COMPARATIVE EFFECTIVENESS OF THREE COMMERCIAL FORMULATIONS OF *BACILLUS THURINGIENSIS* FOR CONTROL OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.)

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ABSTRACT

The efficacies of three aerially-applied *Bacillus thuringiensis* (*B.t.*) products, viz. Dipel 88®, a newly registered oil-based formulation, Thuricide 24B, an experimental water-based formulation and Thuricide 16B® were compared against the spruce budworm, *Choristoneura fumiferana* (Clem.). All *B.t.* products were applied at the same dosage rate (20 billion international units/hectare) but Thuricide 24B was sprayed at 4.7 ℓ/ha, and all the others at 9.4 ℓ/ha. The Dipel vehicle (Dipel 88 without *B.t.*) was applied as a check.

The following conclusions were drawn from the results: 1) The *B.t.* treatments in these tests were not especially effective in terms of immediate insect kill but were highly effective against budworm defoliation. Dipel 88 is as effective against the budworm as are Thuricide 16B and 24B applied at similar dosage levels. 2) The efficacy of Thuricide 24B applied at 4.7 ℓ/ha was equivalent to that of Thuricide 16B applied at 9.4 ℓ/ha. 3) There was no significant correlation between ground level droplet density and defoliation. A precise tree deposit assessment technique is sorely needed. 4) Dipel vehicle was not toxic to budworm larvae under field application conditions. 5) Millipore filter membranes were more efficient collectors of atomized *B.t.* than were Kromekote cards. Oil-sensitive cards can be used as deposit collectors for oil-based *B.t.*

Preliminary biomass studies showed that measurements of population density in the year of treatment alone provide an incomplete assessment of the effects of *B.t.* on a budworm population.

RÉSUMÉ

On a comparé l'efficacité trois préparations de *Bacillus thuringiensis* (*B.t.*), nommément le Dipel 88®, préparation huileuse récemment homologuée, le Thuricide 24B, préparation aqueuse expérimentale, et le Thuricide 16B®, employées par épandage aérien contre la tordeuse des bourgeons de l'épinette (*Choristoneura fumiferana* [Clem.] à la même dose (20 x 10^9 U.I./ha); cependant, le Thuricide 24B a été pulvérisé à raison de 4,7 L/ha et tous les autres à raison de 9,4 L/ha. Le solvant du Dipel 88 a aussi été épandu comme témoin.

De cette expérience, on a tiré les conclusions suivantes: (1) Les traitements ne se sont pas avérés particulièrement efficaces à tuer immédiatement l'insecte, mais ils ont été très efficaces à enrayer la défoliation. À des doses similaires, les trois préparations ont eu des effets équivalents sur l'insecte. (2) L'efficacité du Thuricide 24B épandu à raison de 4,7 L/ha a été comparable é celle du Thuricide 16B épandu à raison de 9,4 L/ha. (3) Il n'y a pas eu de corrélation significative entre la densité des gouttelettes au sol et la défoliation. On a grandement besoin d'une technique précise d'évaluation de la densité des gouttelettes sur le feuillage. (4) Le solvant du Dipel 88 n'a pas été toxique pour les larves dans les conditions d'épandage. (5) Les filtres Millipore ont été de meilleurs collecteurs du *B.t.* pulvérisé que les cartes Kromekote. On peut utiliser des cartes sensibles à l'huile pour récolter les dépôts des préparations huileuses.

Des études préliminaires de la biomasse ont montré que les mesures de la densité de la population effectuées pendant l'année du traitement seulement produisent une évaluation incomplète des effets du *B.t.* sur une population de tordeuses.

ACKNOWLEDGEMENTS

We wish to thank Michel Auger, Michel Doré, Paul Brouillette and other members of the Quebec Department of Energy and Resources for their cooperation and help during the course of this study at Rivière-du-Loup. We are grateful to Abbott Laboratories, Sandoz, Inc. and Forshaw Chemical for supplying the biocide and oil-sensitive cards as well as for their financial support. We thank P. Fast (FPMI) for bioassaying the drum samples and A. Sundaram (FPMI) for spread factor analysis.

TABLE OF CONTENTS

INTRODUCTION

It is generally agreed that acceptable protection of coniferous trees from spruce budworm, *Choristoneura fumiferana* (Clem.), damage can be achieved with commercial *Bacillus thuringiensis* (*B.t.*), provided a high enough dosage of the biocide reaches the target. To enhance effectiveness, such a dosage should remain active at the feeding site for several days because the budworm feeds for much of its time in sites sheltered from the insecticide.

One of the limiting factors to the consistent effectiveness of commercial formulations of *B.t.* is the relatively low deposit efficiency of water-based formulations. Such formulations are always subject to the vagaries of spray weather conditions. Considerable evaporation of spray droplets emitted from the aircraft can take place before they reach the target tree, resulting in loss of active ingredient by drift, lower-than-desirable deposit at the feeding site and consequently lower effectiveness. The addition of extra anti-evaporants to tank mixes of commercial *B.t.* has not been proven to enhance deposit efficiency under field conditions.

In 1980, the deposit efficiency and effectiveness of two new commercial formulations of *B.t.*, viz. Dipel 88® and Thuricide 24B, were compared with that of a standard formulation, Thuricide 16B®. According to the manufacturer, Dipel 88®, an oil-based formulation, was designed with improved atomization and deposit efficiency at the tree canopy level in mind. Thuricide 24B is a more highly concentrated version of the water-based Thuricide 16B and was designed to be applied at low volumes. The Dipel vehicle (i.e., Dipel 88® without *B.t.*) was also tested for its toxicity to the budworm under field conditions.

METHODS AND MATERIALS

Plot Preparation

The spray plots of mixed white spruce, *Picea glauca* (Moench) Voss, and balsam fir, *Abies balsamea* (L.), located near Rivière-du-Loup, Quebec, ranged from 40-200 hectares in size. Fifty sampling stations were selected on trails at right angles to the proposed flight lines, and one white spruce or balsam fir 15-20 m in height was selected for sampling at each station in 40-80-hectare blocks. In each 200-hectare block, 70 trees were sampled.

A truck-mounted, Heathkit Digital Weather Computer, Model #ID40001 was installed in one of the plots to measure weather conditions during spray applications. In addition, a recording, tipping-bucket rain gauge, a hygrothermograph and an Eppley Solar Ultraviolet radiometer equipped with a digital recorder, located near the plots, recorded weather conditions at spray time and during the efficacy assessment period following spray applications. Pre- and post-spray test tree

conditions were estimated by counting the number of current buds per unit area of branch surface.

Spray Formulations and Applications

The spray formulations used, lot numbers, application rates, plot sizes and the number of each tree species per treatment block are summarized in Table 1. Note that Thuricide 24B, which was especially designed for low volume application, was applied at 4.7 ℓ/ha and all others at 9.4 ℓ/ha. The mixing sequence was in the order: water, *B.t.* and sticker. The differences in the numbers of white spruce and balsam fir trees was due to uneven composition of the stands.

Table 1. Experimental *Bacillus thuringiensis* applications criteria, Rivière-du-Loup 1980.

Plot No.	Formulation*	Lot. No.	Application rate	Plot Size (ha)	No. of trees** wS	bF
1	Thuricide 16B,	2W00531	20 BIU/9.4 ℓ/ha	80	8	43
2	Thuricide 24B, 66%	91301	20 BIU/4.7 ℓ/ha	40	43	7
3	Dipel vehicle, 25%	SME26590	9.4 ℓ/ha	80	9	41
4	Dipel 88, 25%	14-783CF,	20 BIU/9.4 ℓ/ha	200	9	61
5	Untreated check	-	-	200	50	50

* 0.1% Chevron spray sticker added to all tank mixes.
** Two 45-cm branches sampled per tree for population assessment and 4/tree for defoliation.
Thuricide is manufactured by Sandoz Inc., San Siego, California, and Dipel by Abbott Laboratories, North Chicago, Illinois.

The spray aircraft was a Pawnee PA25-235 equipped with 4 Micronair AU3000 emission units which were calibrated beforehand to deliver the desired application rates. The droplet volume median diameters were 117 ± 23 µm for Thuricide 16B, 122 ± 21 µm for Thuricide 24B and 164 ± 63 µm for Dipel 88. Kromekote cards were used for Thuricide and oil-sensitive cards for Dipel.

The sprays were applied in the morning or evening of June 5-7 (Table 2), when budworm development was 50% L_3 and 50% L_4 (Table 3), with a Micronair blade setting of 35° and flight speed of 150 km/h. Swath widths were 30 m for the 4.7 ℓ/ha rate and 15 m for 9.4 ℓ/ha with the plane flying 15 m above the tree tops.

Table 2. Weather conditions at time of application - *B.t.* experimental trials, Quebec 1980.

Plot	Spray Date, June	Time (Hrs)	Temperature °C at 12m/2m	Wind Speed (km/h) ± SD
1	5	1945 - 2005	14/16	1.6 ± 0.5
		2040 - 2100	12/11	0.4 ± 0.5
2	6	0602 - 0628	7/7	4.9 ± 1.2
3	7	0450 - 0510	6/6	5.0 ± 0.7
		0730 - 0750	NC	NC
4	6	1855 - 2114	13/11 - 19/22	3.5-3.8 ± 0.5-1.5

NC = NOT RECORDED

Table 3. Records of spruce budworm larval development prior to spray application.

Date		Percentage larval population at					Development Index*
		L_2	L_3	L_4	L_5	L_6	
May 29	bF	64	36	0	0	0	2.4
	wS	18	73	9	0	0	2.9
June 2	bF	1	52	47	0	0	3.5
	wS	1	58	40	2	0	3.5
June 5	bF	0	50	50	0	0	3.5
June 8	wS	9	13	37	47	6	4.6
	bF	0	9	39	56	1	4.7

* Mean instar.

Quality Control

Quality control checks of the commercial products were carried out in three ways. Drum samples of 100 ml were collected immediately following arrival of the *B.t.* products from the manufacturers and sent to Dr. P. Fast (FPMI) for bioassay with spruce budworm. The LC_{50} of each field sample was compared to that of the North American standard, HD-1-S-1971. Relative potency was reported as the ratio of LC_{50} standard/ LC_{50} field sample.

Five 200-ml samples of each tank mix were collected just before filling the aircraft, and were frozen. One was sent, packed in dry ice, to each of Sandoz, Inc., Homestead, Florida; Abbott Laboratories, North Chicago, Illinois; and the U.S.D.A., Brownsville, Texas for bioassay. One sample was retained for reference and one for spread factor studies. The potencies of the tank mixes were compared with their theoretical potencies and with each other. These bioassays were part of a cooperative testing program carried out with American colleagues.

The fifth tank mix sample was submitted to Dr. K.M.S. Sundaram (FPMI) for chemical analyses of possible carbamate and organophosphate contamination of the tank mixes. A Tracor Model 550 gas-liquid chromatograph, equipped with a nitrogen phosphorous detector, was used for analysis of carbamate residues. For organophosphate residues, a Hewlett-Packard 5730 A GLC, equipped with a flame photometric detector, was used.

Deposit Analysis

A ground deposit sample unit consisting of two 37-mm diameter Millipore filter membranes and one 10-cm x 10-cm Kromekote or oil-sensitive card, Forshaw Industries, Charlotte, N.C., was placed in a clearing at each sample site and retrieved 30-40 min. after spray application. Millipore filters were incubated on trypticase soy agar overnight at 29°C and the number of colonies developing per unit area was used to estimate deposit density of drops containing *B.t.* Drop size, drop density and deposit volume were estimated from droplets on the cards using a Microcard reader. The spread factors of the various formulations were determined by Dr. A. Sundaram, FPMI, using a droplet generator which was incapable of generating uniform droplets smaller than 77 µm for the Thuricides, 116 µm for Dipel 88® and 151 µm for Dipel vehicle. Drop size distribution analysis was performed on the droplet spectrum of the Dipel 88® by Dr. A. Drummond, National Research Council, Ottawa.

Biological Assessment

Two 45-cm (for population sampling) and four 45-cm (for defoliation assessment) branch tips were collected from the middle to upper third of each sample tree at -2 (2 days before application), 14 and 21

days post application. Branch tips were examined at Rivière-du-Loup to generate the following data:

1. Pre-spray larval densities per 45-cm branch, per 100 buds, per 100 ft^2, and per m^2 of foliage.

2. Population reduction due to treatment.

3. Larval mortality estimates.

4. Residual population densities.

5. Defoliation (Fettes method).

6. Pupal emergence.

Pupae collected from sample branches during the second post-spray sampling and from test plots 5 to 7 days later were reared in a laboratory for emergence studies. The objective was to determine any delayed mortality effect of the treatments on the budworm population.

Biomass Studies

These preliminary studies were intended to determine the effect of the *B.t.* treatments on larval feeding activity. All live insects collected from each sample branch were pooled and killed in a 75% alcohol solution. The insects were pre-dried by decanting the alcohol and drying under an infra-red lamp. After transferal to FPMI, the larval samples were dried in a vacuum oven for 12 hrs at 30°C and pooled samples weighed to the nearest milligram. Observations on the relationship between budworm biomass and defoliation were recorded and analyzed. Observations were also made on a fenitrothion-treated plot from budworm supplied by the province of Quebec.

RESULTS AND DISCUSSION

Pre-spray Plot Conditions

The data on tree conditions in the test plots (Table 4) show no significant difference in shoot density between treatment plots on the basis of mean number of shoots per m^2 or per 45-cm branch tip. White spruce trees on Plot 4 and on the check plots had a slightly higher shoot density than in other plots.

Pre-spray larval densities were generally similar between test plots on balsam fir, but densities were generally lower on white spruce in plots 1 and 2 than on other plots regardless of the criteria used for judging density, i.e., per 45-cm branch, per 100 buds, per 100 ft^2 or per m^2 of foliage (Table 5). Densities on these two plots, however, were high enough to cause more than 50% defoliation if left untreated.

Table 4. Densities of current year shoots on sample trees at Rivière-du-Loup.

No. Buds	Plot 1 wS	Plot 1 bF	Plot 2 wS	Plot 2 bF	Plot 3 wS	Plot 3 bF	Plot 4 wS	Plot 4 bF	Check wS	Check bF	
Per m^2	1270	1075	1090	872	1399	1097	1172	898	1225	816	Pre-Spray
	738	837	785	580	765	719	1327	646	809	594	Post-Spray 1
	869	744	676	605	502	606	910	771	1172	798	Post-Spray 2
	959	885	850	686	889	807	1136	772	1069	702	$\bar{X}$
Per 45 cm br.	186	161	155	126	201	154	183	132	203	139	Pre-Spray
	135	154	131	113	153	142	222	142	168	122	Post-Spray 1
	122	105	95	69	89	88	160	110	188	100	Post-Spray 2
	148	140	127	103	148	128	188	128	186	120	$\bar{X}$

Table 5. Pre-spray larval population densities at Rivière-du-Loup, 1980.

Unit	Plot 1 wS	Plot 1 bF	Plot 2 wS	Plot 2 bF	Plot 3 wS	Plot 3 bF	Plot 4 wS	Plot 4 bF	Check wS	Check bF
Per 45-cm br.	29.6	13.3	21.1	4.9	53.6	17.4	48.2	12.6	49.5	13.7
Per 100 Buds	15.9	8.3	13.6	3.9	21.7	11.3	26.3	9.5	24.4	9.9
Per 100 ft^2	1873	830	1380	317	28185	1148	2869	796	2775	746
Per m^2	202	89	149	34	303	124	309	86	299	80

Trends in larval development in the untreated plot starting May 29 showed generally faster development on white spruce than on balsam fir (Table 3). Development at the final application on June 7 was peak L_4-L_5 on both tree species.

Quality Control

Dr. K.M.S. Sundaram reported that neither aminocarb nor organophosphate contamination was detected by GLC analysis of the tank mixes of Thuricide 16B, Thuricide 24B, Dipel 88 or Dipel vehicle submitted to him.

Data from the bioassay of the drum samples indicated that the potencies of the commercial products used were generally similar to expected potencies (Table 6). The potency differences between Dipel 88 with a ratio of 1.08, and Thuricide with a ratio of 0.7, was not considered significant.

Table 6. Results of bioassay of *B. thuringiensis* drum samples.

Product	Batch No.	Relative Potency*			
		Rep. 1	Rep. 2	Rep. 3	MEAN ± SE
Thuricide 16B	2WO-0531	1.08	0.518	0.48	0.69 ± 0.19
Thuricide 24B	91301	0.957	0.750	0.408	0.70 ± 0.16
Dipel 88	14-783CF	0.99	1.02		1.01 ± 0.01

*Potency expressed as the LC_{50} of HD-1-S/LD_{50} of sample; 2-3 replicates per sample.

The potencies of the tank mixes, as assayed by Sandoz Inc., Abbott Laboratories and U.S.D.A., varied widely for each tank mix (Table 7). The U.S.D.A. values were extremely low, assaying at 27% to 43% of the theoretical potency. Sandoz's potencies ranged from 36% (Dipel) to 68% (Thuricide 16B) of theoretical and Abbott's were 100% (Thuricide 16B), 75% (Thuricide 24B) and 220% (Dipel 88) of theoretical. The reason for this extremely high potency value is obscure. The variations are probably explained by the fact that the dosing techniques used in these bioassays were highly imprecise in terms of known amount of active ingredient ingested.

Deposit Analysis - Aircraft Calibration

The calibration droplet densities of the tank mixes sprayed from the aircraft, flying at about 10 m above the spray cards, were similar for Thuricide 16B sprayed at 9.4 ℓ/ha and for Thuricide 24B sprayed at 4.7 ℓ/ha (Table 8). Volume median diameters were also roughly equivalent but the calculated volumes deposited were 23% and 43%, respectively, of the emitted volume, reflecting the higher concentration of the Thuricide 24B formulation. The droplet density of Dipel 88 was slightly lower than that of the Thuricides but the droplet sizes were significantly larger and total volume deposited significantly higher, at 80% of emitted (Table 8).

Table 7. Results of bioassays of tank mixes: Rivière-du-Loup 1980.

Formulations	Lot No.	Potency (IU x 10^6/ℓ) Theoretical	Sandoz	Abbott	USDA
Thuricide 16B, 50%	2W00.531	2200	1490	2197 ± 705	720 ± 93
Thuricide 24B, 66%	91-301	4400	2370	3308 ± 765	1210 ± 28
Dipel 88, 25%	14-783CF	2200	790	4418 ± 538	875 ± 152
	15-784CF	2200	-	- -	1020 ± 196
Dipel Vehicle, 25%	SME 26590	-	-	NC	NC

NC = Not completed.

Table 8. Aircraft calibration spray card deposit analyses.

Formulation	Plot No.	Droplets per cm^2 ± SD	VMD ± SD (µm)	$\bar{x}$ ℓ/ha ±SD
Thuricide 16B, 50% at 9.4 ℓ/ha	1	25 ± 13	117 ± 23	2.2 ± 1.1
Thuricide 24B, 66% at 4.7 ℓ/ha	2	24 ± 14	122 ± 21	2.0 ± 1.0
Dipel 88, 25% at 9.4 ℓ	4	21 ± 11	164 ± 63	7.5 ± 6.8

Note: Oil-sensitive cards for Dipel 88 and Kromekote cards for Thuricide.

The mean spread factors (± SD) were 1.53 ± 0.06 for Thuricide 16B, 1.46 ± 0.04 for Thuricide 24B, 1.89 ± 0.05 for Dipel 88 and 2.28 ± 0.34 for Dipel vehicle (Tables 9-12). There was an apparent negative correlation between droplet size and spread factor for all formulations. It is evident that the Dipel formulation spread on impact more efficiently than the Thuricide, which may offer some advantage in terms of coverage. Also, the more highly concentrated Thuricide 24B spreads somewhat less than Thuricide 16B (Tables 9 and 10). This difference in spreading is more evident between Dipel vehicle (no solids) and the fully formulated Dipel 88.

Table 9. Spread factor data for Thuricide 16B spray mix at 20 BIU/hectare in 9.4 litres.

Drop size (µm)	Stain size (µm)	Predicted drop size from graph (µm)	Spread factor
77	135	82	1.65
102	165	104	1.59
115	168	106	1.58
116	195	126	1.55
130	195	126	1.55
142	225	148	1.52
157	225	148	1.52
167	255	170	1.50
168	255	170	1.50
193	285	192	1.48
200	285	192	1.48
345	495	347	1.43
$\bar{x}$ 159 ± 69			1.53 ± 0.06

Droplet distribution in the calibration tests (Figs. 1, 2, 3) showed that the percentages of droplets less than 100 µm in diameter were 78% (Thuricide 16B), 65% (Thuricide 24B) and 29% (Dipel 88). Analysis of data by A. Drummond (NRC, Ottawa) showed that less than 0.11% of the total number of Dipel droplets were less than 10 µm in diameter (Appendix I). This finding has significance for workers operating in the spray zone since only droplets which measure less than 10 µm in diameter have been judged to pose any potential health risks if inhaled.

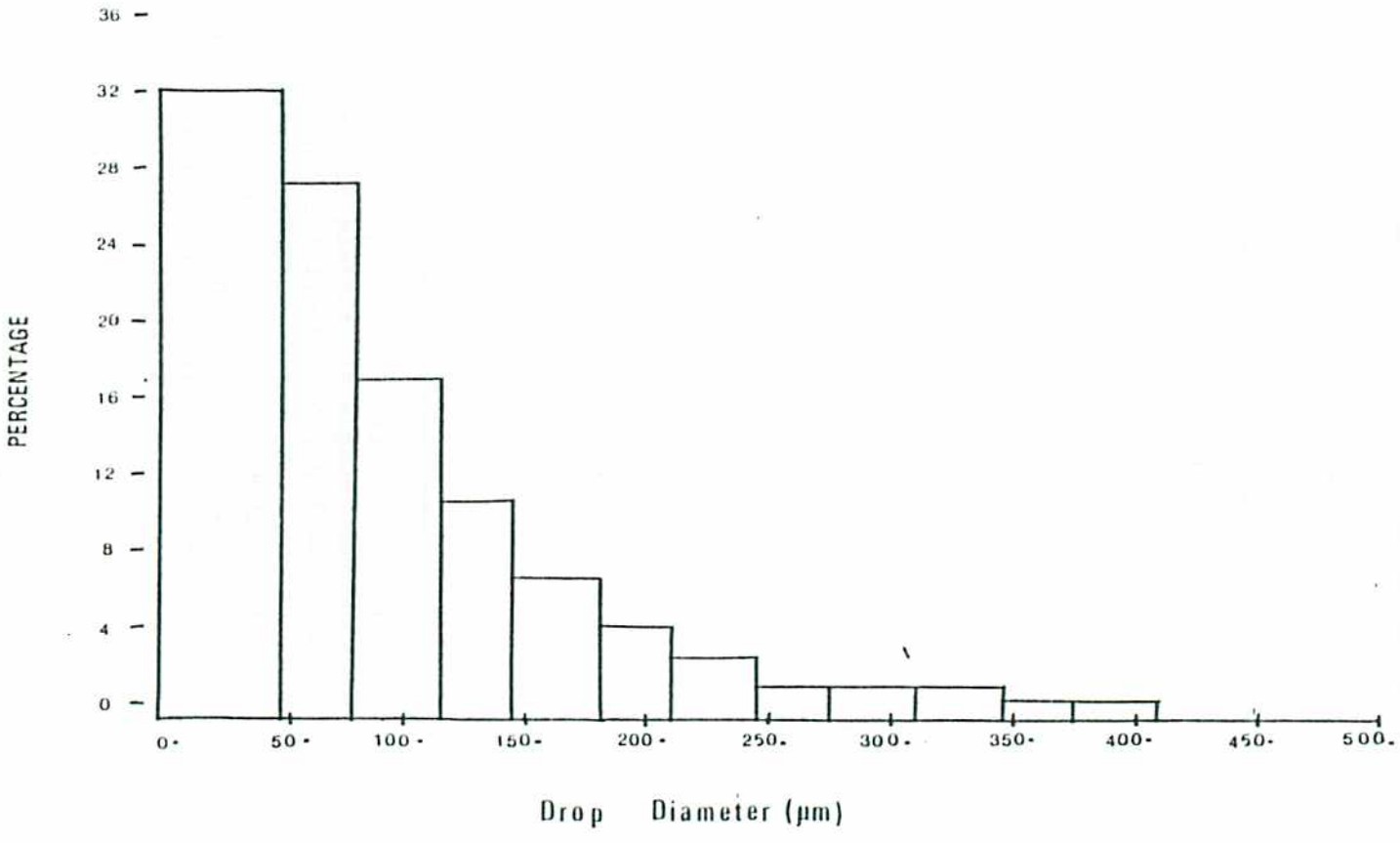

Fig. 1. Droplet size spectrum of Thuricide 16B pre-spray aircraft calibration.

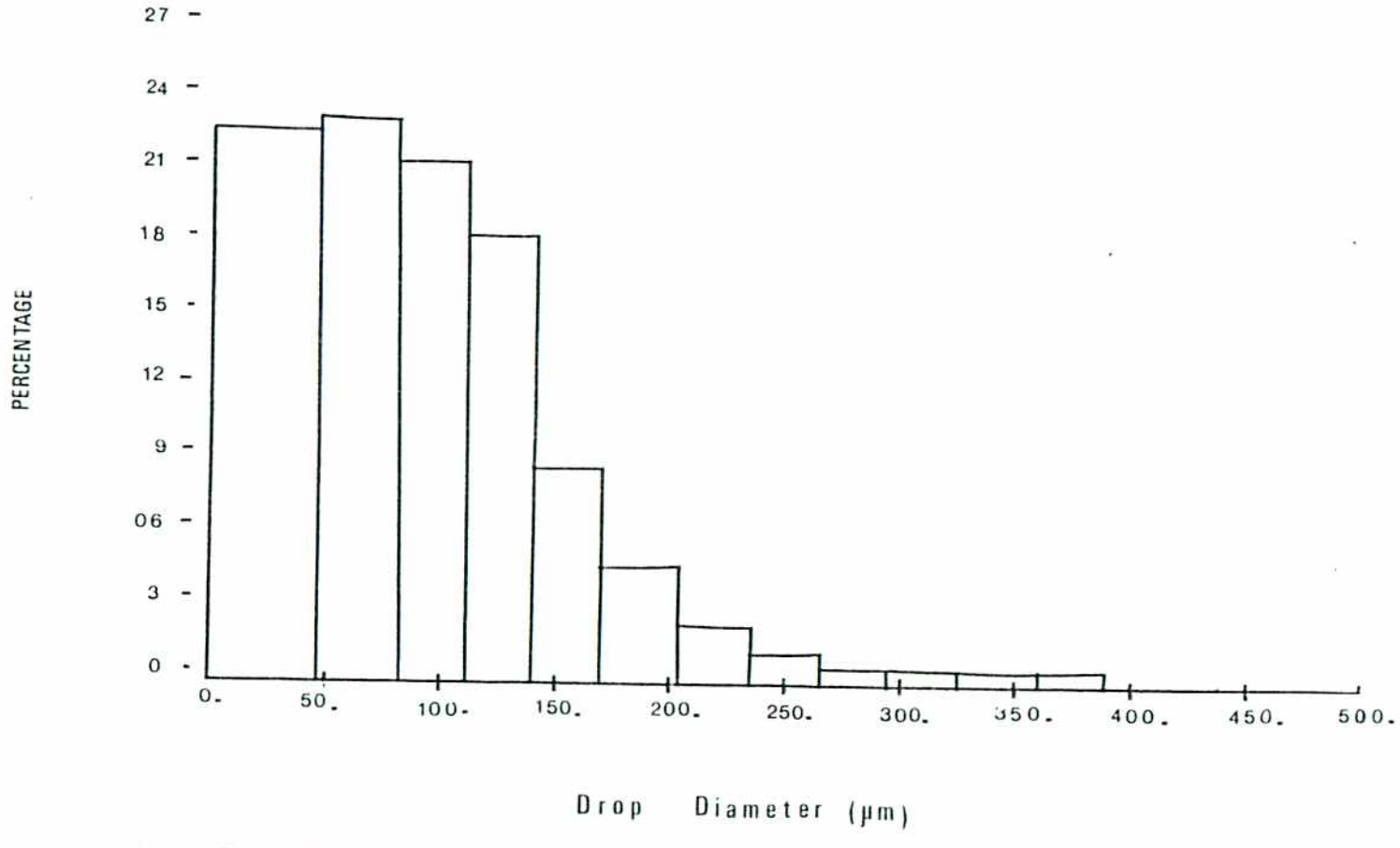

Fig. 2. Droplet size spectrum of Thuricide 24B in pre-spray aircraft calibration.

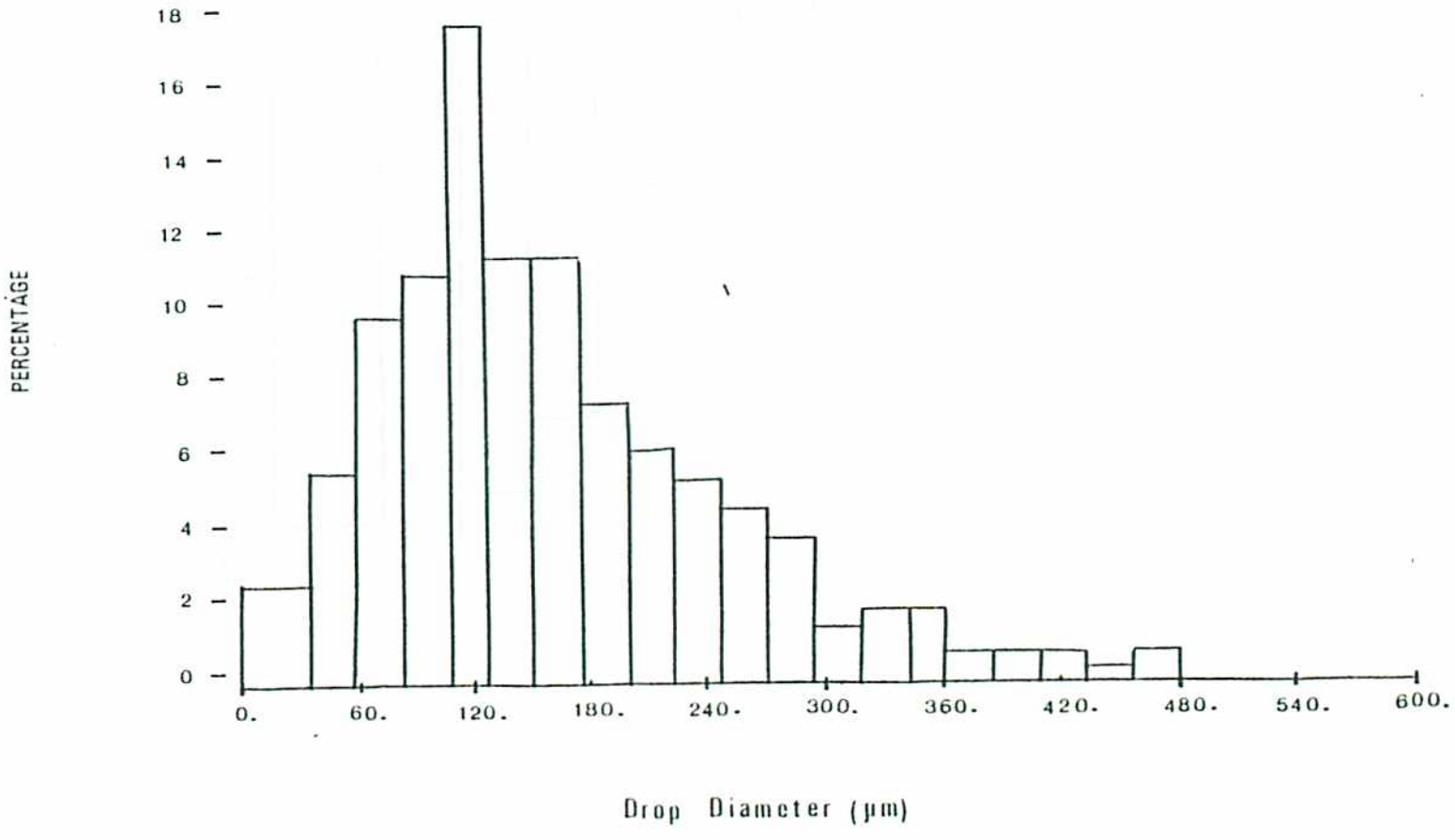

Fig. 3. Droplet size spectrum of Dipel 88 in pre-spray aircraft calibration.

Table 10. Spread factor data for Thuricide-24B spray mix at 20 BIU/hectare in 4.7 litres.

Drop size (µm)	Stain size (µm)	Predicted drop size from graph (µm)	Spread factor
77	103	72	1.46
87	135	92	1.47
102	150	102	1.47
115	174	119	1.46
130	195	133	1.47
142	195	133	1.47
182	270	185	1.46
245	355	243	1.46
$\bar{x}$ 1.35 ± 55			1.46 ± 0.04

Table 11. Spread factor data for "Dipel-88" spray mix at 20 BIU/hectare in 4.7 litres.

Drop size (µm)	Stain size (µm)	Predicted stain size from graph (µm)	Spread factor
116	255	132	1.93
139	312	164	1.90
155	240	123	1.95
232	450	243	1.85
245	435	235	1.85
271	480	261	1.84
$\bar{x}$ 193 ± 64			1.89 ± 0.05

Table 12. Spread factor data for "Dipel vehicle" spray mix at 9.4 ℓ/hectare.

Drop size (μm)	Stain size (μm)	Predicted frop size from graph (μm)	Spread factor
151	402	143	2.81
163	414	155	2.67
188	468	211	2.22
196	471	214	2.20
240	486	230	2.11
265	513	258	1.99
281	528	273	1.93
$\bar{x}$ 2.2 ± 5.1			2.28 ± 0.34

Droplet Analysis - Field Tests

Droplet densities on Millipore filters and on water-sensitive cards were similar for Thuricide 16B and Thuricide 24B applications, despite the higher volume rate of 16B (Table 13). This is due to the finer atomization of 24B as evidenced by the respective VMDs. The droplets of the more highly concentrated 24B formulation will have at least as much, and probably more active ingredient per droplet, so that there seems to be little advantage in applying the higher volume. Droplet densities of the Dipel formulations were moderately high (25-30/cm^2), but significantly lower than those of the Thuricide formulations. Dipel droplets were not detected on the oil-sensitive cards in the field trials despite good deposits on the Millipore filters placed next to them. The reason for this is unknown.

A comparison of the droplet counts on the two collecting surfaces placed side by side at ground level showed that counts on Millipores were significantly higher than on Kromekote cards (Table 14). The correlations were not high, however. The most likely reason for this is the higher sensitivity of the Millipore filters as droplet collectors. Extremely small drops or even single bacterial spores will register as colonies on Millipores but would not be visible on cards using Microcard readers. Also, small droplets would adhere to the filter surface better than to a smooth Kromekote card.

Table 13. Analysis of ground deposits - *B.t.* aerial applications, Rivière-du-Loup, 1980.

Plot No.	Formulation*	Droplet/cm^2 ± SD**		
		Millipore	Oil sensitive or Kromekote	VMD ± SD
1	Thuricide 16B, 50% at 9.4 ℓ/ha	40 ± 12b	30 ± 14b	132 ± 28
2	Thuricide 24B, 66% at 4.7 ℓ/ha	38 ± 17b	33 ± 13b	94 ± 13
3	Dipel Vehicle, 25% at 9.4 ℓ/ha (No *B.t.*)	-	25 ± 11a	96 ± 35
4	Dipel 88, 25% at 9.4 ℓ/ha	31 ± 15a	ND***	- -

* Droplets from plot 4 were not detected on the oil sensitive cards.

** Means within a column followed by the same letter are not significantly different at 5% level (SNK test).

*** No droplets were recorded on cards.

Table 14. Comparison of droplet counts on Millipore filters and Kromekote cards.

Plot No.	Counts/cm^2		Pairwise difference- Mean ± SD (n)	Correlation (r)
	Millipore-Mean ± SD (n)	Kromekote-Mean ± SD (n)		
1	40 ± 12(49)	30 ± 14(49)	9 ± 13(48)*	0.52
2	38 ± 17(45)	33 ± 13(46)	6 ± 18(44)*	0.31

* Significantly different from zero at 5% level (t-test).

Biological Assessment

Weather conditions during the biological assessment period were normal for the area (Table 15). There was relatively little rainfall, so that loss of *B.t.* from the foliage was minimal due to rain.

Table 15. Weather Data: Rivière-du-Loup, June 6-27, 1980*.

Observation Period	Temperature (°C) Mean Max.	Mean Min.	Rainfall (mm)	UV Radiation (cal/cm^2; 295-385 nm)
Prespray-Postspray I				
June 6-20 (Incl.)	21	9	31	340
Prespray-Postspray II				
June 6-27 (Incl.)	21	10	50	510

*Data for relative humidity and total solar radiation are not available due to instrument problems.

None of the treatments caused any apparent change in population density reduction, due partly to the relatively high natural mortality (Table 16). However, the Thuricide treatment apparently had a slightly greater impact on larval population than did the Dipel. Reductions in the Dipel and Dipel vehicle plots were similar to the reduction in the untreated check plot. These data emphasize the fallacy of using insect mortality as the only criterion of effectiveness in pest management operations.

The low budworm kill is reflected in the high residual larval population densities as recorded by the final population assessment (Table 17). Even when the late larval counts were adjusted for natural pupal mortality (Table 18), the residual population densities remained high. This may not be significant, however, since sublethal dosages of *B.t.*, which may have been ingested by the survivors, are known to have physiological effects such as reduced adult fecundity (Soliman et al. 1970, Abdullah and Abdul-Nasr, 1970; Morris and Armstrong 1975), adult teratogenesis (Morris 1969) and decreased pupal weight (Solman et al. 1970; Dulmage and Martinex 1973; Hamed 1978 and Schesser and Bulla 1978). There was no obvious difference between *B.t.*-treated and untreated residual population densities on white spruce but there was on balsam fir.

Table 16. Population reductions in *B.t.* treatment plots, Rivière-du-Loup 1980.[1]

Plot No.	Formulation	Percent population density reduction[2]	
		wS	bF
1	Thuricide 16B	19a	10b
2	Thuricide 24B	9a	1ab
3	Dipel vehiclt (No *B.t.*)	37b	3ab
4	Dipel 88	42b	9ab
Untreated check		40b	0a

[1]Based on number of larvae/45-cm branch.

[2]One-way analysis of variance. Means within a column followed by the same letter are not significantly different at 5.0% level (SNK test).

Table 17. Residual population densities on test plots at final post-spray assessment.

Plot No.	Budworm density per 45-cm branch unadjusted (per m^2)		Budworm density per 45-cm branch adjusted for pupal mortality (per m^2)	
	wS	bF	wS	bF
1	11.6(82)	4.3(30)	8.7(62)	3.2(23)
2	12.2(87)	4.0(34)	6.7(19)	2.2(10)
3	6.9(39)	15.1(104)	3.6(20)	7.9(54)
4	6.3(35)	3.6(25)	4.9(22)	2.3(16)
Check	9.7(60)	14.1(98)	6.0(37)	8.7(61)

Table 18. Emergence of field collected pupae on *B.t.*-treated and untreated plots, Rivière-du-Loup 1980.

Plot No.	Early Collection				Late Collection				Totals			
	No. reared		% emerged		No. reared		% emerged		No. reared		% emerged	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
1	?	?	?	?	103	67	81	85	106	72	78	70
2	51	35	35	29	106	112	63	66	156	147	64	57
3	67	52	64	37	89	72	57	50	146	124	60	44
4	?	?	?	?	18	30	67	66	19	32	63	63
Check	177	198	64	27	80	78	56	65	257	276	75	48

? Numbers collected too small for data analysis.

The data on emergence of pupae collected from the test plots and reared in the laboratory (Table 18) indicated no significant effect of the treatments on pupal mortality.

The percentages of dead larvae collected from the branch samples (Table 19) indicate that the *B.t.* treatments caused substantially greater budworm mortality than did the Dipel vehicle or no treatment. The latter two were similar. It is evident that the high population density reduction reported earlier on the check plot (Table 16) was not due entirely to larval mortality but, more probably, to downward migration or fall from the trees.

Effects on Budworm Biomass

The mean dry weights of budworm larvae and pupae are reported for several dates in June in Tables 20 and 21 and Figures 4-8. The following trends in budworm mass are apparent from these data:

1) Budworm feeding on white spruce are heavier than those feeding on balsam fir. In 21 out of 24 pairs of samples taken on the same dates, insects collected on white spruce were heavier than those collected on balsam fir. In the prespray samples, budworm feeding on white spruce were 45% heavier than those feeding on balsam fir.

2) Growth of budworm larvae is retarded by *B.t.* treatments. This effect is more dramatic on balsam fir than on white spruce (Figs. 4, 5). While a slight decrease in budworm weight appears in the plot treated with the Dipel vehicle alone, this is not considered significant. There is some indication that *B.t.*-infected survivors may recover by about 2 weeks

post-treatment, after which a normal rate of weight increase resumes. In contrast (Fig. 6) survivors in the fenitrothion-treated block show no indication of growth retardation when compared to those in the check plot.

Table 19. Percentages of larvae collected dead on sample branches of white spruce and balsam fir.

Plot	Pre-spray	Post-spray I	Post-spray II	PS 1 + PS II
Check	12.1	3.1	5.8	4.1
1	6.7	37.8	16.0	31.8
2	3.6	38.0	4.7	23.4
3	4.0	6.2	4.4	5.5
4	1.5	57.8	15.9	43.5

For the purpose of this report, budworm biomass is defined as the dry weight of the total spruce budworm population in terms of milligrams per bud. This is equivalent to the mean weight of the insects multiplied by the population density.

The observed reduction of mean budworm dry weight on white spruce in the check plot, from 19 mg to 13 mg between June 26 and 30 (Table 20), is unexplained. This coincides with an unusually large reduction in population density (71%) during the 7 days between the first and second post-spray sample. One may conclude that the larger insects were removed from the sampled population, probably by migration due to lack of food.

Figures 7 and 8 show the changes in budworm biomass on white spruce and balsam fir. In the plots treated with *B.t.* (P1, P2, P4) there is a dramatic decrease in the budworm biomass when compared to the check plot (CP), and to P-3 which was sprayed with Dipel 88 vehicle.

Population densities were unavailabe for the fenitrothion block and, therefore, budworm biomass could not be calculated. Table 22 gives the population density and budworm biomass averaged over the period between the pre-spray sample and the post-spray sample on *B.t.*-sprayed plots.

On white spruce, there is a much better correlation between biomass and defoliation ($r = 0.97$) than between population density and defoliation ($r = 0.55$). On balsam fir, there is a high correlation in both cases ($r = 0.97$ for each).

Table 20. Average dry weight (in mg) of budworm collected on white spruce. Values in parentheses are average dry weight per bud.

June	CP	P1	P2	P3	P4
1		0.40			
2		0.24 (0.04)	0.27 (0.04)		
3					0.32
4		0.49			0.38 (0.10)
5	0.51				
6					
7				0.69 (0.15)	
8	1.03 (0.25)	0.82			
10	1.69	0.96			
11					
12					
13					
14					
15					
16					
17					
18					
19		1.96 (0.40)			
20			3.79 (0.30)		6.71 (0.44)
21					
22				0.57 (1.48)	
23	11.82 (2.10)				
24					
25					
26	18.57	11.89 (1.12)	12.89 (1.65)		
27					8.58 (0.34)
28					
29					
30	12.88 (0.67)	14.91		13.00 (1.02)	

Table 21. Average dry weight (in mg) of budworm collected on balsam fir. Values in parentheses are average dry weight per bud.

June	CP	P1	P2	P3	P4	Block III
1		0.14				
2		0.16 (0.01)	0.23 (0.01)			
3					0.26	
4	0.22	0.46			0.23 (0.02)	
5						
6						
7				0.42 (0.05)		
8	0.80 (0.08)	0.48				
9						
10	1.32	0.31				
11						0.43
12						
13						
14						3.51
15						
16						
17						
18						4.09
19		0.76 (0.04)			1.17 (0.44)	
20			3.92 (0.06)			
21						
22				3.89 (0.46)		3.07
23	6.00 (1.01)					
24						
25						15.99
26	13.89	5.05 (0.21)	4.98 (0.29)			
27					7.42 (0.21)	
28						
29						
30	17.14 (2.42)	12.50		14.97 (2.57)		

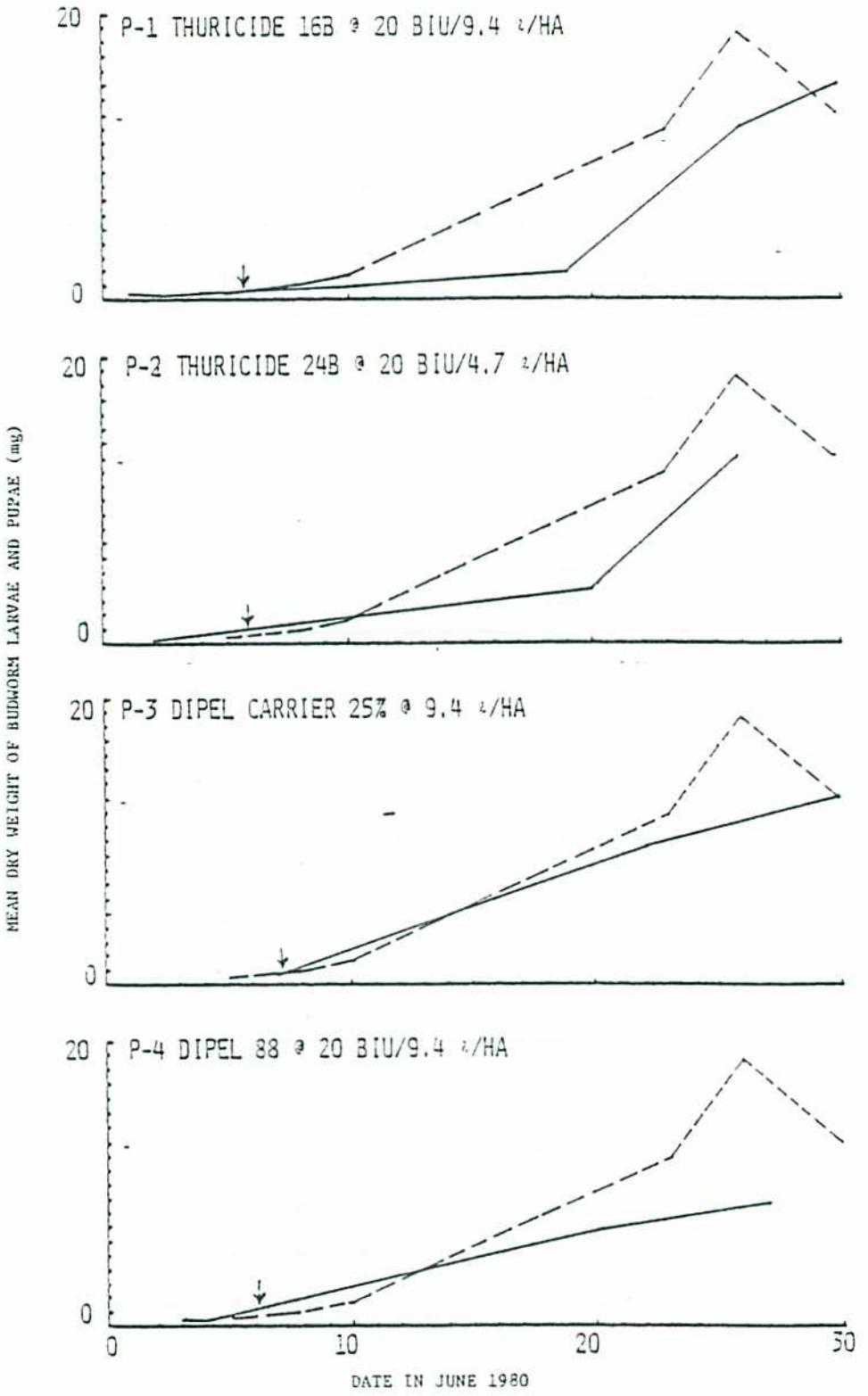

Fig. 4. Mean dry weight of budworm larvae and pupae collected from white spruce. The dotted lines show the trend in the check plot. Spray applications are indicated by arrows.

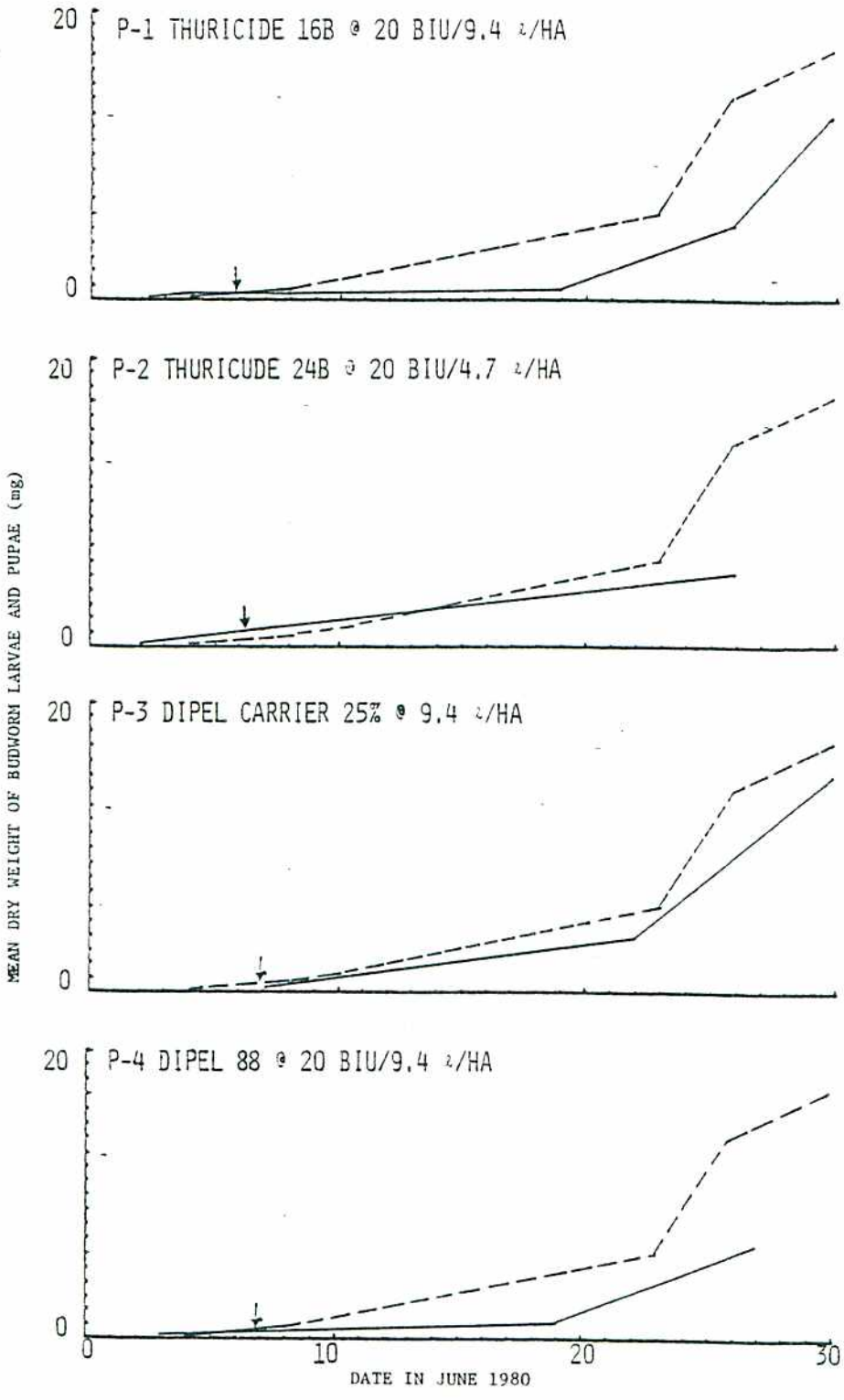

Fig. 5. Mean dry weight of budworm larvae and pupae collected from balsam fir. The dotted lines show the trend in the check plot. Spray applications are indicated by arrows.

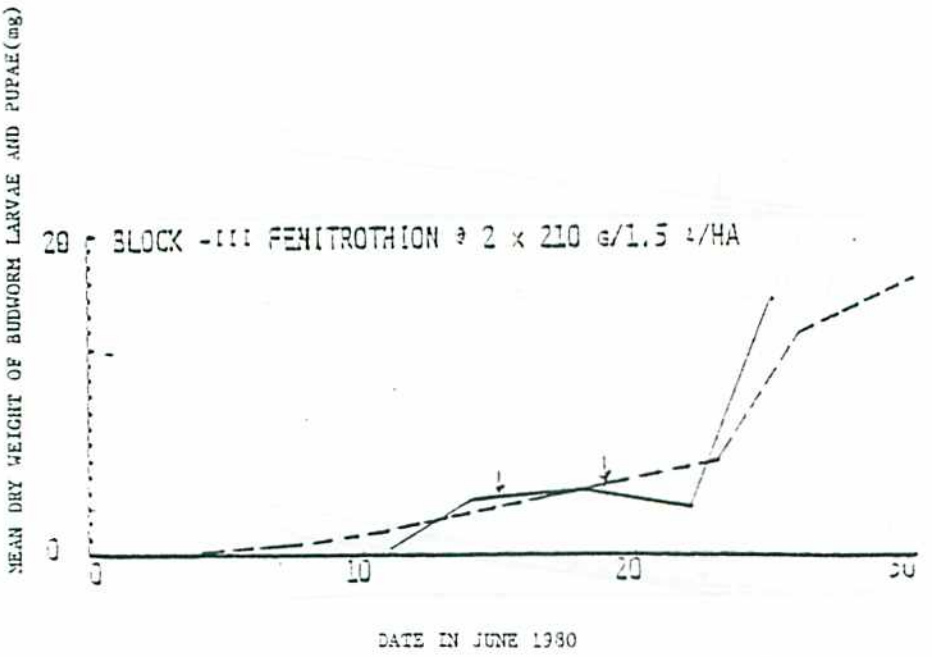

Fig. 6. Mean dry weight of budworm larvae and pupae collected from balsam fir. The dotted lines show the trend in the check plot. Spray applications are indicated by arrows.

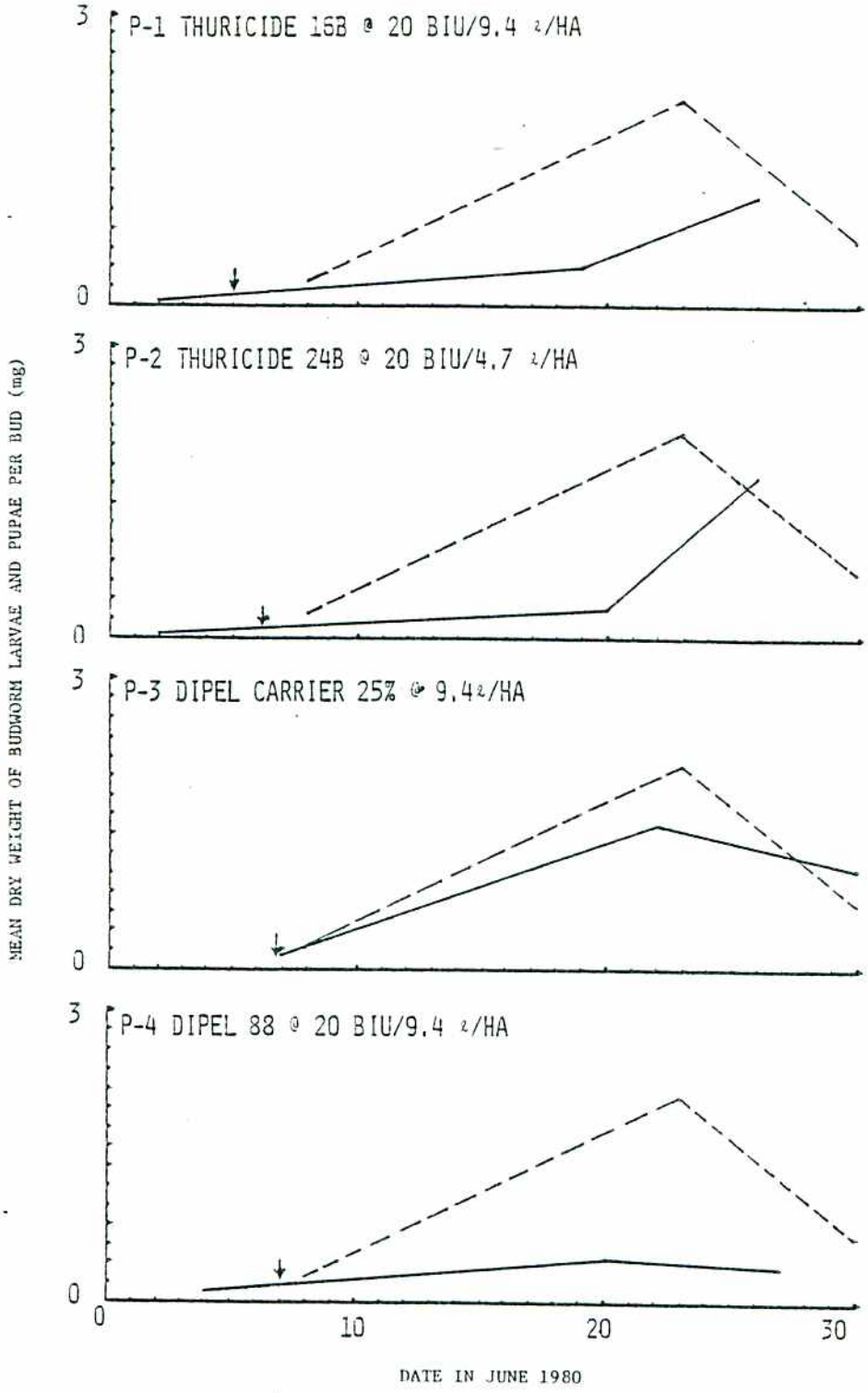

Fig. 7. Mean dry mass of budworm larvae and pupae per developing bud on white spruce. The dotted lines show the trend in the check plot. Spray applications are indicated by arrows.

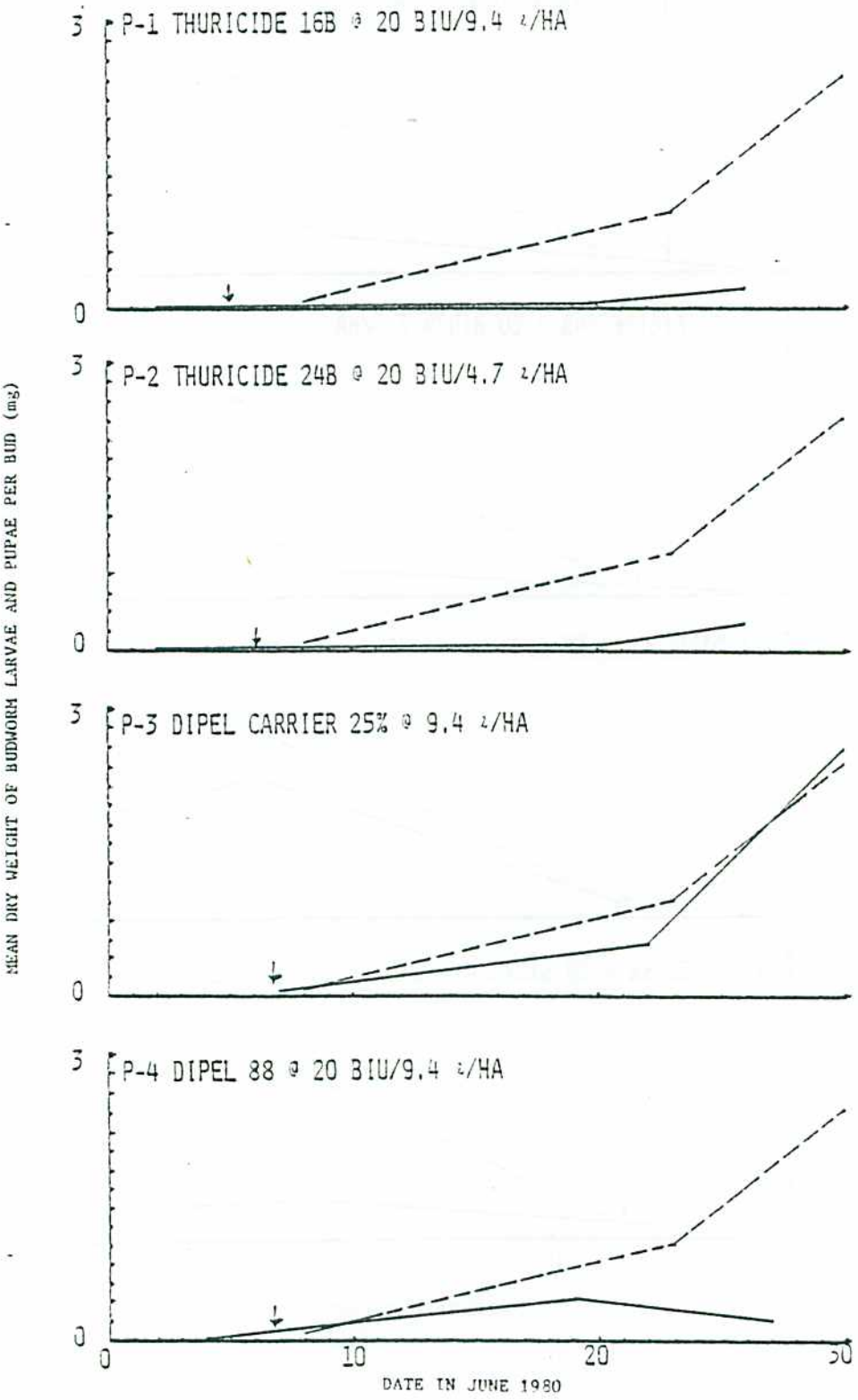

Fig. 8. Mean dry mass of budworm larvae and pupae per developing bud on balsam fir. The dotted lines show the trend in the check plot. Spray applications are indicated by arrows.

Table 22. Relationship of population density, budworm biomass and defoliation on *B.t.*-treated plots.

Plot	Population Density (Larvae per 1,000 Buds)	Budworm Biomass* (µg/Bud)	% Defoliation
White Spruce			
Check	180	1241	50
P1 - TH 16B	173	379	22
P2 - TH 24B	103	371	25
P3 - Vehicle	161	966	51
P4 - Dipel 88	130	304	31
Balsam Fir			
Check	140	918	65
P1 - TH 16B	60	54	20
P2 - TH 24B	28	71	22
P3 - Vehicle	139	743	66
P4 - Dipel 88	46	261	21

*Average population density and biomass between pre-spray sampling and 2nd post-spray sampling inclusive.

Note that, in all cases, there is a greater difference between the biomass in the check plot and that in the treated plot than can be accounted for by the difference in population density. For example, in the case of white spruce in plot 1, there is a 69% difference in biomass, yet only a 4% difference in population density. Therefore, only 4% out of the 69% can be accounted for by reduction in population density. The remaining 65% must be due to sublethal effects expressed as a reduction in the average weight of survivors. This deduction is based upon the premise that, if there are no sublethal effects, the reduction in biomass will equal the reduction in population density. Table 23 shows the relative contributions of lethal and sublethal effects towards the observed reduction in budworm biomass. Note that, when data from all the *B.t.* plots are pooled, there is a net reduction of 78% in budworm biomass, of which 46% is due to lethal effects and 32% is due to sublethal effects.

The aim of any spruce budworm control program is to minimize defoliation of the tree. *B.t.* treatments achieve this aim in two ways: by reducing the budworm population density, and by affecting the growth (and presumably the rate of consumption) of survivors. The above data suggest that sublethal effects provided about 40% of the total reduction in budworm biomass. It is evident that measurements of population density alone provide an incomplete assessment of the effects of *B.t.* on

a budworm population. Measurement of budworm biomass involved no increase in sampling, and only a small increase in sample processing time in return for a more comprehensive assessment of the total effect of treatments on budworm populations. This improvement in efficacy assessment produces a higher degree of correlation with the impact of populations on the foliage, i.e., defoliation. Budworm biomass measurements would be useful in studying the effect of any control method which has a significant, sublethal component, such as slow-acting insecticides, insect growth regulators, and low potency pathogens.

Table 23. Relative contributions of lethal and sublethal effects to reduction in budworm biomass.

	Mean Budworm Biomass		% Difference in Biomass		
Plot	Treated	Untreated	Total	Due to Lethal Effects	Due to Sublethal Effects
White Spruce					
P1 - TH 16B	379	1241	69	4	65
P2 - TH 24B	371	1241	70	43	27
P3 - Vehicle	966	1241	22	11	11
P4 - Dipel 88	304	1241	76	28	48
Balsam Fir					
P1 - TH 16B	54	918	94	57	37
P2 - Th 24B	71	918	92	80	12
P3 - Vehicle	743	918	19	80	12
P4 - Dipel 88	261	918	72	67	4
Both Species					
All B.t. plots	240	1080	78	46	32
Carrier	854	1080	21	6	15

Effects on Defoliation

Defoliation of both white spruce and balsam fir in the B.t. treatment plots was significantly lower (20-31%) than in the Dipel vehicle (51-66%) and check plots (59-65%) (Table 24). The data indicate that the newly-registered Dipel 88 formulation was equivalent in effectiveness to the Thuricide treatments and that 4.7 ℓ/ha of Thuricide 24B was as effective as 9.4 ℓ/ha of Thuricide 16B. It is interesting to compare the effectiveness of Thuricide 16B (Plot 1) and Dipel 88 (Plot 4), both of which were applied at the same rate under similar meteorological and tree conditions (Tables 2, 4). Dipel was applied against a much higher population density on white spruce

(48 vs 30/branch, Table 5) and its ground deposit rate was significantly lower than that of Thuricide (31 vs 40 drops/cm^2, Table 13). In spite of these limitations, the Dipel residual population density was much lower than that of Thuricides (4 vs 9/branch or 22 vs 62/m^2, Table 17), the percentage of the larvae recovered dead was higher with Dipel (75% vs 54, Table 19) and defoliation was similar. It is conceivable that Dipel would have performed even better under more favourable conditions of population density. The similar results obtained with the two Thuricides indicate a need for volume-response field tests for *B.t.* in general.

Table 24. Effect of *B. thuringiensis* treatment on defoliation, Rivière-du-Loup 1980.

Plot No.	Mean percent defoliation ± SD *	
	wS	bF
1	22 ± 11a	20 ± 11a
2	25 ± 7a	22 ± 14a
3	51 ± 13b	66 ± 16b
4	31 ± 8a	21 ± 9a
Check	59 ± 14b	65 ± 19b

* Means followed by the same letter with a column are not significantly different at 5% level (SNK test). Four branches per sample tree, one from each cardinal point.

Lastly, statistical analysis of the ground level droplet density on Millipore filters and percentage defoliation indicated no significant correlation, at the 5% level, between the two on either tree species. The finding emphasizes the need to develop a more precise measurement of tree coverage in relation to efficacy of *B.t.*

CONCLUSIONS

The following conclusions were drawn from this study:

1. The *B.t.* treatments were not especially effective in terms of immediate insect kill but were effective in terms of foliage protection. There was no significant difference between the efficacy of Thuricide 16B, Thuricide 24B and Dipel 88 under the conditions of the tests.

2. A volume rate of 4.7 ℓ/ha appears to be as effective as 9.4 ℓ/ha at the same dosage rate of active ingredient.

3. There appears to be no significant correlation between droplet density at the ground level and defoliation of the corresponding tree.

4. Dipel vehicle was not toxic to budworm larvae under field application conditions.

5. Millipore filters are more efficient collectors of *B.t.* droplets than Kromekote cards. Oil-sensitive cards are effective collectors of oil-based *B.t.* formulations.

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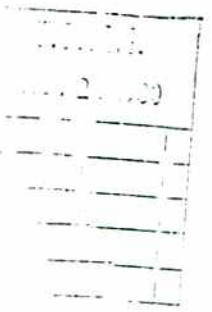
APPENDIX

APPENDIX A

National Research Council Canada
Conseil national de recherches Canada

National Aeronautical Establishment
Établissement aéronautique national



Your file / Votre référence

18 November 1980

Dr. J.A. Armstrong,
Forest Rest Management Institute,
P.O. Box 490,
Sault Ste. Marie, Ontario,
P6A 5M7

Dear Dr. Armstrong:

Your communication of 7 November 1980 requested answers to the following three question's with regard to Dipel 88:

i) how many drops are less than 10μ in diameter?

ii) what proportion of the total number of drops is less than 10μ in diameter?

iii) what proportion of the total volume comes from drops less than 10μ in diameter?

Drop spectrum data was supplied. An Appendix to this letter details the calculations necessary to answer the question's but a few words of explanation regarding the method of solution are appropriate here.

The given drop spectrum was fitted with a Nukiyama - Tanasawa distribution function. This particular function was chosen because formulae for cumulative relative frequency and cumulative relative volume can be obtained by analytical means and because this function has been used with some success in my own research on drop spectra from aerial sprays. The parameter of the distribution function yields an analytical estimate of the volume mean droplet diameter equal to 195.8μ while the volume mean diameter of the given data was 190.3μ. The close agreement of these diameters serves to justify the accuracy of the N.T. fit and the application of derived quantities obtained from the analytical form of the distribution function.

With reference to the Appendix, the answers to the questions are:

i) there are 1.3×10^{5} drops less than 10μ per acre from the given volume of one gallon per acre;

ii) there is 0.11% of the total number of drops with diameters less than 10μ;

iii) there is 7×10^{-6}% of the total volume occurring from drops less than 10μ in diameter.

.....2

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The first solution regarding the total number of drops is the least reliable, but the remaining two solutions are quite accurate from the given droplet diameter data.

I trust that this letter with the accompanying Appendix is sufficient but if further clarification is required, please feel free to call again.

Yours truly,

A.M. Drummond,
Flight Research Laboratory.

AMD/pac
encl.

The following data for the drop spectrum for Dipel 88 from Micronair atomizers at one gallon per acre were supplied by Dr. J.A. Armstrong.

Class No.	Class Diameter Min. μ	Max. μ	RF Relative frequency	D_c central class dia. μ	
1	0	35.09	.0240	17.55	-13.0177
2	35.09	58.48	.0528	46.79	-13.7849
3	58.48	81.87	.0959	70.18	-13.9989
4	81.87	105.26	.1079	93.57	-14.4563
5	105.26	128.66	.1775	166.96	-14.4048
6	128.66	152.05	.1103	140.34	-15.2450
7	152.05	175.44	.1127	163.74	-15.5319
8	175.44	198.83	.0719	187.12	-16.2483
9	198.83	222.22	.0600	210.52	-16.6649
10	222.22	245.61	.0528	233.91	-17.0034
11	245.61	269.01	.0432	257.30	-17.3947
12	269.01	292.39	.0360	280.68	-17.7510
13	292.39	315.79	.0120	403.08	-19.0097
14	319.79	339.18	.0144	327.46	-18.9756
15	339.18	362.57	.0144	350.85	-19.1136
16	362.57	385.97	.0048	374.24	-20.3412
17	385.97	409.36	.0048	397.64	-20.4625
18	409.36	432.75	.0024	421.03	-21.2700
19	432.75	456.14	0	444.43	-
20	456.14	479.53	.0024	467.80	-21.4807

The Nukiyama - Tanasawa distribution function has the form

$$dn = aD^2 e^{-bD} dD \qquad (1)$$

where dn is the number of drops with diameter between D and D+dD with a and b being two parameters.

Equation (1) is written in a form suitable for the above classified data:

$$L = \frac{\ln \Delta n/n}{D_c^2 \Delta Dc} = \ln \frac{a}{n} - bD_c \qquad (2)$$

The quantity L is plotted versus D_c on the attached graph. A least squares fit to the first 18 classes provided a slope b = .0200. A similar fit to classes 4 to 15 inclusive also gave b = .0200.

From (1),

$$CF(D) = \frac{\int_o^D D^2 e^{-bD} dD}{\int_o^\infty D^2 e^{-bD} dD} = 1 - 3^{-bD} \quad 1 + bD + \frac{(bD)^2}{2} \qquad (3)$$

$$CV(D) = \frac{\int_o^D D^5 e^{-dD}}{\int_o^\infty D^5 e^{-bD} dD} = 1 - e^{-bD} \quad 1 + bD + \frac{(bD)^2}{2} + \frac{(bD)^3}{6} + \frac{(bD)^4}{24} + \frac{(bD)^5}{120} \qquad (4)$$

where CF(D) and CV(D) are the cumulative relative frequency and volume respectively of drops 0 to D. It is seen that with b known and D = 10μ, the calculation of answers to questions (ii) and (iii) is direct from (3) and (4).

From equation (1),

$$V = \frac{\pi}{6} a \int_o^\infty D^5 e^{-bD} dD \qquad (5)$$

$$\text{and } n = a \int_o^\infty D^2 e^{-bD} dD \qquad (6)$$

from (5) and substituting in (6) yields

With V = 1 gallon, and assuming Dipel 88 has the same density as water,

$$V = 4.54 \times 10^{15} \mu^3$$

From (7), with $b = .02 \mu^{-1}$,

$$n = 1.16 \times 10^9$$

Hence, the number of drops below 10 μ is n times CF(10):

$$= .0011 \times 1.16 \times 10^{9}$$

$$= 1.3 \times 10^{6} \text{ per acre}$$

The volume mean diameter is obtainable from (1) as 3.9148/b. Hence,

$$\text{Volume Mean Diameter} = \frac{3.9148}{.0200} = 195.74\ \mu$$