AERIAL APPLICATION OF NUCLEAR POLYHEDROSIS VIRUS AGAINST DOUGLAS-FIR TUSSOCK MOTH, *ORGYIA PSEUDOTSUGATA* (McDUNNOUGH) (LEPIDOPTERA: LYMANTRIIDAE): I. IMPACT IN THE YEAR OF APPLICATION

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Abstract

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Four 10-ha plots located in Kamloops Forest District, British Columbia, containing Douglas-fir trees infested with Douglas-fir tussock moth were aerially sprayed with nuclear polyhedrosis virus (Virtuss) in 1982 when most larvae were in the first instar. A dosage of 2.5×10^{11} polyhedral inclusion bodies (PIB) per hectare was applied in an emulsifiable oil tank mix to one plot and the same dosage in an aqueous tank mix containing molasses was applied to a second plot. The remaining two plots were treated with dosages of 8.3×10^{10} and 1.6×10^{10} PIB per hectare, respectively, in the oil mix. The treatments were applied with a fixed-wing aircraft fitted with boom and nozzle equipment and calibrated to deliver 9.4 L/ha. A further four plots were selected as checks.

Population reduction at 6 weeks post-spray (calculated using a modified Abbott's formula) was 65% in the plot receiving the lowest dosage and from 87 to 95% in the remaining three plots. Incidence of virus infection, determined microscopically, peaked at 5–6 weeks post-spray with 85–100% of the larvae scored as positive. Levels of naturally occurring virus remained low in the check plots. Adult emergence from the pupae collected in the treated plots ranged from 4 to 19% and from 28 to 43% in the check plots. Reduction in egg-mass density attributed to the treatments was 97% in one plot, 99% in two others, and not determined for the fourth.

A virus dosage of 8.3×10^{10} PIB per hectare, which is one-third of the previously recommended dosage, is adequate, and either tank mix is acceptable.

Résumé

En 1982, quatre placettes de 10 ha situées dans le district forestier de Kamloops, en Colombie-Britannique, qui renfermaient des douglas taxifoliés infestés par la chenille à houppes du douglas ont été traitées par des épandages aériens de diverses préparations du virus de la polyédrose nucléaire (Virtuss) au moment où la plupart des larves se trouvaient au premier stade. Une première placette a reçu une dose de $2{,}5 \times 10^{11}$ corps d'inclusion polyédriques (CIP) par hectare sous forme d'une préparation huileuse émulsionnable, et une autre, la même dose, mais dans une préparation aqueuse contenant des mélasses. Les deux autres placettes ont reçu une préparation huileuse contenant $8{,}3 \times 10^{10}$ CIP/ha dans un cas et $1{,}6 \times 10^{10}$ CIP/ha dans l'autre. Les traitements ont été réalisés à l'aide d'un avion muni d'une rampe de pulvérisation étalonnée pour un débit de 9,4 L/ha. Quatre autres placettes ont été utilisées comme témoins.

Six semaines après les arrosages, la réduction de la population due au traitement (calculée suivant la formule modifiée d'Abbott) s'élevait à 65 % dans la placette ayant reçu la dose la plus faible et elle variait entre 87 et 95 % dans les trois autres placettes. L'infection virale, déterminée au microscope, a été maximale vers la 5e ou 6e semaine après l'arrosage, de 85–100 % des larves étant alors infectées. Le niveau d'infection naturelle dans les placettes témoins est demeuré faible. On a observé des taux d'émergence d'adultes variant entre 4 et 19 % chez les chrysalides récoltées dans les placettes traitées et entre 28 et 43 % chez celles des placettes témoins. La réduction de la densité

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des masses d'oeufs attribuable au traitement a été de 97 % dans une placette et de 99 % dans deux autres; elle n'a pas été déterminée dans la quatrième.

On estime qu'une dose de 8,3 × 10^{10} CIP/ha, soit le tiers de la dose déjà recommandée, est suffisante et que les deux préparations sont acceptables.

Introduction

The bionomics of Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough), in British Columbia and the western United States (U.S.A.) have been reviewed by Wickman and Beckwith (1978) and Shepherd *et al.* (1984*b*). Naturally occurring nuclear polyhedrosis virus (NPV) epizootics usually terminate Douglas-fir tussock moth outbreaks (Dahlsten and Thomas 1969; Mason and Luck 1978), but not before trees are severely defoliated or killed (Wickman 1978).

Dissemination of virus as a biocontrol agent was considered as early as 1962 in British Columbia (Morris 1963), but it was not until 1975 that large-scale aerial spray experiments were conducted in collaboration with U.S.D.A. Forest Service personnel in British Columbia (Stelzer *et al.* 1977; Shepherd 1980; Cunningham and Shepherd 1984). Two NPV morphotypes were tested: a unicapsid type with virus particles embedded singly in inclusion bodies and a multicapsid type with bundles of virus particles occluded. Subsequently, the multicapsid type was registered by the Environmental Protection Agency in the U.S.A. in 1976. The same virus, propagated in whitemarked tussock moth, *Orgyia leucostigma* (J.E. Smith), was granted a temporary registration in Canada in 1983 under the name Virtuss. This product contains lyophilized, virus-infected larvae ground to a fine powder.

Virtuss was applied both from the air and the ground in 1981 at the early phase of a Douglas-fir tussock moth outbreak in south-central British Columbia before the occurrence of a natural virus epizootic. From the air, a dosage of 2.2 × 10^{11} polyhedral inclusion bodies (PIB) per hectare in 11.3 L/ha was applied; from the ground, a dosage of 2.4 × 10^{10} PIB in a volume of 4.5 L was applied to each tree. All treatments prevented outbreaks (Shepherd *et al.* 1984*b*). Aqueous tank mixes with the addition of 25% (v/v) molasses were used in 1981; this mix has been widely used in previous tests in both Canada and the U.S.A. (Cunningham 1982). However, with the low relative humidity in the interior of British Columbia, spray deposits have often been poor.

In 1982, the second year of the Douglas-fir tussock moth outbreak at another location, Virtuss was again applied from the air, using two different tank mixes. In the water mix, the full dosage (2.5 × 10^{11} PIB per hectare) of the virus was used; in the oil mix, the virus was applied at the full dosage, as well as at two reduced dosages. The aim of these trials was 2-fold: (*a*) to compare an emulsifiable oil tank mix with an aqueous tank mix; and (*b*) to test reduced dosages of virus in the oil tank mix in an effort to reduce treatment costs. The impact of these treatments in the year of application, assessed by several different methods, is described.

Materials and Methods

Experimental plots. Four 10-ha plots containing Douglas-fir, *Pseudotsuga menziesii* var. *glauca* (Beissner) Franco, and a few scattered ponderosa pine, *Pinus ponderosa* Lawson, were selected for treatment near Veasy Lake, about 15 km northwest of Cache Creek, B.C., at elevations ranging from 700 to 900 m in the Kamloops Forest District. Four check plots (no treatment) were established in the same area (Fig. 1). Treatment plots were separated by a minimum buffer zone of 300 m and check plots were 300 m to 1 km away from the treated plots.

Treated plots were numbered T1, T2, T3, and T4 and matched to untreated check plots which had comparable insect population densities. Plot C4 was selected 3 days after the spray application because the pre-spray counts revealed that C1, C2, and C3 had much

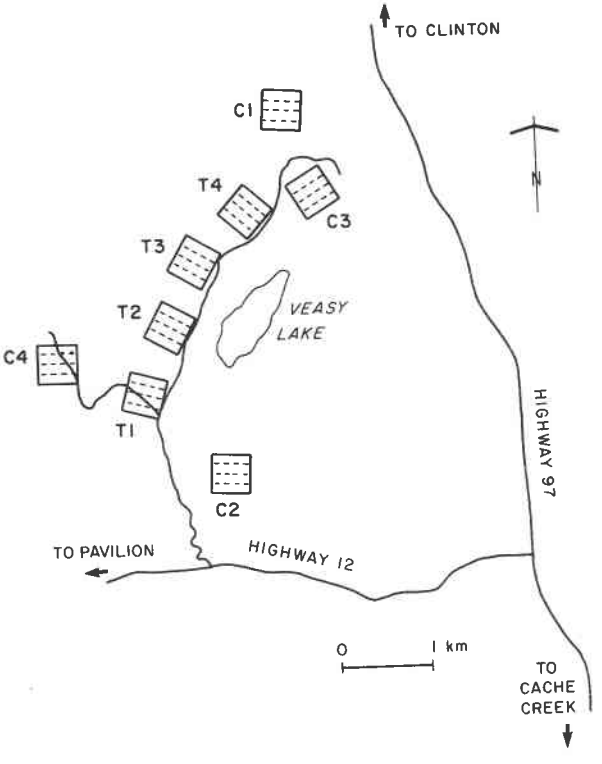

FIG. 1. Location of study plots for the experimental application of Virtuss on Douglas-fir tussock moth infested stands near Veasy Lake, Kamloops Forest District, B.C., 1982. Plots T1–T4 were treated and matching check plots were designated C1–C4. Broken lines through plots indicate lines of sample trees; spray aircraft flew at right angles to these lines.

higher population densities than spray plot T4 and a search was made for a plot with a comparable insect population density.

Spray application and monitoring deposit. Three dosages of Virtuss, 1.6×10^{10}, 8.3×10^{10}, and 2.5×10^{11} PIB per hectare, were tested in an emulsifiable oil carrier. The carrier was supplied by Abbott Laboratories and is the vehicle used to formulate their *Bacillus thuringiensis* Berliner variety *kurstaki* isolate HD-1 product called Dipel 88®. Virtuss was suspended in water and emulsifiable oil was added until the final ratio was one part of oil to three parts of water. A fourth plot was treated with 2.5×10^{11} PIB per hectare in an aqueous tank mix containing one part of animal-feed grade molasses to three parts of water. Rhodamine B dye at 0.04% was added to all tank mixes to monitor spray deposit.

Treatments were assigned randomly to the blocks. Timing of the spray application was determined by monitoring 50 Douglas-fir tussock moth egg masses per plot daily for larval hatch and dispersal. The spray was applied 1 week after 90% of the larvae had dispersed from these egg masses; the larvae were mainly in the first instar although a few second-instar larvae were present. A Cessna AgWagon was calibrated to deliver 9.4 L/ha through 42 Tee-Jets with 8005 nozzles mounted on the boom. Flight speed was 180 km/h at a height of 15 m above the tallest trees and the swath width was 30 m. Spraying was conducted at dawn when wind speed was about 2 km/h. The three treatments of Virtuss in emulsifiable oil were applied on 16 June, and the water and molasses tank mix was

applied on 17 June. Relative humidity on the ground was measured with a sling psychrometer. A rain gauge was used to measure precipitation for 1 week after application at the sites.

Spray deposit was monitored on Kromekote® cards held in wire holders about 50 cm above ground level placed about 10 m apart in openings in the forest canopy. Two lines of cards at right angles to the flight path were set 30 m from both ends of each treated plot. All cards were collected about 1 h after spraying and later analyzed for droplet density.

Egg-mass, larval, and pupal surveys. Fifteen Douglas-fir trees were selected as sample trees in each of three lines per plot giving a total of 45 sample trees per plot. There was about 110 m between lines and about 15 m between sample trees. None of the sample trees was closer than 50 m to a plot boundary. Sample trees were about 12 m tall and had ample foliage for weekly branch sampling. Egg-mass surveys (Shepherd *et al.* 1984*a*) were conducted in each plot in early spring, before eclosion and virus treatment, and again in late fall after oviposition was completed. The mean number of Douglas-fir tussock moth egg masses on three lower whole branches was recorded for each of the 45 sample trees. Pre-spray larval counts were made from two, 45-cm branch tips cut from the mid-crown of each sample tree in each plot: hence, counts were made from 90 branch samples per plot. Counts were made at weekly intervals until 6 weeks after spraying when pupation commenced. For each branch, foliated length, average foliated width, and number of live and dead larvae were recorded. The foliated area was calculated for each branch; the density of larvae for that area was converted to number of larvae per square metre and used to calculate standard deviation and standard errors. Sampling crews and equipment were assigned to either treated or check plots for the duration of the experiment to avoid contamination of the check plots with virus. Population reduction due to treatment was calculated using a modified Abbott's formula (Abbott 1925) described by Fleming and Retnakaran (1985). These calculations used mean numbers of live Douglas-fir tussock moth larvae derived by dividing the total number of larvae counted from the branch samples by the total foliated area (in square metres) of these branches. Approximately 1 week after pupation was first observed, cocoons were collected from all plots and reared to determine adult emergence and sex ratio.

Virus impact assessment. This was done by rearing larvae collected before the spray and by microscopic examination of larvae collected after the spray. Larvae, collected from the pre-spray population samples, were placed individually on artificial diet and reared until death or adult emergence. Dead larvae and pupae were examined microscopically to determine the cause of death. It was planned to collect 100–200 larvae per treated plot at weekly intervals commencing 2 weeks after spraying. However, after week 4, sample size dropped to as low as 60 in some treated plots due to virus-caused mortality and it was difficult to find living larvae; in T4 at week 7 only 20 were collected after intensive searching. The larvae were smeared on slides and examined microscopically for NPV. Larvae from check plots were examined for NPV 3, 5, 6, and 7 weeks after spraying.

Defoliation surveys. Defoliation due to Douglas-fir tussock moth larvae could not be estimated accurately because a low to moderate population of western spruce budworm, *Choristoneura occidentalis* Freeman, was also present in the plots.

Results

Spray deposit and meteorological conditions. At the time of spraying, wind speed was about 2 km/h, temperatures ranged from 11.5 to 21°C, and relative humidity from 46 to 63% (Table 1). Spray deposit was observed on all the cards. Best coverage was obtained with the aqueous tank mix containing molasses, with 27.3 droplets per square centimetre. Coverage on the plots treated with the emulsifiable oil tank mix ranged from 4.4 to 12.0 droplets per square centimetre, which is light considering that the emitted volume was

Table 1. Dosages, meteorological conditions during spray application, and spray deposits of Virtuss applied against Douglas-fir tussock moth at Veasy Lake, Kamloops Forest District, B.C., 1982

Treatment	Tank mix	Dosage (PIB/ha)	Temperature (°C)	Relative humidity (%)	Wind speed (km/h)	Droplet/cm² ($\bar{x} \pm SD$)
T1*	Oil	1.6×10^{10}	13.0	59	2	4.4 ± 2.5
T2	Oil	8.3×10^{10}	16.0	63	2	12.0 ± 9.8
T3	Oil	2.5×10^{11}	21.0	46	2	9.7 ± 5.5
T4	Aqueous	2.5×10^{11}	11.5	52	1	27.3 ± 9.0

*T, treatment.

9.4 L/ha (Table 1). No precipitation was recorded for 1 week after spray application; therefore no leaching of the virus from the needles occurred.

Egg-mass, larval, and pupal surveys. Spring egg-mass densities (Fig. 2) were greater than 0.7 per three lower branches on plots T1, T2, T3, and C1, C2, C3; noticeable defoliation would be expected if these plots were left untreated (Shepherd *et al.* 1984*a*). Plot T4, with a value of 0.52, was not expected to show noticeable defoliation and no survey was conducted on plot C4 as it was selected after eclosion and larval dispersal.

Egg-mass surveys conducted in the fall of 1982 (Fig. 2) showed that egg-mass densities in all treated plots were reduced from their spring outbreak values to endemic levels of 0.089 or less. In plots T3 and T4, which received the highest dosages of NPV, egg-mass density was 0.02. When Abbott's formula was applied to correct for natural mortality, reduction in egg-mass density attributed to treatment was 97% in plot T1 and 99% in plots T2 and T3. Egg-mass reduction in plot T4 could not be calculated because no spring egg-mass survey was conducted on its paired check (plot C4). The pre-spray population densities of Douglas-fir tussock moth larvae and the count taken 6 weeks after spraying are shown in Table 2, along with the population reductions. Applications of Virtuss in an oil tank mix gave population reductions of 65–95%; this suggests a direct

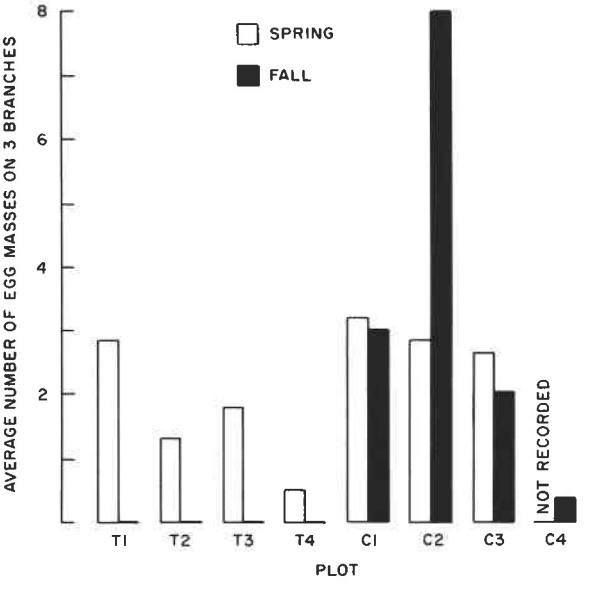

FIG. 2. Spring and fall egg-mass densities in treated and check plots at Veasy Lake, Kamloops Forest District, B.C., 1982. Values for fall collections were 0.089, 0.04, 0.02, and 0.02 for T1, T2, T3, and T4, respectively.

Table 2. Population densities of Douglas-fir tussock moth larvae in plots treated with Virtuss and in matching check plots at Veasy Lake, Kamloops Forest District, B.C., 1982

Plot No.	Treatment (PIB*/ha)	Tank mix	Larvae per m² foliage ($\bar{x} \pm$ SE) Pre-spray	6 weeks post-spray	Population reduction due to treatment† (%)
T1‡	1.6×10^{10}	Oil	182.8 ± 12.6	6.7 ± 1.8	65
C1‡	Check	—	197.5 ± 18.0	20.5 ± 2.9	—
T2	8.3×10^{10}	Oil	145.8 ± 12.2	2.8 ± 0.7	91
C2	Check	—	136.9 ± 9.4	28.7 ± 2.8	—
T3	2.5×10^{11}	Oil	302.0 ± 28.7	1.0 ± 0.4	95
C3	Check	—	360.6 ± 34.6	24.1 ± 4.6	—
T4	2.5×10^{11}	Aqueous	41.8 ± 5.3	2.0 ± 0.6	87
C4	Check	—	81.2 ± 16.5	28.9 ± 4.3	—

*PIB, polyhedral inclusion bodies.
†Calculated using a modified Abbott's formula (Fleming and Retnakaran 1985).
‡T, treatment; C, check.

relationship to dosage. The 2.5×10^{11} PIB per hectare dosage in the oil tank mix gave 95% population reduction, one-third of this dosage 91%, and one-sixteenth 65%. The 2.5×10^{11} PIB per hectare dosage in the aqueous tank mix with molasses reduced the population by 87%. The pattern of changes in larval population densities and NPV infection rates between 2 and 7 weeks after spraying are shown in Figure 3. Emergence from field-collected cocoons from treated plots T1, T2, T3, and T4 was 18, 4, 10, and 20%, respectively; those from check plots C1, C2, C3, and C4 showed 43, 28, 33, and 35% adult emergence, respectively (Table 4). The reduction in adult emergence due to NPV treatment, using Abbott's formula, ranged from 44 to 87%. The sex ratios of the emerging adults were inconsistent.

Virus impact assessment. From 73 to 89% of the larvae collected before spraying from the eight plots were successfully reared to adults (Table 3). Microscopic examination of larvae that died during rearing showed a low incidence of naturally occurring virus except in check plot C4 where 9.7% died from NPV. However, subsequent samples from this plot showed a lower incidence of NPV infection (Fig. 3), indicating that this sample, which was collected post-spray, may have been contaminated during handling. Microscopic examination of the larvae showed that the incidence of NPV infection in the treated plots reached a peak of about 85–100% of the larvae at 5–6 weeks after spraying (Fig. 3). In contrast, NPV in untreated check plots at 5 weeks after spraying only reached infection levels ranging from 0.7 to 10.3%. In the final sample taken 7 weeks after spraying, the highest infection level was 43.4% in C3. This was attributed to naturally occurring NPV. In the other check plots, infection ranged from 1.4 to 23.4%.

Percentage infection and development of an epizootic among the larvae in the four treated plots was directly related to dosage with the exception of plot T4. Although this plot received 2.5×10^{11} PIB per hectare, in the aqueous formulation, the epizootic developed slower than in the other three plots. This slower development was probably due to a lower initial Douglas-fir tussock moth population density. However, by 5 weeks after the spray, percentage infection in plot T4 was higher (97.7%) than in plots T1 and T2 (68.5 and 84.5%, respectively) receiving the reduced dosages. The results in plot T4 show that even at relatively low population levels (41.8 larvae per square metre) it is possible to cause a viral epizootic by application of Virtuss.

Defoliation survey. Although heavy defoliation was observed in most plots, a formal evaluation was not possible because of the light to moderate western spruce budworm population in some of the plots. However, Douglas-fir tussock moth larvae continued to

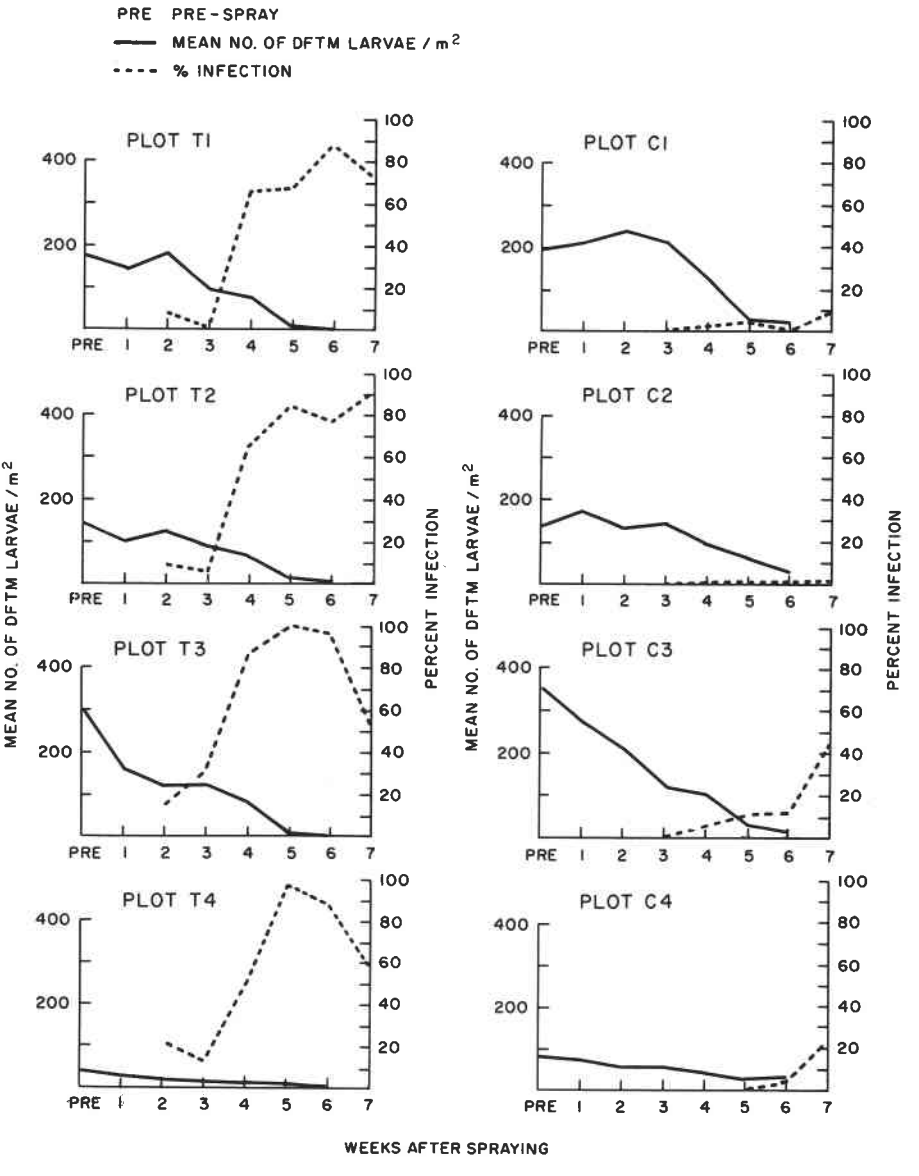

FIG. 3. Douglas-fir tussock moth larval density per square metre of foliage and percentage NPV infection of larvae following application of Virtuss on four treated plots compared with four matching untreated check plots.

feed for several weeks after the spray application. Except on plot T4, the current year's foliage was destroyed on many trees as well as most of the older foliage, with upper crowns particularly heavily defoliated. Plot T4 had an egg-mass density in the spring slightly below the outbreak threshold, whereas plots T1, T2, and T3 were well above this threshold. The apparent foliage protection in plot T4 was probably due to the low Douglas-fir tussock moth population and not the Virtuss treatment.

Table 3. Mortality of individually reared larvae collected before spraying from plots at Veasy Lake, Kamloops Forest District, B.C., 1982

Treatment	Total no. reared	% adult emergence	% Mortality by	
			NPV	Other causes
T1*	167	73.1	0.6	26.3
T2	172	83.1	2.3	14.5
T3	161	80.8	0	19.2
T4	168	82.7	0.6	16.7
C1*	175	89.1	0.6	10.3
C2	180	86.1	1.7	12.2
C3	170	87.1	0.6	12.4
C4	155	78.1	9.7	12.3

*T, treatment (for detail see text or Table 1); C, check.

Discussion

The amount of deposits recorded on Kromekote cards did not reflect the subsequent impact of Virtuss on the Douglas-fir tussock moth population. Two probable reasons for this are that the amount of spray reaching the foliage is not indicated accurately by the amount of spray on the cards and that secondary infection occurred among the larvae. Weekly microscopic examination of samples of larvae revealed NPV infection in about 10–30% of the larvae 2–3 weeks after spraying. When these larvae died, they ruptured and released massive quantities of PIBs onto the foliage. Secondary infection probably resulted from these virus foci and caused an epizootic that decimated the Douglas-fir tussock moth population. Douglas-fir tussock moth is an ideal candidate for control using a virus because it spends about 8 weeks in the larval stage. This allows ample time for the development of an epizootic if the virus is applied when larvae are in the early instars. However, if foliage protection is the principal objective, this may not be an acceptable strategy where high larval populations will completely defoliate trees before an epizootic can develop. To protect foliage, Virtuss should be applied before the Douglas-fir tussock moth population reaches outbreak levels. In most cases this would require treatment the year before defoliation can be observed; a population detection and monitoring system would be essential.

The emulsifiable oil tank mix and the aqueous tank mix containing molasses both gave acceptable results at a dosage of 2.5×10^{11} PIB per hectare. This dosage, originally recommended by the U.S.D.A. Forest Service, is also the dosage on the Virtuss label.

Table 4. Emergence and sex of adult Douglas-fir tussock moth from field-collected pupae from Virtuss-treated plots and check plots, Veasy Lake, Kamloops Forest District, B.C., 1982

Treatment	Number* of pupae reared	Ratio (males:females)	Adult emergence (%)	Emergence reduction† (%)
T1‡	107	5.2:1	17.8	58
C1‡	219	2.1:1	42.9	—
T2	108	1:1	3.7	87
C2	181	1:2.2	28.2	—
T3	105	1:2.3	9.5	71
C3	117	1:1.4	33.3	—
T4	52	1.5:1	19.5	44
C4	265	1.4:1	35.0	—

*Collection included some larvae that pupated shortly after collection.
†Attributed to treatment and calculated using a modified Abbott's formula.
‡T, treatment; C, check.

Present production cost of this dosage in Canada (at Sault Ste. Marie, Ont.) is about $50 (Canadian) per hectare. Virtuss at 8.3×10^{10} PIB per hectare in the emulsifiable oil tank mix (T2) gave a similar degree of control as the recommended dosage. If this dosage gives consistently good results, cost of virus material would be reduced to $17 per hectare. The lowest dosage, formulated and used in the emulsifiable oil tank mix, 1.6×10^{10} PIB per hectare (T1), had less impact than the other treatments when population reduction of both larvae and pupae was considered, but it was still markedly different from the untreated checks and reduction in egg-mass density was impressive at 97%. At this concentration, the production cost would be only $3.20 per hectare, but because experience with Virtuss is limited, we cannot recommend it for operational use at this dosage.

The adult sex ratio is usually 1:1 in Douglas-fir tussock moth (Wickman and Beckwith 1978), but changes of sex ratio in favor of males have been noted following NPV applications (Cunningham 1982). In many Lepidoptera and Hymenoptera, females pupate later than males giving females more time to ingest virus, become infected, and die. With six larval instars in the female and five in the male, this was expected to be evident with Douglas-fir tussock moth. However, a significant change in sex ratio, 5.2 males to 1 female, was found only in plot T1 which received the lowest dosage. There is an interesting discrepancy between adult emergence from the pre-spray larval samples from all eight plots reared in the laboratory, where emergence figures ranged from 73.1 to 89.1%, and adult emergence from cocoons collected in the four check plots, where figures ranged from 28.2 to 42.9%. Perhaps natural control factors, including starvation, reduced pupal viability in the field.

Fall egg-mass densities can be one of the most meaningful measurements of efficacy because one can use these values to predict population densities in the following year (Shepherd *et al.* 1984*a*). This survey showed high Douglas-fir tussock moth populations would occur on the four untreated check plots and low or endemic populations on all treated plots. Although plot T1 had the highest egg-mass density of all treated plots at 0.089 per three lower branches, we predict that this density will be below the damage threshold level of Douglas-fir tussock moth, based on the findings of Shepherd *et al.* (1984*a*). Hence, even the lowest dosage may have provided sufficient control to return the population to an endemic level.

Surveys were conducted in 1983, 1984, and 1985 on all the treated and check plots to determine virus spread, virus carryover, fate of the residual Douglas-fir tussock moth population on the treated plots, incidence of naturally occurring NPV in the check plots, and effect of the Virtuss treatment in terms of tree damage and mortality (Otvos *et al.* 1987). The latest outbreak of Douglas-fir tussock moth in British Columbia collapsed in 1984 and another outbreak is not expected until 1989 at the earliest. When it occurs, we intend to treat blocks (400 ha or larger) with Virtuss at 2.5×10^{11} and 8.3×10^{10} PIB per hectare to determine if the lower dosage found effective here is equally effective on an operational scale.

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