AERIAL APPLICATION OF ENTOMOPOXVIRUS AND NUCLEAR POLYHEDROSIS VIRUS AGAINST SPRUCE BUDWORM AT CHAPLEAU, ONTARIO, 1972.

by

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Grumman Agcat spray plane at Chapleau airport with mixing tanks in the background. Inset: Micronair spray unit.

ABSTRACT

Two viruses of the spruce budworm, Choristoneura fumiferana (Clemens) were tested in an aerial application near Chapleau, Ontario in 1972. A Grumman Agcat aircraft fitted with Micronair equipment was used for the applications and rates of 1 and 2 U.S. gal./acre were tested. Three plots with a total area of 2 square miles were sprayed with entomopoxvirus and two plots with a total area of 1 square mile with nuclear polyhedrosis virus. Larvae were in the second and third instar at the time of application and 40 billion virus inclusion bodies per acre were sprayed on all five plots. In four of the five plots a formulation of 10% molasses, 2.5% IMC sunlight protectant and 0.2% Biofilm® was added to the aqueous virus suspension.

Unfortunately a late frost which occurred 6 days after the application killed all the current year's foliage on balsam fir and most of that on white spruce. The spruce budworm population was decimated but some results were obtained. It appeared that 1 U.S. gal./acre caused almost as much virus infection as 2 U.S. gal./acre and that the formulation used may have inhibited rather than enhanced virus infection.

Almost no virus could be found in the year following application (1973) and the insect population was low on both control and sprayed areas. Nevertheless, the numbers of budworm were significantly lower on the sprayed plots.

INTRODUCTION

The eastern spruce budworm, Choristoneura fumiferana (Clemens) (Lepidoptera: Tortricidae) is the most important forest insect pest in eastern Canada and, to date, the use of chemical insecticides is the only practical method of controlling this pest. Alternative methods of control have been considered including the use of viruses but, until recently, the propagation of sufficient quantities of virus to conduct field trials has hindered this research. However, improved budworm rearing methods (Grisdale, 1970) have made the mass propagation of viruses possible in the laboratory (Cunningham et al, 1972).

Ground spray trials were conducted in 1959 and 1960 and again in 1969 and 1970 (Stairs and Bird, 1962; Bird and McPhee, 1970). The first aerial spray trials with spruce budworm virus were conducted in 1971 near Pembroke, Ontario (Bird, Cunningham and Howse, 1972; Howse et al, 1973). Four different viruses have been studied which infect spruce budworm and the two which showed the greatest potential for control in laboratory tests were used in the aerial spray trials. These were an entomopoxvirus (EPV) and a nuclear polyhedrosis virus (NPV).

In the 1971 aerial spray trials aqueous suspensions of the viruses were sprayed from a helicopter, fitted with boom and nozzle equipment, at a rate of 3 U.S. gal./acre. The EPV was applied at 1 billion, 10 billion and 100 billion inclusion bodies/acre and the NPV at 300 billion inclusion bodies/acre. The applications were tested at two times: at the peak of the second larval instar, and at the peak of the fourth instar, making a total of 8 test plots.

The EPV plots were 4.8 acres each and the NPV plots about 6 acres. In the studies following the application it was found that the EPV suspension was contaminated with cytoplasmic polyhedrosis virus (CPV) and NPV, and that the NPV was contaminated with CPV. It has been shown that mixtures of NPV and CPV inhibit the development of NPV (Bird, 1969). Substantial population reduction and high levels of virus infection were obtained in these trials but no significant foliage protection was noted. Population reduction studies indicated that the late spray application gave better results on both EPV and NPV plots whereas virus infection studies indicated that the early spray was superior on the EPV plots.

One of the objectives in using a virus to control forest insect pests is the establishment of the virus in the insect population so that it will persist from one year to the next and continue to have an impact in the years following application. Of course, when plans were formulated for the 1972 spray operation, the results of virus carryover from the 1971 operation were not known. Hence some educated guesses had to be made when planning the strategy. It was later found that the NPV carryover was better than the EPV.

It was decided that the 1971 results were sufficiently encouraging to justify spraying larger areas in 1972. It was thought that large areas would be best for long-term studies because there is less likelihood of the insect population becoming inundated with moths from the adjacent unsprayed area. The dosage of NPV, 30 billion inclusion bodies/acre, used in 1971 gave 34% infection in the early application and 71% in the late application. These rates

are considered sufficiently high to initiate a virus epizootic but the dosage is so high as to be unrealistic economically. The application rate of 3 U.S.gal./acre was also considered to be high for a forest application and it was decided to test application rates of 1 and 2 U.S.gal./acre applied with Micronair equipment. This equipment gives a very fine breakup of the droplets and should give excellent coverage with low volume.

A formulation had been developed in the laboratory and it was hoped that it would enhance the virus infection in the budworm population. When viruses were produced in the laboratory in the winter of 1971-72 every attempt was made to ensure that they were free from other contaminating viruses.

An area of 2 square miles was selected as an EPV spray plot and an area of 1 square mile as an NPV spray plot. This report describes the spray operation, impact of the viruses in the year of application and in the following year.

MATERIALS AND METHODS

Virus production

In the winter of 1971-72, 991,000 larvae were infected with EPV and a total of 16,838 gm of freeze-dried infected material harvested. For NPV production 770,275 larvae yielded 7,606 gm of freeze-dried infected material (Cunningham et al, 1972). Batches of virus were bioassayed to establish the absence of foreign viruses.

Experimental plots

The EPV plots were located in Borden Twp. near Chapleau, Ontario. Three contiguous plots of 640, 420 and 210 acres designated P, Q and R were set out so that Hwy 101 went through the middle of them (Fig. 1). The plots were on rolling terrain with balsam fir (25-30 ft.) as the dominant species comprising 50% of the stand. White spruce (50-70 ft.) comprised 30% of the stand, the remainder being white birch 10%, white pine 7%, trembling aspen 1%, white cedar 1% and jack pine 1%. In low areas there were pockets of pure black spruce.

Two NPV plots were located in Chewett Twp. also near Chapleau. They were about 1/2 mile apart, had areas of 420 and 210 acres and were designated S and T (Fig. 2). These plots were on slightly rolling terrain with a few wet areas containing 100% black spruce. The dominant species were balsam fir (25-30 ft.) comprising 75% of the stand with 10% white spruce (50-70 ft.), 10% overmature white birch, and the remaining 5% composed of white pine and trembling aspen.

Plot marking

Highway 101 was located in the centre of all the plots and at right angles to the flight paths. The highway was measured off in 100 ft. intervals through the plots and these points were marked with stakes which were numbered. The corners of the plots

Fig. 1 EPV plots located in Borden Twp. near Chapleau, Ontario.

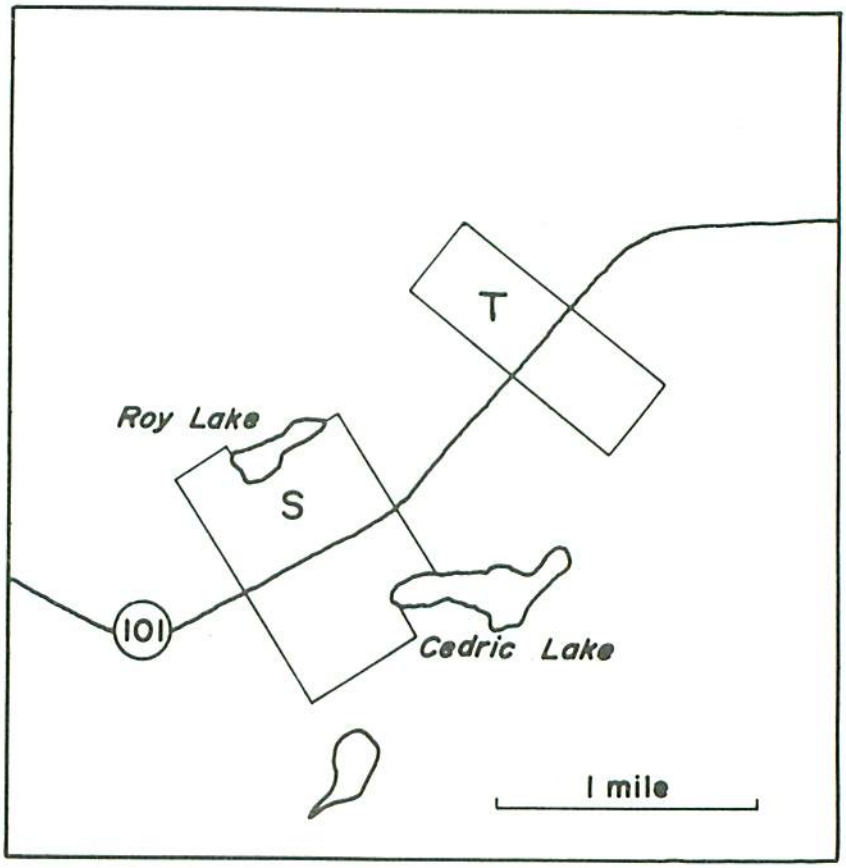

Fig. 2 NPV plots located in Chewett Twp. near Chapleau, Ontario.

were established by using a compass and tape and were 1/2 mile on either side of the highway. Trails were marked to them using flagging tape.

Red meteorological balloons were used to guide the aircraft. Prior to spraying small helium cylinders were carried to the plot corners and balloons put up 30-40 ft. above the forest canopy. A balloon was also put up on the road and was moved from one stake to the next after each pass made by the aircraft.

Formulation and application rates

On 4 of the 5 plots the following formulation was used:

2.5% (wt) IMC sunlight protectant

10% (vol.) molasses

0.2% (vol.) Biofilm® 2

The IMC sunlight protectant was used to protect the virus against UV radiation and extend its viability on the foliage, the molasses as a humectant in the aqueous suspension to prevent evaporation during the application and also, perhaps, as a feeding attractant, and the Biofilm® as a wetter-sticker to prevent wash-off by rain. All three ingredients had been tested individually and together in the laboratory to ensure that they were compatible with the viruses: no loss of virulence resulted.

One EPV plot which will be referred to as "unformulated" contained only the IMC sunlight protectant. This substance has a very dark colour and is useful as a marker dye for monitoring the deposit on spray cards.

1. Manufactured by International Minerals and Chemical Corporation, 5401 Old Orchard Road, Skokie, Illinois 60076.
2. Sample obtained from Colloidal Products Corporation, P.O. Box 621, Petaluma, California 94952.

The type of virus, concentration of freeze-dried material, plot number, gallonage/acre and area are given in Table 1. All the plots received the same number of viral inclusion bodies per acre and this figure was calculated to be 40 billion.

Table 1

Plots sprayed near Chapleau, Ontario, 1972.

Plot	Acreage	Virus and formulation	Concentration of freeze dried material gm./gal.	U.S. gal./ acre
P	640	EPV(unformulated)	5	2
Q	420	EPV(formulated)	5	2
R	210	EPV "	10	1
S	420	NPV "	5	2
T	210	NPV "	10	1

Aircraft

A Grumman Agcat biplane fitted with 4 Micronair AV3000 units, and owned by General Airsprays Ltd. was contracted for the application. It was found necessary to remove the pump filters and line filters as they clogged rapidly with the material. Application rates were calibrated at 1 U.S. and 2 U.S. gal./acre assuming a 100 ft. swath width. The tank in the aircraft could hold a maximum of 250 U.S. gal. but 120 U.S. gal. was the maximum load attempted during this operation due to the limitations of the Chapleau landing strip.

Communications

Johnson portable transceivers were used to communicate between the plots and air strip; the radio in the aircraft was tuned to the

same channel. Because the distance between the plots and airstrip exceeded 8 miles, a relay post was established using a third transceiver.

Monitoring the deposit

Kromekote spray cards on 1/4" plywood backings were placed along the shoulder of Hwy 101 at 25 ft. intervals in the plot areas and to a distance of 500 ft. outside the plot boundaries.

Mixing the formulation

The freeze-dried infected insect powder was weighed and put into suspension using a Kalish homogenizer. The amount of powder required for 100 acres was put into 5 U.S. gal. of water. This was done a day or two before application and the material was filtered through a 20 mesh sieve and then a 50 mesh sieve. It was stored in 5 gal. plastic gasoline cans. The IMC sunlight protectant was added to water at 10 times the final concentration in 40 gal. drums and it went into suspension over a period of 2-3 hr. The final mixing was done in two 400 gal. animal feeding troughs when the virus suspension, sunlight protectant, molasses and correct volume of water were added. Finally the Biofilm® was added slowly and the mixture stirred with a paddle.

Spray operation and larval development

Spraying commenced on the evening of May 18th on plot R. On this date 95% of the larvae were in the second instar and 5% in the third instar; all larvae were needle-mining. It was too windy to spray on the morning of May 19th but spraying was attempted on the evening of that day. Poor deposits were obtained on plot R due to blockage of the filters in the aircraft; it was decided to remove them.

The weather was unsuitable for spraying on May 20th and on the evening of May 21st part of plot Q was sprayed. On the morning of May 22nd 2 loads finished the application on plot Q and 3 loads were sprayed on Plot P. During the spray period, the daytime temperatures reached 80°F+ and this made evening spraying difficult as an inversion did not occur until after dark and the humidity was low. An attempt was made to spray one load on the evening of May 22nd but the deposit was extremely poor. The temperature was 67°F and the humidity 55%. The same area was resprayed on the morning of May 23rd and two more loads finished the application on plot P.

With the high temperatures the insect development had advanced considerably and by now mainly third instar were present. Samples were taken from balsam fir and white spruce and the percentages of different instars and their location on the foliage are given in Table 2.

Table 2

Spruce budworm development when spraying on EPV plots was finished and spraying commenced on NPV plots on May 23, 1972.

On white spruce (30 larvae)		
II	Mining in needles	10%
II	Wandering on buds	7%
III	Inside buds	20%
III	Wandering on buds	63%
On balsam fir (50 larvae)		
II	Mining in needles	30%
III	Mining in needles	22%
III	Wandering on bud	40%
III	Inside buds	8%

On the evening of May 23rd it was too windy to spray and on the morning of May 24th, 4 loads were sprayed on NPV plot S and 2 loads on NPV plot T thus completing the operation. The temperature was 60°F and the humidity 100%. A few drops of rain fell as the last load was applied but an excellent deposit was obtained.

Weather following the application

Six days after the application was completed 4 to 6 in. of snow fell on the plots, accompanied by night temperatures of down to 26°F. All the current year's foliage was killed on balsam fir and most of it was killed on white spruce. The spruce budworm population was decimated and larvae which survived the low temperatures were forced to back-feed on old foliage. As a result they were unhealthy and stunted.

Sampling stations:

Sample trees were selected and tagged on both sides of Hwy 101, 25-50 ft. from the edge. Trees completely exposed on the edge were considered atypical and were avoided. A total of 116 trees in 5 plots were selected with about equal numbers of white spruce and balsam fir. After the first sample was examined this system was abandoned and random samples were taken from each plot. Sampling on the EPV plots commenced about 3 weeks after the spray application and a total of 5 samples at about weekly intervals were taken. The sampling dates were 15th June, 22nd June, 26th and 27th June, 5th July and 16 July. Sampling in the NPV plots commenced about 2 weeks after the application and 4 samples were taken on the following dates: 8th June, 16th June, 21st and 22nd June and 28th June. Samples

were taken from 2 control areas, one west of the EPV plots and the other between the EPV and NPV plots, on the 29th June. The first set of foliage samples was taken at mid-crown using pole clippers, but due to the low insect population following the post-spray freezing weather the random samples were mainly taken at eye level where feeding could be detected. In the laboratory all the larvae and pupae were picked off the foliage for microscopic diagnosis.

In 1973, plots P, S and T and one control were resampled to determine the incidence of viruses in the year following application. Plots Q and R were not sampled as the level of virus was very low in these plots in the year of application. Random samples were taken from balsam fir at eye level on 28th June. When these samples were taken it was observed that the insect population seemed to be lower on the sprayed plots than the control area. To establish population levels, samples were taken at midcrown from both balsam fir and white spruce using pole clippers fitted with baskets. Fifty branch tips (18 in.) of each species were collected from EPV plot P, 25 of each species from plot S and the same from plot T and from 2 control plots. These samples were taken on July 10th and counts were made of the number of larvae or pupae present.

Microscopic diagnosis

All spruce budworm (living or dead; larvae or pupae) were removed from the foliage samples. Living larvae were dissected and squash preparations were made of the gut and portions of the fat tissue. Dead larvae were smeared on a microscope slide; if they were desiccated they were ground up in a drop of water using a glass rod. The slides were observed under a Leitz Ortholux microscope using phase contrast optics and EPV, NPV and CPV were recorded.

Deposit analysis

From each plot 10 spray cards were randomly selected. A 5x5 grid of 1 cm. squares was ruled on each card and a count made of the number of droplets in each of the 4 corner squares and in the centre square using a binocular microscope. The droplets in the centre square were then measured using a micrometer eyepiece and divided into 7 size categories. Some droplets had a halo around them due to spread and this was disregarded when measurements were made.

RESULTS

Deposit analysis

The mean number of droplets per cm^2 are given in Table 3. Satisfactory deposits were obtained in plots Q, S and T. The number was low in plot R due to blockage of the filters in the spray system. An explanation cannot be found for the lower deposit on plot P. The percent distribution of 7 size categories of droplets are shown in Table 4. There was no significant difference between the plots, the majority of the droplets being less than 120µ. The category with the largest percentage of droplets was 50 to 80µ.

Table 3

Mean number of droplets per cm^2 on spray cards

Plot	Number of droplets	Standard deviation
P	41	17
Q	93	35
R	21	17
S	98	27
T	131	60

Table 4

Percentage of droplets on spray cards in different size categories

Plot	Numbers measured	Microns						
		Up to 40	40-80	90-120	130-160	170-200	210-400	410-600
P	41	22.8	35.6	22.5	3.4	4.5	9.9	1.3
Q	93	14.6	36.6	29.6	12.2	2.7	3.7	0.6
R	21	26.8	28.8	32.0	3.9	2.0	5.2	1.3
S	98	25.8	37.4	25.2	3.9	2.9	4.5	0.3
T	131	18.4	45.9	25.6	5.5	2.7	1.7	0.2

Incidence of viruses in the year of application

The results of impact of the EPV spray are shown in Table 5. The first EPV infection was detected in samples collected on June 22nd, which was 34 days after the spraying commenced in plot R and 29 days after it finished in plot P. Only very low levels of EPV were found in plots Q and R, with 4.1% and 2.8% maximum infection respectively on white spruce and 1.1% and none on balsam fir. Higher levels of infection were found on plot P where the EPV was not formulated: on white spruce 20.2% infection was recorded and on balsam fir 6.4%. No NPV was found in the EPV plots but low levels (maximum 1.6%) of CPV were found in both the plots and control area.

The results of the impact of NPV are shown in Table 6. The first NPV infection was detected in samples collected on June 8th from plot S, 15 days after the application. NPV was not detected in plot T until the second sample was collected 23 days after the application. The maximum NPV infection level was 27.7% of the population, found in plot S on balsam fir and 26.3% on white spruce at the time of the first sample. In plot T the highest infection levels were 14.8% and 23.8% respectively. Higher levels of CPV were found than in the EPV plots, 8.3% on balsam fir and 5.2% on white spruce in plot S and 1.1% and 4.0% in plot T, but the levels were also correspondingly higher in the NPV control with 5.1% on balsam fir and 0.5% on white spruce.

Incidence of viruses in the year following application, 1973

Larvae were hand-picked from balsam fir on plots P, S and T on June 28th, 1973. Samples were also taken from the control plot between plots P and S. The numbers of insects examined from each plot are shown in Table 7. Only 1 NPV infected larva was found in plot T and no EPV or CPV were found.

Table 5

Incidence of viruses in plots sprayed with EPV near Chapleau, 1972.

Plot	Sample date	Tree species*	Number of insects examined	% Virus infection EPV	CPV
P	15th June	Fb	11	0	0
		Sw	62	0	0
	22nd June	Fb	24	0	0
		Sw	25	12.0	0
	27th June	Fb	203	6.4	0
		Sw	76	15.8	0
	5th July	Fb	322	3.7	0.9
		Sw	247	20.2	1.6
	16th July	Fb	219	5.9	0
		Sw	307	9.1	0.3
Q	15th June	Fb	2	0	0
		Sw	31	0	0
	22nd June	Fb	82	1.2	0
		Sw	50	2.0	0
	26th June	Fb	89	0	1.1
		Sw	118	0	0
	5th July	Fb	261	2.3	0
		Sw	232	1.3	0
	16th July	Fb	497	1.8	0
		Sw	415	4.1	0.7
R	15th June	Fb	11	0	0
		Sw	19	0	0
	22nd June	Fb	68	0	0
		Sw	9	0	0
	26th June	Fb	97	2.1	0
		Sw	73	2.7	0
	16th July	Fb	169	1.8	0
		Sw	157	1.9	0
Control	29th June	Fb	144	0	1.4
		Sw	240	0	0.8

*Fb = Balsam fir
Sw = White spruce

Table 6

Incidence of viruses in plots sprayed with NPV near Chapleau, 1972.

Plot	Sample date	Tree species	Number of insects examined	% Virus infection NPV	CPV
S	8th June	Fb	65	27.7	0
		Sw	38	26.3	0
	16th June	Fb	63	12.7	0
		Sw	185	22.2	0.5
	22nd June	Fb	72	19.4	8.3
		Sw	77	14.3	5.2
	28th June	Fb	92	13.0	2.2
		Sw	39	12.8	5.1
T	8th June	Fb	26	0	0
		Sw	27	0	0
	16th June	Fb	27	14.8	0
		Sw	101	23.8	1.0
	21st June	Fb	94	11.7	1.1
		Sw	62	14.5	3.2
	28th June	Fb	69	10.1	0
		Sw	25	20.0	4.0
Control	29th June	Fb	215	0	5.1
		Sw	198	0	0.5

Table 7

Incidence of viruses in plots one year after spray application

Plot	Treatment	Number of insects examined	% Virus infection EPV	NPV	CPV
P	EPV	130	0	0	0
S	NPV	127	0	0	0
T	NPV	112	0	0.8	0
Control	-	180	0	0	0

Population levels on sprayed plots and controls in the year following application, 1973.

The average number of insects per 18 in. branch tip on both white spruce and balsam fir on plots P,S,T and 2 controls are given in Table 8. The average densities of insects on the 2 controls and 2 NPV plots were calculated. When these figures were compared it was found that there were 3.4 times more insects on balsam fir and 2.7 times more on white spruce in the control plots than in the NPV plots. When the same calculation was made for the EPV plots there were 2.3 times more insects on the balsam fir controls and 2.7 more on the white spruce controls.

Table 8

Average number of spruce budworm per 18 in. branch tip in 1973 on plots sprayed in 1972 and in control areas near Chapleau, Ontario.

Plot	Treatment	Number of samples of each species	Number of insects/sample Fb	Sw
P	EPV	50	0.80	0.48
S	NPV	25	0.72	0.52
T	NPV	25	0.36	0.48
Control No. 1	-	25	1.64	0.96
Control No. 2	-	25	2.08	1.72

Foliage Protection

In 1972 defoliation could not be measured due to the frost damage and the entire area had a grey appearance when observed in an aerial survey. This was due to the high number of dead balsam fir in the area. In 1973 there was no visible damage to either the sprayed areas or the controls.

DISCUSSION

The unseasonal weather conditions after the spray application make it well-nigh impossible to draw any definitive conclusion from the 1972 spray trials but several useful facts were established.

The viruses used in this experiment, unlike the preparations used in 1971 (Howse *et al*, 1973), appeared to be free from other contaminating viruses and it seems that the quality control checks made in the laboratory were adequate. CPV was found in all but one of the plots (R) and in both control areas and it was concluded that this virus was present in the insect population prior to the spray application. CPV was also found in spruce budworm populations near Pembroke, Ontario (Howse *et al*, *loc. cit.*) and it is probable that CPV is naturally widespread.

A deposit analysis showed that on the NPV plots 1 U.S. gal./acre gave as good coverage as 2 U.S.gal./acre. The coverage was very poor on EPV plot R sprayed with 1U.S.gal./acre but there were problems at this stage of the operation with blockage of the line and pump filters thus accounting for the poor deposit. Deposit on plot Q was better than plot P; they were sprayed on several different days with both morning and evening applications on plot Q. Micronair spray equipment is ideally suited for the application of suspensions of freeze-dried virus infected larvae, and better coverage was obtained than with a boom and nozzle system (Howse *et al*, *loc. cit.*). Keeping the gallonage per acre to a minimum is an important economic factor in forest spraying and it is considered that the slightly better results obtained in plot S with 2 U.S.gal/acre as compared to 1 U.S. gal. on plot T do not merit recommending the higher application rate.

The formulation which was used appeared to inhibit rather than enhance the infectivity of the virus. When EPV plots P and Q are compared the results are very much better on the unformulated plot which also got poorer coverage. Unfortunately, there was no NPV plot which was treated with unformulated virus. The same viruses and same formulation were applied in another experiment where virus alone and in combination with sub-lethal doses of insecticide were applied at Rankin, Ontario in 1972 (Morris et al, 1972). The NPV was applied at double the amount of active ingredient (20 gm./gal.) in these tests and high levels of virus infection were obtained indicating that the formulation had no adverse effect in this experiment.

With the exception of plot S, infection of spruce budworm was better on white spruce than balsam fir. The same phenomenon was noted in trials near Pembroke in 1971 and at Rankin in 1972 (Howse et al, loc. cit.; Morris et al, loc. cit.). A possible explanation was that spruce budworm mine several needles on white spruce and usually only one on balsam fir (McGugan, 1954). Hence, when an early application of virus is applied on second instar larvae there is more chance of them ingesting it on white spruce foliage than on balsam fir. A later application time, when the buds are flushing and when larvae are mainly in the fourth instar, may be preferable on balsam fir stands or stands where balsam fir is the dominant species.

Population reduction studies were scheduled by Dr. G. M. Howse of G.L.F.R.C. in 1972 and a pre-spray sample was taken from the plots but these studies were abandoned when the

extent of the frost damage was realized. The population counts made in 1973 indicated that both viruses had had a marked impact in the year of application and that there had been no influx of moths into the area so that the post-spray situation remained unaltered.

It has not yet been established what level of virus infection is required in the year of introduction to initiate a virus epizootic in the following years. This figure can only be determined in long term experiments and, from that point of view, the 1972 spray trials at Chapleau were not satisfactory and it will be necessary to repeat the experiment.

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