at higher population densities, when the number of available buds became limiting. Therefore, the effect of B.t. on insect density was analyzed on the basis of insect density per 100 buds. The bud data were not available for the virus study and the analysis was based on insect density/m^2 of branch area.

There was a close relationship between the variance and the mean of insect density expressed on both bases, thereby not meeting one of the assumptions of parametric tests. A transformation was made, following Iwao and Kuno (1968), which not only stabilized the variance but also improved the normality of the distribution and the additivity of effects. The mean crowding index, $\overset{*}{m} = \bar{x} + (v/x) - 1$, was regressed to the sample mean for each plot and the a and b parameters from the regression were utilized to give the following transformation:

$$f(x) = \log_e (0.65\ \sqrt{x} + \sqrt{0.42x + 1}).$$

All data were subjected to this transformation before analysis.

Population reductions are usually expressed on the basis of Abbott's formula (Abbott 1925), which allows for the reduction of populations because of natural causes.

$$\% \text{ control} = \left(\frac{S_C - S_T}{S_C}\right) \times 100$$ where S_C is the survival ratio in the check plots and S_T is the survival ratio in the treated plots. This can be reduced to $\left(1 - \frac{S_T}{S_C}\right) \times 100$ and a test of significance, assuming an approximate normal distribution can be as follows:

$k = \frac{S_C - S_T}{SE_d}$ where SE_d is the combined standard error of the difference between the two ratios.

The standard error of the ratio depends on the combined variability of the data collected before treatment (T) and after treatment (t), less the sum of the cross products of the two samples, for each tree (n_i) (Cochrane 1963). Samples taken at the same time in the check plot (C and c) are also required. The formulae, without a finite population correction factor, are as follows:

$$SE_{S_T} = \frac{1}{\sqrt{n \cdot \bar{T}}} \sqrt{\frac{\Sigma t_i^2 - 2 S_T (\Sigma t_i T_i) + S_T^2 (\Sigma T_i^2)}{n - 1}}$$

$$SE_{S_C} = \frac{1}{\sqrt{\bar{n} \cdot \bar{C}}} \sqrt{\frac{\Sigma c_i^2 - 2 S_C (\Sigma c_i C_i) + S_C^2 (\Sigma C_i^2)}{n - 1}}$$

RESULTS OF VIRUS TREATMENTS

(i) Deposit Analysis

Spray deposit on plot 1 was judged adequate during application, but a sudden rain shower prior to collection of cards rendered them impossible to interpret. Spray coverage was acceptable on virus plot 3, with a mean and standard deviation of 26 + 6 drops/cm^2 and with 80% of the spray droplets under 150μ (Table I). Poor coverage was obtained on virus plot 2, with 8 + 14 drops/cm^2 and with 24% of the spray droplets under 150 μ.

(ii) Meteorological Conditions

Weather conditions were satisfactory when application commenced (Table II), but a brief, unexpected rain shower (0.1 mm) fell on plot 2 during the application and plot 1 received similar precipitation (0.4 mm) 10 min after aerial treatment. A steady rain also fell on plot 3 (3.4 mm) approximately 7 hours after treatment.

Density of spray droplets observed on broad leaved vegetation after application appeared less the following day (June 13), and it was feared that precipitation had washed some of the viral formulation from target trees. However, on plot 1, many droplets appeared to have been diluted and spread by the light shower.

(iii) Larval Development

Cool weather delayed bud-break, but permitted larvae to continue development. When the majority of Douglas-fir buds flushed, 45 to 50% of the larvae had reached the fifth instar. The proportion of insects in different stages of development on the day before treatment and 8 days and 15 days after treatment is illustrated in Figure 2. This time of bud-flush relative to spruce budworm development may be the usual pattern on Douglas-fir hosts in B.C.

Possible effects of the NPV retarding larval development are shown in Figure 3. When spray blocks are compared to their corresponding check blocks, it is evident that larval development was

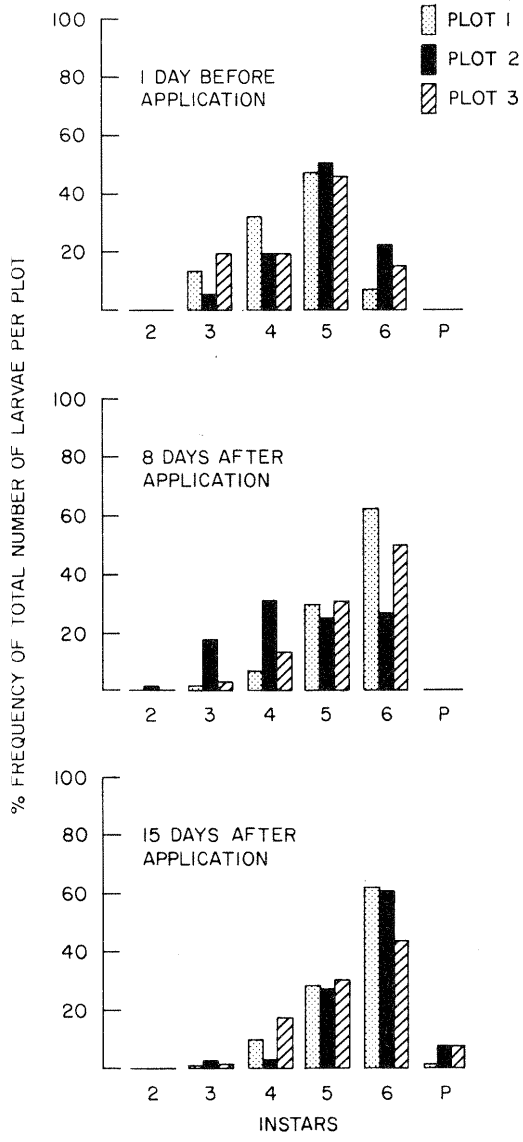

Fig. 2. Frequency of occurrence of each developmental stage of western spruce budworm at pre- and post-spray sample times, virus-treated plots, 1978.

Table I. Percent of droplets in each size classes in microns[1/]

Plot no.	< 75	76-150	151-250	251-350	351-450	451-550	551-650	651-750	>750
V 2	1.4	22.9	17.1	13.6	12.1	11.4	4.3	4.3	12.9
V 3	33.6	36.0	18.2	6.7	2.4	1.3	0.7	0.4	0.6
Bt 1	9.9	12.2	30.5	15.3	8.4	12.2	7.6	1.5	2.3
Bt 2	29.5	26.3	23.0	13.4	5.5	1.8	0.0	0.5	0.0
Bt 3	21.7	29.6	27.0	10.5	6.1	2.0	2.5	0.5	0.3
Bt 4	4.0	26.5	31.8	11.3	10.6	6.6	6.6	1.3	1.3

1/ Size as measured on Kromekote® cards. Spread factor was not determined.

Table II. Meteorological conditions at time of spray application, virus plots, 1978

Plot no.	Date	Application commencement time	Wind speed km/h	Sky	Temperature °C	Relative humidity (%)
1	June 12	0555	4-6	overcast	7°C	92%
2	June 12	0620	6	overcast	7°C	90%
3	June 12	0453	4-5	overcast	6°C	85%

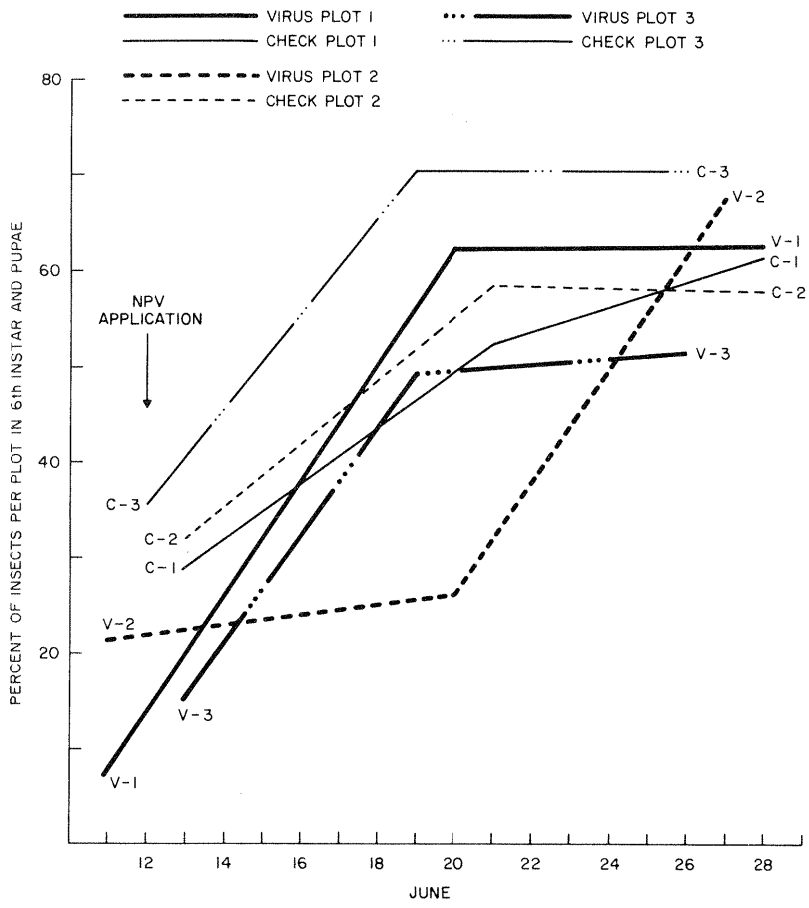

Fig. 3. Cumulative development of western spruce budworm larvae in virus-treated and check plots, 1978.

retarded on plot 2 by 5.6 days at the stage when 50% of the larvae were in the sixth instar. No such effect occurred on plot 1 or 3. Delay of larval development accords well with the greatest population changes and highest levels of NPV infection which were recorded on plot 2 (Tables III and IV).

(iv) Population reduction owing to treatment

Percent change in population density calculated according to Abbott's formula indicated a reduction of 48% in plot 2 at 15 days' post-spray which was statistically significant when compared to the check plot. The probability of the 26% reduction in plot 3 approached significance, having a 13% probability of being due to chance compared with the check at 15 days' post-spray. No reduction was detected in plot 1 (Table III).

(v) Incidence of Pathogens and Parasites

In the larval collection made on June 22, many of the insects were in poor condition following shipment to Sault Ste. Marie, Ontario. From virus plots 1, 2 and 3, 46, 28 and 25 larvae were set up on a synthetic diet, each larva in a separate container. Mortality attributed to NPV in these small samples was 65, 75 and 48%, respectively. Larvae from check plots were handled in the same manner with 46, 39 and 20 larvae from check plots 1, 2 and 3 and no death from NPV was recorded. The dead and dying larvae in these samples were examined

Table III. Effects of NPV on survival of western spruce budworm larvae, 1978

Treatment (plot)	Population density[a]			Percent population reduction attributed to treatment[b]	
	Pre-spray	Post-spray (8 day)	Post-spray (15 day)	Post-spray (8 day)	Post-spray (15 day)
Virus 1	56.7	73.6	28.5	0	0
Virus 2	42.8	53.3	34.7	34	48 **
Virus 3	14.0	13.6	7.9	0	26
Check 1	60.0	61-6	57.8	–	–
Check 2	36.0	67.8	56.4	–	–
Check 3	24.7	19.4	17.8	–	–

[a] Number of budworm larvae and pupae/m^2 foliage.

[b] Adjusted by Abbott's formula.

** Significant reduction at p = 0.01.

Table IV. Incidence of NPV in larvae and pupae collected at various dates from check plots and plots treated with virus on June 12

		Larvae collected June 22		Larvae collected June 28		Pupae collected July 4-6	
		no. insects	% NPV	no. insects	% NPV	no. insects	% NPV
Treated	1	123	39	143	55	289	6
	2	57	56	100	87	248	10
	3	37	35	57	25	–	–
Check	1	85	1	98	0	278	1
	2	73	4	83	0	244	0
	3	50	4	34	3	–	–

microscopically on arrival and the combined results of the larvae reared on the diet and larvae diagnosed on receipt of the shipment are given in Table IV. The incidence of virus infection in the combined samples were 39, 56 and 35% in virus-treated plots 1, 2 and 3, respectively, and 1, 4 and 4% in check plots 1, 2 and 3, respectively.

A further insect collection was made on June 28; no attempt was made to rear the larvae and all the insects were examined microscopically at F.P.M.I. for the presence of pathogens. In virus treated plots 1, 2 and 3, the incidence of NPV infections were 52, 87 and 25%, respectively (Table IV). In check plots 3, 3% NPV infection was found, and in check plots 1 and 2, no virus and 1 and 2% microsporidia were detected.

A pupal collection was made between July 4 and 6. These pupae were kept until adult emergence and the dead pupae were examined microscopically for the presence of pathogens (Table IV). In the treated plots, 6% NPV mortality was found in pupae from plot 1 and 10% from plot 2. In check 1, 1% NPV mortality was recorded and none in check 2. Treated plot 3 and check 3 were not sampled because of low population density of the insects. Pupal parasites were counted and an incidence of 8, 10, 24 and 14% parasitism was recorded in virus-treated plots 1 and 2 and check plots 1 and 2, respectively.

(vi) Effect of NPV Treatment on Pupae and Adults

Pupae were collected from virus-treated plots 1 and 2 and check plots 1 and 2 between July 4 and July 6. As an adequate collection could not be made on virus-treated plot 3, check 3 was also deleted from the pupal rearing schedule. The mean adult emergence of 40% from pupae collected from the treated plots was significantly lower than the 52% emergence from pupae collected on the check plots (Table V). The mean level of virus infection in the pupae was 8% in treated plots and 0.5% in check plots, indicating a significantly higher level of infection in the treated plots (Table IV).

The sex ratio of male to female moths which emerged from the pupae reared at Ashcroft was approximately 2:1 in the treated blocks as compared to the expected 1:1 ratio found in the check blocks. The average number of egg masses laid per emerged female on virus plot 2 was considerably less than in the corresponding check plot, as was the average number of eggs laid (Table V). The same effect was not recorded when virus plot 1 and check plot 1 were compared.

(viii) Defoliation Assessment

Loss of current foliage by crown thirds on all sample trees is summarized in Table VI. The distribution of defoliation varied over the crown; therefore, defoliation averages per tree were calculated from the crown level estimates of severity weighted by the proportion of current foliage located in each crown level. Only in virus plot no. 1 was the mean tree defoliation (15%) significantly less than defoliation in the corresponding check plot 1 (87%). This significance may have been due to the condition of the trees in check plot 1 which had been severely defoliated the previous year, resulting in poor growth of current shoots that were difficult to assess.

RESULTS OF B.t. TREATMENTS

(i) Deposit Analysis

Spray deposit on Kromekote cards was marginal on B.t.-treated plots 1 and 4, with mean number of droplets/cm^2 and standard deviations of 8 $\pm$ 6, and 11 $\pm$ 8, respectively. Deposits of B.t. on plots 2 and 3 were considerably better with means and standards deviations of 21 $\pm$ 12 and 31 $\pm$ 20, respectively. The spectrum of droplet sizes was wide, extending from less than 75μ to 750μ (Table I), but most of the droplet sizes were still smaller than the effective size found by Thompson et al. (1977), even though relatively large nozzles were used.

(ii) Meteorological Conditions

Meteorological conditions at the time of application were judged to be ideal, with cool, humid air, no rain and low wind speeds (Table VII). No rain fell on any plots for at least 10 days after treatment. Partly cloudy skies occurred on June 16, the day of application, and on June 18. Skies were essentially clear on June 17, 19, 20 and 21 for 15 hours per day.

(iii) Larval Development

Budworm larvae were mainly in the fifth

Table V. Effect of NPV treatments on moth emergence and oviposition recorded from pupae reared at Ashcroft

Treatments	Number of pupae caged		Percent adult emergence			Avg. no. of egg masses per emerged female	Avg. no. of eggs per emerged female
	males	females	males	females	Total		
Virus 1	100	100	55	23*	39.0	1.65	68.8
Virus 2	96	100	55	29*	42.0	0.65	29.4
Check 1	100	100	52	65	58.5	1.11	41.3
Check 2	100	100	48	44	46.0	1.50	66.2

* Significantly different from check sex ratio of $p < .0005$.

Table VI. Loss of current foliage of Douglas-fir on NPV treated plots after cessation of budworm feeding

Virus plots	Percent defoliation at 3 crown levels			Mean percent defoliation of tree[a/]
	upper	middle	lower	
1	4	13	28	15**
2	49	42	50	46
3	12	18	33	19
Check plots				
1	83	88	89	87
2	64	48	50	54
3	18	19	26	21

[a/] Severity weighted by size of each crown level.

** Significant reduction at $p \leq 0.01$.

instar, with about 20% sixth instar present at the time of pre-spray sampling, on June 12 (Fig. 4). Rainy weather prevented application but did not retard development. The B.t. was applied 4 days later, when, it was estimated, about 30% of the larvae were in the sixth instar. By the first post-spray sampling on June 23, more than half the population was in the sixth instar or had pupated.

(iv) Population Reduction Owing to Treatment

The only plot to show a noticeable reduction in population density by 8 days was B.t. 3 (Table VIII), suggesting that the treatment probably had not had time to take effect in all plots. Density data collected after 15 days were more promising and an analysis of variance was performed on the transformed densities of larvae per 100 buds. No significance could be detected between dates, but a variation ($P \leq 0.05$) among plots and a difference, at a probability of 0.01, for the critical Dates X Plots interaction was found. Using Tukey's test of all comparisons among Dates X Plots interaction means (Snedecor 1956), it was found that a significant drop in population density between the pre-spray sample and the 15-day post-spray sample occurred only in B.t. plot 3 (Table VIII). The lack of significant change in other plots was partly due to population redistribution within the crown which occurred during the test and is reflected in the check plots. Therefore, a test of survival ratios was made comparing the population changes of the treated plots with those of the check plots. A significant difference was found for B.t. plot 1 ($P \leq 0.05$) and for B.t. plots 2, 3 and 4 ($P \leq 0.01$). Hence, there was a statistically significant reduction in insect population density in the treated plots as compared to the check plots.

Another criterion of success is population density remaining after treatment. U.S. Forest Service personnel indicated that a residual population of 1 budworm larva per 100 shoots would be the maximum acceptable for satisfactory treatment (Anon. 1976). Using this criterion, B.t. 2 and 3 are close to acceptance but populations remaining in B.t. 1 and 4 were too high and could lead to severe defoliation the next year.

(v) Effects of the B.t. Treatment on Pupae and Adults

Field-collected pupae from treated plots and

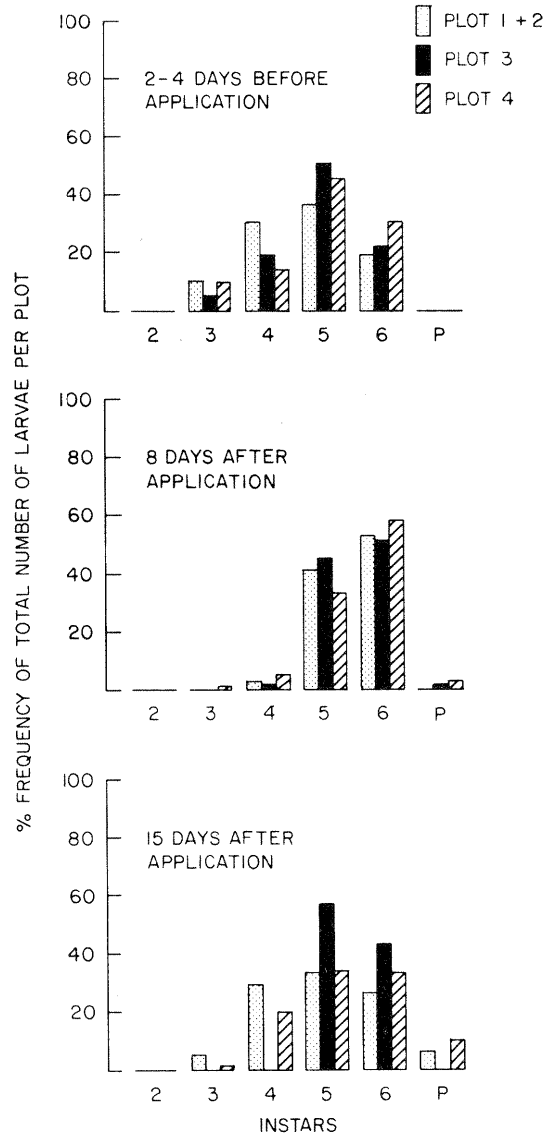

Fig. 4. Frequency of occurrence of each developmental stage of western spruce budworm at pre- and post-spray sample times, B.t.-treated plots, 1978.

Table VII. Meteorological conditions at time of spray application, B.t. plots, 1978

Plot no.	Date	Application commencement time	Wind speed (km/h)	Sky	Temperature	Percent relative humidity
1	June 16	0605	3-5	clear	7°C	72
2	June 16	0715	3-5	clear	8°C	75
3	June 17	0527	3-6	slight overcast	7°C	93
4	June 16	0526	2-3	clear	6°C	95

Table VIII. Effects of *Bacillus thuringiensis* on survival of western spruce budworm larvae in 1978

Treatment (plot)	Population density[1]			Percent population reduction due to treatment[2]	
	Pre-spray	Post-spray (8 day)	Post-spray (15 day)	Post-spray (8 day)	Post-spray (15 day)
Bt 1	7.8	15.5	7.7	0	32*
Bt 2	4.0	3.7	1.2	8	79**
Bt 3	10.5	5.9	1.5	68	91**
Bt 4	15.2	14.1	7.8	47	66**
Check 1	20.3	20.3	29.7	–	–
Check 2	8.5	14.9	12.8	–	–

[1] Number of budworm larvae and pupae/100 buds.

[2] Adjusted by Abbott's formula.

*,** Significant reduction at $p \leq 0.05$ and 0.01 respectively.

check areas in each region were reared and emergence and oviposition recorded. Fewer female moths emerged from pupae from the treated than the check areas (Table IX). There also appeared to be a difference in the number of egg masses laid between moths from treated and check plots, but when egg mass size was taken into account, treated plots were only slightly lower than the corresponding check plots. There was no evidence of virus or microsporidian infection in insects that died as pupae.

Percent pupal parasitism was recorded at 3, 15, 24 and 14% in B.t. plots 1 and 4 and check plots 1 and 2, respectively (Table IX). In check plot 1, a higher incidence of hymenopterous parasites was noticed. Ichneumonid species recovered from the rearings and, identified to date, are as follows: *Phaeogenes hariolus* Cress, *Glypta fumiferanae* Vier, *Apechthis ontario* Cress, *Itoplectis quadricingulata* Prov., *Mesopolobus verditer* Norton and *Dirophanes spirocous* Vier (M. Doganlar, pers. comm.). Two species of Tachinidae were also identified: *Edesia auricaudata* (Tnsd.) and *Madremyia saundersii* (Will.) (D.M. Wood, pers. comm.).

(vi) Defoliation Assessments

Loss of current foliage in treated plots was less than check plots and, except for B.t. 3, roughly corresponded to population levels (Table VIII). The difference in defoliation was statistically significant for B.t. 1 and 2 but not for B.t. 3 or 4. Again, this significant difference was partly due to the poor foliage condition of check plot 1. Although B.t. plot 3 had a large population reduction and a nearly acceptable post-spray density, it did not receive adequate foliage protection. Only in B.t. 2 was defoliation less than 15%, the maximum level acceptable by U.S. Forest Service standards (Anon. 1976).

DISCUSSION

The three replicates of the NPV treatment did not yield consistent results and neither did the four replicates of the B.t. treatment. The same virus treatment used in B.C. was replicated on six plots infested with eastern spruce budworm on white spruce, *Picea glauca* (Moench) Voss, and balsam fir, *Abies balsamea* (L.) Mill., in 1978 in Ontario (Cunningham et al. 1979). Here, population reduction on white spruce hosts was as high as 92% and on balsam fir hosts ranged from 37 to 76%. In Ontario, as in British Columbia, insect development was more advanced than desirable for effective spray treatment, with almost equal numbers of fifth and sixth instar present at the time of treatment.

The bud flush of Douglas-fir in B.C. relative to budworm development was later than expected; in Ontario, bud-flush on balsam fir and white spruce normally occurs when larvae are mainly in the fifth instars, with few sixth instar present. Under field conditions, NPV takes 2 to 3 weeks to kill larvae; hence, the 15-day post-spray sample taken in B.C. just before pupation probably did not reflect the full impact of the treatment. The NPV infection of 87% recorded in larvae in plot 2, 15 days after the application, is the highest level of infection reported for B.C. or Ontario in spruce budworm virus spray treatments to date; but that treatment only resulted in a larval population reduction of 48%. Therefore, all infected larvae did not die or they died as pupae or adults. In 1971, 71% maximum recorded virus infection in eastern spruce budworm resulted in a population reduction of 80% (Howse et al. 1973), and in 1977, a level of 61% maximum recorded NPV infection resulted in 92% population reduction using the same dosage as in B.C. (Cunningham et al. 1978).

The observation that the NPV caused greater mortality in female pupae than in male pupae is interesting. Similar selection against females has been observed in several other species of insects infected with NPV (Campbell 1963). One factor affecting this difference in incidence of virus is the longer development time for females than males, thus providing more time for virus to develop and kill females.

Very low incidence of microsporidia was encountered in the western spruce budworm population in the course of this study. This is in direct contrast to the eastern spruce budworm in Ontario, where the microsporidian, *Nosema fumiferanae* (Thom.), is extremely abundant. This chronic, debilitating pathogen may reduce host vigor, longevity and fecundity. Surveys in Ontario have shown that as the age of a budworm infestation increases, so does the incidence of microsporidosis, which may reach levels of over 70% in some cases (Cunningham et al. 1979). When running a virus production operation in the laboratory, it was found that eastern spruce budworm larvae infected with *N. fumiferanae* were not as susceptible to NPV infection as uninfected larvae (McPhee and Cunningham, unpublished).

Table IX. Effects of B.t. treatment on moth emergence and oviposition recorded from pupae reared at Ashcroft

Treatments	Number of pupae caged		Percent adult emergence			Percent pupal parasitism	Avg. no. of egg masses per emerged female	Avg. no. of eggs per emerged female
	males	females	males	females	total			
Bt 1	100	100	57	40*	48.5	3.0	0.65	36.5
Bt 4	100	109	49	34*	41.5	14.8	0.53	34.1
Check 1	100	100	52	65	58.5	24.0	1.11	41.3
Check 2	100	100	48	44	46.0	13.5	1.50	66.2

* Significantly different from check sex ratio at $p < .0005$.

Table X. Loss of current Douglas-fir foliage on plots treated with B.t., recorded after cessation of budworm feeding

Bt plots	Percent defoliation at 3 crown levels			Mean percent defoliation of tree[a]
	Upper	Middle	Lower	
1	11	19	50	27**
2	1	2	4	3**
3	44	37	50	41
4	28	36	48	40
Check plots				
1	83	88	89	87
2	64	48	50	54

[a] Severity weighted by size of each crown level.

** Significant reduction at $p \leq .01$.

From these observations, it was postulated that NPV and microsporidia are antagonistic in spruce budworm larvae and that the very low levels of microsporidia found in western spruce budworm may have rendered them more susceptible to NPV infection than the eastern species.

Budworm population density apparently increased between the prespray count and the 8-day post-spray count in most of the virus, B.t. and check plots (Tables III and VIII). This increas was probably due to a redistribution of the insect population within the tree crowns which could not be detected by the single-crown sampling schedule used in this experiment. It is suggested that, with high populations, insects feeding in the top crown descended to mid-crown as foliage in the top became scarce. This hypothesis was substantiated by the results from virus plot 3 and check 3, where the insect population density was considerably lower. In these plots with a low population density, a steady decline was observed.

There was no consistent foliage protection in the virus-treated plots, which may have been due to the insect developmental stage at the time of application. Similarly, no significant saving of foliage was noted in any plots treated in Ontario in the year of application. More important, perhaps, than the immediate population reduction and foliage protection is the persistence of the virus in the insect population, and its ability to keep the insect density below damage thresholds in future years.

The spore counts on the B.t. formulations showed that all treatments fell short of the planned 20 BIU/ha. Discrepancies between labels and spore counts have been a contentious issue in recent years. A reduction in spore count does not necessarily mean a reduction in toxin dosage and bioassay methods are being sought to establish the potency of B.t. preparations (Fast, pers. comm.).

The population reductions owing to the B.t. treatments were statistically significant in all the treated plots. However, except on B.t. plot 3, these reductions were not biologically significant because neither a reduction of damage below economic thresholds nor a suppression of insect populations sufficiently to give at least a year's respite from high insect population densities were not achieved. In B.t. applications on eastern spruce budworm in Ontario, high larval mortality was obtained by application on a late instar, but that foliage protection was poor (Tripp 1972).

Nuclear polyhedrosis virus is not at present an operationally available alternative to chemical insecticides for spruce budworm control and it is still at the experimental stage. The major constraint to its widespread use is the production cost. B.t. is an operationally available alternative to chemical insecticides and is registered in Canada for control of eastern spruce budworm. A good deposit is required and, as a rule of thumb, a deposit of at least 50 droplets/cm^2 is recommended (Harper 1974). It has also been shown that effective control can be obtained with droplets having a large mass median diameter (Thompson et al. 1977). The droplet sizes obtained in this trial were much smaller than expected, in spite of using nozzles with large orifices. The reason for this poor deposit is not completely understood, but it may be due partly to the difficulty in flying under conditions encountered in mountainous terrain.

It was judged that under the circumstances, one out of three replicates of the virus treatment gave satisfactory results and one out of four B.t. treatments was deemed adequate. The virus treatments were applied too late to allow the NPV adequate time to kill maximum numbers of larvae before the onset of pupation. If these biological control agents are to be used successfully in B.C. on western spruce budworm, a more detailed study is required to correlate bud phenology and larval development, and expected foliage saving that can be expected with treatment. It is necessary to establish if applications on earlier instars (peak of the fourth instar or 50% fifth instar) are technically feasible.

SUMMARY

Three 20 ha plots of Douglas-fir trees infested with western spruce budworm, *Choristoneura occidentalis,* were aerially treated at a rate of 750 billion polyhedra/ha with a nuclear polyhedrosis virus (NPV) which had been propagated in eastern spruce budworm, *C. fumiferana.* The aqueous formulation, emitted at 9.4 l/ha, contained molasses, Sandoz Shade® and Triton 1956B®spreader-sticker. Late bud flush delayed application until larvae were mainly in the fifth instar and the full impact of the virus treatment was not obtained before pupation occurred. At 15 days' post-spray, NPV infection incidence, determined microscopically, was found to be 25, 55 and 87% in insects from the three plots and population reductions owing to treatment on these

plots were calculated to be zero, 26 and 48%, respectively. Moth emergence was lower in treated plots and the male:female ratio was 2:1 in treated plots compared to 1:1 in check plots.

Four 40 ha plots were aerially sprayed with a commercial preparation of *Bacillus thuringiensis* (B.t.), Thuricide 16 B®, at a rate of 20 BIU/ha in 9.4 l/ha, when spruce budworm larvae were mainly in the fifth instar with about 20% sixth instar present. Statistically significant population reductions, owing to the treatment, were obtained in all four plots 15 days' post-spray and were calculated to be 32, 66, 79 and 91%. Moth emergence and number of eggs laid were only slightly less in the treated than in the check plots.

No consistent foliage protection was detectable in either the plots sprayed with NPV or B.t. and this is attributed to the late timing of the application.

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