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Experimental aerial application of forest tent caterpillar baculovirus

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Northern Forest Research Centre

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EXPERIMENTAL AERIAL APPLICATION OF FOREST TENT CATERPILLAR BACULOVIRUS

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ABSTRACT

Infestations of the forest tent caterpillar (*Malacosoma disstria* Hübner) on aspen (*Populus tremuloides* Michaux) in Alberta were subjected to small-scale experimental aerial applications of a nuclear polyhedrosis virus in 1978-80. In 1978 a range of dosages was applied when most of the larvae were in the second instar. The higher dosages gave significant increases in early and mid-season larval mortality. In 1979 two dosages were applied to the eggs, first-instar larvae, and third- to fourth-instar larvae. Both dosages increased mortality throughout the larval period when applied to the eggs and increased early and mid-season mortality when applied to first-instar larvae. Only mid-season larval mortality was increased when third- to fourth-instar larvae were treated. In 1980 high dosages were applied when the larvae were in the first and second instars and gave significant increases in early, mid-season, and late larval mortality. Some of the above treatments increased total larval mortality by as much as 30%, but none provided any foliage protection. The virus appeared to carry over to subsequent generations in an area that had been treated with a hydraulic sprayer in 1976 but did not carry over in areas sprayed from the air in 1978-80.

RESUME

En Alberta, de 1978 à 1980, on a épandu du haut des airs, à titre d'expérience à petite échelle, un virus de la polyédrose nucléaire sur des peuplements de peuplier faux-tremble (*Populus tremuloides* Michaux) attaqués par la livrée des forêts (*Malacosoma disstria* Hübner). En 1978, on en a épandu différentes doses lorsque les larves en étaient à leur deuxième stade de croissance. Les doses élevées, épandues au début et au milieu de la saison, ont fait augmenter sensiblement la mortalité des larves. En 1979, on en a épandu deux doses sur les oeufs, sur les larves du premier stade, ainsi que sur les larves des troisième ou quatrième stades. Appliquées sur les oeufs, ces deux doses ont fait augmenter la mortalité larvaire dans l'ensemble des stades et l'ont fait augmenter au début et au milieu de la saison lorsqu'elles ont été appliquées sur les larves du premier stade. Seule la mortalité larvaire du milieu de la saison a augmenté lorsqu'on a traité les troisième ou quatrième stades. En 1980, on a utilisé de fortes doses aux premier et deuxième stades larvaires, ce qui a donné une mortalité accrue au début, au milieu et à la fin de la saison. Certains traitements ont fait augmenter la mortalité larvaire de 30%, mais aucun n'a protégé le feuillage. Le virus a semblé s'attaquer aux générations successives dans une zone traitée au moyen d'un pulvérisateur hydraulique en 1976, mais non dans les zones traitées du haut des airs de 1978 à 1980.

CONTENTS

TABLES

INTRODUCTION

The recent outbreak of the forest tent caterpillar, *Malacosoma disstria* Hübner, in the prairie provinces has prompted a renewed interest in methods for controlling this insect. Attacks by the forest tent caterpillar, which persists in Alberta and Saskatchewan, are more annoying than damaging, and the use of chemical insecticides becomes more and more difficult to justify. Consequently, we began a series of small-scale experiments to determine if a naturally occurring nuclear polyhedrosis virus could be used in place of insecticides to control infestations of the pest. Early experiments in 1976 using a hydraulic sprayer and a mist blower to apply the virus to second-instar larvae and unhatched eggs, respectively, gave very promising results (Ives and Muldrew 1978). Larval mortalities of 100% and 97% were obtained in plots sprayed when the larvae were mainly in the second instar. Larval mortalities of 100%, 94%, 81%, and 68% were obtained in small plots sprayed with concentrations of 1×10^8, 1×10^7, 1×10^6, and 5×10^5 polyhedra/mL, respectively, before the larvae hatched.

These results encouraged us to evaluate the effectiveness of aerial applications in controlling forest tent caterpillar infestations. In 1978 we applied various concentrations of the virus to aspen (*Populus tremuloides* Michaux) stands near Sundance, Alberta. The spray was applied when the larvae were in the second instar because older larvae become less susceptible and require large dosages before becoming infected (Bird 1969, Stairs 1965b). In 1979 we applied a moderately high dosage of virus (1×10^7 polyhedra/mL) to three widely separated stands of aspen near Flatbush, Buford, and Partridge Hill, Alberta, during three periods of the insect's development: eggs, first-instar larvae, and third-to-fourth instar larvae. This was an attempt to determine whether the effective period for application of the virus could be extended, because application during the first or second instars severely limits the amount of time available for spraying. Finally, in 1980 we applied what might be termed massive doses of virus (1×10^9 and 2.5×10^8 polyhedra/mL) to two different stands of aspen near Sundance in an attempt to obtain satisfactory population reductions.

In addition, we attempted to assess the amount of virus carry-over from year to year by determining subsequent mortality in three different areas: from 1976 to 1979 in the vicinity of Joussard (along the south shore of Lesser Slave Lake) near a plot that was sprayed in 1976; in 1979 in the Sundance area in some of the stands that were sprayed in 1978; and in 1980 in the Partridge Hill area, which was sprayed in 1979 when the larvae were in the third and fourth instars.

This report presents the results of the initial spraying experiments and assesses the amount of virus carry-over in the areas sprayed in previous years.

METHODS

Aqueous suspensions of forest tent caterpillar nuclear polyhedrosis virus were applied with a Bell 47-G3-B1 helicopter fitted with saddle tanks and a 12-m boom equipped with 26 Diaphragm Teejet nozzles (D8-45) and operating at a pressure of 14 g/cm^2. The helicopter flew at an airspeed of about 95 km/h at a height of about 3 m above the tree tops, which produced a swath width of approximately 18 m. The virus concentrations varied, but all spray solutions contained 25% stock molasses by volume, 50 g/L of Shade (a sunlight protectant), and 6 cc/L Atplus spreader-sticker to improve adhesion. The virus used in 1978 was prepared by Environment Canada's Forest Pest Management Institute, Sault Ste. Marie, Ontario. In 1979 and 1980 the virus was obtained from 1977 and 1978 field collections of fifth-instar larvae that had been sprayed with the virus when the larvae were in the fourth instar. The dead larvae were placed in water, put through a Waring Blender, and allowed to decompose for several months in large vats. The virus was allowed to settle out and then was stored in distilled water at 2.5°C.

The dates, concentrations, and dosages applied in 1978, 1979, and 1980 are given in Table 1, together with the temperature, relative humidity, and wind speed conditions during application. In 1978, frequent showers occurred before, during, and after the spraying operation, which was conducted when some of the aspen leaves were about

Table 1. Summary of weather conditions prevailing during the application of various concentrations of the virus in 1978, 1979, and 1980

Year	Date	Concentration (polyhedra/mL)	Dosage (polyhedra/ha)	Temp. (°C)	Relative humidity (%)	Wind speed (km/h)
1978	May 9	1×10^5	4.4×10^9	8	66	5-8
		5×10^5	3.1×10^{10}	12	54	5-11
	May 10	1×10^6	6.1×10^{10}	8	80	Calm
		1×10^7	4.6×10^{11}	9	76	Trace
		1.5×10^7	3.6×10^{11}	14	63	10-13
1979	May 9	1×10^7*	1.5×10^{11}	6	61	8-13
	May 25	1×10^7*	1.5×10^{11}	16	52	0-10
	June 7	1×10^7*	1.5×10^{11}	5	98	0-3
1980	May 2	1×10^9*	1.8×10^{13}	15	50	Trace
		2.5×10^8**	9.0×10^{12}	16	45	3-5

* Part of plots sprayed twice.

** All of plot sprayed twice.

1 cm in diameter and the remainder were in the expanding bud stage. In 1979 the first plot was sprayed before the foliage flushed, the second when the leaves were about 1 cm in diameter, and the third when the leaves were fully expanded. In addition, parts of each plot were sprayed twice on the same day to obtain more thorough coverage. Weather conditions were almost ideal on all three occasions. In 1980 the foliage was again in the expanding bud stage, or about 1 cm in diameter, when spraying occurred. The plot receiving a concentration of 2.5×10^8 polyhedra/mL was sprayed twice on the same day to obtain more thorough coverage. The weather at the time of application was almost ideal, although daytime conditions tended to be hot and dry, which may have had an adverse effect on the virus.

Kromekote cards were placed in stand openings during all trials to assess spray coverage. Counts of spray droplets were not made, but visual inspection indicated that the coverage was adequate, except for occasional strips that were missed. The sizes of the plots varied from 1 to 10 ha. These are small for aerial application, but the size was dictated by the limited amount of virus available.

Mortality in each of the sprayed areas and in nearby unsprayed areas was assessed by the method outlined by Stairs (1965a), although larvae were not examined microscopically. Consequently, some of the mortality may have been due to causes other than virus infection, although the appearance of the larvae seemed to indicate that the majority were killed by the virus. In each area, one or more collections (usually four) of 300 or more larvae were made at weekly intervals and were placed in paper bags with a supply of foliage in an improvised insectary. The bags were opened 3 to 5 days later, and the numbers of living and dead larvae were determined. From these figures, daily rates of mortality and crude estimates of total larval mortality were calculated. The total larval mortality from hatching until cocoon formation was estimated by summing the mortality during each interval, after adjusting to allow for mortality that had occurred prior to the collec-

tion of each sample[1]. In some cases, sampling was not begun as soon as the eggs hatched, and various approximations of presampling mortality had to be made in order to allow for unmeasured mortality. (The sources of these approximations are given in the tables.)

Mortality in areas sprayed in previous years was determined by conducting less intensive sampling in each of those areas. Generally speaking, sampling was restricted to individual collections from each area at intervals of about 1 week.

RESULTS

The results have been divided into two principal sections: the assessment of each year's spray operation, and the assessment of the incidence of the virus in areas sprayed previously. The effectiveness of the treatments is assessed by calculating Chi-square values for the amount of mortality during the early, mid, and late larval stages. Crude estimates of total mortality are also given in order to provide some idea of the overall effectiveness of the treatments.

AREAS SPRAYED IN 1978

The results of the 1978 aerial applications near Sundance are shown in Table 2. These tests were conducted in six different stands, because no single stand was large enough to accommodate all of the plots. Unfortunately, a natural virus was present in the area at various levels of incidence. Consequently, it seemed advisable to limit comparisons to those plots occurring in the same stands. On this basis, there were no differences in the early, mid, or late larval mortalities in Stand 1, which had been sprayed with concentrations of 1×10^5 and 5×10^5 polyhedra/mL. In Stand 2, the early larval mortality was higher in plots sprayed with concentrations of 1×10^6 and 1×10^7 polyhedra/mL than in the plot sprayed with a concentration of 5×10^5 polyhedra/mL. Mid-season larval mortality was higher in the plot receiving the heaviest dosage. In Stand 3, early larval mortality was marginally higher in the plot receiving the heavy dosage, but mid-season mortality was much higher. In Stand 4, the heavy dosage of virus gave much greater early and mid-season mortality than that which occurred in the unsprayed portion of the stand, even though the natural incidence of the virus was very high. Finally, although no Chi-square values were calculated, the mortality in Stand 5, which was not sprayed, was consistently less than that in Stand 6, which was sprayed with the virus at a concentration of 1×10^6 polyhedra/mL.

AREAS SPRAYED IN 1979

The results of the 1979 aerial applications near Flatbush, Buford, and Partridge Hill are shown in Table 3. Early, mid-season, and late larval mortalities were all greater in the plots that had been sprayed once or twice when the insects were in the egg stage. Larvae collected from plots sprayed once or twice when the larvae were in the first instar suffered greater early and mid-season mortality than those collected from unsprayed areas. Finally, larvae sprayed once or twice when they were in the third and fourth instars suffered greater mid-season mortality than did the unsprayed larvae. (The significant difference in early larval mortality is due to random

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[1] An example may clarify the procedure. Suppose the first sample was collected just after the larvae had hatched and that these larvae were reared for 4 days, at which time they had suffered 10% mortality. Further suppose that a second sample was collected a week after the first sampling date and also reared for 4 days, during which time 20% of the larvae had died. The daily rate of mortality for the first collection was therefore 2.5%, while for the second collection it was 5.0%. There is a 3-day period between the two collections when no direct estimate of daily mortality is available. The two above rates were averaged, so that the daily rate for the unsampled period in this case was considered to be 3.75%. The total mortality occurring in the second period was therefore $(3 \times 3.75) + (4 \times 5.0) = 31.25\%$. However, this must be adjusted to allow for the 10% mortality that had occurred previously, so that the total mortality to the end of the second rearing period was $10.0 + (1 - 0.10)(31.25) = 38.1\%$ rather than $10.0 + 31.25 = 41.25\%$.

Table 2. Percent daily mortality and crude estimates of percent total larval mortality for forest tent caterpillar larvae collected from aspen stands in the Sundance area that were subjected to aerial applications of different concentrations of the virus in 1978

Stand	Concentration (polyhedra/mL)	Larval stage: Early	Larval stage: Mid	Larval stage: Late	Total larval mortality[1] (%)
		(% daily larval mortality)			
1	1×10^5	5.2	2.3	0.8	64
	5×10^5	4.2	2.8	0.6	63
	Chi-square[2]	3.47	1.83	0.68	
2	5×10^5	3.8	4.7	2.4	78
	1×10^6	6.2	4.5	3.3	81
	1×10^7	6.8	6.4	2.4	87
	Chi-square	19.87**	20.74**	4.63	
3	1×10^5	7.4	5.2	2.5	84
	1.5×10^7	8.8	10.5	2.7	97
	Chi-square	3.99*	125.20**	0.33	
4	None	1.6	4.3	1.8	68
	1.5×10^7	4.8	7.6	1.4	92
	Chi-square	42.64**	49.87**	1.05	
5	None	3.0	6.3	2.2	80
6	1×10^6	5.4	8.9	3.2	92

[1] Based on an assumed presampling mortality of 20%, the corresponding average of five sampling areas near Lesser Slave Lake.

[2] A single asterisk indicates significance at the 0.05 level, while double asterisks indicate significance at the 0.01 level.

variation, as this period preceded the spray application.)

AREAS SPRAYED IN 1980

The results of the trials conducted in 1980 near Sundance are shown in Table 4. Two concurrent applications of the virus at a concentration of 2.5×10^8 polyhedra/mL or a single application of the virus at a concentration of 1×10^9 polyhedra/mL resulted in significantly greater early, mid-season, and late larval mortality than occurred in unsprayed areas.

INCIDENCE OF MORTALITY IN AREAS PREVIOUSLY SPRAYED

Joussard area

The Joussard area is the only one in which we attempted to determine the amount of carry-over of virus for more than 1 year following the initial spraying (Table 5). Collections were made from 1976 to 1979 in and around an area sprayed in 1976. Our initial intent was to determine the amount of spread from the treated area, but occurrence of the natural virus made this an impossible task. We have therefore presented data for three loca-

Table 3. Percent daily mortality and crude estimates of percent total larval mortality for forest tent caterpillar larvae collected from aspen stands near Flatbush, Buford, and Partridge Hill that were subjected to aerial applications of the virus (at a concentration of 1×10^7 polyhedra/mL) in 1979

Stage of development when virus applied	Number of applications	Larval stage (% daily mortality) Early	Mid	Late	Total larval mortality[1] (%)
Eggs	None	1.0	0.8	0.3	25
	One	1.9	2.0	1.2	50
	Two	2.6	1.6	0.8	49
Chi-square[2]		25.37**	22.31**	22.55**	
First-instar larvae	None	1.3	0.6	3.2	48
	One	2.2	1.8	3.2	64
	Two	4.5	2.5	3.2	76
Chi-square		86.72**	86.61**	0.20	
Third- and fourth-instar larvae	None	0.7	0.1	1.6	36
	One	0.4	0.6	1.8	36
	Two	0.9	0.9	2.0	54
Chi-square		15.60**	50.75**	2.74	

[1] Based on the assumption that presampling mortality equalled 10%, the corresponding mean value for the last treatment listed.

[2] The double asterisks indicate significance at the 0.01 level.

Table 4. Percent daily mortality and crude estimates of percent total larval mortality for forest tent caterpillar larvae collected from aspen stands in the Sundance area that were subjected to aerial applications of the virus at various concentrations during 1980

Concentration (polyhedra/mL)	Larval stage (% daily larval mortality) Early	Mid	Late	Total larval mortality[1] (%)
None	0.28	0.15	1.56	30
2.5×10^8	1.24	0.91	2.12	44
1×10^9	0.65	1.59	2.60	55
Chi-square[2]	53.64**	130.75**	64.27**	

[1] Based on an assumed presampling mortality of 13%, the corresponding value for larvae in the Partridge Hill area.

[2] The double asterisks indicate significance at the 0.01 level.

Table 5. Percent daily mortality and crude estimates of percent total larval mortality for forest tent caterpillar larvae collected in 1976-79 from an area near Joussard that was sprayed with the virus in 1976 and from nearby unsprayed areas

Year of collection	Location	Larval stage			Total larval mortality (%)
		Early	Mid	Late	
		(% daily larval mortality)			
1976	1 km north	1.1	0.1	0.9	33
	Sprayed in 1976	14.6	12.0	2.7	100
	1.3 km east	-	-	-	-
Chi-square[1]		953.67**	1227.93**	81.05**	
1977	1 km north	0.5	0.2	0.5	12[2]
	Sprayed in 1976	2.4	2.8	4.4	75[2]
	1.3 km east	0.6	1.6	4.8	56[2]
Chi-square		69.01**	110.13**	105.14**	
1978	1 km north	1.9	0.2	1.0	40
	Sprayed in 1976	4.3	3.2	2.2	84
	1.3 km east	1.0	0.3	1.3	34
Chi-square		59.51**	115.49**	16.93**	
1979	1 km north	0.2	0.4	0.3	12
	Sprayed in 1976	1.0	0.5	1.4	31
	1.3 km east	0.1	0.2	0.4	8
Chi-square		17.28**	4.17	31.05**	

[1] The double asterisks indicate significance at the 0.01 level.

[2] Larval development not completed; consequently, total larval mortality is probably underestimated.

tions only: one of the original test plots, and two unsprayed areas on either side of it. In all cases but one, the early, mid-season, and late larval mortalities were significantly higher in the plot sprayed in 1976 than in either of the nearby unsprayed areas. This suggests that the virus persisted in the original spray area, but it also seems to show that there was very little spreading out of the virus infection into surrounding areas.

Sundance area

We did not attempt to determine if there was any spread of the virus following our 1978 spraying in the Sundance area because the high incidence of the natural virus made this impossible. We did, however, attempt to determine if there were any differences in the incidence of the virus in four of the areas in 1979, which would suggest virus carry-over (Table 6). Although there are significant differences in the early, mid-season, and late larval mortalities, there are no apparent trends. Perhaps this is because the 1978 mortality was so high, even in the unsprayed area. Although not all of this mortality was attributable to the virus, most of it probably was, and a large source of inoculum was therefore available.

Partridge Hill area

One of the primary reasons for the late spraying in the Partridge Hill area in 1979 was the hope that the insects might become

Table 6. Percent daily mortality and crude estimates of percent total larval mortality for forest tent caterpillar larvae collected in 1979 from three aspen stands in the Sundance area that were subjected to aerial applications of the virus at various concentrations in 1978 and from one unsprayed stand

Treatment in 1978	Total larval mortality in 1978 (%)	Larval stage (% daily larval mortality in 1979)			Total larval mortality in 1979[1] (%)
		Early	Mid	Late	
None	80	0.45	1.03	1.28	38
1×10^7	87	2.01	0.35	2.00	41
1.5×10^7	92	0.70	0.49	1.83	38
1.5×10^7	97	2.44	1.14	1.34	40
Chi-square[2]		60.78**	33.34**	22.08**	

[1] Based on an assumed presampling mortality of 15%.

[2] The double asterisks indicate significance at the 0.01 level.

sublethally infected and thus increase the amount of carry-over; however, there did not seem to be any evidence of this in 1980 (Table 7). Although there is a significant difference in the early larval mortality, this difference is due to a low incidence of mortality in the plot that was sprayed once, not to any apparent increase in the incidence of mortality in the sprayed plots.

DISCUSSION

The results of this study clearly indicate that the nuclear polyhedrosis virus that we used was of little value in controlling forest tent caterpillar populations, but this does not necessarily mean there is no hope for the virus. The 1978 trials, using laboratory-produced virus, were conducted under extremely adverse conditions. The larvae emerged before the foliage flushed, and they were mining the buds extensively. In addition, the showery weather during and following the virus application may have washed some of the virus from the foliage. The 1979 and 1980 trials, which gave much lower total larval mortality than the 1978 trials, were conducted with field-collected virus. We conducted limited laboratory tests with this virus, which seemed to indicate that it was just as virulent as the laboratory-produced virus, but the possibility still exists that it may not have been.

A more probable explanation may be that none of the virus that we used was particularly virulent. Logic would seem to indicate that such was the case. One problem throughout these trials was that the natural virus seemed to be everywhere. If this natural virus was virulent, its prevalence should have terminated the outbreaks, but it did not. For example, the populations in the Sundance area were nearly as high in 1980 as they were in 1978. In fact, the plots that we used in 1980 were two of the ones that we had previously sprayed in 1978. The populations in these two stands appeared to be very similar in both years, in spite of the high incidence of the virus in 1978.

We are not studying forest tent caterpillar populations, hence our conclusions are

Table 7. Percent daily mortality and crude estimates of percent total mortality for forest tent caterpillar larvae collected in 1980 from aspen stands in the Partridge Hill area that were subjected to aerial applications of the virus at a concentration of 1×10^7 polyhedra/mL in 1979 when larvae were in the third and fourth instars

Number of applications in 1979	Total larval mortality in 1979 (%)	Larval stage (% daily larval mortality in 1980)			Total larval mortality in 1980 (%)
		Early	Mid	Late	
None	36	1.67	0.16	0.54	25
One	36	0.73	0.22	0.54	20
Two	54	1.84	0.02	0.48	24
Chi-square[1]		46.52**	3.12	0.26	

[1] The double asterisks indicate significance at the 0.01 level.

based on first impressions. Hodson (1941), however, also concluded that the virus was not an important mortality factor in the 1933-38 outbreak in Minnesota, and Witter *et al.* (1972), who conducted intensive population studies in Minnesota, concluded that the virus was unimportant during the course of their studies, which lasted from 1967 to 1969.

Perhaps it is naive to expect an omnipresent virus that seems to have such little impact under natural conditions to be able to control infestations when applied from the air. The amount of virus ingested under these conditions may be insufficient to infect more than a small portion of the population. We were able to increase total larval mortality by as much as 30%, but because of the high forest tent caterpillar populations the increased mortality was insufficient to protect the foliage and had little if any effect upon subsequent generations.

In conclusion, it seems safe to say that unless a more virulent strain can be found there is little point in giving further consideration to nuclear polyhedrosis virus for controlling forest tent caterpillar outbreaks in widespread areas. It appears impossible to obtain a thorough coverage of the virus by aerial application. When applied from the ground with either a hydraulic sprayer or mist blower, the foliage is more completely covered with spray, and effective control can be obtained if the larvae are young. Even under these conditions, however, the high production costs would make the virus uneconomical when other, cheaper methods of control are available.

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