AERIAL APPLICATION OF VIRUS-INSECTICIDE COMBINATIONS ACAINST SPRUCE BUDWORM *Choristoneura fumiferana* (Clem) (Tortricidae: Lepidoptera) AT RANKIN, ONTARIO, 1972

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P A R T 1

INTRODUCTION

The use of microbes combined with low doses of chemical insecticides is a promising approach to the integrated control of some forest insect pests. The two kinds of agents may be applied simultaneously or sequentially. Insects, like other animals, are most susceptible to disease when under the influence of stress of one kind or other and theoretically, it should be possible to stress the insect pest with a sublethal dose of chemical insecticide in order to increase its susceptibility to microbial infection. Insects suffering from a sublethal infection by pathogenic microorganisms should likewise be more susceptible to low doses of insecticides. The successes and failures of this approach up to 1968 have been reviewed by Benz (1971). Since then a few authors have reported successful potentiation of pathogenic fungi (Ferron 1970), bacteria (Duvlea *et al* 1968; Creighton *et al* 1971; Smirnoff 1972; Morris 1972b) and viruses (Harper and Thompson 1970; Jaques 1970, 1971; Atger 1971; Watanable 1971; Chapman and Ignoffo 1972) when mixed with low doses

of insecticides. Watanabe found that Bombyx mori L. larvae were more susceptible to mixtures of nuclear or cytoplasmic polyhedrosis viruses and fenitrothion, an organophorous insecticide, than to the viruses or insecticide alone.

During 1972 a nuclear polyhedrosis (NPV) virus and an entomopox virus (EPV) of the spruce budworm, Choristoneura fumiferana, were aerially applied in combination with a low dose (previously determined in the laboratory) of fenitrothion against this defoliator. Earlier mistblower and aerial field trials with the NPV (Stairs and Bird 1962; Bird and McPhee 1970; Howse et al 1973) had shown this virus to be potentially effective in reducing budworm populations on white spruce (Picea glauca). The project was a cooperative one between the Chemical Control Research Institute, Great Lakes Forest Research Centre and the Insect Pathology Research Institute of the Canadian Forestry Service. CCRI assumed responsibility for application, deposit analysis, meteorological monitoring, and intensive larval and post-larval biological assessment on a weekly basis. GLFRC was responsible for live population reduction studies by large scale but less frequent samplings and defoliation estimates. IPRI supplied the virus formulations and provided a diagnostic service for the project in conjunction with GLFRC functions. GLFRC and IPRI results are contained in Parts II and III of this report. Some aspects of the work such as diagnosis of living and dead insects and changes in live bud-

worm populations were deliberately duplicated with the hope that when all sets of figures were compared they would complement each other and make it possible to draw more definitive conclusions.

METHODS AND MATERIALS

Plot Preparation

The test plots consisted of mixed white spruce (Picea glauca) and balsam fir (Abies balsamea) stands about 25 years old. The 30' to 50' high trees, situated at Rankin, about six miles south of Pembroke, Ontario, had been under severe attack by spruce budworm for four or five years. The population was judged to be at peak density during 1972 and a decline was expected thereafter.

The sprayed area was a 1 3/4 x 1/4 mile flat stretch, easily accessible from two main roads. The layout of the plot is schematized in Fig 1. In actuality, Plot E on the schema was slightly off the horizontal line.

For the weekly mortality assessment, five sample stations of three trees each were chosen along the midline of each treatment plot and running in the direction of the virus flight line. An effort was made to choose as many balsam firs as white spruces per line, with as little divergence from the line as possible.

Meteorological Monitoring

A recording tipping bucket rain gauge, a hygrothermograph and a pyronometer were set up on or near the spray plots (Figs 2, 3, 4, 5) for continuous recording of weather data.

In addition, a high tower with meteorological equipment was set up in a clearing adjacent to the spray blocks for determining weather conditions at the time of spray (Figs 6 and 7). The trees in the immediate area of the tower were 15' to 25' high fir and spruce with the closest tree about 20' from the tower. The tower was extended to the 80' height with bivane sensors at 80' and 20' levels. Each bivane sensor measured wind speed, wind direction and turbulence, as indicated by the vertical movements of the bivane. Two sets of paired thermistors were mounted to measure the temperature differentials (ΔT) between the 8' and 20' levels and between the 20' and 80' levels. The relative humidity was measured at 20' and 80' positions.

Continuous records were made on Esterline Angus strip charts and on four-channel FM instrumentation tape from one to two hours prior to the spray application to one hour post-spray. The requirements for spray application were that both ΔT's should indicate an inversion (the temperature at the lower level should be less than that of the higher level) and that the wind speed at the 80' level should be less than 8 mph. The information of ΔT and wind speed was used to calculate a stability ratio S.R. from the formula.

$$S.R. = \frac{T_2 - T_1}{\bar{u}^2} \times 10^5$$

where T_2 is temperature at the higher level, T_1 is the temperature at the lower level (the difference between these two is ΔT), $\bar{u}$ is the mean wind speed in centimeters per second and 10^5 is a factor that brings the number to a manageable value.

More detailed information on the stability ratio and its interpretation is described by Yates et al (1967).

The stability ratio may be positive (indicating inversion) or negative (indicating lapse) and the larger the number the greater the degree of inversion or lapse. A turbulence factor was also calculated based on the frequency and amplitude of movement of the bivane. Table 1 shows average wind speeds, stability ratios, turbulence factors and relative humidities at high and low positions in the tower for each spray application period.

Deposit Analysis

Insecticide deposits were collected on three glass plates and single Kromekote cards set in clearings adjacent to sample trees at each station. These were collected 20-30 minutes after spray application. Spray card analysis was performed by W. Haliburton of CCRI. Droplets on the glass plates were eluted with dimethylformamide (DMF) and stored in a refrigerator for later gas chromatographic analysis.

Microbial deposits were collected on three 47 mm Millipore filters per sample station. The polyhedra were stained, the droplet diameters (total 840) measured and the deposits of inclusion bodies

were counted microscopically according to the method described earlier by Morris (1972a). Deposit was calculated as virus inclusion bodies per acre.

Formulation and Application

The spray formulations are summarized in Table 2. Applications were made in 200' swaths by a Stearman aircraft fitted with 4 Micronair AU 3000 units. For application of the virus suspensions the line filters were removed from the spray system. Laboratory studies had indicated that the desired sublethal effect could be achieved with the application of 0.25 oz fenitrothion applied per acre. The insecticide was applied on May 28 at 5:30 am, nuclear polyhedrosis virus at 8:00 pm the same day and entomopox virus at 5:30 am May 29. Meteorological conditions were nearly perfect for all applications.

The NPV used in these tests was described by Bird and Whalen (1954) and by Bird (1959). The EPV (Figs 8, 9) originated in Choristoneura biennis and was recently described by Bird et al (1971). The methods of propagation of these viruses have been described (Cunningham et al 1972).

Weekly Population Sampling and Recorded Data

Duplicate 18" branch tips were collected from each sample tree by pole pruner and basket, one day pre-spray and 3, 11, 18 and 25 days post-spray. Branch tips were examined at the field laboratory

at Petawawa. Data were recorded throughout the test period for the following studies:

1. Incidence of primary (introduced) and secondary pathogens, such as microsporidia and fungi, among field collected larvae and pupae.
2. Weekly budworm population changes.
3. Effects of treatments on budworm pupation in the field.
4. Changes in population densities of associated or competing species.
5. Larval, pupal and egg parasitism in the field.
6. Effect of treatments on adult emergence (lab data from field collected pupae).
7. Effects of treatments on adult sex ratio (lab data from field collected pupae).
8. Effects of treatments on egg viability (data from egg mass survey).
9. Egg deposition per female (laboratory data from field collected pupae).
10. Oviposition density by tree crown level (upper, middle and lower crowns, egg mass survey).

Diagnostic Studies

All dead insects found in the survey were diagnosed for cause of death by examining wet mounts of the cadavers with a phase

contrast microscope. Virus infection was scored as positive only if mature inclusion bodies were present in the wet mounts.

Virus Transmission Studies

In an attempt to determine whether or not vertical transmission of the introduced viruses occur in nature, 75, 125, 125, 100, 25, and 135 second instar larvae hatched from eggs collected in areas treated with EPV, EPV-fenitrothion, fenitrothion, NPV, NPV-fenitrothion and controls, respectively, were reared on artificial diet through to adults. Out of 400 second instar in hibernacula, only the above numbers successfully emerged and established themselves on diet. Rearing was continued for two months during which time all larvae or pupae which died were diagnosed for cause of death in the usual manner. The rearing method used was earlier described by Morris (1973).

RESULTS AND DISCUSSIONS

Meteorological Conditions at Time of Spray

All sprays were applied under good spray conditions (Table 1). The wind speeds were all within the desired range with the highest wind speed (6.5 mph) being recorded during the application of NPV (28/V p.m.). The stability ratios (S.R.) all indicate stable to very stable conditions. The very large S.R. of 152.3 for 29/V a.m. is the result of the wind dropping to approximately 0.5 mph during the time of spray application. The very small value of wind speed results in a large S.R. The turbulence factor shows an inverse correlation with the stability ratio with the least turbulence (a factor of 0.04) occurring under conditions

of a very large stability ratio (152.3). The measurements of relative humidity show an expected consistency with the R.H. at the low position being greater than that of the high position and the R.H. during the evening spray being less than that during the two morning sprays.

Deposit Analysis

Results of the spray card analysis of fenitrothion deposits are summarized in Tables 3 and 4. According to this method, the dosage of insecticide reaching the ground was about one-half of that emitted from the spray plane. Deposit ranged from 8.30 to 14.0 X 10^{-2} ozs/acre. Results of the gas chromatography analysis of spray droplets (Table 5) show that the deposit rates of the insecticide were about equal for all fenitrothion-treated plots. Deposits ranged from 1.05 X 10^{-2} to 1.61 X 10^{-2} ozs/acre. There was no detectable drift of the insecticide into plots treated with virus alone. Only 5% of the emitted fenitrothion reached ground surface.

Results of the microbial deposit measurements (Table 6) show a considerably higher polyhedra deposit rate for NPV than for entomopox virus treatment. It will be recalled that the nuclear polyhedrosis virus spray suspension contained about 3.5 times as many polyhedra as the entomopox suspension per unit volume.

The mean diameter of inclusion body droplets was 121 microns ranging from 20 - 420 microns. Eighty-eight percent of the drops measured under 200 microns. Thirty-one percent of the emitted NPV and

42% of the emitted EPV inclusion bodies reached ground surface. The higher deposit rate probably reflects the relatively large and heavy inclusion bodies of EPV.

Meteorological Conditions During Test Period

Cumulative meteorological conditions for each sample period are summarized in Table 7. Mean minimum and maximum temperatures and relative humidities varied little between sample periods. It is expected that an increasing degree of deactivation of the viruses occurred as accumulated solar radiation increased in spite of the IMC sunlight protectant added to the spray suspension. Average daily rainfall was heaviest during the first three days post-spray but the sticker-spreader Biofilm®, in the suspension probably prevented wash-off of the virus inclusion bodies.

Larval Development

Larval development at time of spray was 2.9% 2nd, 28.1% 3rd, 36.6% 4th, 27.7% 5th and 5.1% 6th instars. Developmental stadia for each sample period are summarized in Fig 10. The slightly higher proportion of 6th instars for the second sample compared with the third sample is attributable to relatively small sample size.

Effects of Treatments on Larval Mortality

Corrected larval mortality on white spruce and balsam firs combined (Table 8) indicate no additive effect with the entomopox virus insecticide combination but a slight additive effect (7.3%) with NPV-insecticide compared with mortality from virus alone. However, these figures may be somewhat questionable because only white spruce trees

were used for controls. Mortality on balsam firs sprayed with Thuricide(R) have been known to be higher on balsam firs than on white spruce (T. A. Angus, C. Yamvrias and P. Luthy, unpublished data). On the other hand, results of aerial application of NPV and entomopox viruses at Achray indicated a general lack of population reduction on balsam fir but significant population reduction on white spruce (Howse et al 1973). Highest larval mortality (25.5%) occurred on NPV-insecticide treated trees. Corrected mortality on white spruce only was 14.3% (Table 9). These percentages may not, however, reflect the true mortality rates since some dead budworm would be expected to fall from the trees or destroyed by predators. Because of lack of balsam fir controls, mortality on this species is not available.

Changes in live larval density throughout the test period are summarized in Table 10. Entomopox virus + insecticide, NPV alone, and NPV + insecticide, resulted in about 25%, 75% and 75% reduction in larval density, respectively. A large-scale larval reduction assessment is given by Howse and Harnden in Part II of this report.

Incidence of Pathogens Among Larvae

Results of the diagnosis of dead larvae are summarized in Table 11. Larval mortality in the NPV-insecticide and NPV alone treated populations 18 days post-spray were 19.7 and 23.8%, respectively. The incidence of NPV among cadavers on this date were 83.0 and 74.0%, respectively, indicating an additive effect. On the same sampling date, only 0.8% of the control population and 6.6% of the

control cadavers contained virus. The 2.6 to 2.7% incidence of NPV in populations treated with EPV or EPV-insecticide suggests a synergistic effect of the EPV on natural NPV infection. Synergism has been observed between NPV and EPV by Granados (personal communication). A maximum of only 1.1% of EPV-treated insects contained the virus.

Natural infection by microsporidia amounted to 3.8% of the EPV alone treated population and a maximum of 0.8% among controls. This suggests a synergistic effect of the EPV on natural infection by microsporidia.

The incidence of fungus as indicated by the presence of spores in the wet mounts was highest among insecticide-alone treated populations. It is not known with certainty whether these micro-organisms were pathogenic. However, Neilson (1955) has shown that up to 9.4% of some New Brunswick spruce budworm populations are naturally controlled by pathogenic fungi.

Post-larval Effects of Treatments

The data summarized in Table 12 show that NPV alone and NPV-fenitrothion treatments substantially reduced survival through to pupation in the field on both white spruce and balsam fir. This delayed effect was somewhat less noticeable among EPV or EPV-fenitrothion treated populations.

Among the NPV + fenitrothion treated populations about 18% of the pupae were infected with virus compared with 0.3% of the control and 5% of pupae from the NPV-alone treated area (Table 13).

This represents a high incidence of virus among pupae. Total mortality among pupae from EPV-fenitrothion treated areas was also high but the incidence of EPV was low in terms of the diagnostic techniques used. The incidences of microsporidia and fungus were generally very low among pupae.

EPV-fenitrothion and EPV-alone treatments caused 34.3% and 28.8% reductions in adult emergence, respectively (Table 14). NPV + fenitrothion and NPV alone resulted in 19.9% and 13.8% reductions, respectively. In both cases, low doses of the insecticide produced additive effects. The ratio of female to male emergence was 1/1 in all cases (the normal sex ratio among healthy spruce budworm) except 1/2 for EPV-fenitrothion. Adult emergence was considerably higher among insecticide-alone treated populations than among the other treated or untreated populations. Thus, the EPV-insecticide treatment produced a most dramatic effect on adult emergence. The sex ratio effect agrees with Schönherr's (1969) finding that granulosis virus infection in Choristoneura murinana causes a marked reduction in the female population.

Data from the egg mass survey (Table 15) showed that preferred oviposition sites decreased with tree height. Among treated areas, the plots sprayed with EPV alone or NPV alone contained fewest eggs. NPV sprayed areas showed lowest egg hatch (some 47% less than controls). It should be explained that field collected budworm egg masses are easily distinguishable by color and appearance with respect to viability, emergence and parasitism.

The results summarized in Table 16 show that females reared from pupae which had been collected in EPV-insecticide and NPV-insecticide treated plots produced considerably fewer offspring than controls. There was apparently no reduction among adults from virus-alone treated areas. Insecticide-alone treatments appeared to have caused a considerable increase in offspring compared with controls.

The incidence of Dioryctria reniculella, the spruce cone moth was generally low (8-15%) on treated areas but relatively high on the control plot (31%) (Table 17). D. reniculella is competitive with C. fumiferana for food, this may partly explain the low oviposition rate among controls referred to earlier (Table 15). Pupal parasitism was low in all areas but egg parasitism was generally high particularly in the virus-insecticide plot where 42.3% of egg masses were parasitized, probably by Trichogramma spp. It is obvious from these data that egg parasitism can play a major role in the natural control of C. fumiferana.

There is a clear evidence from these data that the delayed effects of the viruses or virus-insecticide mixtures constitute an important mode of action of these pathogens in spruce budworm control. Morris (1969), Nef (1971) and Ignoffo and Gregory (1972), have described delayed effects of Bacillus thuringiensis in infected lepidoptera. Morris (1970) observed acceleration effects of NPV in the precocious development of adult characteristics in virus-infected Lambdina fiscellaria somniaria. Nef (1971) recently reported that in field tests with NPV against Stilpnotia salicis, larval and pupal stadia of infected

insects had a longer duration than normal, pupal mortality was higher than usual and pupae weighed less, adults were malformed and the number of eggs laid and the hatching percentages were decreased. Our present findings support this data.

Latent effects of sublethal doses of insecticide alone appear to be just the opposite. In the present tests, adult emergence (Table 14), oviposition (Table 15) and number of progeny (Table 16) were considerably higher among insecticide treated populations than among controls. This is interpreted to mean that the low insecticide dosage stimulated these aspects of the insect life cycle. Sublethal doses of some insecticides have been shown in the past to increase oviposition and progeny of filial generations of Lepidoptera and Coleoptera (Abo-Elghar et al 1972; Johansson and Johansson 1972). The present data support the recent observation of Blais (1972) that "while chemical treatment of spruce budworm may effectively reduce defoliation, it may also prolong budworm infestation". It is interesting that the stimulatory effects observed in the present study were negated by simultaneous application of virus.

Virus Transmission

Only about 1/5 as many larvae from the NPV-fenitrothion treated area and 1/2 as many from the EPV-fenitrothion treated plot as compared with controls developed from hibernacula and established themselves on media. Success in establishment among EPV, fenitrothion, and NPV treated insects were about equivalent to that among controls.

It is apparent that the virus-insecticide treatments reduced not only egg hatchability (Table 16) but also second generation early instar survival.

Mortality among established larvae occurred mostly in the late larval, prepupal and pupal stages. Mortality among EPV, fenitrothion, and NPV-fenitrothion treated insects ranged from 4-16% with none dying from pathogens. There was 10% mortality among controls with 3% of the total dying from microspordiosis and 19% mortality among NPV-alone treated insects with 14% of the total dying from NPV. This indicates a high level of transovum transmission of NPV and a low level of transovum transmission of microsporidia.

CONCLUSIONS

1. Spray conditions were good to excellent.
2. About 5% of the emitted insecticide spray reached ground surface.
3. About 31% of the emitted NPV and 42% of the emitted EPV reached ground surface.
4. Larval mortality was highest among NPV-insecticide treated populations on white spruce or on mixed spruce and balsam trees.
5. NPV-alone and NPV-insecticide treatments were the most effective in causing population reduction.
6. The incidence of NPV was highest among NPV-insecticide treated populations.

7. NPV + insecticide was the most effective treatment in reducing survival through to pupation in the field.
8. The incidence of NPV among pupae was highest in populations treated with NPV-insecticide combinations.
9. EPV-insecticide and NPV-insecticide treatments caused the lowest rate of adult emergence and insecticide-alone treatment caused the highest.
10. EPV-insecticide treatment reduced female/male sex ratio to 1/2 from the normal 1/1.
11. Populations treated with virus-insecticide combinations produced the fewest viable eggs and fewest progeny. The population treated with insecticide alone produced the largest progeny.
12. Larval and pupal parasitism were low but egg parasitism was high among all treated populations.
13. Early instar progeny survival was lowest among the NPV-fenitrothion treated population.
14. Transovum transmission of virus occurred among about 14% of the progeny of NPV-treated insects.

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TABLE 1

Meteorological Conditions at Time of Spray Applications
Rankin, 1972

Spray Application	Date	Wind Speed (mph)	Stability Ratio	Turbulence Factor	Relative Humidity Low Level	Relative Humidity High Level
Fenitrothion	28/V-am	3.3	9.59	0.20	65%	61%
NPV	28/V-pm	5.1	3.05	0.58	30%	29%
EPV	29/V-am	2.5	152.3	0.04	75%	64%

TABLE 2

Summary of Microbial and Insecticide Formulations
Used in Field Trials at Rankin, 1972

V I R U S	EPV	NPV
Polyhedra/gm of freeze dried material	4 billion	7 billion
PIB/ml of operational spray	2×10^7	7.0×10^7
Application rate of spray	1 gpa (U.S.)	1 gpa (U.S.)
Application rate of active ingredient	10 gm/acre	20 gm/acre
PIB/acre	7.6×10^{10}	2.7×10^{11}
IMC Sunlight Protectant	2.5%	
Molasses	10%	
Biofilm® (sticker-spreader)	0.2%	
pH of final water suspension	5.3 - 5.5	
INSECTICIDE		
0.25 oz wt fenitrothion in 6 fl oz Hysol solvent per acre		

TABLE 3

Summary of Spray Card Analyses - Aerial Virus Insecticide Spray, Rankin 1972[1]

Treatments	Drops/cm^2	Estimated Deposit oz/ac US fl	Estimated Deposit oz/ac (a.i.)
EPV + Insecticide			
A	2.7	.8	.033
B	14.5	4.1	.17
C	9.9	3.5	.15
D	3.1	.9	.04
E	6.4	2.4	.10
Mean	7.3	2.26	.096
Insecticide alone			
A	5.8	1.7	.07
B	2.8	1.0	.04
C	7.4	2.8	.12
D	6.1	1.8	.08
E	9.6	3.0	.13
Mean	6.3	2.0	.083
NPV + Insecticide			
A	2.3	1.8	.08
B	5.1	2.5	.11-
C	8.7	6.7	.28
D	7.8	2.7	.11+
E	3.7	2.5	.10
Mean	5.5	3.3	.14
Mean 3 Plots	6.4	2.5	.10

[1] Plot 1 (check) - no visible deposit. A - E indicate sample stations.

[2] Emitted from aircraft at 0.25 oz active ingredient in 6 fl oz Hysol solvent per acre.

TABLE 4

Summary of Drop Measurements on Kromekote Cards[1]

Stain Class	0	1	2	3	4	5	6
Drop Diam. Class Limit	45	69	96	118	138	156	172
Cumulative Number (%)	13.7	51.3	80.7	96.5	99.2	99.6	100
Cumulative Volume (%)	1.9	17.1	48.0	84.3	94	98	100

[1] Volume median diam. ca 100 µm
Number median diam. ca 68 µm

90% of volume between 50 & 135 µm drop diam.
90% of numbers between 40 & 115 µm drop diam.

TABLE 5

Gaschromatographic Analysis of Fenitrothion Deposits in Ounces Per Acre - Virus Insecticide Spray Rankin, 1972

Sampling Station Designation	Deposits of Fenitrothion Concentration in Ounces Per Acre[1]		
	EPV + Fen	Fen alone	NPV + Fen
A1	8.35×10^{-4}	3.76×10^{-2}	1.50×10^{-2}
A2	8.35×10^{-4}	2.34×10^{-2}	9.19×10^{-3}
A3	1.59×10^{-2}	1.42×10^{-2}	4.01×10^{-2}
B1	1.17×10^{-2}	1.67×10^{-3}	1.00×10^{-2}
B2	1.34×10^{-2}	5.01×10^{-3}	5.01×10^{-3}
B3	8.35×10^{-3}	1.67×10^{-3}	1.75×10^{-2}
C1	1.67×10^{-2}	6.68×10^{-3}	4.18×10^{-2}
C2	2.51×10^{-3}	2.00×10^{-2}	4.10×10^{-2}
C3	4.18×10^{-2}	2.51×10^{-2}	1.25×10^{-2}
D1	5.01×10^{-3}	5.01×10^{-3}	5.01×10^{-3}
D2	2.51×10^{-3}	3.34×10^{-3}	5.01×10^{-3}
D3	1.34×10^{-2}	7.52×10^{-3}	1.09×10^{-2}
E1	1.59×10^{-2}	7.52×10^{-3}	4.18×10^{-3}
E2	3.34×10^{-3}	2.51×10^{-3}	1.67×10^{-2}
E3	5.85×10^{-3}	5.01×10^{-3}	7.52×10^{-3}
Means	1.05×10^{-2}	1.11×10^{-2}	1.61×10^{-2}

[1] No detectable deposits on plots sprayed with EPV alone or NPV alone.

TABLE 6

Microbial Deposit Rates at Sampling Stations
Virus Insecticide Aerial Spray
Rankin, 1972

Treatment	Average number of inclusion bodies deposited per cm^2[1]					Av. number[2] per acre All Stations
	Station A	Station B	Station C	Station D	Station E	
EPV alone	0.0	76.8	801.3	697.1	1165.8	2.2×10^{10}
EPV + Insecticide	1160.4	2096.8	660.1	587.2	693.7	4.2×10^{10}
NPV alone	3628.9	2317.7	3265.4	3652.0	2079.2	1.2×10^{11}
NPV + Insecticide	1841.4	423.5	766.2	1909.3	1419.7	0.47×10^{11}

1 Microscopic counts were done on nine gridded millipore filters placed in the open at each sampling station. Deposits on three squares of 0.096 cm^2 area each were counted on each filter.

2 Mean deposit rates for EPV and NPV plots were 3.2×10^{10} (42% of emitted) and 0.84×10^{11} (31% of emitted) inclusion bodies per acre, respectively.

TABLE 7

Cumulative Meteorological Conditions - Virus Insecticide
Aerial Spray, Rankin 1972

Inclusive Dates	TEMPERATURE (^{o}C) Mean Max	Mean Min	RELATIVE HUMIDITY (%) Mean Max	Mean Min	Solar Radiation (cal/cm^2)	Average Solar Radiation (cal/cm^2/day)	Cumulative Rainfall Inches	Average Daily Rainfall
May 28 - May 31	22.8	10.0	77.5	42.3	1631	408	1.10	0.55
May 28 - June 8	22.2	10.0	77.2	36.5	5375	448	1.79	0.18
May 28 - June 15	21.7	9.4	76.5	36.3	8469	446	2.22	0.13
May 28 - June 22	21.7	10.0	76.8	37.8	11909	458	4.34	0.18
May 28 - July 21	23.9	12.2	75.8	37.9	25529	464	7.02	0.13

TABLE 8

Larval Mortality on White Spruce and Balsam Fir
Virus Insecticide Aerial Spray - Rankin, 1972

Treatment	Cumulative Totals Collected[6] (dead and alive)				Percent Mortality[7] (Uncorrected)			
	S+3	S+11	S+18	S+25	S+3	S+11	S+18	S+25
EPV alone[1]	533	834	1030	1165	13.1	11.2	12.1	12.5(2.1)
EPV + Insecticide[2]	605	919	1131	1218	13.9	13.4	13.0	14.2(4.0)
Insecticide alone[2]	610	872	1124	1233	23.0	24.1	25.4	24.0(15.0)
NPV alone[3]	624	967	1217	1256	21.3	16.3	22.6	27.0(18.3)
NPV + Insecticide[4]	817	1010	1288	1313	27.9	25.5	28.7	33.4(25.5)
Controls[5]	313	605	709	786	8.3	9.6	8.6	10.6

1,2,3,4 4 wS and 11 bF, 8 wS and 7 bF, 9 wS and 6 bF, 14 wS and 1 bF, respectively

5 Eight white spruce

6 Per branch tip

7 Figures in brackets are corrected for natural mortality by Abbott's formula at 25 days post spray. S+3 etc = 3 days etc post spray

TABLE 9

Larval Mortality on White Spruce
Virus Insecticide Aerial Spray
Rankin, 1972

Treatment	Cumulative Totals Collected[6] (dead and alive)				Percent Mortality[7] (Uncorrected)			
	S+3	S+11	S+18	S+25	S+3	S+11	S+18	S+25
EPV alone[1]	223	418	490	515	10.5	8.1	9.8	9.7(0.0)
EPV + Insecticide[2]	484	858	1053	1128	6.6	7.0	7.7	9.0(0.0)
Insecticide alone[2]	452	766	936	999	19.5	15.5	16.9	16.6(6.7)
NPV alone[3]	511	966	1252	1331	11.7	9.9	14.5	17.5(7.7)
NPV + Insecticide[4]	764	1216	1584	1672	25.3	24.1	20.6	23.4(14.6)
Controls[5]	313	605	709	786	8.3	9.6	8.6	10.6

1,2,3,4 4, 8, 9, 14 trees, respectively

5 Eight white spruce

6 Per 18" branch tip

7 Figures in brackets are corrected for natural mortality by Abbott's formula. S+3 as for Table 8.

TABLE 10

Summary of Changes in Live Insect Density[1] Throughout Test Period
Rankin, 1972

Treatments	Average Number Live Larvae per 18" Branch Tip					
	Control[1]	EPV alone	EPV + Insecticide	Insecticide alone	NPV alone	NPV + Insecticide
Pre-spray	35	17	19	29	32	33
S + 3	24	15	17	16	16	19
S + 11	18	12	13	13	16	14
S + 18	16	7	7	8	9	9
S + 24	4	5	3	4	1	1

[1] Twenty-five white spruces and twenty-five balsam fir.

TABLE 11

Incidence of Pathogens Among Larvae - Virus Insecticide Trials
Rankin, 1972

Treatments	Total Larvae Collected[1]	Number Dead	Percent Infected With							
			NPV		EPV		Microsporidia		Fungus[3]	
			Totals	Cadavers	Totals	Cadavers	Totals	Cadavers	Totals	Cadavers
Pre-spray[2]	4688	230	0.0	0.0	0.0	0.0	0.3	6.6	0.1	2.2
EPV alone										
S + 3	533	70	0.0	0.0	0.9*	7.1*	0.4	2.9	4.1	31.4
S + 11	394	23	0.0	0.0	0.0	0.0	3.8	65.2	2.5	43.5
S + 18	228	32	0.0	0.0	0.0	0.0	0.9	6.3	1.8	12.5
S + 25	156	21	2.7	19.0	0.6	4.8	0.0	0.0	1.3	9.5
EPV + Insecticide										
S + 3	605	84	0.0	0.0	0.3	2.4	0.0	0.0	3.8	27.4
S + 11	437	39	0.0	0.0	1.1	12.8	0.2	2.6	2.3	25.6
S + 18	234	23	0.0	0.0	0.9	8.7	0.0	0.0	1.3	13.0
S + 25	114	27	2.6	11.1	0.0	0.0	0.0	0.0	5.3	22.3
Insecticide alone										
S + 3	610	140	0.0	0.0	0.0	0.0	0.3	1.4	3.4	15.0
S + 11	472	70	0.0	0.0	0.0	0.0	0.2	1.4	6.6	44.3
S + 18	327	75	0.3	1.3	0.0	0.0	0.3	1.3	5.8	25.3
S + 25	120	11	0.0	0.0	0.0	0.0	0.0	0.0	2.5	27.3

TABLE 11 (Cont'd)

Treatments	Total Larvae Collected[1]	Number Dead	Percent Infected With							
			NPV		EPV		Microsporidia		Fungus[H]	
			Totals	Cadavers	Totals	Cadavers	Totals	Cadavers	Totals	Cadavers
NPV alone										
S + 3	624	133	0.0	0.0	0.0	0.0	0.8	3.8	2.4	11.3
S + 11	825	52	0.0	0.0	0.0	0.0	0.4	3.8	1.5	15.4
S + 18	340	90	19.7	74.0	0.0	0.0	0.0	0.0	0.0	0.0
S + 25	103	64	34.6	56.3	0.0	0.0	0.0	0.0	1.0	1.6
NPV + Insecticide										
S + 3	817	228	0.0	0.0	0.0	0.0	0.6	2.2	3.9	14.0
S + 11	451	30	0.0	0.0	0.0	0.0	0.2	3.3	4.2	63.3
S + 18	390	112	23.8	83.0	0.0	0.0	0.0	0.0	2.8	9.8
S + 25	93	68	39.8	54.0	0.0	0.0	0.0	0.0	3.5	4.4
Controls[3]										
S + 3	339	21	0.0	0.0	0.0	0.0	0.0	0.0	1.5	19.2
S + 11	312	19	0.0	0.0	0.0	0.0	0.0	0.0	1.0	20.0
S + 18	119	15	0.8	6.6	0.0	0.0	0.0	0.0	1.0	13.3
S + 25	99	22	2.6	35.3**	0.0	0.0	0.8	6.6	3.0	13.6

1 Total dead and alive

2 One hundred and six samples from 53 trees

3 Eight trees

H Unfortunately samples brought in for culture and identification deteriorated en route; probably saprophytic

* Very few inclusion bodies, probably external contamination

** Note that this represents only 7 cadavers

TABLE 12

Cumulative Effects of Treatments on Survival Through to Pupation[1] in the Field Virus Insecticide Trials, Rankin 1972

Treatments	All Trees Combined		White Spruce		Balsam Fir	
	Totals[2]	Pupae	Totals[2]	Pupae	Totals[2]	Pupae
EPV alone	702	22.7	258	21.3	444	23.4
EPV + Insecticide	691	20.0	563	20.1	128	19.5
Insecticide alone	763	21.1	469	26.9	294	11.9
NPV alone	765	10.9	649	11.1	116	9.5
NPV + Insecticide	724	13.5	711	13.6	13	7.7
Controls[3]	-	-	279	33.0	-	-

1 Includes all pupae (emerged and unemerged) collected on last two sampling dates

2 Total larvae and pupae collected

3 Eight trees

TABLE 13

Incidence of Pathogens Among Pupae - Virus Insecticide Trials
Rankin 1972

Treatments	Total Pupae Collected	Percent Dead	Incidence of Microorganisms (%)							
			NPV		EPV		Microsporidia		Fungus	
			Totals	Cadavers	Totals	Cadavers	Totals	Cadavers	Totals	Cadavers
EPV alone	429	49.2	1.4	2.8	0.8	2.4	0.0	0.0	0.6	1.9
EPV + Insecticide	378	60.3	3.3	8.8	4.6	12.3	0.0	0.0	0.5	1.3
Insecticide alone	418	25.6	1.1	5.6	0.0	0.0	0.4	1.9	0.4	1.8
NPV alone	355	67.7	5.1	13.8	0.0	0.0	0.03	0.6	0.3	0.6
NPV + Insecticide	235	59.2	17.8	44.0	0.0	0.0	0.0	0.0	0.2	0.5
Untreated	678	40.4	0.3	0.7	0.0	0.0	0.7	1.8	2.3	5.6

TABLE 14

Effect of Treatments on Adult Emergence
from Field-Collected Pupae
Virus Insecticide Trials, Rankin 1972

Treatments	Total Pupae		Percent Emergence		
	Females	Males	Females	Males	Total
EPV alone	123	154	22.0	26.0	24.8
EPV + Insecticide	135	119	10.4	21.0	16.3
Insecticide alone	127	138	71.7	67.4	71.6
NPV alone	78	111	28.2	30.6	36.8
NPV + Insecticide	129	128	31.8	29.7	30.7
Controls[1]	267	243	47.0	53.0	50.6

[1] Eight trees

TABLE 15

Effects of Treatments on Oviposition and
Egg Mass Viability in the Virus Insecticide Trials
Rankin 1972 - Egg Mass Survey Data[1]

Treatments	Average No. Egg Masses per 18" Branch				Percent Viable
	Upper Crown	Middle Crown	Lower Crown	Total	
EPV alone	8.3	7.9	3.7	19.9	55.0
EPV + Insecticide	13.3	8.3	7.4	29.0	50.0
Insecticide alone	12.6	14.9	6.9	34.4	56.9
NPV alone	9.6	8.4	3.3	21.3	40.4
NPV + Insecticide	9.5	7.4	6.2	23.1	42.2
Control[2]	5.7	4.2	2.2	12.1	87.1

[1] Three 18" branch tips per tree

[2] Twenty-five white spruces and twenty-five balsam firs

TABLE 16

Effects of Treatments on Hatching - Laboratory Reared Adults
Virus Insecticide Trials, Rankin 1972

Treatments	Number Egg Masses[1] per Emerged Female	Percent Hatched Egg Masses	Number Larvae per Emerged Female	Percent Change[2] in Progeny
EPV alone	4.3 (27)	100	36.0	+45.2
EPV + Insecticide	1.4 (14)	84.2	19.7	-20.6
Insecticide alone	5.0 (91)	97.2	46.4	+87.1
NPV alone	3.2 (22)	73.6	33.7	+35.9
NPV + Insecticide	2.5 (41)	83.2	17.6	-29.0
Controls[3]	3.4 (125)	92.5	24.8	-

[1] Number of females examined in brackets

[2] (+) indicates percentage increase; (-) decrease

[3] Eight trees

TABLE 17

Cumulative Incidence of Parasites[1] and Associated Species[2]
Virus Insecticide Trials, Rankin 1972

Treatments	Associated Species (%)	Pupal Parasitism (%)	Egg Parasitism[3] (%)
EPV alone	13.6	0.5	14.4
EPV + Insecticide	14.7	0.9	42.3
Insecticide alone	9.8	0.3	23.3
NPV alone	8.0	0.4	20.5
NPV + Insecticide	10.8	0.3	25.0
Control[4]	31.0	1.35	6.0

1 Mostly hymenopterous parasites; few dipterous

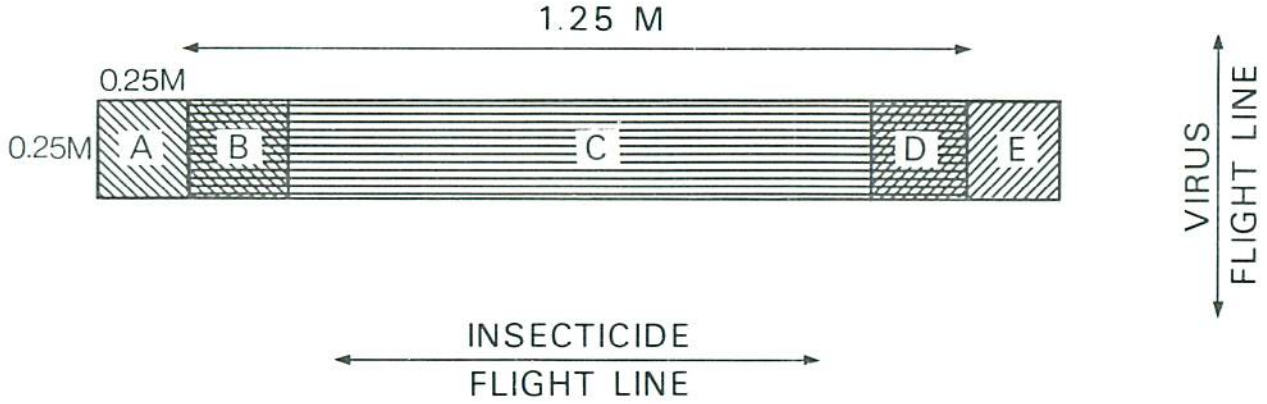
2 Dioryctria reniculella Grate (spruce cone moth)

3 Data from egg mass field survey; upper, middle and lower crowns of 15 trees per treatment

4 Fifty trees in egg mass survey; data for associated species and pupal parasitism are cumulative from 8 trees

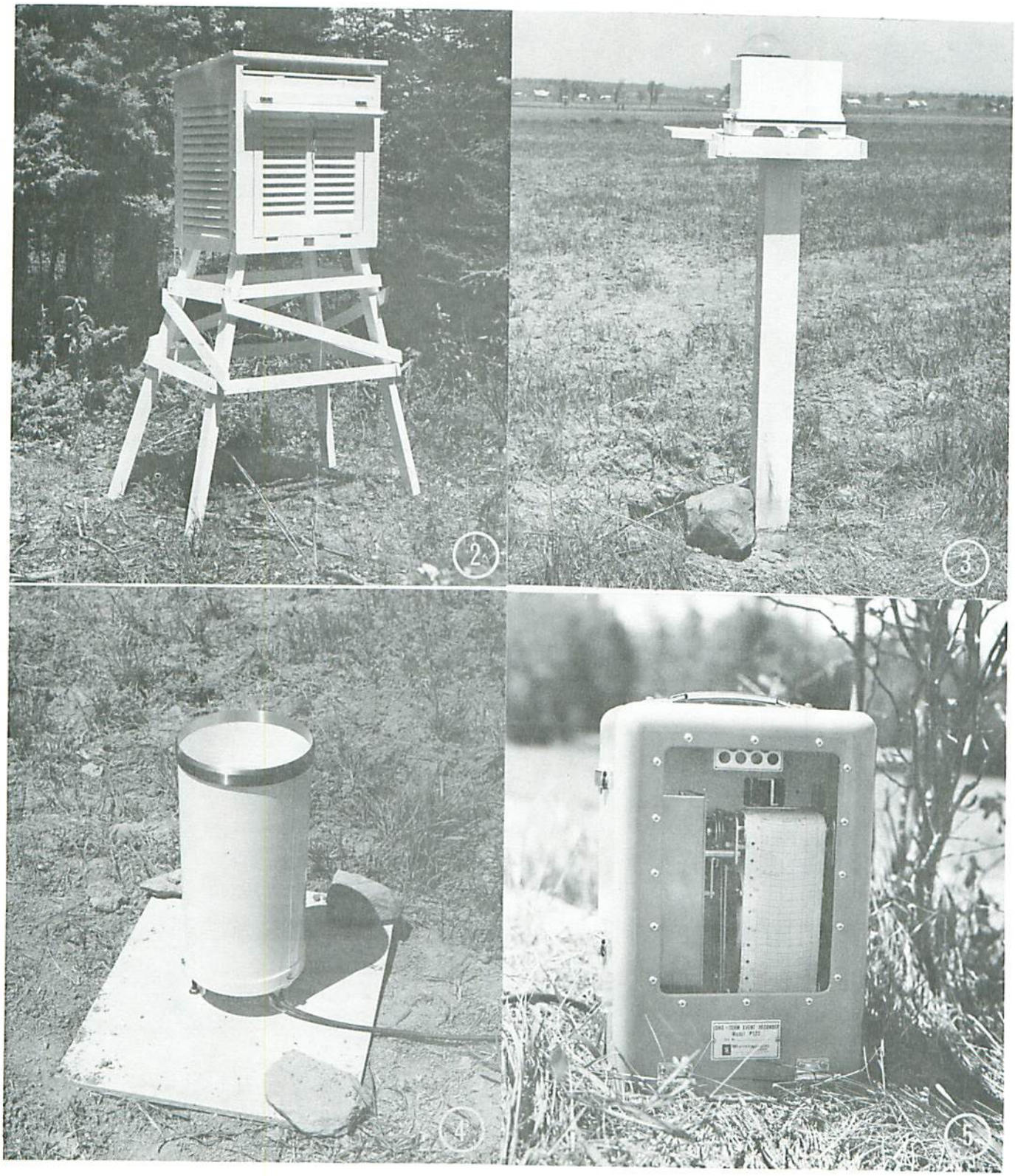
ILLUSTRATIONS

PLOT LAYOUT - RANKIN INSECTICIDE
VIRUS AERIAL TRIALS, 1972



A- POX VIRUS ALONE
B- POX VIRUS + INSECTICIDE
C- INSECTICIDE ALONE
D- NPV + INSECTICIDE
E- NPV ALONE

(1)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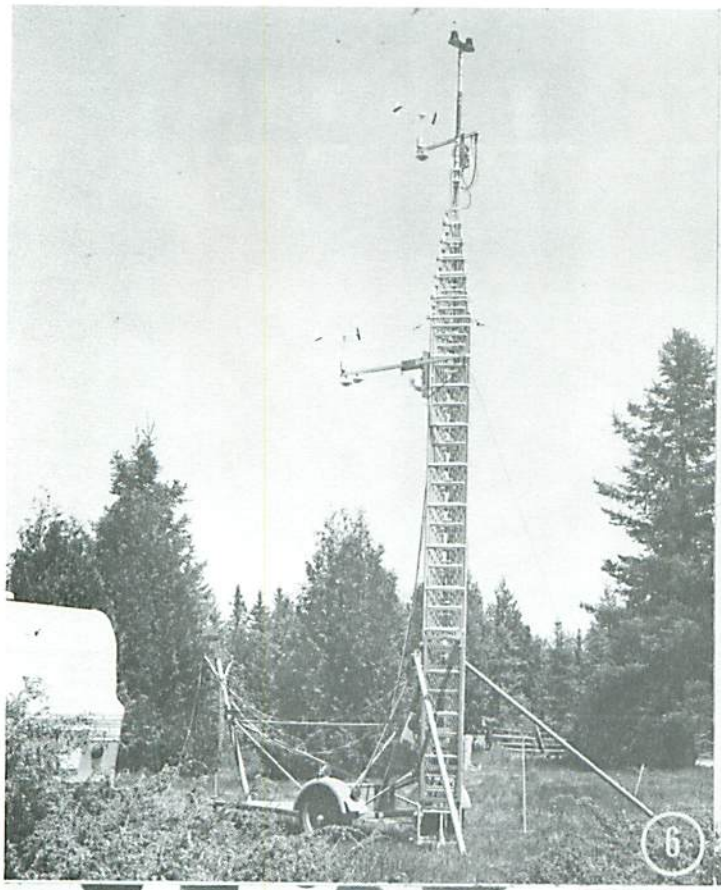

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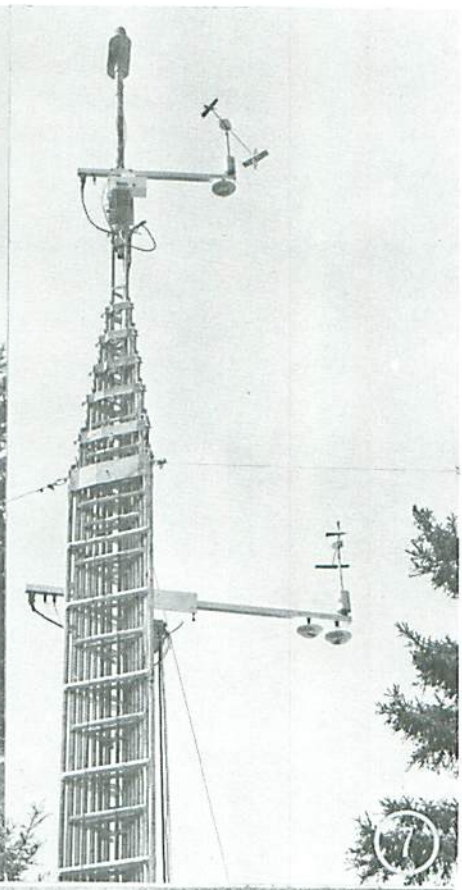

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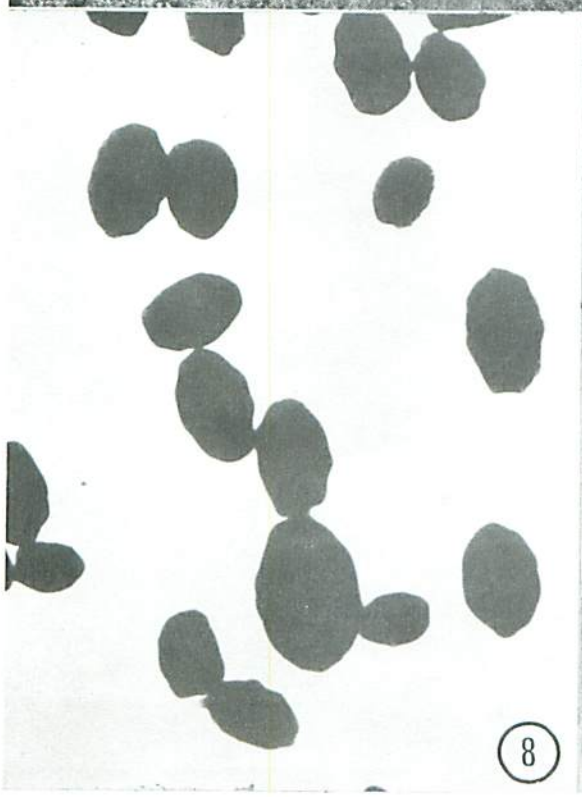

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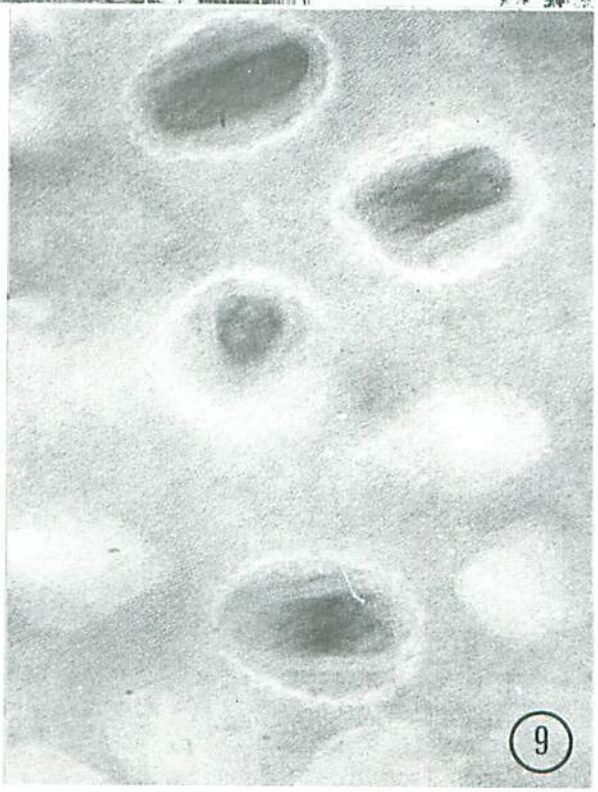
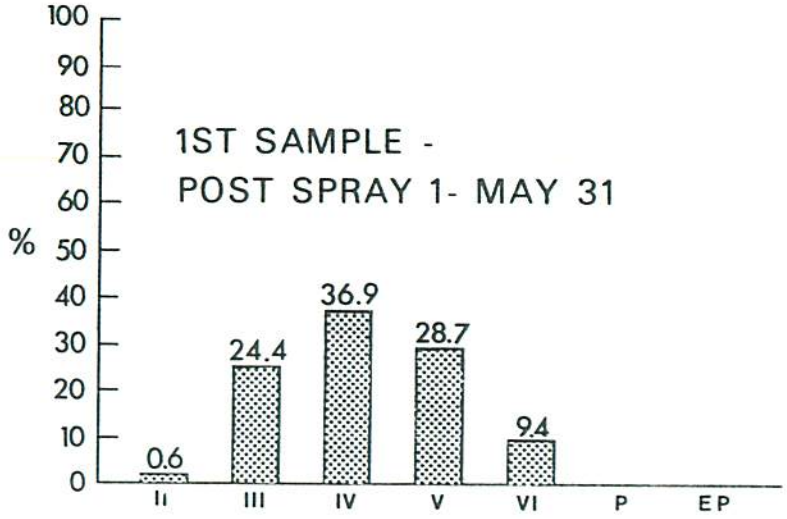
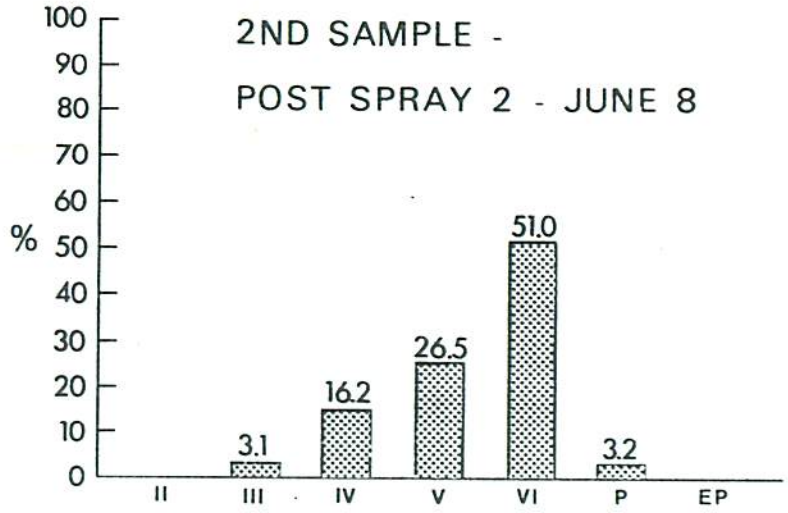
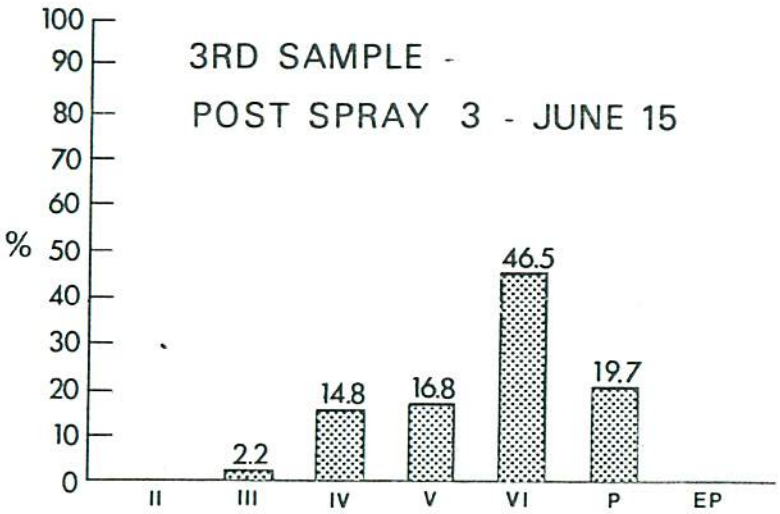


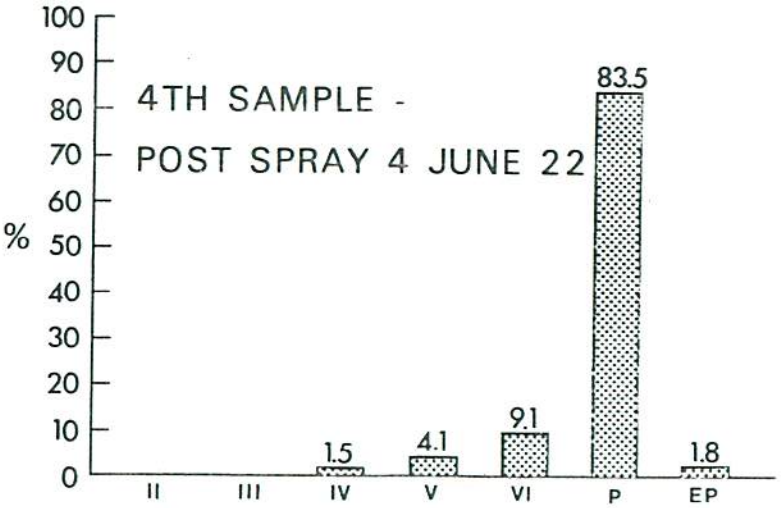
9

LARVAL DEVELOPMENT - AERIAL SPRAY PLOTS - RANKIN 1972









Part II An Operational Assessment of These Trials

by

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INTRODUCTION

The first aerial application of virus against spruce budworm, nuclear polyhedrosis virus (NPV) and entomopox virus was carried out in 1971 as a cooperative project of the Insect Pathology Research Institute (IPRI) and the Great Lakes Forest Research Centre (GLFRC) of the Canadian Forestry Service in Sault Ste Marie (Howse et al 1973). This practical field trial culminated many years of research on budworm viruses at the IPRI.

Analysis of data from these 1971 trials showed that, in general, virus sprays were capable of killing large numbers of budworms, but because of the slow acting nature of the viruses, were not capable of protecting foliage in the year of application. One of the most immediate and potentially practical application of these findings was thought to lie within the context of an integrated control strategy, i.e. some combination of virus and operationally reduced amounts of chemical insecticide. Discussions among representatives of the Chemical Control Research Institute (CCRI), IPRI and GLFRC during the winter of 1971-72 led to the decision to test and assess virus-insecticide combinations in 1972.

This section (Part II) presents the results of the GLFRC studies of these trials which were designed primarily to assess the operational feasibility of using various combinations of virus and insecticide against spruce budworm under Ontario (Howse et al 1971

and 1972). Presently, the main biological criteria used in determining operational effectiveness are the proportions of budworm killed and the degree of foliage protection attributable to treatment.

METHODS

The five treatments that were tested are described in greater detail in Part I of this report but in brief consisted of entomopox virus alone, entomopox virus + fenitrothion and NPV alone. Fifty white spruce (wS) and 50 balsam fir (bF) sample trees (dominants or codominants) were selected in each treatment plot with the exception of the NPV + fenitrothion where only 25 suitable bF sample trees could be found. Three control plots were located four, five and eight miles from the treatment plots in stands similar to the treated stands. Twenty-five wS and 25 bF sample trees (dominants or codominants) were selected in each control plot.

Samples, which consisted of one 18" tip from the midcrown or upper midcrown of each sample tree were collected on three occasions from the treatments and controls. Prespray samples were collected May 25, 26 and 27, the first postspray samples were collected on June 12 and 13 (14 days after spraying) and the 2nd and final postspray samples were collected June 23 and 24 in the treatments (26 days after spraying) and June 26 in the controls. The pre-

spray and 1st postspray samples consisted of one 18" tip from each sample tree whereas the 2nd postspray sample consisted of two 18" tips from each tree. All foliage was carefully examined and the numbers of living budworm were recorded. Many branches were rechecked by experienced personnel to establish correction factors. Additional foliage was collected periodically throughout late May and June from an untreated stand in the vicinity of the treated areas to provide data on larval development.

The 2nd (or final) postspray sample was collected when the budworm were primarily in the pupal stage. All living pupae were saved and their subsequent fate was determined; i.e. whether moths emerged or if the pupae died. In addition, the current degree of defoliation was estimated for each sample.

RESULTS

Budworm emergence occurred May 8 or 9, 1972 in the treatment plots at Rankin. Spraying of all treatment plots was carried out on May 28 and 29 when the budworm were at peak of fourth instar on both wS and bF although development was further advanced on wS with some fifth instars present. Table 1 shows budworm development on wS and bF for sampling and spray dates.

Prespray population densities of living budworm averaged 38.0 per 18" tip and 17.5 per 18" tip on wS and bF respectively for all sample trees in the treatment plots. Prespray densities were somewhat higher in the control areas averaging 59.8 and 22.9 per

18" tip for wS and bF respectively. Three tables summarize the results of GLFRC studies: Table 2, Population Reduction; Table 3, Successful Emergence; and Table 4, Defoliation. Population reduction percentages in Table 2 were based on final successful emergence data and take differential emergence rates (Table 3) into account.

DISCUSSION

It would appear that the best treatment of the five, based on population reduction and foliage protection, was the NPV + fenitrothion. However, this treatment was closely followed by the entomopox virus + fenitrothion primarily from the standpoint of defoliation. These two treatments were particularly outstanding in terms of protecting foliage on bF. NPV alone was quite successful in killing budworm but was not particularly successful in saving foliage. The entomopox virus alone provided poor results in terms of both insect kill and defoliation on both wS and bF. A somewhat surprising result was the amount of population reduction due to fenitrothion alone, - surprising, in the sense that only 1/4 oz of fenitrothion per acre apparently caused population reductions of 35% on wS and 51% on bF. These population reductions were not accompanied by significant foliage protection; in fact, the fenitrothion plot suffered the greatest defoliation on both wS and bF for all treatments.

NPV + fenitrothion and NPV alone had relatively similar results in terms of population reduction but NPV + fenitrothion was

far superior in terms of foliage protection. Entomopox virus + fenitrothion had fair (equal to fenitrothion alone) and entomopox virus alone had poor results in terms of population reduction but entomopox virus + fenitrothion was much superior to entomopox virus alone in terms of foliage protection. Foliage protection by the combined virus-insecticide applications was similar for both host trees. For wS this was 52% in entomopox virus + fenitrothion and 48% in NPV + fenitrothion, and for bF, 18% in entomopox virus + fenitrothion and 14% in NPV + fenitrothion.

The percentage of successfully emerged pupae (those that gave rise to moths as a % of the total live pupae collected in the final sample) appeared normal for all treatments except the two treatments involving the entomopox virus, particularly on bF, where successful emergence was considerably lower than the controls.

Operationally speaking, the results of the NPV + fenitrothion treatment compare favourably with other control methods using only chemical insecticides such as Zectran at 1.2 or 2.4 oz per acre or fenitrothion at dosages ranging from 3 to 9 oz per acre in terms of reducing budworm numbers or saving foliage. However, the cost of producing NPV and spraying this virus at one or more gallons per acre, preceded by an application of chemical, is such that the method cannot be considered practical, particularly over large areas in Ontario, unless additional benefits can be gained through foliage protection one or more years following the year of application.

If our sole criterion was foliage protection, then the entomopox virus + fenitrothion treatment also compares favourably with operational spray results using chemicals alone. However, the same comments concerning the cost of virus production and application and foliage protection gained in subsequent years from virus carry over equally apply here and are equally necessary to justify the use of these techniques on an operational level. Consequently, any possible effects of virus carry over (if it occurs) should be determined for the next several years in the treated and surrounding areas at Rankin. Efforts should also be made to develop more economical methods (reduced dosages and application rates) of developing and applying virus sprays. Consideration should be given to applying virus sprays and chemical insecticide mixed together in a single application.

CONCLUSIONS

It is concluded in terms of the experimental methods (concentrations, rates and application methods) and conditions (weather, etc) which prevailed that;

1. Applications of virus-insecticide combinations cannot be considered operationally practical at present, however the biological results indicate that considerable potential exists for the development of these techniques to a level of operational feasibility, hopefully in the near future.

2. The biological results that help determine operational feasibility can be summarized as follows:

   a) NPV + fenitrothion is the most effective of the five treatments tested in causing population reduction and foliage protection.

   b) Entomopox virus + fenitrothion is less effective than NPV + fenitrothion in causing population reduction but provides a similar level of foliage protection.

   c) Both of the previously mentioned treatments are much more effective in saving foliage on bF and wS.

   d) NPV alone is capable of causing a high population reduction but is not capable of protecting foliage.

   e) Entomopox virus alone causes neither significant population reduction nor foliage protection although it appears that the successful emergence of moths is considerably hindered by this virus.

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The idea and promotion of a study employing combinations of virus-insecticides against spruce budworm, particularly aimed at determining operational feasibility for Ontario, originiated with Dr. W. L. Sippell, Head of the Insect and Disease Survey Unit of the Great Lakes Forest Research Centre.

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TABLE 1

Budworm Development on Key Dates

May 8 or 9	- start of emergence	
May 26	- prespray sample	wS 34% - III, 57% - IV, 9% - V bF 39% - III, 60% - IV, 1% - V
May 28 and 29	- spraying	wS 8% - III, 78% - IV, 14% - V bF 14% - III, 86% - IV
June 12 and 13	- 1st postspray sample	wS 7% - IV, 13% - V, 75% - VI, 5% pupae bF 14% - IV, 25% - V, 61% - VI
June 24 to 26	- 2nd postspray sample	wS 100% pupae (moth emergence starting) bF 15% - VI, 85% pupae

TABLE 2

Percent Population Reduction Due to Treatment
(Adjusted to Account for Natural Mortality)

Treatment	Percent Population Reduction			
	14 days after spraying[1]		26 days after spraying[2]	
	wS	bF	wS	bF
Entomopox virus alone	0	0	22	0
Entomopox virus + fenitrothion	0	21	39	50
Fenitrothion alone	37	18	35	51
NPV + fenitrothion	40	64	72	78
NPV alone	17	0	76	54

[1] 14 days after spraying - primarily sixth instars present with some fifths and pupae

[2] 26 days after spraying - primarily pupae present with moth emergence starting

TABLE 3

Percent Successful Emergence of Moths for
Each Treatment Compared to Controls

Treatment	Percent Successful Emergence[3]	
	wS	bF
Entomopox virus alone	71	66
Entomopox virus + fenitrothion	62	64
Fenitrothion alone	80	77
NPV + fenitrothion	81	88
NPV alone	77	62
Controls - average of three plots	84	78

[3] Percent successful emergence = $\frac{\text{Total Moths}}{\text{Total living budworm}} \times 100$

TABLE 4

Percent Estimated Current Defoliation for
Each Treatment Compared to Controls

Treatment	Percent Estimated Current Defoliation	
	wS	bF
Entomopox virus alone	64	56
Entomopox virus + fenitrothion	52	18
Fenitrothion alone	74	66
NPV + fenitrothion	48	14
NPV alone	66	49
Controls - average of three plots	77	78

Part III An Evaluation of the Incidence of Viruses

by

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INTRODUCTION

The section reports the results of microscopic examination of spruce budworm larvae and pupae performed by staff of IPRI. The incidence of viruses in sprayed and unsprayed areas was determined. These results give an estimate of the impact of the spray on the budworm population and reveal the presence of viruses already present in the population.

The incidence of microsporidia was also determined as it was considered that this may have a bearing on the level of virus infection.

METHODS AND MATERIALS

Two samples were taken; the first 18 days after spray application and the second 25 days after spray application. Twenty random samples of two 18" branch tips were taken from each of the four plots sprayed with virus; ten of the samples were taken from white spruce and ten from balsam fir. There was some variation in the number of samples taken, and this is recorded in Table 1. The plot with fenitrothion alone, an NPV control plot and an entomopox virus plot were sampled once only by the same method.

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 microscopically using phase contrast optics. Dead larvae were smeared without dissection and, if they were dessicated,

they were ground up in a drop of water using a glass rod. The smears or squash preparations were examined for the presence of infected cells containing inclusion bodies of entomopox virus, NPV or CPV and microsporidial spores. When there were large numbers of pupae in the samples, these pupae were kept until adult emergence. If adults emerged they were considered healthy and only dead pupae were examined microscopically.

RESULTS

The results of the diagnosis of budworm larvae from the 5 treated plots and 2 control areas are given in Table 1. The highest incidence of virus was found in the plot sprayed with NPV alone where 74% infection was found in larvae on white spruce and 27% on balsam fir. The entomopox virus, which was disseminated, was contaminated with NPV and higher levels of NPV than entomopox virus were found in the 2 plots sprayed with this virus. No entomopox virus was found in the first sample taken 18 days after spray application but a low level was found 25 days after application. In all four plots sprayed with virus the level of NPV was very much lower in the second sample taken 25 days after spraying than it was in the first sample.

No virus was found in the fenitrothion plot demonstrating that there was no spray drift from the nearby virus plots. In one control sample 4 larvae out of 402 were found to be infected with CPV. The CPV found at the 1% level or less in the NPV plot was therefore

considered to be due to virus already present in the spruce budworm population and not to contamination of the spray formulation.

The level of microsporidia was low in the plots and the control areas and the highest level recorded was 5% of the sample (Table 1).

DISCUSSION

The very low levels of entomopox virus are due to the late application time of the spray. Entomopox virus is very slow to develop in larvae and frequently periods of longer than 25 days must elapse before viral inclusion bodies can be detected microscopically.

The first sample was taken too long after the spray application to observe the increase in incidence of NPV in the population. This sample was taken at the peak of infection or perhaps even after it. The dramatic drop in level of NPV in the second sample can be explained by a loss of dead larvae from the foliage attributable to weathering or predators.

In almost all the samples the incidence of virus was higher on white spruce than on balsam fir. This phenomenon was noted previously in early spray applications when larvae were in the needle-mining second-instar stage. It was found when spray trials were conducted at Achray in 1971 (Howse et al 1973) and at Chapleau in 1972 (Cunningham, Bird and McPhee, unpublished). A possible explanation was that larvae mined more needles on white spruce than on balsam fir

(McGugan 1954) but this theory is not valid for a late spray application when larvae are in the fourth-instar and are open-feeding.

Entomopox virus is difficult to produce without contamination of the stock with other viruses and the entomopox virus sprayed at Achray was contaminated with NPV and CPV. However, with careful bioassay of batches of the mass-produced virus, pure entomopox virus was sprayed at Chapleau in 1972 (Cunningham, Bird and McPhee, unpublished). The carry-over potential of different viruses from one season to the next is of great interest and the use of contaminated suspensions or the presence of virus in the population prior to spray application confuses the results of transmission studies. When mixtures of viruses are used there may be antagonism between different virus, for example CPV interferes with and retards the development of NPV (Bird 1969). It is not yet known whether the same may be true for NPV and entompox virus.

From the results it is difficult to assess if the addition of fenitrothion had an effect, synergistic or antagonistic, on the incidence of virus infection. Better infection was found in the plot with NPV alone than the plot with NPV and fenitrothion but the reverse was true in the entomopox virus plots where the addition of fenitrothion appeared to double the incidence of virus infection.

CONCLUSIONS

1) No contamination with other viruses was found in the NPV virus formulation but the entomopox virus formulation was contaminated with NPV.

2) Satisfactory levels of NPV infection were found in the NPV plots and moderate levels of NPV were found in the entomopox virus plots.

3) The incidence of entomopox virus was very low because this virus does not have time to develop when applied as a late spray.

4) Better virus infection was found in spruce budworm larvae on white spruce than on balsam fir.

5) A very low level of CPV was present naturally in the spruce budworm population.

6) The level of microsporidia in the spruce budworm population was low.

7) No clear-cut assessment could be made of the advantage or disadvantage of the addition of fenitrothion. Infection appeared to be enhanced in the entomopox virus plots and reduced in the NPV plots.

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TABLE 1

Viruses and microsporidia recorded in spruce budworm on plots sprayed with entomopox virus, NPV and fenitrothion and combinations of virus and fenitrothion at Rankin, Ontario in 1972

Treatment	Days after spray when sampled	Tree species	Number of samples	Number of insects examined	Percent Virus Infection			Percent microsporidia infection
					Entomopox	NPV	CPV	
NPV alone	18	BF	10	106	0	27	0	2
	18	SW	10	146	0	74	1	3
	25	BF	10	285	0	1	<1	1
	25	SW	10	216	0	7	0	0
NPV + fenitrothion	18	BF	10	59	0	27	0	0
	18	SW	10	165	0	26	0	1
	25	BF	12	248	0	1	0	1
	25	SW	10	277	0	7	0	5
Entomopox virus alone	18	BF	9	159	0	8	0	4
	18	SW	10	124	0	15	0	0
	25	BF	10	361	5	1	0	0
	25	SW	10	296	1	2	0	1
Entomopox virus + fenitrothion	18	BF	8	47	0	17	0	0
	18	SW	11	119	0	29	0	4
	25	BF	10	92	2	4	0	3
	25	SW	12	187	4	3	0	2
Fenitrothion alone	18	BF	9	127	0	0	0	2
	18	SW	10	411	0	0	0	1
Controls No. 1 NPV	18	BF	10	159	0	0	0	2
	18	SW	10	131	0	0	0	1
No. 2 Entomopox virus	25	BF	8	402	0	0	1	0
	25	SW	7	260	0	0	0	0

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary and Conclusions

During 1972, spruce budworm infested white spruce and balsam fir trees were aerially sprayed with a low dose of fenitrothion, entomopox virus (EPV) alone, nuclear polyhedrosis virus (NPV) alone or each virus-fenitrothion-combination. The project was a cooperative one between the Chemical Control Research Institute, Great Lakes Forest Research Centre and the Insect Pathology Research Institute of the Canadian Forestry Service. CCRI assumed responsibility for application, deposit analysis, meteorological monitoring and intensive larval and post-larval biological assessment on a weekly basis. GLFRC was responsible for population reduction studies by large scale but less frequent samplings and defoliation estimates (Part II). IPRI supplied the virus formulations and provided a diagnostic service for the project in conjunction with the GLFRC functions (Part III). Insecticide deposits on Kromekote cards and glass plates were analyzed by drop size measurements and by gas chromatography, respectively. Microbial deposits on Millipore filter membranes were measured by a microscopical counting method described elsewhere by the senior author. Dead insects were diagnosed to determine death.

The general conclusions drawn from the CCRI, GLFRC and IPRI data are:

1. Spray conditions were good.
2. Fenitrothion (active ingredient), NPV and EPV deposited at ground

surface at 5%, 31% and 42% respectively, of the amount emitted.

3. NPV + fenitrothion was the most effective of the five treatments in causing population reduction and foliage protection. NPV alone is capable of causing a high population reduction but not capable of protecting foliage.
4. A higher virus infection rate was found on white spruce than on balsam fir.
5. The incidence of entomopox among larvae was very low because this virus does not have time to develop when applied as a late spray.
6. Entomopox virus + fenitrothion was less effective than NPV + fenitrothion in causing population reduction but provided a similar level of foliage protection.
7. Both virus-insecticide combinations were more effective in protecting foliage on balsam fir than on white spruce.
8. NPV + insecticide treatment caused highest larval mortality and incidence of virus, lowest survival through to pupation, highest incidence of virus among pupae, the lowest rate of adult emergence among surviving pupae, a very low progeny rate and the lowest rate of progreny survival of all treatments.

9. Transovum transmission of the virus occurred in about 14% of the successful progeny of NPV treated populations.

10. EPV + insecticide treatment reduced female/male sex ratio to 1/2 compared with the normal 1/1 ratio.

11. Larval and pupal parasitism were low in all populations but egg parasitism was high in all sprayed areas.

Recommendations

The use of nuclear polyhedrosis virus combined with a low dose of fenitrothion is a promising approach to the control of the spruce budworm, particularly in high value stands and areas where regular chemical insecticide treatments are hazardous. The Micronair emission system is recommended in aerial application of these agents. Large scale aerial trials with this combination are warranted if the operational cost can be reduced.