AERIAL APPLICATION OF VIRUSES AGAINST SPRUCE BUDWORM, 1971

PART A: IMPACT IN YEAR OF APPLICATION (1971)

PART B: IMPACT IN YEAR FOLLOWING APPLICATION (1972)

G. M. HOWSE, C. J. SANDERS AND A. A. HARNDEN

GREAT LAKES FOREST RESEARCH CENTRE

SAULT STE. MARIE, ONTARIO

J. C. CUNNINGHAM, F. T. BIRD AND J. R. MCPHEE

INSECT PATHOLOGY RESEARCH INSTITUTE

SAULT STE. MARIE, ONTARIO

INFORMATION REPORT O-X-189

CANADIAN FORESTRY SERVICE

DEPARTMENT OF THE ENVIRONMENT

DECEMBER 1973

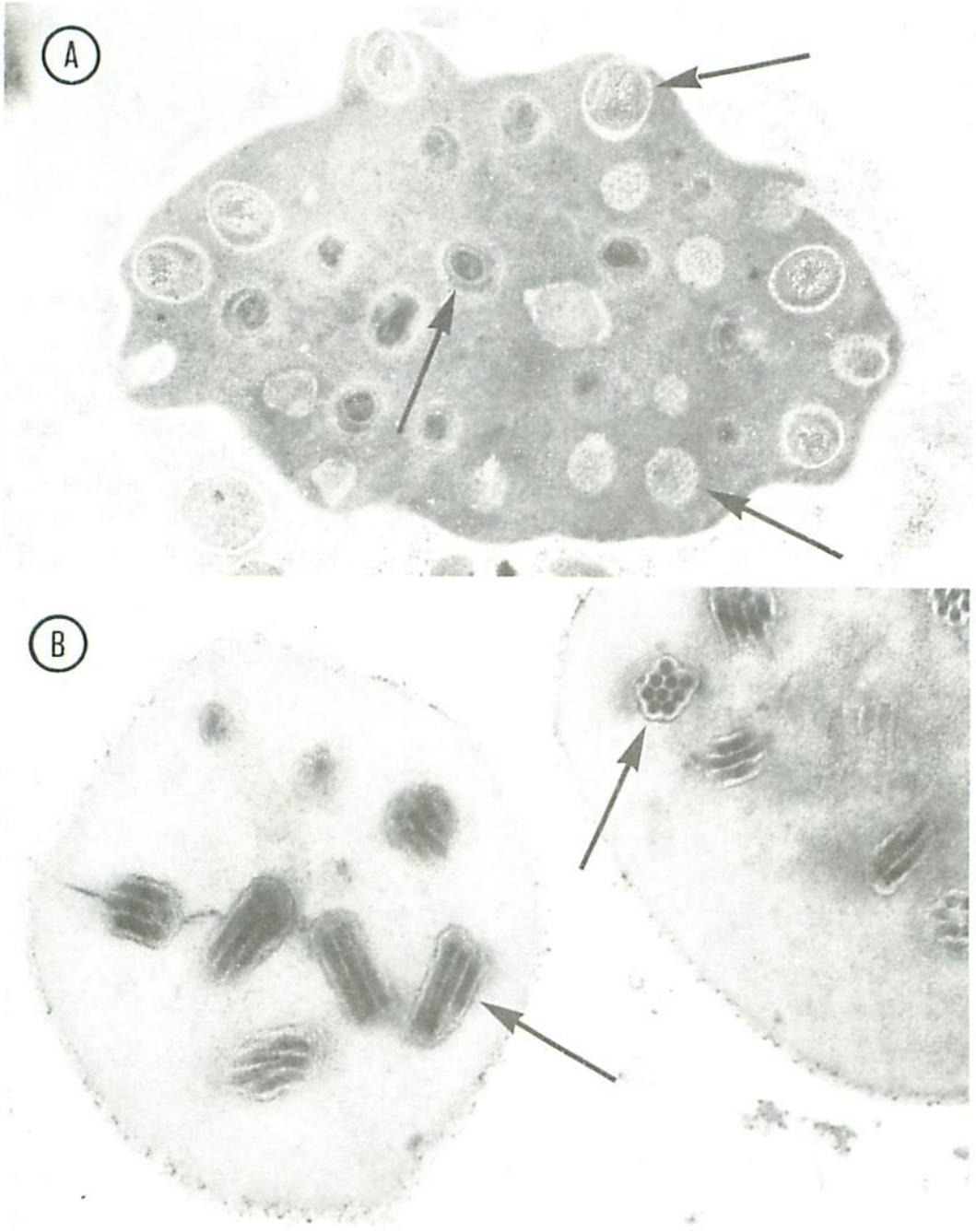
Copies of this report may be obtained from

Director,
Great Lakes Forest Research Centre,
Canadian Forestry Service,
Department of the Environment,
Box 490, Sault Ste. Marie, Ontario.
P6A 5M7

Director,
Insect Pathology Research Institute,
Canadian Forestry Service,
Department of the Environment,
Box 490, Sault Ste. Marie, Ontario.
P6A 5M7

ACKNOWLEDGMENTS

We wish to thank the Ontario Ministry of Natural Resources, in particular Mr. T. W. Heuston, formerly District Forester, Pembroke District, and Mr. K. B. Turner of the Forest Management Branch, Toronto, for various forms of assistance provided while this project was being carried out. Mr. W. Haliburton of the Chemical Control Research Institute kindly established spread-factor calibrations allowing calibration of impinging droplet size. Mr. D. W. MacLean (formerly Director of Petawawa Forest Experiment Station) and members of his staff provided laboratory facilities and other assistance.



Frontispiece. Electron micrographs of sectioned virus inclusion bodies showing virions (arrows) occluded in a protein matrix.

A) Entomopoxvirus developing in a cell. Virions are oval. (36,000 X)

B) Mature nuclear polyhedrosis virus. Bundles of rod-shaped virions are occluded. (70,000 X)

ABSTRACT

Two viruses of the spruce budworm, *Choristoneura fumiferana* (Clem.), a nuclear polyhedrosis virus (NPV) and an entomopoxvirus (EPV) were compared in field tests near Pembroke, Ontario in 1971. Aqueous suspensions of the viruses were sprayed from a helicopter at a rate of 3 U.S. gal per acre, the EPV being applied at 1 billion, 10 billion and 100 billion inclusion bodies per acre and the NPV at 300 billion inclusion bodies per acre. The applications were tested at two different times: at the peak of the second larval instar and at the peak of the fourth, making a total of eight plots.

The efficacy of the spray was evaluated by population-reduction studies, defoliation estimates and microscopic diagnosis of samples of insects to determine the level of virus infection in the population.

In the following year (1972) the plots were sampled again to assess the potential of the virus to carry over from one generation to the next. Very little EPV was found but significant levels of NPV were noted together with considerable population reduction and some foliage protection. The results were considered to be most encouraging.

TABLE OF CONTENTS

Cover photo shows a helicopter spraying entomopoxvirus during the early morning of May 31, 1971 at Achray.

INTRODUCTION

The eastern spruce budworm, *Choristoneura fumiferana* (Clem.), is the most injurious forest insect in eastern Canada. Periodic outbreaks of this pest have caused the destruction of millions of acres of spruce-fir forests throughout Ontario, Quebec and New Brunswick. Considerable time, money and effort have been spent by the Canadian Forestry Service in trying to find methods of controlling this insect. To date, the only practical means of suppressing outbreaks or preventing damage to trees is through the use of chemical insecticides. Generally speaking, the immediate value of chemical insecticides is beneficial but the side and/or long-term effects may be harmful. Therefore, suitable alternatives to chemical insecticides that will control the spruce budworm in an ecologically acceptable manner must be found. One type of alternative is a bioinsecticide containing a disease agent such as a virus.

Four different viruses have been found to infect the eastern spruce budworm. Three of these viruses were isolated from *C. fumiferana* larvae: a nuclear polyhedrosis virus - NPV (Bergold 1951), a cytoplasmic polyhedrosis virus - CPV (Bird and Whalen 1954) and a granulosis virus - GV (Bergold 1950). The fourth virus was isolated from the 2-year-cycle budworm, *C. biennis*, and belongs to the group called entomopoxviruses - EPV (Bird, Sanders and Burke 1971).

These four types of virus share a feature unique to insect viruses: the virions are embedded in protein inclusion bodies which are large enough to observe in a light microscope. It is necessary to use an electron microscope to see individual virions. Thin sections of EPV and NPV inclusion bodies as seen in an electron microscope are shown in the Frontispiece. The NPV inclusion bodies contain bundles of rod-shaped virions and the EPV inclusion bodies contain single oval virions. When ingested by a spruce budworm larva, the inclusion body protein dissolves in the alkaline gut juice and the virions are liberated and penetrate the gut cells. The inclusion bodies facilitate recognition of the viruses in infected insects and make it simple to count and standardize the concentration of suspensions used in field applications.

Stairs and Bird (1962) conducted ground spray trials on single trees using NPV and GV. They concluded that the practical use of viruses in the biological control of spruce budworm depended on 1) the development of efficient methods of virus production, 2) a knowledge of the most efficient concentration of virus to apply, 3) determination of the most vulnerable stage of the life cycle of the insect and 4) whether once established the virus will persist from year to year.

With improved methods of rearing spruce budworm on artificial diet (McMorran 1965, Grisdale 1970), large-scale virus production became practical (Cunningham *et al.* 1972). Ground spraying trials were

conducted in 1969 and 1970 using NPV (Bird and McPhee 1970). It was found that an application of virus when second-instar larvae were emerging from hibernacula resulted in a sufficiently high primary infection to initiate by natural transmission of the virus a secondary infection of epizootic proportions. However, the incubation period of the virus was much longer after early sprays (20-29 days) than after late sprays (11 days) when the weather was warmer and larvae were in the third and fourth instar.

On the basis of ground spraying results in 1969 and 1970 and because of a seriously worsening budworm situation in Ontario (Howse, Harnden and Sippell 1971) and elsewhere throughout eastern Canada, it was decided to spray NPV experimentally from an aircraft in 1971. About this time the EPV was discovered (Bird, Sanders and Burke 1971) and found to be much more infectious than NPV in the laboratory. Therefore, it was decided to test EPV as well as NPV.

In May, 1971 field tests designed to determine the operational feasibility and biological effectiveness of applying EPV and NPV to spruce budworm populations from an aircraft were carried out in southern Ontario. EPV was applied to a mixed stand in three different concentrations and at two different stages of budworm development, at a site near Achray in Algonquin Park. NPV was applied in a single concentration, at two different times, to two white spruce (*Picea glauca* [Moench] Voss) plantations along Deluthier Road near the Petawawa Forest Experiment Station (PFES), Chalk River, Ontario.

The tests and assessments were carried out as a cooperative project among personnel from the Great Lakes Forest Research Centre (GLFRC) and the Insect Pathology Research Institute (IPRI) in Sault Ste. Marie, Ontario. The operational aspects and impact of the virus sprays in the year of application, 1971, are described in Part A of this report. The impact of the viruses in the year following application, 1972, is described in Part B.

PART A: IMPACT IN YEAR OF APPLICATION (1971)

I. OPERATIONAL ASPECTS

by

G. M. HOWSE and A. A. HARNDEN

Aircraft

A Hughes 269 helicopter equipped with a 32-ft[1] boom and two spray tanks with a total capacity of 55 U.S. gal was contracted from Twinn Pest Control Aerial Ltd., Ottawa, Ontario. It was fitted with 16 No. 6504 TEE JET nozzles. Pressure was 40 psi and aircraft velocity was 20 mph. It was assumed that an effective swath width of 50 ft would produce 3.0 U.S. gal per acre.

Experimental plots

a) *Achray* (Algonquin Park, Ontario). The species composition of the stand in which the entomopoxvirus (EPV) experimental plots were located consisted of white spruce, balsam fir (*Abies balsamea* [L.] Mill.), black spruce (*Picea mariana* [Mill.] B.S.P.), trembling aspen (*Populus tremuloides* Michx.), white birch (*Betula papyrifera* Marsh.), eastern white pine (*Pinus strobus* L.), jack pine (*P. banksiana* Lamb.), and red pine (*P. resinosa* Ait.). White spruce was the dominant species comprising about 50% of the total stand volume. Stand density and closure were not uniform and ranged from open with scattered trees to fairly closed and dense stocking.

Six treatment plots, each 700 ft x 300 ft, and three control plots were established. Treatment plots were designated A, B, C, D, E and F and control plots were numbered 1, 2 and 3. A minimum of 600-ft spacing was maintained between plots. Seventy white spruce and fifty balsam fir trees (except in plots C and D which had less balsam fir - see Table 1) were selected in each treatment and control plot for budworm mortality and defoliation studies. Sample tree statistics are contained in Table 1. The plot design is shown in Figure 1.

---

[1] A conversion chart, in which S.I. equivalents are given for the basic English measurements used in this report, is found in the Appendix.

Table 1 The number of sample trees and average values of dbh, height and ages for white spruce (wS) and balsam fir (bF) sample trees in each treatment and control plot at Achray, 1971

	No. of sample trees		Dbh (in.)		Height (ft)		Age (yr)	
	(wS)	(bF)	(wS)	(bF)	(wS)	(bF)	(wS)	(bF)
Plot A	70	50	7.5	5.5	40	38	40	35
B	70	50	8.1	6.4	46	48	41	42
C	70	29	8.3	5.3	41	34	40	31
D	70	40	9.1	4.9	46	35	40	32
E	70	49	8.4	5.5	40	37	41	36
F	70	50	7.4	5.3	46	39	39	36
Control 1	70	50	7.8	6.5	--	--	44	44
2	70	50	7.4	4.6	--	--	41	31
3	70	50	4.3	2.7	--	--	38	30
Total =	630	418	$\bar{\bar{X}}$ =	7.6	5.2	43.0	38.8	40.3	35.4

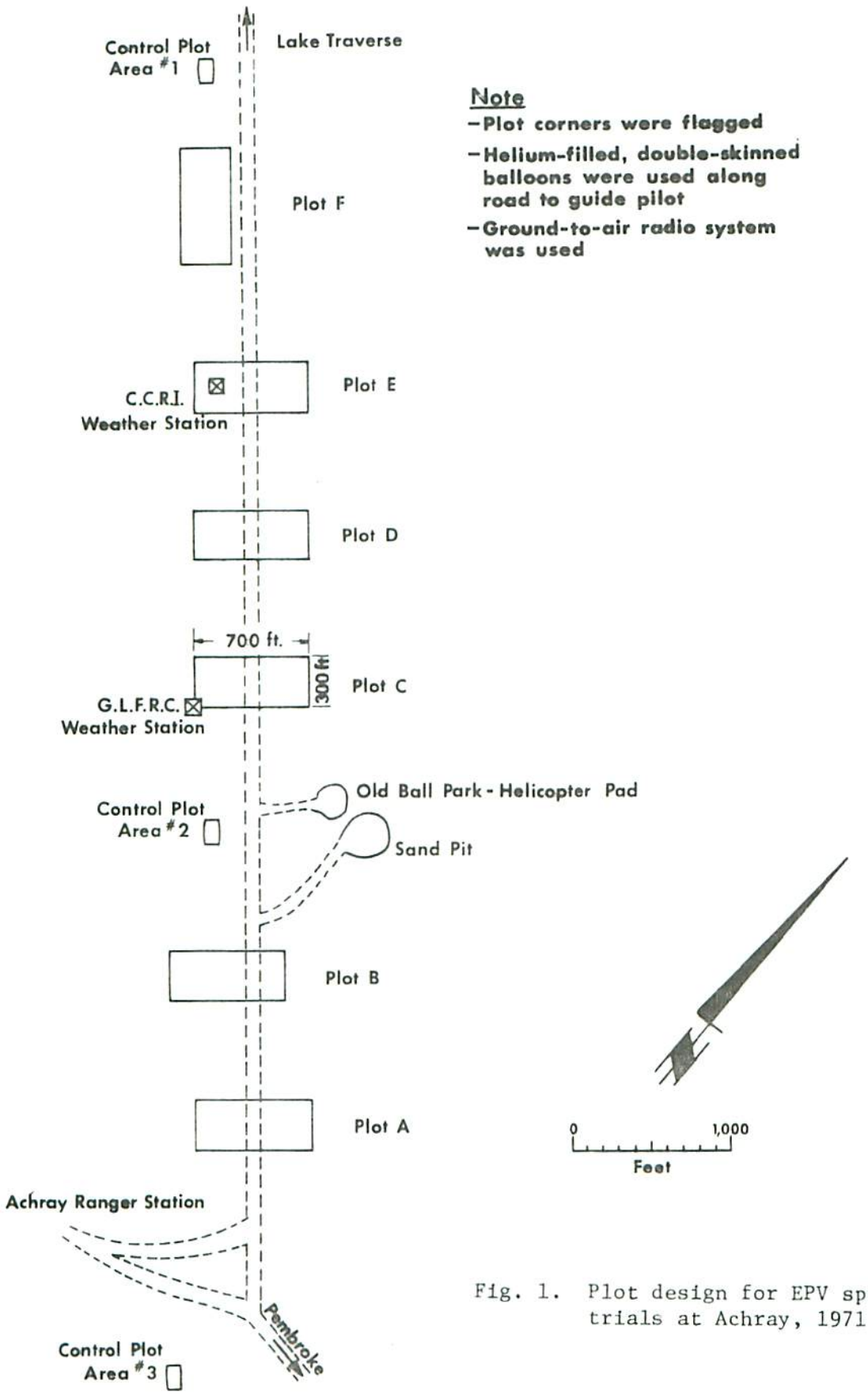


Fig. 1. Plot design for EPV spray trials at Achray, 1971

b) *Deluthier Road.* Two white spruce plantations, approximately equal in area and totalling 11.5 acres, were selected as the NPV test sites. The two plantations were adjacent to each other but divided by a stream flowing between them. Fifty white spruce trees, randomly selected, were sampled in each plantation for budworm mortality and defoliation studies. Most of the trees in the plantations were large with dbh's of 10 in. - 12 in. and heights of 50 ft - 60 ft. Crown closure was virtually complete and the stands were very dense.

Plot Preparation

a) *Achray.* In order to measure spray deposit, it was necessary in some cases to create openings in stands. Thus, within each treatment plot, trees that were overshadowing or enclosing the selected sample trees were removed. The Ontario Ministry of Natural Resources (OMNR) cooperated in this aspect of the work by providing a crew of axemen and a supervisor.

b) *Deluthier Road.* The plantations on Deluthier Road were not altered in any way since it had been decided not to measure spray deposit for the NPV tests.

Plot Marking

a) *Achray.* Flags secured in trees to extend above treetop levels and helium-filled weather balloons were used to mark boundaries and corners of each treatment plot.

b) *Deluthier Road.* The plantations were marked by balloons.

Communications

A two-way radio enabled ground crews to communicate with the pilot of the helicopter.

Treatments, Formulations and Timing of Applications

a) *Achray.* The first aerial applications of EPV were made on the evening of May 15, 1971. On this date, budworm development on white spruce was still primarily second instar but some thirds were starting to appear. Table 2 shows budworm development on white spruce and balsam fir hosts at Achray for 1971. Three concentrations, 0.1 g virus material per U.S. gal, 1 g per U.S. gal and 10 g per U.S. gal were applied to Plots E, D and C respectively, at a rate of 3 U.S. gal per acre. The numbers of viral inclusion bodies per acre were calculated to be 1 billion, 10 billion and 100 billion, respectively.

Table 2 Percentage of spruce budworm in developmental stages from May 14 to June 25, 1971 on white spruce (wS) and balsam fir (bF) hosts at Achray

Date		II[a]		III		IV		V		VI		Pupae		Emerged pupae[b]	
		(wS)	(bF)	(wS)	(bF)	(wS)	(bF)	(wS)	(bF)	(wS)	(bF)	(wS)	(bF)	(wS)	(bF)
May	14	99	100	1											
	15	92	80	8	20										
	18	69	46	31	54										
	21	14	21	78	77	6	2	2							
	22		7		93										
	25	16		74	83	10	17								
	26	7	1	82	76	11	23								
	28			30	33	61	67	9							
	29			40	24	51	70	9	6						
	31			23	13	59	74	18	13						
June	1			15		63	79	22	21						
	2			4	3	71	81	25	16						
	3			6	6	71	79	23	15						
	4			6	6	61	80	31	14	2					
	7					35	50	32	20	33	30				
	10					9	18	19	26	72	56				
	14					4	19	22	17	71	64	3			
	15					4	12	14	14	62	74	20			
	16					4	5	19	21	67	73	10	1		
	17						5	14	22	55	71	31	2		
	19							4	6	47	68	49	26		
	22							4	4	18	22	78	74		
	23							1	2	15	11	74	87	11	1
	25									7	4	93	96	20	20

[a] II-VI indicate larval instars.

[b] Emerged pupae as a percentage of the total number of pupae in the sample.

A later application of the same three concentrations (i.e., .1 g per U.S. gal in Plot F, 1 g per U.S. gal in Plot B and 10 g per U.S. gal in Plot A) at a rate of 3 U.S. gal per acre was made at the peak of the fourth instar on the morning of May 31.

The EPV formulation consisted of a mixture of water and virus material (freeze-dried, infected budworm larvae) to which was added I.M.C. (International Minerals Corporation) sunlight protectant at a concentration of 2.5% by weight. The sunlight protectant also acted as a dye enabling the recording of spray droplets as stains on conventional KromeKote spray cards.

b) *Deluthier Road.* An NPV spray at a concentration of 25 g of virus material per U.S. gal was applied during the second instar on the evening of May 16 at a rate of 3 U.S. gal per acre. The number of viral inclusion bodies per acre was calculated to be 300 billion.

A similar treatment was applied to the second plantation on the evening of May 29 when the budworm were third or fourth instars. Table 3 shows 1971 budworm development on white spruce and balsam fir hosts at PFES.

The NPV spray formulations consisted of water plus virus material (freeze-dried, infested budworm larvae). No I.M.C. sunlight protectant was included in either spray.

Spray Cards

a) *Achray.* In order to measure deposit and drift, spray cards were placed along the road that ran through the middle of each treatment plot (with the exception of Plot F); flight lines were at right angles to the road. The spray cards were placed at 10-ft intervals through the middle of, and for a considerable distance on either side of, each treatment and control plot. Plot F was oriented in such a way that the spraying flight lines were parallel to the road which also served as a boundary. Therefore, the cards located along the road, in this latter case, provided only a rough estimate of the spray deposit received by the plot.

In addition to the road cards, spray cards were placed in open spaces in association with the sample trees within each treatment plot. Altogether, a total of 1,900 spray cards were used to measure spray deposit, drift, pattern and droplet size.

Figure 2 illustrates the patterns and density of spray droplets as measured by spray cards located along the road. It is evident that most of the spray that reached the ground went into the treatment plots

Table 3 Percentage of spruce budworm in developmental stages from May 14 to July 5, 1971 on white spruce (wS) and balsam fir (bF) hosts at Petawawa Forest Experiment Station

Date		II		III		IV		V		VI		Pupae		Emerged pupae	
		(wS)	(bF)	(wS)	(bF)	(wS)	(bF)	(wS)	(bF)	(wS)	(bF)	(wS)	(bF)	(wS)	(bF)
May	13	100	100												
	16	100	66		34										
	18	97	43	3	57										
	20	75	3	25	95		2								
	23	28		67	91	5	9								
	25	20		69	80	9	20	2							
	26	10	5	82	80	8	15								
	27	10	1	84	52	6	47								
	28	5		70	51	18	49	7							
	29			54	38	31	58	15	4						
	31			22	9	47	79	29	12	2					
June	2			18	2	65	78	15	20	2					
	4			8	1	50	57	38	42	4					
	7			2		24		64		10					
	8						28		54		18				
	10					8	14	46	43	46	43				
	12					1	2	32	17	65	81	2			
	14					4	7	15	23	76	69	5	1		
	16					11	1	20	19	68	78	1	2		
	18					2		13	15	52	71	33	14		
	21							18	3	35	13	47	84		
	23							10	8	16	19	74	73	13	4
	24									23	13	77	87	9	1
July	5									3	1	97	99	90	90

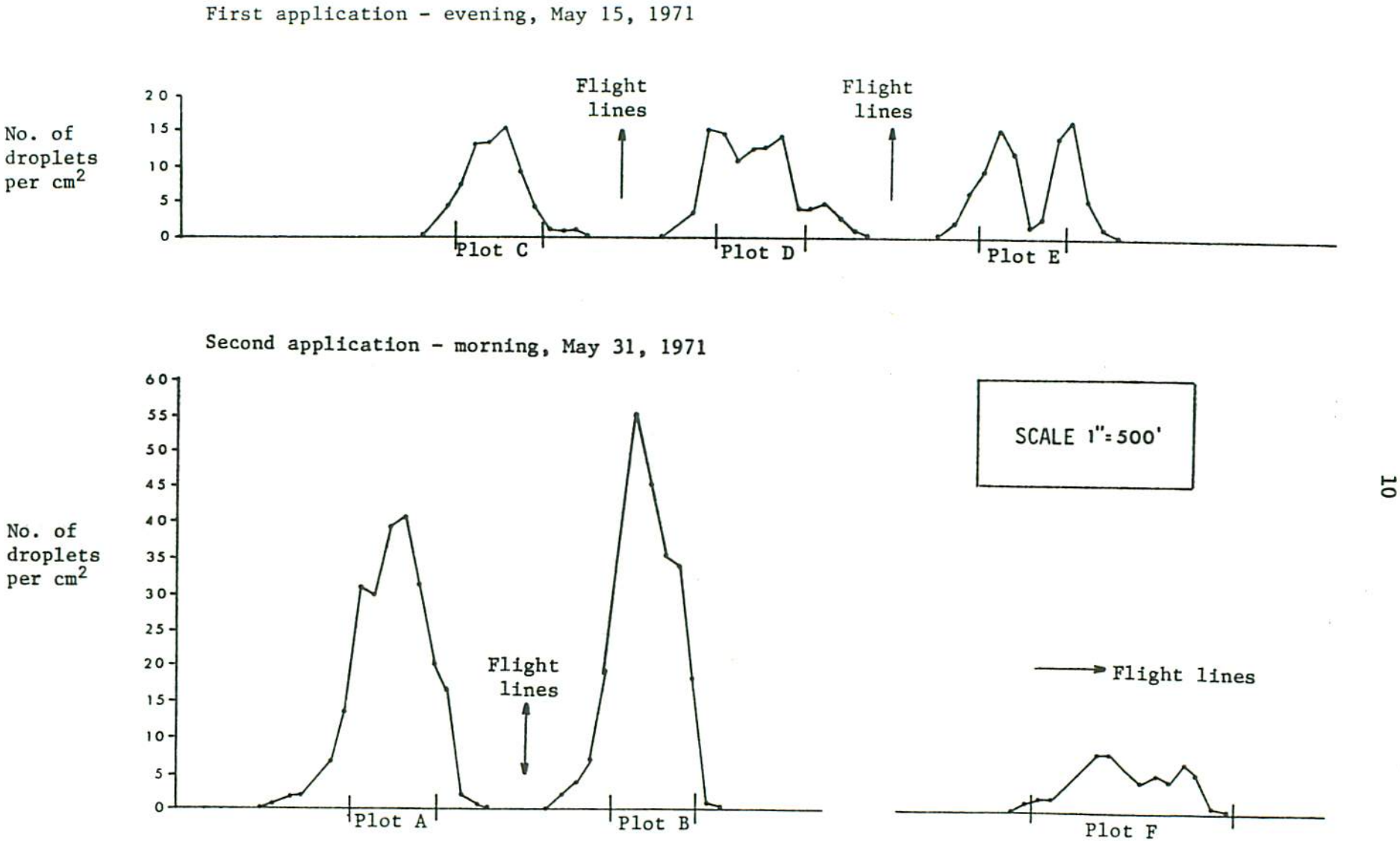


Fig. 2. Pattern and density of spray droplets as measured by spray cards located along the road during EPV applications at Achray.

and there was very little drift or spray deposited between treatment plots. No spray droplets were found on cards placed in and around the control plots.

It is interesting to note the depression in the spray deposit pattern in Plot E (Figure 2). This was due to a 60-ft meteorological tower, operated by the Chemical Control Research Institute (CCRI), that was located in a clearing just off the road in the middle of Plot E. The spray helicopter was forced to stay to either side of the tower when making this application and the spray deposit reflects the flight pattern.

Tables 4 and 5 summarize pertinent data obtained from the spray cards which were located along the road and within each treatment plot. The droplet size measurements are for droplets just before impact on the spray card.

The outstanding features of these data are the differences between the early (May 15 - plots C, D and E) and late (May 31 - plots A, B and F) applications and the differences between measurements obtained from cards located on the road and those obtained from cards located within the treatment plots. Differences between the May 15 and May 31 applications are primarily attributable to the much cooler temperature, higher relative humidity and complete lack of air movement on the morning of May 31 compared with the warm, dry evening of May 15 which would result in less evaporation of spray droplets on May 31. Differences recorded between cards placed along the road and cards located within the treatment plots are probably due to a screening effect of the stand, even though cards were placed only in clearings. Cards along the road probably measured spray deposit more accurately (because of less screening effect) than cards within the plots.

Another perhaps even more unusual difference is evident in Table 6. Droplet densities on cards associated with white spruce sample trees are generally greater (for five of the six treatment plots) than those on cards associated with balsam fir sample trees within each treatment plot. Stand structure and composition may be factors in causing this difference.

Another major difference between the early and late spray applications is evident when frequency distributions of droplet sizes are examined. For example, in the first sprays, about 55% of the droplets were 200 μ or less in diameter whereas in the second spray, nearly 70% of the droplets were 200 μ or less in diameter. The figures for the numerical mean diameter (NMD) values in Tables 4 and 5 also reflect this difference. This assumes even greater significance in view of the much higher droplet densities achieved in the second set of spray applications.

Table 4 Droplet deposit by plot at Achray (1971) on spray cards placed along the road perpendicular to flight lines

Plot	Avg no. of droplets per cm^2	Gal (U.S.) per acre actually deposited	Droplet size measurements		
			NMD[a] (μ)	AMD[b] (μ)	MMD[c] (μ)
C	10.0	1.4	155	206	425
D	9.1	1.3	170	231	360
E	8.5	1.0	160	208	360
A	29.3	4.2	135	200	520
B	30.8	3.4	130	192	445
F[d]	--	--	--	--	--

[a] NMD - Numerical mean diameter: the value that divides the number of droplets of the spray into two equal parts, one half above and one half below the median or 50% cumulative.

[b] AMD - Arithmetic mean diameter.

[c] MMD - Mass mean diameter: the value that divides the volume of the spray into two equal parts.

[d] There are no comparable road card data for Plot F because it was not possible to obtain a cross section of the spray deposit.

Table 5 Droplet deposit by plot at Achray (1971) on spray cards placed in clearings within the spray plots

Plot		Avg no. of droplets per cm^2	Gal (U.S.) per acre actually deposited	Droplet size measurements		
				NMD (μ)	AMD (μ)	MMD (μ)
C	May	5.5	1.2	187	249	505
D	15	4.4	.7	163	229	420
E		4.9	.9	187	245	486
A	May	12.9	2.1	144	208	500
B	31	14.2	1.9	148	205	408
F		6.7	1.1	138	208	595

Table 6 Differences in spray droplet density on cards exposed adjacent to either white spruce (wS) or balsam fir (bF) trees in clearings in Achray plots, 1971

Date	Plot	No. of droplets per cm^2		Average (wS + bF)
		wS	bF	
May 15	C	6.1	4.0	5.5
	D	5.0	3.3	4.4
	E	5.5	4.1	4.9
May 31	A	12.3	13.6	12.9
	B	16.2	11.4	14.2
	F	6.9	6.4	6.7

b) *Deluthier Road.* There were no measurements of spray deposit of the NPV applications.

Meteorology

a) *Achray.* A meteorological station was established by GLFRC in the southwest corner of Plot C to provide continuous records of temperature, relative humidity, wind velocity and direction from April 25 to June 30. The weather station consisted of a hygrothermograph in a Stevenson screen 48 in. above the ground and an anemometer mounted on an extension ladder 40 ft above ground level. Station altitude was approximately 750 ft above sea level.

The following data were recorded on the afternoon and evening of May 15. Spraying started at 1930 hr and finished at 2010 hr.

Time (hr)	Temperature (^{o}F)	Relative humidity (%)
1200	72	23
1300	73	23
1400	74	24
1500	75	26
1600	77	27
1700	78	28
1800	77	29
1900	74	29
2000	70	32
2100	64	40
2200	62	52

On May 15 the average wind speed from 1900 to 2000 hr was 3.1 mph with a range from 0 to 7 mph. Average wind speed from 1930 hr to 2010 hr was 2.4 mph and ranged from 0 to 6 mph. The average wind speed while each plot was being sprayed was as follows: Plot E - 4 mph, Plot D - 1 mph, and Plot C - 1 mph. The wind direction for the period 1900 hr to 2010 hr was from the northwest and west (about 50 - 50).

A second meteorological station was established by Dr. Armstrong of CCRI to measure meteorological conditions during the early spray application on May 15. This station, which consisted of a portable high tower with sophisticated instrumentation was located in Plot E, about 2000 ft from the GLFRC meteorological station. Data recorded by the CCRI meteorological station confirmed the above observations and

showed that strong inversion conditions existed during the application period (Armstrong, personal communication).

The following data were recorded on the morning of May 31 by the GLFRC meteorological station. There was no air movement registered by the instrumentation from 0500 hr to 0700 hr.

Time (hr)	Temperature (^{o}F)	Relative humidity (%)
0100	53	48
0200	49	52
0300	44	57
0400	41	62
0500	38	65
0600	35	72
0700	40	77
0800	43	65
0900	46	58
1000	50	52

Precipitation data were obtained from the OMNR station at Achray on Grand Lake, 3 miles southwest of the experimental spray sites. These data were substantiated by relative humidity data at the virus test site.

Date	Precipitation (in.)
May 14	.03
18	.01
19	.15
25	.53
26	.59
27	.02
June 2	.03
3	.18
8	.37

Note: No precipitation was recorded from June 9 to June 22.

Consideration of the above results in 1971 appeared to justify the following conclusions:

1. There are no operational constraints that prevent the application of water suspensions of EPV or NPV sprays to spruce budworm populations in natural stands or plantations from an aircraft.

2. The most satisfactory deposits of water-based virus sprays can be obtained when temperatures are cool, relative humidities are high and there is no air movement.

3. Spray cards exposed along the road received a heavier deposit than similar cards exposed in clearings within the treated areas and thus may provide a more accurate measure of spray deposit.

II. POPULATION REDUCTION AND FOLIAGE PROTECTION

by

G. M. HOWSE and A. A. HARNDEN

Introduction

This section of the report presents the results of the GLFRC studies which were designed primarily to assess the operational feasibility and biological effectiveness of using viruses against spruce budworm under Ontario conditions and to compare the results of these trials with those of operational spraying being carried out against spruce budworm in Ontario (Howse *et al.* 1971, 1972). In addition to the technical factors discussed in Section I, the principal criteria for determining operational effectiveness are biological in nature, i.e., proportions of budworm killed (population reduction) and the degree of foliage protection attributable to treatment. However, these particular criteria constitute one way of measuring the effectiveness of a particular treatment and the trials described in this report have also been assessed by other researchers using different evaluation techniques.

Methods

The population reduction and defoliation studies of the virus sprays were similar in design to methods used for the assessment of chemical control operations against spruce budworm in Ontario. Densities of living budworm, expressed as number per 18-in. branch tip, were determined for the treated and untreated (control) plots before and after spraying. At Achray, 70 dominant or codominant white spruce and 50 balsam fir (with the exception of plots C and D where, respectively, only 29 and 40 suitable balsam fir could be found) were selected in each of the six treatment plots and three control plots. At PFES (Deluthier Road), 50 white spruce were sampled in each of the two treatment plots and 25 white spruce were sampled in each of two control plots.

Prespray samples, which were collected when budworm were early- or middle-instar larvae, consisted of one 18-in. branch tip from the midcrown of each sample tree. All foliage was carefully examined and many branches were rechecked to ensure an accurate count. Postspray samples were collected when the budworm had reached the pupal stage and consisted of two 18-in. branch tips from each tree. All living pupae were saved and their subsequent fate was determined, i.e., whether moths emerged or the pupae died. Abbott's formula (Abbott 1925) was used to calculate the effectiveness of each treatment.

Estimates of the degree of damage or percent current defoliation were obtained by two methods. Treatment and control plots were examined by experienced observers from an aircraft (Turbo Beaver, OMNR) in late June and from a military helicopter (Canadian Forces Base, Petawawa) in early July. Overall estimates of the degree of damage and obvious differences between treatments and controls or among treatments were noted. These aerial observations were supported by detailed examination and estimates of the degree of defoliation sustained by the 18-in. branch tips collected for the postspray sample (pupal sample) from treated areas and control plots after budworm had ceased feeding.

Results

Prespray population densities of living budworm averaged 40 and 16 per 18-in. tip for white spruce and balsam fir, respectively, for all sample trees in all treatment plots at Achray, compared with average densities of 37 and 19 per 18-in. tip for white spruce and balsam fir, respectively, for all sample trees in all control plots. Prespray densities were considerably higher in the NPV plots at PFES where they averaged over 90 per 18-in. tip on white spruce. The PFES control plots averaged 65 living budworm per 18-in. tip.

Results of analyses are presented in Tables 7-9. Population reduction data in Table 7 are based on counts of emerged pupae and include any differential effects of virus treatments on pupal survival as shown in Table 9. Aerial observations of treated and control areas did not reveal any noticeable differences in the degree of defoliation among treatment plots or when treatments were compared to the untreated control plots. These observations are verified by the more detailed examination of branches (collected for pupal counts) for which defoliation data are presented in Table 8.

Examination of Tables 7-9, inclusive, reveal the following salient points:

1. Both EPV and NPV, at the concentrations used, are capable of causing population reduction of spruce budworm on white spruce.
2. The general lack of population reduction on balsam fir is surprising but may be explained by differences in spray coverage between white spruce and balsam fir.
3. There is little correlation between population reduction and infectivity for the late EPV application but relatively good correlation for the early application of EPV and the NPV applications (refer to Section III for infectivity data).
4. It would appear that of the two application times tested, both viruses are more effective when applied at the peak of the fourth instar.

Table 7 Population reduction by EPV on white spruce (wS) and balsam fir (bF) at Achray and by NPV on white spruce at Deluthier Road, 1971

Application date	Concentration (g/gal)	Theoretical application rate (gal/acre)	Plot	Avg no. of droplets per cm^2 (wS)	Avg no. of droplets per cm^2 (bF)	% population reduction due to treatment (wS)	% population reduction due to treatment (bF)
		EPV - Achray					
May 15 (pm) (95% II instar 5% III instar)	10	3	C	6.1	4.0	40	0
	1	3	D	5.0	3.3	61	0
	0.1	3	E	5.5	4.1	25	0
May 31 (am) (peak of IV instar - some III's and V's present)	10	3	A	12.3	13.6	79	30
	1	3	B	17.2	11.4	57	48
	0.1	3	F	6.9	6.4	59	0
		NPV - Deluthier Road					
May 16 (pm)	25	3	G	--	--	69	--
May 29 (pm)	25	3	H	--	--	80	--

Table 8 Defoliation of white spruce (wS) and balsam fir (bF) sprayed with EPV and NPV, 1971

Location	Plot	% defoliation of 1971 foliage (wS)	(bF)
Achray - EPV	C	59	87
	D	93	92
	E	87	96
	A	74	94
	B	88	99
	F	93	94
	1 (control)	90	95
	2 (control)	72	94
	3 (control)	96	60
Deluthier Road - NPV	G	99	--
	H	96	--
	Bypass (control)	99	--
	Racehorse (control)	86	--

Table 9 Pupal survival on white spruce (wS) and balsam fir (bF) sprayed with EPV and NPV, 1971

Location		% successful pupal emergence[a] (wS)	(bF)
Achray - EPV			
Plot	C	59.0	73.4
	D	72.2	70.8
	E	82.1	76.8
	A	72.6	64.6
	B	70.8	56.6
	F	83.6	66.1
Controls	1	82.2	76.8
	2	83.2	78.5
	3	79.9	70.0
Deluthier Road - NPV			
Plot	G	67.7	--
	H	80.8	--
Controls	Bypass	82.5	--
	Racehorse	84.0	--

[a] successful pupal emergence = $\frac{\text{emerged budworm}}{\text{budworm alive on sample date}} \times 100$

5. There is no significant preservation of foliage by NPV on white spruce or EPV on either white spruce (except perhaps in Plot C) or balsam fir.

6. Pupal survival appears to be adversely affected by the virus sprays, particularly EPV, and in a pattern related to the concentration of virus used.

The data were examined further, and although they were inadequate to provide conclusive confirmation, the following trends and relationships are suggested, and may warrant future intensive investigation:

7. When the relationship between percent population reduction and spray coverage (droplets per cm^2) at a given concentration of virus are examined, it appears that as few as five droplets of spray per cm^2 may be as effective as 20 droplets per cm^2. If this can be confirmed, it could result in reduced operational application rates and/or concentrations.

8. Examination of the relationship between percent population reduction and prespray larval density suggests that effective kill increases in direct proportion to larval density to a point where competition or starvation becomes the dominant factor and the effect due to virus declines. It may be that up to a certain population level (*ca.* 80 or more fifth-instar larvae per 18-in. tip on white spruce) the virus becomes an increasingly more effective killing agent.

9. When the relative heights of white spruce and balsam fir in the spray plots at Achray were examined, it was found that the white spruce sample trees were, in general, 4-5 ft taller than the balsam fir sample trees (Table 1). It was also observed that, in general, the individual white spruce trees were more exposed than individual balsam fir trees. This may explain the greater spray coverage obtained on white spruce than on balsam fir (Table 6).

10. Some general trends appeared when the relationships between tree height and prespray larval population density and between tree height and percent population reduction were examined. For example, larval population densities appeared to increase with tree height for white spruce but not for balsam fir. Similarly, percent population reduction appeared to increase with tree height but very little trend was apparent for balsam fir.

11. Finally, there was some evidence that, in addition to direct pupal mortality, the development of the late larval and pupal stages of budworm sprayed with EPV and NPV is significantly retarded. In some cases, budworm development in the high virus concentration treatment plots was 3-6 days behind development in the control plots. This prolonged development could result in increased mortality due to predation and/or adverse environmental factors.

Consideration of the data presented above for 1971 appears to justify the following conclusions:

1. At the spray concentrations used significant numbers of budworm may be killed by EPV and NPV sprays.

2. NPV does not prevent serious defoliation of white spruce of budworm in the year of application. EPV may provide some protection, but only marginally so on both white spruce and balsam fir in the year of application.

III. INCIDENCE OF VIRUSES

by

F. T. BIRD, J. C. CUNNINGHAM and J. R. MCPHEE

Introduction

In 1971 six plots were sprayed with entomopoxvirus (EPV) and two with nuclear polyhedrosis virus (NPV). The areas sprayed, application rate, time of application, and operational details have been described fully by Howse and Harnden (Section 1). Three of the EPV plots and one NPV plot were sprayed when larvae were mainly in the second instar and the remainder of the plots were sprayed when larvae were mainly in the fourth instar. These application times will be referred to as "early spray" and "late spray" in the discussion which follows.

In virus spray trials one method of measuring the impact of the application is to compare the incidence of virus in larvae on the sprayed plots and on unsprayed (control) areas. Logically, the results of such studies should correspond to the population-reduction trend. Infection studies also reveal if viruses are already present in the insect population, and if the virus which has been disseminated is causing infection which will eventually lead to mortality. In all probability the percentage infection figure will be lower than the population-reduction figure because, as the season advances, larvae which die may be lost from the foliage and diseased larvae may be more susceptible to predation. Therefore, sampling becomes biased in favor of healthy larvae and, by using infection figures alone, a conclusion more pessimistic than is merited can sometimes be drawn.

Methods

In each of the six EPV plots four white spruce trees were studied intensively. Two 18-in. branch tips were collected at the top, midcrown and bottom of each of these four trees. Towards the end of the spruce budworm season when pupation was commencing, random samples (18-in. branch tips) were taken throughout the plots from both white spruce and balsam fir.

In the NPV plots 10 random samples of two 18-in. branch tips were taken from both plots at approximately weekly intervals commencing 2 weeks after spraying. Untreated foliage samples were collected from three sites close to the EPV plots and one site, Young Creek Road, about 2 miles from the NPV plots was sampled twice during the season. These samples were taken between June 16 and June 28.

All spruce budworm, living or dead, larvae or pupae, were removed from the foliage. Living larvae were dissected and squash preparations were made of the guts and portions of fat tissue. They were examined under a Leitz Ortholux microscope fitted with phase-contrast optics. Dead larvae were smeared without dissection, or if they were desiccated, they were ground up in a drop of water using a glass rod.

The smears and squash preparations were examined for the presence of inclusion bodies of EPV, NPV or CPV and the result of the diagnosis of each insect was recorded. When there were large numbers of pupae in the samples they were kept until adult emergence. If adults emerged they were considered healthy. The dead pupae were examined microscopically.

Results

a) *EPV plots.* Examination of larvae from all the plots revealed that the EPV suspension must have been contaminated with NPV and CPV since these viruses were not found in the untreated controls but were present in sprayed plots. A comparison of the incidence of infection from the early and late sprays for the heaviest concentration of EPV used (10 g/U.S. gal on plots C and A) is given in Table 10, the middle concentration (1 g/U.S. gal on plots D and B) in Table 11 and the lightest concentration (0.1 g/U.S. gal on plots E and F) in Table 12. The untreated control samples are recorded in Table 13.

Double and even triple infections with all three viruses present in one larva were sometimes observed; hence, the percentages of the single viruses are not additive to give the percentage of the total virus. This point should be kept in mind when examining the data presented in Tables 10-15. Generally the percent virus infection increased with time from spray date and the samples with the highest percent infection were taken in the late larval and pupal stages near the end of the season. The maximum total virus infection percentages followed the concentrations applied: 44%, 26% and 12% in the 10 g/U.S. gal (C), 1 g/U.S. gal (D) and 0.1 g/U.S. gal (E) plots, respectively, for the early spray and 12%, 2% and 6% for the late spray plots (A, B and F) on white spruce. The maximum infection on balsam fir was considerably lower and the corresponding figures were 9%, 1% and 3% for the early spray and 10%, 2% and 4% for the late spray. The incidence of the three viruses individually is shown in Tables 10, 11 and 12.

The first EPV-infected larvae were found 21 days after spraying in the late-spray plot with the heaviest concentration (A) and both NPV and CPV were found in this sample. Development of virus was slower in the early spray plot although not enough samples were taken to pinpoint the exact length of time. NPV and CPV were found in the heaviest plot (C)

Table 10 Virus infection resulting from dissemination of EPV at a concentration of 10 g freeze-dried material per U.S. gal against the spruce budworm at Achray, Ontario in 1971

Plot	Date of spraying	Type of sample	Tree species[a]	No. of days after spray when sampled	No. of insects examined	EPV %	CPV %	NPV %	Total virus infected %
		four trees	wS	15	206	0	0	0	0
			wS	24	205	0	1	1	1
			wS	30	463	23	4	1	26
			wS	37	479	26	7	10	37
C	May 15		wS	44	570	20	5	8	24
		random samples	wS	37	305	27	5	8	33
			wS	44	660	38	0	6	44
			bF	44	381	9	0	1	9
		four trees	wS	22	99	5	1	7	12
			wS	28	741	2	1	3	5
A	May 31	random samples	wS	28	246	2	0	1	3
			bF	28	301	6	0	4	10

[a] wS = white spruce
bF = balsam fir

Table 11 Virus infection resulting from dissemination of EPV at a concentration of 1.0 g freeze-dried material per U.S. gal against the spruce budworm at Achray, Ontario in 1971

Plot	Date of spraying	Type of sample	Tree species[a]	No. of days after spray when sampled	No. of insects examined	EPV %	CPV %	NPV %	Total virus infected %
D	May 15	four trees	wS	15	123	0	0	0	0
			wS	24	218	1	1	1	1
			wS	30	429	8	3	1	11
			wS	37	294	16	2	8	22
			wS	44	292	16	4	12	26
		random samples	wS	37	185	15	1	4	19
			wS	44	319	16	1	3	15
			bF	44	377	3	1	0	3
B	May 31	four trees	wS	28	358	2	0	0	2
		random samples	wS	28	317	1	0	1	2
			bF	28	275	2	0	0	2

[a] wS = white spruce
bF = balsam fir

Table 12 Virus infection resulting from dissemination of EPV at a concentration of 0.1 g freeze-dried material per U.S. gal against the spruce budworm at Achray, Ontario in 1971

Plot	Date of spraying	Type of sample	Tree species[a]	No. of days after spray when sampled	No. of insects examined	EPV %	CPV %	NPV %	Total virus infected %
E	May 15	four trees	wS	37	324	7	0	2	9
			wS	44	333	9	0	1	10
		random samples	wS	37	218	12	0	0	12
			wS	44	329	0	0	0	0
			bF	44	435	1	0	0	1
F	May 31	four trees	wS	29	157	6	0	0	6
		random samples	wS	29	280	5	0	0	5
			bF	29	525	4	0	0	4

[a] wS = white spruce
bF = balsam fir

after 24 days and EPV after 30 days, although EPV was found after 24 days in the middle concentration (D).

b) *NPV plots*. In both NPV spray plots a considerable incidence of CPV (28-35%) was found (Table 14) in comparison with the untreated (control) samples (Table 15) in which very little (2-3%) was found. There is a high probability that the NPV suspension was contaminated with CPV.

In the early spray plot the first virus infection was noted 14 days after spraying and the highest NPV infection, 21%, was noted 36 days after spraying. In contrast, in the first sample taken from the late spray plot (H) 16 days after spraying, 46% NPV infection was recorded. This first sample was taken too late to follow the development of the infection which had progressed much more quickly in the late spray plot. The percentage NPV in the late spray plot (H) had declined when the second sample was taken 23 days after spraying. It had also declined by the time the fourth and final sample was taken 36 days after spraying in the early spray plot. This decline was considered to be due to the loss of dead or diseased larvae from the foliage. Two larvae infected with natural NPV were found in one of the control samples.

Discussion

The EPV preparation was found to be lightly contaminated with NPV and presumably with CPV; the NPV preparation contained no EPV but was heavily contaminated with CPV. This is undesirable as it is known that CPV interferes with and retards the development of NPV (Bird 1969); it is probable that there is an antagonistic reaction between NPV and EPV. Every effort should be made to produce pure viruses for field spray trials.

Higher infection was obtained in the early EPV spray plots than in the late. The early spray was applied in the evening and the late spray in the morning. The morning is an unfavorable time for spraying viruses since they are exposed to solar radiation for the remainder of the day. Both NPV plots were sprayed in the evening and, in contrast to the EPV plots, better infection was obtained with the late spray. The EPV develops much more slowly than NPV and it is possible that pupation commenced before inclusion bodies were formed which could be diagnosed microscopically.

The results obtained from the EPV plots were disappointing when compared with data obtained for this virus in the laboratory. The results from the late spray NPV plot were extremely encouraging but it must be stressed that a very high dosage of virus was applied. On an operational basis this would be economically unacceptable. Nevertheless,

Table 13 Virus infection in spruce budworm collected from untreated white spruce samples at Achray, 1971

Sample number	Sample date	No. of insects examined	EPV %	NPV %	CPV %	Total virus infected %
1	June 21	263	0	0	0	0
2	June 28	1301	0	0	0	0
3	June 28	1306	0	0	0	0

Table 14 Virus infection resulting from dissemination of NPV at a concentration of 25 g freeze-dried material per U.S. gal at Petawawa Forest Experiment Station, Deluthier Road, Ontario

Plot	Date of spraying	No. of days after spray	No. of insects examined	NPV %	CPV %	Total virus %
G	May 16	14	286	3	1	4
		22	250	2	8	10
		29	187	8	28	34
		36	542[a]	21	21	31
H	May 29	16	200	46	35	71
		23	116	41	22	59

[a] Only 212 pupae and larvae were examined microscopically. Owing to the large number of pupae in the sample, 94 were examined and the scores adjusted proportionately to maintain the balance of the sample.

Table 15 Virus infection in spruce budworm collected from untreated white spruce samples at Young Creek Road, 1971

Sample date	No. of insects examined	NPV %	CPV %	Total virus infected %
16 June	209	1	2	3
23 June	221	0	3	3

this application proved that a high percentage of larvae could be infected by virus disseminated from the air and that microscopically detectable infection occurred in less than 16 days. Now that it has been established that infection can be obtained with a dosage of 25 g per U.S. gal at 3 U.S. gal per acre, lower and more realistic application rates should be tested.

In the early-spray EPV plots (C, D, E) there was a marked difference in the percentage of virus infection on white spruce and balsam fir, the infection being considerably higher on the white spruce than on balsam fir. This marked difference was not noted on the late-spray plots (A, B, F) and white spruce was the only species present in the NPV plots. A possible explanation is that spruce budworm mine several needles on white spruce and only one on balsam fir when they are in the second instar (McGugan 1954). As a result virus is more likely to be ingested on spruce than on balsam fir. However, it should be noted also that spray deposits appeared to be heavier on white spruce than on balsam fir (Table 6).

Virus carryover from one year to the next and spread from the sprayed areas must be investigated in the year following application. This subject forms the second part of the report.

The data presented above for 1971 appear to justify the following conclusions:

1. The early EPV application gave a higher percentage of infection than the late application. It is possible that this slow-acting virus did not have time to develop before the larvae pupated when it was applied as a late spray.

2. In the early EPV application a higher percentage of infection was found on white spruce than on balsam fir. This difference was not found in the later application.

3. The late NPV application gave a higher percentage of infection than the early application. Results were considered to be extremely encouraging for this treatment.

4. Very low levels of natural virus, 1% NPV and 3% CPV, were found in one of the control plots.

5. Every effort should be made to reduce cross contamination of the virus suspension such as is presumed to have occurred in 1971, since antagonism between the viruses may reduce their effectiveness in the field.

IV. DEBILITATION STUDIES

by

C. J. SANDERS

Introduction

It was recognized during the planning stages of the operation that, in addition to causing outright mortality, the viruses might produce delayed effects, or sublethal effects. Such debilitation would not be detectable in infected larvae, but might show up as lower pupation success and survival, and lower mating success and fecundity. Furthermore, virus present in the adults might infect the eggs, causing mortality in the following generation. Larvae were therefore collected from the treated areas and reared through to maturity. The adults were mated and the resulting larvae were kept for subsequent rearings to compare their performance with that of insects from untreated control plots.

Methods

A. *Sampling*

(i) *EPV.* White spruce foliage containing late-instar larvae was collected from Achray on June 11, 1971. The plots sampled were Plot C (early application at highest rate), Plot A (late application at highest rate) and Control 2, located between plots B and C. All samples were of 18-in. branch tips collected in a basket mounted on a pole pruner.

The following day the foliage was taken in paper bags to Sault Ste. Marie where it was kept at 42°F until examination and pick-off on June 14 and 15.

At the time of collection it was noted that the damage caused by the feeding budworm was less intensive near the tops of the trees. To document this, samples were taken from four crown levels. At each level (A highest, D lowest) two branch tips were taken, four trees were sampled in plots A and C and two were sampled in the Control. This intensity of sampling was too low to detect possible differences among the plots, but collectively the pattern was as follows:

Crown level	Budworm distribution (%)	Damaged buds (% of total buds)
A	24	33
B	35	51
C	25	79
D	16	88

Thus, although most budworm were found in level B, followed by A and C, damage was highest at the lower levels, leaving the trees with green apices.

(ii) *NPV.* Foliage was collected on June 12 (using hand clippers) from the early-application NPV plot (Plot G). It was taken directly to Sault Ste. Marie where it was stored at 42°F until pick-off on June 15 and 16. No collections were made from the late application plot (Plot H), or from an unsprayed area in the vicinity.

B. *Rearing*

At the time of pick-off, 83% of the insects from Achray were sixth instar. Development in the NPV plot was slower, but many of the earlier-instar larvae subsequently proved to be parasitized.

All living larvae were reared on synthetic diet following the technique of Grisdale (1970), three larvae per creamer cup. Cups were checked daily. The following information was recorded: date of pupation, sex, pupal weight, date of emergence. Dead larvae and pupae were kept, and parasites were identified as they emerged.

After successful emergence, adult male and female budworm from the same plot were placed together in pairs in 8-oz jars, with balsam fir foliage for mating and oviposition. After 10 days the eggs were counted and placed in petri dishes with gauze for larval spin-up. The following data were recorded: female weight, number of eggs laid, success of mating (i.e., whether or not eggs hatched). Second-instar larvae from successful matings were held at 32°F until diapause requirements were satisfied, after which several families from each treatment were reared on synthetic diet to assess their survival and the possible transovarial transmission of the virus.

Results

A. *Larval survival to pupation*

The relevant data are summarized as follows:

	Control	EPV Plots A	EPV Plots C	NPV (G)
Total number collected	360	995	941	682
% pupating	75	68	64	30
% parasitized	10	7	8	51
% other mortality	15	25	28	19

(i) *Parasitism.* Rates of parasitism among the three EPV plots were not significantly different, and of a total of 94 parasites reared, 76 were *Glypta*, 16 *Diptera* and 2 *Apanteles*.

In contrast, parasitism in the NPV plot was extremely high (51%). Of 349 parasites, 170 were *Glypta*, 167 *Apanteles* and 12 *Diptera*. The high incidence of *Apanteles* suggests that the rate of parasitism may have exceeded the 51% recorded here, since *Apanteles* emerge early and some may have emerged before the collections were made. In addition, larvae parasitized by *Apanteles* are usually small and shrivelled, and could easily have been missed during pick-off.

(ii) *Other mortality.* Mortality due to factors other than parasitism was twice as high in the plots treated with EPV as in the control, giving 12% population reduction in Plot A and 15% in Plot C. The causes of this were not determined.

B. *Pupal survival and sex ratio*

The results are summarized as follows:

	Control	EPV Plots A	EPV Plots C	NPV (G)
% pupae producing adults	87	54	57	85
% females	48	58	56	46

Compared with the controls the increased mortality in the plots treated with EPV showed a population reduction of 38% in Plot A and 34% in Plot C.

In this study it must be remembered that pupal parasitism and predation are both absent, since the pupae were not exposed to attack.

This fact may, in large part, explain the discrepancies in the percentage of pupae producing adults compared with the data recorded by Howse (Table 9).

The unusually high proportion of female moths in plots A and C is of interest. It arises from a higher rate of mortality among both male larvae and male pupae. This tends to offset the effects of higher pupal mortality rates in plots A and C.

C. *Pupal weights, pupation date and duration, mating success and fecundity*

The relevant data are summarized as follows:

	Control	EPV Plots A	EPV Plots C	NPV (G)
Avg pupal weights (mg)				
♂	66	65	62	58
♀	96	93	94	77
% matings successful	87	73	70	71
Avg fecundity	178	178	142	124

Among the EPV plots no differences were found in pupal weights or in date and duration of pupation (not tabulated here).

The fecundity of insects from the C plot was significantly lower than that of insects from the controls, but no effect was found in Plot A. Mating success, as measured by the percentage of matings producing fertile eggs, was lower in both plots A and C, representing a population reduction of 16% in A and 20% in C. This presumably represents a debilitating effect caused by the virus.

The low female pupal weights in the NPV plot were presumably due to partial starvation owing to competition for food.

D. *Survival of small larvae in following generation*

Diapause requirements were satisfied in January, 1972. Larvae from each treatment were reared by Dr. J. C. Cunningham and examined for infection by the viruses. However, none was found, and it is concluded that there was no transovarial transmission of either NPV or EPV.

Discussion

The results may be summarized by compiling partial life tables for each area as follows, where x = the relevant interval in the life cycle, S_x = the survival rate (i.e, 1.0 means all larvae surviving and no mortality, and 0.6 means 60% surviving with 40% mortality) and N_x = the number surviving at the beginning of each interval x.

	EPV Plots							
	Control		A		C		NPV (G)	
	S_x	N_x	S_x	N_x	S_x	N_x	S_x	N_x
Larvae		100		100		100		100
Pupae	.75	75	.68	68	.64	64	30	30
Adults	.87	65	.54	37	.57	36	85	26
Sex ratio (% ♀)	.48	31	.58	21	.56	20	46	12
Mating success	.87	27	.73	15	.70	14	71	8.5
Fecundity	178		178		142		124	
No. of eggs expected		4806		2670		1988		1054
% population reduction	--		44		59		--	

The data presented above suggest the following conclusions:

1. The effects of the different rates of mortality (reflected in the S_x values) are multiplicative, and together, between the late larval stage and the following first-instar stage, indicate a 44% reduction in Plot A and 59% in Plot C when compared with the controls. Such effects can be attributed to the EPV.

2. Although survival was considerably poorer in the NPV plot, in the absence of a suitable control it is impossible to determine how much of this may be attributed to NPV.

PART B: IMPACT IN YEAR FOLLOWING APPLICATION (1972)

V. INCIDENCE OF VIRUSES

by

J. C. CUNNINGHAM, F. T. BIRD and J. R. MCPHEE

Introduction

An important aspect of the use of insect viruses in control of forest insect pests is that some viruses persist from one season to the next and can spread from the original point of introduction. Although the subject of transmission and spread of insect viruses requires considerably more study, there are thought to be three possible methods by which viruses can persist in an insect population from one generation to the next: transovarial transmission in which the virus is carried within the egg, transovum transmission in which the virus is carried on the surface of the egg, and contamination of the environment with virus which is shielded from solar inactivation. The first two methods are discussed by Smith (1967). Contamination of the soil is the means of transmission of *Trichoplusia ni* NPV in cabbage fields (Jaques 1964, 1967). Bird (1961) states that the NPV's of three sawflies, *Diprion hercyniae*, *Neodiprion sertifer* and *N. lecontei* are transmitted via the eggs of infected females and kill young larvae at an early stage of larval development. This has been questioned by Neilson and Elgee (1968) who have demonstrated that the transmission between generations of the NPV of *D. hercyniae* is a result of foliage contamination by diseased and/or externally contaminated adults. No studies have been made on the transmission of any of the viruses of the spruce budworm (EPV, NPV and CPV).

The plots which were sprayed in 1971 were sampled again in 1972 to determine if carryover of virus had occurred. Areas around the Achray EPV plots, which had not been treated in 1971 and were used as control plots, were also sampled in 1972 to establish if spread had occurred. It was not possible to do this around the NPV plots as they were discrete white spruce plantations surrounded by nontarget-tree species.

Methods

Only the heaviest concentration (10 g/U.S. gal) of EPV plots (A and C) were sampled. Four samples were taken which coincided with the following stages of insect development: mainly second instar, third and fourth instar, fourth and fifth instar, sixth instar and pupae. About five (4-6) random samples were taken from balsam fir on the early-application and late-application plots and consisted of two 18-in. branch tips. About 30 (23-38) samples of the same size and type were taken from

white spruce on the same plots. Three control plots close to the spray plots were sampled when pupation commenced and five random samples of two 18-in. branch tips were collected from both white spruce and balsam fir on each of the plots. The sampling dates, numbers of samples and numbers of spruce budworm collected from these samples are given in Table 16.

The two NPV plots were sampled on about the same dates. About 20 (13-21) random samples were taken from each plot consisting of two 18-in. branch tips. Three control plots, about 2 miles from the sprayed area, were sampled when pupation commenced. Ten random samples were taken from white spruce only. The data are presented in Table 17.

The methods used for determining virus infection have been described in Section III.

Results

a) *EPV plots.* In the early spray plot one CPV-infected larva was found when second-instar larvae were sampled, more CPV- and three NPV-infected larvae were found in the third sample, and it was not until the fourth sample of sixth-instar larvae and pupae that EPV was found along with a fairly high incidence of NPV and CPV. The results of all the samples are shown in Table 16. The highest incidence of virus was found in the final sample when 7% NPV and 2% CPV were recorded from insects on balsam fir and 5% EPV, 19% NPV and 5% CPV from insects on white spruce.

A similar result was found in the late-spray plot except that no poxvirus whatever was present. Small amounts of NPV and CPV were found in the second and third samples and in the final sample 17% NPV and 10% CPV were recorded from insects on balsam fir and 20% NPV and 4% CPV from insects on white spruce.

Samples from the controls, two taken at the ends of the series of treatment plots and one taken in the middle, showed a low level of CPV only.

b) *NPV plots.* In the early spray plot no virus was found in the first sample, a very low level was found in the second sample, an increase was found in the third and the highest level was found in the final sample. In this sample 28% NPV and 5% CPV were recorded although the CPV was higher at 10% in the third sample. The results are shown in Table 17.

In the late-spray plot no virus was found in the first or second sample, 10% NPV and 2% CPV were found in the third and 24% NPV and 3% CPV

Table 16 Virus infection recorded in 1972 in spruce budworm in plots sprayed in 1971 with EPV (contaminated with NPV and CPV) at a concentration of 10 g per U.S. gal and a rate of 3 U.S. gal per acre at Achray, Ontario

Plot	Time of application in 1971	Sample date (1972)	Tree species[a]	No. of samples	No. of insects examined	% virus infection		
						EPV	NPV	CPV
C	early spray	19 May	bF	6	52	0	0	0
		19 May	wS	23	132	0	0	1
		29 May	bF	5	51	0	0	0
		29 May	wS	32	288	0	0	0
		8 June	bF	5	50	0	0	4
		8 June	wS	34	334	0	1	1
		21 June	bF	5	54	0	7	2
		21 June	wS	32	367	5	19	5
A	late spray	19 May	bF	4	20	0	0	0
		19 May	wS	33	237	0	0	0
		29 May	bF	5	34	0	0	3
		29 May	wS	38	284	0	1	3
		8 June	bF	5	46	0	2	0
		8 June	wS	36	358	0	2	1
		21 June	bF	5	48	0	17	10
		21 June	wS	33	368	0	20	4
Controls	1	21 June	bF	5	88	0	0	1
		21 June	wS	5	170	0	0	0
	2	21 June	bF	5	60	0	0	0
		21 June	wS	5	87	0	0	0
	3	21 June	bF	5	101	0	0	3
		21 June	wS	5	116	0	0	0

[a] bF = balsam fir
wS = white spruce

Table 17 Virus infection recorded in 1972 in spruce budworm on white spruce plots sprayed in 1971 with NPV (contaminated with CPV) at a concentration of 25 g per U.S. gal and a rate of 3 U.S. gal per acre at Petawawa Forest Experiment Station, Deluthier Road, Ontario

Plot	Time of application (1971)	Sample date	No. of samples	No. of insects examined	% virus infection	
					NPV	CPV
G	early spray	19 May	18	192	0	0
		28 May	20	203	1	1
		7 June	21	216	3	8
		20 June	20	485	28	5
H	late spray	19 May	13	22	0	0
		28 May	14	92	0	0
		7 June	17	115	10	2
		20 June	20	507	24	3
Controls	1 (Racehorse)	21 June	9	213	0	2
	2 (Orange Rd)	21 June	10	297	0	2
	3 (Bypass Rd)	21 June	10	172	0	5

were found in the fourth. The controls in this experiment were not directly comparable as they were approximately 2 miles away but 2% CPV was recorded in two samples and 5% in the third.

Discussion

An interesting comparison can be drawn between the samples taken in the year of application and those taken in the following year, because an increase or decrease in incidence of the different viruses can be determined. A comparison of the 1971 and 1972 results for the 10 g/U.S. gal early- and late-application EPV plots is given in Table 18. It can be seen that there was a very marked decrease in EPV and a marked increase in both NPV and CPV.

The same kind of comparison was made between the early and late NPV sprays and the results are shown in Table 19. In the early spray plot the NPV increased from 21% to 30% and the CPV decreased from 21% to 8%. In the late spray plot the NPV decreased from 41% to 24% and the CPV from 22% to 3%.

It is concluded that EPV does not carry over well from one year to the next. In contrast NPV carried over well in all the plots studied and increased in three out of four plots. The results with CPV in the two sets of plots conflict. This virus markedly increased in the EPV plots and dramatically decreased in the NPV plots although it was recorded in all plots. The situation with CPV is confused by the incidence of this virus in the controls. It was not found in the EPV controls in 1971 but was recorded at a low level in 1972. It was recorded in both 1971 and 1972 in the NPV controls. It is impossible to establish its status with this limited information. It is known that CPV is not uncommon in natural populations and it cannot be determined if virus spread from the treated plots at Achray to the untreated controls or if natural virus was responsible for the infection.

The absence of EPV and NPV from the Achray controls was disappointing as it was hoped that these viruses would spread from the sprayed areas. The mechanism of spread is poorly understood but it is possible that virus-infected or virus-contaminated moths, predators, parasites and birds play a role. This study will continue. It is possible that spread is a slow process and may require more than one year. The NPV plots were unsuitable for studies of spread because they were surrounded by nontarget-tree species.

It was surprising that the viruses were found so late in the season following the year of application. When carryover occurs one would expect that young larvae would become infected and die and that secondary infection would occur as a result of foliage contamination by

Table 18 A comparison of the maximum infection of three viruses found in spruce budworm on plots sprayed with EPV (contaminated with NPV and CPV) at a concentration of 10 g freeze-dried material per U.S. gal and a rate of 3 U.S. gal per acre in the year of application (1971) and the following year (1972)

Plot	Time of application (1971)	Year sample taken	Tree species[a]	% virus infection		
				EPV	NPV	CPV
C	early spray	1971	wS	38	10	7
		1972	wS	5	19	5
C	early spray	1971	bF	9	1	0
		1972	bF	0	7	2
A	late spray	1971	wS	5	7	1
		1972	wS	0	20	4
A	late spray	1971	bF	6	4	0
		1972	bF	0	17	10

[a] wS = white spruce
bF = balsam fir

Table 19 A comparison of the maximum virus infection found in spruce budworm on plots sprayed with NPV (contaminated with CPV) at a concentration of 25 g freeze-dried material per U.S. gal and a rate of 3 U.S. gal per acre in the year of application (1971) and the following year (1972)

Plot	Time of application (1971)	Year samples taken	% virus infection	
			NPV	CPV
G	early spray	1971	21	21
		1972	30	8
H	late spray	1971	41	22
		1972	24	3

the virus liberated from their cadavers. The appearance of virus late in the season suggests that transmission of virus is due to contamination of the foliage with virus which has been shielded from solar inactivation for a year. This virus could also be protected in desiccated cadavers which have remained on the foliage throughout the winter. Later-instar larvae which have eaten the current year's growth and commenced back-feeding would then possibly ingest viral inclusion bodies. Another possibility is that rain spreads the virus from the old foliage or cadavers onto the current year's growth.

The foregoing data appear to justify the following conclusions:

1. NPV persists in the field from one year to the next with the incidence of this virus remaining the same or increasing during the year following application.

2. The incidence of EPV appears to decline drastically from the year of application to the next year.

3. CPV persists from one year to the next and its status is uncertain owing to the presence of natural CPV in the field.

4. NPV does not appear to spread from the sprayed areas one year after application.

5. It is suggested that carryover of virus is by contamination of the foliage with viral inclusion bodies or by virus liberated from cadavers which have remained on the trees. This theory is based on the fact that virus-infected larvae are found only late in their development.

VI. POPULATION REDUCTION AND FOLIAGE PROTECTION

by

G. M. HOWSE and A. A. HARNDEN

Introduction

Several reasons for studying the impact of viruses in the year following application are discussed in the introduction to Section V of this report. In short, they are to determine if the viruses persist from one season to the next and if they spread from the original point of introduction. The implications of virus carryover in developing an effective and practical method of budworm control are highly significant. For example, it was shown in Part A of this report that while virus sprays were capable of killing many budworm, foliage was not satisfactorily protected in the year of application. If virus persists from one season to the next and has an impact on the budworm population in terms of insect mortality and foliage protection, then the original cost per acre is reduced by a factor proportional to the number of years that the virus continues to persist and have an impact.

In 1971, two white spruce plantations on the Deluthier Road (PFES) were sprayed with high dosages of NPV using a helicopter. One plantation was sprayed during the second instar and the other during the fourth instar. Other sprays of EPV were applied at Achray in Algonquin Park.

Areas sprayed with NPV and EPV in 1971 were re-examined in 1972 to determine if carryover of the viruses had occurred, and if so, what the impact was and if the viruses had spread. Carryover was studied by scientists at IPRI from the standpoint of virus diagnosis and incidence of the viruses (Section V). Scientists at GLFRC assessed population reduction and the degree of foliage protection attributable to virus carryover.

Methods

Three of the EPV plots at Achray were sampled: Plot C, which in 1971 had been sprayed during the second instar (early spray) at a concentration of 10 g/U.S. gal; Plot A, which had been sprayed during the fourth instar (late spray) at a concentration of 10 g/U.S. gal; and Plot B, which had also been sprayed during the fourth instar at a concentration of 1 g/U.S. gal. Both NPV plots (G and H) on Deluthier Road were sampled.

Nine control plots (untreated areas) were selected in infested stands in the general vicinity of the virus plots. Data from these control plots were also used for the assessment of chemical spraying at PFES and virus-insecticide sprays at Rankin (south of Pembroke) in 1972.

Densities of living budworm, expressed as number per 18-in. branch tip, were determined for both white spruce and balsam fir for the treated plots and controls at three stages of budworm development:

1. third and fourth instar - last week of May
2. peak of sixth instar (some fifths present) - mid-June
3. pupal stage with moths emerging - last week of June and first week of July

It should be noted that the first foliage collection corresponds to the prespray sample, the second to a first postspray sample and the third to the final postspray sample.

In general, 10 white spruce and 10 balsam fir dominant or codominant trees were selected in each treatment and control plot, except for the NPV plots on Deluthier Road where only white spruce was available. Twenty-five trees of each species were sampled in three of the nine control plots. One 18-in. branch tip was collected from each tree for the first and second samples and two 18-in. tips were taken from each tree for the pupal sample. All living pupae from the final sample were saved and their subsequent fate was determined, i.e., whether moths emerged or the pupae died. Abbott's formula was used to calculate the population reduction in each treatment (carryover) plot. Methods of estimating the degree of damage or percentage of current defoliation were described in Section II.

Results

Early larval population densities varied among treatment plots; however, this range in densities was covered by the control plots. In general, the early living larval densities averaged 50 and 20 per 18-in. tip for white spruce and balsam fir, respectively, in the carryover plots, compared with average densities of 55 and 21 per 18-in. tip for white spruce and balsam fir, respectively, in the control plots.

The reduction in survival of living budworm in each carryover (treatment) plot was compared with the reduction in survival of one or more control plots that had comparable early-instar larval densities. The resulting population-reduction figures and the degree of current defoliation are listed in Table 20 for the plots sampled at Achray and Table 21 for the two plots on Deluthier Road. The population-

Table 20 Population reduction (adjusted for natural mortality) and current defoliation in three plots at Achray in the year following application of EPV sprays

Plot	Time of spray	Concentration of freeze-dried material (g/U.S. gal)	% population reduction		% current defoliation	
			white spruce	balsam fir	white spruce	balsam fir
C	early	10.0	82	64	33	20
A	late	10.0	68	16	41	39
B	late	1.0	42	19	48	37
Controls					81	72

Table 21 Population reduction (adjusted for natural mortality) and current defoliation on white spruce in two plots on Deluthier Road, Petawawa Forest Experiment Station, in the year following application of NPV sprays

Plot	Time of spray	Concentration of freeze-dried material (g/U.S. gal)	% population reduction	% current defoliation
G	early	25	20	82
H	late	25	65	30
Controls				81

reduction figures in Tables 20 and 21 are based on emerged pupal counts and include any differential effects of virus treatments on pupal survival as shown in Table 22. Population-reduction figures were therefore calculated in the same manner for both 1971 and 1972 and are directly comparable (Tables 23 and 24).

Aerial observations revealed a considerable difference in the degree of defoliation between the two adjacent white spruce plantations that had been sprayed with NPV in 1971 on Deluthier Road. Defoliation figures were 82% for the early spray plot (G) compared with 30% for the late spray plot (H). Plot G had more third- and fourth-instar larvae than plot H, 70 per 18-in. tip compared to 50 per 18-in. tip, but the population density in H should still have been high enough to cause severe defoliation if there had not been virus carryover.

Discussion

As is evident from the data in Table 20, there was both significant population reduction and foliage protection owing to virus carryover in the EPV spray plots at Achray. The population reduction is greater on white spruce than on balsam fir but better foliage protection occurs on balsam fir than on white spruce. This phenomenon is a result of the much higher initial larval populations on white spruce than on balsam fir. Population reductions also occurred in both of the NPV plots on Deluthier Road. However, Plot H had much greater mortality and lower initial population density than Plot G, and this resulted in good foliage preservation.

The June 13-14 sample showed that there was population reduction in some of the plots that could be attributed to virus carryover. The amount of mortality varied from plot to plot and ranged from 0% to 20%. Virus (NPV) could be diagnosed as early as June 7-8 (Tables 16 and 17), which was the peak of the fifth instar. Therefore, it appears that most of the mortality occurred between June 14 and June 30 when the budworm were fifths, sixths or pupae.

It is pertinent to mention that NPV was essentially the only virus found in significant quantities in 1972 (Tables 16 and 17). This would be expected for the Deluthier plots since NPV was sprayed there, but not for Achray where EPV (slightly contaminated with NPV and CPV) was applied.

Pupal survival data (Table 22) for 1972 do not present as clear a picture as the 1971 data (Table 9). It appears that NPV does not have as adverse an effect on pupal survival as EPV. This is probably because NPV is a faster-acting virus than EPV. Consequently, the fate of larvae ingesting NPV is more quickly decided than that of larvae consuming EPV.

Table 22 Pupal survival in treatment plots (compared with survival in controls) in the year following the application of virus sprays

Plot	Tree species[a]	% successful pupal emergence[b]	
		treatment	control
Achray - Plot C	wS	82	86
Plot A	wS	100	88
Plot B	wS	84	84
Deluthier - Plot G Road	wS	73	91
Plot H	wS	72	82
Achray - Plot C	bF	62	87
Plot A	bF	83	87
Plot B	bF	84	77

[a] wS = white spruce
bF = balsam fir

[b] % successful pupal emergence = $\frac{\text{emerged budworm}}{\text{budworm alive on sample date}} \times 100$

Table 23 Comparison of population reduction and defoliation in the year of application (1971) and the following year (1972) for three EPV plots at Achray

Plot	Tree species[a]	Year	% population reduction	% current defoliation
C (early spray)	wS	1971	40	59
		1972	82	33
	bF	1971	0	87
		1972	64	20
A (late spray)	wS	1971	79	74
		1972	68	41
	bF	1971	30	94
		1972	16	39
B (late spray)	wS	1971	57	88
		1972	42	19
	bF	1971	48	99
		1972	19	37
Controls (average)	wS	1971	--	86
		1972	--	81
	bF	1971	--	83
		1972	--	72

[a] wS = white spruce
bF = balsam fir

Table 24 Comparison of population reduction and defoliation of white spruce in the year of application (1971) and the following year (1972) for the two NPV plots on Deluthier Road

Plot	Year	% population reduction	% current defoliation
G	1971	69	99
	1972	20	82
H	1971	80	96
	1972	65	30
Controls (average)	1971	--	93
	1972	--	81

More budworm that have been exposed to NPV die before reaching the pupal stage than do those that have been exposed to EPV. In any case, it would appear from Table 22 that in only three instances was pupal mortality due to NPV, i.e., in Plot G, Plot H and on balsam fir in Plot C. It is unclear why there is no pupal mortality due to virus in other plots. It may simply be due to chance or a sampling artifact. The pupal mortality in Plot G is significant because it accounts for virtually all of the population reduction in this plot. Thus the high degree of defoliation in this plot (control was the same as the treatment) is explained by the virtual lack of larval mortality due to virus even though the incidence of NPV was eventually slightly higher in Plot G than in Plot H.

A comparison of population reduction and defoliation in 1971 (the year of application) and 1972 for Achray and Deluthier Road reveals several interesting facts (Tables 23 and 24). At Achray, the rate of mortality increased on both white spruce and balsam fir for one plot (Plot C) and decreased on both white spruce and balsam fir for the other two plots (plots A and B). One difference between Plot C and the others is the original time of application of the sprays. All plots showed marked increases in the degree of foliage protection from one year to the next. On Deluthier Road population reduction decreased in both plots, but considerably more so in G than in H. This difference in population reduction was accompanied by a considerable increase in the level of foliage protection in Plot H.

The foregoing data suggest the following conclusions:

1. A virus (identified by J. C. Cunningham as NPV) is capable of persisting in the forest environment for at least one year.

2. NPV possesses the capability of effecting a satisfactory degree of budworm control and protection among host trees in the first year following application of virus sprays.

3. There is apparently very little relationship between the original concentration of virus sprays and impact in the year following application.

4. Considering the impact of NPV in the year following application (particularly in light of the Achray plots where NPV was introduced as a minute contaminant in minute amounts of EPV sprays), practical and economical budworm protection programs are now possible using NPV.

GENERAL DISCUSSION

Owing to the contamination of the EPV with NPV and CPV a true comparison of the efficacy of these viruses could not be made. A good correlation between infection studies and population-reduction studies was obtained from the NPV plots, and with the heavy concentration of virus applied the late spray application was better. Conflicting results were obtained in the EPV trials when diagnostic studies indicated that better infection was obtained with the early spray, and population-reduction studies showed that the late spray was better. None of the treatments gave satisfactory foliage protection in the year of application and this fact must be accepted in any spruce budworm virus-control program.

To date, two of the four criteria for the use of viruses in budworm control suggested by Stairs and Bird (1962, see Introduction) have been established. Efficient methods of virus production have been developed and it is now known that NPV will persist from one year to the next. The most efficient concentration of virus has yet to be determined. Very high dosages of NPV were applied in 1971 and such dosages, on an operational basis, would be economically unacceptable. The NPV infection obtained in the EPV plots where this virus was present only as a contaminant indicate that very small dosages will give a satisfactory introduction of NPV and lower dosages should be field tested.

The other criterion suggested by Stairs and Bird was the determination of the most vulnerable stage in the life cycle of the insect. It is generally accepted that the smaller the larvae the more susceptible they are to virus infection but this fact must also be correlated with the habits of spruce budworm larvae. It is necessary for larvae to ingest virus inclusion bodies to become infected; hence, virus sprays must be regarded as stomach poisons.

Second-instar larvae mine needles after leaving their hibernacula, and their behavior differs on white spruce and balsam fir (McGugan 1954). On balsam fir they usually mine only one needle and then move into the buds, but on white spruce they mine several needles. Hence there is a greater possibility of infection resulting from an early virus application on white spruce than on balsam fir.

By the time larvae have reached the fourth instar they are considerably more resistant to virus infection but by this time buds are flushing on both white spruce and balsam fir and the larvae are exposed to a spray deposit. Hence, a different approach should be taken to application time depending on the stand composition: an early spray might be effective on a white spruce stand, but on a balsam fir stand or a mixed stand, a late spray would probably give better results.

It was disappointing that in the year following application virus was found only in the plot areas and there did not appear to be any spread. A spray protocol had been envisaged where the virus could be seeded into a small part of the budworm population by widely spaced spray swaths, whence it would spread naturally to the remainder of the population. Of course, spread may be very slow and detectable only two or three years after application. The lack of transovarial transmission is a factor militating against rapid spread of NPV and EPV and dispersal is limited to such agents as predators, parasites and birds. It is postulated that transmission of the virus from one year to the next is by contamination of the foliage with viral inclusion bodies or by virus liberated from cadavers remaining on the trees.

Generally the results were considered encouraging, particularly with NPV on white spruce. Dramatic foliage protection was not obtained with NPV or EPV but if sufficient mortality occurs and foliage protection is obtained for several years the insect population will eventually collapse and the virus-treated stands will retain sufficient foliage to remain alive.

RECOMMENDATIONS

1. The EPV and NPV plots should be examined again in 1973 and for as many years as are necessary to determine the persistence of the viruses.

2. More trials should be conducted with NPV to ascertain the minimum dosage required to establish this virus in the population.

3. A critical evaluation should be made of the best time of application of virus on both white spruce and balsam fir.

4. Batches of virus should be carefully screened after production to ensure that there are no contaminating viruses present.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Bergold, G.H. 1950. The multiplication of insect viruses as organisms. Can. J. Res. E28: 5-11.

Bergold, G.H. 1951. The polyhedral disease of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. J. Zool. 29: 17-23.

Bird, F.T. 1961. Transmission of some insect viruses with particular reference to ovarial transmission and its importance in the development of epizootics. J. Insect Pathol. 3: 352-380.

Bird, F.T. 1969. Infection and mortality of spruce budworm, *Choristoneura fumiferana*, and forest tent caterpillar, *Malacosoma disstria*, caused by nuclear and cytoplasmic polyhedrosis viruses. Can. Entomol. 101: 1269-1285.

Bird, F.T. and Mary M. Whalen. 1954. A nuclear and a cytoplasmic polyhedral virus disease of the spruce budworm. Can. J. Zool. 32: 82-86.

Bird, F.T. and J.R. McPhee. 1970. Susceptibility of spruce budworm to pure nuclear polyhedrosis virus (NPV) sprays. Can. Dep. Fish. Forest., Bi-mon. Res. Notes 26: 35.

Bird, F.T., C.J. Sanders and J.M. Burke. 1971. A newly discovered virus disease of the spruce budworm, *Choristoneura biennis*, (Lepidoptera: Tortricidae). Invertebr. Pathol. 18: 159-161.

Cunningham, J.C., F.T. Bird, J.R. McPhee and D. Grisdale. 1972. The mass propagation of two viruses of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. Forest. Serv., Sault Ste. Marie, Ont. Inf. Rep. IP-X-1. 19 p.

Grisdale, D. 1970. An improved laboratory method for rearing large numbers of spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Can. Entomol. 102: 1111-1117.

Howse, G.M., A.A. Harnden and W.L. Sippell. 1971. The spruce budworm situation in Ontario, 1970. Can. Forest. Serv., Sault Ste. Marie, Ont. Inf. Rep. O-X-147. 41 p.

Howse, G.M., A.A. Harnden and W.L. Sippell. 1972. The 1971 spruce budworm situation in Ontario. Can. Forest. Serv., Sault Ste. Marie, Ont. Inf. Rep. O-X-163. 56 p.

Jaques, R.P. 1964. The persistence of a nuclear-polyhedrosis virus in the soil. J. Insect Pathol. 6: 251-254.

Jaques, R.P. 1967. The persistence of nuclear polyhedrosis virus in the habitat of the host insect, *Trichoplusia ni* II. Polyhedra in soil. Can. Entomol. 99: 785-794.

McGugan, B.M. 1954. Needle-mining habits and larval instars of the spruce budworm. Can. Entomol., 86, 439-453.

McMorran, A.R. 1965. A synthetic diet for the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. Entomol. 97: 58-62.

Neilson, M.M. and D.E. Elgee. 1968. The method and role of vertical transmission of a nucleopolyhedrosis virus of the European spruce sawfly, *Diprion hercyniae*. J. Invertebr. Pathol. 12: 132-139.

Smith, K.M. 1967. Insect virology. Acad. Press, New York and London. 256 p.

Stairs, G.R. and F.T. Bird. 1962. Dissemination of viruses against the spruce budworm, *Choristoneura fumiferana* (Clemens). Can. Entomol. 94: 966-969.

APPENDIX

APPENDIX

The following conversion chart gives the S.I. equivalents of the basic English measurements used in this report:

1 inch = 2.54 centimeters

1 foot = 30.48 centimeters

1 mile = 1.61 kilometers

1 mile per hour = 1.61 kilometers per hour

1 acre = 0.40 hectares

1 U.S. gallon = 3.79 liters

1 gram per U.S. gallon = 0.26 grams per liter

1 U.S. gallon per acre = 9.35 liters per hectare

1 ounce = 31.10 grams

1 billion inclusion bodies per acre = 2.50 billion inclusion bodies per hectare

$1^{o}F = 5(t-32)/9^{o}C$