Aerial applications of *Bacillus thuringiensis* formulations against eastern blackheaded budworm in Newfoundland in 1990

R. J. West and J. Carter

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Cover Photos

Left: Eastern blackheaded budworm, *Acleris variana* (Fern.)
Right: Balsam fir bud infested with *A. variana*.

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AERIAL APPLICATION OF *BACILLUS THURINGIENSIS* FORMULATIONS AGAINST EASTERN BLACKHEADED BUDWORM IN NEWFOUNDLAND IN 1990

by R.J. West and J. Carter

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ABSTRACT

Oil- and water-based formulations of *Bacillus thuringiensis* (*B.t.*), Dipel 176 and Futura XLV, were applied twice at a dosage of 30 Billion International Units (BIU) per ha on four 45 ha plots in a balsam fir, *Abies balsamea* (L.) Mill., forest infested with eastern blackheaded budworm, *Acleris variana* (Fern.). Spray deposit was better than one droplet per needle. Population reductions 10 days after the second application were 52 and 94% for the two treatments of Dipel 176 and 84 and 85% for the two treatments of Futura XLV. Analysis of upper-crown branch samples indicated foliage savings of 0 and 19% for the Dipel treatments and 8 and 50% for the Futura treatments. Whole-tree estimates of current-year defoliation indicated that no foliage was saved in the plots treated with Dipel and that savings of only 1 and 7% resulted from the treatments with Futura. This lack of efficacy was attributed to the feeding behaviour of larvae; the blackheaded budworm feeds within buds and is less likely to ingest a lethal dose of *B.t.* than a defoliating species that feeds openly. Neither product can be recommended for control of blackheaded budworm on the basis of results from these aerial spray trials.

RÉSUMÉ

Des formulations huileuses et aqueuses de *Bacillus thuringiensis (B.t.)*, le Dipel 176 et le Futura XLV, ont été appliquées à deux reprises, au taux de 30 milliards d'unités internationales (MUI) par ha, sur quatre lots de 45 ha d'une forêt de sapin baumier, *Abies balsamea* (L.) infestée de tordeuses à tête noire de l'epinette, *Acleris variana* (Fern.). Le dépôt des pulvérisations a été supérieur à une gouttelette par aiguille. Dix jours après la seconde application, on a relevé des réductions des populations de 52% et de 94% pour les deux traitments de Dipel 176, et de 84% et 85% pour les deux traitements de Futura XLV. L'analysse d'échantillons de branches prélevées dans la partie supérieure du houppier a indiqué que les traitments au Dipel ont permis de préserver 0% et 19% des aiguilles, et ceux au Futura de 8% et de 50%. Pour l'année visée par la présente étude, des estimations de défoliation portant sur l'arbre complet indiquent au'aucun feuillage n'aura été préservé étude, dans les traités au Dipel, et qu'une préservation de 1% à 7% aura été réalisée grâce aux traitements au Futura. Ce manque d'efficacité a été attribué au comportement alimentaire des larves; la tordeuse à tête noire se nourrissant à l'intérieur des bourgeons, elle est moins susceptible qu'une espèce défoliatrice qui se nourrit à l'air libre d'ingérer une dose mortelle de B.T. Compte tenu des résultats de ces essais de pulvérisation aérienne, aucun des deux produits ne saurait être recommandé pour lutter contre la tordeuse à tête noire de l'épinette.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

ACKNOWLEDGMENTS

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AERIAL APPLICATION OF *BACILLUS THURINGIENSIS* FORMULATIONS AGAINST EASTERN BLACKHEADED BUDWORM IN NEWFOUNDLAND IN 1990

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INTRODUCTION

Outbreaks of eastern blackheaded budworm, *Acleris variana* (Fern.), periodically occur in balsam fir, *Abies balsamea* (L.) Mill., forests in eastern Canada. Damage in past outbreaks has included one or two years of severe defoliation with slight losses in radial increment but no tree mortality (Miller 1966). The biology of the blackheaded budworm also was described by Miller. Eggs overwinter and larvae hatch in late May and early June, feeding until they pupate in August. The total number of instars is four for males and five for females. The pupal stage lasts about 20 days and adults mate in August and September. Eggs are laid singly from mid-August to mid-September on the underside of needles. Hosts besides balsam fir include white spruce, *Picea glauca* (Moench) Voss, red spruce, *P. rubens* Sarg., and black spruce, *P. mariana* (Mill.) B.S.P. (Miller 1966). Western hemlock, *Tsuga heterophylla* (Raf.) Sarg., is the preferred host of the western blackheaded budworm, *A. gloverana* Wlshm (Miller 1966).

The present outbreak of the blackheaded budworm in Newfoundland began in 1987 between Plum Point and Roddickton on the Northern Peninsula. Since then the outbreak, alone and in association with eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Guen.), has caused extensive defoliation of mature balsam fir, particularly in the upper crown. Counts of eggs by the Forest Insect and Disease Survey indicated that 89 000 ha of forest would be severely defoliated in 1990 (Clarke and Carew 1990). A consideration to use control measures resulted from this forecast. However, no insecticide is registered for operational use against eastern blackheaded budworm. Forestry Canada and the Newfoundland Department of Forestry and Agriculture cooperated to field-test Futura XLV (ChemAgro, Mississauga, Ont.) and Dipel 176 (Abbott Laboratories, Chicago, IL), formulations of *Bacillus thuringiensis (B.t.)* currently registered and used operationally to control populations of hemlock looper, to provide efficacy data for registration purposes. If effective against blackheaded budworm, *B.t.* formulations would be particularly useful in areas where populations of the looper are also present; both species could be treated at the same time because the feeding periods of larvae coincide. Laboratory bioassays with sprayed foliage indicated that blackheaded budworm is as susceptible to *B.t.* as is hemlock looper (K. van Frankenhuyzen, Forest Pest Management Institute, pers. comm.). The results of field trials in British Columbia against blackheaded budworm with Thuricide SO-75 (Kinghorn *et al.* 1961), Thuricide S7-150 (Morris 1969) and Dipel 176 (Otvos[1], unpublished 1989 data) also indicate that *B.t.* formulations might be effective in aerial application against *A. variana* in eastern Canada.

We report the effectiveness of double applications of Futura XLV and Dipel 176 at 30 BIU/ha in 2.1 and 1.8 L/ha, respectively, against blackheaded budworm to reduce larval numbers and protect fir foliage.

MATERIALS AND METHODS

Plot Layout

Test plots were 45 ha (450 by 1000 m) in area and bisected by a road in a balsam fir, *Abies balsamea* (L.) Mill., forest 20 km east of Plum Point (Fig. 1). Two plots were used to evaluate each of the two *B.t.* formulations. Trees about 8 to 10 m in height, near the road and at least 150 m from the plot edges were chosen for insect population monitoring (Fig. 1). Similarly-sized trees were sampled across

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1 Dr. I. Otvos, Forestry Canada, Pacific and Yukon Region.

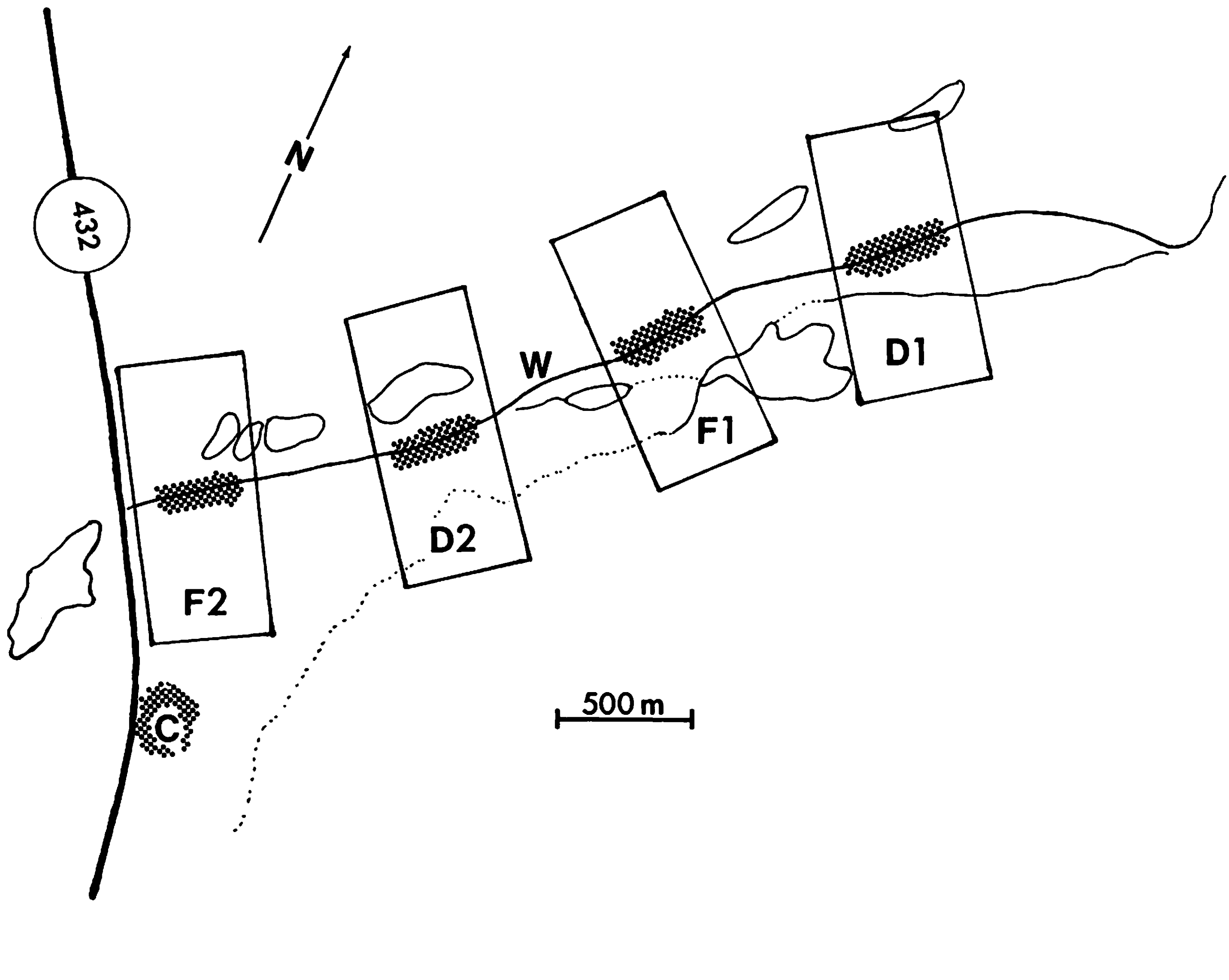

Figure 1. Plot locations for testing *Bacillus thuringiensis* formulations against blackheaded budworm near Plum Point, Newfoundland 1990. D1 and D2, Dipel 176; F1 and F2, Futura XLV; C, control plot; W, weather station. Flight lines during spray application were parallel to the long borders of the plots. Shaded areas were sampled for population and defoliation assessments.

the entire plot for spray-deposit assessment. An untreated area near the treated plots was designated as a control plot (C, Fig. 1). The blackheaded budworm was the main defoliating species present. Larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.), balsam fir sawfly, *Neodiprion abietis* (Harr.), and hemlock looper also were present, but their numbers were very low and did not affect assessments of efficacy.

Spray Formulations and Applications

Dipel 176 and Futura XLV were pre-mixed with a 1% concentration of the tracer dye, Day-Glo fluorescent pigment, and supplied by Abbott Laboratories and ChemAgro Ltd., respectively. The formulations were applied undiluted from a Grumman AgCat aircraft equipped with six Micronair AU4000 rotary atomizers. The plane flew along flight lines 1000 m long and 80 m apart at 140 km/h and at an altitude of about 20 m above the canopy.

Treated plots were sprayed twice at 30 Billion International Units (BIU)/ha, in 1.8 L/ha (Dipel 176) and 2.1 L/ha (Futura XLV) (Table 1). First applications were on 13 July when larval development was 3% first instar, 60% second instar and 37% third instar. Second applications were 8 days later on 21 July when larval development was 51% third instar, 41% fourth instar and 8% fifth instar. Applications were made between 0530 and 0730 h.

Weather during the applications was monitored within the experimental area (Fig. 1) with a 21X data micrologger, two temperature probes, a wind speed and direction sensor, a psychrometer and a tipping-bucket rain gauge (Campbell Scientific, Edmonton, Alta.). Instruments with the exception of the rain gauge were mounted on a 12 m tower. Temperature at 1.5 and 12 m, wind speed and direction at 12 m and relative humidity at 1.5 m above the ground were recorded during spray periods. Rain accumulation was recorded daily at 0000, 0600, 1200, and 1800 h.

Spray Deposit

Spray deposit was determined by counting spray droplets on upper-crown foliage sampled within 2 h of application. Current year needles were examined on both sides under a black light (Raytech Industries Inc., Stafford Springs, CT). Seven hundred needles were examined for each application: 7 needles per shoot, 5 shoots per branch, 2 branches per tree and 10 trees per plot. One tree was sampled every 50 m across each spray plot. Shoots were stored in paper bags in a refrigerator and examined within one week of application.

Effect of Treatment on Population Density

Upper-crown 45 cm branch tips were used to estimate larval numbers. Three clusters of 5 trees

Table 1. Number of droplets per needle on upper-crown balsam fir branches after application of *Bacillus thuringiensis* formulations at 30 BIU/ha near Plum Point, Newfoundland, 1990. Seven hundred needles were examined for each application.

Formulation/plot	Rate applied (L/ha)	Droplets/needle ± SE	
		First application	Second application
Dipel 176/1	1.8	1.4 ± 0.3	1.6 ± 0.3
Dipel 176/2	1.8	1.3 ± 0.4	1.7 ± 0.3
Futura XLV/1	2.1	2.0 ± 0.3	2.5 ± 0.3
Futura XLV/2	2.1	2.0 ± 0.6	3.2 ± 0.6

were used for sampling each plot with one branch sampled per tree. The tree clusters were located at points 150, 225 and 300 m across the test plots and were about 50 to 75 m apart in the control plot. Samples were taken 4 days before each application; a final post-spray sample was taken 10 days after the second application. Samples were stored indoors at 20°C and processed on the day after collection. The following information was recorded for each branch sample: number of buds (N), number of buds attacked by the blackheaded budworm (N_B), and number of live blackheaded budworm larvae dissected from 10 attacked buds (N_{B10}). The number of larvae per 100 buds (L_{100}) was calculated by:

$$L_{100} = \frac{(N_B)\ (N_{B10})\ x\ 10}{N}$$

Population reduction due to the *B.t.* treatment was calculated using Abbott's (1925) formula modified by Fleming and Retnakaran (1985).

Effect of Treatment on Preventing Defoliation

The 10 buds dissected to determine larval numbers also were used to determine defoliation levels. The amount of defoliation to the nearest 10% was estimated prior to bud dissection. Current-year defoliation per branch (DEF_{BR}) was calculated by:

$$DEF_{BR} = (DEF_{B10})\ (\%_B),$$

where DEF_{B10} = mean defoliation on 10 attacked buds

$\%_B$ = percentage of attacked buds on branch.

The amount of current-year defoliation on entire tree crowns was estimated to the nearest 10% from the ground on 21 August for all sample trees.

The percentage of foliage saved (F) as a result of treatment was calculated by:

$$F = \frac{DEFOLIATION\ (CONTROL) - DEFOLIATION\ (TREATMENT)}{(DEFOLIATION\ CONTROL)}\ x\ 100$$

Mortality from Treatment, Disease and Parasites

Twenty-five larvae per plot per application were reared singly on balsam fir foliage in 28-ml cups (Bioserve Inc. Frenchtown, NJ) for 6 days at 20°C to determine mortality from treatment. Foliage was obtained from the treated and control plots and larvae were obtained from the control plot on the application dates. The numbers of living and dead larvae were counted on the sixth day of rearing. A further 50 larvae from each spray plot and the control plot were collected on 31 July and reared on unsprayed foliage until death or emergence of moths or parasites. Fresh foliage was provided after 4 to 5 days. Cadavers were examined microscopically at 400 X magnification to identify pathogens and unemerged parasites.

RESULTS

Weather Conditions

Weather conditions during the applications were windy and humid. Winds were from the southwest and ranged from 18 to 20 km/h for the first applications and from 7 to 8 km/h for the second applications. Differences in temperature measured at 1.5 and 12 m above the ground were less than 1°C and indicated a slight inversion bordering on neutral conditions. Temperatures ranged from 9 to 11°C during the first applications and from 12 to 13°C during the second applications. Relative humidity ranged from 95 to 100%. There were rain-free periods of 48 h following the first applications and 12 h following the second applications. Rain accumulation after 24 h after the second application was 5.6 mm.

Spray Deposit

Spray coverage was considered excellent across the plots with an average of over one droplet per needle (Table 1, Fig. 2). Tree-to-tree variation in the number of droplets per needle was high but few trees in the areas sampled for population and defoliation measurements had deposits under one droplet per needle (Fig. 2).

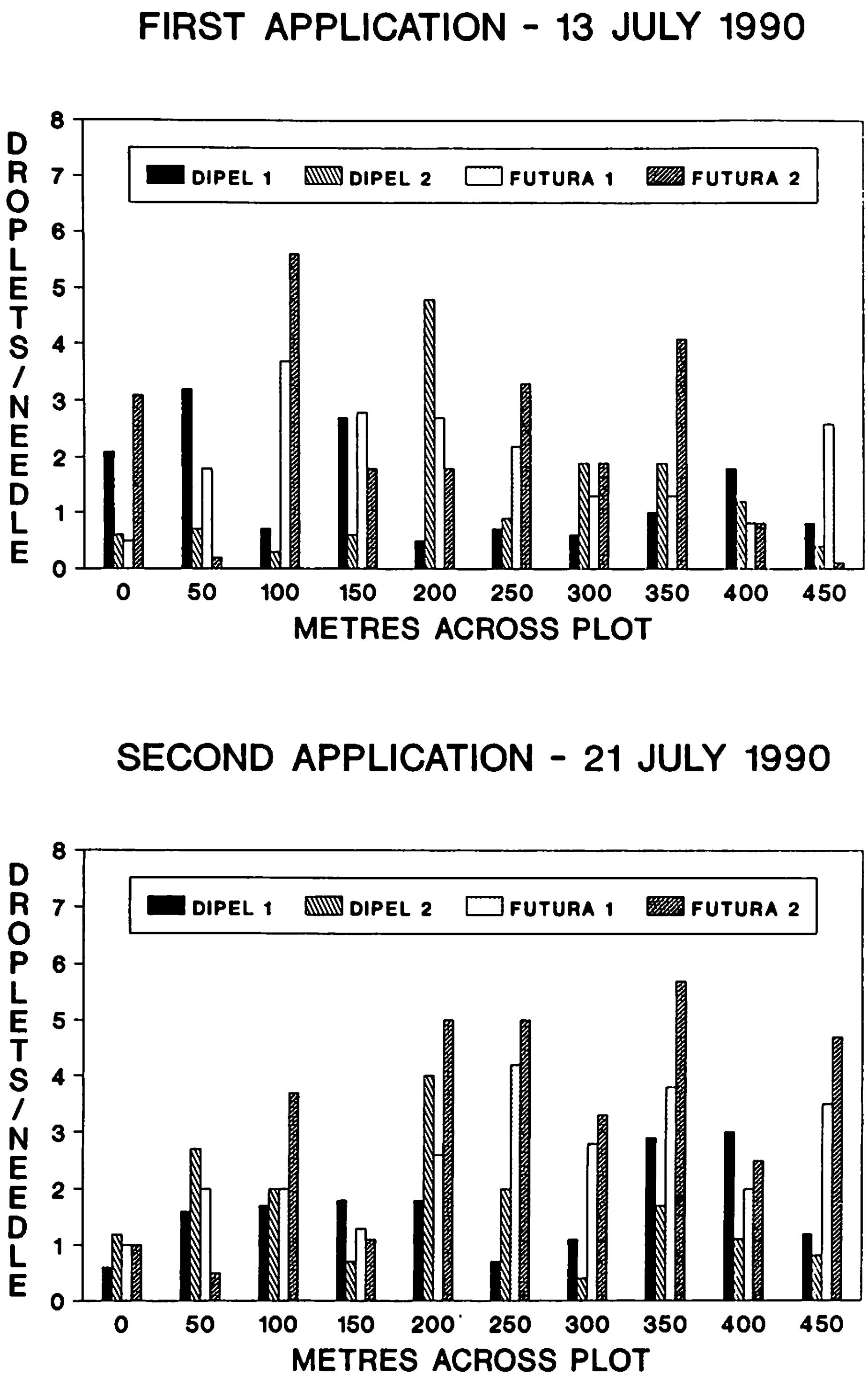

Figure 2. Spray deposits on upper-crown foliage following application of *Bacillus thuringiensis* formulations near Plum Point, Newfoundland in 1990.

Effect of Treatment on Population Density

Counts prior to the first application ranged from 33 to 75 larvae per 100 buds (Table 2) with 31 to 62% of current-year buds attacked (Table 3) indicating high population levels. Population reduction after the first application ranged from 38 to 50% for Dipel 176 and from 0 to 71% for Futura XLV. Absolute decreases in larval numbers in the Dipel-treated plots 5 days after the first application were less than 25%. Five days after the first application of Futura, larval numbers decreased by 55% in one plot but doubled in the other. Population reductions after both applications due to treatment ranged from 52 to 94% for Dipel 176 and from 84 to 85% for Futura XLV.

Effect of Treatment on Preventing Defoliation

The percent of buds infested increased and defoliation increased following the first application of the *B.t.* formulations (Tables 3 and 4). Similar increases in defoliation in 3 of the treated plots occurred following the second application, however, these increases were not as marked as in the control plot (Tables 3 and 4). The amount of defoliation of infested buds did not increase for one of the plots treated with Futura after the second application, but an increase in the percent of infested buds resulted in slightly increased branch defoliation (Tables 3 and 4). There was no apparent foliage protection 5 days after the first application for either formulation, but foliage saved 10 days after the second application was 8 and 50% for the Futura treatments and 0 and 19% for the treatments of Dipel (Table 4). Foliage protection in tree crowns was zero for the plots treated with Dipel and was negligible at 1 and 7% for the plots treated with Futura (Table 5).

Mortality from Treatment, Disease and Parasites

Mortality of larvae reared for 6 days on foliage sprayed in the first applications ranged from 56% for one of the plots treated with Futura to 100% for one of the plots treated with Dipel (Table 6). Mortality of larvae collected from the control plot was only 8%. Mortality of larvae reared on foliage sprayed in the second applications was 80% to 92%, but mortality in the control was 72% (Table 6.) Starvation was likely a mortality factor in the second set of 6-day rearings because most or all of the shoots were eaten by the time the foliage was changed.

Survival of larvae collected 10 days after the second application ranged from 0 to 16% for the treated plots and was only 22% for the control plot. The incidence of parasitism and disease ranged from 32 to 46% and 18 to 46%, respectively (Table 7). Of the parasite species reared, 36% were tachinids (Diptera) and 64% were braconids (Hymenoptera).

Black-yeast fungi representing a complex of at least two species, *Hormonema* sp. (possibly *dematoides* Lagerberg & Melin) and *Aureobasidum pullulans* var. *pullulans* (de Bary) Arn. were consistently present in diseased larvae or pupae. Entomophtoraceous fungi, bacterial cocci and bacilli were occasionally present.

DISCUSSION

Neither Dipel 176 nor Futura XLV were effective in protecting balsam fir foliage from defoliation in spite of excellent spray deposits of between 1 and 3 droplets per needle. Deposits as high as these protect well against hemlock looper (West *et al.* 1989), but the looper is an open feeder and more likely to encounter and ingest a spray droplet than a blackheaded budworm. Hemlock looper larvae feed haphazardly on many needles and shoots but blackheaded budworm larvae can live on the needles of one shoot for their first three instars (Miller 1966). We did not observe blackheaded larvae feeding openly. Larvae fed between needles matted together with silk and only the outer layers of these feeding sites would be expected to receive spray droplets. The discrepancy between the results of these field trials (Table 2) and laboratory bioassays (Table 6) can be attributed to this feeding behaviour. Larvae placed on sprayed foliage in a bioassay are more likely to consume a lethal dose than an undisturbed larva within its feeding site in the field. A blackheaded budworm larva in the field would most likely encounter a lethal dosage of *B.t.* when moving to and creating a new feeding site. Larvae were in their third or fourth instars when the second application was made and the drop in post-spray

Table 2. Effect of double applications of *Bacillus thuringiensis* on larval populations of blackheaded budworm at Plum Point, Newfoundland, in 1990.

Treatment/plot	Larvae/100 buds ($\bar{x}$ ± SE) Pre-1st spray	Pre-2nd spray	Post-spray	Reduction (%) After first application	After both applications
Dipel 176/1	75.7 ± 8.4	72.3 ± 10.6	8.0 ± 3.8	38.1	93.9
Dipel 176/2	43.7 ± 17.0	33.7 ± 3.9	36.7 ± 7.2	50.0	51.7
Futura XLV/1	49.5 ± 15.4	102.0 ± 13.1	14.1 ± 5.6	0	83.6
Futura XLV/2	65.8 ± 5.7	29.5 ± 7.5	16.8 ± 3.8	70.9	85.4
Control	32.9 ± 3.9	50.6 ± 9.2	57.2 ± 8.9		

Table 3. Defoliation of buds in the upper crown of balsam fir trees infested by blackheaded budworm and treated with *Bacillus thuringiensis* formulations near Plum Point, Newfoundland, 1990.

	% Defoliation attacked bud ($\bar{x}$ ± SE)			% of buds attacked on a 45 cm branch ($\bar{x}$ ± SE)		
Treatment/plot	Pre-1st spray	Pre-2nd spray	Post-spray	Pre-1st spray	Pre-2nd spray	Post-spray
Dipel 176/1	16.3 ± 0.6	47.6 ± 5.0	75.3 ± 3.6	62.4 ± 3.4	75.4 ± 4.2	91.1 ± 1.9
Dipel 176/2	11.2 ± 0.8	23.3 ± 1.4	42.5 ± 4.2	31.1 ± 4.3	59.9 ± 3.6	92.8 ± 1.0
Futura XLV/1	13.8 ± 1.4	40.9 ± 3.9	56.4 ± 5.3	32.8 ± 4.0	69.8 ± 5.6	79.3 ± 4.2
Futura XLV/2	13.5 ± 0.6	34.1 ± 4.3	28.4 ± 3.7	47.1 ± 2.5	62.8 ± 5.8	86.2 ± 2.1
Control	10.2 ± 0.1	27.5 ± 3.5	61.5 ± 3.7	35.0 ± 3.7	45.9 ± 3.6	78.8 ± 3.0

Table 4. Current-year defoliation on upper-crown branches and foliage saved in balsam fir trees infested by blackheaded budworm and treated with *Bacillus thuringiensis* formulations on 13 and 21 July 1990 near Plum Point, Newfoundland.

	% Defoliation/branch			% Foliage saved	
Treatment/plot	**9 July**	**17 July**	**31 July**	**17 July**	**31 July**
Dipel 176/1	10.2	35.9	68.6	0	0
Dipel 176/2	3.5	14.0	39.4	0	18.6
Futura XLV/1	4.5	28.0	44.7	0	7.7
Futura XLV/2	6.4	21.4	24.5	0	49.5
Control	3.6	12.6	48.5		

Table 5. Estimates of current-year defoliation in crowns of balsam fir trees treated with *Bacillus thuringiensis* formulations near Plum Point, Newfoundland, in 1990.

Treatment/plot	**% Defoliation/trees ($\bar{x} \pm$ SE)**	**% Foliage saved**
Dipel 176/1	61.0 ± 4.4	0
Dipel 176/2	50.7 ± 4.9	0
Futura XLV/1	44.7 ± 5.2	1.3
Futura XLV/2	42.0 ± 6.4	7.3
Control	45.3 ± 6.7	-

Table 6. Mortality of blackheaded budworm larvae reared in laboratory bioassay for 6 days on foliage sprayed with *Bacillus thuringiensis* formulations near Plum Point, Newfoundland, in 1990. Fifty larvae were collected per plot.

	% Mortality	
Treatment plot	**First application**	**Second application**
Dipel 176/1	100	92
Dipel 176/2	60	80
Futura XLV/1	72	88
Futura XLV/2	56	84
Control	8	72

Table 7. Fate of mature blackheaded budworm larvae collected 10 days after the second applications of *Bacillus thuringiensis* formulations near Plum Point, Newfoundland, in 1990.

	% Survival			% Mortality	
		To adult			
Treatment/plot	**To pupa**	**Male**	**Female**	**Parasites**	**Diseases**
Dipel 176/1	23	2	6	35	42
Dipel 176/2	28	12	0	42	30
Futura XLV/1	22	10	6	32	46
Futura XLV/2	24	0	0	46	30
Control	42	10	12	40	18

larval numbers (Table 2) suggests that some movement to new feeding sites was occurring at this time.

The first *B.t.* application targeted against immature larvae did not reduce damage (second pre-spray samples, Tables 3 and 4), but reductions in the numbers of mature larvae after the second application provided some protection in 3 of the 4 treated plots (Table 4). Treatments also appeared to reduce survival to the pupal and adult stages (Table 7). Based on these results, the operational use of *B.t.* against the blackheaded budworm using current practices is not warranted. A control method is needed to effectively reduce numbers of immature larvae before appreciable damage occurs. Possibly higher dosages of *B.t.* in a more concentrated form would provide adequate protection. Van Frankenhuyzen (1990) suggests that a key to treatment success is "to use highly concentrated formulations to deliver a maximum dose in the first droplet encountered by feeding larvae." Single droplets of Futura XLV and Dipel 176 encountered by some blackheaded budworm larval may have been too small to deliver an efficacious dose resulting in either death or prolonged feeding inhibition. With more potent formulations, even small droplets are more likely to be more efficacious. Before *B.t.* is dismissed as a control option for the blackheaded budworm, we recommend double applications of formulations twice as potent as those used in the current study at dosages of 40 BIU/ha. Such formulations are available from the manufacturer for testing but are not currently registered for forestry use.

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