Aerial Application of *Bacillus thuringiensis* – Orthene® Combinations against the Spruce Budworm, *Choristoneura fumiferana* (Clem.)

by

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ABSTRACT

Mixtures of a commercial formulation of *Bacillus thuringiensis* (B.t.) + low concentrations of Orthene®, an organophosphate insecticide, were aerially applied to white spruce and balsam fir trees infested with spruce budworm, *Choristoneura fumiferana* (Clem.). An application rate of 8 billion international units (BIU) of B.t. + 0.6 oz. active ingredient of Orthene® was highly effective in reducing the budworm population on balsam fir only but provided significant protection of both tree species. There was apparently no effect on non-target associated insect species (mainly *Dioryctria reniculella* Grate) and a drastic reduction in moth emergence and oviposition occurred.

INTRODUCTION

The concept of integrated control stresses the utilization of all suitable techniques and methods in as compatible a manner as possible in maintaining pest populations at levels below those causing economic injury without causing significant environmental damage. The simultaneous or sequential applications of insect pathogens and low or sublethal doses of chemical insecticides was proposed by Steinhaus (1956), Franz (1961) and Hall (1963) and has been shown in laboratory and field trials to be more effective in reducing spruce budworm damage than *B. thuringiensis* (B.t.) or nuclear polyhedrosis virus (NPV) alone (Morris 1972a, Morris 1972b, Morris et al 1974, Morris and Armstrong 1973). Recent laboratory studies (Morris 1974 a,b) have shown that Orthene®, an organophosphate, was compatible with B.t. at high concentrations (10,000 ppm) of the chemical insecticide and produced a pronounced additive effect with the pathogen even at sub-lethal chemical insecticide (no mortality) doses against the spruce budworm.

In 1974, aerial applications of B.t.-Orthene® combinations were conducted against this insect species to test the validity of the laboratory results under field conditions. This paper presents the results of the field trials.

MATERIALS AND METHODS

The test plots consisted of mixed white spruce, *Picea glauca* (Moench) and balsam fir, *Abies balsamea* (L.) Mill. stands located on the Petawawa Forest Experiment Station, Chalk River, Ontario. The 30-50 ft. high trees had been infested by spruce budworm for the previous 4 or 5 years and had been operationally sprayed with fenitrothion (sumithion) yearly for the previous three years. The present test plots varied in size from 100 to 267 acres (Table 1).

Twenty-five white spruce (wS) or 25 wS and 25 balsam fir (bF) were selected in each plot for periodic assessment of the efficacy of the treatments. Deposit sample units consisting of a Kromekote card, 2 glass plates and 2 Millipore filters were installed at each sample station (1 per tree).

The suitability of spray conditions was determined using wind speed and wind direction sensors mounted on a 97 ft tower and temperature differential sensors were mounted on the Branstead tower at the station. Dry bulb temperatures and relative humidities were recorded with standard recording units at the Branstead site. The criteria for suitability of spray conditions were a wind speed less than 8 mph and temperature differentials indicating inversion conditions to give a positive stability ratio. Weather conditions were measured for a period of 40 min. after application to give an indication of the conditions during the time that the spray cloud was settling.

The sprays were applied during the mornings and evenings of May 25 to June 3 using a Cessna Agtruck aircraft fitted with 4 AU 3000 Micronair emission units calibrated to deliver droplets in the 50 to 100 micron range. The swaths were about 200 ft with the plane flying 200 ft above the tree tops. Larvae were mainly L_2 (second instar) at the start and mainly L_3 at the end of the spray period.

The commercial B.t. used was Dipel WP (Abbott Laboratories, North Chicago, Illinois) with a potency of 16000 International Units of activity/mg and 25 billion viable spores/gm. The tank mix formulations are summarized in Table 1 and the spray application schedule in Table 2. The Erio Acid Red XB (Ciba-Geigy, Etobicoke, Ontario) used in the spray as a tracer dye had been previously shown in the laboratory to be compatible with B.t. (Table 3). Both the dye and the insecticide were added to the tank mix 15 min. before application. Plots were sprayed with 12 BIU B.t. plus 0.9 oz. AI Orthene®, 8 BIU B.t. plus 0.6 oz. Orthene®, 8 BIU B.t. alone or 4 BIU B.t. plus 0.3 oz. Orthene® per acre.

Deposit units were collected 20-30 min. after spray. The Kromkote cards were analysed for droplet size and density. Volume deposit on the glass slides were estimated by colorimetric analysis and deposits of the viable spores were estimated by counting the number of bacterial colonies developing on the Millipore filter membranes which had been placed on trypticase soy agar media for 24 hours at 29^{o} C.

Two 18-inch branch tips were collected from the top third of each sample tree on May 17-20 (pre-spray), June 17-18 and June 25-26. The number of dead and live insects taken from the foliage was recorded for each sample period to determine the effects of the treatments on larval survival and parasitism. Dead insects were diagnosed for the presence of B. thuringiensis and other pathogens using phase contrast optics or stained smears.

At the completion of pupation in the field, 200 male and 200 female pupae were collected from each plot for moth emergence and parasitism studies. Defoliation and oviposition assessments were conducted at the end of the field season.

The results were compared with those from a plot treated with 3 oz. AI of Orthene®/acre applied at the same spray period by the junior author.

RESULTS AND DISCUSSION

The data from the laboratory studies on compatibility of the tracer dye with B.t. showed that Erio Acid Red had no substantial effect on the pathogenicity of the bacteria (Table 2). It had been shown in a previous study (unpublished data) that this dye did not reduce the bacterial replication rate when mixed with B.t. in liquid culture broth for up to 160 hrs.

The meteorological conditions at spray times varied considerably but all sprays were applied under inversion conditions (Table 3). The last spray (June 3) was applied under the most desirable conditions as indicated by the relatively high stability ratio (23.6) and high relative humidity. During this spray session a rain shower moved across the spray block. It showed on the records as an increase in RH to 99%. The effect of the shower was to modify wind speed and temperature differentials to give the high stability ratio. This high ratio would suggest almost ideal spray conditions but in reality the effect of the rain was to wet the foliage just before and just after application probably washing off some deposits from the trees. The presence of 10% molasses in the spray mix probably increased the chances of wash off under the showery conditions.

Results of the deposit analysis (Table 4) showed no clearcut relationship between amounts emitted and deposited. For example, although the meteorological conditions under which 8 BIU alone and 8 BIU + Orthene® were sprayed were comparable, the deposit in the latter application was more than twice that of the former. However, because of the limitations on cutting trees on the station, screening of ground deposit by trees may partly have accounted for this discrepancy. In general, the coverage (indicated by the number of drop/cm^2) was lower than desirable and drop diameters (Table 5) were higher than expected. The number of viable spores deposited showed no relationship to actual deposit of active ingredient. Twenty-five percent fewer spores reached ground surface from the 12 BIU spray than from the 8 BIU spray. This lack of direct relationship has been reported before (Morris and Hildebrand 1974) and further documents the fact that spore deposit alone cannot be used to estimate B.t. deposit efficiency.

The data in Table 6 show that solar radiation and rainfall were lower and higher respectively than usual over the biolical assessment period. Temperature and relative humidity were close to normal.

Results of spruce budworm development on the sprayed and untreated blocks at the two sampling periods are summarized in Table 7. The object was to determine if any of the treatment decelerated development compared with untreated insets. During the larval stage, development on balsam fir was always faster than on white spruce. The reverse was true, however, on the final day of assessment when most budworm had pupated. The only substantial difference in developmental rate between treated and untreated budworm was on balsam fir at the pupal stage. Budworms in plots treated with 8 BIU B.t. with or without Orthene® developed at about half the rate as those in the untreated check plot. In a previous report (Morris and Hildebrand 1974) a retardation of development among B.t. treated budworm in balsam fir was shown. Dulmage and Martinez (1973) also made similar observation on B.t. fed tobacco budworm, <u>Heliothis virescens</u> (F.) and showed that development time was directly related to the amount of endotoxin consumed by this budworm.

Corrected percentage population reductions were low in all treatment plots except on the 8 BIU B.t. + 0.6 oz. Orthene® plot and then only on balsam fir trees (Table 8). The reduction here was about equivalent to that in a plot treated with 3 oz. Orthene® alone (2.13 oz. AI deposited). The pre-spray population densities for these two plots were also almost similar. The percent current growth defoliation on the two plots (45.1 and 43.8 on white spruce and 18.8 and 8.2 on balsam fir) reflected their similarity in population reduction. Further details on the effect of higher doses of Orthene® alone on population reduction and defoliation are reported by Armstrong and Nigam (1974). In the present tests, the large difference in population reduction on balsam fir between Dipel alone and Dipel plus Orthene® (27.9 and 78.0) was not reflected in the defoliation. Comparing these two treatments, it is apparent that the addition of Orthene to the B.t. spray significantly enhanced the protective effect of the pathogen on both tree species.

It must be stressed, however, that no reliable method has been devised for assessing defoliation of white spruce and these figures as well as all others based on the Fettes (1941) method originally designed for balsam fir may not be meaningful for white spruce. In the present trials, visual estimates in the plot sprayed with 12 BIU + Orthene® indicated about the same level of defoliation as on that sprayed by 8 BIU + Orthene and about 90% on the untreated plot. These inconsistencies point to a need for standardization of efficacy assessment techniques in the chemical and in the biological or integrated control of the spruce budworm.

The data on the incidence of B.t. among treated larvae (Table 9) showed that in plots where both white spruce and balsam fir were assessed, B.t. infection rate was always higher on the former tree species. Results of previous trials at P.F.E.S. with B.t.-fenitrothion mixtures (Morris and Armstrong, 1973) showed a similar trend. However, considering the high application and deposit rate especially of the 12 BIU treatment, the incidences of B.t. in the populations were generally low. The natural incidence of nuclear polyhedrosis virus in the test populations was low as in the previous year (Morris and Armstrong 1973). Only one plot showed any natural incidence of this disease. The natural incidence of microsporidia, on the other hand, increased considerably from the previous year when a maximum of 2% of all larvae contained this parasite. With the exception of the untreated check plot where the incidence on both trees species was about the same, the rates of infection was always higher on white spruce than on balsam fir. The added stress placed on the population by microsporidia may partly explain the higher incidence of B.t. on white spruce. However, this was not reflected in population reduction or foliage protection. Whether anti-bacterial exudates from balsam fir (Smirnoff, 1972) come in to play here is an interesting possibility. It is noteworthy that the incidence of NPV is also usually higher on white spruce than on balsam fir trees sprayed with this pathogen (Morris _et al_, 1974). Data on changes in associated insect species (mainly _Dioryctria reniculella_ Grate) (Table 10) show no apparent trend in reduction of the non-target insects due to treatments.

With the possible exception of the population treated with 12 BIU B.t. + 0.9 oz. Orthene®/acre, the treatments had no substantial effect on pupal weights (Table 11). The 12 BIU B.t. + Orthene® and 8 BIU + Orthene® treatments, however, had pronounced effects on moth emergence reducing it by 60% and 37% respectively. The higher dosage rate also reduced oviposition of viable eggs by 42% on white spruce and the lower rate reduced it by 61% on balsam fir and 31% on white spruce (Table 12).

Data on the effects of the treatments on the incidence of parasitism (Table 13), show that the 12 BIU B.t. + 0.9 oz. Orthene® and 3 oz. Orthene® treatments apparently reduced larval parasitism by 60% and 73%, respectively. These parasites included Hymenoptera and Diptera. Pupal parasitism by Diptera and Hymenoptera were apparently eliminated by 3 oz. Orthene® but there was no apparent effect by the other treatments. Egg parasitism by Trichogramma minutum Riley was apparently not affected by any of the treatments.

SUMMARY AND CONCLUSIONS

White spruce and balsam fir trees infested with spruce budworm were aerially sprayed with various dosages of Bacillus thuringiensis - Orthene® combinations and efficacy of the treatments were assessed on population reduction, defoliation of current year's growth, incidence of introduced and naturally occurring pathogens, changes in density of associated insect species, pupal weights, moth emergence, oviposition and parasitism.

The results indicated that:

1. The dosage rate of 8 BIU B.t. plus 0.6 oz. Orthene® which deposited at ground level at the rate of 2.29 BIU B.t. plus 0.17 oz. active ingredient of Orthene® was highly effective in terms of population reduction on balsam fir and significantly reduced defoliation of current year's growth on white spruce and balsam fir.

2. The incidence of B.t. was generally low for all treatments but that of microsporidia was generally high. The incidence of both pathogens were usually higher on white spruce than on balsam fir.

3. Associated species of insects (mainly Dioryctria reniculelia) were apparently not affected by the sprays.

4. Deposits of 3.67 BIU B.t. + 0.28 oz. active ingredient Orthene® and 2.29 BIU + 0.17 oz. Orthene® markedly reduced moth emergence and oviposition.

5. A deposit rate of 3.67 BIU B.t. + 0.28 oz. active ingredient Orthene® drastically reduced larval (but not pupal or egg) parasitism. Orthene® alone at 3 oz. AI/acre apparently eliminated pupal parasitism and greatly reduced larval parasitism.

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TABLE 1

Summary of Formulations Used in Bacillus thuringiensis - Orthene Aerial Sprays, Petawawa 1974

Treatments B.t.-Orthene/A	Plot Size (acres)	Spray Application Dates	Total BIU of B.t. + Orthene Applied/Acre	Dipel[1] (lbs)	Orthene[2] AI (gm)	Chevron[3] Spray Sticker (ml)	Erio Acid Red XB (gms)	Water (US gallons)	CIB[5] Molasses (US gallons)	Total[6] gallons
3x(4 BIU + 0.3 oz Orthene)	230	May 25-26 May 30-31 June 3	12 BIU + 0.90	115.0	69.0	1380	655.5	155.25	17.25	172.5
2x(4 BIU + 0.3 oz Orthene)	267	May 25-26 May 30-31	8 BIU + 0.60	133.5	80.1	1602	761.95	180.0	20.0	200.25
2x(4 BIU alone)	100	May 25 May 30	8 BIU alone	50.0	--	600	285.0	67.5	7.5	75.0
1x(4 BIU + 0.3 oz Orthene)	260	May 25-26	4 BIU 0.30	130.0	78.0	1560	741.0	175.5	19.5	195.0
Untreated Check	100	--	--	--	--	--	--	--	--	--

[1] 0.5 lbs. (4 BIU) per gallon spray applied at 0.75 gallons/acre.

[2] Approximately 3000 ppm in final spray mixture.

[3] 8 ml/US gallons

[4] 0.1% w/v or 3.8 gm/gal

[5] 10% Conc.

[6] pH was 3.8 for all tank mixes.

TABLE 2

Effect of Erio Acid Red[1] Dye on Pathogenicity of *Bacillus thuringiensis* for Spruce Budworm

Treatment	Number Larvae	Corrected % Mortality	% of Treated Insects B.t. positive
Erio Acid Red (0.1%) alone	50	0.0	0
Thuricide 16B alone	50	89.7	78
Thuricide 16B + Erio Acid Red (0.1%)	50	82.0	67

1 This dye does not decrease germination of B.t. when in contact for up to 160 hrs. in liquid culture.

TABLE 3

Meteorological at Time of Application - B.t. - Orthene Spray, PFES 1974

Spray Dates	Time of Application	Wind Speed (MPH)	Wind Direction	Stability Ratio	Relative Humidity (%)	Temperature °C
25/5	1923 - 2118	5.7	NW	+0.4	52-75	9.5
26/5	1949 - 2024	4.0	NW	+1.6	75-85	11.1
30/5	1958 - 2055	2.1	N-NE	+15.4	70-90	12.8
31/5	0606 - 0700	7.6	SE	+0.12	75-90	12.8
3/6	0630 (approx.)	1.0	NW	+23.6	95-99	11.6

TABLE 4

Deposit Rates on Plots Treated with *B. thuringiensis* - Orthene

Treatments B.t. + Orthene	Fluid oz/acre Deposited B.t.	Fluid oz/acre Deposited Orthene	SR* Range	Percent Deposited	Calculated Billions International Units of B.t./acre Deposited	Drops/cm^2	Number Viable Spores/acre $X10^9$ wS	Number Viable Spores/acre $X10^9$ bF	Number Viable Spores/acre $X10^9$ wS + bF
12 BIU + 0.9 oz. Orthene	88.6	0.28	0.4-23.6	30.0	3.67	43	3.04	-	3.04
8 BIU + 0.6 oz. Orthene	53.6	0.17	0.4-15.4	28.6	2.29	25	2.37	1.72	4.09
8 BIU alone	24.2	-	0.4-15.4	12.5	1.0	25	1.39	1.51	2.90
4 BIU + 0.3 oz. Orthene	17.9	0.06	0.4-1.6	18.8	0.75	20.0	1.41	-	1.41
Orthene alone at 3.0 oz AI	-	2.13	7.9	40.0	-	-		-	

* Stability Ratio

TABLE 5

Average Volume Drop Diameters on

Kromekote Card - PFES 1974

	Diameter (Microns) of Drop of Average Vol.				
	wS-1	wS-2	wS-3	bF-1	bF-2
12 BIU + 0.9 oz. Orthene	180	109	130	--	--
8 BIU + 0.6 oz. Orthene	118	148	--	119	149
8 BIU alone	105	115	--	106	108
4 BIU + 0.3 oz. Orthene	102	--	--	--	--

wS-1 = white spruce 1st spray etc.

TABLE 6

Meteorological Conditions During Biological Assessment Periods

Petawawa 1974

Inclusive Dates	Temperature °C [1]		Relative Humidity %		Cumulative Solar Radiation (Cal/cm^2)	Average Solar Radiation $Cal/cm^2/day$	Cumulative [1] Rainfall (Inches)	Average Daily Rainfall (Inches)
	Mean Min.	Mean Max.	Mean Min.	Mean Max.				
May 25-June 18	6.4	18.3	38.9	95.2	11,100	442	1.80	0.072
May 25-June 26	6.7	18.4	39.1	94.7	14,900	453	3.02	0.092

[1] Normal mean temperature and rainfall for May and June Petawawa is about 14° C and 3.99 inches, respectively.

TABLE 7

Spruce Budworm Development on Sprayed and Unsprayed Plots on the Final Day of Population Density Assessment

Treatments	Total No. Budworm.		% L_2		% L_3		% L_4		% L_5		% L_6		Pupae	
	wS	bF	wS	bF	wS	bF	wS	bF	wS	bF	wS	bF	wS	bF
12 BIU + 0.9 oz. Orthene	446	--	0.0	--	0.0	--	0.0	--	1.1	--	26.3	--	72.7	--
8 BIU + 0.6 oz. Orthene	381	51	0.0	0.0	0.0	0.0	0.0	1.9	2.1	11.3	9.9	54.7	88.0	32.1
8 BIU alone	579	249	0.0	0.0	0.0	0.0	0.0	1.2	1.2	10.0	13.6	51.6	85.2	37.2
4 BIU + 0.3 oz. Orthene	557	--	0.0	--	0.0	--	0.2	--	2.1	--	12.7	--	85.0	--
Untreated Check	704	399	0.0	0.0	0.0	0.0	0.1	0.6	0.7	3.2	14.6	31.1	84.6	65.2

TABLE 8

Corrected[1] Percentage Population Reduction Due to Treatment with B. thuringiensis - Orthene and Defoliation Estimates - Petawawa 1974

Treatments	Pre-Spray[2] Density		Percent Reduction 32 Days After[2] 1st Application		Percent Current Growth Defoliation	
	wS	bF	wS	bF	wS	bF
12 BIU + 0.9 Orthene	28.3	--	13.6	--	62.6	--
8 BIU + 0.6 Orthene	10.4	5.5	0	78.0	45.1[a b]	18.8[a b]
8 BIU alone	15.8	10.5	0	27.9	52.4[a b]	27.7[a b]
4 BIU + 0.3 Orthene	18.7	--	0	--	54.5[b]	--
Orthene 3.0 oz.	25.3	4.2	0	71.0	43.8	8.2
Untreated Check	34.4	11.8	(58.7)	(40.7)	65.4	32.9

[1] Corrected by Abbott's Forumulation

[2] Average number larvae/18" branch tip

'a' indicates significant differences at 99% level of confidence between B.t. treatments with and without Orthene; 'b' indicates significant differences at 99% level of confidence between treated and untreated check (t-test).

TABLE 9

Incidence of Pathogens among Larvae and Pupae Collected in Post-Spray Biological Assessment Samples

Treatments	Total Budworm Collected (Larvae & pupae)	Total Number Dead	Percent Infected With					
			B.t.		NPV		Microsporidia	
			Total	Cadavers	Total	Cadavers	Total	Cadavers
12 BIU + 0.9 Orthene - wS	1272	239	5.2	27.5	0	0	5.1	27.0
8 BIU + 0.6 Orthene - wS	753	108	6.8	47.5	0.3	1.7	4.4	30.5
- bF	149	9	2.7	38.5	0	0	0.9	15.4
8 BIU alone - wS	1143	107	1.2	12.7	0	0	1.7	18.0
- bF	442	23	0.2	2.8	0	0	0.3	5.6
4 BIU + 0.3 Orthene - wS	1371	55	2.0	50	0	0	1.1	28.4
Untreated Check - wS	2221	206	0	C	0	0	2.9	30.8
- bF	1044	71	0	0	0	0	3.4	50.0

TABLE 10

Population Density of Non-Target

Species[1] Associated with Spruce Budworm Following B.t. - Orthene

Spray, PFES 1974

Treatments	Pre-Spray[2] Density	Post Spray Density: 24 Days After 1st Application	Post Spray Density: 32 Days After 1st Application
12 BIU + 0.9 oz. Orthene	0.0004	0.02	0.05
8 BIU + 0.6 oz. Orthene	0.003	0.02	0.02
8 BIU alone	0.0	0.08	0.07
4 BIU + 0.3 oz. Orthene	0.004	0.03	0.04
Orthene 0.3 oz.	0.001	0.0	0.008
Check	0.002	0.05	0.06

[1] Mainly dioryctria reniculella

[2] Per 18-inch branch tip.

TABLE 11

Effect of Treatments on Moth Emergence and Oviposition - Pupal Rearing

Treatments	Total Pupae Caged		Average Pupal Weights (mg)		Percent Moth Emergence		
	Males	Females	Males	Females	Males	Females	Totals
12 BIU + 0.9 Orthene	200	200	67 ± 2	88 ± 1	25	36	31
8 BIU + 0.6 Orthene	200	200	68 ± 2	104 ± 6	49	49	49
8 BIU alone	200	200	79 ± 5	108 ± 2	73	69	71
4 BIU + 0.3 Orthene	200	200	66 ± 5	99 ± 8	70	89	80
Orthene 3.0	119	219	62 ± 1	81 ± 1	99	98	98
Untreated Check	200	200	76 ± 1	107 ± 1	88	68	78

TABLE 12

Effect of Treatments on Oviposition (Egg Mass Survey)

Treatments	Average No. Egg Masses/100 Sq. Ft. Foliage					
	Total			Viable		
	wS	bF	wS + bF	wS	bF	wS + bF
12 BIU + 0.9 Orthene	332	--	332	163	--	163
8 BIU 0.6 Orthene	527	332	431	192	70	132
8 BIU alone	533	280	406	307	104	204
4 BIU + 0.3 Orthene	465	--	465	237	--	237
Untreated Check	517	506	512	279	215	262

Note: There was considerable moth invasion from surrounding area so that these figures are probably inflated in relation to spray efficacy

TABLE 13

Larvae, Pupae and Egg Parasitism in B.t. - Orthene Treated Plots

Treatments	% of Larvae Parasitized	Total Number Pupae Reared	% of Pupae Parasitized			% Egg Parasitism (Egg Mass Survey)		
			Diptera	Hymenoptera	Total	wS	bF	wS + bF
12 BIU + 0.9 Orthene	0.6	400	0.3	3.0	3.3	50.8	--	50.8
8 BIU + 0.6 Orthene	3.1	400	0.3	4.0	4.3	63.5	79.1	69.4
8 BIU alone	2.2	400	0.8	4.0	4.8	42.7	62.9	49.7
4 BIU + 0.3 Orthene	2.8	400	0.5	4.0	4.5	46.2	--	46.2
Orthene 3.0	0.4	338	0	0	0	38.1	61.7	50.1
Untreated Check	1.5	400	1.0	3.5	4.5	46.0	57.5	51.1

[1] From larvae collected in population density assessments. In the case of Orthene (3.0 oz.) samples from plots treated with 0.5 and 1.0 gallon/acre were pooled since insufficient samples were taken from either treatment plot to give a reasonably good indication of parasitism, even so only 229 were found.