AERIAL APPLICATION OF NUCLEAR POLYHEDROSIS VIRUS AGAINST DOUGLAS-FIR TUSSOCK MOTH, *ORGYIA PSEUDOTSUGATA* (McDUNNOUGH) (LEPIDOPTERA: LYMANTRIIDAE): II. IMPACT 1 AND 2 YEARS AFTER APPLICATION

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Abstract

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Following aerial application of a Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough), nuclear polyhedrosis virus (NPV) product called Virtuss on four plots in 1982, observations were made to determine the impact of these treatments in 1983 and 1984. Treated plots as well as buffer zones between and adjoining the treated plots, and three of the four check plots established in 1982, were monitored. The NPV appeared to have spread from the treated plots to adjoining areas in 1982, effectively reducing the Douglas-fir tussock moth population. This observation suggests that a strategy of spraying alternate swaths of Douglas-fir tussock moth infested stands with this viral insecticide may effectively initiate an epizootic that would control the population at a reduced cost.

A naturally occurring NPV epizootic decimated the Douglas-fir tussock moth population in the three check plots in 1983, but severe tree mortality occurred in two of these plots with 60 and 62% of sample trees dead in 1984. Light tree mortality was noted in 1984 in two of the four treated plots with 4 and 7% of sample trees killed. It is concluded that the virus treatments in 1982 were successful in preventing tree mortality.

Résumé

Un épandage aérien d'un produit appelé Virtuss et contenant le virus de la polyédrose nucléaire (VPN) de la chenille à houppes du douglas, *Orgyia pseudotsugata* (McDunnough), a été effectué sur quatre placettes en 1982. En 1983 et 1984, on a cherché à déterminer les répercussions de ces traitements dans les placettes traitées de même que dans les zones tampons de chaque côté des placettes et on a aussi examiné trois des quatre placettes témoins établies en 1982. Il semblerait que le VPN se soit propagé aux zones voisines où une nette réduction de la population de la chenille a été observée. On peut donc penser qu'une stratégie consistant à alterner les bandes arrosées et non arrosées dans les peuplements infestés pourrait être efficace en déclenchant une épizootie qui permettrait de réduire la population à un coût réduit.

Une épizootie du VPN d'origine naturelle a décimé la population de la chenille dans les trois placettes témoins en 1983; on a cependant observé en 1984 une mortalité très importante des arbres dans deux de ces placettes, soit de 60 et 62 % des arbres d'échantillonnage. Par contre, une faible mortalité des arbres a été observée en 1984 dans deux des placettes traitées où 4 et 7 % des arbres d'échantillonnage avaient été tués. On en conclut que les traitements viraux appliqués en 1982 ont permis d'empêcher une mortalité élevée des arbres.

Introduction

In the spring of 1982, a Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough), nuclear polyhedrosis virus (NPV) product called Virtuss was applied from the air to four 10-ha plots near Veasy Lake, Kamloops Forest District, British Columbia. Three dosages, 2.5×10^{11}, 8.3×10^{10}, and 1.6×10^{10} polyhedral inclusion bodies (PIB) per hectare, were applied in an emulsifiable oil tank mix (one part oil : three parts water) and

a dosage of 2.5×10^{11} PIB per hectare was applied in an aqueous tank mix (one part molasses : three parts water). Four untreated plots were monitored as checks. Population reductions due to treatments, calculated using a modified Abbott's formula (Fleming and Retnakaran 1985), were 65% in the plot receiving the lowest dosage and from 87 to 95% in the remaining three plots. Incidence of virus infection, determined microscopically, in larvae examined at 5–6 weeks post-spray reached 85–100% in the treated plots and a naturally occurring NPV infection reached maximum levels ranging from 0.7 to 43.4% of larvae in the four check plots at 7 weeks post-spray. Reduction in egg-mass density attributed to the virus spray ranged from 97 to 99% in three of the treated plots and was not determined for the fourth.

Protection of the 1982 foliage was negligible because the virus is slow acting and Douglas-fir tussock moth has a long larval development stage. Consequently, all but one of the treated plots (T4, with the lowest larval density) were severely defoliated. All the check plots, except one with a low Douglas-fir tussock moth population (C4), were severely defoliated as well. Western spruce budworm, *Choristoneura occidentalis* Freeman, larvae were also present in both the treated and check plots, thus making the partitioning of the total defoliation contributed by the two species impossible as the insects are competitors for food. Virtuss does not infect the western spruce budworm in the laboratory (Cunningham, unpublished data) and the treatment had no effect on this species in the field. Details and results of the 1982 spray operation were reported by Otvos *et al.* (1987).

The effects of strategies aimed at regulating insect populations may extend beyond the year of application and these longer-term effects may profoundly influence decisions taken by forest managers. For this reason, following the virus application in 1982, studies were continued in 1983 and 1984 and the results are presented in this report. The primary objective was (*a*) to determine if the virus treatments prevented tree mortality. Other parameters examined were (*b*) population densities of both Douglas-fir tussock moth and western spruce budworm in treated plots, buffer zones, and check plots in 1983, (*c*) the development and progress of Douglas-fir tussock moth NPV epizootics in 1983 in treated plots, buffer zones, and check plots, and (*d*) defoliation in 1983 and 1984.

Materials and Methods

Experimental plots. The four experimental plots, designated T1–T4, and their corresponding check plots, C1–C4, in 1982 are shown in Figure 1. Details of the treatments are given by Otvos *et al.* (1987). During the winter months of 1982/1983, a corridor for a hydro-electric line was cut through plot C2 removing two-thirds of it; therefore studies on this plot were discontinued. The buffer zones adjoining and between the treatment plots were designated as BA, BB, BC, BD, and BE (Fig. 1).

Sampling techniques. Due to manpower shortages and low Douglas-fir tussock moth numbers, the original 45 sample trees per plot (Otvos *et al.* 1987) were not sampled; instead, sample lines were run diagonally across each of the four treated plots and medially across the remaining three check plots, and one tree was sampled at each 25-m interval (Fig. 1). Straight lines were run in the buffer zones at right angles to the treated plots and a sampling station established at each 25-m interval. For population counts, a pair of 45-cm branch tips were clipped from the mid-crown of each of the sample trees. Douglas-fir tussock moth and western spruce budworm larvae were counted and the tussock moth larvae subsequently examined microscopically for evidence of NPV infection. When numbers of tussock moth larvae were low in the branch sample, additional larvae were collected by beating larvae from adjacent trees. In 1983, two surveys were made: the first between 8 and 24 June, when Douglas-fir tussock moth larvae were in the third and fourth instars, and the second between 14 and 28 July, when larvae were in the fifth and sixth instars. In

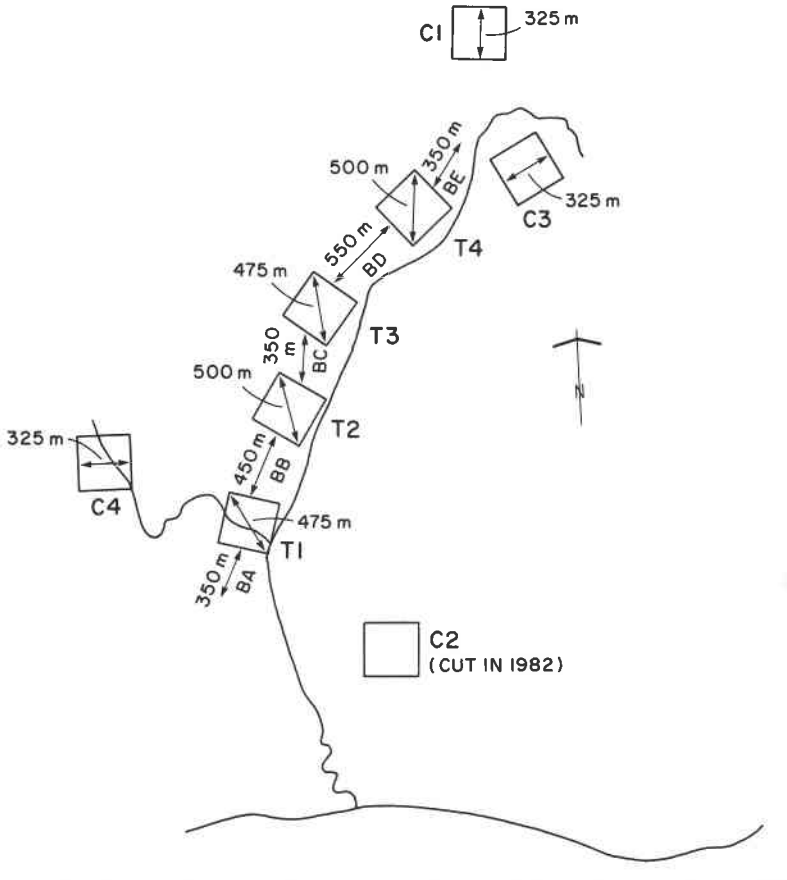

FIG. 1. Diagram showing sample lines used for insect population and virus infection studies in 1983 in areas treated with Virtuss in 1982 and corresponding check plots near Veasy Lake, Kamloops Forest District, B.C. T, treated plot; C, check plot; B, buffer zone.

1984, no Douglas-fir tussock moth larvae could be found in either the treated or check plots, so population counts were discontinued.

Microscopic examination of larvae. Larvae collected during population studies were smeared on glass slides and examined microscopically for the presence of PIBs. Those containing PIBs were scored as positive. The aim was to examine at least 100–200 larvae per plot and per buffer zone per survey date, but this was not always possible, particularly when the second sample was collected, due to very low Douglas-fir tussock moth population densities.

Tree defoliation. Defoliation estimates were made on the original 45 sample trees in each treated and the remaining three check plots which had been used in the 1982 assessment. The overall defoliation of the tree, taking into account the amount of both current and old needles missing, was estimated by a method similar to that of Wickman (1978) in which tree defoliation was assessed to the nearest 10% according to the proportion of crown length that was 100% defoliated. The defoliation estimates of each tree were added and the mean for the plot calculated. Trees classified as dead were excluded from the calculations. Defoliation measurement was conducted in the spring of 1984, before any insect

feeding, and it reflected the cumulative defoliation by Douglas-fir tussock moth and western spruce budworm as of the end of the 1983 feeding season. Although only western spruce budworm larvae were found in 1984, a second defoliation measurement conducted after the 1984 feeding season reflected the defoliation resulting from the earlier combined Douglas-fir tussock moth and western spruce budworm feeding on old foliage plus the feeding of the western spruce budworm on the 1984 current foliage.

Tree mortality. The cumulative number of dead trees was recorded on the original 45 sample trees in each of the four treated and the three remaining check plots in 1983 and 1984 and re-checked in 1985. Douglas-fir trees have a great ability to survive and some trees with no needles in 1 year may put out shoots the following year and recover; for this reason a tree was classified dead only when it had no needles for 2 consecutive years.

Data analysis. Significant differences in mean plot defoliation were tested by ANOVA with percentages transformed to arcsin. The numbers of trees killed by defoliation in the treated and check plots were analyzed using the χ^2 test.

Results

Population densities of Douglas-fir tussock moth. In the first sample, in June 1983 when larvae were in the third and fourth instars, populations were low in both the treated plots and buffer zones, ranging from 1.5 to 10.7 per square metre in the treated plots and from 2.2 to 9.5 larvae per square metre in the buffer zones (Fig. 2). Populations were much higher in check plots; C1 had 162.5 larvae per square metre, C3 had 70.6 larvae per square metre, and C4, which also had a much lower Douglas-fir tussock moth population density than C1 and C3 in 1982, had 23.6 larvae per square metre. In the second sample, in July, when larvae were in the fifth and sixth instars, there was a dramatic drop in population density in both the treated and check plots (Fig. 3). Population density in the treated plots

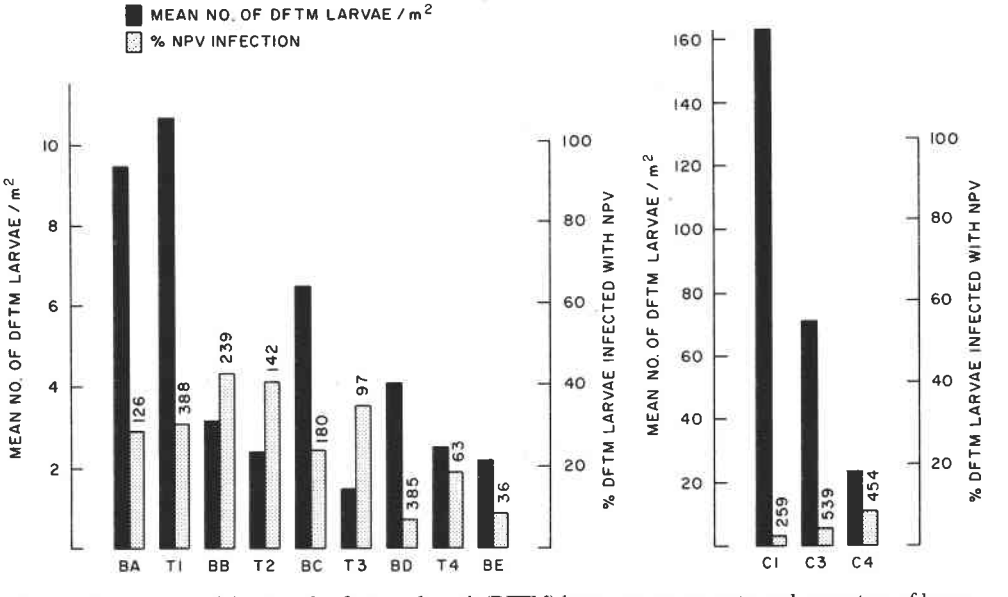

FIG. 2. Mean number of live Douglas-fir tussock moth (DFTM) larvae per square metre and percentage of larvae infected with NPV in the treated plots (T1–T4), buffer zones (BA–BE), and check plots (C1, C3, and C4) sampled between 8 and 24 June 1983. The number of larvae examined microscopically is indicated above the percentage infection bars.

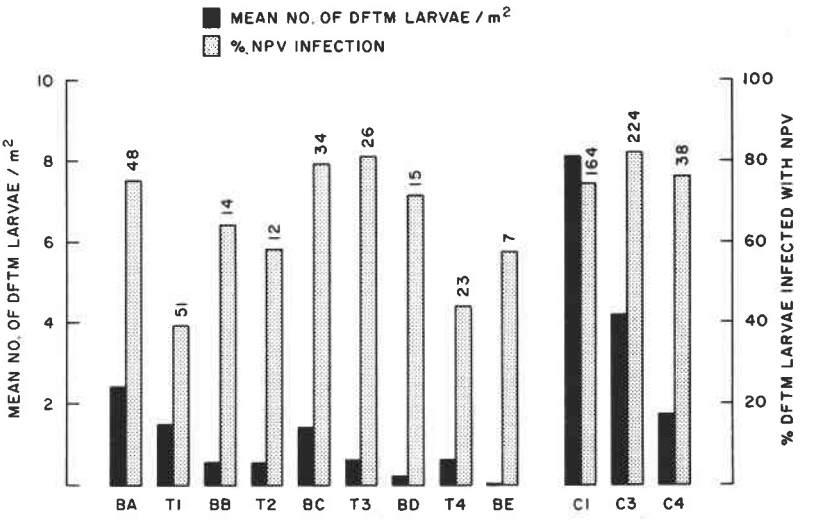

FIG. 3. Mean number of live Douglas-fir tussock moth (DFTM) larvae per square metre and percentage of larvae infected with NPV in the treated plots (T1–T4), buffer zones (BA–BE), and check plots (C1, C3, and C4), sampled between 14 and 28 July 1983. The number of larvae examined microscopically is indicated above the percentage infection bars.

ranged from 0.5 to 1.5 larvae per square metre and in the buffer zones from 0 to 2.4 larvae per square metre. In the check plots it ranged from 1.7 to 8.1 larvae per square metre.

Population densities of western spruce budworm. Western spruce budworm and Douglas-fir tussock moth compete as both insects preferentially feed on the current year's growth. For this reason, western spruce budworm population densities probably reflected the amount of available foliage on Douglas-fir trees. In the first sample, population densities in the buffer zones and treated plots ranged from 15.6 to 71.0 and from 33.6 to 107.3 larvae per square metre, respectively. Check plots C1 and C3 had 8.4 and 6.2, respectively, and C4, in which trees were well foliated, had 86.2 larvae per square metre (Fig. 4). Plot C4 had the lowest pre-spray Douglas-fir tussock moth population density among the check plots. In the second sample, western spruce budworm population declines were not as severe as Douglas-fir tussock moth (Figs. 3 and 4), except for C1 where the population dropped from 8.4 to 0.4 larvae per square metre, probably due to starvation.

Incidence of virus infection in Douglas-fir tussock moth. In the first sample taken in 1983, the incidence of virus infection in the treated plots ranged from 19.0 to 40.8% and in the buffer zones from 7.3 to 43.5% (Fig. 2). The check plots had a much lower incidence, ranging from 1.9 to 7.9% of the larvae infected with NPV. In the second sample, larvae were scarce in both the treated plots and buffer zones. The incidence of NPV-infected larvae ranged between 39.2 and 80.8% and 57.1 and 75.0%, in the treated plots and buffer zones, respectively. Incidence of NPV in the check plots had risen dramatically to range between 74.4 and 81.7% (Fig. 3).

Tree defoliation. Because of the reduced Douglas-fir tussock moth larval population, foliage recovery in surviving trees was noticeable in 1983 and 1984. A low-level western spruce budworm population partially consumed the new foliage produced in 1983 and 1984. Average defoliation in the check plots (pooled) was significantly higher (16.1% in 1983 and 6.4% in 1984) than in treated plots (0–6% in 1983 and 0–1.6% in 1984) (ANOVA $F = 8.1$ in 1983 and $F = 6.2$ in 1984, both $P < 0.001$). This difference reflected the

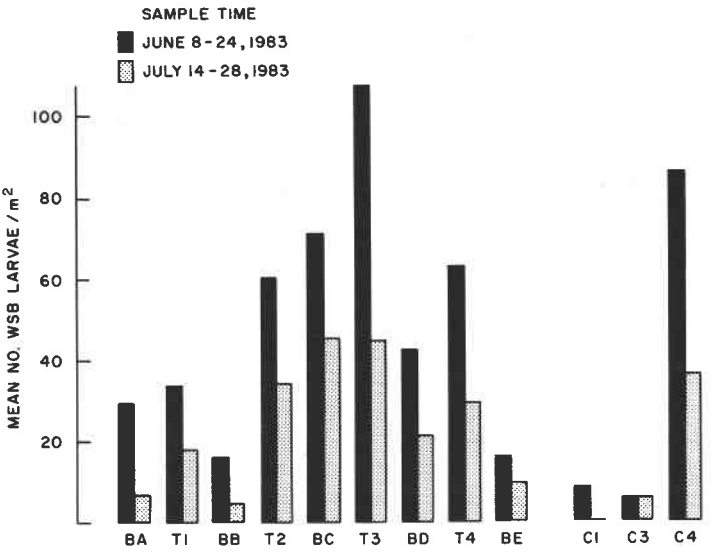

FIG. 4. Western spruce budworm (WSB) larval density in the treated plots (T1–T4), buffer zones (BA–BE), and check plots (C1, C3, and C4) in 1983.

overall foliage protection due to the NPV spray. When individual check plots were tested against the treated plots, only check plots C1 and C4 in 1983 and only C4 in 1984 had significantly higher defoliation than the treated plots (ANOVA and Duncan's multiple range test, $P < 0.001$) (Table 1). Foliage recovery on check plot C3 occurred in 1983 and in plot C1 in 1984.

Tree mortality. The 1983 survey showed heavy losses in two of the check plots, C1 and C3, with 53 and 60% mortality of the sample trees, respectively (Table 1). No tree mortality occurred in check plot C4, probably because of the low initial Douglas-fir tussock

Table 1. Defoliation estimates and cumulative number of trees killed by Douglas-fir tussock moth (DFTM) larvae in four plots treated with Virtuss in 1982 (T1–T4) and in three check plots (C1, C3, and C4) near Veasy Lake, Kamloops Forest District, B.C., in the 2 years following application

		1983					1984			
			No. of trees					No. of trees		
Plot No.	1982 pre-spray DFTM density/m²	% defoliation	Total	Alive	Killed by DFTM (cumulative) No.	%	% defoliation	Alive	Killed by DFTM (cumulative) No.	%
C1	197.5	14.8a*	45	21	24	53	2.2b	18	27	60
C3	360.6	10.3ab	45	18	27	60	0b	17	28	62
C4	81.2	19.1a	45	45	0	0	10.4a	45	0	0
T1	182.8	3.5b	45	45	0	0	1.1b	45	0	0
T2	145.8	6.0b	45	44	1	2	0.9b	42	3	7
T3	302.0	2.9b	45	45	0	0	1.6b	43	2	4
T4	41.8	0b	45	45	0	0	0b	45	0	0

*Mean defoliation figures followed by the same letter are not significantly different ($P>0.05$) using Duncan's multiple range test.

moth population in this plot (Table 1). Mortality was negligible in the treated plots with only one dead tree (2.2%) by 1983 in plot T2 (Table 1). In 1984, tree mortality increased to 60 and 62% of the trees in check plots C1 and C3, and to 7 and 4% in treated plots T2 and T3 (Table 1). No mortality occurred in check plot C4 or treated plot T4.

Chi-square tests indicated a significant difference in tree mortality between the two check plots in which mortality occurred and the treated plots ($\chi^2 = 148$, $P<0.001$) showing that the virus application at the concentrations and formulations applied prevented tree mortality. The number of dead sample trees was not significantly different among the treated plots in 1984 ($\chi^2 = 5.6$, $P>0.05$) suggesting that all four treatments were equally effective in preventing tree mortality. A survey in 1985 showed that trees recorded as dead in 1984 had not recovered and that no additional trees died as a result of previous Douglas-fir tussock moth defoliation in 1982 and 1983.

Discussion

As indicated by the percentage infection in larval samples, a virus epizootic developed earlier in the treated plots and buffer zones than in the check plots in 1983 as a direct result of the application of Virtuss in 1982 (Fig. 2). Although naturally occurring NPV was present in the check plots, it occurred at much lower levels at the beginning of the season and it only increased to epizootic levels toward the end of the larval period almost reducing Douglas-fir tussock moth densities to the same low level as in the treated plots. Artificially disseminated virus, carried over from 1982, was present in the treated plots, but presumably some naturally occurring NPV was present as well. The incidence of NPV in the tussock moth population in the buffer zones was not monitored in 1982, but 1983 figures indicate that virus had spread from the treated plots in 1982 and reduced population densities to levels similar to those on the treated plots. In the second sample, taken in 1983, the naturally occurring virus in the check plots reached levels as high as or even higher than the artificially disseminated virus in the treated plots and buffer zones (Fig. 3). From these data, we conclude that the 1982 virus application brought about the collapse of the population in the treated plots earlier than if it had been left to the naturally occurring NPV epizootic and hence prevented tree mortality in these plots.

The question of spread of artificially disseminated NPV from treated to untreated areas is extremely difficult to answer and has never been unequivocally resolved. It appears that virus from treated plots spread to the buffer zones; this could be partially due to spray drift or larval migration. However, our data indicate that Douglas-fir tussock moth population densities and levels of infected larvae in the buffer zones were similar to the treated plots, and markedly different from those in the check plots. If Douglas-fir tussock moth NPV does, in fact, spread from sprayed to unsprayed areas, then a novel strategy can be used to apply Virtuss. Instead of blanket spray coverage of Douglas-fir tussock moth infested stands, widely spaced swaths, 100–200 m apart, may be used to "seed" the virus into the insect population. Normally, 30-m swaths are sprayed for blanket coverage and this strategy of spraying widely spaced swaths would reduce the cost of operational spray programs 3- to 6-fold.

Populations of western spruce budworm larvae in the experimental area made it difficult to evaluate the impact of Douglas-fir tussock moth larvae on the trees. However, the presence of western spruce budworm in the experimental plots confirmed laboratory results that Virtuss does not infect western spruce budworm larvae. Another NPV that infects western spruce budworm is available, but its performance in field trials was less than satisfactory (Cunningham 1985). Even if potentially useful, an application of a mixture of two viral insecticides would not be economically feasible at this time because of the cost of production of the two. Treatment with most chemical insecticides or with a *Bacillus thuringiensis* Berliner preparation would have had an impact on both Douglas-fir tussock moth and western spruce budworm, although disappointing results have been obtained

with *B. thuringiensis* on both species in earlier trials conducted in British Columbia (Shepherd and Cunningham 1984; Cunningham and Shepherd 1984).

Population densities of western spruce budworm recorded in 1983 probably reflect the amount of current year's foliage available in that year in the different plots and possibly the effect of starvation in the previous year. At the first count, western spruce budworm densities were generally higher in the treated plots and buffer zones than in the check plots supporting the idea that there is competition between Douglas-fir tussock moth and western spruce budworm and starvation of the latter species occurred in the check plots where most of the foliage had been consumed by Douglas-fir tussock moth.

Because Douglas-fir tussock moth NPV is slow acting, the progress of an epizootic peaks 5–6 weeks after spraying; therefore foliage protection is often negligible in the year of virus application (Shepherd *et al.* 1984; Otvos *et al.* 1987). However, if the trees are not killed, subsequent growth loss may be the only consequence in stands treated with Virtuss. In addition to growth loss, untreated stands will sustain heavy mortality as indicated by the 60.0 and 62.2% dead trees in check plots C1 and C3, respectively. As western spruce budworm populations in general were low and because this species is not as ravenous a defoliator or as wasteful a feeder as Douglas-fir tussock moth, the heavy tree mortality in check plots C1 and C3 was assumed to be due to Douglas-fir tussock moth feeding. It has been observed that Douglas-fir tussock moth can severely defoliate and kill trees in 1 year (Canadian Forestry Service, Forest Insect and Disease Survey, unpublished data) but it takes 2–3 years of defoliation by western spruce budworm to kill trees (Alfaro *et al.* 1982).

It is evident that the Virtuss treatments prevented significant tree mortality in the treated plots in spite of the western spruce budworm population also present in the area. Virtuss was applied in the 2nd year of the Douglas-fir tussock moth outbreak; had treatments been delayed until the 3rd year, they would have been virtually useless and a naturally occurring NPV epizootic probably would have had the same effect in terms of collapse of the Douglas-fir tussock moth infestation. Early detection of outbreaks of Douglas-fir tussock moth is therefore vital if NPV is to be used as a control agent (Shepherd *et al.* 1984). Monitoring with pheromone traps has been advocated as a means of early detection providing evidence of the presence of Douglas-fir tussock moth populations before visible defoliation is observed, thereby providing sufficient lead time for various control measures (Shepherd and Otvos 1986).

The lowest dosage of 1.6×10^{10} PIB per hectare had less impact on Douglas-fir tussock moth larval densities than the two higher dosages in the year of treatment (Otvos *et al.* 1987). However, observations in 1983 and 1984 indicated no real differences between the three dosages in preventing tree mortality except that the plot receiving the lowest dosage had slightly higher Douglas-fir tussock moth larval populations in 1983 than the other three plots. Reluctance to recommend the lowest dosage for Douglas-fir tussock moth control was expressed by Otvos *et al.* (1987). Virtuss is expensive to produce but this cost could be greatly reduced by applying lower dosages or by introducing the NPV by spraying widely spaced swaths. More research is required before these strategies can be recommended with confidence, and we hope to test them when the next Douglas-fir tussock moth outbreak occurs in British Columbia.

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