Efficacy of single applications of *Bacillus thuringiensis* and diflubenzuron formulations against the hemlock looper in Newfoundland in 1988

R. J. West, J. P. Meades and P. L. Dixon

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EFFICACY OF SINGLE APPLICATIONS OF *BACILLUS THURINGIENSIS* AND DIFLUBENZURON FORMULATIONS AGAINST THE HEMLOCK LOOPER IN NEWFOUNDLAND IN 1988

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FOREWORD

The experimental sprays in Newfoundland in 1988 were a joint research effort by the Forest Protection Division of the Newfoundland Department of Agriculture and Forestry and the Newfoundland and Labrador Region of Forestry Canada. Funding included support from the Province through the Federal-Provincial Forestry Agreement, Abbott Laboratories, ChemAgro Limited, Corner Brook Pulp and Paper and Abitibi-Price Limited. We thank R. Fusco (Abbott Laboratories) for his assistance in calibrating spray equipment, A. Sundaram for Kromekote card analysis and K.M.S. Sundaram for determining diflubenzuron concentrations in foliage samples, J. Bissett (Biosystematics Research Centre, Ottawa) for *Hormonema* sp. identification, the summer students hired to help in the field and laboratory, and the staff of the Port Saunders Forest Unit Office for their cooperation and kind hospitality. W. Sutton, D. O'Brien, D. Stone and E. Banfield helped in locating plots and collecting egg samples. D. Rowe assisted by obtaining equipment and supplies. Helpful comments on the manuscript were made by K. van Frankenhuyzen, J.C. Cunningham, A.G. Raske and K.P. Lim.

DISCLAIMER

ABSTRACT

The efficacy of single applications of high-potency formulations of *Bacillus thuringiensis* Berliner (*B.t.*); Dipel 176®, Dipel 264® and Futura XLV®, and diflubenzuron, Dimilin ODC®, was tested against eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Guen.), in a balsam fir, *Abies balsamea* (L.) Mill., forest in Newfoundland. The *B.t.* formulations were applied against various stages of larval development whereas diflubenzuron was applied against first-instar larvae. Application rates were 30 or 40 Billion International Units (BIU) per ha for the *B.t.* formulations and 70 or 120 g.ai. per ha for the diflubenzuron treatments.

Larval numbers were reduced by 80% or more following the applications of Dipel 176 and Dipel 264, and by 68% following the application of Futura XLV. The reduction in larval numbers following the diflubenzuron applications ranged from 51 to 98%. Foliage protection provided by the early treatments of Dipel 176 and Dipel 264 was 80% or more but less for the late treatments. Treatments of Futura XLV and diflubenzuron failed to provide adequate foliage protection.

The optimal spray period began when second-instar larvae first appeared and concluded at the onset of the third instar.

Natural mortality in the experimental area was high and attributed to infection by various fungi, and to parasitism by tachinids.

RÉSUMÉ

L'efficacité d'applications uniques de formulations très puissantes de *Bacillus thuringiensis Berliner (B.t.)*, le Dipel 176®, le Dipel 264® et le Futura XLV®, et de diflubenzuron, le Dimilin ODC®, a été testée contre l'arpenteuse de la pruche, *Lambdina fiscellaria fiscellaria* (Guen.), dans une forêt de sapin baumier, *Abies balsamea* (L.), de Terre-Neuve. Les formulations de *B.t.* ont été appliquées contre les larves à divers stades de leur développement, alors que le diflubenzuron a été appliqué lors du premier stade larvaire de l'arpenteuse. Les taux d'application étaient de 30 ou de 40 milliards d'unités internationales (MUI) par ha pour les formulations de *B.t.* et de 70 ou de 120 grammes de matière active par ha pour les traitements au diflubenzuron.

Les populations larvaires ont été réduites de 80 % ou plus par l'application de Dipel 176 et de Dipel 264, et de 68 % par le traitement au Futura XLV. La réduction du nombre de larves après les applications de diflubenzuron variait de 51 % à 98 %. La protection apportée aux feuillages par les premiers traitements de Dipel 176 et de Dipel 264 était de 80 % ou plus, mais a diminué avec les traitements ultérieurs. Les traitements au Futura XLV et au diflubenzuron n'ont pas réussi à protéger les feuillages de façon adéquate.

La période de pulvérisation idéale a débuté avec l'émergence du deuxième stade larvaire et s'est terminée à l'amorce du troisième stade larvaire.

Le taux élevé de mortalité naturelle dans la zone expérimentale a été attribué aux infections causées par divers champignons et au parasitisme des tachinidés.

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by

R.J. West, J.P. Meades and P.L. Dixon

INTRODUCTION

Double applications of formulations of the biological insecticide, *Bacillus thuringiensis* var. *kurstaki* (*B.t.*), were shown to be effective in protecting balsam fir, *Abies balsamea* (L.) Mill., foliage against infestations of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria* Guen., in Newfoundland (West *et al.* 1989) . However, single applications of *B.t.* against low to moderate infestations may provide adequate protection and lower treatment costs by reducing flying time and the quantity of insecticide. However, timing of the single application is crucial; it is important to define the period in larval development ('spray window') when one application of *B.t.* results in the best foliage protection.

Diflubenzuron is a benzoylphenyl urea that upon ingestion by insect larvae selectively inhibits chitin synthesis. Double applications of Dimilin WP® in aqueous formulations have reduced numbers of hemlock looper larvae by as much as 60 to 80% and pupae by over 90% in field trials in Newfoundland in 1985, 1986 and 1987 (Retnakaran *et al.* 1988; Raske *et al.* 1987, 1990). However, the foliage protection demonstrated in these trials has generally been below 50%. Raske *et al.* (1991) stated that the use of oil rather than water as a base for diflubenzuron formulations might improve spray deposit and efficacy.

This paper reports the results of field trials carried out in 1988 to evaluate the effectiveness of single applications of three formulations of *B.t.*: Dipel 176®, Dipel 264®, and Futura XLV®; and one oil-based formulation of diflubenzuron, Dimilin ODC®. The applications of Dipel 176 and Dipel 264, both oil-based, were made against the hemlock looper at several stages in larval development whereas Futura XLV, a water-based formulation, was tested only once when the majority of larvae were in their second instar. The diflubenzuron applications were made against first-instar larvae.

MATERIALS AND METHODS

Plot Layout

Test plots of 30 ha (300 by 1 000 m) were located in a forest of balsam fir, *Abies balsamea* (L.) Mill., near Hawkes Bay, Nfld.; six untreated areas were designated as control plots (Fig.1). Some of the test plots had been used in an experimental spray program in 1987 and treated with either *B.t.*, diflubenzuron or fenitrothion. They were used again in 1988 because alternative areas were lacking and egg sampling in the

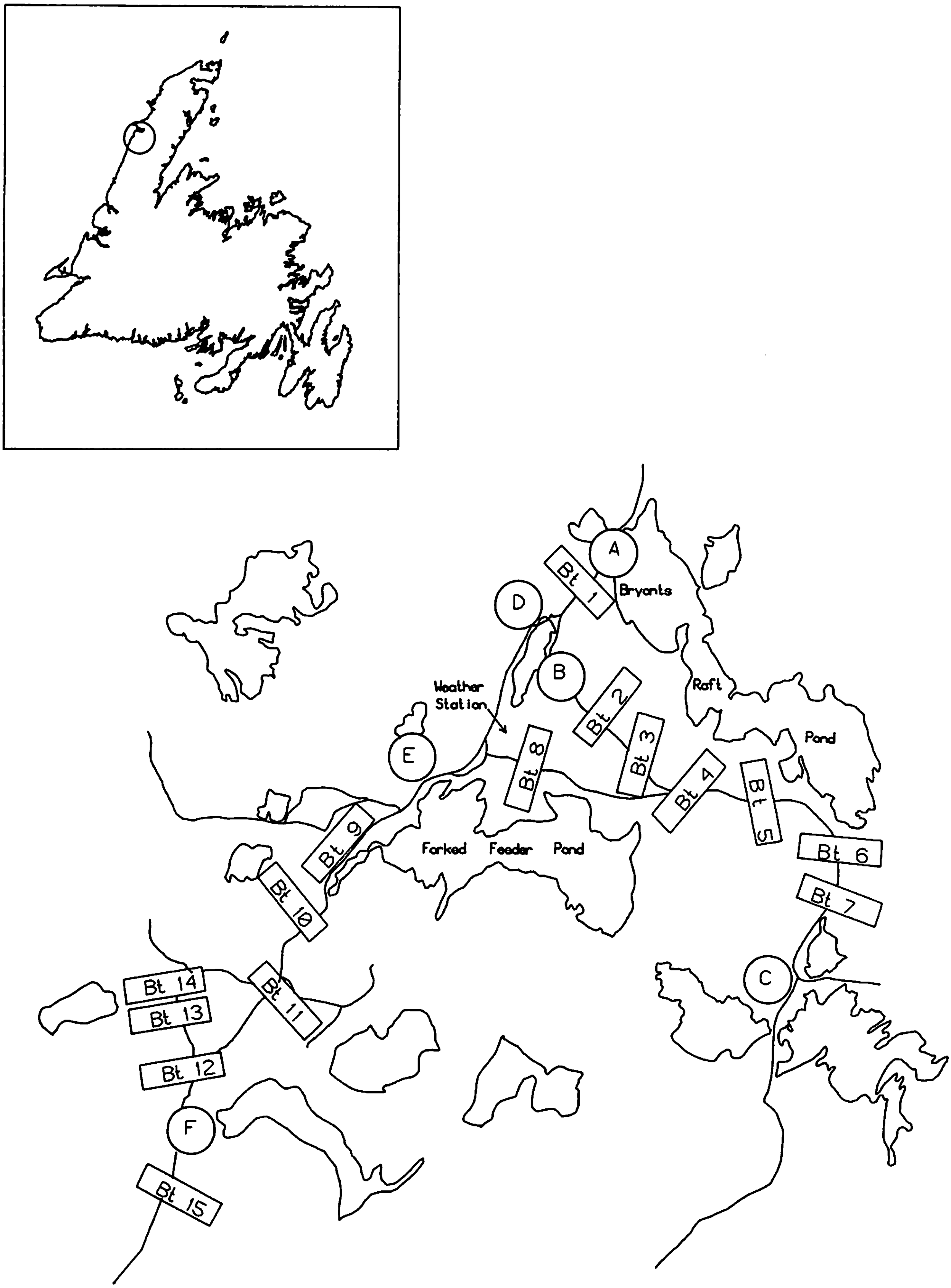

Figure 1. Location of plots treated with *Bacillus thuringiensis* or diflubenzuron formulations in Newfoundland in 1988. Plots 1-5 = treated, Plots A-F = control areas.

fall of 1987 indicated that the hemlock looper infestation would continue in 1988. Trees used for sampling were about 8 m in height and located along a logging road or cleared line crossing the centre of each plot. Trees were not sampled within 60 m of the plot boundaries.

Spray Formulations and Application

Volume rates ranged from 1.2 to 2.4 L per ha for the *B.t.* applications and were 2.5 L per ha for the diflubenzuron treatments (Table 1). Dipel 176 and Dipel 264 were supplied by Abbott Laboratories (Chicago, IL) and applied in dosages of 30 or 40 BIU per ha; Chemagro Ltd. (Mississauga, ONT) supplied Futura XLV and it was applied at 30 BIU per ha. Chemagro Ltd. also supplied Dimilin ODC applied at 70 and 120 g.ai. per ha. The formulations were mixed with a 1% w/v concentration of a Day-Glo® fluorescent pigment; Fire Orange for the oil-based formulations and Rocket Red for the water-based formulation. Formulations of *B.t.* were applied undiluted; Dimilin ODC was diluted in Superior 70 oil (Table 1). Applications were made from a Grumman AgCat aircraft equipped with four Micronair AU4000 atomizer units set with a blade angle of 25°. The plane flew at a speed of 160 km/h in flight lines 30 m apart and 20 m above the tree tops. Flight lines were parallel to plot length. The applications were made within 2 h of either sunset or sunrise.

Temperatures at 2 and 10 m above the ground, wind speed, relative humidity (RH) and daily rainfall were monitored with a 21x datalogger (Campbell Scientific, Edmonton) set up in the experimental area (Fig. 1). Wind direction was determined on site during the applications by observing the movement of a helium-filled aerial balloon and the spray cloud.

Table 1. Formulations, application rates and spray equipment settings used in applications of *Bacillus thuringiensis* (Dipel, Futura) and diflubenzuron (Dimilin) formulations in Newfoundland in 1988.

Formulation	Dosage/ha	Volume rates (L/ha)	Pump Pressure (PSI)	Micronair Setting	Flow rate
Dipel 176[1]	30 BIU	1.8	40	7	16.2
	40 BIU	2.4	30	9	21.6
Dipel 264[1]	30 BIU	1.2	30	5	10.8
	40 BIU	1.6	25	7	14.4
Futura XLV[1]	30 BIU	2.1	20	9	18.2
Dimilin ODC[2]	70 g.ai.	2.5	35	9	22.5
	120 g.ai.	2.5	35	9	22.5

[1] Applied neat; dye premixed by supplier.
[2] Tank mixes contained the following amounts:
8.00 L of (70 g.ai./ha) or 13.5 L (120 g.ai./ha) of Dimilin ODC 45
6.25 L Fire Orange Stock
110.75 L (70 g.ai./ha) or 105.25 (120 g.ai./ha) Superior 70 oil
Fire Orange Stock: 800 g DayGlo Fire Orange dye, 4 L Dowanol TPM, 50 ml glycerol.

Spray Deposit

Kromekote® cards and foliage samples were used to monitor spray deposits at ground and mid-crown levels, respectively (West *et al.* 1989). Twenty-one cards across each plot were used for each application and 5 cm^2 were examined per card. Foliage samples were taken 1 and 48 ha after application: 5 one-year old shoots per branch, 2 branches per tree and 10 trees (spread out across the plot) for each sampling occasion. Shoots were stored in paper bags in a freezer until examined. Seven current-year and 7 one-year-old needles from each shoot were examined on both sides under a hand-held black light (Raytech Industries Inc., Stafford Springs, CT) to count droplets. Student's t-test (SAS Institute 1985) was used to compare mean droplet counts on foliage collected 1 and 48 h after application.

Concentrations (ppm) of diflubenzuron on sprayed foliage were determined from samples taken 1 h after application in plots 12 to 15; plot 12 also was sampled 48 h after application. Three groups of 8 balsam fir trees were sampled on each sampling occasion: 10 one-year old shoots per tree were pooled and processed following the method of Sundaram (1986).

Effect of Treatment on Population Density

Larval numbers in treated and untreated areas were estimated by taking "beating samples" from 20 trees per plot (West *et al.* 1987) 1 to 2 d before and 10 to 12 d after application. An additional 21-d post-spray sample was taken from plots treated early in larval development. A tree once sampled was not resampled.

Burlap traps were placed at a height of 1.5 m on the trunks of 20 trees per plot in late July prior to pupation and removed in late August to sample pupal populations (Otvos 1974).

Population reduction due to treatment was determined with Abbott's (1925) formula (Fleming and Retnakaran 1985). Treated plots were matched with control plots with the closest pre-spray population densities.

Mortality from Pathogens and Parasites

Larvae and pupae were reared from the prespray and final post-spray samples to determine mortality from pathogens and parasites following the method of West *et al.* (1989). Larvae were fed only foliage from the plots from which they were collected. Thus larvae collected in pre-spray samples from the treated plots were initially fed unsprayed foliage but within 24 h of spray application were fed sprayed foliage. Smears of cadavers were examined microscopically at 400X to identify pathogens and unemerged parasites.

Effect of Treatment on Preventing Defoliation

Defoliation (DEF) of current-year and year-old foliage was estimated to the nearest 10% by visually scanning entire trees from the ground on 15-16 August 1988. The trees evaluated were located along the logging roads or cut lines crossing the plots. Forty trees within each plot and 20 trees on either side of the plot within 200 m of the plot border were evaluated. The percentage of foliage saved (F) as a result of treatment was calculated as:

$$F = \frac{DEF_{CONTROL\ (OR\ BORDER)} - DEF_{TREATMENT}}{DEF_{CONTROL\ (OR\ BORDER)}} \times 100$$

RESULTS

Weather

Weather during the applications was generally favourable (Table 2). Air stability ranged from inversion to slightly lapsed conditions. Temperature ranged from 5 to 12°C for morning applications and from 12 to 18°C for evening applications. RH ranged from 76 to 100%, and windspeed from 1 to 12 km/h.

Spray Deposit

Droplet densities were generally uniform across the plots (Appendix I). The number of droplets per cm^2 ranged from 35 to 69 for the *B.t.* applications and from 56 to 79 μm for the applications of diflubenzuron (Table 3). Droplet number generally increased with the volume rate. An exception was for the treatment of Futura XLV made at the relatively high rate of 2.1 L per ha which resulted in a deposit of only 35 droplets per cm^2, the lowest recorded (Table 3). Droplet sizes ranged from 5 to 165 μm for the applications of Dipel and Dimilin and from 15 to 116 μm for the application of Futura (Appendix I). Number median diameter (NMD) ranged from 40 to 50 μm; volume median diameter (VMD) ranged from 69 to 97 μm (Appendix I).

Densities of at least one droplet per needle were common for samples taken 1 and 48 h after treatment (Table 3). A significant ($P \leq 0.05$) decrease in the number of droplets per needle did not occur within 48 h for the *B.t.* applications despite several instances of post-spray precipitation. However, droplet numbers significantly declined within 48 h for the application of Dimilin in plot 12 (Table 3). The number of droplets per needle in plots 5 and 6 was unexpectedly higher for the 48 h samples than for the 1 h samples (Table 3). There was no significant difference in droplet numbers on current and one-year-old foliage sampled at the same time.

Concentrations of diflubenzuron on balsam fir foliage averaged 8.43 ppm in samples taken from plot 12. This was more than twice the concentrations of those from plots 13, 14 and 15 where averages were 1.97, 2.02 and 3.74 ppm, respectively (Appendix II).

Effect of Treatment on Population Density

Average prespray counts ranged from 33 to 806 larvae per beating sample (Table 4) indicating population levels that were capable of causing light to severe defoliation. Larval population reduction 10 to 12 days after treatment was more than 78% for all but two of the Dipel applications, 24% for the application of Futura XLV and 50% or less for the applications of diflubenzuron (Table 4). Larval population reduction 21 days after treatment ranged from 50 to 98% (Table 4). Pupal numbers were essentially reduced to zero for all treatments. Mortality from natural causes, especially in the late larval stages, was very high in the control plots resulting in low numbers of pupae (Table 4). Pupal numbers were very low or nil in the treated plots (Table 4).

Table 2. Weather conditions and larval development at time of applications of *Bacillus thuringiensis* (Dipel, Futura) and diflubenzuron (Dimilin) formulations against hemlock looper larvae in Newfoundland in 1988.

Plot No.	Time of Application		Larval Development	Temperature (°C)		Wind speed (km/h) and direction	RH (%)	Accumulation of rain (mm)		
	Date	Hour	(L_1:L_2:L_3)	10 m	1 m			6h	24h	48h
Dipel 176 (30 BIU/ha)										
1	2-VII	0722	82:18:00	9.2	9.1	3.2 E	97	5.5	7.1	13.8
5	5-VII	2110	29:71:00	18.2	18.0	12.3 W	77	0	0.9	0.9
8	14-VII	0710	05:60:35	5.8	4.9	1.2 SE	94	0	2.1	7.9
Dipel 176 (40 BIU/ha)										
2	2-VII	0806	82:18:00	10.0	9.9	6.3 E	94	5.5	7.1	13.8
9	14-VII	0807	02:55:43	8.8	5.6	1.0 SE	89	0	2.1	7.9
Dipel 264 (30 BIU/ha)										
3	4-VII	0645	90:10:00	11.9	12.1	10.6 S	97	0	0	0.9
7	5-VII	2202	47:53:00	16.3	16.3	11.4 SW	80	0	0.9	0.9
10	14-VII	2107	05:71:24	13.5	14.7	1.6 SE	94	0.1	7.9	7.9
Dipel 264 (40 BIU/ha)										
4	4-VII	0725	81:19:00	12.2	12.3	11.9 S	94	0	0	0.9
11	14-VII	2145	03:58:39	12.4	13.2	1.1 SE	99	0.3	7.9	7.9
Futura XLV (30 BIU/ha)										
6	5-VII	2240	33:67:00	15.1	15.3	8.8 SW	83	0	0.9	0.9
Dimilin ODC (70 g.ai./ha)										
12	22-VI	2125	98:02:00	10.0	10.4	2.0 NE	95	1.0	3.6	5.4
14	25-VI	0750	99:01:00	5.7	5.4	0.6 E	95	0	0	3.2
Dimilin ODC (120 g.ai./ha)										
13	24-VI	2140	99:01:00	7.0	7.0	6.2 W	89	0	0	2.0
15	29-VI	2135	82:18:00	12.9	13.3	3.1 NE	96	0	6.4	8.4

Table 3. Spray deposit on Kromekote cards at ground level and on mid-crown foliage following application of *Bacillus thuringiensis* (Dipel and Futura) and diflubenzuron (Dimilin) formulations in Newfoundland in 1988.

Plot No.	Kromekote cards (droplets/cm²)	Current-year foliage (droplets/needle)		Year-old foliage (droplets/needle)	
		1h	48h	1h	48h
Dipel 176 (30 BIU/ha)					
1	53	0.9	0.5	1.7	0.6
5	55	0.8	3.7*	1.0	4.6*
8	68	1.4	1.2	1.5	1.3
Dipel 176 (40 BIU/ha)					
2	69	0.9	0.9	1.4	1.0
9	65	4.3	5.5	3.2	4.9
Dipel 264 (30 BIU/ha)					
3	40	2.9	2.6	3.4	3.1
7	42	1.3	0.9	1.4	1.4
10	51	2.7	2.5	2.6	2.1
Dipel 264 (40 BIU/ha)					
4	63	1.1	1.0	1.3	1.1
11	58	3.4	3.6	3.2	3.2
Futura XLV (30 BIU/ha)					
6	35	1.1	2.5*	1.5	3.5*
Dimilin ODC (70 g.ai./ha)					
12	79	5.6	1.6*	9.4	3.8*
14	67	1.4	-	1.8	-
Dimilin ODC (120 g.ai./ha)					
13	56	1.0	-	1.6	-
15	68	1.2	-	1.6	-

* Indicates a significant difference ($P \leq 0.05$) in mean number of droplets sampled 1 h and 48 h after treatment (t-test, SAS Institute 1985).

Table 4. Effect of *Bacillus thuringiensis* (Dipel and Futura) and diflubenzuron (Dimilin) treatments on hemlock looper populations in Newfoundland in 1988. Only one post-spray sample was taken from plots treated late in larval development (plots 8, 9 10 and 11).

Plot No.	Hemlock Looper/Tree Sample				Population Reduction Due to Treatment		
	Larvae						
	Pre-spray	Post-spray			Post-spray		
	1-2d	10-12d	21d	Pupae	10-12d	21d	Pupae
Dipel 176 (30 BIU/ha)							
1	63.8	25.4	3.6	0.1	37.5	90.1	97.0
control	124.5	79.3	71.2	6.5			
5	154.7	8.1	1.0	0	85.3	96.5	100
control	191.4	68.3	35.8	2.7			
8	130.6	8.3	-	0.1	78.6	-	98.7
control	126.1	37.5	-	7.6			
Dipel 176 (40 BIU/ha)							
2	51.9	5.6	3.1	0	82.9	89.4	100
control	124.5	79.3	71.2	6.5			
9	78.6	13.4	-	0.3	81.0	-	95.3
control	79.3	71.2	-	6.5			
Dipel 264 (30 BIU/ha)							
3	69.0	8.2	4.6	0.1	81.3	88.3	97.2
control	124.5	79.3	71.2	6.5			
7	33.3	4.5	9.5	0.3	78.8	50.1	82.7
control	124.5	79.3	71.2	6.5			
10	122.7	17.7	-	0	68.7	-	100
control	94.3	43.4	-	3.2			
Dipel 264 (40 BIU/ha)							
4	479.6	5.8	4.2	0.3	96.1	95.9	52.9
control	451.4	140.4	96.9	0.6			
11	44.0	4.5	-	0.1	80.5	-	94.3
control	68.3	35.8	-	2.7			
Futura XLV (30 BIU/ha)							
6	286.2	55.3	12.0	0.3	24.4	64.3	87.9
control	369.1	94.3	43.4	3.2			
Dimilin ODC (70 g.ai./ha)							
12	399.5	105.5	3.9	0	49.7	90.6	100
control	366.5	192.5	38.0	6.5			
14	201.5	147.2	13.7	0	1.6	50.7	100
control	257.9	191.4	35.6	2.4			
Dimilin ODC (120 g.ai./ha)							
13	133.4	59.9	9.1	0	37.7	88.0	100
control	124.5	79.3	71.2	6.5			
15	805.9	223.4	3.3	0	12.1	98.1	100
control	451.4	140.4	96.9	6.5			

Mortality from Disease and Parasites

Few larvae collected in prespray samples and subsequently reared on treated foliage survived (Table 5). Survival also was low for larvae collected at the same time from the plots and reared on unsprayed foliage (Table 5). Survival of larvae from postspray samples ranged from 0 to 41% on treated foliage and from 7 to 28% on unsprayed foliage. Very few pupae were available for rearing from the treated plots but most of these survived (Table 5). Pupae sampled from control plots had survival rates of 27 to 81%.

Natural mortality agents included members of the family Entomophthoraceae, black yeast fungi and tachinid parasites (Fig. 2). Spores of black yeast fungi (Hyphomycetes: Dematoides) were present in a large proportion of dead hemlock looper larvae (Fig. 1). These fungi were identified as a complex of at least two species of black yeasts, *Hormonema* sp. possibly *dematoides* Lagerberg & Melin, and *Aureobasidium pullulans* var. *pullulans* (de Bary) Arn. *Entomophaga aulicae* (Reichardt in Bail) Humber was the most common member of the Entomophthoraceae although *Erynia radicans* (Bref.) Humber, Ben-ze'ev and Kenneth occurred occasionally. When only resting spores were present, species identification was not possible, but it is likely that they were produced by *E. aulicae* or *Z. radicans* as these were the only species of Entomophthoraceae recorded. All the parasitoids were recovered as tachinid larvae (Diptera: Tachinidae). These could not be reared to the adult stage for identification, but might be either *Winthemia occidentis* Rnd. or *Madremyia saundersii* (Will.), both common tachinid parasites in Newfoundland.

The mortality pattern in the prespray and postspray larval samples was similar for the treated and control plots where the incidence was BLACK YEAST > ENTOMOPHTHORACEAE > PARASITOIDS (Fig. 2). A pattern of pupal mortality was less discernable for the treated plots, probably because of the low number of pupae available. Infection of pupae by the Entomophthoraceae was common in the control plots but not in the treated plots, parasitism of pupae was higher than for larvae and pupae generally were not infected with black yeast fungi (Fig. 2). There was a higher percentage of death due to unknown causes for pupae than for larvae.

Effect of Treatment on Preventing Defoliation

Little or no defoliation occurred in the plots treated with Dipel before third instars were first recorded and the amount of foliage saved generally was more than 80% (Appendix III, Table 6). For late applications of Dipel at 30 BIU/ha, defoliation of current year's growth was 32% for Dipel 264 (Appendix III); foliage saved ranged from 0 to 100% (Table 6). There was no defoliation for late applications of Dipel at 40 BIU/ha, but the areas bordering these plots were not defoliated either. In the plot treated with Futura (Plot 6), defoliation was 55% on current-year's growth and 47% on year-old growth (Appendix III); foliage saved was 34% when compared to the matched control plot but 0% when compared to the obrdering area (Table 6).

Defoliation ranged from 10 to 40% in the plots treated with diflubenzuron (Appendix III). The amount of foliage saved was 27% when compared to the

Table 5. Number and percent survival of hemlock looper larvae and pupae collected and reared from plots treated with *Bacillus thuringiensis* (Dipel, Futura) or diflubenzuron (Dimilin) formulations and from untreated plots in Newfoundland in 1988. Larvae were reared on balsam fir foliage collected every 3 to 4 d from their respective plots.

	Larval Samples						Pupal Sample	
	Pre-spray			Final post-spray				
		Survival (%)			Survival (%)			
Plot No.	No. reared	To pupa	To adult	No. reared	To pupa	To adult	No. reared	Adult emergence (%)
Dipel 176 (30 BIU/ha)								
1	100	3	3	46	28	6	1	100
5	100	0	0	15	6	6	0	-
8	100	0	0	100	5	2	1	100
Dipel 176 (40 BIU/ha)								
2	100	0	0	37	41	38	0	-
9	100	0	0	100	17	12	4	50
Dipel 264 (30 BIU/ha)								
3	100	0	0	49	20	14	1	0
7	100	0	0	100	21	14	4	50
10	100	0	0	95	3	2	0	-
Dipel 264 (40 BIU/ha)								
4	100	0	0	44	2	2	4	25
11	100	0	0	25	0	0	1	100
Futura XLV (30 BIU/ha)								
6	100	3	1	89	3	3	3	0
Dimilin ODC (70 g.ai./ha)								
12	100	0	0	37	3	3	0	-
14	100	3	1	100	0	0	0	-
Dimilin ODC (120 g.ai./ha)								
13	100	26	12	100	7	5	0	-
15	100	0	0	31	0	0	0	-
Control								
A	100	39	23	100	17	13	100	27
B	100	24	21	100	28	21	43	56
C	100	0	0	100	7	4	100	81
D	100	18	14	100	18	17	100	23
E	100	6	2	100	18	4	69	54
F	100	5	5	100	24	19	10	80

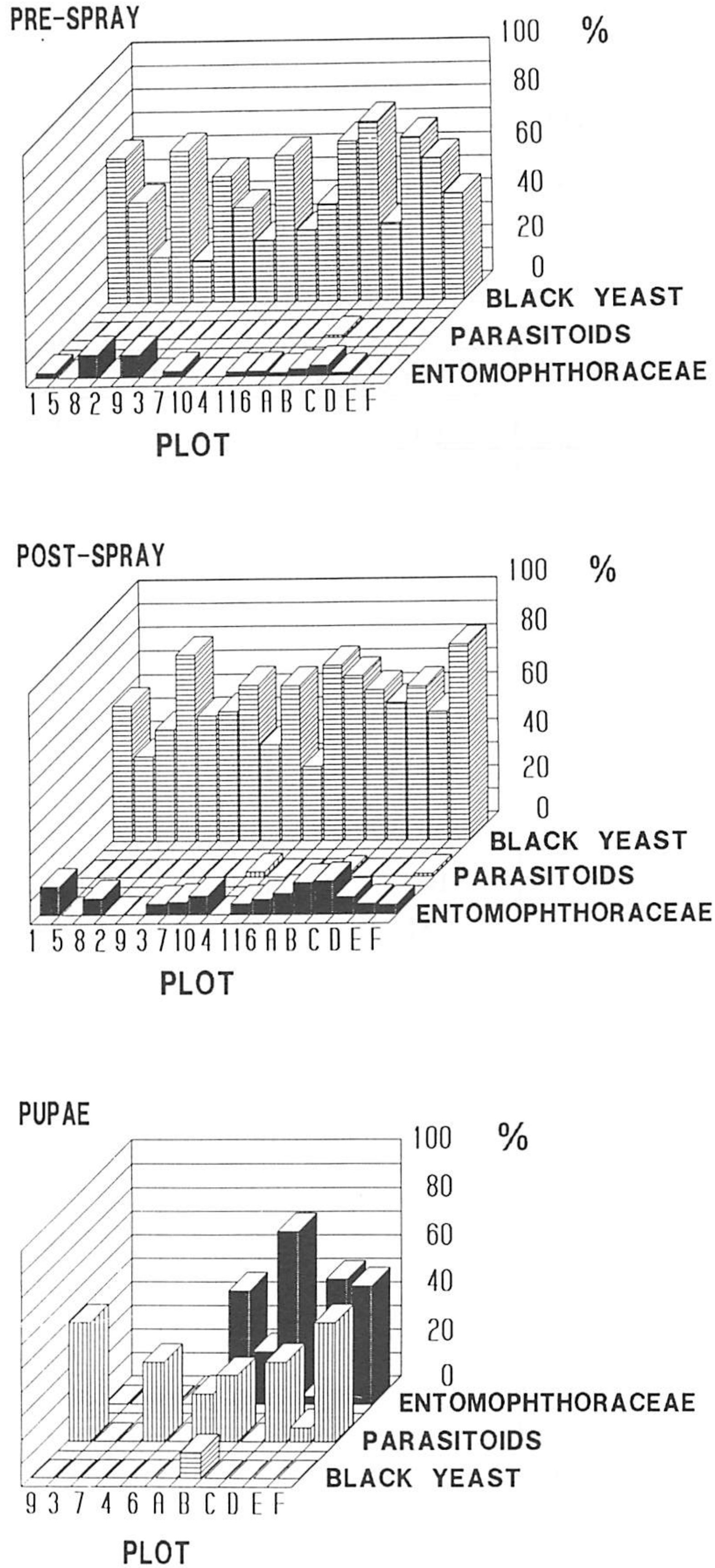

Figure 2. Mortality patterns in *Lambdina fiscellaria fiscellaria* collected from plots treated with *Bacillus thuringiensis* formulations (plots 1 to 11), diflubenzuron (plots 12 to 15), and control plots (plots A to F) in Newfoundland in 1988. **BLACK YEAST** = infection by black yeast fungi; **ENTOMOPHTHORACEAE** = infection by *Entomophaga aulicae* or *Erynia radicans*; **PARASITOIDS** = parasitism by tachinids.

Table 6. Current-year (CURR) and one-year-old (1 Yr) foliage saved in plots treated with *Bacillus thuringiensis* (Dipel, Futura) or diflubenzuron (Dimilin) formulations in Newfoundland in 1988. Calculations were made by comparing defolation estimates in the treated plots to those in matched control plots and areas bordering the treatment plots. n/a = no defoliation in either the bordering area or control plot.

Plot No.	Foliage saved (%) in comparison to:			
	Control plot		Border areas	
	CURR	1 YR	CURR	1 YR
Dipel 176 (30 BIU/ha)				
1	66	0*	89	52
5	100	100	100	100
8	0	0*	55	52
Dipel 176 (40 BIU/ha)				
2	100	100*	100*	100
9	100	100*	n/a*	n/a*
Dipel 264 (30 BIU/ha)				
3	100	100*	100	100
7	81	100*	98	100
10	86	57	76	38
Dipel 264 (40 BIU/ha)				
4	100	100	100	100
11	82	0	n/a*	0*
Futura XLV (30 BIU/ha)				
6	34	21	0	0
Dimilin ODC (70 g.ai./ha)				
12	27	0	42	28
14	0	0*	0	0
Dimilin ODC (120 g.ai./ha)				
13	0	0	0	4
15	0	0	9	11

* Defoliation in matched control plot or areas bordering treated plot 1% or less.

matched control plot and 42% when compared to the bordering area for plot 12; there was little or no foliage saved for plots 14, 13 and 15 (Table 6).

DISCUSSION

Assessment of spray efficacy was complicated by extensive mortality from natural causes. We feel that the use of Abbott's formula adequately represents larval population reductions due to treatment because the incidence of disease in the treated and control plots was similar (Fig. 2). The pupal population reductions calculated probably are inaccurate because the sample sizes were generally small.

Populations in the control plots collapsed but generally did not do so before defoliation had occurred. In some control plots defoliation was as high as 83%. Therefore, forest protection measures may still be required in declining stages of looper outbreaks.

Single treatments at 70 and 120 g. ai. per ha of the oil-based formulation of diflubenzuron, Dimilin ODC, failed to result in adequate foliage protection despite good deposit and early application. As observed in studies testing the field efficacy of water-based formulations of diflubenzuron (Retnakaran *et al.* 1988, Raske and Retnakaran 1987, Raske *et al.* 1992), larval mortality did not reach high levels until considerable defoliation had occurred. All results to date indicate that diflubenzuron is not a practical option to control larval populations of the hemlock looper.

Single applications of *B.t.* were most effective in preventing defoliation by the hemlock looper during the period beginning with the first appearance of second-instar larvae and ending with the appearance of third-instar larvae. This defines the optimum spray window. Earlier applications may not persist long enough to affect late-hatching larvae; later applications provide reduced protection due to pre-spray defoliation.

The decision whether to use one or more applications of *B.t.* is affected by a number of factors including the density of hemlock looper populations, weather, stand value, expected natural mortality levels, and available funds. In areas with mostly low to moderate populations, one application should suffice. Where populations are high, double applications are recommended. The first application should be made early in the spray window to prevent defoliation. A second application should be made within one week unless monitoring in the spray block indicates sufficient population reduction.

Post-spray precipitation affects the persistence of applications of aqueous *B.t.* formulations (Leong *et al.* 1980, Van Frankenhuyzen 1987, Van Frankenhuyzen and Nystrom 1989), but information is needed on the persistence of the newer and highly potent oil-based formulations. Exposure to 8 mm of rain within 24 h (Plots 10 and 11) and 14 mm in 48 h (Plots 1 and 2) following treatments of the Dipel formulations (Table 3) did not significantly reduce the number of dyed droplets on current or one-one-year-old foliage. However, for the applications of Dipel 176 and Futura XLV the number of droplets recorded for the 48-h samples was significantly higher than for the 1-h samples (Table 3, plots 5 and 6). The light shower occurring within 24 h of these applications may have contributed to smearing and fragmentation of dyed droplets resulting in the higher number of smaller droplets recorded. Van Frankenhuyzen (1987) noted disintegrated droplets on misted balsam fir foliage treated with Thuricide 48LV, an aqueous *B.t.* formulation. The relationship between the persistence of dyed droplets and residual activity of *B.t.* was not established in our study, but is worth pursuing if there is continued use of tracer dyes to measure spray deposits.

Van Frankenhuyzen and Nystrom (1989) reported that 6 mm of rain falling within 7 h of application resulted in a 50% loss of residual toxicity of Thuricide 48LV on potted balsam fir trees. However, the same accummulation of rain within 6 h of applications of Dipel 176 at 30 and 40 BIU per ha (Table 3) on 2 July failed to prevent excellent population reduction of late-instar larvae (Table 4, 21 d Post-spray). The application at 30 BIU per ha provided only a 38% reduction of early-instar larvae suggesting that some wash-off of *B.t.* occurred yet at 40 BIU per ha the larval reduction was 83% (Table 4, 10 to 12 d Post-spray). When 8 mm of rain fell within 24 h of application for late treatments of Dipel 264, better larval reduction was obtained at 40 BIU per ha (81%) than at 30 BIU per ha (69%) (plots 10,11; Tables 3,4). Therefore, higher doses should be considered if rain is threatening. The 0.9 mm of rain within 24 h of application at 30 BIU per ha on 5 July did not appear to affect the efficacy of Dipel 176 and Dipel 264, but may account for the poor performance of Futura XLV (plots 5,6,7; Tables 3,4,5).

Rain is frequent in the coastal fir forests of Newfoundland where looper infestations are most common. Thus treatments may have to be made under sub-optimal conditions to remain within the spray window. Therefore, it is important to use a product which continues to provide protection after rain occurs. Our results support the use of *B.t.* in oil-based formulation, but additional field-testing of highly potent water-based formulations of *B.t.* is warranted.

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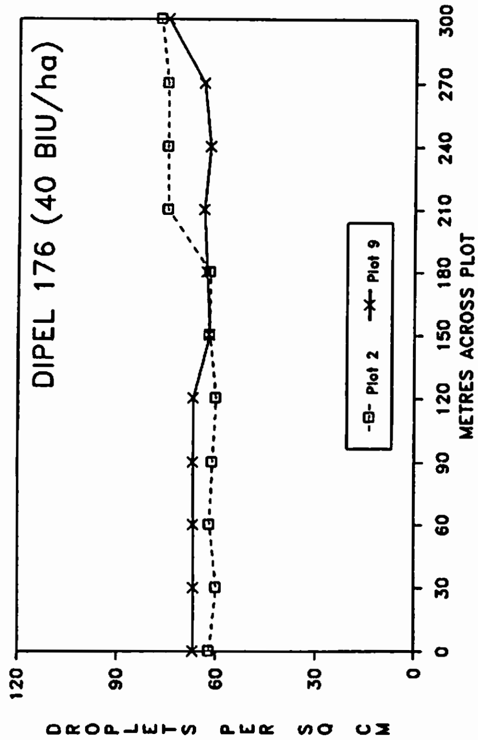
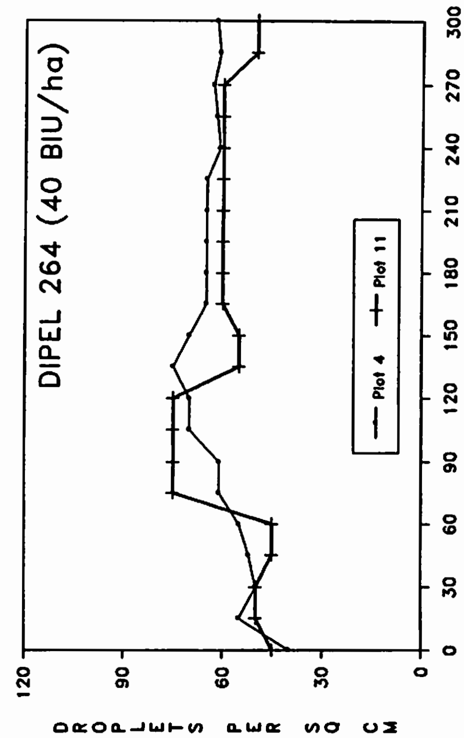
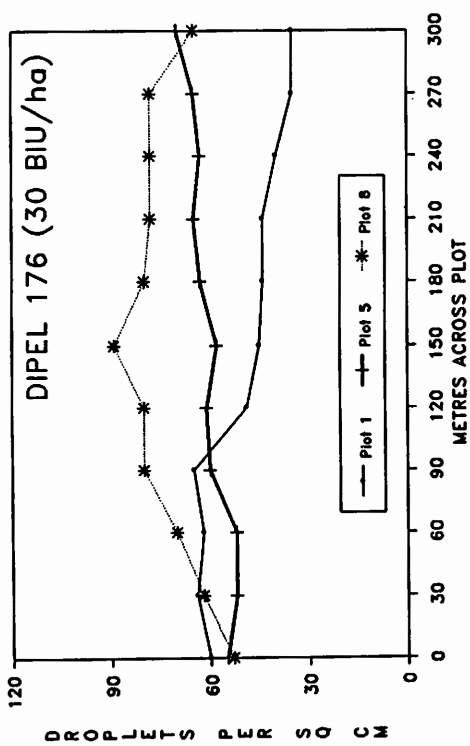
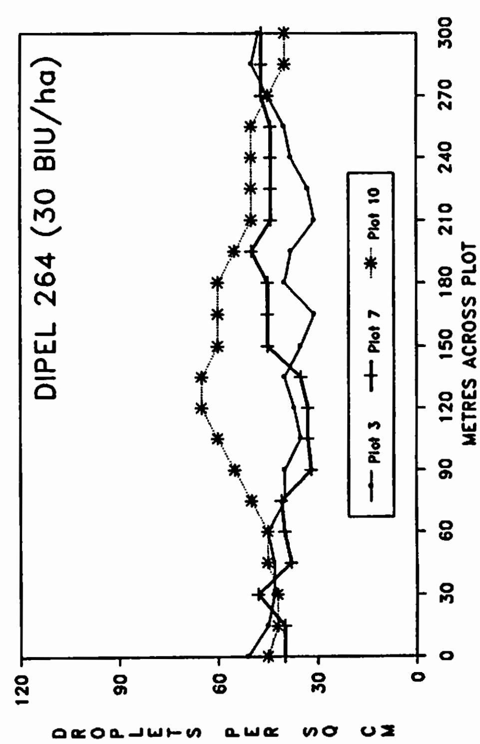
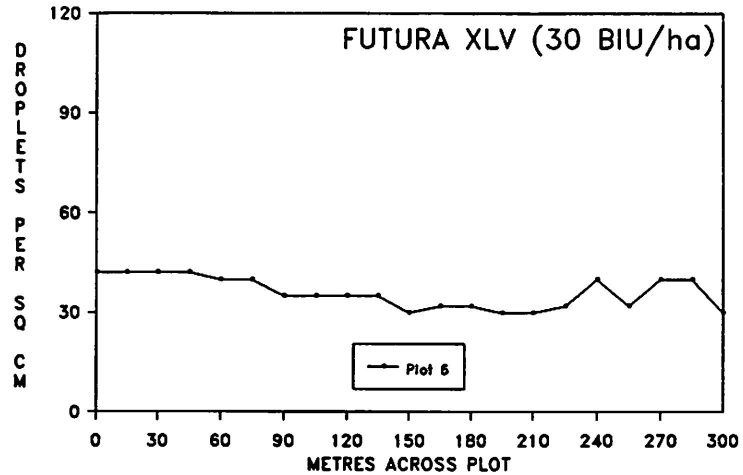


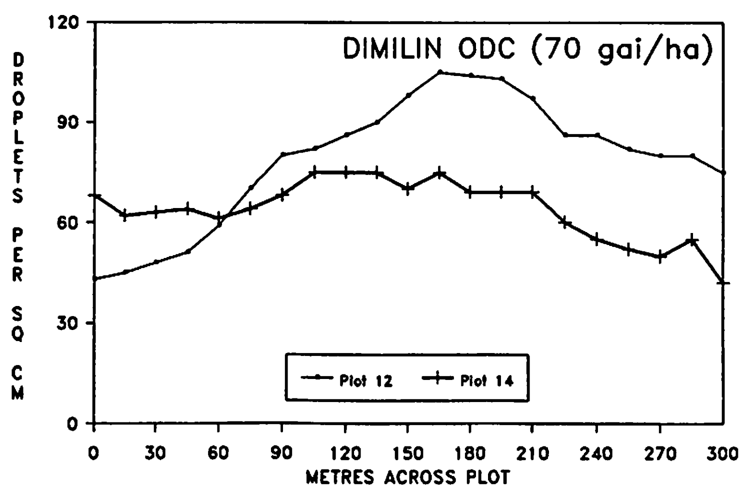


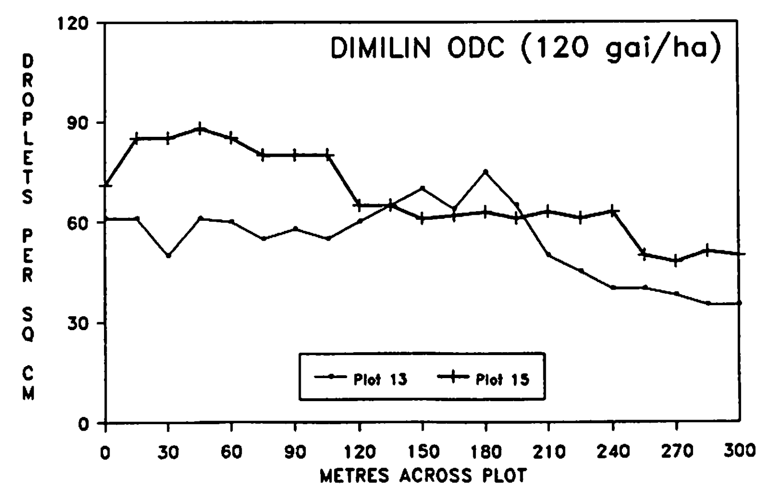
Appendix I. Droplet densities across plots sprayed with *Bacillus thuringiensis* or diflubenzuron formulations in Newfoundland in 1988.






FUTURA XLV (30 BIU/ha)
DROPLETS PER SQ CM
METRES ACROSS PLOT
Plot 6
0
30
60
90
120
150
180
210
240
270
300


DIMILIN ODC (70 gai/ha)
DROPLETS PER SQ CM
METRES ACROSS PLOT
Plot 12
Plot 14
0
30
60
90
120
150
180
210
240
270
300


DIMILIN ODC (120 gai/ha)
DROPLETS PER SQ CM
METRES ACROSS PLOT
Plot 13
Plot 15
0
30
60
90
120
150
180
210
240
270
300

Appendix II. Concentrations of diflubenzuron in foliage sampled from the mid-crown of balsam fir trees in plots treated with Dimilin ODC in Newfoundland in 1988. Three replicate samples were analyzed for each cluster of 8 trees.

Plot No.	Average Concentrations of Diflubenzuron (ppm)			
	Cluster 1	Cluster 2	Cluster 3	Average
Dimilin ODC (70 g.ai./ha)				
12	5.4	10.6	9.3	8.4
14	1.2	1.6	3.1	2.0
Dimilin ODC (120 g.ai./ha)				
13	3.2	1.6	1.3	2.0
15	2.1	4.1	5.1	3.7

Appendix III. Defoliation of current-year (CURR) and one-year-old (1 YR) foliage in plots treated with *Bacillus thuringiensis* (Dipel, Futura) or diflubenzuron (Dimilin) formulations (TRT), matched control plots (CONT) and areas bordering the treatment plots (BORD).

	Mean Defoliation/Tree (%)							
	TRT		CONT		BORD 1		BORD 2	
Plot No.	CURR	1 YR	CURR	1 YR	CURR	1 YR	CURR	1 YR
Dipel 176 (30 BIU/ha)								
1	2	8	6	1	37	33	0	0
5	0	0	10	5	62	63	7	14
8	32	27	30	20	67	48	74	62
Dipel 176 (40 BIU/ha)								
2	0	0	6	1	1	3	0	6
9	0	0	6	1	0	0	0	0
Dipel 264 (30 BIU/ha)								
3	0	0	6	1	2	2	66	59
7	1	0	6	1	46	37	50	53
10	8	14	56	34	3	2	60	46
Dipel 264 (40 BIU/ha)								
4	0	0	38	28	58	45	17	17
11	2	6	11	5	0	0	1	1
Futura XLV (30 BIU/ha)								
6	55	47	82	59	56	40	46	37
Dimilin ODC (70 g.ai./ha)								
12	22	28	30	20	0	0	75	79
14	8	10	6	1	0	0	7	11
Dimilin ODC (120 g.ai./ha)								
13	54	49	11	5	75	80	27	23
15	47	40	38	28	45	50	57	39